Biotechnology for Zero Waste

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Emerging Waste Management Techniques

Edited by Chaudhery Mustansar Hussain and Ravi Kumar Kadeppagari

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Contents

Foreword

This book reveals innovative biotechnology tools for *Zero Waste Drives*, providing an integrated approach for biotechnology tools, methodology, and indicators for waste management practices and evaluating the advanced biotechnology and other transformational options. The new concept of *Zero Waste* is a sustainable approach to minimize the waste and making the world better and currently is being adopted in various sectors like mining, urbanization, manufacturing, agriculture, etc. Zero waste approach looks wastes as salvageable resources, which contain valuable nutrients, bioactives, industrial chemicals, and precious metals. Most of the zero waste drives are nowadays focused on optimum recycling, reuse, and resource recovery, ideally leading to the zero waste manufacturing as a futuristic approach. Among them, biotechnological approaches for reaching zero waste are more eco-friendly and sustainable, being based on the recovery of energy and biofuels from agricultural, urban, and food wastes. In whole, bioconversion technologies like bioleaching, biosorption, and bioremediation can be used to obtain valuable products from different wastes and these technologies use different organisms and enzymes. Classic examples are the enzyme-based technology for the recovery of ethanol from lignocellulosic waste, bio-H_2 production by dark fermentation process and recycling of used cooking oil as fuel, microbial-enzymatic degradation of plastic, creation of biodegradable polymers or bioremediation of pesticides, energy generation from biowastes, among many others, described in this book. Economic aspects and commercialization of zero waste biotechnologies are also discussed.

I consider this monograph as "all-in-one" handbook in the area of zero waste approach, discussing emerging biotechnological and nanobiotechnological approaches for futuristic greener and sustainable future with zero emissions and production of marketable products from wastes.

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Preface

Zero waste should be a sustainable approach to minimize or nullify the waste and making the world better. This concept is being adopted in various sectors like mining, urbanization, manufacturing, agriculture, etc. Though zero waste manufacturing is believed to be the best and futuristic approach, most of the zero waste drives are currently focused on optimum recycling, reuse, and resource recovery. Manufacturing scrap, e-waste, discarded constructional materials, plastics, domestic, agri-food waste, and sewage have been haunting because their disposal affects the environment. Different physical and chemical methods to tackle these wastes by recycling and resource recovery in turn generate hazardous chemicals, emissions, and accessory wastes which are not eco-friendly.

Biotechnological approaches for reaching zero waste are more eco-friendly and sustainable. Research has been conducted on the recovery of energy and biofuels from agricultural, urban, and food wastes since long, and it has been practiced quite well, though enzyme-based technology was developed recently for the recovery ethanol from lignocellulosic waste. Bio-H_2 was produced by dark fermentation process, and recycling of used cooking oil as fuel is gaining momentum. Zero waste approach should look wastes as salvageable resources, which contain valuable nutrients, bioactives, industrial chemicals, and precious metals. Bioconversion technologies like bioleaching, biosorption, and bioremediation were used to obtain above valuable products from different wastes, and these technologies use different organisms and enzymes. However, composting has been used for converting agro-food waste into biofertilizers since long time. Submerged and solid-state fermentation technologies were used for the biotransformation of agro-food wastes into useful biochemicals and biopolymers which can be used for making biodegradable packaging materials. Plastic waste is one among the major current threatening problems to environment. Recently, Microbes and their enzymes were explored for the degradation of plastics, and microbes were used for the production of biodegradable plastics, though it was not economical. Microbes were also used in the bioremediation of pesticides which originate as accessory contaminants of agricultural practices. Biopulping and biofiltration were also applied for processing agro wastes. In this book, biotechnological approaches for reaching zero waste will be discussed in detail.

This book was divided into several parts focusing on recent advancements in biotechnology for zero waste drives. Biotechnological approaches like anaerobic co-digestion, integrated biosystems, immobilized enzymes, zero waste biorefineries for circular economy, membrane bioreactors, microbial fuel cell technology for energetic valorization, biosorbents, bio-diesel, biofunctionalized nanomaterials for bioremediation, etc. for zero waste drive were brought in.

Part I

Modern Perspective of Zero Waste Drives

1

Anaerobic Co-digestion as a Smart Approach for Enhanced Biogas Production and Simultaneous Treatment of Different Wastes

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1.1 Introduction

The world has witnessed tremendous growth over the past hundred years fueled by richness of earth's natural resources, but now we stare at the bleak prospects of exhaustion due to overutilization. With future economies balanced precariously on cost of fuel, with increasing demand for energy, ever-increasing annual fuel consumption, limited natural resources, volatility and disruption in fossil energy supplies, need of clean technologies has certainly driven us toward a pragmatic approach for optimized and proper use of natural resource for a sustainable ecosystem. Insightful planning and innovative methods are essential to enhance energy production in order to meet surge in future energy demands. Another scourge of the modern society is waste management; especially in the developing economies punctuated by improvement in individual purchase parity, it has led to tripling of waste generation per person just over the last one decade. An attempt is made in this chapter to link these two possible issues of fuel generation and waste management through a biotechnological intervention. The era of biotechnology as a futuristic technology strives to tap the service of the potential saprophytic microbes, which not only hastens the recycling of dead organic matter but can provide the fuel for running the future economy.

1.1.1 Biodegradation – Nature's Art of Recycling

The elemental components of our periodic table have finely blended the earth into molecules of infinite diversity. The organic forms of molecules are the basis of life existence in which the principal elements carbon, hydrogen, nitrogen, and oxygen have a subtle role in the formation of living system. The photosynthetic forms of life are one of the biggest producers of the organic matter, and it comes with an inherent clause of undergoing natural degradation over a period of time. This biodegradation is a very important invention of the nature, for, without recycling, a continuous existence of new life over millions of years would have been impossible. Microorganisms

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play a pivotal role in this process of biodegradation, without it recycling would have been unimaginably slower.

1.1.2 Anaerobic Digestion (AD)

Naturally existing anaerobic ecosystems such as paddy fields, swamps, lakes, ponds, intestine of ruminants, and ocean sediments rich in dead organic matter have paved way for microbes especially the archaeal obligate anaerobes-methanogens, mutual togetherness with other prokaryotic anaerobe leading to the production of methane. Though it can be attributed as a natural process, it leads to release of methane, a potential greenhouse gas capable of global warming far many times higher than carbon dioxide (CO_2). Anaerobic digestion (AD) as a technology refers to a provision of a closed condition for efficient digestion of the organic waste and to collect the by-product, methane.

The benefits of AD are immense for both the economy and ecosystem:

- Firstly, the digestion takes place in a closed environment, thereby preventing air pollution from obnoxious gases or disease-spreading germs.
- There is no issue of leachate escaping into water bodies and thus prevents open water body pollution.
- No underground seepage and pollution of groundwater.
- Faster degradation of organic matter compared with composting (aerobic).
- The AD process can be easily monitored circumventing the problems, for example, seasonal variation in temperatures.
- A microbial consortium can be developed, and it would aid in continuous and efficient digestion of waste.
- Biogas production with a range of fuel applications.
- Downstream processing is not required as biogas collects in the head space and is siphoned off for clarification and usage.
- Further effluent treatment would not be necessary as the slurry can be used as organic manure.
- Pathogens are inactivated, thus rendering the digestate harmless and safe.

The drawbacks are few, but critical enough to be highlighted:

- Limited access to high-quality feedstock that is free of contamination
- Non-perennial aspects of feedstock
- Transportation costs
- Long-term sustainable biomethanation
- Unexpected digester failures
- Maintenance of high fuel quality
- Issues of multistakeholders (in case of co-digestion)

The first four issues are related to feedstocks and its management, while the last three issues are related to lack of good microbial inoculum. Thus in this chapter, these two aspects of feedstock and real-time monitoring of operational parameters are dealt in detail.

Technical issues could be overcome by reliable public–private partnership, government initiatives, financial supports followed by technological advancement.

1.1.3 Sustainable Biomethanation

Sewage water treatment plants mandatorily follow AD for sludge treatment, and the ensuing methane-based gas is used for running wastewater treatment plants (WWTPs), though this is in principle, but the scenario is that many WWTPs struggle to maintain sustainable digesters, which are progressively jeopardized by frequent reactor failures. Biogas plants were ideally found to be an alternate source for renewable energy and were operated widely in rural areas of India; however, over the last few decades, it has taken a back seat, partially attributed to:

- digester operational instability,
- nonhomogeneous substrate,
- lack of good microbial inoculum,
- promotion and easier availability of LPG,
- deeper reach of electricity to remote rural areas,
- dip in active promotion of AD and their significance, especially in rural areas.

Renewed interest in AD stems from the problems of rapid urbanization and urgent need of waste management. Running successful biogas digesters depends mainly on two important factors: nature of substrate and the quality of inoculum. Real-time monitoring emphasizes on the following factors:

- balanced micro- and macronutrients,
- efficient microbial inoculum,
- digester design optimization,
- optimized organic loading rate (OLR),
- efficient monitoring of critical parameters (pH fluctuations, temperature range, total solids (TSs) utilization rate, volatile solids (VSs) accumulation and dispersal rates, microbial profiling: that is, eubacterial versus archaeal load ratio),
- continuous evaluation of digester performance [rate of biogas production, methane percentage, reduction in total solids, reduction in chemical oxygen demand (COD)],
- Reducing inhibitor concentrations.

1.2 Anaerobic Co-digestion (AcD)

Biogas technology is a perfect example to emphasize on zero waste concept, conversion of waste into fuel, and even the final digested remnant slurry's immense value as organic manure, which is potentially free of pathogens. Mono-digestion refers to the classical way for biogas production from a single type of feedstock while a co-digestion refers to mixing of two different feedstocks in a digester for biogas production. Co-digestion was initially planned to balance a carbon-to-nitrogen

(C/N ratio) content of the feedstocks, as few feedstocks are either rich in carbon (agricultural) or found to be rich in nitrogen (animal waste). High C/N ratio of feedstock will ultimately lead to reduction in microbial load due to overall nitrogen deficiency while lower C/N can result in ammonia poisoning that could particularly affect methanogens leading to lower biogas production. Excess of carbohydrates in feedstocks needs shorter retention time (RT) in digesters attributed by its quick oxidation, while excess protein content leads to lesser biogas production ascribed to accumulation of toxic levels of ammonia; on the other hand, excess lipids though results in higher biogas production but RT nearly doubles [1] further characterized by high concentrations of volatile fatty acids (VFAs) and low pH, thus leading to a consensus that excess of any nutrient cannot be beneficial for biogas production [2]. The anaerobic co-digestion (AcD) thus offers an opportunity to modify the composition of the waste to our need that suits our microbial consortium very well, and in this regard, C/N ratio can be altered to the optimum range. WWTPs around the world have increasingly opted for co-digestion to increase biogas output, and a WWTP in Mesa, USA, has successfully evaluated co-digestion of commercial solid food waste with sewage sludge in pilot-scale anaerobic digesters [3]. Lipid-rich restaurant waste has been co-digested with sewage sludge [4].

1.2.1 Zero Waste to Zero Carbon Emission Technology

The biogas as renewable energy can contribute in a big way to meet an overzealous future goal of zero emission economy by supplying fuel to major contributors of greenhouse gas emissions such as transportation and heavy industries (power plants, steel and cement industry, to name a few). Presently the biogas, which is rich in methane, burns clean and helps in the cutdown of carbon emissions at a domestic level. It is evident now as many countries have taken initiatives in setting goals for tapping the renewal energy resources, the Australian water industry is said to have generated 187 GW/year of electricity from biogas via WWTPs and an additional 5.5 GW/year through AcD [5]. Channeling of organic wastes from land fill, restaurants, other urban wastes toward existing and time-tested WWTPs is advocated by many countries and has envisioned zero carbon emission by the year 2040. Figure 1.1 summarizes the scope of AD.

1.2.2 Alternative Feedstocks

Feedstock refers to the particular form of organic waste available for AD but if left unattended can lead to environmental pollution. United State Environmental Protection Agency (USEPA) has assigned each feedstock a unique RIN (renewable identification number) that helps to rate how much of greenhouse gas it can emit in comparison to fossil fuel [3]. Cattle dung has been traditionally preferred as the typical substrate for AD; however, in terms of substrate quality it represents the semi-digested material excreted by ruminants. However, the advantage of cattle dung as a substrate is that it has inherent microbes catered from intestines of ruminants specialized in AD and biogas production. Any substrate for AD is

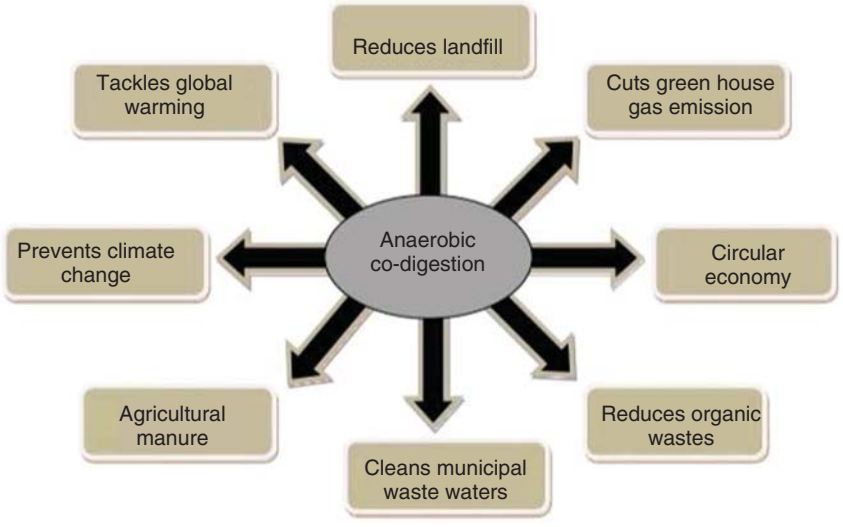

Figure 1.1 Applications of anaerobic co-digestion.

basically referred to as organic wastes generated at its source; it can be available in many forms and its characteristic depends on the source. It can be available from a single crop agricultural waste to a blended form as municipal solid waste (MSW/urban waste) categorized in terms of complexity in defining the exact composition of waste. Emphasis has been laid on alternative feedstock such as:

- agricultural residues (energy crops),
- commercial food waste (canteen/mess/restaurant),
- retail wastes/fruits and vegetable wastes (peels, press cake),
- animal waste (ranch waste/poultry waste/livestocks processing wastes),
- effluent treatment in industries (dairy wastes, bioprocess industry, sugar industry),
- garbage waste (MSW),
- sewage sludge (WWTP), etc.

It is still contradictory to classify based on source/origin because some untreated waste such as food waste may ultimately end up in land fill or may be diverted to WWTP. The wastes are characterized based on principal nutrient content for microbes, namely carbohydrates, proteins, and fats. Animal wastes are protein-rich, while agricultural wastes are carbon-rich with cellulose, hemicelluloses, lignin, etc. Dairy-industry-generated wastes are fats and protein-rich. Thus each type of feedstock is unique in composition and based on that requires different approach for digestion. Feedstock composition should be assessed for certain inhibitors of methanogenesis, such as nitrates, sulfates as they could support growth of denitrifiers and sulfate reducers at the expense of methanogens [6, 7]; this tends to have a drastic effect on hydrogen foraging methanogen population leading to suboptimum biogas production. Though the organic waste is abundant in nature, its

availability at a particular location could vary on a daily basis. Moreover, substrate heterogeneity, seasonal variation, and feasibility of transportation of waste from source are also to be coordinated. The idea of setting up the AD at the source of waste generation is a viable option; still the supplies could be erratic or inconsistent. The opportunity to go for co-digestion not only helps in circumventing the problem of nonavailability of single substrate but also helps in managing different wastes generated at source efficiently.

1.2.3 Microbiological Aspects

The emphasis of the role of microbes is well documented in every successful biogas digester. There is a systematic and sequential breakdown of complex organic waste into methane carried out by four metabolically distinct bacterial groups:

- hydrolyzing bacteria: complex carbohydrates, fats, and proteins converted to simple sugars, long-chain fatty acids (LCFAs) and amino acids;
- acidogens: lead to the accumulation of VFAs, alcohols, and carbonic acids;
- acetogens: further degradation results in acetic acid, hydrogen, carbon dioxide with trace amount of ammonia, H_2S, etc.; and
- methanogens: scavenge on H_2 and C1 and C2 carbon compounds for energy leading to production of methane.

Each of the aforementioned groups plays a pivotal role in AD and inactivation of any one group could possibly lead to accumulation of intermediate compounds impacting the outcome of the digester performance, while methanogen biomass ratio is miniscule in comparison to other groups [8]; still their influence is immense and found to be critical for sustainable biomethanation [9].

1.2.4 Strategies for Inoculum Development

It is highly impossible to define the exact microbial composition of any anaerobic digester, culturing techniques in coordination with molecular diagnostics can aid in identification, but never have we deduced the true potential population of AD. Inoculum for any biogas digester is usually sourced from ruminant fluid, municipal WWTPs, landfill leachate, or sludge collected from any preexisting active biogas digester. It is primarily important to relate inoculum with its role in biogas digesters, for example, an inoculum collected from WWTP may have few cellulolytic bacteria and thus may not lead to a sustainable biomethanation of agricultural wastes. Ruminant intestines harbor a natural population of methanogens, hydrolytic and other fermentative anaerobes, which cater to efficient biogas production and general success only for cattle-dung-based digesters; the same success is difficult to reproduce when inoculum from cattle-dung-based digester is added to digest poultry waste or dairy-waste-based digesters. Microbial population may vary even between sample inoculum and digester, for example, fresh cattle dung is rich in hydrogenotrophs (93–80%) [10] compared with acetoclastic methanogens (6–20%) [10] (Reasons being nonavailability of acetates, which are being reabsorbed by ruminant intestines along

with other VFAs leading to the formation of animal fat) [10] while active digesters exhibit higher load of acetoclastic methanogens in comparison to hydrogenotrophs.

Even within digesters the microbial population may change, which can be attributed to the complex metabolic processes leading to accumulation of various intermediates that continuously influence the dynamics of microbial population. Hence, there is need for inoculum development, which involves acclimatizing a set of microbes to the digester environment; this could be done by pooling in a set of potential dominant anaerobes isolated from successfully running digesters to form a working consortium. Such microbial consortium had proven to give higher yield of biogas and better degradation of biological waste [11].

Consortium development is mostly targeted on methanogens as they are found to be the sole reason for biogas digester failure. The consortium has to be tested under lab-scale digesters for their efficiency before implementing in larger-scale biogas digesters. Care should be taken while developing consortium to select potential strains capable of withstanding digester environment fluctuations in pH and temperature, resistance to inhibitors, nutritionally diverse, and can syntrophically coexist. Potential strains of methanogens have been mostly identified to be hydrogenotrophic methanogens, acetoclastic and methylotrophic methanogens. The most abundant species among hydrogenotrophic methanogens are *Methanobacterium*, an hydrogen foraging methanogen that is known to dominate rumen intestinal environment while its role in a typical biogas digester is overshadowed by acetate utilizing methanogens (*Methanosaeta*, *Methanosarcina*, and *Methanospirillum*) that represent nearly 75% of the methane produced in digesters, still hydrogenotrophs are crucial for interspecies hydrogen transfer between syntrophic bacteria that could help diminish the concentrations of fatty acids in digesters [1], especially propionic acid as its presence can upset digester performance.

As mentioned earlier, there are four groups of bacteria in a synergetic action in digesters, each group of bacteria have their own physiological requirements and show varying degree of growth efficiency and wide range of sensitivity to environmental parameters. Acidogenic bacteria are among the fastest-growing organisms, generally leading to quick accumulation of acid end products. While acetogenic bacteria and methanogens are slow-growing organisms, to further complicate the matter, the methanogens are found to be very sensitive to changes in environmental parameters, which is detrimental for sustained biomethanation. Hence, inoculum is a critical parameter for determining the efficiency of anaerobic digesters. There is still diverse population of microbes that could not be cultivated and assessed from AD, and hence, any potential microbial consortium that is developed in laboratory should be considered as an supplementary feed and cannot by itself regarded as sole group of organisms that could digest waste in a digester [12].

1.2.5 Real-Time Monitoring of AcD

Real-time monitoring is essential for sustainable biogas production, will help us to continuously evaluate the digester performance, and help us to take immediate

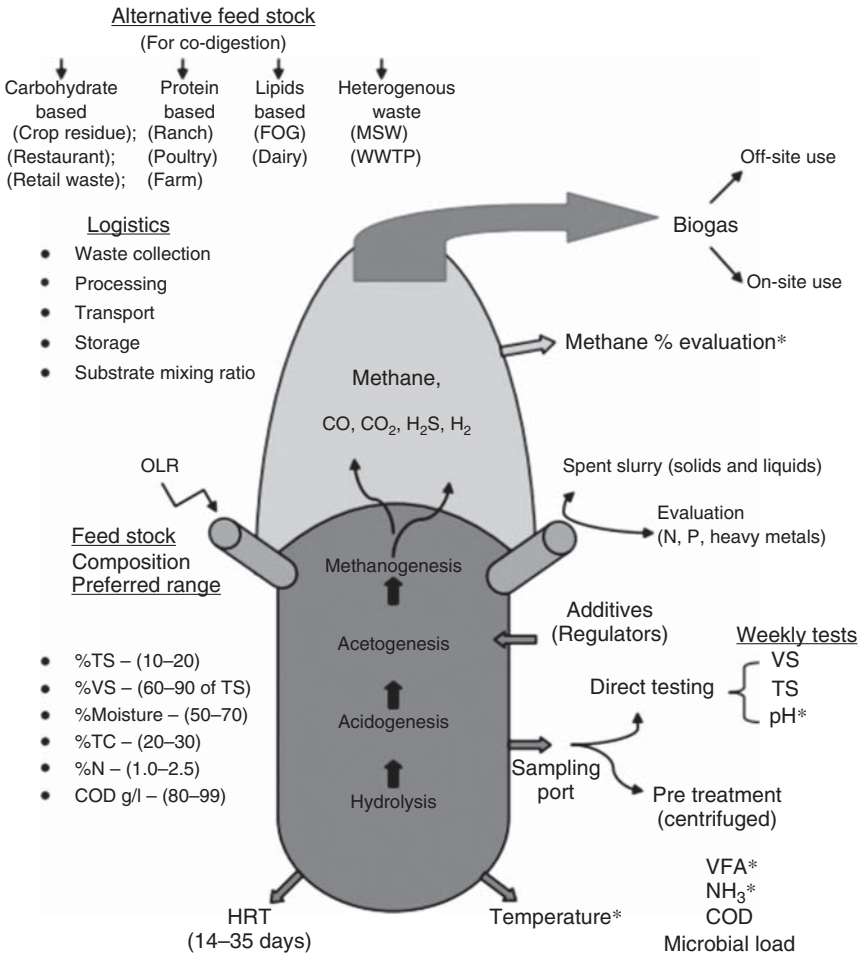

Figure 1.2 Real-time monitoring of anaerobic digesters. * Daily tests. FOG – fat, oil, and grease; P – phosphorus.

remedial action to circumvent the problem and prevent digester failures (Figure 1.2). Direct monitoring of microbial growth is not always a feasible option, as it requires an equipped anaerobic laboratory for studies, further the problems are compounded by slower growth rate of methanogens as it takes days to evaluate the exact microbial content of the digester. Molecular techniques such as fluorescence *in situ* hybridization (FISH), 16S rRNA, real-time polymerase chain reaction (RT-PCR), and denaturing gradient gel electrophoresis (DGGE) aid in assessment of microbial load feasible mostly for laboratory studies and applicable to large-scale biogas digesters.

1.2.5.1 The pH Fluctuations

There are other ways of monitoring bioreactor performance; these parameters are simple and can efficiently diagnose the current status of the working reactors. pH is one such factor that can be readily checked at regular intervals; neutral pH is

preferred for sustainable biomethanation; and any variation in pH can drastically cut down methane production. Fluctuations in pH are one of the biggest problems associated with AD and mostly shift toward lower pH, which is directly attributed to accumulation of VFAs. Sometimes pH may shift toward alkalinity contributed by accumulation of ammonia. This pH problem is due to microbial metabolism, especially by higher growth activity of acid-producing bacteria, compounded by the absence of buffering agents. Simultaneous degradation of proteins can lead to formation of ammonia that could help in balancing of pH in a digester averting shift toward acidic range. As mentioned earlier, too much of protein degradation in digesters can lead to excessive ammonia shifting pH toward 8.0 that shuts down microbial activity. The pH fluctuations should be seriously dealt with and a delay could permanently alter the microbial population of the digesters and sometimes cause irreversible damage to digester performance. Either way the methanogens are said to very sensitive to pH change and the problem can be overcome by neutralizing the pH with an alkali or a weak acid, but could turn to costlier affair to invest on alkali treatment, which is not generally recommended. A robust and an efficient microbial population of VFA converters are essential, while few digesters have adopted for dual digesters/two-stage digestion for circumventing the pH problem.

1.2.5.2 Carbon–Nitrogen Content

It is essential to know the total carbon (TC) and nitrogen (N) content of the feedstock while the optimum C/N ratio for AD should preferably be in a range of 20–30. And increase in the value signifies the problem of nitrogen shortage leading to lesser load of microbes and process of AD getting delayed while lower ratio could imply higher microbial growth but the biogas could abruptly stop due to problems associated with by-products of protein degradation significantly changing the digester balance toward inactivity. The AcD thus plays a crucial role as we can finely balance the carbon–nitrogen ratio for optimum biogas production.

Anaerobic digesters can work in a wide range of temperature; however, it been noted that temperatures below 20 °C can affect the efficiency of digesters by considerably slowing down the process; still in natural habitats, methanogenesis is found to happen significantly at low temperatures and over a period of time has contributed to global warming [13].

1.2.5.3 Temperature

Eightfold reductions in COD can be observed with mesophilic and thermophilic digestion at hydraulic retention time (HRT) of 35 days, while digesters at lower temperature are stable for a longer period of time more than 45 days [12]. Digesters around the globe are mostly operated in mesophilic conditions with recommended temperatures of around 35 °C, while faster digestion is generally reported at thermophilic temperatures of 55 °C but that comes with an inherent need of heat exchangers for temperature maintenance that can either shoot up or drastically fall reflecting microbial metabolism. Here biogas can be self-employed for heating the digesters, and thus it could be self-sustained process without much investment. It has been noted that the microbial population dynamics vary greatly between

mesophilic and thermophilic digesters, for example, at 55 °C, hydrogenotrophs are found to dominate and if properly supplemented by syntrophic acetate-oxidizing bacteria [14] could even lead to sustainable biogas production in complete absence of acetoclastic methanogens.

1.2.5.4 Volatile Fatty Acids

Efficient monitoring of digesters can also be carried out by constant evaluation of VFA content of the digesters. Though VFA accumulation above 2000 mg/l leads to digester failures, still it should be kept in mind that the same VFA gets finally converted to methane, in fact carbon atom of VFA is the principal source for methane production. The answer lies in the nature of VFA that accumulates in the digesters; most preferred form of VFA is acetic acid as it is the essential substrate for methanogens.

Fatty acid oxidizing bacteria breakdown LCFA to acetic acid, and these bacteria are inherently resistant to the toxic effects of accumulated LCFA. It has been noted that microbial load of fatty acid oxidizing bacteria fluctuates within the digesters directly influencing LCFA conversion rate, and their total absence in digesters leads to digester failures. Fatty acids oxidizing bacteria have been identified to be either producer of hydrogen (obligate hydrogen-producing acetogens [OHPAs]) or hydrogen consumer (homoacetogens) but certainly lead to the formation of acetic acid. Not all VFA contributes to methane, certain volatile acids have a deleterious effect on the overall process especially propionic acid, and its accumulation decreases the pH to an extent of inhibiting the growth of methanogens, leading to fall in biogas production.

1.2.5.5 Ammonia

High protein content-based feedstocks on AD can trigger an alkaline shock with accumulation of ammonia or ammonium ions, at about pH 8.0 the drastic reduction in microbial activity can be noted and with pH reaching 8.5 can completely deactivate methanogens thereby completely stopping methane production. The problem can be circumvented by balancing C/N ratio of the feedstock; immediate actions would be to reduce loading rate and further diluting the digester content. This corrective action can quickly adjust the pH to optimum range, it is imperative that the microbial consortia play a significant role in AcD.

Both ammonia and VFA thus play a crucial role and are intricately related to pH fluctuations; a VFA/ammonia ratio of 0.1 is preferred for a balanced sustainable digesters and increase to 0.5 indicates that the digesters could fail and further rise can completely stop biogas production.

1.2.5.6 Organic Loading Rate

Continuously operated digesters require balanced input of feedstock, (feedstocks/organic) loading rate (OLR) refers to the rate at which the feedstocks are fed into the digesters. OLR depends on the waste composition and is directly correlated to microbial growth rate, substrate conversion rate and evaluated by the rate of methane production. Excess OLR can dilute the microbial load, reduce

digestion, foaming, and lesser yield of methane. OLR is further related to HRT, which implies the time taken by the digester for maximum gasification of the feedstocks. Shorter RT is preferable to avoid accumulation of fatty acids and toxins but way less than shorter RT can lead to microbial washout. Minimum one day RT is enough for stable buildup of fermentation bacteria especially for protein and nonfiber carbohydrates-based feedstocks; cellulose and hemicelluloses may require two to three days to establish the process, while fat-based feedstock may require longer RT of five days.

Complete gasification of waste can be achieved in a digester by increasing RT to 35 days (in case of batch digestion); the process is influenced by temperature: higher the temperature, shorter the RT, and RT of more than 35 days is required for psychrophilic temperature. Longer RT leads to improvement in quality of biogas in terms of methane concentration, shorter RT may generally exhibit 70% methane content while the percentage of methane tends to increase with longer RT. Total solid (TS) of more than 30% is not preferred for AcD as it leads to the problem of mixing concentrated pockets of temperature and pH burst in a continuously operated digesters depends on feedstock composition. The volatile solid (VS), which is a part of TS, is generally preferred in a range of 60–90% for efficient biogas production and for optimum microbial growth.

Pretreatment of feedstock is essential to minimize the natural flora on the surface of substrate as it will hinder the role of potential consortium developed for the purpose that is already active inside the digesters.

1.3 Digester Designs

The earliest digesters were simple in design with a digestion chamber, an inlet for feedstocks, and two outlets, one for spent slurry and one for biogas. The appropriate modeling of anaerobic digesters is imperative for biogas production. Digesters are designed with the view of maintaining strict anaerobic conditions and for collection and retrieval of biogas. The digesters can be operated in batch or continuous phase. Anaerobic biogas digester such as the one used in WWTP is distinct as it is continuously fed with heterogeneous liquid wastes, microbes agglomerate to form the granules (sludge) that set in to form a layer/blanket with a constant upflow hydraulic regime [15]. WWTPs around the world have opted for upflow anaerobic sludge blanket (UASB) digester for anaerobic treatment, which has been found to be cost-effective and emphasizes the role of microbial granules (solid phase) that knit into a group of specialized agglomerated bacterial biofilm [16].

Expanded granular sludge beds (EGSBs) are a modified version and next-generation biogas digesters with enhanced flow rate of liquid waste that could result in mixing of sludge particles establishing contact with nutrient for the purpose of breakdown. Further efforts have been taken to make thin, lighter-weight biofilm of uniform thickness (granular sludge) for better fluidization and at lower energy expenses in the form of inverse fluidized bed reactors (IFBR), which would reduce HRT at a higher OLR that was initially carried out for distillery effluent [17].

Digesters with constant mixing can take up higher OLR, and it has been reported that OLR increased up to 300 kg COD/m^3/d using super high rate anaerobic bioreactor (SAB) that works on a principle of spiraling baffle running through the middle of the digester body [15].

Mixing helps in uniform distribution of feedstocks during AcD and provides access of metabolic intermediates, microbial interaction; prevents stratification and release of trapped methane that has been observed with completely stirred/mixed tank reactors (CSTRs) [4]. Mixing of digester content can occur naturally to some extent by rise of methane bubbles, which is by itself not sufficient for optimum biogas production, hence auxiliary mixing is essential. It has been reported that intermittent mixing leads to better biogas production in comparison to continuous mixing [4].

As we know that four groups of microbes are responsible for biogas production, an attempt has been made to build two-stage digesters basically dividing microbial role of hydrolysis/acidogenesis and acetogenesis/methanogenesis [18]. The first-stage hydrogenic reactor (HR) and the second-stage methanogenic reactor (MR) are linked but operated at different pH [19] and only recommended for digesting sugar-rich feedstocks [20]

1.4 Digestate/Spent Slurry

The effectiveness of AcD can be evaluated based on the quality of the digestate/spent slurry of the digester. The composition of the digestate will naturally differ from initial feedstock, there should have been a drastic reduction in total solids content and COD. With richness in nitrogen and potassium and low on carbon content, the digestate can be an excellent source for organic manure for crop production, could support by minimizing usage of chemical fertilizers, and bedding can prevent soil erosion and help to retain soil fertility [21]. There have been few concerns on long-term impact on usage of manure as fertilizer:

- chances of altering preexisting and natural soil microflora,
- impact of excessive nitrogen emissions from manure applied farm lands,
- presence of recalcitrant compounds, and
- slow degrading remnant organic matter contributed by manure.

There has been considerable research over the aforesaid drawback, and we have conclusive results with reports stating minimal or of minor relevance with no major impaction on overall soil fertility [22]. Manure can be packed and stored over of period of few months without much loss in nitrogen content and has been evaluated for storage during different seasons for their efficacy [23]. The grade of the manure would vary and generally rely on the nature of feedstocks digested, for example, AD of agricultural feedstocks may yield manure with less nitrogen content while livestocks waste or dairy waste manure may be nitrogen-rich, especially liquid compost; accordingly soil management plan is essential to determine the quality and quantity of manure and its influence on appropriate soil type before any large-scale application of manure over farm land [24].

1.5 Conclusion

Circular economy is mooted to loop in the excess energy dissipated from human activities, which gets dispersed into environment in the form of greenhouse gases leading to global warming. International Renewable Energy Agency (IRENA) has called for a global energy transition toward complete de-carbonization of energy sector by the year 2040. Water treatment boards around the world have partnered with various environmental technological companies and have initiated zero waste movement, an ambitious plan to divert organic waste from landfills and incinerators to AcD. Steps have been taken to reduce carbon foot print by investing in infrastructural upgradation of AcD especially for treatment of commercial food waste with existing wastewater anaerobic sludge treatment plants. AcD has been identified as a key technology to attain net zero. Many countries have even linked bio-methane produced from AcD to the national grid for gas transmission. Few nations have reported more than 100% growth in popularity of AD and have set up hundreds of digester plants and are operating them successfully.

Steps are being taken by the scientific community to address the issue of natural methane emission into atmosphere from organics-rich land environment, water bodies and ocean sediments, substantial livestocks population, and man-made landfills. Methane mitigation efforts are taken on all frontiers to cut the flow of methane into the atmosphere that is presently contributing to global warming. One such technology is being reviewed for methane mitigation from cattle by supplementing feed with anti-methanogen IgY antibodies [25], while AcD is way forward envisaged for zero waste. Few logistics issues pertaining to feedstock and its transportation have already been highlighted earlier in this chapter, and this has to be addressed in future. In this regard, it can be noted that WWTPs are the best examples for case study to see through the reason for its success and it can be chiefly attributed to continuous supply of wastewater, sewage treatment plant (STP) generating uninterrupted solid sludge (feed stocks), digesters designed for retaining microbial granules, thus reducing energy and cost for transportation. And yet again linking other feedstock (like food waste) with WWTP leading to AcD has further enhanced the scope of the key technology for visualizing a world of net zero waste.

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2

Integrated Approaches for the Production of Biodegradable Plastics and Bioenergy from Waste

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2.1 Introduction

Production of biomaterials and bioenergy from the waste has reduced the environmental burden with respect to waste. Organic waste is highly valuable renewable source for the production of bioethanol or bioplastics which can be derived by fermenting organic waste with specific microorganisms. Technologies have added value to the waste by leading to biopolymers, biogas, biohydrogen, industrial chemicals, etc., from organic waste. Few biopolymers are getting produced through the accumulation of exopolysaccharides (EPS) on some microorganisms. Biopolymers are used in the manufacturing of packaging materials for food, chemical, cosmetics, and other industries. Biopolymers can also be used as absorbents and lubricants [1].

Biopolymers are produced by living cells and can be classified into three main classes such as polynucleotides, polypeptides, and polysaccharides. Polypeptides and proteins are polymers of amino acids and polysaccharides are linear or branched polymeric carbohydrates (starch, cellulose, alginate, etc.). The polyhydroxyalkanoates (PHAs), polylactides, and aliphatic polyesters are identified as bioplastic polymers due to their similarity in physical and chemical properties to conventional synthetic plastic. The production of PHA can be done using bacteria [1–3]. The promising results were obtained when wastewater and organic wastes like molasses, starch waste, dairy waste, food waste, etc., used for the production of biopolymers and bioenergy (biomethane and biohydrogen). Such waste substrates can be simultaneously used for the production of bioenergy and biopolymers [1]. Deriving of biodegradable plastics and bioenergy from waste is shown in Figure 2.1.

2.2 Food Waste for the Production of Biodegradable Plastics and Biogas

Sugarcane, potato, corn, and mixed food wastes can be efficiently used for the production of biodegradable plastics (PHAs). Biogas can be produced through the

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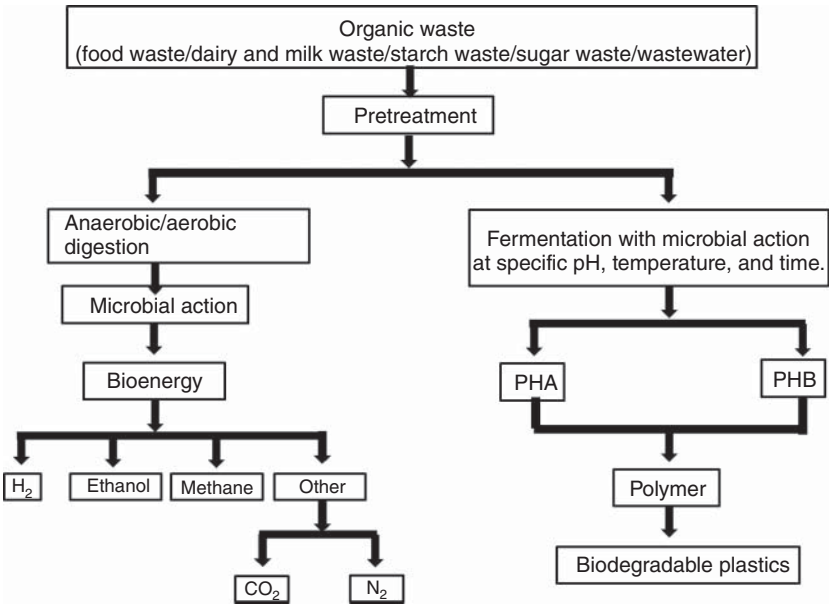

Figure 2.1 Schematic representation of deriving biodegradable plastics and bioenergy from waste materials.

anaerobic digestion (AD) of food waste and biogas can be subsequently used for the production of electricity or thermal energy (http://www.eesi.org/papers/view/fact-sheet-biogasconverting-waste-to-energy) [4].

2.2.1 Biodegradable Plastics from Food Waste

Food wastes are rich sources of oil, fat, mineral, protein, carbohydrates, and other components. Food waste can be converted into value-added products like PHAs, alcohols, gases, organic acids, etc., by fermentation and conditions like microbial composition, pretreatment, temperature, pH, humidity, oxygen levels, etc., need to be optimized for the efficient production of products from waste. For the commercial production of PHA from different kinds of sources, few bacteria were identified out of 250 natural producers of PHA. The *Pseudomonas oleovorans*, *Bacillus megaterium*, *Alcaligenes latus*, and *Cupriavidus necator* are the microbial strains most widely utilized for the production of PHA [5]. The food waste containing cellulose, fat, fatty acid, protein, and starch was used as substrate for growing bacteria, *Halomonas campaniensis* LS21 and *Halomonas hydrothermalis*, and approximately 70% of PHB production was observed at pH 10 and 37 °C. The PHB yield of 0.1 g/g of dairy waste was observed by the action of *B. megaterium* SRKP-3 strain [4]. An activated sludge can be used as source of microbes while fermenting food waste for the production of PHA. In contrast to the pure culture, the use of activated sludge as mixed culture removes the necessity for the aseptic conditions, which intern reduces the operating cost [6].

2.2.2 Food Waste and Bioenergy

2.2.2.1 Ethanol from Food Waste

Food waste should be pretreated to obtain simple waste since it will have complex lignocellulosic biomass. Pretreatment can be done by thermal process, enzymatic process, acid or alkali treatment to improve the digestibility of cellulose, lignocellulose, pectin, and starch present in the food waste. After the hydrolysis, the acquired mash can be subjected to the ethanol fermentation by inoculating with the yeast. After the fermentation, distillation process can be carried out for obtaining the pure ethanol. Food waste treated with amylase enzymes helped to obtain ethanol yields of 29.1–32.2 g/l [7, 8].

2.2.2.2 Food Waste to Biohydrogen

The dark fermentation is mostly used in biorefineries for the production of H_2 due to low energy requirement for the process. The macromolecules present in the waste need to be broken down into amino nitrogen and glucose before H_2 production by microbial fermentation. The hydrolysis of food waste can be completed by enzymes and heat treatment without harming bacteria which result in high H_2 production. The sonification of food waste enhanced H_2 production without having additional inoculum, and this study suggested that pretreatment is essential parameter to enhance H_2 production [7, 9]. The optimum production of H_2 was 120 ml/g of carbohydrate with 35.69 ml/h at controlled chemical oxygen demand (COD) of 200 g/l of food waste, and similar value of H_2 yield was also obtained at controlled moisture content of food waste. For optimal production of H_2 the required C : N ratio is up to 20. Food waste is very suitable feedstock for the production of H_2, due to the presence of high carbon content and indigenous microbial consortium [10].

2.2.2.3 Production of Biogas from Food Waste

The food waste is most promising for the production of biogas, due to its wide availability and heterogeneous composition with high energy content. The processing of food waste and a shredded municipal solid waste (MSW) by AD with an operation period of 20–40 days yielded 0.18 m^3 of CH_4/kg of volatile solid (VS) added. The 1 m^3 of biogas produced via AD is equivalent to about 21 mJ energy that is efficiently converted to electrical energy (2.04 kWh) at 35% process efficiency. A batch study on methanization of food waste for 10 and 28 days was conducted and observed the optimum CH_4 yield (0.435 m^3/kg VS) after 28 days of digestion with VS removal of 81%. A yield of 0.348 m^3/kg VS was observed after 10 days of digestion. Different research studies related to AD have proved that co-digestion of food waste with MSW has enhanced biogas yield by 40–50% in comparison to digestion of food waste alone [11]. Co-digestion batch tests with different combinations of sugar beet leaves and potato waste were conducted, and highest CH_4 yield of 0.68 m^3/kg VS added was observed for mixing at 16% : 24% total solid. The observed CH_4 yield from potato waste alone was 0.42 m^3/kg VS [7, 12].

2.3 Dairy and Milk Waste for the Production of Biodegradable Plastics and Biogas

Milk waste comprise of casein protein and lactose sugar. Two wild-type microorganisms, Lava DSM1034 and methyl bacterium sp. ZP24, are extremely efficient in acquiring PHAs from the lactose [13]. Various biopolymers can also be attained from dairy industrial effluents. Numerous microorganisms such as *Bacillus licheniformis* and *B. megaterium* can be used for the production of polyhydroxybutyrate (PHB). The PHB is a common member of PHAs family with monomers consisting of about four or five carbon atoms [14].

2.3.1 Biodegradable Plastics and Dairy Waste

The gram-positive bacterial strain, SRKP-3, which is similar to *B. megaterium* could potentially accumulate PHAs and it was isolated from brackish water. This organism could use dairy waste containing production medium for the accumulation of PHA granules. The strain, SRKP-3, produced maximum amount of PHB after 36 hours of inoculation into the medium containing dairy waste (350 ml/l), rice bran (40 g/l), and sea water (350 ml/l) at pH 9.0 [14]. *B. megaterium* is the first organism in which the synthesis of PHB was reported.

2.3.2 PHB Production in Fermenter

For the production of PHB in fermenter, excess carbon level was maintained by feeding dairy waste as the carbon source at 12th and 24th hours. Initially, the PHB yield was low and as the dairy feed was given the accumulation of PHB was increased. The pH was maintained at 9.0 consistently, during the accumulation of PHB. The maximum production of PHB obtained was 11.32 g/l at 36th hour and the synthesis of PHB decreased afterward [14].

2.3.3 Bioenergy from Dairy and Milk Waste

The hydrogen and methane can be mainly produced from dairy waste by aerobic and anaerobic bacteria (lactic acid bacteria) which are commonly available in dairy waste in high concentration and produce lactic acid by fermentation process (heterolactic or homolactic). The microorganisms of Lactobacillaceae and Streptococcaceae are most relevant for increasing the production of hydrogen gas, so increase in the population of lactic acid bacteria will increase the production of H_2. When the production of hydrogen was increased, the concentration of lactic acid will decrease. When *Clostridium* spp. (*C. clariflavum*, *C. thermopalmarium*, and *C. tyrobutyricum*) and *Sporanaerobacteracetigenes* join with members of *Tissierellaceae*, the production of CH_4 and H_2 detected. The *Clostridium clariflavum* fermentation results in the production of lactate, ethanol, acetate, CO_2, H_2, and also a small amount of formate. Fermentation of sugar by *Clostridium thermopalmarium* will yield acetate, ethanol, lactate, H_2, and CO_2 [15].

The AD of a mixture of buttermilk and mozzarella cheese whey amended with 5% (w/v) of industrial animal manure pellets with a culture of lactic acid bacteria (Lactobacillaceae and Streptococcaceae) for about 14 days increased the amount of hydrogen production (more than 10 ml H_2/g VS). During the incubation, a gradual decrease of lactic acid bacteria was observed with a simultaneous increase of *Clostridia* families (Clostridiaceae and Tissierellaceae). In inoculated sample of dairy waste, several archaeal genera were identified as compared to non-inoculated same samples of waste mixture. The *Methanoculleus* (methanogenic archaea) was a dominant genus during the production of methane, and relative abundance was increased to 99% at the end of the incubation time. This suggested that methane was formed from dairy wastes primarily by the hydrogenotrophic pathway in the reactors [15].

2.4 Sugar and Starch Waste for the Production of Biodegradable Plastics and Biogas

2.4.1 Sugar Waste

Sugar waste can be employed by the microbes as energy source which can be accumulated intracellularly. Sugar-rich wastes can also be used for the production of ethanol. Cellulosic sugar can be used for bioethanol production. This cellulosic material can be obtained as waste during extraction and mashing of the juice from cane sugar, beetroot, etc. [16, 17].

2.4.1.1 Sugar Waste and PHA

Bacterium (*Pseudomonas fluorescens* A2a5) was used to produce high amounts of PHB (up to 70% of dry cell weight) in sugarcane liquor medium. Bacterial cells in single or clusters were able to accumulate massive amounts of PHB. The doubling time for the strain A2a5 is around six hours and the sugarcane liquor medium was optimal for growth. The optimum temperature was around 20–25 °C and the strain A2a5 would not able to grow over 30 °C. The optimum growth was ensured at pH 6.5–7.0 with the PHB concentration of 31 g/l. In the 5-l bioreactor, a maximum cell dry weight (CDW) of 32 g/l with the concentration of PHB of 22 g/l has been obtained [18]. The M5 strain of *Bacillus cereus* was used in sugar beet molasses to produce PHB. This strain produces higher PHB (73.84% of dry cell mass) and higher amount of dry cell mass (0.44 g/l) in 1% and 4% molasses [19].

A *recombinant Escherichia coli* strain (HMS174/pTZ18u-PHB) uses glucose as a sole carbon source and produces PHB. The process of fermentation with the molasses is cheaper than with the glucose. The final dry cell weight, PHB productivity, and PHB content of 39.5 g/l, 1 g/l/h, and 80% (w/w), respectively, were obtained in 5-l stirred tank fermenter just after 31.5 hours in fed-batch fermentation. Recombinant *E. coli* cells could efficiently utilize fructose (97%), glucose (99%), and sucrose hydrolyzate (96%) for the production of PHB. However, utilization efficiency on sucrose was very low (20%). But, beet molasses generally contain 30–50% of (w/v) sucrose. Therefore, beet molasses must be hydrolyzed before use. The production of greater PHB obtained when cell density was higher on molasses. The highest cell mass of 72.6 g/l and PHB content of 42% of a CDW were observed

in 24 hours of cultivation time, with improved productivity of PHB (1.27 g/l/h). The system was suboptimal due to limiting dissolved oxygen and capable of further improvements for PHB production rate [16, 20].

2.4.1.2 Bioenergy from Sugar Waste

Alcoholic fermentation of sugar waste will lead to ethanol fuel production [21].

The general chemical equation for the alcoholic fermentation is

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Step-by-step process has been given in Figure 2.2.

2.4.2 Starch Waste

Starch waste can be obtained from cassava, corn, and other cereal crop residues. The mixed culture of *Clostridium butyricum* and *Enterobacter aerogenes* could produce hydrogen from starch at a yield of about 2 mol-H_2/mol glucose, and hydrogen production of about 6 mol-H_2/mol glucose could be obtained from the starch using a mixed culture of *C. butyricum* and *Rhodobacter* sp. M-19 [22, 23].

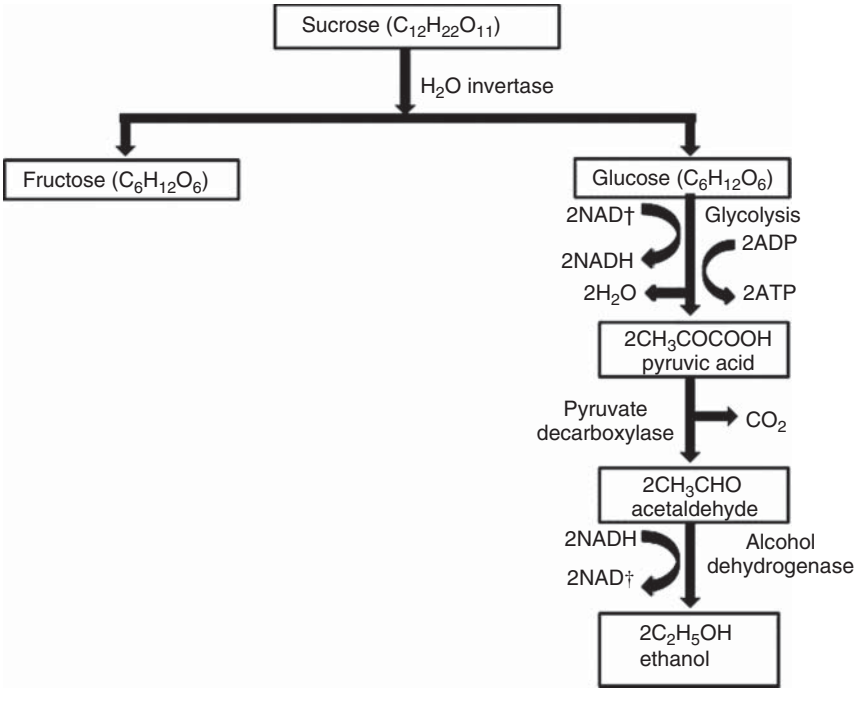

Figure 2.2 Pathway that leads to ethanol from sucrose.

2.4.2.1 Biodegradable Plastics and Starch Waste

The isolated strain *B. cereus* (CFR06) is able to accumulate PHAs in the medium made from soluble starch and PHB was produced at the concentration of 0.48 g/l. The observed result was less promising than that found in another study. The saccharified waste potato and starch is used as a carbon source by *C. necator* NCIMB 11599 which produced PHB at the concentration of 94 g/l [24, 25]. The strain, *Cupriavidus* sp. KKU38, accumulated PHAs up to 65.27% (at concentration of 2.8 g/l) in the cassava starch hydrolysate medium. However, this process is not cost-effective since the hydrolysis of starch into glucose is a two-step process (saccharification and liquefaction) which makes the feedstock less economically viable [26]. The *recombinant E. coli* strain SKB99 sheltering plasmids containing genes for the starch hydrolysis (from *Paenibacillus* sp.) and the PHB synthesis (from *Ralstonia eutropha*) utilizes starch as an exclusive carbon source, with a maximum production of PHB at 1.24 g/l (with 40% of PHB content) at 2% (w/v) starch. The production of PHB in engineered *E. coli* strain SKB99 is not regulated by stress response in contrast to *R. eutropha* and other microbes [27].

2.4.2.2 Bioenergy from Starch Waste

Liquefaction and saccharification are the general processes used for the conversion of starch into oligosaccharides and glucose. In the first liquefaction stage, dextrin will be obtained from gelatinized starch by the action of thermophilic α-amylase at high temperature (95–105 °C) and pH of 6–6.5. In the next saccharification process stage, cooled liquefied starch slurry will be adjusted to pH 4–4.5 and mixed with glucoamylase enzyme to hydrolyze dextrins at relatively lower temperature (60–65 °C) into glucose. The glucose can be used for the production of bioenergy through the action of microorganisms [28].

Several microorganisms produce highly effective hydrogen from starch-manufacturing waste. Mixed culture of *C. butyricum* and *E. aerogenes* HO-39 is used in the starch waste medium prepared from sweet potato starch residue (carbon source) and corn steep liquor (nitrogen source) for high yield production of hydrogen (2.7 mol-H_2/mol glucose). In a repeated batch culture (pH 7.5) of *C. butyricum*, *E. aerogenes* HO-39, and *Rhodobacter* sp. M-19, a yield of 4.5 mol-H_2/mol of glucose was obtained [23].

2.5 Wastewater for the Production of Biodegradable Plastics and Bioenergy

The P(3HB) can be produced from excess sludge obtained in waste-water treatment plants. Methane can be produced from wastewater activated sludge by the action of bacteria in two-stage treatment [29–32].

2.5.1 Biodegradable Plastics from Wastewater

2.5.1.1 Production of PHA from Wastewater

Number of related co-polymers and *Alcaligenes* spp. were identified in activated sludge obtained from wastewater. The particular polymer yield has been increased to about 0.39 g/g dry cells. The yield can be increased by increasing the C/N ratio from 20 to 140. Once the C/N ratio has been maintained at the nitrogen-deficient level of about 100, the highest polymer production was achieved. The particular polymer yield in the isolated *Alcaligenes* spp. reached as high as 0.7 g/g dry cell mass. This approach not only reduces the cost of production of biodegradable plastics but also reduces the amount of the excess sludge which was generated from the wastewater treatment by around 39% [32].

2.5.1.2 Production of PHB

The fresh activated sludge can be collected from the water treatment plant for the production of PHB. Bacterial strains enriched were isolated by the spreading of the sludge on the different nutrient agar plates. Five different types of bacterial strains were obtained on the basis of colony characteristics. It was found that the PHB granules are produced in all the five different strains. In the normal conditions, the bacteria will synthesize proteins as they grow. During the limited nutrient conditions, the bacteria will move their proteins for the synthesis of PHB in order to survive [33]. As per an increase in the C/N ratio (24–168), the accumulation of the PHB in the cell mass also increased. The maximum PHB (33%) was accumulated at C/N ratio of 144 after an incubation period of 96 hours. For the optimization of production of PHB, the various concentrations of the activated sludge (biomass) ranging from 0.5 to 3.5 g/l was also used and the maximum production of the PHB was attained at 3 g/l [33].

2.5.2 Production of Bioenergy

The composition, potential, and efficiency of bioenergy from the sludge of wastewater mainly depend on high-rate algal pond (HRAP) and combination of HRAP with intensified oxidation ponds and an algal reactor. During the operation of HRAP, the hydraulic retention time (HRT) of 48 hours results in highest biomass (54 ± 12 g ash-free dry weight/m^2/d) with good settling properties and algal-bacterial population compared to a HRT of 72 hours. The two-stage process was found more efficient than one-stage treatment in the removal of nitrogen and increase of methane yield (up to 30%, from 267 to 340 ml CH_4/gVS).

The change in the composition of algal–bacterial biomass leads to variation in total energy output (nearly 40%), net energy ratios (1.5–2.2), and efficiencies (60–68%). However, energy output of only 15–20% of energy available with biomass and methane yields of only 40–50% of theoretically available with biomass were achieved. These values can be improved from algal–bacterial biomass of wastewater in the coming years [34, 35].

2.6 Integrated Approaches for the Production of Biodegradable Plastics and Bioenergy from Waste

Integrated approaches for the production of the biodegradable plastics and bioenergy are flexible and aim (i) to use the mixed cultures or microbial strains which show better capacity for the accumulation of PHA under the specific feeding conditions, (ii) to produce organic acids from a complex organic solid wastes which are rich in carbohydrates, and (iii) to produce bioenergy or PHA by microorganisms from the acidogenic effluents.

For the valorization of the waste, biomass derived from municipal organic waste, food processing factory wastes, agricultural wastes, etc., can be used for the production of both biogas and biohydrogen by microbial processes. The advancement of high-performing microbial strains and the use of the byproducts and wastes as the substrates make the production cost of biodegradable polymers lower and can promote their use. Several bacterial strains can synthesize biopolymers from waste material and store intracellularly (PHA) and extracellularly (EPS). Large number of bacteria, such as *Bacillus* spp., *Pseudomonas* spp., *Rhizobium* spp., Methylotrophs, *Nocardia* spp., *Alcaligenes eutrophus*, *Azotobacter vinelandii*, *Azotobacter chroococcum*, *A. latus*, *Azotobacter beijerincki*, and recombinant *E. coli*, have been efficiently used for the production of PHAs at an industrial scale from various types of organic byproducts [1].

Usually, PHA represents intracellular energy and carbon storage, whereas EPS and the biosurfactants can be produced as extracellular substances for the protection of cells from desiccation and predation. Biosurfactants will be produced by several varieties of bacterial strains like *Bacillus*, *Rhodococcus*, *Arthrobacter*, *Enterobacter*, and *Acinetobacter*. Several microbes such as *A. beijerincki*, *A. eutrophus*, *P. oleovorans*, *B. megaterium*, *Nocardia*, and *Rhizobium* are involved in the integrated systems for the production of bioenergy from the agricultural and industrial wastes, which also utilize formic acid, acetic acid, and propionic acid as substrates for the production of PHA. They show the accumulation of PHAs up to 70% of CDW under the nitrogen- and phosphorous-limited conditions. However, *Pseudomonas* spp. and *Rhizobium* spp. accumulated (PHAs) approximately 60% of CDW. Several other bacterial strains have also showed the production of PHAs under various conditions with different yields. Among these species, purple non-sulfur bacteria have shown the production of both H_2 and PHA under nutrient-limited conditions (for example, species such as *Bacillus* spp., *Rhodopseudomonas palustris*, *Rhodopseudomonas sphaeroides*, and *Rhodospirillum rubrum*) [1, 36].

A study on the metabolic activities of *Bacillus* strains in the transformation of glucose into PHB and H_2 has been conducted in two different stages [37]. During the first three days in a batch-mode operation, *Bacillus thuringiensis* EGU45 and *B. cereus* EGU44 have reached 1.67–1.92 mol-H_2/mol glucose. In the next two days, *B. thuringiensis* EGU45 culture has been added with the residual medium which contains glucose, residual nutrients, and fatty acids, and it produced a PHB yield of 11.3% of CDW. *R. palustris* WP3-5 has been studied for the estimation of the competition between the H_2 production and PHB synthesis [38]. They tested six different

substrates, such as glucose, lactose, propionate, acetate, malate, and lactate, and it was found that the strain WP3-5 utilizes lactate, propionate, malate, and acetate which lead to the production of H_2, whereas it was able to synthesize PHB on propionate and acetate. Under specific pH stress conditions, PHB synthesis can also decrease the H_2 production [39]. However, such a decrease was not observed in *R. palustris* under limited amount of nitrogen. Under a nitrogen-limited growth condition, *R. palustris* synthesized 40 mg/l/day of PHB and around 200 ml/l/day of H_2 was also produced when the studies were supplemented with 60 mg/l/day of nitrogen [1].

2.7 Conclusions

Both biodegradable plastics and bioenergy were produced separately from different wastes like food, dairy, starch wastes, and wastewater itself. However, separate processes and systems should be set up for the production of plastics and bioenergy which are cumbersome, not eco-friendly and not economical. Hence, integrated production of bioenergy and bioplastics will be an advantageous process. However, further improvement of microbial strains and more integrated studies on different wastes or their derived products for the production of bioenergy and bioplastics will definitely augment the existing processes for the economic production of both the products at industrial level.

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3

Immobilized Enzymes for Bioconversion of Waste to Wealth

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3.1 Introduction

Waste can be defined as an unwanted material which has no value as it is viewed as unusable material that can be disposed or discarded. At present, we survive in the world where the exhaustion of resources is going beyond the control. The production of diverse wastes can lead to severe pollution and environmental degradation. Solid wastes are unwanted substances that originate from animal and human activities. Solid wastes can be categorized into biodegradable and non-biodegradable. Biodegradable wastes can be totally decayed by biological processes in the presence or absence of oxygen (e.g. kitchen waste, agriculture waste, animal dung, etc.). Non-biodegradable wastes are meant to be the waste products which cannot be completely decomposed or decayed. They are mainly of two types, recyclable and nonrecyclable. Recyclable can be reused or recovered such as paper, plastic, cloth material, etc. Some of the nonrecyclable wastes are carbon paper, thermo coal, etc., which does not have an economic value of recovery. The environmental hazards caused due to increase of wastes can be reduced by managing wastes appropriately. Different waste management technologies will definitely favor the planet, and integrated approach in waste management that include recycling, reuse, and recovery will facilitate the waste reduction.

Waste-to-wealth concept (Figure 3.1) literally means the transformation of waste to a susceptible or desirable product. The idea of obtaining wealth from waste is important to ensure that even the poorest countries will also be benefited from all the waste management technologies. Waste management also led to a major sector of occupation that provides livelihood to the vast majority in the growing population. Waste management helps the society on several counts, as mainly it reduces the pressure induced by the waste on the environment and converts it into wealth. It helps to bring back the useless or discarded waste into valuable economic products. It can impact the value of life, and concept of 5Rs (Reduce, Reuse, Recycle, Recover, and Refuse) is essential in an integrated approach of waste management.

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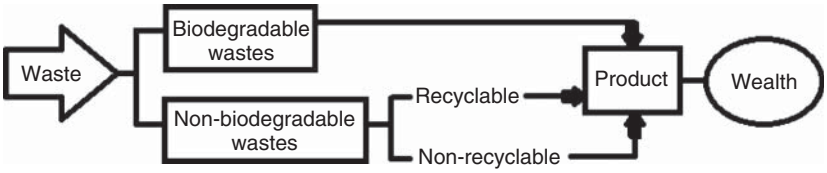

Figure 3.1 Schematic representation of waste to wealth.

Wastes are produced in agricultural, household, and industrial sectors including food processing activities. Agricultural activities will produce several types of wastes in the daily operations, such as hazardous wastes, solid wastes, and wastewater. There are several advantages of recycling wastes like reduction in the amount of waste disposals, saving natural resources including nonrenewable ones like petroleum, reduction in the amount of energy desirable to manufacture new products, reducing pollution, and several more [1]. During the industrial treatments of agricultural products, agro-industrial wastes can be generated. These can also be considered as most abundant renewable resources on the earth. Large amounts of such wastes can be generated throughout the year. Solid wastes like garbage, sewage sludge, ashes, discharged wastes, and trash of any solid or semi-solid materials have become another major concern, since humans started living in large permanent settlements [2]. Myriads of the organic wastes including the agro-waste can be exploited as a substrate in the production of sustainable energy or other desirable products at a fraction of the standard cost using enzyme technologies. Immobilized enzymes are advantageous economically and performance wise. In this chapter, immobilization methods of enzymes and how immobilized enzymes are used for the conversion of waste into useful products are discussed. In addition, applications of nanotechnology for the immobilization of enzymes and bioconversion are brought in.

3.2 Enzymes as Biocatalysts

Enzymes are versatile biocatalytic proteins, which have applications in many areas including organic synthesis [3]. The main benefits of using enzymes at industrial scale are generally the high reaction rates and the specificity of the reactions they catalyze. The major advantages of using enzymes in biocatalyst transformation are their region-, chemo-, and steriospecificity as well as the mild reaction conditions those can be used.

For the usage at industrial level, the free enzymes will pose several disadvantages such as low stability, low activity, non-native activity, and so on. In the last few decades, applications of enzymes have been rapidly increased in several fields like food modification, biofuel production, biomedical, agro-industrial waste transformation, pharmacy, laundry, etc. [3]. Enzymes are also applied in paper, leather, and textile industries which effects a significant cost reduction. As a substitute of traditional chemical catalysts, the demand for new biocatalysts is greatly increasing.

Biological processing techniques can be engaged in a variety of ways using enzymes in the conversion of solid waste into a value-added products or forms of energy or can be improved to the materials which provide fuels and energy.

3.3 Immobilization of Enzymes

Enzyme immobilization generally characterizes the integration of enzyme molecules onto or into larger and non-active structures by several methods such as covalent coupling, special encompassment, and physical adsorption. The immobilization of enzymes provides several advantages such as easy parting from reaction mixture, prevention of protein contamination into the product, enhanced stability, repeated or continuous use, and possible modulation of catalytic property [4].

Immobilized enzymes can be reused in medical and analytical applications. The reusability of biocatalyst reduces the production costs due to efficient process control and recycling. Enzyme immobilization will help micro-devices in controlled release of protein drugs. Immobilization also effectively helps in solid-phase protein chemistry. During any biochemical reaction, the maintenance of structural stability is highly challenging and this issue can be addressed by immobilization. Immobilized enzymes provide higher functional efficiency and enhanced reproducibility. The immobilized biocatalyst can be an enzyme or whole cell [5]. Enzyme immobilization procedures are developed with the objective of conversion of biocatalysts into reaction catalysts. Enzyme immobilization is suitable and a powerful tool to decrease the cost of production and or to develop the novel industrial processes which are based on biotransformation. Approaches those are carrier-bound or carrier-free offer novel alternatives for the extensive and intensive use of enzymes. A unique example can be chitosan and its derivatives. Several schemes have been developed to produce various varieties of the chemically modified chitosan materials for the immobilization of enzymes.

3.3.1 Enzyme Immobilization Methods

Due to better turnover over a significant period of time, enzyme immobilization can also provide an efficient increase in the ease of access of an enzyme to the substrate. The present demand of world's biotechnological industries is the development of novel techniques to increase the shelf life and productivity of enzymes. Several varieties of methods are practiced for an efficient enzyme immobilization.

3.3.1.1 Adsorption

Hydrophobic interactions and salt linkage result in an enzyme adsorption, where enzyme can be either dried on the surface or physically adsorbed onto the surface by immersion. Adsorbed enzymes can be shielded from several physical problems such as proteolysis, aggregation, and the interactions with hydrophobic interfaces. Silanized molecular sieves are successfully used as supports for enzyme adsorption due to the presence of silanols on the pore walls

which will help during enzyme immobilization by forming hydrogen bonds. Various modified methods are currently used to obtain a better immobilization. Lipase from *Yarrowia lipolytica* has been immobilized on octadecyl-sepa beads by physical adsorption [6]. Lipase of *Candida rugosa* has been adsorbed onto poly(3-hydroxybutyrate-*co*-hydroxyvalerate) and it has a reusability till 12 years. The eco-friendly supports of biological origin will help to cut down the cost and also prevent the ethical issues. Biocompatible mesoporous silica nanoparticle supports have been introduced in the biocatalysis of energy applications for long-term efficiency and durability.

3.3.1.2 Covalent Bonding

Sustaining structural and the functional properties of enzymes during the immobilization process is generally the key role played by the cross-linking agents. One such bifunctional cross-linker which is popularly used is glutaraldehyde. They are soluble in the aqueous solvents and form stable intra- or inter-subunits. The covalent bonding to supports occurs through the side chains of the amino acids like aspartic acid, arginine, and histidine present in the enzyme. The degree of reactivity during bonding is based on several functional groups like phenol, hydroxyl, imidazole, indolyl, etc. Peptide-modified surfaces were used to obtain greater specific activity and the stability with a controlled protein orientation. The cross-linking of enzymes to electrospun nanofibers has shown a greater residual activity due to an increase in the surface area and porosity. Enzymes can be immobilized onto magnetic nanoclusters through covalent bonding, and immobilized enzymes will have applications in pharmaceutical sector due to their longevity, stability, reusability, and activity enhancement [7].

3.3.1.3 Affinity Immobilization

It supports enzyme immobilization under different physiological conditions. Affinity immobilizations can be achieved by two ways: (i) enzyme can be conjugated for developing affinity toward the matrix and (ii) the matrix can be precoupled to the affinity ligand which can target the enzyme. Affinity matrices are also used for the purification of enzymes. Due to the existence of several non-covalent forces such as hydrogen bonding, van der Waals force, etc., bioaffinity layering can also be considered as an efficient technique which will increase the enzyme binding capacity and also reusability [8].

3.3.1.4 Entrapment

It is generally considered as the caging of the enzymes by non-covalent or covalent bond within the gels or fibers. Hybrid material, alginate–gelatin–calcium, has shown an efficient encapsulation which prevents enzyme leakage and provides an increase in the mechanical stability. The enzyme immobilization has been revolutionized with an effective entrapment by nanostructured supports like electrospun nanofibers. This method has wide range of applications in the fields of chemistry, biosensors, biomedical, and biofuel. Entrapment by mesoporous silica was recognized by its high surface area, high adsorption capacity, and uniform pore size

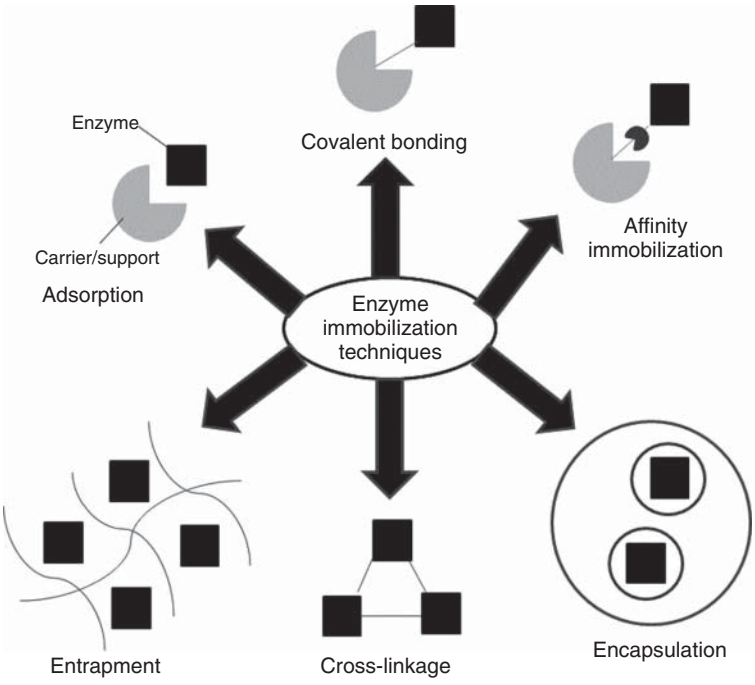

Figure 3.2 Diagrammatic representation of various enzyme immobilization methods.

[9]. Lipase entrapped in carrageenan showed high thermostability and tolerance to organic solvent. Various enzyme immobilization methods were illustrated in Figure 3.2.

3.3.2 Advantages of Immobilizing Enzymes

Advantages are described here, though they are mentioned earlier.

3.3.2.1 Stabilization

Immobilization need not to lead significant stabilization always. Both storage and operational stability of the enzyme should be achieved by immobilization. The stability of correctly folded enzymes will be better than the corresponding unfolded structure. Hence, enzymes are found to be more fragile catalysts than chemical catalysts, and immobilization will make enzymes more robust.

3.3.2.2 Flexibility of Bioreactor Design

Enzyme immobilization allows one to choose among three fermentor designs, packed bed, batch, and fluidized bed column reactor. Some of the most frequently used carriers for the enzyme immobilization are porous micro-sized particles or beads of dextran, cellulose, agarose, etc. The internal surface of beads is larger than their outer surface. Large amount of enzyme can be immobilized on the surfaces of beads, since enzyme dimensions are much smaller than that of the pores of the

beads and they can get onto the internal surfaces of beads. Hence, adsorption or the covalent couplings can also be used for the enzyme immobilization. During the process of encapsulation or entrapment, the enzymes can be found inside the beads. However, enzyme molecules cannot have free access during encapsulation or entrapment due to their presence at the interior of the beads.

3.3.2.3 Reusability and Recovery

Recovery can be distinguished from the reusability and considered as a removal or separation from the reaction component from reuse. The use of smart carriers to develop stimuli-sensitive immobilized enzymes made them to separate as homogeneous catalysts. However, heterogeneous catalysts can be easily separated out suitably from the reaction mixture. Magnetic stirrers can be used to separate the enzymes immobilized on the solid supports using magnetic field. Immobilized enzymes can be reused in many cycles of the reaction due to their stability.

3.4 Bioconversion of Waste to Useful Products by Immobilized Enzymes

Biodegradable wastes are generally established in municipal solid waste (MSW). In the urban MSWs, the organic substances are commonly present in the range of 75–85% [10]. The pretreatment methods with the use of dilute acid (H_2SO_4, 3%) and alkali (NaOH, 3%) will effectively increase the production of ethanol from the MSW. The enzymatic hydrolytic process can be carried out with the help of microorganisms like *Aspergillus niger*, *Aspergillus fumigates*, and *Trichoderma reesei* which produce various amylolytic, pectinolytic, and proteolytic enzymes those degrade the organic content of waste into sugars and simple molecules. The final fermentation process can be carried out with ethanogenic yeasts like *Saccharomyces cerevisiae*, *Pichia stipitis*, and *Candida shehatae* for the production of ethanol. The waste pretreated with alkali yields more sugar and ethanol compared to the acid pretreatment during enzymatic hydrolysis of waste [11].

Generally in food processing industries, the wastes with undesired byproducts will be separated from target products. Nowadays, enormous research has been made to produce high-value byproducts while handling the food processing waste. Generally, food waste streams are rich in carbohydrates, lipids, proteins, etc. Certainly, protein, fat, and high-sugar food waste streams will become an attractive feedstock for the enzymatic valorization [3]. The food processing wastes can be produced in solid, liquid, or semi-solid form. Solid food wastes are commonly cooking wastes and waste products like spoiled food, grape/apple pomace, potato/tomato waste, etc., will also form solid waste. Solid wastes generally consist of starch, cellulose, lignin, pectin, and monosaccharaides (i.e. fructose and glucose).

Liquid food wastes contain nutrients in diluted form. Liquid wastes are generated due to the use of huge quantities of water for the various purposes like sanitization, cleaning, temperature regulation, cooking, etc. The resulting effluents will contain nitrogenous compounds, fats, oils, suspended solids, organic matter, and many

other organic materials. Liquid effluents from agri- and food processing industries will contain various vegetable-processed water, whey from the manufacturing of cheese, starch and sugars from bakeries, beverage units and soda industries, fat materials from yogurt processing units and oil mills, and so on [3]. Immobilization of enzymes increases the yield of products where it helps to reduce the cost and efforts. Biotechnology is facilitating the production of many of the chemicals by environmental-friendly and energy-effective ways. Several chemical methods which are used in the synthesis of various products are energy-exhaustive and can cause environmental issues like polluted effluent, high-temperature discharge, etc. Numerous compounds such as citric acid, amino acids, vinegar, etc., can be manufactured using waste food stocks. Food protein shortage can be addressed by the bioconversion of the fruit waste into single-cell protein. For example, the bioconversion of single-cell protein from pineapple can be considered as a promising method for the waste utilization. Single-cell protein can be obtained from cheap agro-waste materials [12].

Bioconversion of waste to wealthy products was achieved using immobilized enzymes, and some of these processes are discussed under utilization of protein, carbohydrate, polysaccharide, and lipid wastes.

3.4.1 Utilization of Protein Wastes

Several varieties of protein-rich wastes from food industries such as oilseeds, dairy, soybean, and poultry can be converted into valuable chemicals (e.g. polymer precursor) using proteolytic enzymes. Dairy waste products, mainly whey protein, can be hydrolyzed by immobilized trypsin. Glutaraldehyde-activated agarose maintaining aspartic protease was shown to hydrolyze whey protein concentrates into antioxidant peptides [13]. In addition to enhanced thermostability at 40–50 °C, the immobilized enzyme also offered a significant reusability, which preserves more than 50% of the original activity after 10 repeated cycles. The α-lactalbumin protein shows higher affinity to the immobilized enzyme compared to β-lactoglobulin in the hydrolysis reaction. It indicates that the immobilization can change the cleavage affinity and selectivity of biocatalyst [13].

Alcalase alkaline protease was immobilized on chitosan-coated magnetic nanoparticles using glutaraldehyde as cross-linking agent. The immobilized enzyme was used for soy protein hydrolysis [14]. The immobilized enzyme showed the enhanced activity and better thermostability compared to the free enzyme. Immobilized enzyme retained about 86% of its initial catalytic activity after 10 continuous reaction batches suggesting it as a favorable candidate for the soy protein hydrolysis [14].

3.4.2 Carbohydrates as Feedstock

Food processing wastes are mainly rich in carbohydrates and can be readily made vulnerable to the enzymatic valorization by amylases, isomerases, and hydrolases. Carbohydrates can be mainly converted into simple sugars. Carbohydrates are

demonstrated to be leading carbon sources in many processes. They play a major role in the microbial metabolisms which will yield abundant fermentative products like lactose, oil, hydrogen, bioethanol, etc. [15].

Carbohydrates containing crude fibers and free sugars can be converted into different varieties of products. Free sugars illustrate the naturally occurring monosaccharaides which are found in honey, fruit extracts, and fruit waste streams, etc. The enzyme immobilization can be utilized and adapted for the conversion of carbohydrate into value-added compounds. Industrial food wastes are highly concentrated with numerous polysaccharides like starch, cellulose, hemicellulose, pectin, etc. Immobilized cells of *Aspergillus awamori* and *S. cerevisiae* produced amylase and simultaneously caused the hydrolysis of cassava starch and production of ethanol in the alternating liquid–air phase culture system [16].

Titania–lignin hybrid material was used as a novel support for immobilizing α-amylase, and immobilized enzyme showed improved thermal and chemical resistance [17]. Magnetic beads immobilized with α-amylase were used for fishing amylase inhibitors from the extract of *Ginkgo biloba* [18]. Same beads can be used for recovering the inhibitors from agri- and food wastes. Catalytic activity and stability of α-amylase were improved by immobilizing the enzyme on bioactive phosphosilicate glass, lignin from bamboo shoot shells, and so on. Immobilized amylases can be used to convert waste streams rich in carbohydrates into simple sugars which can be used for various purposes.

3.4.3 Utilization of Polysaccharides

Polysaccharides are abundantly present in the waste streams from the processing of fruits and vegetables. Polysaccharides can be considered as an attractive substrate applicants for the enzymatic transformation. Several enzymes mainly, cellulases, hemicellulases, pectinases, and xylanses have been shown to have a potential to convert polysaccharide containing waste into a value-added products like biofuel, bioplastics, sweeteners, etc. Starch-rich waste streams can be found during the processing of potato, corn, rice, sweet potato, and so on. In the case of potato, around 16% of starch is being lost by several processes such as washing and slicing. It can also be used to produce texture plasticizers and modifiers by lipase-catalyzed acylation reaction [19].

Pectinase enzyme was immobilized on silylated montmorillonite clay through covalent bond. This immobilized enzyme showed high resistance to highly acidic conditions, and it was used for the clarification of pineapple juice [20]. Pectinase enzyme was immobilized on calcium alginate beads, chitosan magnetic particles, alginate–graphene oxide composite beads, and so on to improve its stability and reusability. Pectinase from *Aspergillus ibericus* was immobilized on the functionalized nanoporous-activated carbon, and the stability of the enzyme was improved [21]. This immobilized enzyme was used for treating citrus processing industrial wastewater, and it cleared the 94% of pectin [21]. Immobilized cellulase enzyme on economical carrageenan gel disks was shown to disintegrate the cellulose fibers into nanofibers which are useful in biomedical and food packaging applications

[22]. Bioconjugation of cellulase to graphene oxide hydrogel showed higher stability and activity. This bioconjugated enzyme was effectively utilized for the hydrolysis of lignocellulosic biomass, and it increased the hydrolysis of sugar beet pulp [23]. Hydrogel-based ionic liquid-tolerant immobilized cellulase system was built [24]. This system improved the *in situ* saccharification of biomass [24]. Calcium alginate immobilized cellulase exhibited high reusability and easy recovery during the hydrolysis of carboxymethyl cellulose [25]. Hence, immobilized enzymes can be applied onto the polysaccharide wastes for the easy recovery of antioxidants, sugars, and other metabolites.

3.4.4 Lipids as Substrates

The valorization of lipid waste is highly significant with respect to environmental impact and economy. High value-added products like lubricants, biodiesel, surfactants, and so on can be obtained from waste oils by enzymatic treatment. Polymeric resins have also been practiced to obtain biodiesel, surfactants, fatty acids, etc., from waste oils by immobilizing lipases on them [3]. The higher levels of biodiesel were obtained from the waste cooking oil using multi-enzyme system based on covalently immobilized lipases from *Rhizomucor miehei* and lipase B from *Candida antarctica*. These enzymes were immobilized onto epoxy-functionalized silica. Very high production (91.5%) of fatty acid methyl esters (FAME) has been recorded after 10 hours of reaction time [26].

Near carbon dioxide neutrality makes biodiesel as environmental-friendly fuel. Immobilized lipases will become the sustainable catalysts for the production of biodiesel due to their reusability, efficiency, and easy separation. Lipid wastes from different sectors will drive the need for immobilized lipases. Lipase from *C. rugosa* was immobilized onto composite of Fe_3O_4 and poly(glycidyl-methacrylate-*co*-methacrylic acid). This immobilized enzyme gave the 92% yield of biodiesel from the transesterification reaction of soybean oil, and this lipase was easily recovered by applying external magnetic field [27]. Immobilized lipase EQ3 was used along with commercial lipozyme RMIM for the conversion of coconut oil into liquid wax esters which can be used in the manufacture of cosmetics and skin care products [28]. Lipase immobilized on the activated carbon was used for the synthesis of aromatic esters [29].

3.5 Applications of Nanotechnology for the Immobilization of Enzymes and Bioconversion

Nanoparticles generally have two drawbacks while they have been used as carriers for the enzymes: first one is clump formation due to temporary dispersion with sonication process and second one is difficulty during separation due to their small size. Superparamagnetism is one solution for these problems, where a material becomes magnetic only in the existence of a magnetic field. Particles of such materials can be easily dispersed in the solution and recovered by the usage of a

simple magnet. The immobilization of an enzyme on nanoparticle can able to place excess of biological activities on a very small surface area, and it can also create hybrid assemblies. When nanomaterials are used as solid supports, all the benefits of the immobilized enzymes on nano-sized particles are inherited. The methods of immobilization, for example, adsorption, covalent bonding, encapsulation, or entrapment which are used with the solid supports of conventional sizes can also be used for the immobilization on the nanomaterials.

Iron oxide (Fe_3O_4) nanoparticles are extensively used as superparamagnetic supports. The immobilization of enzymes on Fe_3O_4 was done by several approaches. Commonly, iron particles are coated with other materials which can be functionalized with different groups and these can be used for coupling to enzymes. Covalent coupling can result into certain loss of enzyme activity. In the case where the coating material is porous, various enzyme molecules can be immobilized inside the porous coating.

Adsorption of enzymes by non-covalent interaction and with or without coating will be gentler, and the enzymes will generally retain higher biological activity. In the event of bioaffinity method, the fusion tags will be made to have specific affinity to either iron oxide or silica coat on the nanoparticles. Both the single- and multi-walled nanotubes (MWNTs) have been generally used for the enzyme immobilization process. Poly-nanofibers have been also used as carriers for the enzymes, and these fibers can be produced by electrospinning. Additionally, nanotechnology will offer various alternatives for the enzyme encapsulation like nanosheets, nanovesicles, etc. Silica particles are of great interest for the enzyme immobilization since they provide an opportunity to introduce chemical functional group on their surfaces which in turn provide biological molecular interaction [30]. Nanoparticulate materials provide wide advantages as the supporting materials for the enzyme immobilization which include higher surface area allowing more enzyme loading, lower mass transfer resistance, and improved stability.

Maltogenic amylase and α-amylase were co-immobilized by a method based on nano-magnetic combi cross-linked enzyme aggregates [31]. These co-immobilized enzymes were used for the production of maltose from corn starch and they retained original activity for 10 cycles with improved thermostability [31]. Nanocomposite beads of chitosan–montmorillonite were used for the immobilization of α-amylase, and immobilized enzyme showed high pH and thermal stability in addition to retaining its 64% of original activity after 40 days [32]. Reusability and retention of α-amylase activity were improved by immobilizing the enzyme in nanoporous composites of polyacrylamide–graphene [33]. Similarly, β-amylase was immobilized onto graphene oxide nanosheets, carbon nanotube composite, and iron oxide nanoparticles to improve the retention of activity at higher temperature.

Pectinase enzyme was immobilized on magnetic nanoparticles grafted with trichlorotriazine-functionalized polyethylene glycol [34]. This immobilized enzyme functions as robust nanobiocatalyst for the clarification of fruit juice [34]. Pectinase and cellulase were co-immobilized onto magnetic nanoparticles in order to extract antioxidant from waste fruit peels [35]. Immobilization of chitosan–cellulase nanohybrid onto alginate beads was done, and these beads were successfully used

for the hydrolysis of sugarcane bagasse [36]. Obtained hydrolysates were used for the production of ethanol [36].

Mesoporous silica nanoflowers grafted with amino groups were used for the immobilization of lipase from *C. antarctica* [37]. This immobilized lipase was used for the selective production of ethyl levulinate (a biofuel) from biomass-derived levulinic acid [37]. A novel one-pot synthesis method was developed for making functional oil having diacyl glycerols, α-linolenic acid, and phytosterol esters. In this method, Fe_3O_4 nanoparticles co-immobilized with *C. rugosa* lipase and *Thermomyces lanuginosus* lipase were used as nanobiocatalysts [38]. Lipase from *Bacillus atrophaeus* was immobilized onto graphene oxide nanosheets modified with amine groups and coated with maleic copolymer. This immobilized enzyme showed (96.3%) better esterification of valeric acid compared to free enzyme (34.5%) [39].

3.6 Challenges and Opportunities

Circular economy-based ecological development has attained a significant role globally. The idea of circular economy is based on several factors such as valorization, waste minimization, resource efficiency, recycling, etc. Food industry waste can be generally considered as a key-focused area in circular economy which can be converted into several useful products [30]. Though immobilized enzymes became unique technological instruments for addressing economical, environmental, and waste problems, several challenges remain as such with their large-scale applicability. Pilot-scale research studies are required to overcome these obstacles. The cost is another impeding future while accepting immobilized catalystic system in the waste valorization. Almost, 47% of the cost is related to the immobilization support system or matrix. Yet another issue is change in the behavior of different enzymes upon their immobilization.

The usage of purified enzymes instead of whole cells or crude extract can also raise the cost of biocatalysis. Hence, economical carriers or carrier-free immobilization systems like cross-linked enzyme aggregates or systems utilizing whole cells or crude extracts have to be explored. Compared to single enzymatic systems, multi-enzymatic biocatalytic systems are more promising for higher conversion efficiencies and effective catalysis of waste into value-added products. Certainly, interdisciplinary approaches in terms of molecular biology, enzyme engineering, biochemistry, agricultural economics, biotechnology, food technology, waste management, regulations and laws, etc., are required to facilitate the enzyme-assisted applications to the commercial-scale valorization of water stream [3].

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Part II

Bioremediation for Zero Waste

4

Bioremediation of Toxic Dyes for Zero Waste

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4.1 Introduction

Currently, water contamination is one of the major global problems, due to the improper discharge of industrial wastewater into the environment, the high utilization rate of chemical fertilizers in the agricultural sector, the construction of roads, buildings, etc. There are many chemical industries that are processing dyes. Among them, a large amount of dye utilization and wastewater discharge after treatment are resulted by the textile industries. During the process of converting fibers to yarns, from yarns to fabrics, dyeing and finishing, the textile industry uses a lot of water, a lot of chemicals, auxiliary chemicals, dyes, and sizing materials. The use of such hazardous materials has caused water contamination and environmental pollution. The colored dye wastewater released from textile processing industries not only reduces the aesthetic value of the water body but also enhances the toxicity of the water, making it unsafe to drink. Discharge of colored wastewater in the textile industry is one of the most noticeable indicators of water pollution, and it is reported that when the concentration is higher than 1 mg/l, the color is visible [1]. Obviously, dyes containing water can interfere with the penetration of sunlight and hinder photosynthesis. In addition, it inhibits the growth of aquatic animals and plants by interfering with gas solubility. As the use of synthetic dyes in industrial processes has greatly increased, and humans use them more and more, water pollution caused by these dyes is a key issue from the perspective of human health and serious ecological consequences. Therefore, there is an urgent need to remove these dyes from the industrial effluents [1].

There are several methods that can be used to remove these dyes from wastewater, including biological, physical, and chemical or a combination of these methods. Some of them are uneconomical, while others are not 100% effective. In addition, these processes generate a large amount of sludge, which is a secondary source of pollution that requires new treatment, and therefore leads to high costs. Microbial-mediated destruction is an effective way to solve a large amount of

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dye-contaminated waste, because microorganisms change the chemical structure in an environmentally friendly and cost-effective way. The microbial treatment produces less sludge and requires less water, and the final product is less toxic when compared to various physical and chemical treatment processes. The microbial remediation method has several advantages because it can be performed on-site, is cost-effective, has few problems, and can be integrated with physical and chemical methods. However, all these methods differ in terms of efficiency, cost, and environmental impact. Therefore, there is a crucial need for all researchers to look for efficient, inexpensive, and environmentally friendly systems to reduce the dye concentration in wastewater to acceptable levels [2]. This chapter summarizes the latest research on the use of biodegradation methods to remove dyes, including microbial treatment, recombinant DNA (rDNA) technology, enzyme-mediated dye removal, immobilization technology, and phytoremediation. The secondary focus is to discuss the combination of appropriate technologies to form an eco-friendly system.

4.2 Background to Dye(s)

Colorants are chemicals that impart color to the materials in which they are used. Colorants can be divided into pigments and dyes, and the main difference between them is their solubility. The pigment retains its granular nature during application and always combined in a medium applied to the surface. On the other hand, the dye is soluble and diffuses into the material, becoming an indispensable part. Among all types of synthetic dyes used for commercial purposes, azo dyes are the most widespread used and most toxic among all. This dye is an aromatic compound with one or more —N=N— groups in the chemical structure. They are extensively used in many industries, such as textile printing and dyeing, food, cosmetics, paper printing, etc., among which the textile industry is the largest consumer. A dye has at least one chromophore group which possesses the color. Besides chromophores, most of the dyes also contain groups called auxochromes, examples of which are sulfonic acid, carboxylic acid, hydroxyl, and amino groups. Even though these are not responsible for the color of dye, their presence can change its color and is most commonly used to affect its solubility.

4.3 The Toxicity of Dye(s)

The toxicity of dyes has been studied by many researchers and their acute toxicity is usually low. US regulatory agencies believe that only a few dyes and pigments are carcinogenic. Except for some azo dyes with free amino groups, azo dyes are rarely mutagenic or carcinogenic. Under reducing conditions, the azo group can be broken down to form two aromatic amines, and these intermediate products cause serious harmful effects on humans and aquatic life. For humans, these intermediates can damage important organs such as the liver, brain, kidneys, reproductive system, and central nervous system. They are also known to cause cancer of the human bladder,

Table 4.1 Some examples of azo dyes and their toxic effects.

Sl. no.	Dye	Toxic effects
1.	Disperse red 13	It demonstrates mutagenic potential in human lymphocytes by causing chromosomal damage
2.	Tartrazine	Oxidative stress can be caused by free radicals forming
3.	Benzidine	Carcinogenic
4.	Pigment red 3	Weakly mutagenic
5.	Acid violet 7	It has got potential to cause chromosomal aberrations, lipid peroxidation, and inhibitory effects of acetylcholinesterase
6.	1-amino-naphthalene	Carcinogen
7.	*p*-dimethylaminobenzene	Cytotoxic and genotoxic effects on the bone marrow cells and rat spermatozoids
8.	Disperse red 1 and disperse orange 1	Increase the micronucleus level in human lymphocytes and HepG2 Cells
9.	Scarlet RR	Cytotoxic and mutagenic effects in time- and dose-dependent manners showed in *Allium cepa* root tip cells
10.	Malachite green	Genotoxic and carcinogenic and also affects immune and reproductive system
11.	CI disperse blue	Base pair substitution and frame-shift mutation in *Salmonella*

Source: Saini et al. [3].

spleen, and liver in laboratory animals and are also known to cause chromosomal abnormalities in mammalian cells. Some azo dyes can also induce the formation of liver nodules in experimental animals. Table 4.1 summarizes the different toxic effects of the azo dyes [3].

4.4 Bioremediation Methods

4.4.1 Types of Approaches: *Ex situ* and *In situ*

Bioremediation methods are mainly divided into *in situ* and *ex situ*. The *ex situ* method refers to the treatment that involves the physical excavation of pollutants from polluted sites and then transporting them to another site for treatment, while the *in situ* technology involves the removal of pollutants at the site of pollution. The information of *in situ* and *ex situ* bioremediation is as follows:

i. **Land-dwelling**: This method can use solid-phase treatment solutions for contaminated soil.
ii. **Bioreactors**: The biodegradation in a large reactor can be used to treat liquid or slurry.

iii. **Composting**: This is an anaerobic and high-temperature treatment process in which contaminated substances are mixed with fillers.
iv. **Bioventing**: This method treats contaminated soil by supplying oxygen to stimulate microbial activity.
v. **Biofilters**: In this method, a microbial stripper is used to treat air emissions.

4.4.2 Microbial Remediation

Microorganisms already exist in wastewater treatment feeds, and the complex substances in it will be converted into their simpler forms, thereby improving the treatment effect. Nowadays, biological treatment is a common technology for dye wastewater treatment. Several reports indicate that a large number of species have been used to remove and fully mineralize different types of dyes. The main advantages of this method are cheap, low operating cost, and nontoxic final product. However, these processes may be aerobic, anaerobic, or a combination of aerobic and anaerobic. Bacteria and fungi are commonly used in aerobic treatment due to their ability to treat dye wastewater [4].

4.4.2.1 Aerobic Treatment

In aerobic treatment, enzymes secreted by bacteria present in wastewater decompose organic compounds. Since more than two decades, the work of identifying and isolating aerobic bacteria that can degrade various dyes has been ongoing. *Kurthia* sp. has been discovered to effectively decolor (92–100%) various triphenylmethane dyes such as malachite green, crystal violet, magenta, ethyl violet, and brilliant green. Since the past two decades, various researchers have conducted extensive studies on *Phanerochaete chrysosporium* among various fungi to enable it to decolor many dyes. In addition, the microbial decolorization using *Rhizopus oryzae*, *Corio cyanobacteria*, *Trichoderma harzianum*, *Laetiporus thiourea*, *Streptomyces*, and *Aspergillus multicolor* was also tested. In order to improve the treatment of dye effluents, the treatability of wastewater by other microbes can be improved. Obviously, these techniques are applicable to certain dyes. However, most dyes are resistant to biodegradation or cannot be transformed under aerobic conditions [4].

4.4.2.2 Anaerobic Treatment

Anaerobic effluent treatment is quiet promising and can well prove and establish methods to degrade many synthetic dyes. Since the past few decades, it is reported that the mordant granular sludge can reduce and decolorize azo orange 1 and azo disalicylate in anaerobic environment. Another study proved the possibility of using anaerobic granular sludge to completely decolorize 20 azo dyes. The anaerobic pretreatment is inexpensive alternative when compared with the aerobic system because it does not require expensive aeration and can avoid the problem of sludge expansion. Researchers have certainly reported that the anaerobic effluent treatment can be effectively performed to remove dyes [4].

4.4.2.3 Aerobic–Anaerobic Treatment

So as to achieve better removal of dyes from textile effluent, the combination of aerobic and anaerobic treatment may bring encouraging results. This is advantageous

because complete mineralization is achieved due to the synergy of different organisms. According to reports, the reduction of azo bonds can be achieved under the reducing conditions of an anaerobic bioreactor. As a result, a colorless aromatic amine can be formed, which is further mineralized under aerobic conditions. Therefore, it is usually recommended to perform anaerobic decolorization first and then to perform aerobic posttreatment to treat dye wastewater. This combined approach is cost-competitive and applicable to various dyes [4].

4.4.3 Decolorization and Degradation of Dyes by Fungi

Fungi can quickly adapt their metabolism to various carbon and nitrogen sources by producing a large number of intracellular and extracellular enzymes that can degrade a variety of complex organic pollutants. This ability of fungus to degrade various organic compounds is caused by the relative non-specificity of their lignin-decomposing enzymes, such as manganese peroxidase, lignin peroxidase, and laccase [5]. Most research on the biodegradation of azo dyes has focused on fungal cultures derived from white-rot fungi that have been used to develop biological processes for the mineralization of azo dyes. *P. chrysosporium* is the most widely studied white-rot fungus, but others have also received considerable attention, such as *Aspergillus ochraceus*, *Bjerkandera adusta*, *Trametes versicolor*, species of *Phlebia*, and *Pleurotus*, *Peyronellaea prosopidis*, and many other isolates. However, the application of white-rot fungi to remove dyes from textile wastewater has some inherent disadvantages, such as long growth cycles and the need for nitrogen-limiting conditions.

4.4.4 Decolorization and Degradation of Dyes by Yeast

There is very little work to explore the decolorization ability of yeast, and it has been used mainly for the study of biosorption. Some yeast species, such as *Debaryomyces polymorphus*, *Candida zeylanoides*, and *Candida tropicalis*, have been used to perform putative enzymatic biodegradation and subsequent decolorization of different azo dyes [6]. Recently, it has been reported that *Saccharomyces cerevisiae* MTCC-463 plays a role in the decolorization of malachite green and methyl red [7]. In addition, *S. cerevisiae* cells also showed the bioaccumulation of reactive textile dyes (*Remazol Black B*, *Remazol Blue*, and *Remazol Red RB*) during growth in molasses [8]. Recently, the decolorization of Reactive Black 5 has been studied in detail using a salt-tolerant yeast strain *Sterigmatomyces halophilus* SSA-1575, and the enzymatic mechanism and toxicity of the degradation products have also been reported [9].

4.4.5 Decolorization and Degradation of Dyes by Algae

Photosynthetic organisms are ubiquitous, distributed in many habitats around the world, and are receiving more and more attention in the field of wastewater decolorization. Literature surveys indicate that algae can degrade azo dyes through an induced form of azo reductase. Several species of *Chlorella* and *Oscillatoria* are able to degrade azo dyes into their aromatic amines and can further metabolize

aromatic amines into simpler organic compounds. According to reports, *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Oscillatoria tenuis*, *Anabaena flosquae* UTCC64, *Phormidium autumnale* UTEX1580, and *Synechococcus* sp. PCC7942 can decompose and decolor more than 30 kinds of azo compounds into simpler aromatic amines [10, 11]. Therefore, the above results may mean that algae play an important role in the removal of azo dyes and, moreover, this biosorption process can be used as a cost-effective method for wastewater decolorization.

4.4.6 Bacterial Decolorization and Degradation of Dyes

Generally, the decolorization of azo dyes occurs by different kinds of bacteria under conventional anaerobic, facultative anaerobic, and aerobic conditions. The work to isolate pure bacterial cultures capable of degrading azo dyes began in the 1970s, and *Bacillus subtilis*, *Aeromonas hydrophila*, and *Bacillus cereus* were reported. Recently, a large number of studies have been conducted on decolorization using pure strains of bacterial cultures such as species of *Enterobacter*, *Enterococcus*, *Acinetobacter*, *Proteus*, *Pseudomonas*, etc. In addition, there are several studies describing the decolorization mechanisms of reactive azo dyes mediated by pure bacterial cultures. The use of a pure culture system ensures reproducible data, so the interpretation of experimental observations becomes easier. The results of bacterial species reported as dye degraders are summarized in Table 4.2 [12–16].

Due to their ability to function by consortia or synergistic alliances that function as biological inducers, bacterial activity in the degradation of azo dyes is enhanced. The combination of the catabolic function of each microorganism makes them a more useful substitute, which can increase the decolorization rate. The most important competitive advantages that place bacteria as the most successful microorganisms in the degradation of azo dyes are summarized in Table 4.3 [12].

There are also some drawbacks to the use of bacteria to remove dyes: (i) the decolorization process does not depend solely on these microorganisms, but also on external variables such as aeration, agitation, pH, temperature, concentration of the dye, structure of the dye, sources of carbon and nitrogen, electron donor, and redox mediator; (ii) under anaerobic conditions, the dye penetrates through the cell membrane with difficulty, affecting the degradation rate; (iii) they produce noxious and recalcitrant aromatic amines as a result of the anaerobic degradation process; and (iv) pure strains of bacteria do not degrade the azo dyes completely, so bacterial consortia are required to make the process more efficient. Bacteria, however, are the most resilient microorganisms, which become possible degraders of recalcitrant pollutants such as azo dyes because of their structure and genome. Among other things, the competitive advantages of bacteria are their ability to adapt and their metabolic activity, short life cycle, and capable of degrading and detoxifying the secondary metabolites produced during the process of decolorization.

4.4.6.1 Factors Affecting Dye Decolorization and Degradation

Changes in different physicochemical parameters, namely aeration, agitation, pH, temperature, concentration of the dye, structure of the dye, sources of carbon and

Table 4.2 Bacterial species reported as dye degraders.

Sl. no.	Degraded dye(s)	Bacteria	Percentage removal of dye at 100 mg/l concentration (%)
1.	Novacron super black G	*Alcaligenes faecalis*	90
2.	RY107, RB5, RR198, and DB71	*Brevibacterium* spp.	99
3.	Direct red-22	*Bacillus cohnii*	95
4.	RV-5R and RBO-3R	*Bacillus* spp.	63.33, 96.15
5.	Orange 10	*Pseudomonas putida*	70
6.	Malachite green	*Enterobacter* spp.	100
7.	Yellow 107	*Staphylococcus arlettae*	99.5
8.	Synazol red 6HBN	*Alcaligenes aquatilis*	82
9.	Crystal violet	*Aeromonas hydrophila*	99
10.	Direct red 81	*Enterococcus faecalis*	100
11.	RO-16, DB-19	*Acinetobacter junii*	90
12.	Acid red 337	*Bacillus megaterium* KY848339	98.9
13.	Reactive red 198, Congo red	*Acinetobacter baumannii*	>95
14.	Reactive red 35, 198, 106, 120, 111, 141, and 152 Reactive black 5 Reactive blue 160 and 28	*Enterococcus gallinarum*	>91

Source: Paba et al. [12]; Hossen et al. [13]; Roy et al. [14]; Ayman et al. [15]; Ajaz et al. [16].

nitrogen, electron donor, soluble salts, and redox mediator, can highly affect the bacterial degradation of different toxic dyes. Therefore, the growth of industrial bioreactors demands that these abiotic conditions should be optimized.

pH of the Medium Due to the dependence of enzyme activity on pH, the pH of the medium is one of the most important factors in the microbial decolorization of dye. The pH of the dye effluent can be alkaline, acidic, or neutral depending on the type of dyes and salts used. Often, at neutral pH, the efficiency of bacterial decolorization is stronger and a pH between 6.0 and 10.0 is optimal for color removal. The color removal rate is highest at optimum pH, and at highly acidic or highly alkaline pH it is likely to decrease. This problem can be solved by (i) changing effluent pH to help dye degrading bacteria growth or (ii) choosing microbial species which can grow at the pH of the effluent. It is considered that the movement of dye molecules across the cell membrane is correlated with pH change and this may affect their transport, which is a rate-limiting step for the decolorization process.

Effect of Temperature Another essential element involved in the bacterial decolorization of dye is temperature, which can affect the growth of bacteria and enzyme

Table 4.3 Competitive advantages of bacteria for the degradation of azo dyes.

Sl. no.	Identified bacteria	Advantages
1.	*Aerococcus* sp., *Carnobacterium* sp., *Enterococcus* sp., *Lactobacillus* sp., *Lactococcus* sp., *Pediococcus* sp., *Streptococcus* sp., and *Weissella* sp.	To carry out their metabolic activities, they use complex organic compounds
2.	*Proteus vulgaris*	They have short life cycles, creating faster processes of discoloration
3.	*Staphylococcus equorum* and *Psychrobacter alimentarius*	When used in consortiums, their degradation ability is enhanced
4.	*Escherichia coli*, *Klebsiella aerogenes*, *firmicutes* sp., *Staphylococcus aureus*, *Pseudomonas putida*, *Bacillus* sp., *Streptomyces* sp., and *Arthrobacter viscosus*	Heavy metal resistance is identical to the mechanisms of antimicrobial resistance
5.	*Bacillus* sp., *Proteus mirabilis*, *Aeromonas hydrophila*, *Pseudomonas* sp., *Escherichia coli*, and *Klebsiella* sp.	They have a higher rate of growth and adaptability
6.	*Bacillus* sp., *Aeromonas hydrophila*, *Proteus mirabilis*, and *Pseudomonas* sp.	Their use is more natural, cost-effective, and ecological
7.	*Aeromonas hydrophila*	They degrade anaerobic degradation-generated aromatic amines
8.	*Micrococcus glutamicus*, *Pseudomonas* sp., *Enterococcus gallinarum*, *Klebsiella* sp., *Lysinibacillus* sp., and *Micrococcus* sp.	The efficacy of the degradation of dyes has to do with the existence of enzymatic genes that, in the presence of toxic substances, can be expressed or over-expressed in an innate way

Source: Paba et al. [12]. Licensed under CC BY 4.0.

activity. By giving the bacterial culture an optimum temperature that is typically stated as 30–40 °C for most bacteria, a faster rate of dye degradation can be achieved. There are, however, few thermophilic bacteria recorded for high-temperature azo dye degradation. It has been reported that the thermophilic bacteria *Anoxybacillus rupiensis* could degrade 75% effluent at 60 °C [17].

Structure of Dyes The decolorization potential of bacteria is greatly affected by variations in the chemical structures of the azo dyes. Studies have shown that it is easy to decolorize the low molecular weight and basic structure containing dyes. Whereas there is a low decolorization rate of high molecular weight and complex structure containing dyes. Azo compounds containing hydroxyl or amino groups are more vulnerable to degradation than those containing other functional groups. Likewise, as compared to diazo and triazo dyes with high molecular weight, bacteria decolorize monoazo dyes quicker. Owing to their inability to travel through the bacterial cell membrane quickly, sulfonated azo dyes are thought to be more recalcitrant than

carboxylated azo dyes. Also, the chemical composition of the dye determines the induction of the enzyme in bacteria for dye decolorization.

Oxygen and Agitation Different groups of bacteria under anaerobic and aerobic conditions are strictly involved in the decolorization of azo dyes. In order to increase the biomass and also transfer oxygen between bacterial cells and the nutrient medium, aeration and agitation are typically necessary. Moreover, it also increases enzyme activity during the aerobic growth, but reductive enzymes are mostly susceptible to oxygen presence. However, oxidative enzymes play an important role in aerobic dye degradation, requiring the presence of oxygen [17].

Carbon and Nitrogen Supplements For the fast degradation of contaminants, microorganisms require nutrient supplements. In order to achieve high and rapid dye degradation rates for both pure and mixed cultures, organic sources such as peptone, yeast extract or a combination of carbohydrates and complex organic sources have been reported. The efficiency of dye degradation can be improved by adding glucose as the major carbon source and phosphorus has been identified as a significant growth factor [18]. Lignocellulosic agricultural waste has also been used by some researchers as a substitute for successful decolorization, thereby making the process commercially profitable and theoretically useful. Through adequate production of lignolytic enzymes in the presence of lignocellulosic substrates can enhance color removal efficiency.

Dye Concentration Enzymes that are secreted by dye degrading bacteria may not detect low dye concentration. High dye concentration, on the other hand, is harmful to bacteria and also affects dye degradation by blocking active sites of the enzyme. However, it was noted that this increasing dye concentration effect was reduced when bacterial coculture was used instead of pure culture, possibly due to the combined effect of both microorganisms.

Electron Donor and Redox Mediator Electron donors and redox mediators play a major role in achieving a successful anaerobic decolorization process, as azo dye and various other organic textile wastewater material are not adequate substrates for the growth of anaerobic bacteria. The application of electron donors, such as sodium succinate, sodium formate, sodium acetate, sodium citrate, and sodium pyruvate, has shown to increase the decolorization efficiency. Flavin-based compounds such as flavin adenine dinucleotide (FAD) and flavin adenine mononucleotide (FMN) and quinone-based compounds such as anthraquinone-2,6-disulfonate, riboflavin, and cyanocobalamin are the majority of the recorded redox mediators.

Soluble Salts Dye industry wastewater has high electrical conductivity due to the use of high salt concentration. In the dye treatment plant, salts like NaCl, Na_2SO_4, and $NaNO_3$ are typically added to increase ionic strength and fix the dye on fabric. Therefore, salts are also released into industrial wastewater when dye pollutants are released. Effluents containing high salt concentrations may reduce the rate of biodegradation by inhibiting the biological movement [19].

4.4.7 Microbial Decolorization and Degradation Mechanisms

The microbial treatment of dye-rich wastewater may follow two main mechanisms: biosorption and enzymatic degradation. It can also happen through a combination of the above two methods.

4.4.7.1 Biosorption

The biosorption potential of the selected bacterial species depends on the characteristics of the lipid component and heteropolysaccharides on the cell wall. They have several functional groups such as $-NH_2$, $-COOH$, $-OH$, $-PO_4$, etc., and other charged groups. Several pretreatment processes may cause modifications in the cell surface and change the capacity of the binding site. Compared with the living form, the lack of nutrition, long-term barrier-free storage and use, and the convenient regeneration of organic solvents and detergents make dead cells more suitable for biosorption.

4.4.7.2 Enzymatic Degradation

Owing to the presence of azo bonds (—N=N—), azo dyes are essentially electron defective and are related to other electron withdrawing moieties (such as sulfonic acid groups) in many cases. Under optimal conditions, these dyes may be degraded by various enzymes (such as reductase, laccase, oxidase, etc.). Among these laccase-producing species have higher bioremediation potential, due to their nonspecific oxidizing ability, non-requirement for cofactors, and use of oxygen as an electron acceptor [20].

4.4.8 Decolorization and Degradation of Dyes by Plants (Phytoremediation)

Phytoremediation refers to the use of plants to remove environmental pollutants. The use of living plants as bioremediation agents is promising for the degradation of various dyes and other organic and inorganic pollutants. Due to cost-effective technology, effectiveness, and environmentally friendly technology, it is an *in situ* biological treatment method that pays more attention to the treatment of dye-contaminated sites. Plants are highly sensitive to pollutants and have the potential to degrade textile dyes [21]. The use of plant systems for bioremediation is still limited because of the poor understanding of the basic mechanisms and processes involved. Many green plants, including herbs, shrubs, and trees (terrestrial and aquatic plants), have a good potential to regenerate and rebuild the contaminated ecosystem, according to research. These plant species can remove the pollutants by acting as excluders, accumulators, and hyper accumulators. The excluders accumulate contaminants from the substrate into the roots but restrict their transportation into the aerial parts such as shoots. Compared with other plant species, accumulators can concentrate pollutants and convert them into inert forms in air tissues, while hyper accumulators can accumulate unusually large amounts of pollutants.

The indigenous population of *Phragmites australis* has been extensively studied for textile wastewater remediation and primarily for the removal of acid orange 7 dye [22]. Aquatic plants have the ability to degrade azo dyes, such as *Azolla pinnata* and *Lemna minor*. Some studies have showed improved phytoremediation capabilities in the presence of plant microbes and their associated microorganisms, due to their transformation ability of organic and inorganic compounds, biological weathering, element cycling, formation of fungal minerals, and interaction between fungi and clay [23]. Combination technologies include the effects of plants and microorganisms, which may also contribute to the degradation of certain textile dyes. According to reports, when used in combination with the salt-tolerant bacteria *Gracilibacillus* sp., both *Sesbania cannabina Pers* and *alfalfa* plants can degrade effluent containing acid scarlet GR or acid red B dyes [24]. However, most of the research on dye phytoremediation is carried out on the laboratory bench, and there are few reports on the pilot scale of dye wastewater treatment. Table 4.4 lists the potential of different wild/native plants and their dye remediation properties [25, 26].

Table 4.4 Phytoremediation performances of various indigenous/wild plants for textile dyes and effluents.

Sl. no.	Name of the plant	Dye/effluent	Decolorization (%)
1.	*Alternanthera philoxeroides*	*Remazol Red*	100
2.	*Pogonatherum crinitum*	Effluent	74
3.	*Nasturtium officinale*	Acid blue 92	78
4.	*Ipomoea hederifolia*	Scarlet RR	96
5.	*Typha angustifolia*	Reactive blue 19	70
6.	*Bouteloua dactyloides*	Effluent	92
7.	*Petunia grandiflora*	Brilliant blue G	86
8.	*Azolla filiculoide*	Basic red 46 and Acid blue 92	90 and 80
9.	*Lemna minor*	Methylene blue and Acid blue 92	80.56 and 77
10.	*Portulaca grandiflora*	Reactive blue 172	98
11.	*Glandularia pulchella*	Green HE4B and *Remazol Orange 3R*	92 and 100
12.	*Aster amellus*	*Remazol Red*, *Remazol Orange 3R*	96 and 100
13.	*Typhonium flagelliforme*	Brilliant blue R	65
14.	*Blumea malcolmii*	Malachite green, Red HE4B, Methyl orange, Reactive red 2, and Direct red 5B	96, 76, 88, 80, and 42
15.	*Phragmites australis*	Acid orange 7	68 and 98
16.	*Bacopa monnieri*	Reactive and direct azo dyes	90–100

Sources: Rahul et al. [25]; Shanmugam et al. [26].

4.4.8.1 Plant Mechanism for Treating Textile Dyes and Wastewater

Plants can absorb pollutants remaining in the environment through roots, and roots provide a larger surface area and promote the mobilization, removal, or detoxification of pollutants in plants through several mechanisms. Such plant characteristics have been used to effectively treat the wastes containing phenolic compounds, metals, azo dyes and colorants, and numerous other organic and inorganic pollutants [27]. Information on the dye metabolism mechanism of plants is very limited. Plants are autotrophic and are believed to absorb xenobiotics during their absorption of natural minerals and water. In the process of evolution, plants have adapted to adversity mechanisms and enzyme synthesis. Plants mainly remove textile dyes through adsorption and accumulation, and subsequent degradation is mediated by enzymes in different parts.

4.4.8.2 Advantages of Phytoremediation

Compared with other physical and chemical remediation methods, phytoremediation methods to remove the contaminants have the advantage of low cost. This is mainly because it requires cheaper equipment, is easy to implement, and does not require personnel to handle it. This phytoremediation technique can be used without disturbing the location of pollutants.

4.4.9 Integrated Biological, Physical, and Chemical Treatment Methods

In order to better remove the dyes in textile wastewater, combined use of biological, physical, and chemical treatments may produce encouraging results. This is advantageous because complete degradation is achieved due to the synergetic effect of the different treatments. Biodegradation and radiation treatment are considered to be the most suitable methods to remove toxic compounds in natural water. Research has shown that combining biological methods with physical methods and chemical oxidation processes will increase efficiency and reduce operating costs. The latest research on the treatment of textile dye wastewater using combined methods is discussed in Table 4.5.

4.4.10 rDNA Technology

Synthetic dyes are now produced in such a way that they resist degradation and become time and effort consuming due to this degradation of dye by conventional techniques. A big revolution in the area of bioremediation has taken place in genetic engineering. Under environmental conditions, dye degradation/decolorization may be enhanced using genetically modified organisms. Functional genes of different bacterial strains such as *Escherichia coli*, *Sphingomonas desiccabilis*, *Pseudomonas putida*, *Ralstonia eutropha*, *Mycobacterium marinum*, and *Bacillus idriensis* have been used to design genetically modified organisms (GMOs) and transferred to other species [36]. A few studies on the mechanisms of dye decolorization at the genetic level have been published. Sandhya et al. [37] developed *E. coli* by transferring the azoreductase gene from *Bacillus laterosporus* to *E. coli* for the

Table 4.5 Application of integrated approaches for textile dye wastewater treatment.

Sl. no.	Method	Wastewater	Results	References
1.	Combined radiation and biological treatment	Reactive red-120 dye	The gamma radiation treatment of textile dye significantly decreased the concentration of RR-120 before the start of the microbial treatment (*Pseudomonas* sp. SUK1) and the radiation-induced fragmented products showed a variety of enzyme activities	[28]
2.	Combined biological and advanced oxidation treatment	Composite wastewater samples were collected from inlet of sewage treatment plant	Integration of advanced oxidation processes with activated sludge process yielded 98% and 100% chemical oxygen demand (COD) and color removal	[29]
3.	Combined sub-filtration and biological process	Dyeing and printing wastewaters	The wastewater is first treated by a biological process, and then the sub-filter method is used. The average turbidity, color, and COD reach 90.9%, 92.5%, and 91%, respectively	[30]
4.	Combined biological and photocatalytic process	Azo dye (Reactive Black 5)	The photocatalytic process was used as a posttreatment for biological dye degradation. The combined process was more effective than the photocatalytic and biological process only in aromatic byproduct remediation	[31]
5.	Combined ozonation and anaerobic treatment	Synthetic wastewater prepared using Reactive Black 5	The combined process has achieved a 90% reduction of the total COD and 84% of the total organic carbon (TOC) in the dye wastewater	[32]
6.	Combined chemical and biological process	Textile reactive azo dye	The integrated biological processes (*Clostridium oleophila*) and Fenton's reagent-yeast has decolorized 91% of Reactive Black 5 dye with an initial concentration of 500 mg/l	[33]
7.	e-Beam irradiation and activated sludge system	Textiles effluent	The radiation treatment destroyed the molecular structure of organic compounds and converted them into biodegradable compounds. Thus, the degradation of dye become easier in biological reaction	[34]
8.	Integrated biological biofilm and ozonation	*Remazol Black B*	The ozonation process is used as a pretreatment for dye degradation. Under the condition of 500 mg/l of *Remazol Black B dye* and a pH of 3–11, a dye removal rate of about 96% can be achieved	[35]

degradation of remazol red dye [38]. It is reported that the genetically modified *E. coli* has shown the decolorization of Direct Blue 71 [39]. It has been reported that the remazol red dye was degraded with the help of azoreductase gene replicated from *B. laterosporus* and incorporated into *E. coli* [16].

4.4.11 Enzyme-Mediated Dye Removal

The use of different enzymes for the dye degradation is in the initial stages of growth, but their revolutionary applications are increasingly growing and expanding through all textile processing sectors. According to reports, enzymes from both anaerobic and aerobic systems can effectively decolorize dyes, and most of the results come from the white-rot fungi *Phanerochaete* and *Trametes*. These species generate nonspecific extracellular lignin-degrading enzymes (copper-containing laccases and manganese/lignin peroxidases) which can cleave the azo bond. These lignin-decomposing enzymes are usually produced by white-rot fungi, when nutrient levels such as carbon, sulfur, or nitrogen become limited [22]. They are capable of oxidizing different compounds in large number and are thus intensively studied in the treatment of effluent from textile industries. White-rot fungi have shown great potential to degrade azo dyes and related effluents because of the production of lignin-degrading enzymes. Laccases have tremendous potential for the bioremediation of these dyes due to their ability to oxidize a wide variety of substrates. The laccases ability to degrade phenolic compounds makes them ideal for the degradation of dye effluent containing xenobiotic compounds.

However, before industrial-scale enzyme-mediated dye removal can take place, there are numerous technical and economic hurdles that have to be addressed. A significant upstream challenge remains the selection and successful large-scale strain cultivation for maximum enzyme production. On the other hand, for efficient fermentation processes, the production of an effective genetic-engineered strain is crucial. The variables affecting recombinant strain are not well known, despite the regular use of laboratory-scale cloning, and no industrial-scale process is currently developed.

4.4.12 Immobilization Techniques

Immobilization of microorganisms or enzymes has been widely documented for the biological treatment of wastewater. There are different bacterial cell immobilization methods. Four key groups can be categorized into the vast majority of the methods: microencapsulation, matrix entrapment, covalent binding, and adsorption. Among them, due to easy use, low cost, low toxicity to the device, and greater operational stability, trapping in polyvinyl alcohol gel beads is the strongest.

When applied in a vertical bioreactor system, the immobilized enzymes from *T. versicolor* and *Pestalotiopsis* spp. have been documented to show high decolorization efficiency. The durability of the beads can be increased by the 0.6% glutaraldehyde reaction that is necessary for the beads to be reusable. This research indicates that there is a great potential strategy for the treatment of textile dye effluents for the

application of double-layered immobilized enzymes in a vertical bioreactor system [40]. Despite the unusual degradation properties of peroxidases, the commercialization of enzyme treatment for industrial wastewater treatment is often hindered by the lack of long-term stability in service and storage, as well as the inability to recover and reuse enzymes. Recent years have centered attention on the immobilization of peroxidases in order to solve the free enzyme disadvantages. Immobilization enhances the enzyme's stability toward high temperature and high pH and renders the enzyme less susceptible to inhibitors. This justifies the widespread deployment of immobilized peroxidases in applications for wastewater treatment.

4.5 Conclusion

Since ages and until today, no single, environmentally benign, and economically feasible process has been established that can effectively treat dye-rich wastewater for reuse, and it has been a major challenge. Different physical and chemical approaches have been used, and these approaches typically have several drawbacks, such as secondary waste generation, high costs, poor performance, and insufficient resources. On the other hand, for dye effluent treatment, bioremediation is an eco-friendly, effective, inexpensive, and biologically benign technique. The use of bacteria, fungi, algae, yeast, and plants has shown that they are capable of detoxifying different dyes. In addition, because of its fast growth rate and high hydraulic retention time, microbial degradation does not create a significant amount of sludge and may be very successful in the treatment of high-strength organic wastewater. In this respect, it may be of added benefit to the use of genetically modified organisms to increase the process efficiency of degradation. Another significant factor is the convergence of innovations, which may bring future benefits. Integration of different degradation methods is yet another significant aspect, which may bring potential benefits. In order to disclose the desirable mechanism of dye degradation, more information on the biochemistry of degradation is needed. Attempts should be made to develop and apply these methods of treatment for bacterial decolorization in real industrial discharges, based on favorable laboratory conclusions. The combination of biochemistry and molecular biology, together with recent proteomics and genomics studies, has the potential to increase the bacterial degradation of wastewater containing azo dye.

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5

Bioremediation of Heavy Metals

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5.1 Introduction

Human habitable ecosystems are rich in heavy metals since ancient time; it is the effect of spontaneous geogenic and modern-day anthropogenic activities, which are responsible for contemporary environmental heavy metal contamination [1]. Heavy metals could also be derived from both direct sources such as sludge dumping, industrial effluents, and mine trailing and indirectly through highway runoffs, which in turn lead toward the exploration of metal–microbe interactions that could recover or stabilize heavy metals in soils and effluents. In recent times, the heavy metal contamination caused biomagnifications that ultimately resulted in a major human health hazard globally.

Essential heavy metals, for instance, iron, zinc, and copper, are required by living organisms in trace amounts, but their presence above a threshold concentration often observed to be toxic. Among the heavy metals, cadmium (Cd), chromium (Cr), and arsenic (As) are reported to act as a carcinogen as designated by the International Agency for Research on Cancer (IARC) and the US Environmental Protection Agency (USEPA) [2]. It has also been observed that various metals such as iron (Fe), zinc (Zn), nickel (Ni), and copper (Cu) are considered essential metals for growth and other functions, if their level remains within the threshold level [3].

There are an array of techniques, for instance, filtration, chemical precipitation, reverse osmosis, membrane technology, oxidation and reduction, ion exchange, and electrochemical treatment, for the removal of heavy metals from a contaminated environment. However, these techniques have some serious demerits associated with them. The most important one is their inability to remove heavy metals found at lower concentration ($\leq$100 mg/l) [4]. These traditional techniques are expensive and require energy sources and still often alter the properties of soil without complete removal of metal contaminants. Furthermore, the pollutant may also be displaced to other sites in the environment where they can accumulate and may cause the same issue. The presence of an array of traditional decontamination

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techniques could not serve ecosystem-friendly heavy metal decontamination, which leads to the development of environment-friendly strategies that may be employed to clean up the environment. Thus, the conventional decontamination techniques which often accelerates removal of all microbial activities including the ecologically important microbial symbionts, for instance, nitrogen-fixing bacteria and mycorrhizae, enhancing reduction of biodiversity, ecologically sustainable bioremediation strategies developed in recent time [5, 6]. The bioremediation strategies utilize metabolism of life forms for viable, safer, more efficient, and less expensive physiochemical methods for metal decontamination.

Interestingly, microorganisms do require certain metal ions, such as those of Cu^{2+}, Zn^{2+}, Co^{2+}, and Ni^{2+} in very low concentrations, as essential micronutrients as components of important cofactors in enzymatic reactions. Numerous findings have reported that some microbes are tolerant of heavy metals with an ability to either remove them from the environment or breakdown them to a less toxic or comparatively benign forms [6]. Microbial resistance or tolerance to pollutants is vital for the process of environmental bioremediation.

The potential for bioremediation of heavy metals by microorganisms is very much dependent on the nature of the site and the chemicals in the environment. It remains the most cost-effective process that reduces pollutants to non-hazardous materials. Over the past decade or so, the use of microorganisms in treating wastewaters contaminated with heavy metals has become an attractive technique. Currently, much work is being done in the removal of nitrogen, phosphorous, and metal ions from commercial and municipal waste, by bioremediation. Most microorganisms have their origin in soil and play a direct or indirect role in maintaining the biogeochemical cycles within the soil ecosystem. They play an important role in recycling mineral nutrients such as nitrogen, phosphorous, sulfur, and numerous metallic ions of copper, mercury, iron, and aluminum, thereby contributing substantially to life forms and also influencing various microbial populations and their related functions.

5.2 Ubiquitous Heavy Metal Contamination – The Global Scenario

Although geogenic activities are the primary cause of the ubiquity of environmental heavy metals in world, but in recent times anthropogenic activities have become a serious concern [5]. Anthropogenic activities such as mining, refinement of ore, fuel combustion, metal-working industries, battery manufacturing, paints and preservatives, insecticides, and fertilizers have led to the emission of heavy metals and their accumulation in human habitable ecosystem causing serious threat to the environment [1].

When considering the anthropogenic contribution to heavy metal pollution, it has been reported that anthropogenic emissions of Cd are in the range of 30 000 ton/year. In unpolluted soil, Cd is present at a concentration of 0.1–0.5 mg/kg, but in heavily polluted soils of sewage sludge, concentrations of up to 150 mg/kg have been found. Arsenic, the metalloid ranking twentieth in abundance of elements in the

earth crust, is present in soil, air, and water as chemical compounds in both inorganic and organic forms. Environmental arsenic pollution is increasing due to its mobilization from geological sources and anthropological and industrial activities. One of the major sources of As is its potential mobilization and transport in the ground water and drinking water supplies. In India, West Bengal state is most affected from arsenic contamination in ground water. An estimated 6 million people in West Bengal and 57 million people in Bangladesh have been exposed to arsenic through contaminated wells. Lead (Pb) has been introduced in the environment from a variety of sources such as storage battery, lead smelting, tetraethyl-lead manufacturing and mining, plating, ammunition, ceramic, and glass industries. It has been used in piping, building materials, solders, paint, type metal, ammunition, and castings since the medieval times. Thus, human activities are a major cause of increase in the concentrations of these heavy metals in the soil. Scientific investigations demonstrated that the concentration of heavy metals in several sites, assessed in water, soil, and sediment samples, are affected by different anthropogenic pollution sources.

5.3 Health Hazards from Heavy Metal Pollution

Heavy metal affects health in myriad ways, which include heart disease, liver damage, cardiovascular, neurological diseases, and cancer (Table 5.1). The contamination of drinking water supplies is of particular concern; soils and sediments are the major sinks for metals. Heavy metal contamination in soil can accumulate in crop and therefore transferred and resulted in bioaccumulation. Heavy metals are ever persistent in the earth and consequently are difficult to remove from the environment. The chemical nature and bioavailability of a metal can be changed through oxidation or reduction; however, the elemental nature remains the same because the metals are neither thermally decomposable nor degradable. Because of the toxicity and the ubiquity of the metals in environment, microbes have evolved various unique adaptations to deal with high concentrations of metals [1]. Microorganisms have been previously reported to sequester

Table 5.1 Health hazards caused by environmental heavy metal pollution.

Heavy metal	Health hazard(s)
Nickel (Ni)	Hypersensitivity; cancer; pulmonary cancer; nasal sinus cancer; neurological disorders; abortion of pregnancy
Chromium (Cr)	Oral toxicity; respiratory problems (e.g. asthma); acute tubular necrosis; kidney failure
Arsenic (As)	Blackfoot disease; skin, bladder, liver and lung cancers; arsenicosis
Lead (Pb)	Anemia; central nervous system and neuromuscular ailments; chronic renal problems; abnormal sperm production
Cadmium (Cd)	Acute gastrointestinal effect; pneumonitis; kidney damage; interference in progesterone and testosterone production; osteoporosis; prostate cancer; renal cancer

and immobilize metals, whereas others actually enhance metal solubility in the environment, thus converting them to non-toxic forms [6].

The bio-concentration factor (BCF) of several heavy metals in the crop–soil interface, particularly in major global staple crops such as wheat and corn, has been documented earlier [7]. The intake of metal-contaminated vegetables has been previously reported to cause gastrointestinal cancer, fragile immunological mechanisms, mental growth retardation, malnutrition, etc. [8]. Human health hazards are closely linked to the intake of metal-contaminated food crops. Heavy metals can accumulate in human bones or fatty tissues through dietary intake, thereby leading to the depletion of essential nutrients and weakened immunological defenses. Certain heavy metals (e.g. Al, Cd, Mn, and Pb) are further suspected to cause intrauterine growth retardation [9]. Lead contamination adversely affects mental growth, causing neurological and cardiovascular diseases in humans, especially children [10]. Certain heavy metals can also lead to bone fractures and malformation, cardiovascular complications, kidney dysfunction, hypertension, and other serious diseases of the liver, lung, nervous system, and immune system. Excessive levels of As in soil, food crops, and groundwater can cause cancer, dermal problems, respiratory complications, and many other diseases in the cardiovascular, gastrointestinal, hematological, hepatic, renal, neurological, developmental, reproductive and immune systems [1].

Excess Zn levels in the human body can affect the concentration levels of high-density lipoproteins and disturb the immune system. Likewise, excess Cu intake can induce liver damage and other gastric-related problems in humans [11]. Heavy metals (e.g. Cr, Cu, and Zn) in soil can cause non-carcinogenic human health hazards such as neurologic complications, headaches, and liver disease [11]. Chromium(VI) is more hazardous than Chromium(III) and other ionic forms in terms of its stability. As such, the former form is suspected to have enhanced potential to cause lung cancer compared with the latter form [11]. Cadmium is highly carcinogenic, typically ingested by humans through contaminated food crops, especially rice, and causes postmenopausal breast cancer [12].

The inhalation of soil and dietary intake of fruits, crops and vegetables contaminated with metals or metalloids can lead to gastrointestinal cancer. The concentrations of heavy metals were measured in several leafy (e.g. lettuce and spinach) and non-leafy vegetables (e.g. radishes and carrots) to determine the bioavailability of the metals in the human gastrointestinal tract. Health risk studies on the intake of food crops in a developing country were conducted to assess 30 agro-ecological zones in terms of health indices. The results revealed that the consumption of vegetables contaminated by heavy metals (especially Mn and Cu) was more deleterious to human health than the consumption of contaminated fruits [13]. Scientific studies earlier reported that vegetables grown near a Pb–Zn mine were contaminated with heavy metals, especially Pb and Mn, which can lead to Alzheimer's disease and manganism, a toxic condition resulting from chronic exposure to manganese.

Heavy metal contamination influences human health in a negative manner through the alteration of food chain even at very low concentrations. The health hazards caused by heavy metals often mediated by oxidative stress through the formation of free radicals [14], for instance, the enhanced generation of reactive

oxygen species (ROS), which could ultimately lead to cell damage or death or by replacing metals in pigment molecules or the activators of other metallo-proteins such as enzymes disrupting their function.

5.4 Decontaminating Heavy Metals – The Conventional Strategies

The conventional decontamination strategies could be *in situ* and *ex situ* in nature, but none of these proven to optimize the sustainable environmental decontamination of heavy metals. The heterogeneous and multidimensional nature of environment makes most remediation efforts economically demanding. Traditional decontamination strategies of heavy metal remediation have a wide range of strategies (Table 5.2).

Table 5.2 Various conventional metal decontamination strategies.

Method	Key features	Advantages	Disadvantages	References
Heat treatment	Substrate heated, heavy metals exposed to very high temperature, making them evaporate and later recovered by condensation	• Shorter treatment time • Complete removal of metals (e.g. Cd and Cu)	• Need of very high temperature leading to more leaching of the metals	[15]
Electroremediation	Based on the principle of electrokinesis, involving application of low electric current to contaminated substrate for recovering the pollutants	• Shorter time interval	• Lower extraction of heavy metals from soil	[16]
Vitrification	Bringing contaminated soil to a very high temperature until they melts and vitrified	• Volume reduction of natural soils • Cost effective	• The treatment soil is limited to a maximum of 7–10% organics by weight	[17]
Precipitation	Traditional chemical precipitation method for effective elimination of heavy metals	• Simple, cost-effective, and non-toxic procedure	• Requirement of an array of chemicals • Secondary waste generation	[18]
Chemical leaching	This process involves dissolving heavy metal ions into the leaching liquid followed by extraction	• This is the method of choice when the concentration of heavy metals is significant	• Requirement of large amount of acid to maintain the pH for solubilization, followed by its neutralization	[19]

Prior to the application of biological processes for the removal of heavy metals from the environment, conventional methods like chemical precipitation, chemical redox reactions, ion exchange, filtration and reverse osmosis were the method of choice. These techniques had some demerits, for instance, if the concentration of heavy metals was below 100 mg/l, then it cannot be removed by these techniques. Similarly, these techniques were expensive, were difficult to operate, and produce some secondary contaminants. The field application of conventional metal remediating methods is often observed to be expensive and inefficient, which lead towards the development of new methods. Keeping in mind the demerits of these methods, biological removal of heavy metals can be efficient, easy, cost-effective and environmentally friendly strategy.

5.5 Bioremediation – The Emerging Sustainable Strategy

Bioremediation is a sustainable, environment friendly strategy that explores the cellular resistance of microorganisms and plants to clean-up contaminated environment. It achieves contaminant decomposition by existing metabolic potential of microorganisms. Bioremediation as a technology may be introduced in the removal of xenobiotic compounds from agrochemical and petrochemical industries, oil spills, heavy metals in sewage, sludge and marine sediments, etc.

Decontamination of heavy metals from polluted environment is of great significance to local agriculture and the population elsewhere in the affected area. The disadvantage of the traditional metal decontamination techniques includes lesser accuracy, particularly, in very low heavy metal concentration and secondary environmental pollution due to the chemicals used in the remediation process. The cost that is involved restricts the utilization of the prevailing techniques.

Bioremediation techniques are often broadly divided into *in situ* and *ex situ* bioremediation strategies. The *in situ* technique deals with the treatment of soil and associated ground water in its original place without displacing the material, whereas the *ex situ* process involves removal of the entire contaminated material for treatment at different places where the activity of bioremediating agent could be controlled. But the field selection of the choices depends on three basic principles: the responsiveness of environmental pollutant to biological transformation; the accessibility of the contaminant to bioremediation agent (bioavailability); and the possibilities for the optimization of biological activity (bioactivity).

5.5.1 Intervention of Metal Contamination by Microbial Adaptation

Microbe-assisted bioremediation of heavy metal involves uptake of heavy metals by microorganisms either by bioaccumulation, which is an active process, and/or through adsorption, which is a passive process. Microbial cell wall comprises various functional groups such as carboxylate, hydroxyl, amino, and phosphate. The metal ions can easily bind to such groups and be separated from the environment.

Table 5.3 Adaptive mechanisms in microorganisms resulting in metal resistance physiology.

Adaptations	Features	References
Extrusion system	Metals are pushed out through the cells using mechanisms such as chromosomal or plasmid-mediated events	[4]
Biotransformation	Microorganisms convert the toxic metal to non-toxic forms	[6]
Degradation enzymes	Using enzymes such as oxidases and reductases: microbes produce these enzymes to convert pollutants to metabolic products	[20]
Exopolysaccharides (EPS)	Microorganisms get adapted to the contaminated surrounding by secreting EPS, which develops as an outer hydrophobic cell membrane comprising efflux pumps against the cell membrane disrupting contaminants (e.g. solvents)	[4, 21]
Metallothioneins	The metal-binding proteins to which metals form a complex	[22]

Metal ions bind to the bacterial cell surface via different interactions such as covalent bonding and electrostatic and van der Waals forces. Microorganisms that act as metal accumulators possess an inherent property of converting toxic form of metal contaminants to non-toxic or less toxic form. The life cycle of microorganisms are intricately associated with the biochemical cycle of different heavy metals, which also influences the process of redox transformations of environmental heavy metal leading to different oxidation states with different solubility and mobility, therefore influencing the toxicity factor.

Certain microorganisms in nature have evolved genetic machinery that encodes cellular circuitry that orchestrates to ensure heavy metal resistance for the metal-contaminated ecological niche (Table 5.3). Scientific studies have previously reported myriad microorganisms having heavy metal remediation capabilities. Efficient Ni removal was observed with *Escherichia coli* AS21 previously [23]. Arsenic remediation was observed in *Micrococcus* sp. isolated from the paddy field of West Bengal, India [6]. Earlier studies have also reported Cd, Cr, Hg, and Pb decontamination in a microbe-assisted way in *Bacillus subtilis* 38 (B38) [24].

An array of resistance strategies have been reported in microorganisms that could resist high metal concentrations, for instance, extracellular sequestration, alteration in cell morphology, altered permeability, precipitation of heavy metals, and biosorption of heavy metals [1]. Microbes could accumulate heavy metals within the cell by utilizing different metabolic pathways that have been extensively studied and observed in a wide range of microbes [6]. Both Gram-positive and Gram-negative bacteria have some cellular components such as teichoic acid, polypeptide, and protein, such as metallothionein, which helps in cellular accumulation and conversion to less toxic form. Earlier studies have reported that *Pseudomonas aeruginosa* was

Table 5.4 The environmental heavy metal pollution and the responsible genes conferring the metal resistance.

Heavy metal	Resistance operon	References
Arsenic	*arsRBC* or *arsRDABC*	[1, 6]
Cadmium	*cadCA*	[25]
Chromium	*chrBACF*	[26]
Mercury	*mer* operons	[27]
Lead	*pbr* operon	[28]
Nickel	*ncc* operon	[29]

able to accumulate Cu in the form of copper sulfide and was also able to accumulate Ni in the form of phosphide salts. Metallothionein, a cysteine-rich soluble protein, has been reported to help in the accumulation of Cd by *Pseudomonas putida*. This bioaccumulation strategy can be exploited for heavy metal removal or recovery process from contaminated water or soil samples.

5.5.1.1 Genetic Circuitry Involved in Microbial Bioremediation

Environmental heavy metal pollution became a serious health hazard globally in contemporary times [1, 5]. The metal-resistant phenotype of microorganisms conferred by the presence of microbial operons is well evident in scientific literature. Although the resistance genes were originally discovered on plasmids, they have also been found on the chromosomes of a diverse group of organisms. Metal-resistant genes are common in microbial communities growing in contaminated environments (Table 5.4). Heavy metals exert a strong selective pressure on microorganisms, resulting in major changes in the structure and diversity of the microbial community.

The resistance genes conferring heavy metal resistance often arranged in operons [1]. Microbial arsenic resistance genes are organized as *ars* operons, which may involve three genes constituting the *arsRBC* or five genes forming *arsRDABC* operon. Cells expressing the five genes *arsRDABC* are more resistant to arsenic than those expressing only the *arsRBC* genes. The cadmium resistance operon *cadCA* has been isolated and characterized previously in *Staphylococcus* sp.

5.5.1.2 Different Heavy Metal–Resistant Mechanisms

Microbes exert myriad different heavy metal–resistant mechanisms.

Intracellular Accumulation Transport of the metal across the cell membrane yields intracellular accumulation, which is a metabolism-dependent process. Many bacteria have the ability of sequestering metals from the environment. After entering into the cell by this energy-dependent process, heavy metals may be compartmentalized and/or converted to innocuous form by binding with cellular metabolic components such as carbide, sulfide, phosphide or hydroxide.

Extracellular Precipitation Microbes are able to produce a wide variety of extracellular, specific and non-specific substances, which can bind to the heavy metal in the ambient environment by converting them to less toxic nature. Extracellular polymeric substances (EPSs) produced by the biofilm-producing bacteria play a crucial role in heavy metal removal. EPS comprising a mixture of polysaccharide, proteins, mucopolysaccharide, and nucleic acids are able to bind with the heavy metals and can effectively remove metals from the environment.

Adsorption on the Cell Surface Microbes can also sequester toxic heavy metals by cell surface adsorption mechanism. It is a rapid physicochemical process involving both live and dead cell biomass and has been considered as an effective biotechnological process for the removal and/or recovery of toxic heavy metals. In this process, metal ions get quickly attached to the cell within a few minutes. Several mechanisms such as ion exchange, chelation, and diffusion through cell walls and membranes contribute to bioadsorption process [1, 5].

Volatilization Microbes can often convert metals to its less toxic and less soluble form by volatilization process. Volatilization can be achieved by a variety of processes such as reduction, oxidation, methylation, and demethylation of the compounds.

Metal Efflux A wide variety of metal efflux transport systems are present in microbes that are involved in the excretion of metals out of the cell. Most of them are non-specific being involved in efflux of a wide range of molecules. These transporters are originally identified as multidrug transporters but are also involved in transportation of heavy metals, organic acids, and many other non-specific compounds. The ABC transporters are involved in the efflux of Mn in *Streptococcus gordonii* [30]. The P type ATPase is another efflux protein associated with the efflux of Cd in *Staphylococcus aureus*.

5.5.2 Plant-Assisted Bioremediation (Phytoremediation)

Since contamination of soils and waters by toxic heavy metals is a serious environmental problem, therefore effective remediation methods are necessary. Phytoremediation is the use of plants and associated soil microbes to reduce the concentrations or toxic effects of ambient heavy metal contamination. It can be used for the removal of heavy metals and as well as for organic pollutants. It is a novel, cost-effective, efficient, environment and eco-friendly, *in situ* applicable and solar-driven remediation strategy. The method is reported to be economically sustainable than traditional physical and chemical methods, which are categorized into phytoextraction, phytodegradation, phytostabilization, phytovolatilization, rhizodegradation, rhizofiltration, and other methods (Table 5.5). The effects of phytoremediation were previously reported to be observed in short time.

The plants, categorized as metal hyperaccumulators and wild, are able to remove heavy metals many times higher compared to the ones that are cultivated. Exploration of hyperaccumulators as a potential agent of phytoremediation is the most

Table 5.5 Glimpses of different categories of phytoremediation strategies to decontaminate environmental heavy metal pollution.

Techniques	Description	Heavy metals	References
Phytoextraction	• Accumulation of organic and inorganic pollutants in harvestable biomass i.e. shoots • Plants able to absorb metals are chosen to remove contaminants from soil with the harvesting or removal of the plant • Time consuming	Gold and nickel	[31]
Phytodegradation (vegetal degradation)	• Degradation of organic xenobiotics by plant enzymes through metabolic processes within plant tissues • Applied to soil, clay, sediment, and underground waters • Reduction and degradation occur inside the plant as a physiological process and do not depend on microorganisms	Mercury and lead	[32]
Phytostabilization (root stabilization)	• Stabilizing the mobility and bioavailability of pollutants in soil by plant roots • Phytostabilization plants are able to tolerate heavy metal levels and immobilize the metals through sorption, sedimentation, complexation, or reduction of metal valences	Copper	[32]
Phytovolatilization (vegetal evaporation)	• Conversion of heavy metals to volatile form and their subsequent release to the atmosphere • The most important aspect of this method is transformation of the excessive toxic compounds into less toxic forms • The contaminants can be removed from the plant by transpiration or evaporation	Selenium	[33]
Rhizodegradation (the use of roots for degradation)	• Degradation of organic contaminants in rhizosphere by microorganisms in soil • The most important benefit is the dissolution of the contaminants in their natural environment	Cadmium	[32]

Source: Based on Ali et al. [34].

effective strategy for successful phytoremediation of heavy metals. Recently, myriad plant species have been reported as metal hyperaccumulators for effective phytoremediation. It has been earlier reported that phytoremediation can cost as less as 5% of alternative clean-up methods. The establishment of vegetation on polluted soils also helps prevent erosion and metal leaching.

An improved understanding of heavy metal uptake by plants from soil will also help in promoting phytomining – a plant-based eco-friendly mining of metals, which can be used for the extraction of metals even from low-grade ores. High biomass-producing crops, such as *Helianthus annuus*, *Cannabis sativa*, *Nicotiana*

tabacum, and *Zea mays*, have been reported to effectively remove heavy metals from contaminated soil through phytoextraction. Phytoextraction of Cd, Pb, Cu, and Zn has been observed in *Trifolium alexandrinum* earlier [32] due to its fast growth, resistance to pollution, high biomass, and other favorable parameters. It has also been speculated that phytoextraction of heavy metals will be a commercially viable technology for phytoremediation and phytomining of heavy metals in future. The combinatorial approach involving genetic engineering, microbe-assisted approaches, is essential for highly effective and sustainable phytoremediation of environmental heavy metal.

5.5.3 Algae-Assisted Bioremediation (Phycoremediation)

Algae also perform well in the field of bioremediation. The term "phycoremediation" is used to denote the remediation that includes either removal or degradation and assimilation, using various types of algae and cyanobacteria. Similar to bacteria, algae have various chemical moieties on their surface such as hydroxyl, carboxyl, phosphate, and amide, which act as metal-binding sites. In a study, different species of brown algae were examined for their metal uptake activity. Among them, *Padina* sp. and *Cystoseira* sp. were reported to have effective metal uptake capabilities [35].

5.5.4 Fungi-Assisted Bioremediation (Mycoremediation)

It has been shown previously that bioleaching is a biological process in which microorganisms (fungi, algae, and bacteria) uptake heavy metals from the environment. It has been reported that fungi are considered the most suitable candidates for bioremediation due to their high tolerance to heavy metals with higher surface-to-volume ratio. The main advantage to use an indigenous fungal strain is that they are adapted not only to the presence of contaminants but also to the environmental condition of the site. Thus, there is a need to develop new strategies to utilize indigenous fungal strains for heavy metal removal from contaminated soil.

Fungi are also being utilized as a contrivance for the remediation of heavy metal–contaminated areas because of their ability to accumulate toxic metals. *Coprinopsis atramentaria* is studied for its bioaccumulation capacity of Cd and Pb. Hence, it has been recognized as a potential accumulator of heavy metal ions and a very important tool for mycoremediation.

Fungi have the ability to uptake both essential and non-essential heavy metals [36]. Fungal survival in heavy metal–contaminated environments mainly depends on intrinsic structural and biochemical properties, genetical and/or physiological adaptation, morphological changes, environmental modification of heavy metal, its toxicity, and availability. Biological mechanisms associated with fungal existence include extracellular precipitation, crystallization, transformation, and complexation of metal species by using mechanisms such as reduction, oxidation, dealkylation, methylation, biosorption to cell walls, extracellular polysaccharide and pigments, impermeability or decreased transport, efflux, intracellular compartmentation and sequestration [37].

5.6 Conclusion

Environmental heavy metal pollution is the contamination of soil, water and air with heavy metals that now became one of the most serious global environmental problems. As metals are non-biodegradable, can accumulate in living organisms via the ecological food web and some of these metals are extremely toxic in trace concentrations and can cause devastating health problems worldwide. The persistent nature of heavy metals makes the environmental removal of the heavy metal a realistic problem especially in case of the industries using heavy metals in their productions. The devastating scenario of environmental heavy metal pollution is more intense for the population of developing countries, relying mostly on growing necessity of industries, making the contamination of environment with heavy metals a great concern due to the fact that it consequently affects the health of animals and plants [1].

Several bioaccumulating agents have been reported earlier; for instance, fungi and bacteria could serve as potential candidate of bioremediation by bioaccumulation [1, 5, 6]. *Penicillium* and *Aspergillus* were reported to be the efficient bioaccumulators, whereas bacteria such as *Bacillus* spp., *Pseudomonas* sp., *Enterobacter* spp., and *Aeromonas* spp. have an ability to decontaminate heavy metals [38]. Similarly, *Penicillium rubens* was found the second best Cd bioaccumulator and *Aspergillus fumigatus* showed remediation potential for Cd and Cr removal. *Metarhizium anisopliae*, *Saccharomyces cerevisiae*, *Fusarium oxysporum* and two species of *Penicillium* have also been reported for their bioremediation potential against Cd and other heavy metals [39]. Studies also shown that *Aspergillus* sp., *Penicillium* sp., and *Yarrowta* sp. can remove both soluble and insoluble heavy metal species from solution [40]. The biosorption of Cr(VI) and Fe(III) has been shown in *Streptococcus equisimilis* and *S. cerevisiae*. Significant Cr(VI) removal was observed using growing cells in batch and continuous modes of operations and using non-living biomass in a batch bioreactor. They conducted the study to evaluate the potential of the resting cells of the *Fusarium solani* for Cr(VI) removal from aqueous solution with an aim to develop a suitable operational strategy for the treatment of Cr(VI)-contaminated wastewaters. According to Jiang et al. [41], the microbial isolates *Chryseobacterium indoltheticum*, *Pseudomonas helmanticensis*, *Bacillus mycoides*, *Bacillus almalaya* and *Acinetobacter* showed high tolerance to Cd, Pb, Cr and Zn. Thus, the use of microbial biomass may therefore be considered as remedy for the removal of toxic substances from the environment. Not only being cost-effective, the indigenous microorganisms isolated also detoxify the contaminated site itself, to exercise their natural power and remedy the situation. Industrialization is the best known cause for heavy metal pollution of the soil and bioleaching is the most efficient, cost-effective and environmentally friendly method. Fungi could be the most suitable bioaccumulating agents for the removal of cadmium and chromium from contaminated soil. Considering the threat of these heavy metals to human health, the future challenge is to remove toxic metalloid from our habitable ecological niche. The myriad arrays of resistant adaptations in contemporary life forms are the evolutionary tools for the sustainable environmental bioremediation [5, 6]. Furthermore, deeper investigations for linking the

myriad-resistant properties of organisms with respect to their life history and the environmental factors are essential for successful bioremediation.

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6

Bioremediation of Pesticides Containing Soil and Water

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6.1 Introduction

Pesticides are defined as substances intended to kill, prevent, or regulate defined forms of plants or pests. They include weeds, rodents, insects, rodents, and fungi [1]. Some of the important types of pesticides used include herbicides, insecticides, fungicides, and disinfectants. They are used to destroy weeds, unwanted vegetation, growth of molds, mildew, and bacteria. Based on chemical nature, pesticides can be classified as organo chlorine pesticides, organo phosphorous pesticides, carbamates, neonicotinoids, and miscellaneous pesticides of biological origin like spinosad and abamectin [2].

Loss of pesticidal residues from one environmental compartment to another due to either degradation or transformation is defined as pesticide dissipation. The pesticide dissipation comprises various processes like adsorption, transformation, breakdown, and degradation. Releasing of pesticides into the environment can be either constructive or destructive as not the entire applied chemical reaches the target site [3]. Health effects of pesticides may either be acute such as headache, abdominal pain, nausea dizziness, and vomiting. Along with these, problems related to skin and eye also persists. Cancer, nerve illness, contrary effects on reproductive tract, chronic kidney diseases of unknown etiology, etc. are few to add to the list [4–9].

Insecticides like methyl parathion, dichloro-diphenyl-trichloroethane (DDT), and particularly pentachlorophenol will interfere with the chemical signaling between legume and rhizobium. This leads to reduced crop yields due to reduced nitrogen fixation. Root nodule development in these plants guards the world economy roughly US10 billion every year through artificial nitrogen fertilizer [10]. According to the

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United States Fish and Wildlife Service (USFWS) and United states Department of Agriculture (USDA) estimation, US farmers face condensed crop cross-pollination since the pesticides eradicate honeybee colonies [11].

Pesticide usage has been regulated in several countries through biodiversity action plans due to their harmful effects on animals. For example, in England, increased utilization of pesticides in farmland and gardens witnessed a decreased number of chaffinches [12]. Food chain of species gets affected the most, since certain pesticides bioaccumulate to toxic levels over time.

6.2 Pesticide Biomagnification and Consequences

Biomagnification is a cumulative rise in the concentrations of enduring substances like metals and pesticides as they move up the food chain. Mercury, cadmium, arsenic, and few pesticides such as polychlorinated biphenyls (PCBs) and DDT are the major contributors [13]. The lower food chain organisms are prone to the exposure of the toxins at a faster rate and when higher food chain organisms consume them, the toxins get gathered [14]. Mechanism to sequester and defecate metals is highly displayed by organisms that are subjected to greater levels of exposure to metals. When organisms are imperiled to higher concentrations than normal levels, it leads to difficulty in excretion of these metals and also portray danger to the organism's reproductive system [15].

Suedel and his co-group concluded that toxaphene, DDT, PCBs, dichlorodiphenyldichloroethylene (DDE), and organic forms of arsenic and mercury will efficiently bio-magnify in nature. The accomplishment of bird retrieval (bald eagles and peregrine falcons) in North America was seen after imposing ban on DDT usage in agriculture which proves the effect of biomagnification [16–18]. Herbicides, fungicides, pesticides, and inorganic fertilizers will infiltrate into the soil and run off in to rivers, ponds, and lakes through rains or natural calamities [19]. The industrial effluents and agricultural wastes also have a major impact on biomagnifications [20].

In agricultural lands, the used pesticides which consist of toxic chemicals and heavy metals will be captivated by zooplankton and plants. Once these are swallowed by consumers, the chemicals are circulated into the body tissues leading to hazardous health disorders. Algal blooms are caused by eutrophication which is due to the excessive use of organic substances such as manures and biosolids in the fields. They contain carbon, nitrogen, and phosphorous which act as causative agents in the depletion of the aquatic animal's oxygen leading to their death [2].

Pesticide's toxicity is decided based on its dose and how long the chemical is applied. Primary exposure of these pesticides to wildlife animals causes problems during their nesting and nursing of young ones. Pesticides will reduce the insect population leading to destruction of food chain thus harming the entire vegetation [15].

6.3 Ill Effects of Biomagnification

In current years, the ingestion of seafood has been associated with few types of cancer. This is due to the accretion of mercury and certain polycyclic aromatic

hydrocarbons in the tissues. Moreover, mercury is identified to display antagonistic effects [21] such as deterioration in the nervous system, impaired vision, gait, hearing and speech, leading to involuntary muscle actions and deterioration of mucosa and skin. Consumption of plants or aquatic animals with heavy metals and toxic substances is a major reason for the consequences of different kinds of cancers, respiratory illnesses, kidney failure, brain impairment, heart diseases, and birth defects [22]. The addition of metals in the tissues of aquatic organisms will have an ill effect on their growth and reproduction.

Accumulation of material that leads to biomagnification can interrupt the usual food chain that is vital for the existence of all categories of animals in the ecosystem. This may have a long-lasting consequence which might not be observed in short period [23, 24]. India reported its first case of poisoning owing to pesticides from Kerala in 1958. Over 100 people died subsequently after the intake of wheat flour which was contaminated with parathion, a pesticide [11].

Also, production workers, sprayers, formulators, loaders, mixers, and farm workers are at major risk of diseases due to maximum pesticide exposure. Throughout the formulation and manufacture, workers are at amplified risk as they manage numerous toxic chemicals to produce pesticides which include toxic solvents, raw materials, and inert carriers [11].

6.4 Bioremediation

Bioremediation is the process where genetically modified or naturally occurring microorganisms or plants [19] are used to degrade hazardous molecules from ecological samples including water and soil. Hence, the bioremediation process plays an important role as an eco-friendly procedure. Pesticides are involved in the destruction of the typical features of soil and also spread into the water, thus damaging the marine environment. Hence, they have to be decontaminated off the polluted areas.

Bioremediation can be performed by two methodologies, "*in situ*" and "*ex situ*." To endure microbial activity when the climate is too cold or the soil is too compressed for the nutrients to dispense, requirement of *ex situ* is most recommended. However, *ex situ* is cost-consuming as the process requires clearing of the soil on the ground and exhuming. Microbes exploit the contaminants which include solvents, oil, and pesticides as their source of energy. Process of utilization of these contaminants leads to water and harmless gases like carbon dioxide. However, if the conditions like temperature and nutrients are not supportive enough, the bioremediation facilitation will slow down. Hence, to improve the process substances like molasses, vegetable oil can be added as amendments and fasten the cleanup. The added substances aid in optimization for microbes to thrive in the surroundings and thus gear up the process of bioremediation [19].

The *Burkholderia, Pseudomonas, Azotobacter, Flavobacterium*, and *Arthobacter* are the bacterial strains that aid in degradation of pesticides. When the in-house microbes cannot involve successfully in the degradation of pesticide, additional strains of bacteria or fungi need to be provided to facilitate the degradation. Favorable conditions like pH, temperature, nutrients, and enzymes are of much

important for the degradation process that involves microbes. But, the anionic moieties in the pesticides hinder the degradation. Concentration of contaminants, soil density, and size of the contaminated area will act as the deciding factors for the duration of the bioremediation which might vary from few months to several years.

Advantages of bioremediation comprise of:

- Transfer of contaminants to a different environment does not occur since complete breakdown of impurities to nontoxic compounds is achievable [15].
- In comparison to other removal/degradation techniques, the equipments involved are extremely minimal, thus making it cost-effective.
- Either of the techniques, *in situ* or *ex situ* approaches, can be conducted based on the parameters.
- Bioremediation is more of a natural process that is publicly accepted and the cost involved per unit volume of ground water or soil is low.

Disadvantages of bioremediation comprise of:

- Toxic byproducts can be obtained in case of partial degradation of the process involving organic pollutants.
- Precise parameters like temperature and pH are mandatory for the microbial activity to sustain and carry out the sensitive process which involves toxins.
- Volatile organic compounds (VOCs) are difficult to regulate during *ex situ* methods.
- In comparison with other techniques, it is time-consuming.
- Uncertain performance regulations are observed since there is no distinct level of "clean" site.

6.5 Methods Used in Bioremediation Process

Bioremediation is a novel method to overcome the problem of contamination of soil and water due to various kinds of contaminants. During the intrinsic bioremediation where microbes that already exist is not adequate to degrade the quantity of pesticides used, it requires a processed bioremediation method where new natural or engineered microbes are added for the effective action [1]. The mechanism usually utilizes microorganisms like fungi, bacteria, actinomycetes, and cyanobacteria to degrade or eradicate noxious pollutants [19]. These microorganisms are naturally appearing and they assimilate the contaminants from the surrounding environment, leading to a province which is nearly contaminant-free. Usually, the pesticides are absorbed within the organism [25], while organic constituents are digested. Methods involved in bioremediation are natural, as they promote the growth and reproduction of these organisms that can successfully eradicate precise contaminants by converting them to nontoxic byproducts. Notably, bioremediation can also be combined with an extensive range of long-established chemical and physical methods to augment their ability [26].

6.5.1 *In Situ* Method

6.5.1.1 Bioaugmentation

The process involves an addition of either indigenous or exogenous microorganisms to contaminated locations [27]. It is favorable for the soils that are bioremediated, but yet have risks, because microbes that are naturally occurring could not do their part of work due to the unfavorable environmental factors (temperature, pH, salinity, etc.) or the changes occur in the microbial population due to mutation. To apply bioaugmentation method, there are few conditions which depend on site size and availability of certain microbes.

6.5.1.2 Bioventing

This method comprises of utilizing native microorganisms for the biodegradation of organic pesticide content absorbed into the soil at the unsaturated zone. Vacuum-enhanced soil vapor extraction method is used where pressure alterations in the subsurface display an influx of oxygen supply which is required for aerobic degradation of impurities. In the case of volatile contaminants, extraction of soil vapor is carried out by the process of adsorption on activated carbon and biodegradation within a biofilter [28].

6.5.1.3 Biosparging

It is a stimulation and exploitation of novel microorganisms for the biodegradation of organic toxins in water-logged soil. To enhance the microbial activity by increasing the oxygen dissolution, air is injected into the saturated region present beneath the water table through the boreholes which will significantly upsurges the aerobic biodegradation of pesticides. Moreover, it is also used to remove petroleum products [29, 30]. An imperative feature for the effective elimination of contaminants is soil porosity.

6.5.1.4 Biostimulation

It is the modification of natural habitat for the stimulation of the existing bacterial population to carry out bioremediation process. Addition of phosphorus, oxygen, carbon, and nitrogen will stimulate indigenous microbes in the soil which act as rate-limiting nutrients and electron acceptors. The advantage of this method is that the bioremediation will take place by natural native microbes [31]. Alternatively, bioremediation of halogenated pesticide contaminants in anaerobic condition can be stimulated by electron donors, thus indigenous microbes use the halogenated contaminants as electron acceptors.

6.5.2 *Ex Situ* Methods

6.5.2.1 Composting

Soil will be treated with aerobic thermophilic microorganisms for the degradation of pesticides. Periodic moistening and mixing are done to promote microbial activity and to diminish the toxicity of metallic residues, pesticides, waste, and byproducts.

Composting is a natural process that occurs in soil where organic waste components are degraded by the microorganisms. During artificial ways of composting, temperatures are kept higher in soil which results in amplified solubility of contaminants and display greater metabolic endeavor. Co-metabolism of organic contaminants can exist at higher levels of substrate in the compost. Mechanical treatment of unwanted nondegradable materials such as metals, plastic, stones, and glass makes it easy for the biological treatment to take place. Nature of organic contaminants, composting standards, protocols, microbial population, and incubation period act as major factors which will impact the total operation of the compost methodology [32].

6.5.2.2 Land farming

It is an overhead ground remediation skill which is also called as land treatment. This method comprises spreading of contaminated soil in slender layers up to 0.4 m thickness on terrain surface and accelerating aerobic microbial activity in the soil by adding nutrients and aeration. It demands a large treatment area as the soil is spread into thin layers. To increase the degradation process, oxygen supply and mixing (plowing, milling, and harrowing) at regular intervals of time are done.

6.5.2.3 Biopiles

It is the combination of composting and land farming. Here, evacuated soils are combined with soil amendments and positioned on treatment areas. They are bioremediated by obligated aeration and thereby completely convert toxins into carbon dioxide and water. The mentioned system comprehends aeration system, treatment bed, nutrient or irrigation organization and leachate assembly. The soil piles are up to 20 ft and enclosed by plastic to avoid the runoff, volatilization, and evaporation. Biopiles offer a favorable atmosphere for the indigenous anaerobic and aerobic microbes.

6.5.2.4 Bioreactors

Here polluted soil is treated in slurry or solid status. The principle of solid-state apparatus is systematic mechanical decomposition of the soil by the intensive mixing and enabling of mechanisms in the locked system. This makes sure that the toxins, microorganisms, water, and nutrients are in lasting contact. The slurry state bioreactors may be elucidated as contaminant system and apparatus is used to produce a three-phase (solid, liquid, and gas) mixing. As compared to *in situ* or solid-state systems, the rate of biodegradation is superior in the slurry bioreactor scheme because the environment is manageable and foreseeable. The contaminated soil requires pretreatment by soil washing and physical extraction before feeding into the bioreactor.

6.6 Bioremediation Process Using Biological Mediators

6.6.1 Bacterial Remediation

Biodegradation of pesticides (including micro-pollutants) comprises the oxidation of parent compound which forms carbon dioxide and water [33]. During the process, the contaminants provide energy and carbon for the growth and multiplication of

microorganisms. Biodegradation involves various steps depending on the contaminant and each step was initiated by specific enzymes which present in bacterial cells. Biodegradation of contaminants by either internal or external enzymes will break at any phase if a suitable enzyme is absent. Lack of specific enzymes is one of the reasons for the accumulation of byproducts of contaminants. Hence, specific enzyme-bearing microorganisms are introduced into the soil and water to improve the activity of biodegradation. Indigenous bacterial cultures are capable of metabolizing contaminants. Biodegradation can be through either aerobic or anaerobic mechanisms [34]. Several pesticide-degrading genes were identified on the plasmids of soil bacteria, which are known as catabolic plasmids. They are found in the species of *Flavobacterium*, *Pseudomonas*, *Rhodococcus*, and *Alcaligenes* which can degrade pesticide contaminants.

6.6.2 Fungal Remediation

Fungi produce a variety of extracellular enzymes and fungal species possess high capacity to degrade pesticides. Branching and filamentous fungal growth will permit for effective colonization and examination of contaminated soil. White-rot fungi are filament-like creatures and propose advantages over bacteria as they are better oxidizers. They are strong organisms and are mostly extra tolerant to high concentrations of polluting chemicals than bacteria. Additional fungi that can be used in bioremediation are zygomycetes, e.g. mycorrhizal and mucoraceous fungi. Anaerobic aquatic fungi can also be used for bioremediation [26].

6.6.3 Phytoremediation

It is the method which uses the living plants and their allied microorganisms for *in situ* elimination and degradation of contaminants present in soil, ground water, and surface water. Plants can accumulate and metabolize organic pollutants (phytodegradation) or stimulate rhizospheric microorganisms (phytostimulation). Phytoremediation is a less cost-consuming, eco-friendly, and easier for the remediation of adulterated soil and water using plants. Plants used will have an exclusive and careful uptake capability of roots, with the translocation, bioaccumulation, and degradation of contaminant. Plant-dependent soil remediation systems are biological, solar-driven, and capable of self-extending uptake network systems which enhance the underground ecosystem for productive use. Plant possesses a favorable microenvironment in the root zone that leads to contaminant degradation.

In the soil, plant-associated bacteria are endophytic (nonpathogenic) and rhizospheric. Endophytic bacteria effortlessly occur in the interior tissues of plants which promote plant growth and degrade soil contaminants. Rhizospheric bacteria have a capacity to degrade various agrochemicals due to enhanced microbial activity. Advancements in recombinant DNA technology have created transgenic plants that exhibit better-quality tolerance and catabolic activity against contaminants present in the soil [35].

6.7 Factors Affecting Bioremediation

Microbial degradation of pesticides depends on various factors which can be divided into external and internal factors. For the complete degradation of pesticide contaminants, physico-chemical parameters such as pH, temperature, water potential, substrate availability, oxygen, etc., should be at optimum level as these parameters influence the biodegradation efficiency.

6.7.1 Soil Type and Soil Moisture

Pesticide contaminants adhere to soil particles (absorption/adsorption) and microorganisms are incapable of using them for degradation. Soil moisture is considered one of the important factors for microbial functioning which helps significantly during pesticide degradation, on the other hand, under dry soils condition the degradation rate will be slow. Water will act as a solvent for pesticide to move it around and enables pesticide biodegradation. The degradation rate mostly rises with water levels. Moisture range between 50% and 80% was found to be optimal for the biodegradation [36].

6.7.2 Oxygen and Nutrients

Availability of molecular oxygen is one of the further most vital factors constraining the rate of biodegradation. For the aerobic degradation of pesticide by microorganisms, oxygen supply can be limited by unfavorable soil porosity. Henceforth, mass transmission from gaseous phase to aqueous phase will be hindered. In water, subdued solubility of oxygen is the restrictive factor. Additionally, while implementing bioremediation methods, development and activity of microorganisms must be quickened. Biostimulation engages in the addition of oxygen and nutrition. Nutrients are elementary building chunks for the life of microorganisms which lead to production of enzymes and such enzymes have the capacity to degrade the pesticides. Macro and micronutrients are essential for the growth of microorganism and degradation of pesticides [37].

6.7.3 Temperature and pH

Temperature disturbs the adsorption by fluctuating the solubility and hydrolysis of pesticides. As adsorption mechanisms are exothermic, it is expected that it will decrease with increase in temperature along with increased solubility of pesticides. Microbial activity will be intensified as there is an upsurge in temperature. The highest growth and activity of microbes will happen in soils at 25–35 °C. Furthermore, soil pH is also a chief factor that affects the biodegradation of pesticides. The biodegradation of compounds will be done by certain enzymes produced by the microbes. The enzyme secreting bacteria will have optimum pH between 6.5 and 7.5 and enzymes are also pH-dependent. Soil pH will also stimulate the pesticide adsorption. It also influences the absorption of pesticide fragments on organic and

clay surface. Considering the susceptibility of pesticides to acid or base catalyzed hydrolysis, the soil pH also affects it [37].

6.7.4 Organic Matter

By offering nutrients for cell growth, organic compounds will also regulate the pesticide movement by adsorption process, thus biodegradation is affected. Soil organic compounds will aid in the biodegradation of pesticides. A decreased microbial degradation by stimulating pesticide adsorption is observed. When organic materials are added to swamp soils, they help in the rise of the bacterial degradation of pesticides. Reports claim to have degraded linuron by microbial activity in non-sterilized soils and it was purely by the addition of organic matter [26].

6.8 Future Perspectives

As compared to different remediation techniques, i.e. thermal disposition, incineration, etc., a brighter future is seen for bioremediation for the degradation of pesticides [38]. A study conducted to understand the degeneration of target compounds in the provinces claimed that the tactics do not stretch the measure of the biological status or functioning of the degrader microbes. The further creation of toxic arbitrates can prevent the process or increase the risk of grade in soil. Such a backdrop happens among the developments in laboratory research. Lack of information to know the complete mechanism of contaminant degradation rate and lack of selected study centers and lack of technology demonstrations act as major backdrops. To rush the development of innovative techniques which are fast, reliable, and cost-effective is urgently needed. Development of accessible, expanded, and well-documented databases in the field of bioremediation is required [28].

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7

Bioremediation of Plastics and Polythene in Marine Water

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7.1 Introduction

The continued increase in anthropogenic activities has led to pollution in almost all the domains of the ecosystem, which has affected all the living creatures on the planet earth. With increasing pressure on the dependencies on the plastic and its products, the available disposal capacities have become insufficient to meet needs. Plastics are a group of materials that are either synthetic or naturally occurring. The malleable nature of plastic makes this most desired material when it comes to applicability. Chemically, it is a polymer composed of repeating units joined via polymerization to form long extensions that form the macro form of plastic. The range of application of plastics has widened to so many fields that it is nearly impossible to count them. Being such a useful material has attributed to its overutilization and mismanagement. The mismanaged and overused plastic can be seen piled up in the environment affecting all forms of life. Today, plastic pollution has increased to the extent that this polymer has started integrating into the food chain of many organisms, including plants.

The global plastic production data is quite astonishing. The oceans are dumped with roughly 8 million metric tonnes of plastic each year. Currently, 150 million measured tonnes of plastic waste are being circulated in our marine ecosystem (http://www.oceanconservancy.org/tarsh-free-seas/plastics-in-the-ocean (accessed 8 September 2020)), making it one of the worst affected. The problem of marine pollution persisted for a long and escalated when globalization and industrialization took place. The marine environments, including flora and fauna, have long been struggling with plastics in the marine waters. As the dependence of humans on plastics is increasing, this has led to the ultimate impact on marine life.

The oceanic currents allow the convergence of plastic material to accumulate overtime at the major vortices forming a huge mass of floating plastic over the surface of the water. All forms of plastic that end up in the ocean are hazardous to marine life

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forms as it has a number of effects ranging from disturbing their food chain to choking them to death. In addition to the existing problem of macroplastics, which is discussed, the emergence of micro- and nanoplastics is also posing an emerging threat.

Plastics are now an integral part of the marine ecosystem, but their interaction with various physical, chemical, and biological processes results in their breaking up into smaller fragments giving rise to micro- and nanoplastics. The only option we are left with to manage such issues is bioremediation.

7.2 Plastic Pollution: A Threat to the Marine Ecosystem

More than a dozen species have succumbed to the poison of plastic. This deadly polymer is taking a heavy toll on the diversity of aquatic life. Around 800 species are affected worldwide by marine debris, all of which are anthropogenic in origin, and the plastic litter accounts for 80% of this debris (http://www.pewtrusts.org/en/research-and-analysis/articles/2018/09/24/plastic-pollution-affects-sea-life-throughout-the-ocean (accessed 12 September 2020)). Humans, on the other hand, are also exposed to plastic pollution as a significant fraction of the population imparts seafood as a part of their daily diet. The incorporation of plastic into the food chain has exposed nearly all life forms of life, including birds, wildlife, marine forms, and humans, to the effects of plastic pollution (Figure 7.1).

The presence of plastic disrupts the natural balance and ambiance if the marine ecosystem by interfering with entire biogeocycles causing all marine life forms to suffer. Various reports suggest the ingestion of plastic by fishes and other marine organisms not only cause digestive problems but also may lead to life-threatening issues such as a blockage in the gut and ulcers in the lining of the gut. Birds, too, got trapped by the attractive look of the floating plastic debris which chokes them, and they end up dying due to suffocation. Around 44% of the seabirds, cetaceans, and sea turtles have been documented to have ingested plastic in one form or another (http://www.marineinsight.com/environment/how-is-plastic-ruining-the-ocean (accessed 12 September 2020)).

Disposal of plastic into the water bodies comes with many toxic and hazardous compounds such as additives, flames retardants, plasticizers, colors, etc. These compounds, when degraded by natural forces in water, consume oxygen resulting in lowering the concentration of dissolved oxygen in that vary environment. A lower level of oxygen has a severe impact on marine animals, especially whales, dolphins, and penguins.

7.3 Micro- and Nanoplastics

The recalcitrant nature of plastics enables them to persist in the environment for a very long time. This property causes the plastic to fragment naturally, resulting in mesoplastics (5–40 mm), microplastics (1–5000 μm), and nanoplastics (0.1 μm or less). The microplastic contamination has emerged as a newer form of pollution

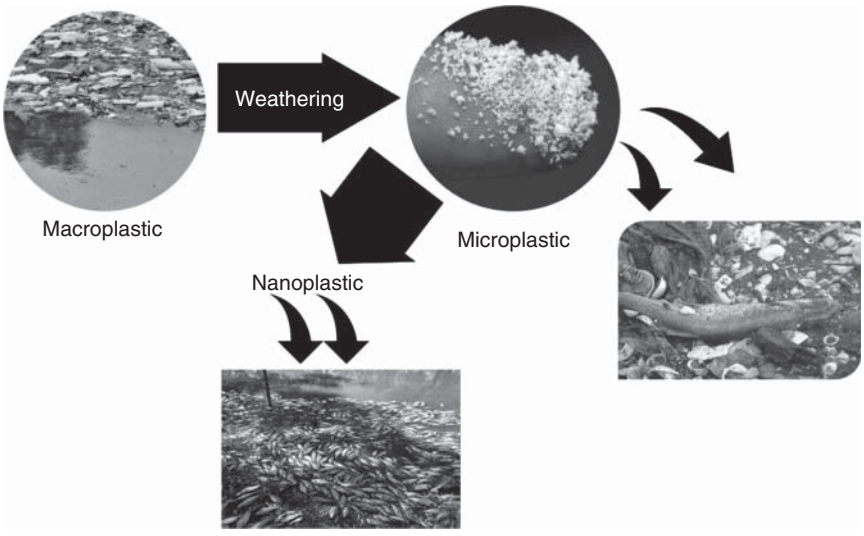

Figure 7.1 Effect of micro- and nanoplastics on marine life.

which has an increasingly damaging impact on the ecosystem. Microplastics have been reported from the water surface of open ocean to estuaries, subtidal segments, and deep oceans. There have been reports of microplastic in considerable concentration in Arctic sea ice.

The major characteristics of micro- and nanoplastics are

1. Being smaller in size, they can travel farther and faster in the environment.
2. They are efficient sorbents of other pollutants and often release sorbates to the surroundings – for example, heavy metals, persistent organic pollutants, antibiotics, etc. The chemicals hence released leads to direct toxic effects on aquatic fauna at the tissue, cellular, and molecular level.
3. Being micro- and nano-sized, they can move through various trophic levels and gets incorporated into the food chain.
4. They also can migrate through tissues.

The existence of micro- and nanoplastics can now be considered as ubiquitous as their widespread distribution, starting from open oceans to marine life from where it travels up the trophic levels to human beings and other landforms. Hence, they have invaded a wide variety of life forms and the ecosystem.

7.3.1 Microplastics

Microplastics can be broadly classified as primary and secondary. Primary microplastics constitute plastic particles that are directly released into the environment as microsized pellets, beads, etc. These include waste scrapings from various production industries and wearing and tearing from day-to-day operations. Secondary microplastics, on the other hand, are the microplastics that are

formed over the course of time in the environment by physical, chemical, and biological forces of nature. These include mechanical stresses, UV, oxidation, and biodegradation.

7.3.1.1 Toxicity of Microplastics

Large surface area to volume ratio of microplastics results in the release of their constituents chemicals into the surrounding water, which includes UV stabilizers, flame retardants, plasticizers, and colors, etc. Chemicals such as flame retardants (polybrominated diphenyl ether [PBDE]) were found in *Puffinus tenuirostris*, and plasticizer (mono-2-ethylhexyl phthalate [MEHP]) was detected in muscle tissue of *Cetorhinus maximus* (basking shark) [1].

The toxicity of microplastics on the marine ecosystem has been studied since the late 1980s and early 1990s. Various studies since then were conducted to establish the toxicity level of microplastics in the marine environment. For instance, a study done on the marine model organism *Mytilus edulis* by Browne et al. (2013) determined the effect of fluorescent polystyrene (PS) microspheres on the uptake, translocation, and cell viability [2]. The major findings of this study came out to be that the short-term exposure of microplastics did not cause a considerable effect on cells. Still, long-term exposure has certainly affected various biological functions, including cell viability. A study by Graham and Thompson (2009) on *Thyonella gemmata*, *Holothuria floridana*, and *Cucumaria frondosa* established that microplastics could transfer between various trophic levels in the food chain and food web [3].

1. Uptake of microplastics by marine animals:
   There are some unique features of microplastics owing to which marine organisms easily take them up. Few of which are as follows:
   a) Due to the attractive appearance of microplastics, lower organisms such as phytoplankton, zooplankton, etc., prey upon them, mistaking it being food and transfer them to higher trophic levels
   b) The low density of microplastics is prone to be eaten by filter feeders and suspension feeders. On the other hand, high-density microplastics are eaten away by marine animals during sinking through the water column.
   c) Attractive colors may be deceived as natural prey by vision predatory marine animals.
   d) The probability of preying upon microplastics is entirely dependent on the enrichment with them in the marine ecosystem.
2. Microplastics in the body of marine life forms:
   Microplastics persist inside the digestive tract for a long when ingested. Studies have shown their presence inside gills, intestines, digestive tubules, and stomach of many animals such as the mussel *M. edulis* and pelagic fish *Platycephalus indicus* [4]. Different kinds of experiments performed, such as acid tissue digestion, fluorescence methods, labeled microplastics, showed the persistence of microplastics inside the body of marine animals.
3. Impact of microplastics on marine animals:
   a) Microplastics can cause ventricular overloading, resulting in blockage in the digestive tracts of birds and ultimately lead to their death. Microplastics also cause malnutrition in animals such as turtles where the structural feature of

esophageal papillae inhibits regurgitation and may end up in its accumulation. An increased concentration of microplastics in the vicinity may cause cytotoxicity, decreased feeding, and decreased phagocytic and increased lysozyme activity in lower marine invertebrates.

b) The ingested microplastics accumulating inside the body may block fluid passage across the body, thus rendering internal organs inflamed and damaged. The long-term accumulation of microplastics may start leaching of additives, plasticizers, flame retardants, which may be fatally toxic.

7.3.2 Nanoplastics

Nanoplastics exhibit enhanced toxicity in comparison with microplastics. Even the short-term exposure of nanoplastics has been reported to cause assimilation and deposition inside the body. Polystyrene nanoplastics cause embryotoxicity and abnormal gene expression in the sea urchin *Paracentrotus lividus* [5]. Carboxylated and amino-modified nanoplastics adversely affect feeding, motility, and cell viability.

Various studies have shown that the trophic transfer of nanoplastics is one of the major routes of pollutant exposure. It has also been concluded to confer behavioral modifications and metabolic issues in large marine animals. The behavioral changes are the result of the invasion of the brain by nanoplastics.

7.4 Microbes Involved in the Degradation of Plastic and Related Polymers

Plastic degradation in the marine environment can occur in multiple ways like abiotic and biotic processes or biological processes like microbial degradation using various microbial entities. The natural phenomenon of degradation is a prolonged process. The natural weathering effects here account for the degradation of plastic. Microbes, on the other hand, aid in the biodegradation of plastic polymer in a speedy and environment-friendly manner. The basic principle behind this process is that the microorganisms directly utilize plastic as a source of carbon and energy for their growth and multiplication, consuming it in the process. The degradation of plastic occurs by an enormous genus of bacteria by both aerobic and anaerobic modes, and fungi by anaerobic mode only. Aerobic bacteria utilize as a terminal electron acceptor and mineralize plastics and other polymers to CO_2 and H_2O. Anaerobic microorganisms (including bacteria and fungi) may use iron, sulfate manganese, and nitrate as a terminal electron acceptor to degrade organic hydrocarbons into simpler molecules like methane, for example (Table 7.1).

7.4.1 Biodegradation of Plastic

Different microbial entities employ a specific mechanism by which they can degrade various forms of plastic. Recent studies revealed that polyethylene (PE), polyethylene terephthalate (PET), and polystyrene (PS), along with polypropylene (PP) constitute a significant portion of plastic debris in the marine system.

Table 7.1 Microorganisms involved in the bioremediation of different types of plastic.

Sr. no.	Type of plastic	Organism	References
1.	Polyethylene	*Rhodococcus ruber*	[6]
		Penicillium simplicissimum	[7]
		Brevibacillus borstelensis	[8]
		Streptomyces sp.	
		Zalerion maritimum	[9]
2.	Polyhydroxy alkanoic acid (PHA)	*Pseudomonas stutzeri*	[10]
		Alcaligenes faecalis	[11]
		Streptomyces sp.	[12]
		Basidiomycetes, deuteromycetes (*Penicillium*, *Aspergillus*), ascomycetes	[13]
3.	Polycaprolactone (PCL)	*Alcaligenes faecalis*	[13]
		Clostridium botulinum	[10]
		Fusarium	[14]
		Pseudomonas sp.	[15]
		Moritella sp.	[16]
		Shewanella	[16]
4.	Polylactic acid (PLA)	*Bacillus brevis*	[17]
		Fusarium moniliforme	[14]
		Penicillium roqueforti	[10]
5.	Polyurethane (PU)	*Fusarium solani*, *Aureobasidium pullulans* sp.	[18]
		Pseudomonas chlororaphis	[19]
6.	Polyvinyl chloride (PVC)	*Pseudomonas putida*	[20]
7.	Polystyrene	*Rhodococcus ruber*	[21]
8.	Polyethylene terephthalate (PET)	*Bacillus cereus*	[22]
		Bacillus gottheilli	
9.	Polypropylene (PP)	*Bacillus* sp.	[23]
		Rhodococcus sp.	

7.4.1.1 Polyethylene (PE)

Polyethylene, also known as daily use plastic, could be largely seen floating at the sea surface. It is the most difficult form of polymer to degrade because of its linear long carbon chain (family: polyolefins), which is very stable and contains balanced charges for the destabilization of local charge enzymatic oxidation via monooxygenases and dioxygenases, which leads to the formation of alcohol and peroxyl group.

The oxidation of reactions makes a polymer more hydrophilic and susceptible to enzymatic attack, further leading to the complete mineralization of the polymer.

7.4.1.2 Polyethylene Terephthalate (PET)

There are very few bacterial isolates known to be involved in the degradation of PET, which generally includes *Ideonella sakaiensis* [24], *Pseudomonas mendocina* [25], and *Thermobifida fusca* [26] along with some fungal communities which include *fusarium* species and *Humicola insolens* [27]. The enzyme PET hydrolase (the best-studied enzyme for PET degradation) has relatively lower turnover rates. The enzymes involved in the PET degradation contain a C-terminal disulfide bond, which helps in the attachment of organism with the hydrophobic surface.

Mechanism:

$$\text{PET} \xrightarrow{\text{PETase}} \text{mono}(2-\text{hydroxyethyl})\text{terephthalate} \xrightarrow{\text{MHETase}} \text{terephthalic acid} + \text{ethylene glycol}$$

Terephthalic acid is then internalized by a TPA transporter protein.

$$\text{Terephthalic acid} \xrightarrow[\text{1,2-dihydroxy-3,5-cyclohexadiene-1,4-dicarboxylate dehydrogenase (DCDDH)}]{\text{TPA-1,2-dioxygenase (TPADO)}} \text{protochatechuic acid}$$

$$\text{Protochatechuic acid} \xrightarrow{\text{PCA-3,4-dioxygenase (PCA34)}} \underset{\text{(hemiacetal form)}}{\text{4-carboxy-2-hydroxymuconic acid}}$$

$$\text{4-carboxy-2-hydroxymuconic acid} \xrightarrow{\text{dehydrogenase}} \text{2-pyrone-4,6-dicarboxylic acid}$$

Finally, 2-pyrone-4,6-dicarboxylic acid enters TCA cycle.

7.4.1.3 Polystyrene (PS)

This high molecular weight, highly hydrophobic polymer, supports only partial degradation. To date, no enzyme is reported to degrade polystyrene completely. However, black-rot fungi *Gloeophyllum Striatum and Gloeophyllum trabeum* were reported to employ hydroquinone-driven Fenton reaction to attack polystyrol moiety. The degradation pathway involved mainly the oxidation of the styrene side chain:

$$\text{Styrene} \xrightarrow{\text{styrene monooxygenase}} \text{Styrene epoxide} \xrightarrow{\text{styrene oxide}}$$
$$\text{Phenylacetaldehyde} \rightarrow \text{Phenylacetic acid} \xrightarrow{\text{yields}} \text{Phenylacetyl Co-A}$$

Phenyacetyl Co-A enters TCA cycle, where the final products are acetyl Co-A and succinyl Co-A.

7.5 Enzymes Responsible for Biodegradation

See Table 7.2.

Table 7.2 Plastic-degrading enzyme with source.

Sr. no.	Type of plastic	Enzyme	Source of enzyme	References
1.	Polyethylene (PE)	Laccase	*Rhodococcus ruber*	[28]
		Alkane hydroxylase	*Pseudomonas* sp. E4 strain	[29]
2.	Polyethylene terephthalate (PET)	Cutinase		[30]
		Lipase		
		Carboxylesterase		
		PETase	*Ideonella sakaiensis*	[24]
3.	Polyurethane (PU)	PueB Lipase	*Pseudomonas chlororaphis*	[31]
		Cutinase	*Thermobifida*	[32]
4.	Polyamide	Cyclic dimer hydroxylase		[33]
		6-Aminohexanoate aminotransferase	*Arthrobacter* sp.	
		Semialdehyde dehydrogenase		
		Manganese-dependent peroxidase	White-rot fungus	[34]

7.6 Mechanism of Biodegradation

7.6.1 Formation of Biofilm

The foremost step toward the biodegradation of plastic is the attachment of microbial cells to the surface of the polymer, forming a film. There are various factors which may affect the formation of a biofilm as follows:

1. **Biotic factor**: biotic factors include nutritional source and formation of the film.
2. **Abiotic factor**: abiotic factors include the topography of polymer, presence of oxygen, chemical characteristics such as temperature, pH, salinity, chemical characteristics, and hydrophobicity, and surface roughness.

Various forces act on the microbial cells which initiate the process. One of which being nutritional deficiency, which leaves plastic as only nutrient sources and hence facilitates the adherence of the cell. The adherence was the initial physical interaction between the cells and the substrate, which cause an irreversible attachment of the cell. These attachment forces are responsible and determine the properties of biofilm hence form.

The formation of an initial layer of biofilm over the surface of the polymer is very crucial and deciding step. This layer determines the degradation efficiency. Chemical properties of biofilm, such as surface modification of the polymer structure of biofilm, all of these factors have an ultimate impact on the growth of subsequent

layers of the cells, its rate of growth, and the production of suitable metabolites responsible for degradation. Any variation in the properties mentioned above has a direct impact on the degradation efficiency. Some examples of biofilm-forming microbes include *Rhodococcus ruber* (mushroom-like structures) and *Alcanivorax borkumensis*, which pioneers in the degradation of low-density polyethylene by the formation of thick biofilm [35].

7.6.2 Biodeterioration

The next stage in the process of biodegradation after surface colonization and biofilm formation is biodeterioration. This stage is augmented by the release of exopolysaccharides (EPSs), a characteristic feature of biofilm-forming microorganisms. The EPS released in large quantities have the microorganisms to stick to the surface more tightly hence forming a more robust biofilm. The EPS, along with enzymes (exo- and endo-enzymes), have a significant impact on biodeterioration.

7.6.3 Biofragmentation

This stage largely includes the breaking up of polymer chains to oligomers, dimers, and monomers. The step mostly utilizes enzymes that distort the basic polymer geometry holding the polymer chain together, easing microbial attack. Microorganisms can directly utilize the resulting oligomer and monomer units as a carbon source, contributing to the production of biomass.

The initial attack occurs on the terminal moieties causing a sequential reduction in the molecular weight. The enzymes involved in the fragmentation of polymer belong to class oxidoreductases and hydrolases.

Hydrolases work by mainly acting on the carboxylic linkages specifically. Examples of hydrolases include esterases, lipases, and cutinases. The presence of three amino acids (aspartate, serine, and histidine) residues in the active site is a characteristic of hydrolases, and these three amino acids aid in the production of nucleophilic alkoxide group (–O), which attacks the ester bond-forming alcohol and acyl–enzyme complex.

Oxidoreductases, on the other hand, add oxygen atoms to alcohol and peroxide groups, which are easier to fragment and consume.

Two factors which affect the oxidation of plastic structure are

1. Length and exposure and type of additive used
2. Type of microbial species involved in the process

7.6.4 Assimilation

Biodegradation of plastic is an electron transfer process. The driving force for this process is mainly channeled through the oxidation of molecules that were obtained from chain fragmentation. Major products that are the result of enzymatic attack are amides, alcohols, and organic acids. These compounds are easily assimilated

by microorganisms through the cell membrane and enter the central metabolic pathway.

The electrons released from the substrate are finally consumed by a terminal electron acceptor, which in the case of aerobic microbes, is oxygen, and for anaerobic microorganisms is nitrates and sulfates. For traveling down various metabolic routes to terminal electron acceptors, electrons gain energy from oxidation via β-oxidation.

Degradation of plastic is generally a surface phenomenon where the oxidative and hydrolytic enzymes act on to eject out electrons and other simpler sources of carbon, which can be assimilated by microorganisms into their metabolic pathway, where they contribute toward the growth.

7.6.5 Mineralization

The conversion of all complex forms of polymer moieties into simpler molecules such as carbon dioxide, water, and oxygen, etc., constitute mineralization. This is the final step in the degradation of plastic, and the final product obtained is primarily the microbial biomass.

7.7 Biotechnology in Plastic Bioremediation

Biotechnology has been a boon to the field of biological and environmental science. Its use in the field of bioremediation of plastic has led to various outcomes which have benefitted the environment. The solution based on biotechnology may either be stand-alone, or they may complement the existing technologies. The term "biodegradation" presumes a nearly stand-alone method, but in nature, both abiotic and biotic factors contribute equally to complete degradation of the polymer under consideration. Moreover, abiotic degradation processes occur much before the microbial attack; hence, abiotic factors largely determine how the plastic will be biodegraded. The main drawback of biodegradation of plastic is that it takes a longer time for an initial attack on the polymer chain. This can be overcome either by pretreating the polymer making it more susceptible to microbial attack or genetic modification of organisms to enhance its inherent capability of biodegradation. The pretreatment of the polymer may pose various problems, which are huge capital investment, the involvement of hazardous chemicals, which pose an environmental risk.

Genetic engineering makes it possible to enhance and alter existing properties of the degradative enzymes, to modify and cluster multiple genes coding for enzymes into a single organism. These newer genes hence will produce proteins that will not only be genetically diverse but also be functionally rich and ultimately give us a pool of novel biocatalysts. For example, biosynthetic genes *phbA* (for 3-ketothiolase), *phbB* (NADPH-dependent acetyl Co-A reductase), and *phbC* (PHB synthase) have been cloned to produce PHA (polyhydroxy alkanoic acid) and PHB (poly(3-hydroxy butyric acid)). These genes are clustered in a single operon and have been expressed in *Escherichia coli* and *Pseudomonas* sp. [36].

Pseudomonas putida (an oil-degrading bacterium), the first organism to be engineered for novel catabolic efficiency, degrades different hydrocarbons. *Pseudomonas* sp. are a metabolically diverse group of bacteria which are also active degrader of various types of the plastic polymer. An important aspect of genetic engineering is the careful and detailed characterization of genes responsible for the production and regulation of desired enzymes. To determine the flow of genes and genetic information between microbial communities, ^{13}C stable isotopic markers are utilized. The ^{13}C-DNA gets stably incorporated as normal ^{12}C-DNA and can be traced out. Once characterized, one can genetically modify the desired microorganism for the degradation of plastic waste. The technique, although it seems very simple, it comes with various technical and ethical difficulties.

The bioremediation of plastic is mainly involved in finding and reporting of microbial diversity and the biotransformation pathway. Deciphering metabolic pathways and networks of host cells capable of attacking plastic is an essential step. It will further support the engineering of whole cells and engineering enzymes capable of plastic mineralization. The basic aim is to enhance the biocatalyst nature of enzymes. A prominent example being the enhanced expression of cutinase from *Thermobifida cellulosilytica* toward PET via site-directed mutagenesis in *E. coli* BL21-Gold expression vector [37].

Various modern techniques which are now being used for genetic engineering as follows:

1. 16S rRNA sequencing, microarray profiling, and the NextGen high-throughput metagenome sequencing give insights about the phylogeny and expression systems.
2. The Cre/lox recombinase system can be used to manipulate gene expression levels by inserting specific genes at the recognition site.
3. Zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), which binds to the DNA site associated with the various catalytic domain of enzymes, are also utilized to introduce new functions with reduced error rates.
4. The most advanced and highly specific modern gene-editing tool is CRISPR-Cas (clustered regularly interspaced short palindromic repeats). With this system, one can insert programmed RNA to complementary DNA sequences and hence introduce new genes at any location in the genome.

The complete understanding of genomics, proteomics, and metabolomics data would provide an insight into the processes going on in any living system which could be rewired to address the target. Here, computation biology comes into the picture, which combines complex biological and engineering techniques to design biological systems. It helps in digging out all the possible outcomes along with their results and consequences via comparative algorithms that work at the back-end. *In silico* studies, along with molecular modeling, have till now not been utilized in the field of environmental remediation science. But the computational analysis will prove to be the boon for research in this area and speed up the process to a greater extent.

7.8 Future Perspectives: Development of More Refined Bioremediation Technologies as a Step Toward Zero Waste Strategy

The plastic has entered all domains of the ecosystem. There is a need the hour to develop as many methods as possible to degrade the plastic polymer, so that the environment runs free of this deadly pollutant. As it is known from studies that there exists a small pint of enzymes that are available that can degrade synthetic plastic. The significant drawback here lies in the initial attack on the high molecular weight chain of the highly robust and stable polymer.

The current old school techniques of cultivation of organisms seem inefficient in searching for a method that could degrade plastic at a much faster pace. On the other hand, various modern techniques such as metagenomic analysis, gene-mining, and dark matter proteins offer promising results and solve this growing problem. Various biotechnological interventions in the field of molecular engineering have shortened the path between genes and pathways. The accessibility to different online databases makes it possible to correlate the pathway and functions of key proteins, and cellular metabolism provides an insight to all naturally existing capabilities. *In silico* computing has immensely helped predict and understand the metabolic pathways and the working mechanism that organisms follow during degradation. Genome mapping and protein engineering seem to play a vital role in designing proteins and enzymes, which may simplify the task of cleaving plastic debris.

There is an important need to standardize the existing findings/protocols on plastic degradation. As commercial plastic, which results in plastic waste, it consists of not only plastic alone but also certain additives, plasticizers, and colors, etc., which make the actual process of biodegradation difficult and complicated. Keeping in mind the biodegradation of existing plastic debris, one area should also be considered in the production of environment-friendly polymer (biopolymer), which could be easily degraded naturally. The production of the existing biopolymer needs to be paced up and should replace synthetic polymer for practical applications.

Various research groups are looking for a solution that may reverse the dogma where biodegradation of long-chain polymers to their monomeric units can proficiently be achieved. Through integrated approaches and interdisciplinary work done in a well-organized and disciplined manner, it is very much likely to curb plastic pollution on a few more decades to come.

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Conflict of Interest

Authors declare no conflict of interest regarding the publication of this book chapter.

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Part III

Biological Degradation Systems

8

Microbes and their Consortia as Essential Additives for the Composting of Solid Waste

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8.1 Introduction

The major contributing factors for solid waste (SW) management are population explosion, migration to urban areas, industrialization, urbanization, and indiscriminate disposal of SW. The first two main contributors will result in a surge of megacity population that mandates strategizing a solid waste management (SWM) plan [1]. The major steps toward this shall include collection of solid waste, transportation to spot locations, and processing and disposal of the waste through technologies (biological or chemical).

Indian SWM rules (2000) were drafted by the Ministry of Environment and Forest, providing all the necessary guidelines for efficient collection, processing, and disposal of the solid waste. Even though the present SWM system appears competent enough, deeming the last few decades (changing waste characteristics and volume) fewer modifications in the process might prove vital. These modified SWM practices may promote source material reduction, recycling, energy recovery, and waste stabilization prior to landfilling. However, it might differ and depend on the country, state, rural–urban setup, and government authorities [2].

8.2 Classification of Solid Waste

According to the statistics, the last decade has witnessed a sharp increase in the quantity of solid waste generation. This is the unavoidable outcome of expansion, production, and consumption activities practiced in any economy, clearly reflecting the improvement in socioeconomic status. The rapid expansion of urban, agricultural, and industrial sectors results in population increase that adds up to the SW problems, thereby polluting environment and faster depletion of resources. The quantity of waste generated in any country/state/society mainly depends on the population and lifestyle of the inhabitants. Thus, waste reduction and management becomes a social responsibility and appropriate management of solid waste will

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eventually moderate the harmful impact of SW on environment and human health. The solid waste mainly constitutes of heterogeneous as well as homogeneous wastes discarded from residential, industrial, and agricultural areas. It may be categorized in the following three ways:

- **On the basis of source of origin**: municipal solid waste (MSW), agro waste (AW), industrial waste, bio-medical, e-waste)
- **On the basis of nature of waste**: organic and inorganic material
- **On the basis of toxicity**: hazardous and non-hazardous

The MSW comprises of food waste (FW), rubbish from residences, commercial, institutional, industrial, construction, demolition, and sanitation waste. It also includes recyclables like paper (3–6%), plastic, glass, and metal (each less than 1%); toxic substances such as paints, pesticides, used batteries, and unused medicines. The organic and inorganic fractions encompass kitchen refuse, packaging material, fruit and vegetable waste, clothes, used bottles, paper, cans, batteries, etc., which generally do not carry any value to the primary user [3, 4].

The monitoring and management of solid waste is a grave concern for developed as well as developing economies, posing a bigger challenge for municipal authorities. Further, various processes and techniques that are employed for effective management of such waste must include monitoring, gathering, transportation, processing, recycling/recovery, and appropriate discarding of final residue.

8.3 Role of Microbes in Composting

Composting is considered as a significant approach for treatment and disposal of solid wastes, but with a question of time and efforts required toward processing and degradation. The solution to this lies with the organic composition of the waste as they possess enormous amount of cellulose, hemicellulose, sugar components, and lignin. Composting refers to biological hydrolysis of solid waste (organic fraction) into a stable and sanitized residue called humus [5]. It is an aerobic process wherein microbes implicate the waste decomposition by consuming carbon and nitrogen as energy sources. A sufficient percentage of oxygen (15–20%) and water is directly correlated with an efficacious microbial succession [6]. This is essentially required to ensure a spontaneous rise in temperature to eliminate the pathogens, ultimately generating a good product in the form of soil-enriching compost.

The process of composting can be divided in to three phases: (i) an initial mesophilic phase, where mesophilic bacteria and fungi degrade the simpler compounds such as sugars, amino acids, etc., at a temperature of around 45 °C; (ii) thermophilic phase, where thermophilic bacteria and fungi degrade complex compounds like fats, cellulose, hemicellulose, and lignin at a temperature of around 60 °C; and (iii) cooling phase materializes products (humic-like substances) with limited microbial activity, decreased temperature, and a declined organic matter degradation. A gradual and effectual microbial succession is critical for composting wherein growth of nonspecific microbes extensively affects the waste degradation

rate and compost quality. The waste composition, nutritive supplements, and environmental setup (ambient or trial) support the type of bacteria and fungi that emerge during composting [7]. Furthermore, additives in form of microbes will alter the breakdown process of cellulose, hemicellulose, and lignin during composting. These microbes release substrate-based hydrolytic enzymes to split up the complex compounds to produce water-soluble metabolites. In addition, assessment and monitoring (physiological profiling) of microbial succession within composting can denote the scale of compost maturity.

Suitable microbial addition during composting of solid waste (organic) is known to accelerate the degradation process, thus enriching the nutrient composition of the resulting compost. Microbial additives will speed up the process through nutrient transformation and production of extra-cellular enzymes (lignocellulases, proteases, etc.). When added to a compost mixture, effective microbes will influence the temperature, ammonia balance, and production of volatile organic compounds (VOCs) and nitrogen–sulfur compounds [8, 9]. Hence, microbial inoculation will serve as a positive stimulation in composting and aims to achieve maximum efficiency. The same has been illustrated in the Figure 8.1.

Several studies in the past have revealed microbial addition as a positive approach toward solid waste treatment resulting in enhanced rate of waste degradation [10, 11]. These microbes can be isolated from various sources such as soil, cow dung, straw, or waste mixture depending on the requirement [12]. While in some conditions, a pre-derived mature compost may be applied to waste mixture, in other conditions either a single bacterial or fungal strain or a viable consortium of effective microbes (mixed culture) might be substantial enough [10, 13]. Few examples of potent microbes are *Bacillus* spp., *Cellulomonas*, *Pseudomonas*,

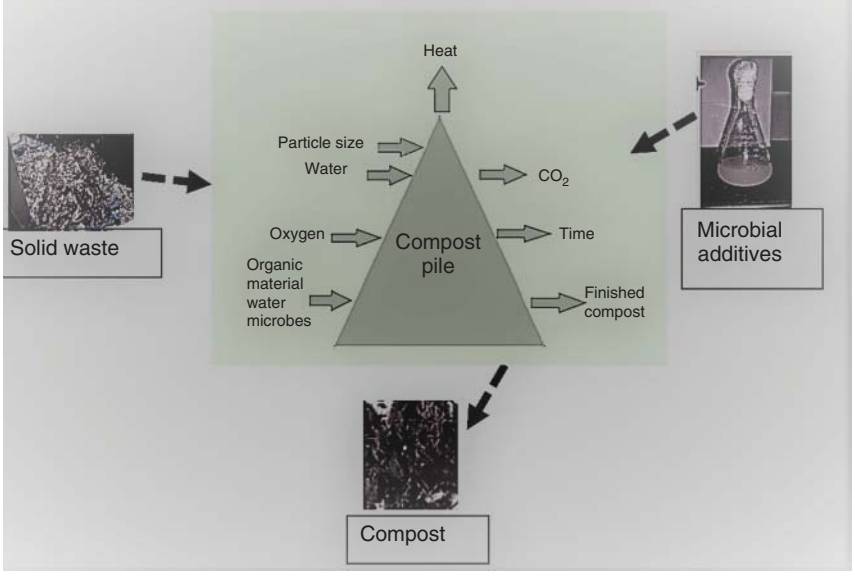

Figure 8.1 Process flow of a SW composting with microbial additives.

Thermoactinomycetes, *Aspergillus* sp., white-rot fungi, *Trichoderma* sp., etc. [14]. These microbial additives will deal with the organic portion of the waste by secreting degradative–oxidative enzymes. The enzymes will shorten the initial lag time and generate a phenomenal rise in temperature that justify the accelerated rate of waste composting. However, not all the microbial strains have the ability to secrete enzymes, good enough for solid waste biodegradation. They significantly affect the physical, chemical as well as biological parameters (pH, C/N ratio, color, humic substances, pathogenic activity, and germination index) within the waste matrix which are indirectly related to the compost quality. Monitoring the temperature, ammonia emissions, C/N ratio, etc., can effectively present a clear picture about the quality of the generated compost [8]. In addition, reduction in composting time, C/N ratio, and organic content will clearly indicate good enriched compost. Some investigations discussing the impact of microbial addition on the degradation of various solid wastes (MSW, FW, and AW) have been summarized in Table 8.1.

8.4 Effect of Microbial Consortia on Solid Waste Composting

While exploring the synergistic action of potent microbes in a consortium on composting of solid waste, a boost in the waste degradation rate and reduction in the composting time was evident. To support this, a MSW composting experiment was performed, where feedstock material was inoculated with a mixed microbial culture of *Phanerochaete chrysosporium*, *Trichoderma viride*, and *Pseudomonas aeruginosa*. Here, decrease in the composting period was observed [14]. In another investigation on passive bin composting of MSW, an inoculum of *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus nakamurai*, and *Bacillus velezensis* was added. Here, rapid degradation of organic substrates was confirmed with maximum humification [35, 36]. A study recorded enhanced mineralization rate when MSW was co-composted using mixed cultures of bacteria and fungi (*Bacillus Casei*, *Candida rugopelliculosa*, *Lactobacillus buchneri*, and white-rot fungi *Trichoderma*) [37]. Following the same approach, transformations in carbon content and C/N ratio were brought in MSW composting due to the addition of fungal consortium [38]. Among three aerated composting bioreactors having *Aspergillus niger* or old compost or control as additive, maximum decrease in C/N ratio was observed in the first one (63.37% or 59.6% or 46%, respectively). Operation time was also reduced in the first case (18 days). Checking the effect of potent microbial consortium (8% inoculum) on waste mineralization represented a stable pH, C/N ratio (30), temperature (27 °C), and carbon dioxide formation (5.28 d/l) [18].

Experimental conditions were modified and an extreme rise in the waste degradation rate was noticed, when microbial additives were inoculated at multiple stages (initial and second stage) of MSW composting [19]. This can be attributed to improved microbial diversity and lesser competition between additives and indigenous microbes [22] with repressed foul odor and better polymerization or humification of waste. Inoculation of fungal consortia during MSW composting

Table 8.1 Composting studies with microbial addition to various solid waste (MSW, FW, and AW).

Compost feedstock	Additives	Results	References
Municipal solid waste	Mixed culture (*bacteria* and fungi)	Improved humification and process efficiency	[15]
	Trichoderma viride, *Aspergillus niger*, and *Aspergillus flavus*	pH, temperature, TOC, TKN, C/N ratio, germination index, degradation, and maturity	[16]
	Cellulolytic consortium of clostridia	Improved anaerobic digestion of cellulosic biomass	[17]
	Bacteria	Higher mineralization, stabilized C/N ratio	[18]
	Aspergillus niger	Stabilized C/N, process time	[19]
	Microbial inoculums originated from sludge and MSW	Higher enzyme activity, C:N ratio, and compost maturity	[20]
	Psychrotrophic bacteria	Stable temperature, moisture content, pH, C/N, nitrogen, and enhanced compost stability	[13]
	White-rot fungi (*Phanerochaete chrysosporium, Trametes versicolor*, and *Fomes fomentarius*)	Accelerated degradation, C/N, pH, electrical conductivity, and a better degree of maturity	[21]
	MI (cellulolytic and lignocellulolytic)	Better compost quality, C/N ratio, temperature, odor, enzymatic activities, and humification	[22]
	Lactic acid bacterium *Pediococcus acidilactici*	Enhanced organic matter degradation	[23]
	Thermophilic lignocellulolytic fungi	Stable compost maturity	[24]
	Trichoderma	Stable C:N ratio, increased nitrogen (N), phosphorus (P), and potassium (K), enhanced soil properties	[25]
	Microbe culture	Accelerated degradation	[26]
	Cellulolytic thermophilic actinomycetes	Increased humic substances	[27]
	Lactic acid bacteria, yeast and phototrophic bacteria	Increase in humic substances, matured compost	[10]
	Mesophilic yeast *Pichia kudriavzevii*	Accelerating the composting process	[6]
	Efficient microbes (EM)	Enhanced humification	[28]
Agricultural waste	*Phanerochaete chrysosporium*	Better C/N ratio, temperature, and organic matter	[29]
	Bacillus subtilis and *haetomium thermophilum*	Accelerated degradation of proteinaceous and degree of humification	[30]

(Continued)

Table 8.1 (Continued)

Compost feedstock	Additives	Results	References
	Bacillus licheniformis 2D55	Increase in CMCase and FPase production	[31]
	Consortia of beneficial microbes like N-fixers, P-solubilizers, or K-mobilizers and biocontrol agents.	Achieved value-added compost for direct application for crop production.	[32]
	Actinomycetes	Increase in nitrogen content and a drop in carbon and organic matter in compost.	[33]
	Trichoderma species and cellulase degrader mixed culture coded as AMB1	Increased cellulase activity by consortia and 50% reduction of hemicelluloses content in rice straw.	[34]

decreased the C/N ratio in four treatment piles 1–4 from 36.12 to 17.12, 31.43 to 17.52, 31.49 to 19.47, and 34.54 to 26.18, respectively, on the 35th day of composting [28]. However, lignocellulosic microbes when inoculated during waste composting were not as effective as expected in small-scale composting.

A modified drum composting conducted to verify the effect of microbial additives and natural air circulation on FW resulted in a mature compost after 60 days [10]. Microbial inoculation to FW fairly paced up the degradation process and achieved a fairly higher temperature with an early maturity. An enhanced degradation rate, better humification, and reduction in odors were shown with the addition of microbiota to the organics. In another study, when an inoculum of thermo-tolerant lipolytic microbes was added to FW, results indicated better decomposition of organic matter in lesser time suggesting good composting. A 60-day FW composting under optimized pH and temperature, wherein a consortium of *Pseudomonas* sp., *T. viride*, and *Trichoderma* sp. was added, showed an accelerated degradation rate. Furthermore, waste volume reduction with total decolorization of fruit waste was observed in the microbe-amended composting with fine good grade compost. FW (rabbit food and cooked rice) when composted with acid-degrading yeast (mesophilic) at preset temperature for different time periods developed a well-stabilized compost. An improved conversion rate of FW into compost was further observed as a result of inoculated effective microbes at day 45 of composting [10]. To sum up, FW and MSW degradation through vital microbial additives is highly significant and relevant, especially in terms of reduced process time and other benefits like no foul odor and pathogen-free compost.

Regarding AW, cocktail of AW and microbes turned out to be a better alternative for waste composting and it was efficient with cellulase production. It was found that a concoction of untreated sugarcane bagasse and pretreated rice husk inoculated with *Bacillus licheniformis* 2D55 showed enhanced degradation which is

remarkable. A good carboxy methyl cellulase (3.7- and 1.4-fold) and FPase (2.5- and 11.5-fold) production was observed in the compost obtained from concoction [39]. A mixed culture proved to be better option for rice straw degradation than single strain, owing to better activation of enzyme production. When three fungal cultures were used as inoculant to degrade rice straw, 50% of the hemicellulose content in rice straw was successfully degraded. The highest cellulase activity (1.5 U/ml) was noted for the mixed culture consortium compared to individual fungal strains, suggesting application of mixed cultures is an effective strategy for composting [31]. A study carried out on the bioconversion of agro-residue into compost by fortifying the residue with individual or a consortium of beneficial decomposers. For this study, N-fixers, P-solubilizers, or K-mobilizers along with biocontrol agents were used as fortifiers. The compost thus derived was enriched with humic acid, amino acids, mineral nutrients, and phyto-hormones [34].

8.5 Benefits of Microbe-Amended Compost

The intensive agricultural practices currently in use cause severe deterioration of soil health and cause damage to the environment. Biological compost derived through an integrated on-farm production can work as magic to remediate them. This can support farmers by providing value-added compost for direct application in the field as a soil conditioner. When compost is added as an organic amendment, it basically enriches the quantitative soil parameters (biomass, enzymatic activities, porosity, water-holding capacity, and nitrogen content). It not only improves soil quality but also increases the soil microbiota that eventually responsible for the plant health. In addition, application of bio-manure generated from SW (FW, MSW, and AW) composting is reported as an economical method for *in situ* removal of metalloids, pesticide immobilization, and getting rid of budding pollutants [32].

Though the current waste management system is good and effective, it has its own implications. Composting with effective microbial additives (general or waste specific) is rendered as an economic and eco-friendly way out. The ease of isolation from source and application to the composting process makes it an appropriate solution for the existing waste treatment and disposal issue. Herein, a prominent role can be played by the government in solid waste collection, segregation, treatment, and disposal by implementing a centralized Solid Waste Management System (SWMS).

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9

Biodegradation of Plastics by Microorganisms

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9.1 Introduction

Biodegradation is a biologically catalyzed mechanism where, before transferring to the environment, the carbon-based material goes through a transition from complex modules into smaller substances. This process is often accomplished with the assistance of living organisms like bacteria, fungi, and protozoa. Such organisms perform the task of breaking the matter up into new objects. In certain contexts, however, biodegradation is frequently used to designate most of the biologically induced alteration in the substrate. It is therefore very important to understand the processes of biodegradation very clearly and the microorganisms that carry out the entire process.

Due to the remarkable lightness, durability, and low-cost properties, plastics have been widely used as a substitute material for paper, wood, and metal for various applications over the last 50 years. Use of plastic materials in every area of life and development has been growing every year. Composition wise, plastics are high-molecular-weight compounds of natural or synthetic origin. From 1950 to 2019, the overall amount of plastics production was 8100 Mt. worldwide, with an increase of around 230% during this time. In 2013 alone, 56 Mt. of polyethylene terephthalate (PET) was produced [1]. Plastic materials have been an environmental catastrophe and one of the most persistent pollutants due to their wide variety of use as well as challenging degradation properties. Plastic wastes are one of the chief environmental pollutants. They enter all habitats and ecotypes, when they get released into the environment. The world's capacity to deal with them is being overwhelmed by the growing production of disposable plastic goods. This pollution can harm and affect human, wildlife, and wildlife habitat adversely. Moreover, plastic contamination can damage soil, rivers, and oceans. Plastic pollution at sea is a global problem and has a concentration of about 580 000 plastic parts/km^2 in everywhere and the entire ocean [2]. Animals living inside the sea and outside of

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it are suffering from various hazardous plastic wastes, either trapped inside the plastic frames or by consuming the leftover materials. The easily devourable, small pieces of plastics lead to malnutrition, obstruction of the small intestines, or slow intoxication of chemical products leached from plastics. Gases like furans and dioxins are produced due to burning of plastics and result in ozone layer depletion. Besides, dioxin adversely affects human health. According to a report published by the Center for International Environmental Law [3], annual emissions of carbon dioxide from the production and ignition of plastics will double from 850 Mt./year by 2030.

The methods which are used to treat plastics have some drawbacks. If plastics are buried in the soil, it destroys the building holding potential of that soil, rendering it too soft. In fact, numerous toxic compounds and/or gases resulting from the incineration of plastics may cause a disturbing contamination of the environment. The biodegradation of plastics by microorganisms, apart from these processes, has proved to be an environmentally safe form of reducing and degrading waste plastics. While recycling and reprocessing is the most preferred process yet, biodegradation is beneficial and efficient for plastics with defined applications. A variety of microorganisms have been discovered that are capable of degrading various plastic materials. Microorganisms are more widespread in nature that may make a significant contribution to the biodegradation of plastics. The application of biodegradable plastics is increasing mainly in the packaging sector, as well as in the agriculture and health industries. However, biodegradation is not commonly used in plastics industries. Complex chemical structure of plastics and lack of optimization conditions for plastics degradation may be the causes of less commercial applications. In order to replace non-biodegradable plastics from different sectors, provision must be made for a proper and effective waste management system. However, a waste control procedure, training in waste management, and as well as establishing a suitable industrial biodegradation techniques also required. The use of biodegradable plastics will certainly provide environmental protection accordingly.

9.2 Definition and Classification of Plastics

9.2.1 Definition of Plastic

The word “plastic” refers to the capacity to bend or deform without breaking. The term “plastic” originated from Greek word “plastikos” and means any material that may be designed in any manner. It is a kind of synthetic or semi-synthetic organic polymer with the presence of carbon and hydrogen. It is usually derived from petrochemicals and can occur in both natural and synthetic forms [4]. This plastic nature makes the material amenable to be molded, pressed, and extruded into different forms. These forms included films, threads, sheets, tubes, bottles, boxes, etc. It is projected that almost 40% of plastics are manufactured for various packaging purposes in the world. Plastics are light in weight, have good electrical strength and corrosive resistance, as well as possess excellent electric insulating

properties. Plastics are economically beneficial compared to other materials and can be made in both opaque and translucent forms.

9.2.2 Classification

9.2.2.1 Based on Biodegradability

Biodegradable plastics are those plastics that are decomposed by the surrounding environment after a period of time. The biodegradation of plastics can be accomplished by allowing the molecular structure of plastic films to be metabolized by microorganisms in the atmosphere to produce a material that is inert and less toxic to humans. They may come in the form of either bioplastics or petroleum-based plastic products. Bioplastics are plastics made from recycled raw materials. Biodegradability and microbial assimilation are the basis for the use of bio- and fossil-based polymers in biodegradable plastics. Biodegradation process includes enzymatic and non-enzymatic hydrolysis. Depending on the presence or absence of oxygen, biodegradation results in the production of H_2O, biomass, energy, CO_2, and methane [5].

Bio-based degradable plastics can be derived from renewable sources. From an ecological point of view, biodegradable polymers are advantageous in some commercial processes due to their ability to be degraded biologically. Cellulose, starch, and many more starch-based degradable plastics such as co-polymers are consumed directly by microorganisms. Several microorganisms such as *Aspergillus fumigatus*, *Variovorax paradoxus*, *Comamonas* sp., *Acidovorax facilis*, and *Paucimonas lemoignei* can be isolated from the environment such as soil. These microorganisms can degrade the bio-based polymers both under both aerobic and anaerobic conditions [6]. Biodegradable fossil-based plastics are used for a variety of purposes, particularly for packaging purposes (Figure 9.1).

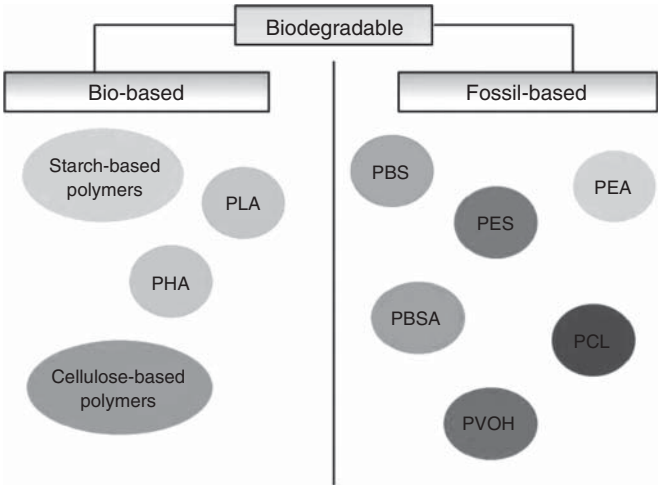

Figure 9.1 Classification of plastics (based on biodegradability).

9.2.2.2 Based on Structure and Thermal Properties

Thermoplastics Thermoplastic materials are those materials that may be cooled and heated several times without altering their chemical or mechanical properties. Polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polystyrene (PS), and polytetrafluoroethylene (PTFE) are some examples of common plastic polymers. Their molecular range varies from 20 000 to 500 000 amu. These polymers can be used for a variety of possible applications, such as wire and lighting systems, matrix for natural or synthetic fibers. Thermoplastics may melt before going to the gaseous state and are soluble in certain solvents. Most thermoplastic materials give high strength, resistance to shrinkage, and stress-free bendability. Thermoplastics may be extracted, processed, and synthesized by chemical processing from plants in vast quantities.

Thermosetting Plastics Thermosetting or heat convertible plastics are substances which cannot be reshaped by heat or pressure once they are in their final state. They differ from the thermoplastic in the way that they are more resistant to temperature and breakage. Due to the permanent chemical structure and oddly related arrangement, these plastics cannot be recyclable. Thermosetting plastics are preferred for many applications in the building materials, due to their long-term characteristics. Common examples include vulcanized rubber, fiberglass, polyester resins, polyurethane (PU), melamine, epoxy resin, and bake lite.

9.2.2.3 Characteristics of Different Biodegradable Plastics

Polyhydroxy-Alkanoates (PHA) Polyhydroxy-alkanoates (PHA) polymers are thermoplastic. They are easily biodegradable, non-toxic, and produced naturally by bacterial fermentation of lipids and sugars. Production of PHA is encouraged by the availability of carbohydrates during the bacterial growth phase. It can be preserved in microorganisms and weigh as much as 80% of the organism's dry weight. The rate of microbial degradation and the nature of end products depend on the soil, environmental conditions, and the nature of PHA. Microorganisms are able to degrade and utilize it for their carbon and energy requirement under restricted energy and carbon sources. Some representative bacteria found responsible for the biodegradation of this kind of plastics that include *Bacillus*, *Nocardiopsis*, and *Cupriavidus* [7]. Again, a variety of fungal genera (*Mycobacterium* and *Micromycetes*) are recognized to incorporate PHA by using aerobic and anaerobic mechanisms. It is less sticky when melted than conventional polymers. They are soluble in halogenated solvents such as chloroform, dichloromethane, or dichloroethane, but insoluble in water. It is resistant to hydrolytic degradation. Despite having good resistance to ultraviolet (UV), it shows low resistance to acids and bases. PHA polymers are used in medical sector, packaging, and pharmaceutical industries due to their biocompatibility and biodegradability. Disposable medical equipment, food packaging materials, and some paints are also widely used PHA products.

Polylactide (PLA) It is biodegradable in nature and thermoplastic aliphatic polyester. It can be obtained from renewable sources such as corn starches, sugarcane,

tapioca roots, etc. **Polylactide** (PLA) has characteristics comparable to PE, PP, or PS. It is likely to be a substitute for low-density polyethylene and high-density polyethylene (LDPE and HDPE), PS, and PET [8]. It can be produced when corn or other carbohydrates are converted chemically into dextrose. Dextrose is fermented into lactic acid and then polycondensed into monomers of lactic acid. There are different stereo-chemical compositions of PLA, namely, L-, D, and L,D-lactide. They have melting temperatures of 170–180 and 55°C for the optical pure L- and D-lactide and the amorphous L,D-lactide, respectively [5]. It has use in the food industry to package sensitive food items. This is also used in medical implants, drug distribution system, tissue engineering etc. due to its capability to be integrated into human and animal bodies.

Starch-Based Polymers These polymers are complex mixtures of starch and biodegradable plastics. Some examples include polybutylene succinate, polylactic acid, polybutylene adipate terephthalate (PBAT), polycaprolactone (PCL), and polyhydroxyalkanoates. Thermoplastic starch (TPS) is formed by the action of thermal and mechanical energy [5]. Low water vapor barrier with reduced mechanical properties, brittleness, and bad process ability are some of the drawbacks [5]. On the other hand, applications include bottle manufacturing, food packaging, disposable table ware, cutlery, and coffee machine capsules.

Polyethylene Succinate (PES) Polyethylene succinate (PES) is a thermoplastic polyester which is made by co-polymerization of succinic anhydride and ethylene oxide. An additional type of production is ethylene glycol and succinic acid polycondensation [9]. A bacterial strain called *Pseudomonas* sp. AKS2 is documented to degrade this polymer in an efficient manner. In contrast to diversity of PCL degrading microorganisms, the distribution of microbes that degrades PES is restricted. Another PES-degrading thermophilic strain named *Bacillus* sp. TT96 has been isolated from the soil. Moreover, the genera *Bacillus* and *Paenibacillus* phylogenetically have many mesophilic microbes that are isolated with the intrinsic ability to degrade PES [10]. Plastics industries use PES to manufacture films for livestock, in the form of paper coating material, and for shopping bags.

Polycaprolactone (PCL) PCL is a fossil-based biodegradable, bio-compatible, and non-toxic polymer. It is partially crystalline and has a low melting point and a glass transition temperature of 60 and –60 °C, respectively [5]. It is made by ε-caprolactone with ring-opening polymerization [5]. Microbial lipases and esterases can degrade this. The causative bacteria for PCL biodegradation can widely be found in the atmosphere. *Aspergillus* sp. ST-01, a fungal strain, has been found to degrade PCL into a wide variety of produces such as butyric, caproic, succinic, and valeric acids [11]. It is also an industrial polymer. It has a wide range of applications in hot-melting glue and laminating bags. It is also found in model making, prototyping, and molds making for reproduction. Due to its low melting point and high biodegradability, pure PCL is mostly used in clinical applications.

Polyvinyl Alcohol (PVOH) It is a biodegradable vinyl polymer. The degrading microorganisms require selective enrichment to successfully mineralize polyvinyl alcohol (PVOH) or PVOH blends. Water solubility depends on the hydrolysis ratio. It is commonly used as coatings (e.g. carbon dioxide barrier of PET), adhesives parts, and additive in the production of paper and board.

9.3 Biodegradation of Plastics

9.3.1 General Outline

Biodegradation is the process where the constituent polymer gets converted into several compounds by the action of the enzyme secreted by microorganisms. Commonly used enzymes are lipases, proteinase k and dehydrogenases [12]. Biodegradable plastics typically decompose in the natural environment. Both synthetic and natural plastic materials can be biologically degraded by bacteria, fungi, and actinomycetes. These microorganisms turn the polymeric materials into their metabolic products by chemical degradation (e.g. H_2O, CO_2, CH_4, biomass, etc.). The process of biodegradation proceeds dynamically under conditions such as soil and its properties. Soil pH, oxygen, moisture, temperature, and light are factors that affect the optimal growth of microorganisms. The degradation characteristics and the rate are strongly dependent on soil pH, oxygen, moisture, temperature, and light. They consume various substances as a source of food in order to eliminate its original form [6]. Plastics with high molecular weight are usually hard to degrade. Different characteristics of plastic materials such as morphology, mobility, presence of functional group, molecular weight, additives, and cross-linking usually control the degradation process [12]. Amorphous plastics are easily biodegradable than crystalline polymers. Moreover, plastics having high melting point also make them less biodegradable. Chemical and physical properties are important as they play a significant role in the biodegradation of plastics. As for example, plastics having side chain are less biologically degradable than those without side chains.

However, the biodegradation of plastic is a steady process. Primarily, it begins by environmental factors, like temperature, pH, and UV rays. Biodegradation of plastics involves following steps:

(a) Attachment of microorganisms on to the polymeric surface area.
(b) Growth and development of microorganisms (by using the polymer as a carbon source), and
(c) Final degradation of plastic material.

Microorganisms can be attached to the surface of plastic until it is hydrophilic. Once attached to the surface, microorganisms proliferate by using plastic as a carbon source. Initially, enzymes (extracellular) secreted by microorganisms cause the main chain to cleave. This leads to the generation of low-molecular-weight fragments, i.e. monomers, dimes, or oligomers. Once transformed into their monomers, they begin to transform into a mineralized form. In the case of large polymers, it creates

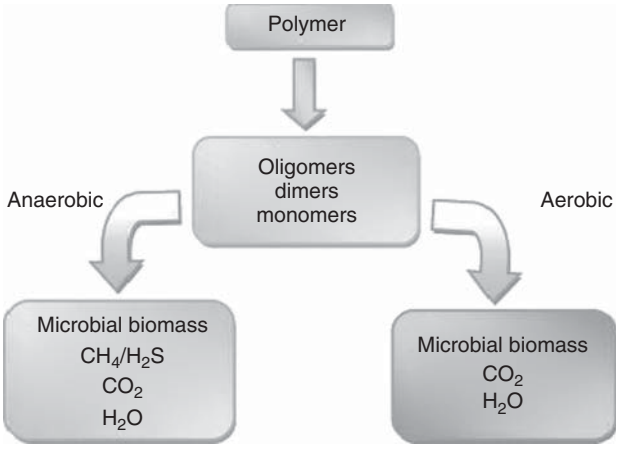

Figure 9.2 Reaction pathways of polymer biodegradation. Source: Gu [14].

problem to pass through the cellular membrane. As a result, depolymerization takes place to create smaller monomers and later, the microorganisms absorb it [12]. These low-molecular-weight compounds are further used by microbes as a source of energy and carbon. Small oligomers can also spread to the microorganism which adapted in the internal environment [13]. The reaction pathways are demonstrated in Figure 9.2.

The following two distinct methods affect the working mechanisms of microorganisms during degradation of different plastics. (i) Direct action method: in this process, metabolites formed during the degradation of plastics act as a nutrient for the growth and development of microorganisms. (ii) Indirect action: where the metabolic substances of the microbes cause further deterioration.

9.3.2 Biodegradation Phases and End Products

Abiotic and biotic are the main two classes of biodegradation. Abiotic biodegradation includes hydrolysis and photolysis. Instead, biotic biodegradation is the microbial degradation. Furthermore, based on organic material, biotic degradation may be further classified into aerobic and anaerobic degradation [15].

9.3.2.1 Aerobic Biodegradation

Aerobic biodegradation is also known as aerobic respiration. It is a significant constituent of the natural reduction of pollutants in the so many hazardous wastes. Usually, this biodegradation reaction occurs in the presence of oxygen due to the microbial breakdown of organic contaminants. The final products of this process are water, minerals, biomass, carbon dioxide, and salt [15]. The presence of oxygen, the causative organisms, surrounding environment, and the chemistry of the system are categorized by oxidative conditions.

In the cellular respiration process, aerobic bacteria use oxygen to obtain energy by the oxidation of sugars and fats. There is no production of pungent gases in the

aerobic digestion, unlike the anaerobic digestion stage. The aerobic biodegradation process helps improve the environment of human beings and animals, thereby controlling the pathogens. Comparatively better and more complete digestion of solid wastes can be obtained through aerobic processes with a reduction of more than 50% accumulation in most cases.

9.3.2.2 Anaerobic Biodegradation

In the anaerobic biodegradation, organic contaminants are quickly biodegraded by microorganisms, under anaerobic conditions. This process produces methane, water, CO_2, minerals, and salt [15]. Anaerobic degradation occurs in a situation when there is a dominance of anaerobic microbes over the aerobic ones. Several anaerobic bacteria use sulfate, iron, nitrate, manganese, and CO_2 as their electron acceptors, thus breaking down the organic compounds to smaller complexes. The anaerobic process is widely known for the biodegradable waste treatment and treatment of wastewater sludge because it helps to reduce the mass and volume of input.

Various bacteria, including acetic acid and methane forming bacteria, are engaged in the anaerobic degradation of plastics. These acetic acid and methane forming bacteria feed upon the primary feed stock that undergoes several progressions changing it into intermediate molecules containing hydrogen, sugar, and acetic acid before being transformed into biogas at the end. To date, scientist found four major chemical and biological stages of anaerobic degradation, namely, hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

9.3.3 Mechanism of Microbial Degradation of Plastic

Microorganisms have different enzymes that enable plastic to be used as a substrate. For this purpose, they are best suited or eco-friendly degradation of plastics. Microorganisms break down polymers bond through redox enzymes. The nature of enzymes and their catalytic activity vary depending on the microbial species and strains. Different types of microbial enzymes can degrade different types of polymers. As for example, *Bacillus* spp. and *Brevibacillus* spp., both can produce protease, which can degrade various polymers [16]. Fungus contains laccase that can degrade lignin and also oxidize aromatic and non-aromatic compounds. Microbial enzymes control the biodegradation of polymers in an efficient and eco-friendly way.

Biological degradation of plastics waste product depends on so many factors. They include molecular weight, surface area, functional groups, hydrophilicity, hydrophobicity, chemical structure, crystallinity, and melting point of plastics. Molecular weight of polymers also influences the digestibility cycle of plastic. If the molecular weight of the polymer is higher, the degradation potential is lesser, due to reduction in solubility and degradation rate. There are four key steps involved in the microbial digestion of polymers namely, bio-deterioration, assimilation, bio-fragmentation, and mineralization. They are briefly described in Figure 9.3. The phase of degradation can be altered by the superficial degradation. Bio-deterioration largely affects the superficial degradation process. Growth of microbial biofilms causes severe physical and chemical degradation on the

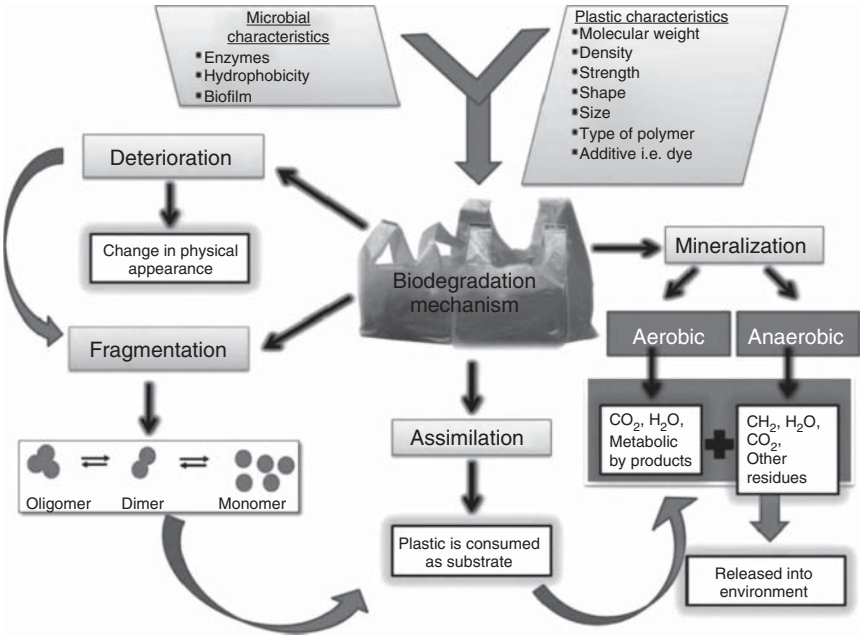

Figure 9.3 Mechanism for the biodegradation of plastics. Source: Jaiswal et al. [17].

polymers. The formation of microbial biofilms depends on the structure and composition of polymers. Upon completion of bio-deterioration, microbial degradation is known as biofragmentation, where microbial enzymes include their catalytic action on polymers.

Bacteria contain oxygenases (mono-oxygenases and di-oxygenases), which have potential to break down and degrade the polymers. They also bind oxygen molecules to a long carbon chain and produce less recalcitrant, less environmental damaging alcohol and peroxyl products. In addition, lipases and esterases as well as amide group endopeptidases catalyze the mechanism of carboxylic group's transformation. *Brevibacillus borstelensis*, *Rhodococcus ruber*, *Pseudomonas chlororaphis*, *Pseudomonas putida* AJ, *Thermomonospora fusca*, *Alcaligenes faecalis*, and *Clostridium* sp., etc. are some microorganisms found to degrade polymers. The ultimate results of microbial polymer degradation are mineralization and assimilation. Plastic monomers formed by biofragmentation which is essential to cross the microbial cell membranes. Few of these monomers which are not able to penetrate through cell membrane generally stay outside of the membrane and do not get assimilated. In the cells, plastic monomers are oxidized by catabolic pathways and energy produced generates a new biomass of a cell. The assimilation cycle requires atom incorporation into the microbial cell for complete degradation.

9.3.4 Factors Affecting Biodegradation of Plastics

Biodegradation of polymers is affected by several factors. These include the characteristics and exposure conditions of plastics, such as flexibility, mobility, molecular

weight, functional groups, and co-polymers present in plastic structure, as well as extra additives and plasticizers. Hydrophilic biodegradation is faster than the hydrophobic degradation. This hydrophobicity is facilitated by the availability of functional groups. Again, amorphous and soft plastics with lower molecular weight and density degrade faster than the higher ones. Biodegradation process of plastics is also affected by the occurrence of easily breakable bonds including ester or amide bonds.

Exposure conditions can also be characterized into abiotic and biotic factors. The foremost chain scission from photodegradation decreases the average molecular weight of the polymer. Microorganisms and moisture get better accessibility to the polymer chain through the reduction in molecular weight. Abiotic factors including moisture, pH, and temperature can influence the rate of hydrolysis during the degradation process. The increase in moisture content and temperature thus increases the levels of hydrolysis reactions and microbial growth. As microorganisms require moisture for their growth, survival, and multiplication, the rate of degradation of polymer is higher in the presence of moisture. Availability of moisture enhances the rate of hydrolysis by producing further chain scission reactions [4]. A change in the pH (acidic or basic condition) modifies the rate of hydrolysis reactions. Degradation of plastic products alters the pH followed by the polymer degradation rate and microbial growth. Equally, enzymatic degradability is inversely affected by the melting point of the polymer as also the temperature of degradation [4].

Different enzymes have specific active sites and are capable of biodegrading polymers. As for example, polyesters with straight chain, assimilated from di-acid monomers containing 6–12 carbons, degraded rapidly by enzymes formed by *Aspergillus flavus* and *Aspergillus niger* as compared to any other polyesters with straight chain monomer [18]. From the biodegradability perspective, molecular weight plays an important role in determining certain properties of polymers. The increase in the molecular weight decreases the degradability [4]. Bio-surfactants are enabling to biodegrade polymers because they contained certain functional groups. Bio-surfactants are known as amphiphilic compounds, formed on living surfaces and very active under high salinity, pH, and temperature.

Environmental factors influencing the degradation of plastics include, in particular, UV light, temperature, humidity, and the incidence of chemicals. Two dimensions influence the microbial degradation of plastics on the influence of the external environment. At the same time, the growth and metabolism of associated organisms, particularly biomass and the degradation process of microbes, can be affected and influenced by the environment. On the other hand, aging and damage to plastics can occur due to the external oxidation environment, which also accelerates the degradation and utilization of plastics by microorganisms.

9.3.5 Microorganisms Involved in the Biodegradation Process

Microorganisms have different enzymes that enable them to utilize environmental pollutants as their energy source. Their tiny nature helps them to encounter waste and contaminants quickly. Thus, they are ideal for the removal of contaminants.

Most plastics are generally polyesters that are normally catalyzed by microbial enzymes such as cutinases or esterases. A particular set of enzymes is involved in the degradation of different plastic materials. There are a number of enzymes found to be involved in plastics degradation process. In the PLA degradation processes, protease enzyme mainly targets PLA and depolymerase is responsible for the first degradation of long-chain polymer. After that, serine proteases, i.e. protease K and trypsin, further degraded it to low-molecular-weight compounds. Research revealed that the proteases (the PLA degrading enzyme) may found in *Amycolatopsis*, *Saccharothrix*, and *Pseudonocardia*, etc. However, these proteases can degrade PLA only [19].

On the other hand, cutinases have the ability to degrade PCL, PLA, and PET, etc. and similarly, lipases may degrade PCL, PLA, and polybutylene succinate (PBS), etc. It is noticeable that a variety of microbial enzymes can degrade plastics. However, a single plastic material can be degraded by different enzymes. Furthermore, a wide variety of plastics may be degraded by plenty of bacteria. Besides bacteria, fungi also use and adhere to plastic materials by decreasing hydrophobicity and forming a number of chemical bonds. These chemical bonds include carboxyl, carbonyl, and functional groups of ester. Some fungus such as *Penicillium funiculosum*, *A. fumigatus*, and *Pseudomonas fluorescens* can degrade 10 or more types of plastics. However, more than 30 species of microorganisms are reported to degrade PE, PU, and PHB. On the other hand, PCL and PLA can be degraded by over 20 types of microbes [19].

9.3.6 Enzymes Involved in the Plastic Biodegradation

Each living cell, including the microorganisms, contains diverse enzymes that vary with different species or strains of same species. Thus, the process of plastic biodegradation involves different enzymes. Several studies report on the use of enzyme extracted from microorganisms in the process of degradation of plastics. Table 9.1 displays the examples including that of lignin-degrading enzymes such as laccase, manganese-depending peroxide and hydrolyase such as urease, protease, and lipase.

Until now, only 79 established microbial enzymes have been recognized to act as degrading agent for plastics. Very few researchers have studied the potential mechanism of this degrading activity of the microbial enzymes. Most of them suggest that the bond cleavage step during hydrolysis process is the main mechanism for degradation of polymers by microbial enzymes. Some studies also indicated mechanism of affection of microbial enzymes to the polymer surfaces, and mechanism of entrance of large molecules of polymers to the active site of enzymes [27].

However, increase in chain flexibility of polymers might increase the rate of hydrolysis of PBAT by lipase form *Rhizopus oryzae*, and cutinase form *Fusarium solani*. Enzyme with higher available active sites has higher hydrolysis tendency against PBAT [27]. A research demonstrated that combination of cutinases and a polymer binding segment might heighten the hydrolysis the polyester poly(1,4-butylene adipate), which recognized as better binding between enzymes and polymers [28].

Table 9.1 Different types of microbial enzymes responsible for the degradation of different types of plastics.

	Characteristics				
Types of plastics	**Recycling**	**Type of polymer**	**Microorganisms involved**	**Enzymes involved**	**References**
PET	Yes	Polyethylene monomer	*Ideonella sakaiensis* 201-F6	PETase, MHETase, α- or β-hydrolase, lipase	[20]
LDPE	Yes	Polyethylene monomer	*Acinetobacter* sp. 351, *Pseudomonas* sp. AKS2, *Pseudomonas stutzeri*, *Brevibacillus*, *Aspergillus*, *Pseudomonas* sp. E4, *Aspergillus japonicas*, *Aspergillus terreus*, *Streptomyces setnoii*, *Streptomyces badius*, *Streptomyces viridosporus*	Hydrolase	[21]
HDPE	Yes	Polyethylene monomer	*Klebsiella pneumonia* CH001, *Bacillus* sp. BCBT21, *Pseudomonas putida* S3A, *Arthrobacter* sp., *Aspergillus flavus*, *Comamonas acidovorans*, *Rhodococcus*, *Penicillium oxalicum* NS4	Cutinase, lipase	[22]
PVC	Yes	Vinyl chloride monomer	*Staphylococcus, Klebsiella*, *Micrococcus*, *Pseudomonas putida* AJ, *Chaetomium*	Catalase, peroxidase, laccases	[23]
PS	Yes	Styrene monomer	*Actinomycetes*, *Pseudomonas* sp.	Alkene mono-oxygenase, Esterase	[24]
PP	Yes	Propylene monomer	*Brevibacillus*, *Rhodococcus*, *Bacillus*, *Aneurinibacillus*, *Rhizopus oryzae*, *Phanerochaete chrysosporium*	Lipase	[25]
Others	Partial	Ethylene monomers, nylon	*Pseudomonas* sp.	Nylon hydrolase	[26]

Source: Jaiswal et al. [17].

9.3.6.1 Cutinases (EC 3.1.1.74)

Cutinases can hydrolyze the cutin, which is an aliphatic polyester originated from plant cuticle. This type of polyester hydrolases is from the superfamily of α/β hydrolases. They are very much active against several polyester plastics. Lipases and cutinases both display traid composed of Ser-His-Asp. Owing to its lack of usual lipase lid structure, the active site of cutinases is exposed to the solvent. Based on origin, structure, and homology, this cutinase enzyme can be divided into two groups, i.e. (i) fungal origin and (ii) bacterial origin [29]. Cutinases from fungus are using for the hydrolysis and structure modification of PET films and fiber [29]. However, cutinases extracted from *Thermomyces insolens* performed higher activity against low crystalline PET due to the thermal stability very close to the glass transition temperature (70 °C) of PET [29]. Cutinases and its homologues from bacteria (*Thermobifida* species) show PET hydrolyzing character. However, cutinases from *Thermomonospora curvata, Saccharomonospora viridis, Ideonella sakaiensis*, and as well as its metagenome isolated from plant compost also show PET hydrolyzing character [30].

9.3.6.2 Lipases (EC 3.1.1.3)

Lipases are similar to cutinases, from the superfamily of α/β hydrolases, and both display traid composed of Ser-His-Asp. Microbial lipases have the ability to hydrolyze aliphatic polyester or aliphatic-aromatic co-polyesters. Lipases from *Thermomyces lanuginosus* degraded PET and poly (trimethylene terephthalate) [29, 31]. Lipases demonstrated lower hydrolytic activity against PET, comparing to cutinases. This might be due to its lid structure covering the buried hydrophobic catalytic center, and it prohibits the contact of aromatic polymeric substrates to the active site of the enzymes [31]. Lipases from *T. lanuginosus* [31] and *Candida antarctica* [32] can also degrade low-molecular-weight PET degradation products. Combination of lipases from *C. antarctica* and cutinases from *T. insolens* improved the production of terephthalic acid resulted from hydrolysis of PET [32].

9.3.6.3 Carboxylesterases (EC 3.1.1.1)

PET oligomers and their analogues can be degraded by carboxylesterases isolated from *Bacillus licheniformis, Bacillus subtilis,* and *Thermobifida fusca* [33]. Carboxylesterases Tfca isolated from *T. fusca* can release water products from high-crystalline PET fibers. Combination of carboxylesterase with polyester hydrolase exhibits inhibitory activity against low-molecular-weight degradation products of PET because of their higher activity against PET oligomers [33].

9.3.6.4 Proteases

Research revealed that proteases isolated from *Pseudomonas chlororaphis* and *P. fluorescens* can degrade polyester PU [34]. Proteases such as papain are very active against PU and may hydrolyze amide and urethane bonds. The porcine pancreatic elastase can release degradation of products from polyester and polyester PU due to the breakdown of hydrolyzable ester, urethane, and urea bonds in the soft segment domains of the polymer.

9.3.6.5 Lignin Modifying Enzymes

Lignin modifying enzymes such as laccases (EC 1.10.3.2), manganese peroxidases (MnP, EC 1.11.1.133), and lignin peroxidases (Lip, EC 1.11.1.14) are known to degrade lignin, a complex cross-linked aromatic polymer of phenypropanoid units [35]. These enzymes are responsible for the biodegradation of PE. In the presence of iron, laccase, a thermo-stable enzyme isolated from *R. ruber* C208 can degrade UV-irradiated PE films both in culture supernatants and in cell free extract. The key mechanism involved in this process includes the increasing of carbonyl groups and decreasing of molecular weight within the amorphous component of PE films. Similarly, laccase isolated from *Trametes versicolor* can degrade high-molecular-weight PE membrane, in the presence of 1-hydroxybenzotriazole, which oxidized non-phenolic substrates by the enzyme. However, high-molecular-weight PE also degraded by a combination of MnP from white-rot fungi (*Phaerochaete chrysosporium* ME-446) and MnP isolated from IZU-154 [36]. This high-molecular-weight PE also degraded by cell free supernatant from *P. chrysosporium* MTCC-787 containing both extracellular LiP and MnP, respectively. The combination of Lip and MnP enzymes permitted the degradation of 70% of the pre-oxidized high molecular weight of PE with 15 days of reaction.

9.4 Current Trends and Future Prospects

There is an emerging trend in the use of environmental-friendly bio-based and fossil-based biodegradable plastics. The proper use of biodegradable plastics in the form of sustainable waste management approaches should be practiced worldwide. A recent research suggested that hydrolysis of PET and its mono-2-hydroxyethyl-terepthalic acid to ethylene glycol and terephthalic acid is occurred by two enzymes isolated from *I. sakaiensis*, 201-F6 strain [19]. Research also illustrated that *Pantoea* spp. and *Enterobacter* spp. have the ability to degrade LDPE [37]. Tan et al. [38] found some microbes convert the organic styrene (an industrial waste material from plastic processing) into PHA. They also recognized that *P. putida* NBUS12 is an efficient and effective styrene degrading bacterium. *Achromobacter xylosoxidans*, a recently characterize bacteria, was found to affect the structure of HDPE. Similarly, a thermophilic bacterium, named, *Anoxybacillus rupiensis* Ir3 (JQ912241), was isolated from soil in Iraq, which confirmed a good capacity and efficiency to utilize aromatic compounds as carbon sources followed by degradation [39]. Extensive research is therefore required worldwide to improve the process of degradation of bio-based and fossil-based plastics in order to recognize their potential eco-friendly applications and waste management plans.

Innovative and eco-friendly biodegradable plastics should be used in the packaging, agriculture, and heath industry which is the simplest strategy to resolve the plastic-related problem throughout the world. Bio- and fossil-based biodegradable polymers should be exploited more proficiently and effectively to degrade in the cells, eco-friendly, or under optimized facilities. At present, however, only non-biodegradable petroleum products are utilized for the processing of plastics,

which can pose a major risk to the environment. As a result, the demand of environment-friendly polymers and plastics is increasing day by day in the certain application such as in the manufacturing of food packaging, packaging stuff, and disposal medical items. The use of bio-based, fossil-based, and biodegradable plastics in the agricultural sectors, fishery materials (fishing nets), bio-absorbable plastics in therapeutics, surgical frameworks, and sterile goods need to be increased for better future of the world. In addition, biodegradable plastics must be used where the diffusion into the environment is imminent or where it is difficult to remove the garbage. However, proper management and arrangement of the plastic waste and littering control of polymers is very much essential for this world. For the next generation, biodegradable plastics should be used to build a sustainable world for specifications. Moreover, this plastic must be biodegradable and recycled in a balanced way to make it reusable. Researches from different area namely, biomass, process engineers, chemists, and microbiologist should make proper use of their expertise, strength either individually or work together to make the society sustainable by producing eco-friendly materials.

List of Abbreviations

CH_4	Methane
CO_2	Carbon dioxide
etc.	et cetera
e.g.	Exempli gratia
H_2O	Water
HDPE	High-density polyethylene
LDPE	Low-density polyethylene
Mt.	Million tonne
sp.	Species
spp.	Several species
PBAT	polybutylene adipate terephthalate
PCL	Polycaprolactone
PE	Polyethylene
PES	Polyethylene succinate
PET	Polyethylene terephthalate
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PLA	Polylactide
PP	Polypropylene
PS	Polystyrene
PTFE	Polytetrafluoroethylene
PU	Polyurethane
PVC	Polyvinyl chloride
PVOH	Polyvinyl alcohol
Ser-His-Asp	Serine-Histidine-Aspartate

UV	Ultraviolet light
%	Percentage
α	Alpha
β	Beta
0°C	Degree Celsius

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10

Enzyme Technology for the Degradation of Lignocellulosic Waste

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10.1 Introduction

Among all plants, cereal plants contribute to nearly 75% of lignocellulosic waste. Cereal straw alone accounts for 2.9 billion tonnes per annum. Maximum residue is produced from sugarcane as bagasse, stalk, leaves, press mud, etc. which are wasted [1]. Cellulose, hemicellulose, and lignin are the primary lignocellulosic components, along with some proteins and phenolic polymers.

Nowadays, many countries are producing "First-generation bioethanol" by fermenting starch or sucrose obtained from the food grains wheat, corn, and sugarcane. The growing demand for bioethanol is increased to 100 billion liters in 2020 [2]. However, reduction in the production cost for bioethanol can be achieved by using the non-food substrates like lignocellulosic materials for the production of "second-generation ethanol." This second-generation ethanol can be blended with fossil fuels to produce biofuels [3].

The plant cell wall polysaccharides mainly consist of cellulose, hemicellulose, pectin, and the phenolic polymer, lignin. Together, they give structural integrity, strength, and complexity to the cell wall [3]. Cellulose forms a linear polymeric chain consisting of 8000–12 000 D-glucose units linked by β-1,4-glycosidic bond. Microfibrils are highly insoluble structures which exist as bundles in the crystalline form of cellulose. The non-crystalline structure, i.e. the amorphous region, is found within the microfibrils [4]. The second rich polysaccharide present in the cell wall is hemicellulose, which is a heterogeneous polysaccharide. The structural backbone of hemicellulose contains many heteropolymers like xylan, galactomannan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan. In the cereals and hardwood, xylan is most prevalent, while in the softwood, glucomannan is the most abundant polymer of hemicelluloses [5, 6]. Pectin is a heteropolysaccharide with α-1,4-linked D-galacturonic acid forming the backbone. It has two regions viz. the smooth region containing D-galacturonic acids which can undergo acetylation or methylation and the hairy region containing both

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D-galacturonic acids and α-1,2-linked L-rhamnose. L-arabinose and D-galactose can form side chains by linking to the rhamnose residue. Substituted phenylpropane units joined by carbon–carbon linkages form the backbone of lignin. Lignin forms a cross-linked network within the cell wall which gives mechanical strength and protection against osmotic lysis to the cell wall [7]. The four bonds viz. ether bonds, ester bonds, carbon–carbon bonds, and hydrogen bonds provide intramolecular and intermolecular linkages within and between various components of lignocellulose.

Degradation of lignocellulosic waste is difficult since it is hard to dissolve lignin and its subunits. Lignin is also linked to cellulose and hemicellulose. Because of the crystalline nature, cellulose is hard to degrade [8]. Fungi and other microorganisms which are able to break the lignocellulosic wastes do so by secreting various enzymes that act synergistically. Some monomers are released during the process which are utilized by the microorganisms as their energy source [3]. The lignocellulosic wastes can be utilized to develop various value-added products like animal feed, composites, enzymes, biofuels, pulp, and paper.

10.2 Enzymes Required for the Degradation of Lignocellulosic Waste

10.2.1 Degradation of Cellulose

Cellulose is a linear chain polysaccharide comprising D-glucose monomers linked by β-1,4-glycosidic bonds [1]. Adjacent polysaccharide chains are linked by van der Waals forces and hydrogen bonds. Cellulose is crystalline in nature, and the different forms are cellulose I, II, III, and IV [9]. Cellulose is insoluble in water, but the solubility increases under specific conditions. Acid and alkali treatment can break down cellulose into glucose units. Cellulose forms aggregates with the rise in temperature and/or becomes recalcitrant with dehydration [10].

10.2.1.1 Microbial Production of Cellulase

Both solid-state and submerged fermentations are used for the production of cellulase from substrates like corn cob, wheat bran, and wheat and rice straws [11, 12]. Solid-state fermentation is more economical than submerged fermentation. The commonly used and most efficient fungus for cellulase production is *Trichoderma reesei*. Other fungi viz. *Trichoderma longibrachiatum* and *Aspergillus niger* are also used for bioprocessing [13]. Water hyacinth can also produce cellulase, and maximum enzymatic activity was observed at pH 5.0 and 40 °C temperature [14]. The substrates used by fungi for cellulase production are shown in Table 10.1. The *Aspergillus japonicus* C03 produces endocellulase via solid-state fermentation [15].

Certain bacteria are also capable of producing cellulase. The *Clostridium*, *Ruminococcus*, *Streptomyces, Pseudomonas fluorescens*, *Bacillus subtilis* CEL PTK1, *Acidothermus* sp., *Rhodothermus marinus*, and *B. subtilis* AS3 are some of the bacteria commonly used in cellulase production [1]. The *Cellulomonas bioazotea, Cellulomonas fimi*, and *Streptomyces* sp. are some of the actinobacteria also used for the same purpose [16].

Table 10.1 Types of substrates used by fungi for cellulase production.

Fungi	Substrates
Aspergillus niger	Corn cob, Sorghum straw
Alternaria alternata	Corn cob
Aspergillus oryzae	Soybean hulls
Trichoderma reesei	Soybean hulls
Aspergillus japonicas URM5620	Castor bean
Thermostable yeast	Bagasse powder
Aspergillus terreus	Rice straw

Source: Modified from Gunjal et al. [1].

Some bacteria can produce cellulase even in extreme conditions. For example, *Pseudoalteromonas haloplanktis* found in sea water of Antarctica produces a cellulase, Cel5G which is psychrophilic in nature. Thermophilic bacteria like *Clostridium* sp., *Fervidobacterium* sp., *R. marinus*, *Geobacillus* sp., *Acidothermus cellulolyticus*, *Thermotoga* sp., *Caldicellulosiruptor* sp., and *Anaerocellum thermophilum* also secrete thermostable cellulase which helps in the degradation of lignocellulosic wastes [1].

10.2.1.2 Enzymes Responsible for Cellulose Degradation

The main enzyme required for cellulose breakdown is cellulase. The enzyme has both catalytic and non-catalytic cellulose binding modules (CBMs). The CBM helps in binding to the surface of the cellulose, and the catalytic domain of the enzymes facilitates the breakdown of the β-1,4-glycosidic bonds of cellulose producing glucose units [17]. The end products of cellulose degradation are CO_2 and water (aerobic condition), and CO_2, water, and methane (anaerobic condition) [18].

Three types of enzymes are used in cellulase degradation viz., β-1,4-endoglucanases (EGL) or Endoglucanases, exoglucanases/cellobiohydrolases (CBH), and Cellobiase or β-glucosidase (BGL). The EGL acts in the amorphous region and breaks internal bonds releasing the cellulose chains (endo-cleaving). The CBH acts at the terminal part of the polysaccharide chain and releases cellobiose units (exo-cleaving) [19]. Finally, BGL breaks down cellobiose and/or remaining oligosaccharide chains into glucose units, which becomes the carbon source for the microorganisms [20]. All the three enzymes, i.e. EGL, CBH, and BGL, must act in a synergistic and sequential way for the complete degradation of cellulose.

10.2.1.3 Physical Pre-treatments to Break down Cellulose

Apart from the biological methods used for the breakdown of lignocellulosic wastes, there are also some physical methods that are adopted by many industries. The pre-treatments' aim will facilitate the breakdown of cellulose by increasing the surface area of the wastes. Surface area is increased by reduction in the particle size, crystallinity, and degree of polymerization. Reduction in the particle size is achieved

by grinding, milling, steam injection, and pyrolysis [21]. It also involves the use of acids (hydrochloric acid, sulfuric acid, or phosphoric acid), alkalis (sodium hydroxide, potassium hydroxide, calcium hydroxide, and ammonium hydroxide), organic solvents (alcohols, organic acids, ketones, phenols, glycols, and ether), and ionic liquids.

10.2.2 Degradation of Hemicellulose

The different sugar molecules which form the hemicellulose include D-glucose, D-xylose, D-arabinose, D-mannose, D-galactose, D-4-*O*-methyl-glucuronic, D-glucuronic, and D-galacturonic acid, and other ester-linked coumaryl, acetyl, and feruloyl moieties. The sugars are linked with each other by β-1,4-glycosidic bonds and β-1,3-glycosidic bonds [22]. Hemicelluloses are not crystalline in nature but are attached with cellulose microfibrils, and together, they constitute the hard fibers and secondary wall of plant cells. Among all the heteropolymers found in hemicellulose, xylan is most abundant [23].

10.2.2.1 Enzymes Responsible for Degradation of Hemicellulose

Due to the complex nature of hemicellulose, it requires a combined effort by endo-enzymes, exo-enzymes, and accessory enzymes for its degradation. The endo-enzymes break the main chains internally, the exo-enzyme produces monomers, and the accessory enzymes breaks the side chains and also the attached oligosaccharides thereby producing monosaccharides and disaccharides.

Xylan degradation is achieved by two main enzymes viz., ß-1,4-endoxylanase and ß-1,4-xylosidase. The ß-1,4-endoxylanase splits the xylan backbone and produces smaller oligosaccharide. The ß-1,4-xylosidase further breaks the oligosaccharides into smaller units, i.e. xylose monosaccharides. Depending upon substrate specificity, the fungal endoxylanases are two types G10 and G11 [24]. Complete degradation of xylan is achieved by ß-xylosidase.

ß-1,4-linked D-glucose forms the backbone of xyloglucan substituted with D-xylose side chains. The enzymes, xyloglucanases and ß-glucosidases, are needed for xyloglucan degradation. Xyloglucanase activity is not same for all substrates. For example, some xyloglucanases break only the glucose backbone of xyloglucan and not the glucose backbone of any other cellulose. Also, xyloglucanase activities derived from different fungi are different. For instance, the *T. reesei* xyloglucanase has substrate specificity for branched glucose chains, whereas the *A. niger* xyloglucanase belonging to GH12 family breaks xylogluco-oligosaccharides having more than six glucose residues with at least one non-branched glucose residue [25].

Mannan degradation is also done by two enzymes viz., ß-endomannanases and ß-mannosidases [4]. Mannans are comprised ß-1,4-linked D-mannose backbone with D-galactose side chains. The ß-endomannanases break down the galactomannans and produce mannobiose and mannotriose, and these were further broken down into mannose by ß-mannosidases.

Many accessory enzymes are required to remove all the substituted side chains from the hemicellulose backbone. A total of nine enzymes belonging to different

Table 10.2 Types of enzymes used to cleave side chains in hemicellulose [3].

Substituent	Enzymes
L-arabinose	α-Arabinofuranosidases arabinoxylanarabinofuranohydrolases
D-xylose	α-Xylosidases
D-glucuronic acid	α-Glucuronidases
Ferulic acid	Feruloyl esterases
Acetyl group	Acetyl xylanesterases
L-fucose	α-Fucosidases
p-Coumaric acid	*p*-Coumaroylesterases
D-galactose	α-Galactosidases

families viz., glycoside hydrolase (GH) and carbohydrate esterase (CE) families are required for complete degradation [26]. There are many substituents found in the hemicellulose structure, such as arabinose, D-xylose, D-glucuronic acid, ferulic acid, acetyl group, L-fucose, and *p*-coumaric acid. The enzymes required for debranching these side chains from the hemicellulose backbone are presented in Table 10.2.

10.2.2.2 Microbial Production of Hemicellulases

The main hemicellulase producing microorganisms are fungi, though many bacteria and actinomycetes are also reported to produce hemicellulases. The *Cladosporium* sp., *Fusarium*, *Penicillium thomii*, *Penicillium canescens*, *Penicillium pinophilum*, *Alternaria alternata*, *Ceratocystis paradoxa*, *Geotrichum*, *A. niger*, *Paecilomyces*, *Trichoderma*, and *Cephalosporium* are some of the fungi producing hemicellulases [1].

The hemicellulase producing bacteria are *Cellulomonas*, *Bacillus* sp., *Micrococcus*, *Thermotoga*, *Staphylococcus*, *Pseudoxanthomonas*, *Arthrobacter*, and *Rhodococcus* [27].

Thermobifida fusca, *Streptomyces flavogriseus*, *C. fimi*, *Cellulomonas flavigena* ATCC 482, *Actinomadura*, *Thermomonospora curvata*, *Thermomonospora alba*, *Microbispora bispora*, *Micromonospora*, *Nocardia*, *Thermoactinomyces*, *Saccharomonospora viridis*, *Streptomyces violaceoruber*, *Streptomyces lividans*, *Streptomyces aureofaciens*, *Microtetrasporaflexuosa*, *Streptomyces thermocyanaeviolaceus*, and *Thermoactinomyces thalophilus* are the actinomycetes producing hemicellulase enzyme [28].

10.2.2.3 Physical Pre-treatments to Break down Hemicellulose

Some of the physical pre-treatments commonly used for hemicellulose are alkali pre-treatment, wet oxidation, acid pre-treatment, steam-explosion pre-treatment, use of green solvents, etc. Hemicellulose hydrolysis is achieved by the physical pre-treatments. The commonly used alkalis are sodium hydroxide, potassium hydroxide, calcium hydroxide, and ammonium hydroxide [29]. Acid pre-treatment helps to convert the hemicellulose into sugars. The commonly used acids are

sulfuric acid, hydrochloric acid, trifluro acetic acid, phosphoric acid, and nitric acid. Disruption of hemicellulose microfibrils occurs in steam-explosion pre-treatment [30]. The wastes are subjected to high pressures and temperatures for a short time followed by depressurization of the system. Sometimes, acid catalysts are used to aid the steam-explosion system. Wet oxidation and steam explosion combined together help in the processing large amounts of biomass [31].

10.2.3 Degradation of Lignin

Lignin is a phenolic polymer and is found in every terrestrial plant. Lignins are derived from 3-hydroxyl-cinnamyl alcohols or monolignols namely, *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. The monolignols form the building blocks of lignin. Two monolignol radicals dimerize to form a starting dimeric unit. It then undergoes cross-coupling with another monolignol/dimeric radical, and thereby polymerization sets in to biosynthesize lignin. Substituted phenylpropanoid building units connected to each other by carbon–carbon linkages forming the backbone of lignin. A precise and simplified definition for lignin is lacking because of its structure, non-repetitive multi-units, diversity in composition depending on origins, etc. [32].

10.2.3.1 Microbial Production of Lignin Degrading Enzymes

Due to the diversity in the interunits of lignin, it is not easy to break the internal bonds. Only a few microorganisms are able to degrade lignins. White-rot basidiomycetes, actinomycetes, *Phanerochaete chrysosporium*, *Trichoderma*, *Streptomyces cinnamomensis*, *Lentinus squarrosulus*, *Schizophyllum commune*, *Bjerkandera adusta*, *Xanthomonas* sp., *Fomitopsis palustris*, and *Aspergillus* are able to efficiently degrade lignin.

Phanerochaete chrysosporium is extensively studied in lignin degradation and also been commercially used for lignocellulosic waste degradation. It produces 10 lignin peroxidases (LiP), 5 manganese peroxidases (MnP), and several other lignocellulolytic enzymes [33]. Another phytopathogenic fungus, *Chondrostereum purpureum* also produces many lignocellulolytic enzymes [34].

10.2.3.2 Enzymes Responsible for the Degradation of Lignin

Lignin degradation is a difficult task to achieve by microorganisms. There are many complexities in the lignin structure. First, lignin is a large polymer, and hence, the enzymes required should be extracellular. Second, since the interunit bonds are carbon–carbon and ether bonds, oxidative degradation rather than hydrolytic breakdown is essential. And third, owing to its irregular structure, the enzymes needed should be less specific in nature [35]. The enzymes utilized for lignin degradation are known as ligninases or lignin-modifying enzymes. Ligninases are grouped into two classes viz., heme peroxidases and phenol oxidases. LiP, MnP, versatile peroxidases (VP), and dye decolorizing peroxidases come under the

heme peroxidases, whereas laccases comes under phenol oxidases. The peroxidase enzymes contain heme molecule and require hydrogen peroxide for its oxidative activity. Accessory oxidases provide H_2O_2 which will be used by peroxidase, whereas laccases contain copper molecules and catalyze oxidation–reduction reactions. LiP, MnP, versatile peroxidases, and laccases are the major ligninases.

LiP removes one electron from the C—C bonds (non-phenolic part) of lignin and releases cation radicals that degrade chemically. However, MnP cannot directly remove electron from the non-phenolic part of lignin. It has to first transfer its oxidizing power to Mn^{3+} (which is a product of MnP reaction), and then, this Mn^{3+} enters the lignin structure and catalyzes the oxidative reactions [36].

The extracellular oxidases/accessory oxidases synergistically oxidize a cosubstrate and reduce O_2 to H_2O_2. This H_2O_2 is then utilized by peroxidases (i.e. LiP and MnP) for their action. Aryl alcohol oxidase and glyoxal oxidase are the most significant accessory oxidase enzymes used in ligninolysis [3].

Versatile peroxidase (VP) like LiP and MnP also contains a heme protein. In addition to the oxidative catalytic activity, VP also shows dye decoloration activity in the presence of Mn(II). Laccase oxidizes the phenolic units of lignin and breaks down into sinapyl alcohol [37]. It also acts as a catalyst in the oxidation of many aromatic substrates and produces water as a byproduct.

10.2.4 Degradation of Pectin

Hydrolytic enzymes viz., GHs and polysaccharide lyases (PLs) are used in pectin degradation.

Endo- and exo-polygalacturonases of the GH family break down the pectin backbone. They do so by cleaving the α-1,4-glycosidic linkages of α-galacturonic acids. Other enzymes of the GH family viz., endo- and exo-rhamnogalacturonases, α-rhamnosidases, xylogalacturonases, unsaturated rhamnogalacturonan hydrolases, and unsaturated glucuronyl hydrolases are involved in the degradation of the "hairy" region of pectin [26]. D-galacturonic acid and L-rhamnose residues of the pectin backbone are present in the "hairy" region of pectin. Rhamnogalacturonases acts upon the α-1,2-glycosidic bonds between the two residues [3]. The xylose residue on the galacturonic acid backbone is cleaved by an endo-xylogalacturonase from the fungus *Aspergillus tubingensis* [38].

The PLs viz., pectin lyase and pectate lyase cleave the α-1,4-linked D-galacturonic acid residues of pectin backbone [39]. The two PLs have different preference for substrate esterification. Pectin lyases prefer high degree of esterification, while pectate lyases prefer low degree. Also, pectate lyases act in the presence of Ca^{2+} ions, while pectin lyases do not have any such requirement. Another enzyme of the PL family, rhamnogalacturonan lyase shows catalytic activity in the "hairy" region of pectin. A set of accessory enzymes are required to cleave the substituted chains of pectin backbone to make way for the main pectinolytic enzymes. Some of the accessory enzymes for pectin degradation are β-endogalactanases, endo- and exoarabinases [4].

10.3 Utilizing Enzymes for the Degradation of Lignocellulosic Waste

Owing to the delignification action of peroxidases and laccases, they are used in biopulping and biobleaching of wood pulp. These enzymes also decolorize the dye wastewater, effluents from textile industry, distilleries, and waste treatment plants. Cellulases are increasingly used in the biofuel production. Amylases help in starch hydrolysis and hence are used in bioethanol production. They also find applications in biofuel, paper, and textile industries. Mannanase is used in the paper, pulp, textile, and pharmaceutical industries. Xylanases improve pulp bleachability when they are applied to treat rice straw pulp [40].

10.4 Conclusion

Lignocellulosic wastes can be converted into renewable resources with the help of microorganisms. Fungi, bacteria, and actinomycetes are able to degrade these wastes with the help of their unique enzyme systems. The enzymes also have wide applications in various industries like paper and pulp, textiles, food and feed, etc.

Increase in energy demand and global climate change have prompted many countries to use biomass residues for sustainable fuels. Large-scale ethanol production from sugarcane and corn is manufactured in the factories of Brazil and USA. But, the main challenge is to obtain biofuel from the sugarcane bagasse and corncob wastes from those factories. The process of producing cellulosic ethanol using cellulase is not easy as biomass recalcitrance happens and this increases the cost of production. To overcome biomass recalcitrance, pre-treatment like steam explosion is used and this helps the microorganisms to penetrate deeply into the biomass.

Using advanced genetic engineering new strains are developed by introducing or removing of genes responsible for metabolic pathways leading to biomass degradation. Multiple customized design of gene insertion has been made possible with integrated advanced techniques like synthetic bioengineering.

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11

Usage of Microalgae: A Sustainable Approach to Wastewater Treatment

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11.1 Introduction

Water is one of the most valuable resources for the sustenance of life on the planet. However, industrialization and high rate of urbanization led to an emanation of large quantities of wastewater from different manufacturing industries, agricultural fields, and domestic activities. Water has been polluted by toxic matters from industrial wastes, mining activities, domestic waste, pesticides, chemical fertilizers, and radioactive wastes. Domestic waste accounts for the highest percentage of water pollutants followed by livestock industries. Shortage of clean water was identified as one of the three most rapidly emerging global challenges by the United Nations Human Development Report. It has been reported that ∼29% of the world's population faced shortage of clean water supply. The most common water pollutants include suspended solids, pathogens, nutrients like nitrogen, phosphorus and carbon, salts, and oxygen demanding materials. Discharge of waste into water bodies highly disturbs environmental sustainability. Poor quality of water has resulted in a high rate of illness and deaths, accounting for ∼50 million deaths per year worldwide, especially in Africa and Asia. An elevated amount of water pollutants such as heavy metal, phosphorus, carbon, and nitrogen species are the major cause of eutrophication in natural waters leading to bigger consequences in the ecosystem including species mortality, biodiversity reduction, and loss of ecosystem [1]. Therefore, treatment of polluted water is crucial to ensure a quality living standard. A typical wastewater treatment process includes preliminary treatment where settleable inorganic solids are removed followed by primary treatment where suspended organic solids are removed. The next process is the secondary treatment where dissolved compounds are removed and finally tertiary treatment involves further elimination of organic pollutants and pathogens in wastewater. Various technologies and techniques include filtration, floccula-

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tion, desalination, fixed biofilms, ultraviolet radiation, and chlorination which are expensive and complex. The major disadvantages of conventional treatment include (i) inconsistency depending on the nutrients to be removed, (ii) high operation cost, (iii) generation of secondary pollution by chemical processes, (iv) loss of valuable potential nutrients, and (v) incomplete utilization of resources.

Wastewater treatment should be efficient and cost effective. For the past few years, research on the use of microalgae emerged as a sustainable approach to eliminate water pollutants [2]. The advantages of biological wastewater treatment using microalgae include bioremediation in situ, zero production of secondary waste, and economic viability.

11.1.1 Microalgae

Microalgae are photosynthetic, unicellular, or multicellular organisms representing diverse species with varied physiology and growth requirements. It is reported that there are ~80 000 different microalgal species on earth and 40 000 species have been studied so far. Microalgae may be prokaryotic or eukaryotic and can be divided into different phyla.

Chlorophyta: They may be marine, freshwater, or terrestrial, which may be unicellular or and multicellular, autotrophic with photosynthetic pigments (chlorophyll *a*, *b*, *c*, *d*, and *e*; carotenoid *a* and *b*; xanthophylls; pheophytin *a* and *b*; and bacteriochlorophylls) and hence also known as green microalgae. Their growth rate is ~100 times higher than terrestrial plants, and their biomass can double in less than 24 hours which is attributed to their simple structure and high cell surface: volume ratio resulting in high rate of nutrient absorption, such as in *Chlorella vulgaris*, *Dunaliella salina*, and *Haematococcus pluvialis*.

Rhodophyta: They may be unicellular and multicellular. Multicellular species are common and are found in marine environments while only a few are freshwater unicellular species. The stromal side of thylakoids are lined with phycocyanin and phycoerythrin as observed in *Porphyridium cruentum* and *Rhodella reticulata*.

Haptophyta: They are mainly marine, unicellular, and existed in colonies. They have one or two chloroplasts containing pyrenoids out of which *Isochrysis galbana* and *Pavlova salina* are the important ones.

Dinophyta: They are unicellular and mainly marine, while some species are found in freshwater. Half of the total species in this phylum are true autotrophs, and the other half are heterotrophs with no chloroplast. Example: *Crypthecodinium cohnii*.

Ochrophyta: They include diatoms that are the smallest unicellular and most abundant marine phytoplanktons.

Microalgae flourish in varying habitats, which may be aquatic or terrestrial, and withstand a wide range of environmental variations including nutrient depletion, salinity, drought, light, osmotic pressure, temperature, and ultraviolet radiation. They can even optimize lipid production that is the raw material for biodiesel

production under stress. Different species of microalgae adapt with changing mode of nutrition. Based on their metabolic process microalgae may be divided into:

Autotrophs: Autotrophic microalgae *C. vulgaris*, *Coenochloris pyrenoidosa*, and *Scenedesmus* sp. can produce hydrocarbons, proteins, lipids, adenosine triphosphate (ATP), oxygen, and 3-phosphoglycerate, by photosynthesis, using light and CO_2, which are utilized for their growth.

Heterotrophs: Heterotrophic microalgae rely on organic carbon-like glycerol and carbon for their energy and cannot utilize atmospheric CO_2 and light. They can convert sugar molecules into biomass-like lipids [3].

Mixotrophs: Mixotrophic microalgae have the metabolic ability of both autotrophic and heterotrophic growth. They can propagate under low light intensity and profits from the aspects of both type of metabolism.

Photoheterotrophs: The source of energy include light and organic carbon. In the absence of light, they use organic carbons as the main source of energy, making them favorable for wastewater treatment overcoming light dependency, which is an important disadvantage of large-scale photobioreactors.

11.1.2 Composition of Wastewater

The composition of wastewater depends on the sources and is an intricate assortment of organic and inorganic compounds. Most organic constituents of sewage are in the form of carbohydrates, lipids, polypeptide, and volatile acids. The inorganic components include high concentrations of calcium, chlorine, potassium, magnesium, sodium, sulfur, phosphate, and heavy metals. The organic water contaminants can be macro (organic acids, carbohydrates, melanins) or micro (xenobiotics-antibiotics, pesticides, and recalcitrant chemicals) pollutants which are highly toxic to the ecosystem. Domestic wastewater has a high concentration of organic macro and micropollutants with increasing discharge of low-molecular hazardous pollutants, including phthalates, polychlorobiphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), bisphenol A originating from pharmaceuticals, which can be excreted in its original or metabolized form. Study has shown that high concentration of these molecules is also present in urban wastewater, sewage from hospitals, groundwater, and drinking water [4]. Total nitrogen (TN) and total phosphorus (TP) have been reported to be 10–60 mg/l in municipal wastewater [5].

Wastewater from agricultural fields has high concentrations of nitrogen and phosphorus derived from manure compared to municipal wastewater. Agricultural and agro-based industries release high volumes of organic contaminants, inorganic nutrients, pathogens, antibiotics, and pesticides [6], which may not contain a high concentration of heavy metals and hazardous pollutants. The amount of TN varies from dairy (185 mg/l) to swine wastewater (3213 mg/l), whereas TP content ranges from 30 mg/l in dairy wastewater to 987 mg/l in swine piggery wastewater [7]. The TN and TP have been reported to be more than 1000 mg/l in agricultural wastewater to 500–600 mg/l in farms [5].

Most of the industrial wastewaters contain high concentrations of heavy metal pollutants and organic chemical pollutants like hydrocarbons, biocides, and surfactants rather than nitrogen and phosphorus. Textile industries generate a massive volume of waste when discharged into water bodies posing risk to the aquatic ecosystem. Heavy metals like chromium (Cr), arsenic (As), copper (Cu), and zinc (Zn) are the common constituents of textile wastewater. The concentrations of nitrogen and phosphorus, however, vary in textile wastewaters depending on the source [7]. In textile wastewater, the amount of TN varies between 21 and 57 mg/l and TP varies from 1.0 to 9.7 mg/l [8]. Besides, chemical oxygen demand (COD) and biological oxygen demand (BOD) also vary due to the structural variations in the dyes and their metabolites used. Water bodies are also highly polluted by leachates from landfills and dump yards, containing high levels of hazardous micropollutants along with nitrogen and phosphorus (~100 mg/l) in their inorganic forms [9].

11.2 Microalgae for Wastewater Treatment

Wastewaters from industries and polluted rivers have an elevated amount of nutrients such as carbon, nitrogen, phosphorous, and other minerals. Some of the major elements required for the propagation of microalgae include nitrogen, phosphorus, and carbon. Studies have been carried out on the treatment of industrial, domestic, agricultural wastewaters, and eutrophicated lakes using microalgae in addition to wastewaters from aquacultures, fish farms, wineries, domestic discharges, and industries [10]. Microalgae can absorb different types of pollutants (biosorption) due to a series of independent metabolic processes, electrostatic interaction, ion exchange, complexation, chelation, and micro-precipitation, and occurs essentially within the dead or inactive cell walls. The microalgal cell composition is fibrous and amorphous complex with different types of polysaccharides and functional groups having the ability to capture heavy metals. Microalgae-based wastewater treatment is driven by unlimited solar energy, CO_2, and nutrients from wastewater itself. Besides, microalgae produce extracellular biosurfactants as a result of metabolic degradation which are employed in tertiary treatment due to its efficiency in sequestering nutrients and heavy metals and production of secondary metabolites preventing pathogen growth.

Phosphorus, a major nutrient leading to eutrophication is removed by precipitation of the effluent to form an insoluble solid fraction or transformed into an activated sludge which is not recyclable. Microalgae are highly efficient in removing nitrogen, phosphorus, and toxic heavy metals and therefore can be employed during the tertiary treatment of wastewater. It is a greener and sustainable alternative to the current energy-intensive and expensive technologies as they produce oxygen *in situ* needed for mineralization of water pollutants. Microalgae-based wastewater treatment is highly recommended for developing countries as the oxygen generated from photosynthetic microalgae reduces the cost of mechanical aeration in the pond treatment. Wastewater treatment using microalgae provides an opportunity for efficient recycling of nutrients. For example, recovered algal biomass enriched with

nitrogen and phosphorus can be further used as low-cost fertilizer or as animal feed. Microalgae-based biofertilizers have the same efficiency as the chemical fertilizers [11]. Moreover, using microalgae-based biofertilizer resulted in lower phosphorus concentration in water runoff as organic phosphorus is slowly mineralized by microalgae and then liberated at a rate almost equal to that of the phosphorus uptake by plants. It was also observed that this type of biofertilizer does not have negative effects on the soil microbiome and biological activity. Microalgae used for biofertilizers include *Chlorella* sp., *Acutodesmus* sp., *Nostoc muscorum*, *Anabaena variabilis*, *Tolypothrix tenuis*, and *Aulosira fertissima*. Cultivation of microalgae in wastewater also results in the capture of CO_2, one of the most abundant greenhouse gases. It also yields biomass convertible into biodiesel, biochemicals having pharmaceutical applications and other value-added products.

Chlorella and *Scenedesmus* are the most common microalgae used for wastewater treatment due to their ease of isolation, propagation, and efficient removal of nutrients. Investigations have been carried out to test the efficiency of microalgae in remediating wastewater [12]. It may have various effects on the characteristics of wastewater as:

11.2.1 Biological Oxygen Demand (BOD)

BOD indicates the amount of biologically degradable organic pollutants in the water bodies. It is the amount of oxygen used by microorganisms to completely disintegrate organic molecules to CO_2 and water. BOD depletes dissolved oxygen in the water leading to the dead of fishes and anaerobiosis, hence, its reduction is the primary objective of wastewater treatment. Microalgae utilize the CO_2 released as a carbon source for photosynthesis, using carbonic anhydrase. BOD values vary with the concentration of organic pollutants. The BOD of pristine waters lies below 1, 2–8 mg/l for moderately polluted waters and 20 mg/l for treated municipal sewage [13]. Studies have shown *Scenedesmus* sp. reduced BOD of a fertilizer wastewater plant in Vietnam by 83.7% [1], while species of *Neochloris*, *Chlorella*, and *Chlorococcum* reduced BOD of river polluted with pharmaceuticals effluents by 91%, 84%, and 83%, respectively [4]. *C. vulgaris* reduced BOD of domestic wastewater by 68.4% [14]. Another study showed that a consortium of microalgae removed 82% of BOD in an untreated sewage wastewater [10].

11.2.2 Chemical Oxygen Demand (COD)

COD is an indicator of the amount of total organic carbon in the water, denoting both the amount of biologically active and inactive organic matter in water. The COD varies with water bodies, wherein the surface water normally ranges from 5 to 20 mg/l [15]. A study showed that COD of domestic wastewater from IIT Kharagpur campus, India was reduced by 95.9% using *Scenedesmus* sp. [2]. COD of primary septic tank effluent from Environmental Sciences and Engineering Department (NUST), Pakistan, was reduced by 84.86% and 95% by *Chlorella* sp. and *Scenedesmus* sp., respectively [12]. Further studies showed that the COD removal efficiency

of *C. vulgaris* was 79.6% and 71.7% in sewage and slaughterhouse wastewater, respectively, whereas *Spirulina* sp. reduced COD by 71.7% in dairy processing wastewater [16]. The COD removal efficiency of species of *Neochloris*, *Chlorella*, and *Chlorococcum* in a river polluted with pharmaceutical wastes was found to be 90%, 84.5%, and 88.4%, respectively [4].

11.2.3 Nutrients (Nitrogen and Phosphorus)

Nitrogen and phosphorus are the two major nutrients in wastewaters, wherein total nitrogen can be removed by nitrification and denitrification using microalgae. Different species of microalgae assimilate different organic and inorganic compounds containing nitrogen in autotrophic and heterotrophic conditions. Inorganic nitrogen species include nitrite (NO^{2-}), nitrate (NO^{3-}), and ammonia (NH^{4+}). Microalgae usually take up nitrogen in the form of ammonia via glutamine synthetase and glutamine-2-oxoglutarate amido transferase pathway (GS/GOGAT pathway). Ammonia mostly in the form of glutamine move across the cell membrane and finally assimilate into amino acids to synthesize proteins, which requires less energy to reduce and assimilate as compared to NO^{2-} and NO^{3-} [8]. Phosphorus is usually present as orthophosphate (PO_4^{3-}) in wastewater that are vital for cell membrane, DNA, RNA, and ATP. Microalgae can also absorb extra phosphorus under in high phosphate condition and stored as polyphosphate which is used as an internal buffer under conditions of low phosphorus.

Several studies have been conducted to investigate the efficiency of microalgae in removing nitrogen and phosphorus from wastewater. *C. pyrenoidosa* removed 62% of TN and 87% of TP in textile wastewater [17], *C. vulgaris* removed 30–95% of TN and 20–55% of TP in agro-industrial wastewater [18], *Spirogyra* sp. removes 95% of TN and 90% of TP in textile wastewater and *Cladophora* sp. removed 93% of TN and 88% of TP in textile wastewater [19]. *C. vulgaris* was used to remove over 90% of TN and 80% of TP from the primary treated sewage [20], whereas an earlier reported the elimination of TN (50.2%) and TP (85.7%) in industrial wastewater using microalgae [21]. *Chromochloris zofingiensis* removed 68–81% of TN and 90–100% of TP in piggery wastewater [22].

11.2.4 Heavy Metals

Microalgae absorb heavy metals (metal biosorption) in two phases. The first phase is called passive biosorption where the cell surface of the microalgae interacts with heavy metals. The functional groups of cell surface have metal-binding groups like amines, hydroxyls, carboxylates, phosphates, and sulfates providing multiple active sites for metal ions. This phase takes place rapidly by ion exchange, physical adsorption, complexation, or inorganic micro-precipitation mechanisms. The second phase, called active biosorption, is where metal ions enter the microalgal cell through the cell membrane. This stage is dependent on the metabolism of the organism and slower compared to the first phase. A study on the cellular distribution of microalgae revealed that large amounts of metal ions attached to

the cell wall and the insoluble fraction gets accumulated intracellularly. Different species of microalgae such as *C. vulgaris*, *Scenedesmus* sp., *Chlorococcum* sp., *Lyngbya spiralis*, *Tolypothrix tenuis*, *Stigonema* sp., *Phormidium molle*, *Aphanothece halophytica*, and *Chroococcus paris* can remove heavy metals like Hg(II), Cd(II), and Pb(II) from water bodies.

Leather tanning, electroplating, and tincture wood preservative industries discharge a considerable amount of Cr(III) into the water bodies. *Dictyosphaerium chlorelloides* can sequestrate a high amount of Cr(III) into its cell wall, chloroplast, vacuoles, and cytoplasm through biosorption, accumulate, and use it to produce sugars and fats. Cr(III) can be oxidized to Cr(VI) by MnO_2 and bacteria. Cr(VI) when compared to Cr(III) is highly mutagenic, carcinogenic, and is 500–1000 times toxic to a living cell. It is reported that *C. vulgaris* can convert Cr(VI) into its less toxic trivalent form [23], whereas *Spirulina platensis* has 60.92% Cr(VI) removal efficiency when cultivated in a mixture of artificial medium and wastewater [24]. *Scenedesmus quadricauda* species was shortlisted from 11 microalgal species to treat synthetic wastewater, reducing 30 mg/l of nickel (Ni) and zinc (Zn) to 0.4 mg/l within 90 minutes which is attributed to its higher surface area compared to the other microalgae [25] Arsenic (As) is a toxic water pollutant and its high concentration can lead to skin and respiratory diseases, neurological, cardiovascular, gastrointestinal, and urinary disorders, increasing the risk of high blood pressure and diabetes. The inorganic forms As(III) and As(V) are more toxic compared to its organic form. A study has shown that *Ostreococcus tauri*, a marine microalga converts inorganic As into its organic form, integrating into biogeochemical cycles and catalyzing As volatilization through the metabolic mechanism of biomethylation [26].

11.2.5 Xenobiotic Compounds

Various industries producing high concentrations of synthetic toxic chemicals should be treated before releasing into water bodies which is expensive and complex. Microalgae are efficient in treating these xenophobic compounds by dispersion, chemical transformation, and bioaccumulation. PAHs are highly toxic with carcinogenic properties, and therefore, their presence and levels need to be checked. Several species of microalgae are reported to assimilate and transform into a less toxic compound. *C. vulgaris*, *Spirulina platydiscus*, *S. quadricauda*, and *Selenastrum capricornutum* were tested for their ability to remove fluoranthene, pyrene, and their mixture. *S. capricornutum* showed the highest removal efficiency ranging from 88% to 98% for different concentrations [3]. Monoaromatic hydrocarbons are highly mutagenic and carcinogenic xenobiotic compounds from production, transportation, and storage of oil and its products, which can be sequestrated using microalgae. Chlorophenols are another class of xenobiotics with carcinogenic properties mostly used in the production of pesticides and wood preservatives. A study showed that *Tetraselmis marina* metabolized 2,4-dichlorophenol, whereas *C. vulgaris* and *C. pyrenoidosa* showed 100% p-chlorophenol removal efficiency [27] and *Chlorella fusca* showed 90% Bisphenol A removal efficiency [28].

11.3 Cultivation of Microalgae in Wastewater

11.3.1 Factors Affecting the Growth of Microalgae

The efficiency of wastewater treatment using microalgae greatly depends on their growth in wastewater and the extent of nutrient uptake depends on pH, TN:TP ratio, pH, and light.

11.3.1.1 TN:TP Ratio

The TN:TP ratio of wastewater is an important factor for the successful cultivation of microalgae as it determines the extent of nutrient assimilation, biomass productivity, and dominance of microalgal species. Depending on the source, ranging from 4 : 1 to as high as 40 : 1. TN:TP ratio of 16 : 1, also known as Redfield ratio was initially considered as a constant for the growth of marine microalgae but 22 : 1 is now regarded as an optimal ratio after a comprehensive study of oceanic organic particulates. For freshwater microalgae TN:TP ratio of 6.8 : 1 to 10 : 1 is considered as an optimal ratio. A nutrient ratio higher than optimal ratio can lead to phosphorus limitation and lower ratio might result in nitrogen limitation as the structure and function of the microalgae are greatly affected by the nutrient concentration. TN:TP ratio, however, varies during microalgal growth.

11.3.1.2 pH

The different cellular processes in microalgae like energy production, composition and function of cell organelles, enzymes, and proteins depend on the pH of wastewater. The optimal pH for microalgae ranges from pH 7 to 9. However, microalgae exhibit a high tolerance to pH variation. *Chlorella ellipsoidea* can thrive in both acidic and basic pH (4–10). A study showed that *C. vulgaris* showed best growth rate at pH 10 [29]. Nutrient uptake is also highly influenced by pH. *C. vulgaris* showed the best nitrogen removal efficiency at pH between 7 and 8. Wastewater treated with microalgae has higher pH due to its high photosynthetic rate, drawing more dissolved CO_2 leading to high concentrations of carbonates and bicarbonates [10].

11.3.1.3 Light

Light is an important component for photosynthetic microalgae. Like green plants, they capture light using chlorophyll and convert it into chemical energy in the form of ATP. Oxygen and other reducing agents convert CO_2 to organic molecules. Different species of microalgae possess different pigments such as chlorophylls, carotenoids, and phycobilins. Green microalgae (*Chlorophyceae*) have chlorophylls *a* and *b* while majority of cyanobacteria (blue-green algae) have chlorophyll *a*, phycocyanin, and phycoerythrin to help them in absorbing light with varying wavelength and intensity. A 50% increase in biomass production and better nutrient removal was reported in *Scenedesmus* sp. using red and blue lights compared to white light [30]. Another study showed that exposure to blue light resulted in higher growth rate in *C. vulgaris* as compared to the wavelengths of other light (white, red, and green). A report showed that the intensity of light affected cell growth,

carbohydrate, and lipid production, along with increased efficiency of CO_2 fixation. A study was conducted where biomass production and CO_2 mitigation efficiency increases with increase in light intensity from 140 to 540 μmol/m^2 s and the peak is obtained at 420 μmol/m^2 s after which a decrease is observed [31]. It was observed that microalgae under 24 hours continuous artificial illumination showed higher TP removal than under a 12 hours light-12 hours dark regime by solar radiation [32].

11.3.2 Algal Culture Systems

The type of culture system depends on the reason for cultivation (biofuels, CO_2 mitigation etc.), nutrient source, final products desired, operation, maintenance, and capital costs. The cultivation system is classified into "open" and "closed" systems. "Open" systems (ponds, lagoons, and deep channels) are constructed outdoors, while "closed" system may be established outdoors under sunlight or indoor under artificial light.

11.3.2.1 Open Systems

The most commonly employed algal cultivation system for commercial scale is the open systems because of simple operation technique and low cost of construction [8]. These systems may be natural like ponds, lagoons, and lakes or artificial water systems like the comprise tanks and man-made ponds. Open systems can be categorized into stirred and non-stirred ponds depending on the mode of aeration and nutrient distribution in the medium. Stirred ponds are suitable for abundant light, good aeration, and nutrients influencing microalgal growth, whereas the non-stirred ponds are easier to manage and are economical.

Stirred Ponds High rate algal ponds (HRAPs), also known as raceway ponds, are circular, largest, and most used forms of stirred ponds, which were devised to combine biofuel production and wastewater treatment on a large scale almost 50 years ago [33]. Oswald and his colleagues developed HRAP technology for wastewater treatment demonstrating efficient removal of organic compounds, and nutrients (P, N) with a reduction in pathogens. HRAPs are shallow (15–25 cm deep) open systems in closed loop with individual or multiple channels with paddlewheel for water circulation [34]. They run on hydraulic retention under varying time intervals according to seasons, organic loading rates of 100–150 BOD/ha day and varying depths from 0.25 to 0.6 m [33]. Raceways are economical in comparison to other forms of closed systems but are low in productivity due to poor mixing efficiency, contamination, shading effect, and inefficient CO_2.

Algae grown in HRAPs assimilate nutrients from wastewater thus helping in recovery of nutrients. HRAP is a type of advanced pond system which includes section like anaerobic digestion pits, algal settling, and maturation ponds in succession. Advanced pond system requires larger land area (~50 times) than activated sludge system, one of the most common waste processing technologies, while the cost of construction of this system is about one-fifth of activated sludge system [33].

Non-stirred Ponds Nonstirred ponds that are 1.5 m deep on an average have the simplest of media to large scale cultivation facilities. The disadvantages of this culture system include zooplankton predation, contamination of algae, as well as pathogen invasion.

11.3.2.2 Closed Systems

The disadvantages of open-pond systems can be minimized or removed in closed pond systems known as bioreactors. A photobioreactor is a reactor utilizing light for cultivation of phototrophic microorganisms (algae and cyanobacteria). Optimization of photobioreactor system increases biomass, whereas better mixing and air delivery can be achieved by shifting to cylindrical ceramic diffusers but optimization of algal density and mixing rate can make this system highly efficient and productive. Better aeration helps in higher mass transfer thereby removing oxygen that can be detrimental at high concentrations. Reduction of algal contamination and water loss due to evaporation are the main advantages of this type of culture system. Although algal biomass can be significantly improved in the photobioreactor culture system, the major limitations are high capital, operating, and maintenance costs. The various types of photobioreactors designed for algal culture are tubular, flat plate, and plastic bag photobioreactor.

In addition to the above culture techniques, there are other alternative culture and treatment systems including hyper concentrated cultures, immobilized system, dialysis cultures, photobioreactor, stabilization ponds and anaerobic ponds, facultative ponds, and maturation ponds.

11.4 Algae as a Source of Bioenergy

Biofuel conversion technology is hindered in microalgae due to the resistant cell wall. Pre-treatment is essential to disintegrate the cell wall and enhance energy output. Pretreatment is of three types – physical (mechanical and thermal), chemical, and biological. Physical pretreatments have been considered as the most effective in breaking down microalgal cells by disintegrating the crystalline structures in cell wall using mechanical tension (microwave and sonication) and heat. Thermal pretreatment is commonly studied among the various types of physical pretreatments, but its efficiency varies according to the species of microalgae. Microwave pretreatment and sonication are independent of the microalgae species but require high energy [35]. Chemical pretreatments use alkali or acid reagents to break down polymers within the cell wall. Chemical pretreatments combined with heat are highly effective. Biological pretreatments use hydrolytic enzymes to breakdown cell wall components. It was proposed that the use of an enzymatic mixture (lipase, cellulose, α-amylase, xylanase, and protease) to pretreat *Rhizoclonium*, resulted in increased methane production over physical pretreatment [36]. They can be used to improve biofuel production from microalgae but, the method depends on the algal species, their growth, and energy demands.

11.4.1 Biodiesel from Microalgae

Microalgae are potential candidate for the production of biodiesel due to their high lipid content. The total lipid content of microalgae is an important criterion for choosing algae for biodiesel production. It is a common attribute that microalgae having high lipid content usually have slow growth resulting in low productivities. Growth study has shown that most species of microalgae with high lipid productivity are not adapted to grow in wastewater. To solve this limitation microalgae were isolated from different wastewater and optimized to grow with different organic carbon substrates and resulted in increased biomass production and lipid productivity as they were already acclimatized to wastewater environment [35].

11.4.2 Bioethanol from Microalgae

Bioethanol are biofuels obtained by fermentation of sugars from various feedstocks. Microalgae have high amount of carbohydrates which can be fermented to bioethanol [35]. Large amount of CO_2 produced by ethanol fermentation can be coupled with the cultivation of carbohydrate-rich microalgae which can be used as feedstock for bioethanol production, mitigating greenhouse gases, and reutilization [32].

11.4.3 Biomethane from Microalgae

Microalgae are used for biomethane production due to its high carbohydrate content [33]. Anaerobic digestion is the process by which organic matter from microalgal cells is converted to biogas through reactions catalyzed by microorganisms. The factors that determine the methane yield in anaerobic digestion of microalgae are the composition of cell wall and its weight contributing to biomass. It has been reported that biomethane production from *C. vulgaris* and *Scenedesmus* requires less energy compared to biodiesel production [37]. Photoautotrophic microalgae capture CO_2 during growth and oxidize H_2S from biogas, generating high-quality biomethane.

11.4.4 Hydrogen Production

Fermentative metabolism results in hydrogen production using microalgae. Biohydrogen is regarded as a sustainable and clean energy yielding high energy (142 M J/kg). Microalgae are suitable feedstocks for hydrogen production as they harbor hydrogenase enzyme that helps in hydrogen production. Many strains of microalgae fix atmospheric N_2 to NH_3 and use sunlight as energy source to generate biohydrogen. Some strains produce hydrogen using sunlight as electron source and water as energy source under specific conditions. There are four different mechanisms of hydrogen production in microalgae- direct biophotolysis, indirect biophotolysis, dark and photo-fermentation. Several microalgae like *C. pyrenoidosa*, *Chlamydomonas*, and *Scenedesmus* sp. have the capacity for producing molecular hydrogen under anaerobic condition [38].

11.4.5 Microbial Fuel Cells

Microalgae can be used for energy production by hydrolysis and fermentation of biomolecules [39]. Bioenergy can be obtained from microalgae using microbial fuel cells (MFCs). An MFC is a bioelectrochemical reactor containing a microorganism that can oxidize both organic and inorganic matter in the reactor generating current. MFCs represent bioelectrochemical systems using exoelectrogenic bacteria for the generation of electricity using wastewater. When MFCs used photosynthetic microorganisms as a catalyst for oxidation–reduction reactions, they are known as photo-MFCs. A microbial electrolysis cell (MEC) are another type of cell they generate hydrogen by applying an electric current on wastewater and works on the same principle as MFCs [39]. MFCs can be used to address various problems like bioremediation, wastewater treatment, and desalination. The cathode in MFCs is stored in an environment with no oxygen where hydrogen is generated as the electron acceptor [35, 39]. Microalgae interact with the photosynthetic bacteria in a healthy manner and serve as electron donors and oxygen suppliers for the removal of organic matter. The wastewater provides nutrients for the microorganisms which is broken down into simple forms and utilized. The COD reduced by microalgae finds an important application in the field of MFC. Studies have shown up to 90% COD removal which makes MFC quite promising and can be scaled up commercially in future [39].

11.5 Conclusion

Although lists of benefits are offered by algae-based fuel, it is exorbitantly expensive. Large amounts of energy are required for algae cultivation and harvesting. Microalgae cultivation is the best approach for CO_2 removal and the cultivation system depends on the microalgae species, scale, and expenditure. Open ponds were projected to be economically favorable than closed photobioreactors. HRAP provides efficient tertiary-level wastewater treatment at a reasonable cost. Although microalgae can be independently used for CO_2 bio-fixation, biofuel production or wastewater treatment, integration of all these processes would make the process economical. In addition, different biomass of microalgae like carbohydrates and lipids can be utilized for bioethanol and biodiesel production. Commercial by-products can also be obtained from proteins, pigments, and vitamins derived from microalgae. This technology can be sustainable technology, if the advancements coupled with research can allow the commercialization of biofuel and wastewater treatment.

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Part IV

Bioleaching and Biosorption of Waste: Approaches and Utilization

12

Microbes and Agri-Food Waste as Novel Sources of Biosorbents

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12.1 Introduction

The term sorption is defined as the attachment to the co-existing solid surface by the charged species of a solution. For removing the metal ion, this technique helps in the separation process. An efficient sorbent should possess a large surface area along with the diverse functional groups present on the surface. Biosorption is an alternative biotechnological method for the wastewater treatment facility as microbes have a high surface area due to their small size. So, to interact with surrounding metals, microorganisms offer a large contact interface [1]. Various organisms such as algae, cyanobacteria, fungi, bacteria, and yeast act as an efficient bio-accumulator. The removal of metal from the solution comprises the following pathway – the first microprecipitation takes place, which enhances the uptake of metal cations after attaching to the surface of the cell. Further, metal ions translocate into the cell usually by active uptake process, which is known as bioaccumulation. Then the bio-precipitation of metal occurs, i.e. metal precipitates after reacting with the anions produced by microbes or extracellular polymers. Finally, biotransformation takes place, which involves the metal volatilization [2].

The process that involves the usage of dead biomass formed from the byproducts of fermentation waste is known as biosorption. The non-living biomass of actinomycetes showed a greater capacity of binding as compared to the living one for the ions of cadmium [3]. For example, to remove the oxyanion, namely, chromates or cadmium, the non-living cells of *Bacillus licheniformis* and *Bacillus laterosporus* were

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showed a higher capacity of sorption, while in contrast, algal species were widely reported for the removal of metal cations [3]. The process of biosorption could reduce the costs of total treatment, operating cost, and capital cost by 28%, 36%, and 20%, respectively, compared to the exchange system of convenient ion. In 1990, various biosorbents at commercial scale were anticipated for removing the heavy metal ions, namely, AlgaSORB, AMTIBIOCLAIM, and BIOFIX [4].

The heavy metals are considered to be major pollutants in the waste effluents discharged by the various industries [3]. Heavy metal pollutants cause toxicity to humans as well as to the ecosystem as they are non-biodegradable. The lead, chromium, zinc, mercury, and cadmium metals are the majorly used compounds by the industries, and according to United States Environmental Protection Agency (USEPA), they are toxic (EPA). Since, these pollutants can lead to many diseases in living beings and affects the cardiovascular system, central nervous system, and gastrointestinal tract [3]. Hence, it is essential to regulate pollutants discharge by treating industry effluents. There are several ways to use biological wastes, i.e. byproducts of agriculture and urban area, to adsorb these heavy metals [4]. For the purification of water, lignocellulosic biomass is considered as a potential adsorbent as well as their raw materials produce the carbons in the activated form [5]. Generally, the byproducts of the vegetables such as cauliflower and broccoli from the agri-food industries offer to be significant biomass for preparing low-cost adsorbents [6]. A substantial amount, around 37.2 million tons of agri-waste biomass, is annually generated worldwide. A probable alternate to adjust these aggravated agri-food industry byproducts can be to use them as raw adsorbents and their transformation into carbon-based materials to control environmental pollution and other applications. This approach could contribute to a reduction in the disposal costs and environmental impact of vegetable waste management [7].

The advantages of using these agri-wastes for the biosorption process include multiple benefits. Along with the treatment of wastewater in large volumes by rapid kinetics, it offers the cheap natural biomaterials with high selectivity for the recovery and removal of heavy metals, along with capabilities to manage the wastes including numerous heavy metals, less requirement of additional reagents, and inexpensive operation cost. Various isotherms are used to describe the kinetics of biosorption. It determines the adsorbent feasibility and also represents the relationship of equilibrium [8].

The agri-food waste-based adsorbents are characterized by their good uptake capacities in many studies. The textile dye RR198 was removed using the pomace residues of untreated olive from the wastewater effluents. The solid residues of olive oil adsorb the zinc ion from the aqueous solution. For the removal of this dye, biosorption method was endothermic and spontaneous [9]. The biosorption capacity of heavy metals using the banana peels was observed to show adsorption efficiency of copper and lead ions, 2.18 and 5.71 mg/g, respectively [10]. The cauliflower leaves were used to produce the magnetic composite of clay biochar and used for the removal of oxytetracycline from the aqueous medium with the 33.31 mg/l capacity of adsorption [11].

12.2 Conventional Methods for Agri-Food Waste Treatment

Generally, the biological, chemical, and physical methods are used for the removal of heavy metals. The conventional methods involved in removing the metal ions are reverse osmosis, extraction of solvent, ion exchange, precipitation of chemical, coagulation of chemical, membrane filtration, and adsorption from an aqueous solution. Every technology has its disadvantages and advantages in the application. Consequently, these traditional processes have certain limitations such as the requirement of the high energy and reagent, toxic sludge/waste production, and partial metal removal. If the concentration of the metal increases than the permissible value (1 mg/l), then some of these approaches become uneconomical.

Microbial biomass nowadays becomes a sustainable option for developing eco-friendly and cost-effective methods for the treatment of wastewater. For removal and controlling the heavy metal pollution, much attention has been given to biotechnological processes. The biosorption is an alternate method as it utilizes different types of biological materials, for example, algae, bacteria, yeast, fungi, etc. [2]. The capability of metal bioaccumulation via the uptake pathway from the aqueous waste solution through biomaterials is regulated by physiochemical or metabolically active processes, known as the biosorption. The absorbent binds with heavy metals and separates them from the source. The biosorbents that have the potential to absorb the metals include yeast, fungi, bacteria, and algae [5].

Biosorption has merits over conventional methods such as minimization of chemical use, less expensive, highly efficient, and the possibility of biosorbent regeneration. Both liquid and solid phases are involved in the process of biosorption along with the sorbent species. The species of adsorbate adsorbent has a high affinity due to which the latter is attracted and bounded using different ways. This process remains continued to establish an equilibrium among the adsorbate species of solid bound and their remaining substances in the solution. The distribution between the phases of liquid and solid is determined by the adsorbent degree of affinity [4]. It can be affected by the physicochemical properties and the bioavailability of the pollutant. This process is passive, independent in metabolism between the physicochemical interaction of microbial surfaces and heavy metal ions. The microorganism's cell wall contains several functional groups that provide the attraction forces to metal ions and removes the pollutants with high efficiency [5]. It is a multistep process that comprises four successive steps mentioned later [5]:

1. Solute transfer on the boundary liquid film
2. Solute transport to the particle surface
3. Transfer of solute to internal active binding sites
4. Solute interaction with binding sites

The biosorbent possesses various functional groups, namely, thioether, amide, imine, carbonyl, phosphate, amine, sulfhydryl, imidazole, sulfonate, phenolic, and phosphodiester groups, which sequesters and attracts the metal ions [8]. There are several key factors to control or characterize the mechanism such as biosorbent

properties, targeted metal characteristics of chemical, coordination, and stereochemical, binding sites availability, and sorbent and sorbate concentration [9]. The agriculture waste enriched with lignin and cellulose content has high efficiency to bind with the metal as it contains the functional polar groups, which donate the lone pair to the metal ions and form the complex with them [8]. Agriculture wastes are readily available and have a unique chemical composition; therefore, it a feasible option for the removal of heavy metals.

12.3 Application of the Biosorption Processes

12.3.1 Removal of Inorganic Pollutants

Numerous biosorbents have been used in biosorption processes that are utilized for wastewater treatment and in the removal of harmful heavy metals that became the subject of focus in the present-day time [9]. Biosorption processes, for instance, just like electrostatic connections, ion exchange, chelation, immobilization, and compartmentation of metals, generally rely upon the physicochemical association between functional groups on the cell surface and metals [12]. Maximum biosorption methods have been employed on various microorganisms, primarily microalgae, bacteria, and fungi and with noxious heavy metals [13]. Waste biomasses of *Pseudomonas aeruginosa* and *Enterobacter cloacae* removed around 72% of Pb as biosorbents [14]. Biosorption methods cause the progression of nutrient independently and quicker, thus correspondingly enhance the metal absorption. Furthermore, biosorbents such as tannin resins also perform as reducing agents and are helpful in the accumulation of high content of important metals, such as gold, platinum, and vanadium, and considerable degradation of anionic water pollutants, for instance, chromium (Cr^{6+}) [13]. Bio-Recovery Systems Inc. company in the United States established a biosorbent Alga SORB™ from an algal species *Chlorella vulgaris* on silica gel polymer matrix, which significantly eliminated metal ions, being used as an alternative of commercial ion exchange resins. Therefore, this biosorbent is utilized by several nuclear sites to decontaminate the water from mercury and uranium [12]. Biosorption method is not only utilized to eliminate heavy metals but also practised for the heavy metal retrieval and cell walls of organisms biomass, which are used for biosorption, primarily made up of carbohydrates, lipids, and proteins that have various functional groups, for instance, aldehyde (–CHO), carboxyl (COOH), sulfate (SO_4^{2-}), phosphate (PO_4^{3-}), and amino groups for association with these heavy metals [13]. Calcined rice husks exhibited greater ability for eradicating Pb^{2+} and Cu^{2+}, where higher biosorption for these metals was observed to be 0.0530 and 0.0573 mmol of metal/g in calcined rice husk, respectively, in batch equilibrium experiments and kinetic sorption method. The Langmuir model well signified the adsorption isotherms of Pb^{2+} and Cu^{2+}. Biosorption potential of peanut husk charcoal, fly ash, and natural zeolite was studied for the treatment of wastewater by the exclusion of cationic Cu^{2+} and Zn^{2+} metal ions. It was found that these biosorbents significantly removed Cu^{2+} and

Zn^{2+} metal ions from industrial wastewater and fly ash possess greater biosorbent potential in comparison to peanut husk charcoal and natural zeolite [12]. The biosorbent potential of coconut tree sawdust (CTS), eggshell (ES), and sugarcane bagasse (SB) was 3.89, 25.00, and 23.81 mg/g for CTS, 34.48, 90.90, and 35.71 mg/g for ES, and 3.65, 21.28, and 40.00 mg/g for SB, for Cu^{2+}, Pb^{2+}, and Zn^{2+} metal ions, respectively [15].

12.3.2 Removal of Organic Pollutants

Release of harmful organic pollutants such as phenolic compounds, polycyclic aromatic hydrocarbons (PAHs), organic pesticides, and herbicides is increasing day by day in the various ecosystems. These harmful compounds have a severe toxic characteristic, and poor biodegradability can cause health and ecological issues. For the eradication of these contaminants, several physical, chemical, and biological techniques are presently being practised including chemical precipitation, extraction, advanced oxidative processes, filtration, electrokinetics, membrane bioreactor, etc. at industrial levels [12]. However, these techniques are expensive, require high energy sources, and are not efficient in small amounts; hence, the demand for inexpensive, harmless, agro-industrial wastes and byproducts has been increasing [13]. In this context, biosorption is becoming a promising, cost-effective technique to substitute the existing remedial methods of organic contaminants, dyes, and organic compounds from wastewater [12]. Tannin-based biosorption has a natural ability to absorb and accumulate dyes, surfactants, and pharmaceutical moieties from polluted water. Tannin rigid foams also act as beneficial biosorbent to accumulate long-chain anionic surfactants from water, such as poly-oxy-ethylene sodium lauryl ether sulfate [13]. In addition to this, biosorption processes are also employed for the enhancement of micronutrients, organic feedstuffs, and fertilizers, which are beneficial to ecosystem organisms directly [14]. They are also helpful in any 1° or 2° biological methods to aqueous clean-up ecosystems and other streams such as domestic, municipal, industrial, and solid wastes [15].

Various plant wastes as biosorbents, i.e. wood chip, ryegrass root, orange peels, bamboo leaves, and pine needles, were investigated through linear isotherms to degrade PAHs and practised partition coefficient (K_d) to calculate their biosorption capacity. It was found that K_d values of pine needles, which are 5306 ± 92.49 l/kg, are maximum among all plant residues used [16]. Carbon extracted from sesame stalks exhibited greater efficiency and considered as a good biosorbent for the degradation of phenanthrene in aqueous solution [17]. Biosorbents obtained from sugar cane bagasse, coconut shells, and rice husk remove PAHs such as naphthalene, acenaphthylene, fluorene, and pyrene significantly. Of them, coconut shells displayed higher PAHs uptake ability in comparison to sugar cane bagasse and rice husk [16]. Raw and modified plant residues of bamboo wood, needles, and bark of pine were reported to be better biosorbents for the efficient removal of PAHs from wastewater [16]. Biosorption by raw plant residues occurred primarily by partition method, whereas, in modified plant residues, biosorption took place by nonlinear isotherms.

12.4 Use of Genetically Engineered Microorganisms and Agri-Food Waste

Alteration of the genetic material of the microorganism to increase the potential of efficient strain for the removal of metal ions is carried out through genetic engineering technology. It has been proved proficient against the wide range of contaminants present in the environment. Their use, along with agri-food wastes, can also be thought to a prolific option. Hpn (UniProt P0A0V6) from *Helicobacter pylori* has been recognized as heavy metal adsorption protein, which has strong binding affinity for Nickel (Ni) [18]. Genetically engineered *Escherichia coli* strains (pMt-Thio) were proved to have increased metal biosorption ability of microbes' biosorbents for lead (Pb) and cadmium (Cd) ions. It was found that pMt-Thio resulted in noteworthy improvement in biosorption ability, particularly for Pb biosorption, hence could be recognized as a promising technique for decontaminating material from Cd and Pb ions [14]. *Deinococcus radiodurans* bacteria have the potential to ingest and exploit toluene and ionic mercury (Hg) from radioactive waste [19].

GolS, a transcriptional regulator, which belongs to MerR group, from *Salmonella* regulates the functioning of two transcriptional factors that are capable of accumulation of gold (Au^{+}), and this GolS protein has the promising capacity to bind Au^{+} while discriminating copper (Cu^{+}) [20]. Expressing recombinant *Oreochromis mossambicus* fish metallothionein (MT) in *E. coli* was utilized as a better biosorbent for Hg exclusion, where cytoplasmically expressed tMT exhibited high Hg adsorption [21]. The recombinant Gram-positive *merP* gene (GB) and Gram-negative *merP* gene (GP) biosorbents resulted in a significant increase in both adsorption capacity and rate for the zinc (Zn^{2+}) and Cr^{3+} metals. It was proved that recombining metal-binding proteins on genetically engineered *E. coli* could be efficient practice for producing well-developed heavy metal biosorbents [22]. The genetically engineered bacterium *Bacillus cereus* BW-03 (pPW-05) has greater potential for the biosorption of inorganic Hg [23].

Agri-food wastes are high-volume, eco-friendly, low-cost, easily applicable, processed, and easy to recover materials that have strong affinity and selectivity for heavy metals as biosorbents [8]. These moieties generally constitute greater amounts of cellulose, hemicellulose, lignin, and proteins and also beneficial as renewable natural resources and ideal for sustainable waste management [9]. Lignocellulosic biomasses are a promising resource and can be employed as biosorbents for water decontamination and can act as feedstocks to generate activated carbons [9]. Agri-food wastes, broccoli stalks, cauliflower cores, and coconut shell wastes are utilized as biosorbents for heavy metals [6]. *Durio zibethinus* rind, which is also recognized as an agri-food waste, significantly acts as a biosorbent for the removal of heavy metals such as Pb, Cd, Cu, Zn, and Ni [11]. Agro-wastes, for instance, charcoal, wheat and rice straw, rice husk, and sludge are better biosorbents by enhancing microbial biomass [9] and also offer nutrients and more exterior surface area for their proper growth [14].

Coffee husks are practised as biosorbents for the elimination of methylene blue dye. Therefore, this agri-food waste can be utilized as a cost-effective and easily

obtainable substitute biosorbent for the exclusion of cationic dyes from the polluted water [15]. Waste obtained from *Artocarpus odoratissimus* fruit acts as a biosorbent for the removal of Cu^{2+} and Cd^{2+} ions. It is possible because of the presence of organic functionalities such as alcoholic, carbonyl, phenolic, amido, amino, and sulfydryl moieties, which have a greater affinity for such metals to induce metal chelation or metal complexes [24]. *Cynara scolymus* agro-waste biomass as a biosorbent is recognized as an alternate to contribute to the elevation of the circular economy, because of its cost-effectiveness, no harmful impact on the ecosystem, and its ability to remove metal ions such as Pb^{2+}, Cd^{2+}, and Cu^{2+} [11]. Cotton stalks, maize stalks, and rice straw removed heavy metals significantly in which maximum exclusion ability is of cotton stalks due to the presence of cellulose, hemicellulose, and lignin in higher amounts in comparison to other crop-residues [24].

12.5 Biosorption Potential of Microbes and Agri-Food Waste

Use of microbes as biosorbents provides a promising strategy and can be advantageous because of their inexpensiveness, capability to regenerate, enhanced pollutants exclusion, and efficient retrieval of some worthwhile metals [12] (Figure 12.1). Utilization of microbial biomass for the removal of noxious metals from polluted areas has become a noteworthy [13]. Microbial cells of *Pseudomonas putida* I3, *Microbacterium* sp. OLJ1, and the fungus *Talaromyces amestolkiae* significantly removed Pb^{2+}, and these biosorbents recognized to have greater adsorption ability and mechanical durability due to the variations in cell walls and cellular organization [14]. Phosphorylated dry baker's yeast cells showed a greater biosorption capacity for the removal of Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} in comparison to non-phosphorylated dry baker's yeast because of the great negative charges added through the phosphorylation mechanism [12]. In the present day time, utilization of algae as sustainable biosorbents has gained huge consideration among scientists [13]. Algae are recognized as a good biosorbent due to the existence of exceptional chemical constituents, large surface area, greater binding affinity, and high uptake efficiency [25]. *Penicillium chrysogenum* biomass showed higher biosorption of 100.41 mg/g dry biomass for Cd^{2+} metal ions according to the Langmuir isotherm; in addition to this, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and X-ray photoelectron spectroscopy (XPS) analysis exhibited that the –OH and –C=O groups on the fungus cell wall are the major binding positions for Cd^{2+} [26]. *Trametes* sp. SC-10 fungus showed maximum biosorption ability of 221.6 mg/g for Acid blue 161 (AB-161) dye; therefore, it is well-thought-out as a promising biosorbent to decontaminate industrial wastes [27].

Garlic waste exhibited greater removal of malachite green, i.e. 232.56 mg/g via the Langmuir model at pH 8.0 and 298 K temperature, and hence, garlic root waste can be utilized as a cost-effective biosorbent to eliminate dyes from industrial wastewater [28]. Bagasse fly ash (BFA) significantly removed 2,4-D. Hence, BFA can be used as a cost-effective and effective biosorbent. Stem and leaves powder of potato

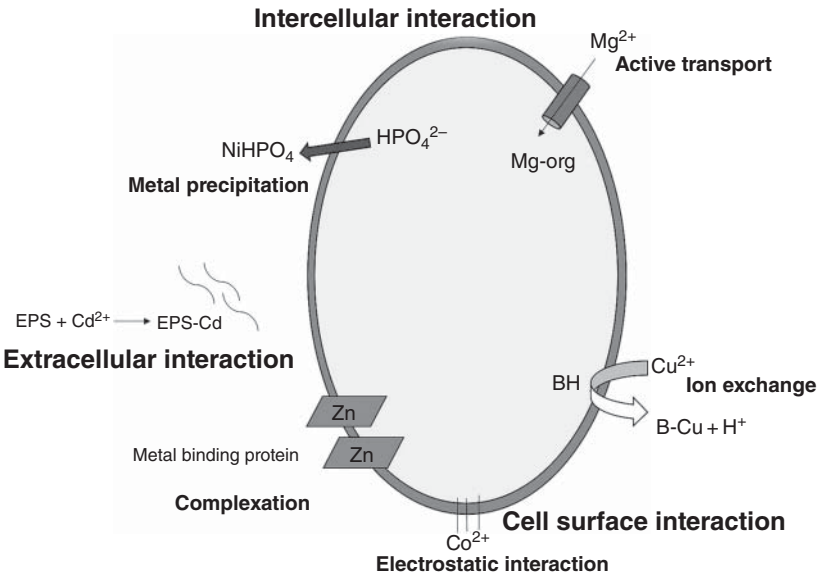

Figure 12.1 Schematic representation of the different mechanisms of microbial (bacterial) biosorption. Source: Based on Demirbas [12].

proved to be a promising candidate for the exclusion of methylene blue and malachite green dyes. Leaves powder demonstrated more biosorption than stem powder, and coarse exterior and functional groups made the potato wastes a better asset for the biosorption [12].

12.6 Modification, Parameter Optimization, and Recovery

The availability and number of the functional groups are mainly responsible for the biosorption potential of biosorbents. These can be modified by changing their surface characteristics through certain processes. Microbial-derived biosorbents are responsive for modification to increase the available binding sites and enhance the biosorption capacity leaving low residual metal concentration. Several methods have been employed for surface modification of microbial biomass. Some parameters were studied on the kinetic model and isotherm of biosorption, which can affect the selectivity [13]. It could be accomplished by using a molecular technique in the synthesis known as molecular imprinting. Numerous parameters can affect the microbes' hydrophobicity. Some of these parameters are contact angle, surface tension, and electrokinetics and have been studied for the toxic metals' removal from aqueous medium [14]. Also, the hydrophobicity and floatability were interrelated, and these physicochemical parameters were confirmed with the laboratory measurements.

The complex interactions between the interfaces of liquid, solid, and air result in the hydrophobicity [15]. During the study of the pH range, the zeta potential value gradually decreases negatively. The pH value of fungal biomass and *Streptomyces* was determined to be around three and zero, respectively, whereas, for yeast, it happened at a low value of pH. It was observed that the zeta potential changes its value toward less negative in the presence of surfactant, i.e. flotation collector and metals. The neutralization of surface charge and reversal to positive value was also perceived after the addition of the cationic polyelectrolyte [12].

The biosorption fixed-bed column process model is similar to the normal adsorption [14]. At the mines of the US Bureau, similar work was carried out with the biomass of sphagnum peat. The separation at the solid or liquid stage was found to be difficult during biosorption, and the possible reason for this might be due to the low biomass mechanical resistance and stability [9].

12.6.1 Modification

The application of the agriculture waste as biosorbents needs modifications as they cause the increment in the chemical oxygen demand and biochemical oxygen demand due to the organic soluble compounds release from the waste of plants. It leads to oxygen depletion in the water. The additive selection plays a vital role in the adsorbent modification to ensure the best usability and good efficiency. This can be enhanced by improving chemical and physical modalities. The adsorbent size comes under physical modification which can be achieved by grinding or chopping, freeze-drying, thermal treatment, stirring, and ultrasonic irradiation. The adsorbent of small size provides the high surface area, which is suitable for the batch process as compared to the column process of adsorption as it causes the clogging of the column. The biosorbent's physical structure might be damaged due to a high temperature, but heating increases the efficiency of biosorption by increasing the adsorbate kinetic energy and surface activity. By decreasing the resistance of mass transfer of the pollutant, the agitation process can enhance the biosorption process. Whereas chemical modifications in different types of chemicals including salicylic acid, sodium hydroxide, methanol, carbon tetrachloride, hydrochloric acid, sodium chloride, sodium carbonate, calcium chloride, phosphoric acid, epichlorohydrin, ammonium hydroxide, citric acid, nitric acid, tartaric acid, acetone, toluene, formalin, ethylenediaminetetraacetic acid (EDTA), iso-butanol, benzene, formaldehyde, etc. include the adsorbents, chemical treatment for washing, co-polymerization of graft enhances the binding group and eliminates the inhibition groups [7, 12] The binding group enhancement includes the addition of ester group saponification, hydroxyl group amination, phosphorylation, thiolation, oxidation, amine group carboxylation, halogenation, xanthanation, and sulfonation. The inhibition group removal is achieved by deamination and decarboxylation, while polymerization of graft involves grafting by chemical initiation, photochemical, and high energy radiation. Hence, it is recommended for having surface modification before using the low-cost agri-waste-derived adsorbents.

12.6.2 Parameters

The decayable wastes produced from the waste/used/overripe vegetables and fruits in the type of shells, epicarps, seeds, cobs, coats, peels, trimmings, stones, bagasse, etc. were having different adsorption potentials. The capacity of adsorption of dyes and metals via these agriculture wastes varies by various factors such as the host, adsorbent structural characters, adsorbate initial concentration, modification in the surface, size of the adsorbent particle, solution pH, temperature, dosage of the adsorbent, and concentration of the pollutant. The adsorbents of the small size represent the high affinity for the removal of metal. The ionization degree and the density of surface charge get affected by the pH of the solution because of the competition between metal and hydrogen ions for binding to sites. Thus, along with sorbent treatment measures, optimization is also important.

12.6.3 Recovery

Through chemical and physical treatment, recovery can be achieved of loaded dyes and metals. Physical treatment can be done in two ways, i.e. heating and microwaving. The chemical processes use the organic solvent, alkali, and acid. Generally, various chemicals are being used for the desorption process; some are sodium hydroxide, potassium hydroxide, sulfuric acid, hydrochloric acid, sodium nitrate, nitric acid, EDTA, etc. Some metals have been recovered in traces amount such as cupric ion, chromium ion, cadmium(II) ion, and nickel(II) ion after optimizing the parameters and protocol. By using the improved method of striping, which involves the treatment of alkali, gold can be recovered from biosorbents [13]. After metal elution, adsorbent regeneration is cheaper and easier because of the presence of numerous carboxyl and hydroxyl groups on the adsorbent surface.

12.7 Immobilization of Biosorbent

For the biosorption process, the liquid- and solid-phase separation step is an important consideration. The commonly used techniques used in the separation are filtration and centrifugation, but at the industrial level, these methods are not suggested. The suitable bed attached to biosorbent is referred to as a continuous system that is advantageous [19]. In this system, using biosorbents as free microbial cells causes many disadvantages, for example, biosorbent loss after regeneration, low rigidity and strength, and difficulty in biomass separation [14]. By using the polymeric/biopolymeric matrix, immobilization of microbial biomass can be done. It is a key element that enhances the biosorbent performance after improving the capacity and mechanical resistant and facilitates biomass separation from pollutants [15]. For the

recovery of non-destructive products, the immobilization process is the best-suited technique. The methods such as encapsulation or entrapment are used to immobilize the biosorbent into particles [14].

Several types of matrices have been considered for immobilization of biopolymers, including polysulfone, calcium alginate, sodium alginate, polyurethane, silica, and polyacrylamide. It is essential to utilize a suitable matrix for immobilization as it indicates the biosorbent particle's chemical resistance and mechanical strength though it should be feasible and cheap [16]. But using the immobilized biosorbents has some limitations. It reduces the number of binding sites and also affects the kinetics of mass transfer [17]. From an aqueous solution, copper, lead, and zinc were removed using the heat and live immobilized beads of *Trametes versicolor* within carboxy methylcellulose [27]. The polyurethane immobilized matrix was used to eradicate the lead, copper, cadmium, nickel, and reactive yellow 2. In contrast, the polyacrylamide and calcium alginate immobilized matrix was used to remove the lead, gold, and uranium [28].

The cell wall of bacteria has a complex structure which plays an important role in selective sorbent [7]. The Gram-positive bacteria show a higher capacity of sorption due to thick peptidoglycan layer. The teichuronic and teichoic acids in Gram-positive bacteria are encapsulated, while the Gram-negative bacteria cell wall constitutes a thin peptidoglycan layer along with lipopolysaccharide phospholipids [12]. In the continuous and batch system, the biomass of *Arthrobacter* species is used to remove Cu(II) ions with polysulfone and inactivated free heat-immobilized biomass [14]. Hence, we can conclude that the microbes have natural potential toward the removal of heavy metals and can act as promising biosorbents (Table 12.1).

12.8 Conclusions

Microbes and agri-food waste are considered as promising biosorbents for the removal of both organic and inorganic contaminants in aqueous solutions. These wastes comprise high cellulose and lignin contents having good sorption capacity. Presence of various functional groups such as amine, sulfonate, phenolic, phosphodiester, carbonyl amide, etc. on their surface has shown a good percentage of adsorption efficiency. Their use as biosorbents contributes to minimizing the environmental impact and disposal cost of agricultural biomass. Various factors characterizing or controlling its mechanism include binding site availability, sorbate concentration, sorbent, coordination, stereochemistry, etc. It is a cost-effective approach for the removal of heavy metal and other contaminants from different sources. Further study needs to check the assessment of agri-based biosorbents, the effect of different conditions such as temperature, pH on removal percentage, and to check the material reusability.

Table 12.1 Biosorption mechanism of various microbes and agri-food waste.

Contaminant	Microbe/agri-food waste	Biosorbent	Results	References
Chromium	Microbe	*Arthrobacter viscosus*	Bacteria, in combination with zeolite, resulted in high removal rate of Cr at higher pH values in which this Cr biosorption was highly pH-dependent	[29]
Lead	Microbe	*Bacillus xiamenensis*	Highest Pb^{2+} uptake of 216.75 and 207.4 mg/g was observed with both live and dead biomass of bacteria, respectively, through surface adsorption mechanism	[30]
Gold	Microbe	*Lysinibacillus sphaericus*	Showed greater efficiency (100%) after 3 h of exposure, for the removal of precious metal	[31]
Gold	Microbe	Cyanobacteria	Exopolysaccharide producing cyanobacteria showed efficient biosorption of Au from the wastewaters	[32]
Malathion	Microbe	*Spirogyra*	*Spirogyra* alga removed 76.34% of Malathion at pH 7, using biomass amount of 75 mg and a contact time of 5 h	[33]
Copper and silver	Microbe	*Sargassum filipendula*	Cu^{2+} ions exhibited a maximum affinity for seaweed from alginate extraction in which external diffusion showed the crucial part in Cu^{2+} biosorption kinetics	[34]
Cobalt and nickel	Agri-food waste	Watermelon rind	Elevation in the pH from 2 to 7 resulted in an escalation of the adsorption potential, however, at greater than 5 pH, adsorption uptake rate reduced due to the establishment of soluble hydroxyl compounds. FTIR analysis showed the involvement of acidic groups and carboxyl and hydroxyl groups in the adsorption	[35]

(Continued)

Table 12.1 (Continued)

Contaminant	Microbe/agri-food waste	Biosorbent	Results	References
Mercury	Microbe	*Polyporus squamosus* fungus	Maximum biosorption yield of 35.37% was observed at 5.30 pH, 20 °C temperature and contact time of 254.9 min	[36]
Methyl orange	Microbe	*Aspergillus flavus*	Fungus removed 53.62% of methyl orange at 5.5 pH, with biomass amount of 2 g/l and contact time of 40 min	[37]
2,4-D, 2,4-dichlorophenoxy propanoic acid (2,4-DP), and 2,4-dichlorophenoxy butyric acid (2,4-DB)	Agri-food waste	Apple shell, banana and orange peel, and millet waste	Efficient removal of pesticides from polluted wastewater by agricultural waste at a pH range of 6 and 7 and contact time of 60 min	[38]
Tebuconazole, triadimenol, cymoxanil, pirimicarb	Agri-food waste	Spent mushroom substrate (SMS)	Biosorption of these pesticides by SMS modified soils enhanced because of the improvement in organic carbon produced by the biosorbent SMS	[25]
Pirimicarb, imidacloprid, acetamiprid, and thiamethoxam	Agri-food waste	Chestnut shells	Chestnut shells waste has optimal biosorption characteristics for these pesticides, in which maximum uptake was observed when chestnut shells were pretreated with citric acid	[39]

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13

Biosorption of Heavy Metals and Metal-Complexed Dyes Under the Influence of Various Physicochemical Parameters

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13.1 Introduction

About three-fourth of the earth is occupied by water, the rich natural resource and vital for all forms of life on our planet. However, the rapid growth of industrialization over the years has led to the gradual depletion of this natural resource. Natural water bodies such as ponds, rivers, lakes, and seas have been highly affected by the discharge of industrial effluents. For instance, chemical processing and mining industries produce large amount of liquid effluents with heavy metals and toxic species. These pose serious ecological risks due to their nondegradable nature [1].

There are several industrial processes like mining, fertilizer production, surface finishing, electroplating, and electrical material production. Industries like mining and smelting of tanneries, atomic energy production, and aircraft production are the major sources of heavy metal pollution in aqueous systems [2]. But the excessive amount of toxic heavy metals are majorly discharged from power production from the steam, mining process with acid mine drainage, electrocoating process, and nuclear power production. In addition to that, textile, printing, petroleum, pesticide, solvent, and paint are major industries contaminating water bodies by organic-based chemicals.

In addition to that, industries such as plastic, fabric, and cosmetics produce a huge amount of synthetic colored products [3]. The dye production and raw material industries discharge 15% of untreated dye complexes along with the effluents into water bodies without any prior treatment. The discharged dyes comprised of different contaminants which are highly acidic or basic and dissolved or suspended. The untreated effluent leads to an increase in the hazardous effect in an aquatic

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system leading to massive health issues for both aquatic species and human beings through the food chain and reduces the photosynthetic activity of the plants. In addition to that, the anionic dyes used in wool, polyamide and polypropylene fibers, and cationic dyes which are highly water-soluble and reactive dyes pose harmful effects such as skin allergies, irritation on skins, mutation of genes, tumors, and cancer diseases [4].

Ozacar and Sengil [5] revealed that the negatively charged (anionic) metal colorants are mostly used in fabric and tanning industries, and polyamide fibers are used to reduce the fading of pigments. The major metals used for producing metalized dyes are copper, cobalt, and chromium. Moreover, the complexed dyes and metal ions lead to the formation of several color shades of dyes. Additionally, the salt-based metal-complexed dyes are mainly used in the dye and finishing industries to reduce the dissolution of dye and enhance the aggregation of the dye molecules. In this process, NaCl is the major salt used as an additive in the dying process which affects the aquatic species when the salt concentration is high in the system.

Heavy metals and dyes become more toxic and cause detrimental effects on the environment when their concentration exceeds the permissible limit in the effluents. To overcome these issues, many conventional methods have been applied to remediate deadly heavy metals and dyes from the industrial run-offs. One method is chemical precipitation where alkylation of metal solution by the coagulants such as sodium bisulfide, potash alum, sulfide, iron base salts, and limestone will be carried out to convert solubilized metal ions into insoluble forms. Electrowinning is another method where recuperation of metals from mining, metallurgical operation, electrical industries, and electronics will be carried out. This process is carried out by passing current from an inert anode to a cathode kept in an electrolyte, which contains metal ions and follows an electroplating process. Reverse osmosis is yet another conventional method to retentate the heavy metals using a semipermeable membrane. The membrane-based process has the advantage for the selective metal separation and highly stable with respect to changes in pH [6].

Two-phase extraction method is another viable method to recuperate selective metals (for example, platinum group metals from spent catalysts). The extractants used to recover the metal ions are organophosphorus compounds, aliphatic amines, and quaternary ammonium salts. But this extraction process is very difficult to convalescence metals from the organic phase, and also the harmfulness of extractants is determinantal factor in this separation process. However, these methods are most expensive and are not preferable for environmental conditions. Gupta et al. [7] stated that surface adsorption is one of the mass transfer processes for an active elimination of dyes compared to other processes. Zeolite, polymer-based porous material, and activated carbons are the selective adsorbents which are used to remediate the dyes because of their huge surface area to volume ratio and the presence of functional groups present on the surface of the zeolite. In this chapter, we have mainly concentrated on discussing the importance of biosorption for removing metal-complexed dyes while using various microbial species such as bacteria, fungi, or yeast and also the new methods which were adopted in industrial processes.

13.2 Mechanisms Involved in Biosorption of Toxic Heavy Metal Ions and Dyes

The elucidation of interaction mechanisms involved in the removal of metal and metal complex dyes (MCDs) by the biosorbents is very important to enhance the biosorption process as well as to recover the metal ions and metal-chelated dyes. The multifaceted structure of biomass indicates that it has different ways to remove the metal contaminants. However, the mechanism of metal and MCD interaction with biosorbent is not yet fully understood. Hence, the mechanisms involved in the bioremediation of both metal and dyes were found to be complicated ones. The mechanisms of metal biosorption were influenced by various factors such as (i) live and dead microbial cells, (ii) kinds of microbes used, (iii) characteristics of metal and metal-complexed dyes, and (iv) physiochemical conditions such as pH and temperature. Biosorption is a metabolic-independent process that is based on the passive sequestration of metal species on the surface of the dead biomass. The bacterial biomass consisting of several chemically active groups that tend to attract metal ions and dyes from aqueous solution get sorbed onto its surface. The sequestration of metal and MCD on the surface of the biomass was followed by physio-chemical interaction with the functional groups present on the microbial surface [8].

The bacterial cell wall as already discussed in the previous section mainly consists of several biomolecules namely polysaccharides, proteins, and lipids. The toxic heavy metals adhere to various functional groups such as phosphate, sulfate, amino, and carboxyl group by electrostatic attraction, ion exchange, complexation, van der Waal's forces, covalent bonding, and micro-precipitation. The magnitude of biosorption is influenced by several factors such as the valance of the metal ions, bacterial genus, and variations in cellular constituents. The biosorption process also depends on several environmental factors such as ionic strength, solubility of the metal ions, solution chemistry, pH, and temperature conditions [9]. One possible way to increase the biosorption process is using less hydrophilic molecules which lowers the affinity between solute and solvent and therefore get biosorbed more easily [10].

13.3 Chemistry of Heavy Metals in Water

In the sorption process, it is very important to study the two phases, solid and liquid. The solute or sorbate must be dissolved in the solution which ultimately interacts with the solid phase. The performance of the biosorption process will be affected by the properties and behavior of both the sorbate and the sorbent. The speciation of metal ions in the solution is mainly reliant on pH which is an important parameter in a sorption process. The metal in the solution might be cationic or anionic when it becomes dissociated in the solution. Most of the metals expose positively charged cationic species when they are dissolved in the aqueous media. Different form of metal ionic species, protonation–deprotonation of active sites on bio-sorbents, is also dependent on the pH of solution [11]. The protonation of functional groups leads to lowering of cationic metal ion sorption. At low pH, all functional groups present on

the biosorbents will get attached with hydronium ion, thereby shielding the metal ions from adsorbing onto the biomass [12].

13.4 Chemistry of Metal-Complexed Dyes

The MCDs and acid dyes comprise of chelating active sites for the coordination of metal ions along with the dyes, and these dyes are particularly used for the coloration of wool, silk, and nylon. These complex dyes are classified into two different kinds such as mordant and premetallized dyes. Generally, the metal-complexed dyes are having less solubility in the water system. The solubility of metal complexes decreases to twofold than the mono-azo-type dyes which are chelated before with the metal ions by carboxyl, hydroxyl, and amine functional groups. In case of divalent Cu^{2+} ions, it has a coordination number of 4 and it can complex with two bidentate ligands in the acid dye. Hannemann [13] stated that the major metals such as Cu^{2+}, Co^{2+}, Ni^{2+}, and Cr^{3+} used for the complexation of acid dyes. These 1 : 1 complexed premetallized dyes are the source for the moderate dye used for wool and silk fibers. In the dying process, the acidic MCDs should be maintained between the pH 2.7 and 4 which helps to increase the rate of dying. At the same time, the fastness property on nylon 6,6 has improved by 1 : 2 MCDs.

13.5 Microbial Species Used for the Removal of Metals and Metal-Complexed Dyes

Biosorption is a process mainly used for detoxifying the heavy metals and metal acid dye solutions using bacteria, algae, and fungi. Different types of microorganisms play an important role in the elimination of toxic metallics and dye contaminants by different mechanisms. The microbial consortium used for the removal of metal and metal-complexed dyes is discussed in Sections 13.7–13.9.

13.5.1 Biosorption of Zinc Using Bacteria

There are various bacterial species having different capability of accumulating metal ions present in aqueous solutions. *Bacillus* species have been identified as the most potential bacterial species for metal sequestration and thus used in commercial biosorbent applications [14]. The sulfate-reducing bacteria, *Desulfotomaculum nigrificans*, was used to bioremediate higher concentration of zinc that resulted in achieving maximum percentage of biosorption (60–70%) [15]. Certain bacterial species such as *Klebsiella oxytoca* (P2), *Pseudomonas veronii* (2E), *Ralstonia taiwanensis* (M2), *Klebsiella ornithinolytica* (1P), and *Delftia acidovorans* have the capability to remediate heavy metals such as zinc, copper, and cadmium by different processes such as biosorption, bioaccumulation, and bioprecipitation. Chen et al. [16] studied the metal uptake by both live cell and nonliving *Pseudomonas putida* CZ1 bacterial cells. The adsorption capacity of live *P. putida* CZ1 was found to be

more than the nonliving cells under the tested conditions. It was found that live cells actively accumulate 40–50% of zinc and copper with the remaining metal ions being passively bound onto the bacterium. Similarly, the desorption efficiency achieved by the living cells was also found to be less than the nonliving cells mainly due to the enhancement of intracellular accumulation of Cu(II) and Zn(II) by the live cells. The Cu, Zn, and Cd metals present in the water waste were treated by immobilized autochthonous microorganism *P. veronii* 2E [17]. The toxic heavy metal ions are retained by beads, and the entrapment of bacterial cells in calcium alginate is futile to progress the heavy metal holding [18]. The performance of biosorption of zinc by *Botrytis cinerea* was improved by treating the bacterial cells with heat, sodium hydroxide, detergent, and acetic acid. The gram-negative microbial species *Pseudomonas aeruginosa* secluded from the petroleum site was found to have more tolerance to the toxic heavy metals such as trivalent chromium, divalent copper, zinc, and manganese and also it can adapt to higher concentrations (300, 150, 100, and 320 mg/l, respectively) [19]. In addition to that, the efficacy of biosorption of zinc by *P. aeruginosa* AT18 was found to be very effective in single metal system than multi-metal solution mixture containing Cr^{3+}, Cu^{2+}, Mn^{2+}, and Zn^{2+}. It was reported that the *Bacillus thuringiensis* could achieve a maximum biosorption of Zn(II) at pH 6 [20].

13.5.2 Biosorption of Heavy Metals by Algae

Algae play a significant role in the biosorption of toxic heavy metals in aqueous systems. Different types of brown, green, and red algae have the capability for the removal of heavy metal effectively. The algal cell wall is mainly composed of cellulosic compounds, glycoproteins, amino acids, and polysaccharides [21]. The removal of copper, zinc, iron, and manganese was investigated using the dead biomass of *Cyanobacterium* and *Phormidium laminosum*, and it was found that the removal process is very rapid for a single metal at an optimum pH of 7. The biosorbents modified by dilute acids showed a better performance compared to those treated with NaOH, NaCl, $CaCl_2$, and ultrapure water. Likewise, the effect of macro algae, *Chaetomorphalinum*, on the removal of zinc ion was studied [22] and found that maximum biosorption of zinc could be achieved at pH 5. Beyond pH 5, biosorption of zinc sharply declined and attributed to the formation of an anionic form of zinc hydroxide complexes which restrict the interaction of metals with active sites. It [20] was reported that maximum biosorption of zinc happens with six types of algae namely *Codium vermilaria, Chondrus crispus, Spirogyra insignis, Asparagopsis armata, Ascophyllum nodosum,* and *Fucus spiralis* at pH 5 and 6. Also, there is the biosorption of zinc by the various algal species such as *Chlorella vulgaris, L. taylorii, L. tayloriiphos, Ankistrodesmus densus,* and *Dunaliella bioculata* occurs. The zinc biosorption studies using *Sargassum muticum, Laminaria hyperborea, Bifurcaria bifurcata,* and *F. spiralis* showed a maximum zinc uptake of 18–32 mg/g at pH 5 at an initial zinc concentration of about 75 ppm [23]. The biosorption capability of other algal species such as *Sargassum filipendula, Caulepra lentilliferra*, and *Codium vermilara* was also reported [24]. The *S. filipendula* showed maximum zinc

biosorption capacity of about 41.84 mg/g of inactive cell at pH 6. Microalgae also have the ability to resist zinc ions which is mainly due to the properties of their cell wall and their ability for phytochelatin production.

13.5.3 Removal of Toxic Heavy Metals by Fungi

Fungal biomass has also revealed a significant potential to remove heavy metals and radio-nuclides from the polluted water system. The *Streptomyces, Rhizopus, Aspergillus, Penicillium*, and *Mucor* are the different genera of fungi used by various researchers for the bioremediation of heavy metals [25]. It [26] was reported that the use of *Aspergillus niger* for zinc biosorption resulted in a maximum zinc removal in the pH range of 3–6.5. A dead biomass of *Streptomyces rimosus* was collected from the antibiotic fermentation industry, and it was used in the biosorption studies of zinc and the efficiency of the biosorption was found to be improved after the modification of the surface of the fungi using 1 mol/l NaOH [25]. Nongrowing *Penicillium spinulosum* showed sixfold higher zinc biosorption compared to the growing cells. The time taken by the nongrowing mycelia for biosorption process was found to be 60–120 minutes, while, in the case of living cells, the accumulation of heavy metals depends on the age of the cells. The amount of zinc biosorbed was high during the lag phase, and the rate of adsorption was decreased at early stages of growth as well as in stationary phase [1]. The fungal species such as *A. niger* and *Trichoderma viride* also found a similar way of uptake analogy. The biosorption capability of *Penicillium, Rhizopus*, and *Aspergillus* toward different metal ions can be represented as Fe > Cu > Zn, Ni > Cd, Pb > UO, UO_2 > Pb > CD > Zn > Cu and Fe^{2+} > UO > Cu > Zn.

13.5.4 Biosorption of Heavy Metals Using Yeast

The yeast *Saccharomyces cerevisiae* attracts more attention among the researchers for the bioremediation of heavy metals due to its distinctive nature despite of its average capability of metal uptake. The yeast biomass, which was collected from the fermentation industries, could bioaccumulate various metal ions such as Ag^{+}, Cd^{2+}, Cr^{3+}, Cs^{+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Sr^{2+}, and Zn^{2+} at different pH [2]. Moreover, the yeast biomass can be easily and quickly parted from processed effluent because of its self-aggregation properties. Several yeast species such as *Candida*, *Clavispora*, *Pichia*, *Rhodosporidium*, *Rhodotorula*, *Sporidiobolus*, *Debaryomyces, Leucosporidium*, *Sporobolomyces*, *Saccharomyces*, *Stephanoascus*, *Trichosporon*, and *Yarrowia* are found to have higher capability to biodegrade organic contaminants and bioremediate heavy metals. The *S. cerevisiae* is used as a model system to study about mechanisms involved in the interactions between the metal and microbe at the molecular level. The *S. cerevisiae* is found to be an excellent biosorbent to remediate toxic heavy metals such as Zn, Pb, Cu, and Cd [10]. It was observed by Mapelelo et al. that a maximum biosorption of multi-metal system comprising Cu^{2+}, Cr^{3+}, Pb^{2+}, Cd^{2+}, and Zn^{2+} by *S. cerevisiae* was occurred at pH 5.

13.6 Industrial Application on the Biosorption of Heavy Metals

The removal or the treatment of contaminants by the specific type of biosorbents was first patented in the year 1980. During 1990s, lot of researchers were focused on the process of understanding the biosorption mechanisms and its fundamentals. But this process is not widely applied for the removal of toxic metal and several compounds [27]. There are various types of reactors used to investigate the biosorption process like stirred tank bioreactors (SBRs), packed bed reactors, fluidized bed reactors (FBRs), and fixed bed reactors (FxBRs). These reactors can be used to remediate the heavy metals and the MCDs either by continuous, batch, or both continuous and batch systems. The major physiochemical properties such as temperature, mixing, agitation, pH, and nutrient availability have to be optimized.

13.6.1 Biosorption of Heavy Metals Using Fluidized Bed Reactor

Generally, the biosorption process is investigated using a fixed bed reactor due to high concentration and uniform residence time. But the solid impurities present in the wastewater will lead to persistence of the solid in the fixed bed and the liquid must be cleared to overcome the column blocking. In the last two decades, the fluidized bed reactors have been used for turbid liquids and also for avoiding channeling issues [28]. In addition to that, the main advantage of using a fluidized bed is to reach a proper mass and heat transfer between the fluid and particles and also between the particle and sidewall of the column. In this process, the liquid or gases flow with a certain velocity through the bed, and simultaneously the pressure drop was emerged to balance the gravitational force on the particles, and finally, the minimum fluidization velocity was achieved by further increasing the velocity of liquid or gas phase [29]. The performance of a fluidized bed for the removal of metal ions mainly depends on particle density, size of the particle, size distribution, and surface characteristics. Illamathi et al. [30] carried out the experiments to study about the biosorption of heavy metals such as Cr(VI), Ni^{2+}, Cu^{2+}, and Cd^{2+} by the liquid–solid fluidized bed using sol–gels as catalysts which contain several bacteria such as *P. aeruginosa, Bacillus subtilis*, and *Escherichia coli* as shown (Figure 13.1). They achieved the maximum percentage of removal of copper, cadmium, chromium, and nickel which was found to be 84.62%, 67.17%, 49.25%, and 61.02%, respectively.

The biosorption of cadmium, copper, and lead using cabbage leaves was done by varying different physiochemical parameters such as specific surface area, the porosity of the particle, and void space. The efficiency of the biosorption process in the fluidized bed has been evaluated by different parameters such as superficial velocity, bed height, and heavy metal concentrations, and the effectiveness of biosorption was elucidated by the breakthrough curves of divalent ions like Pb(II), Cu(II), and Cd(II).

The fluidized bed which is shown in Figure 13.2 made up of glass with an inner diameter of 7.5 cm and a height of about 100 cm, respectively. The influent flow of metal contaminant through the bottom of the reactor by the 2-mm stainless steel distributor and the pressure of the water were measured by the U-tube

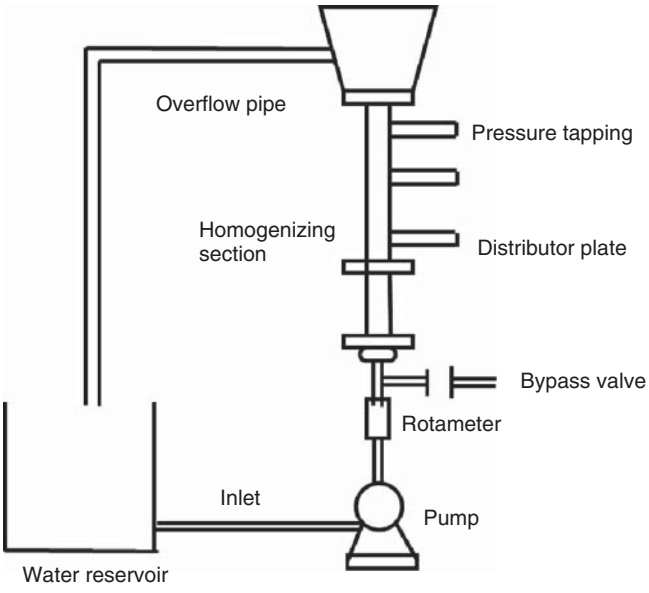

Figure 13.1 Schematic representation of a fluidized bed. Source: Tsezos and Volesky [29].

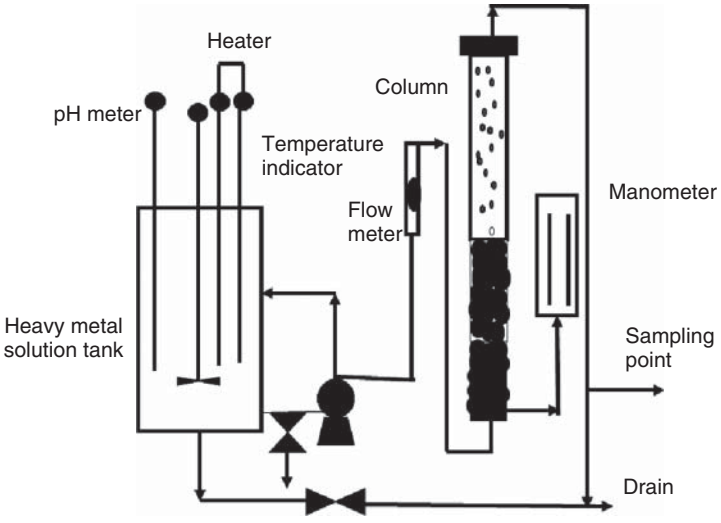

Figure 13.2 Schematic representation of fluidized bed for the removal of Cu, Cd, and Pb metal ions [30]. Source: Kamar et al. [31].

manometer filled with carbon tetrachloride (CCl_4) with a density of 1590 kg/m^3. The one end of the manometer was fitted above the distributor and the left end was open to the atmosphere. Another study [31] investigated on the comparative studies on continuous removal of chromium(VI) present in the aqueous solution using chemically adapted and immobilized *Rhizopus nigricans* on fluidized and

packed bed reactor. The performance of the packed bed reactor was analyzed by varying flow rate, inlet concentration, and depth of packed material in the range of 5–15 ml/min, 50–250 mg/l, and 4.9–22.8 cm, respectively. Eventually, they observed that time taken for the breakthrough point and the adsorption rate is decreased by increasing flow rate, inlet chromium concentration, and depth of sorbent packing. In the case of chromium biosorption by the fluidized bed reactor, the efficacy of the process was analyzed at the initial concentration of 100 ppm, flow rate of 5 ml/min, and fluidized air flow rate of 0.5 kg/cm^2. The maximum adsorption capacity by biomass in packed and fluidized bed reactor was found to be 123.33 and 153.04 mg of Cr/g of biomass, respectively.

13.6.2 Biosorption of Heavy Metals by Using Packed Bed Reactors

Ibrahim et al. [32] examined the removal of heavy metals using a fixed bed bioreactor packed with supporting material. In this research work, the biosorption process was carried out for several divalent metal ions such as Cu^{2+}, Cd^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}, and trivalent Fe^{3+} using *P. aeruginosa* and the luffa pulp as packing material. Figure 13.3 shows the schematic representation of a fixed bed reactor. To overcome the contamination effect, sterile air was collected through the two air filters which will pass through the glass nozzle at the bottom of the column (4) with a fixed flow rate of 1 l/min. The column was packed with 8 g of supporting material (luffa pulp pieces), and the biomass was adsorbed onto the surface material. In this design, two heads are connected by the peristaltic pump at the top and bottom for pumping the wastewater into the reactor and discharge the effluent without heavy metals.

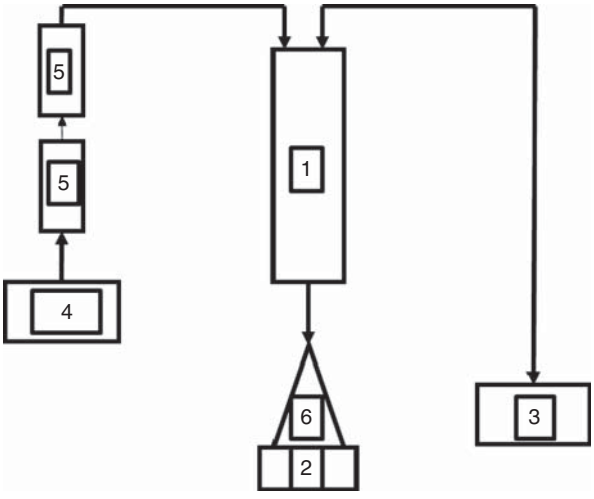

Figure 13.3 Schematic diagram of a fixed bed reactor supported with luffa pulp in a glass column [31]. (1) Hot plate with a magnetic stirrer, (2) peristaltic pump, (3) air pump, (4) two air filters, (5) heavy metal contaminated sample, and (6) flexible hoses for connections. Source: Ibrahim et al. [32].

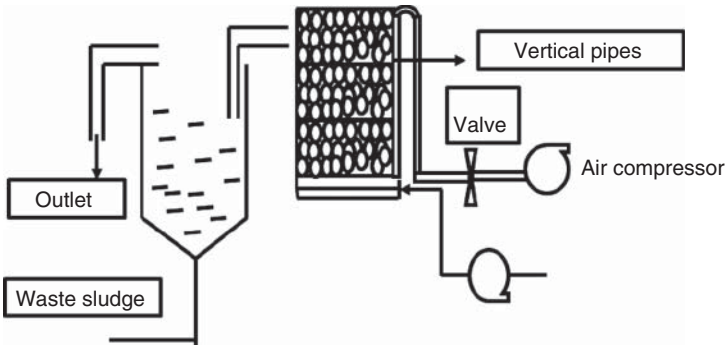

Figure 13.4 Schematic representation of packed bed reactor [32]. Source: Azazi et al. [33].

The obtained results revealed that the heavy metal ions such as Cu^{2+}, Cd^{2+}, and Zn^{2+} can be remediated by the *P. aeruginosa* in the multi-metal system with 100% efficiency. Azizi et al. [33] concentrated on the determination of efficacy on the selective removal of heavy metals such as cadmium, copper, nickel, and zinc using a packed bed bioreactor (PBBR). In this process, PBBR is used to remediate the heavy metals as well as the organic contaminants by the attached-growth process. The performance of the bioreactor was evaluated and assessed by varying different metal concentrations.

Figure 13.4 shows the laboratory unit of the biological reactor (PBBR) to determine the strength of composite heavy metals in the system. The major significance of the PBBR system developed with a different layer with a fixed bed, and the vertical pipeline helps to confirm the effluent flow and simultaneously increases the oxygen transfer in each layer in the PBBR. In addition to that, the void ratio is around 98.18%. Once the sewage sludge entered through the bottom of the reactor, enough upflow velocity has to be maintained to prevent clogging. Polypropylene is used as a carrier media with a density of 0.95 g/cm^3 and an active surface area of 350 m^2/m^3 and is designed with outer grooves to restrict biofilm formation. Similarly, the packed bed bioreactor shows a high retention time of about two hours for copper and zinc metal ions than cadmium and nickel ions. The maximum tolerable limit for the composite material used for the heavy metal is around 20 ppm for about two hours.

13.7 Biosorption of Reactive Dyes

Reactive dyes are the combination of various types of reactive groups such as vinyl sulfone and chlorotriazine with azo-based chromophores. The dyes are impregnated with the textile fibers by covalent bond interaction. These dyes have inevitable characteristics in making optimistic colors, water fastedness, and consumption of low energy. But these dyes are very tough to remediate in the presence of light, higher temperature, oxidizing agents, and also nondegradable by the biological compounds. In most practical applications, activated carbon shows an effective adsorption for the removal of color in the effluents discharged from the textile industries. But, it is very

difficult to use in high-scale process because of its high cost [34]. Many researchers preferred very inexpensive and other natural sources such as bagasse, bark, chitosan, wood, peat, and fly ash. In addition to that, they found microbial sources such as bacteria, fungi, and yeast which have the potential to degrade the dyes. The reactive functional groups of azo-based dyes will impart with the active sites after taking part with acidic polysaccharides, chitin, amino acids, fatty acids, and other constituents of the microbial consortium. In addition, the ionic strength shows a greater impact on the increase of the interaction between the cell surface and dyes. The interaction between the ligands present on the cell surface and dyes is carried out by electrostatic interaction and hydrogen bonding. The efficiency of the color removal by the yeast decreases at higher concentration of dyes due to the nonavailability of active sites to support the biosorption. Moreover, the binding capacity of dyes on the surface of the microbial species depends on several factors such as structure, ligands present on the surface, surface area, and differences observed in morphology and division of the yeast. In another study [35], it was explained about the removal or detoxification of the reactive dyes (Drimarene dyes) in the presence of *Aspergillus foetidus*.

13.8 Metal-Complexed Dyes

The negatively charged highly solubilized metal-chelated dyes are used for improving the light fastedness and dyeing of the polyamide fibers and proteins in the textile and tanning industries. The important metals used for the formation of metal-complexed dyes are cobalt, copper, and chromium. Different types of colored dyes from bright black to greenish-yellow can be developed depending on the usage of metal ions, functional groups of the dyes, and the complexation of metals and dyes. At the same time, the aggregation of dye molecules and decrease in the solubility of the dyes can be achieved by the addition of salts like NaCl in the dyeing and finishing industries. The salts change the salinity of the water bodies and it affects the aquatic species [5]. The biosorption of textile dyes on the microbial surface mainly depends on various factors such as the chemistry of the dye, microbial type, surface properties of the microbes, and physiochemical characteristics such a pH, temperature, ionic strength, and the presence of organic and inorganic ligands in the solution. The fungal biomass *A. niger, Rhizopus arrhizus*, *Neurospora crassa*, and *Phanerochaete chrysoporium* are the economically cheap biomass which was often used in the remediation of dyes. Yellow RL or cobalt complex formazan dye derivatives are used in tannery and fabric industries for coloring the polyamide, natural silk, leather, and wool materials. The molecular structure of cobalt-complexed dye (Formazans) is shown in Figure 13.5.

The formazan dyes are produced from the reduction of aqueous soluble tetrazolium salts which have the capability of formation of complex with metal ions to produce symmetric and unsymmetrical metal (iron)-complexed dyes. Cobalt, copper, and chromium trivalent MCDs are used in several industries, but these synthetic dyes cause environmental toxicity. The iron-complexed formazan dyes are

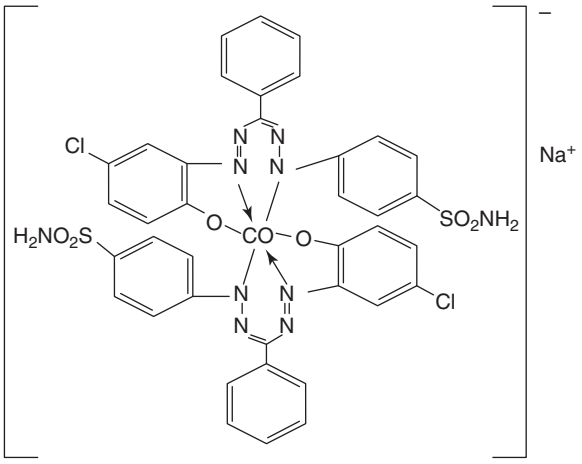

Figure 13.5 Formazan dye. Source: Freeman et al. [36].

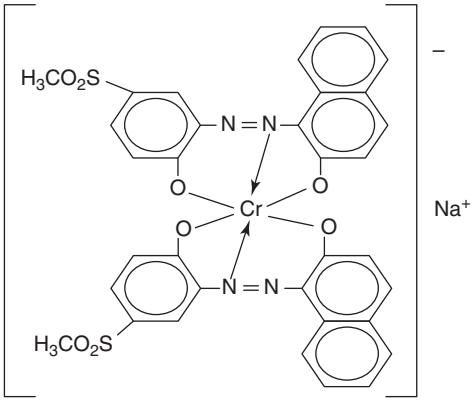

Figure 13.6 Irgalan dye. Source: Based on Freeman et al. [36].

synthesized by the diazotization of *o*-aminophenol derivatives coupled with benzaldehyde derivatives such as benzaldehyde phenylhydrazones and benzaldehyde phenylhydrazone-4-sulfonic acid thrived by iron(II) sulfate [36].

The Irgalan dyes are chromium MCDs formed by complexation of metal and acid dyes. It has six coordination sites in which three coordination atoms are utilized by dye and the remaining atoms complexed by the water molecules at acidic pH or hydroxyl ion at basic pH. To improve the solubility of dyes and fastedness characteristics, it was modified by blending with non-hydrophilic methyl sulfonyl sulfomide. The solubilizing effects were enhanced due to electron sharing (covalent bonding) between water and sulfonyl oxygen groups in Irgalan dyes (Figure 13.6) [36].

13.9 Biosorption of Metal-Complexed Dyes

The fungal biomass is one of the most facilitated microbial sources for the removal of synthetic or metal-complexed dyes. During the biosorption process, the cell

wall acts as a primary source for the removal of dyes. The fungal cell wall consists of a huge amount of chitin and chitosan in addition to proteins, amino acids, and lipids which has the functional groups of carboxylic and amine groups. But the biosorption capacity of the cell wall reduces due to the presence of different ionizable sites such as carboxy and phosphate present in the glucosamine. The ionic strength of the solution affects directly the surface charge of the biomass as well as the solubility of the dyes. The surface charge of the biomass can be evaluated by measuring the zeta potential or isoelectric point to pH. The surface charge of the ligands present on the cell wall becomes negative due to the deprotonation of carboxyl, phosphate, and the amine groups or positive due to imidazole at lower pH. These functional groups are responsible for the attachment of dyes on the microbial surface. At lower pH, the positively charged functional groups will attract the negatively charged MCDs due to the electrostatic interaction between the ligands and dyes [37]. The biosorption of Yellow RL adsorbed on the cell surface at the pH 2 is due to the presence of positively charged molecules. But the textile and tanning industrial dyes contain both salt and dyes. The salt concentration in the effluent directly changes the ionic strength and pH of the solution, and also it resists the equilibrium uptake of dyes in the biosorption process. It [3] was observed that the impact of ionic strength on the biosorption process is enhanced by the ion exchange mechanism at acidic pH conditions. The higher ionic strength or pH of the solution is favorable for the removal of dyes which is very stable or highly solubilized at higher pH conditions. But there is a possibility of denaturation of biosorbents. Muthezhilan et al. [38] isolated strains of *Rhizopus, Mucor, Aspergillus, Cladosporium, Fusarium, Penicillium,* and *Trichoderma* from dye-containing industrial effluents. The other isolated microbial species are *Aspergillus flavus, Fusarium oxysporum, Fusarium moniliforme,* and *Trichoderma* from the soil samples around the textile industries of Nanjanjud, Karnataka (India). They analyzed soil samples from different textile dye industries in Mangalagiri. *Mucor mucedo* was found to be predominant. Additionally, 13 terrestrial strains of fungi for the decolorization and degradation of dyes in the soil were taken. They concluded that effective dye degraders for the remediation of the environment contaminated with recalcitrant dyes can be obtained from soils. The biosorption of chromium-complexed metal dyes using *Pseudomonas* strains was carried out. The study was carried out using the native and heat-treated *Pseudomonas* strain DY1. The results clearly showed that the thermally modified bacteria displayed a significant adsorption capacity of about 2.98 mmol/g of biomass which is 20-fold higher than the adsorption capacity of live cells. Moreover, the adsorption capacity of the live and thermally modified *P. putida* DTZ did not improved at the temperature of 4 and 30 °C. The percentage of biosorption significantly increased between 30 and 100 °C [39]. Akar et al. observed that the maximum amount of Acid Black 172 absorbed by the cones of macro-fungi, *Agaricus bisporus*, and *Thuja orientalis* was around 0.18 mmol/g of biomass. Moreover, the interaction efficacy of sawdust was analyzed by varying different parameters such as pH, particle size, contact time, and initial metal concentration. The amount of metal complex interacted on the sawdust increases with the increase in the surface to volume ratio of the particles. The maximum amount of metal

complex adsorbed by the sawdust was 25.1 and 62.5 mg of dye per gram with respect to metal complexed blue (MCB) and metal complexed blue (MCY). Additionally, there is a possibility of intraparticle diffusion because of the movement of adsorbed molecules through the porous medium present in the sawdust. The adsorption density of sawdust reduces with increased adsorbent dose and due to the presence of unsaturated adsorption active sites on the surface of the sawdust [40]. Erden et al. carried out an experiment for the removal of Siris blue KFCN dye by the lyophilized *Trametes versicolor* biomass. The obtained results revealed that the maximum adsorption capacity of the respective biomass is around 62.62 mg/g. The kinetic and the equilibrium data show that the adsorption process followed the pseudo-second model and Langmuir isotherm model. It is stated that the biosorption followed the biomass concentration and time. Additionally, the Langmuir adsorption defined that the process followed monolayer coverage of adsorption. This biosorption process depicted that the biomass is more effective than the well-known adsorbents such as activated carbon and amberlite. Likewise, the potential of *Aspergillus parasiticus* for the decolorization of textile reactive dye was tested by varying various parameters such as ionic strength, reaction time, biomass concentration, and initial metal concentration in batch studies. The obtained results showed that the highest adsorption capacity achieved by the biomass is around 1.03×10^{-4} mol/g at pH 2.0 with 2.0 g/l of biosorbent concentration. Additionally, the recent studies showed that the material like fly ash shows a noteworthy effect on the removal of several dyes such as methylene blue, rhodamine B, and malachite green from the artificial textile wastewater. The obtained result delineated that the removal of malachite green, rhodamine B, and methylene blue by the selective microbial species is around 0.228–0.814, 0.184–0.618, and 0.219–0.644 mg/1, respectively, when the initial dye concentration increased from 5 to 38 mg/l. The maximum time taken to remove malachite green and rhodamine was around 80 minutes and it was 100 minutes for the methylene blue. Additionally, the Brewer spent grain (BSG) was used as a material for the removal of Acid green (AG 25) by varying different parameters such as initial pH, temperature, initial dye concentration, biosorbent dosage, and contact time. The maximum amount of dye was adsorbed by the biomass at pH 7 and 30 °C, the initial concentration was around 90 mg/l, and the biomass concentration and time were found to be 0.2 g and 75 minutes, respectively. The observed result depicted that the amount of dye removed increased with respect to time and biomass concentration and decreased with respect to the temperature. In the case of isotherm study, the equilibrium sorption capacity increased when the initial dye concentration increased till 90 mg/l. Later, the adsorption capacity was decreased due to the less availability of active sites. Likewise, the ability of adsorption capacity of *Ganoderma lucidum* was tested with dyes present in the wastewater by varying different parameters in response surface methodology. From the results, the optimized conditions are observed at acidic pH of 6.6, temperature of 26.5 °C, agitation speed of 200 rpm, and dye to wastewater ratio of 1 : 2. Under these optimized conditions, the maximum dye decolorized is 81.4% and chemical oxidation demand (COD) reduction is about 90.3%.

13.10 Conclusion

In this chapter, we discussed about the industrial process followed for the biosorption of heavy metal and the metal-complexed dyes using various microbial sources such as bacteria, fungi, and yeast under both batch and continuous processes. The biosorption of heavy metals and metal-complexed dyes depends on functional groups like carboxyl, phosphate, amine, and hydroxyl present on the cell surface. In addition to that, the biosorption process depends on various physiochemical parameters such as pH, metal concentration, biomass concentration, surface to volume ratio, and temperature. The temperature highly influences the degradation of MCDs for the reduction of color present in the dye. The efficacy or the adsorption capacity was enumerated by various models such as Langmuir and Freundlich adsorption model. These two models help to understand about the dynamic equilibrium conditions between the metal ions and microbial consortium.

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14

Recovery of Precious Metals from Electronic and Other Secondary Solid Waste by Bioleaching Approach

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14.1 Introduction

Over the last decade, there is an increased demand for electronic and electrical gadgets due to rapid urbanization and population growth. Technological advancements and development have enabled industries to come up with novel and smart electronic and electrical equipment (EEEs) to meet the growing demand of people. The ever-growing production of EEE products has resulted in the production of a vast quantity of waste electronic and electrical equipment (WEEEs) [1]. WEEE is the amalgam of various metals such as copper, nickel, iron, and aluminum trapped in materials like plastic and ceramic through mechanisms such as mixing or binding. Metal groups (Au, Ag, and Pt) are considered as superior value waste due to their high conductivity and high chemical stability [2]. There is a growing need for recovery of these metals from e-wastes as they have toxic effect to human health and environment apart from their superior value as waste [3]. Equipment such as cell phones, smart phones, light-emitting diode lamps, printers, superior televisions, driers, refrigerators, temperature exchangers, and other devices are the major source of e-waste [4]. Both hazardous and non-hazardous components are present in the e-waste. The annual production of e-waste is estimated at around 20–50 Mt. globally. E-waste is separated from normal municipal waste as it contains toxic or unsafe materials. When left untreated or not treated properly, these materials can pose threat to humans when passed through air, soil, or water. These materials include plastic, lead, mercury, cadmium, arsenic, etc., and they can create various kinds of diseases when present in atmosphere. People working in recycling plants for e-waste can be easily affected with chronic and acute diseases including cancer.

Bioleaching is one of the advanced methods which involves metal leaching from waste materials, and it is of low cost and considered to be a green technology approach [5]. There are several advantages to bioleaching approach in comparison to conventional hydrometallurgy or pyrometallurgy methods which is considered as threat to nature due to usage of chemicals. This chapter explains the mechanism

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of bioleaching, research on the dissolution kinetics, metal leaching yields, and processes to improve the efficiency of bioleaching process.

14.2 What Is Bioleaching?

There is a growing need to dispose e-waste generated from electronic products when they function or life ceases. Recovery of metals from printed circuit board (PCB) is presently done through processes, such as bio metallurgical, mechanical, pyrometallurgical, and hydrometallurgical technologies [6]. There is growing attention toward biohydrometallurgy for the extraction of metals. In this process, microorganisms and their metabolites are found to play a vital role for solubilization of metals into aqueous phase and subsequently their recovery. Microbial agents involved in bioleaching are grouped into two types based on the requirement of metabolism as chemolithotrophs and chemoorganotrophs. The mode of action of these microbes in solutions is as follows: initially, microorganisms bring about oxidation and reduction reactions in the solution. This is followed by formation of acid from either organic or inorganic route and ends with the leaching of metal from the sulfide matrix [7].

The process is carried out under acidic conditions (pH between 1.5 and 3). This pH condition aids the growth of the microbes, thus improving the solubilization and separation of metals. These technologies are easy to regulate and maintain as they work under mild conditions at ambient temperature and pressure. Microbial processes for the metal extraction from low grade ores were first discovered at sulfide mines for field-scale processing and were referred to as bioleaching or biomining. The first scientific proof of metal solubilization by microorganisms was identified by Colmer and Hinkle in year 1947. In 1950s, copper was extracted from mine dump using microorganisms for the first time at Kennecott Copper Corporation [8]. Currently, at the industrial scale, bioleaching is applied to process substantial portion of many minerals/ores. Advancement in the field of molecular biology has helped scientists to recognize the various genera of microbes playing a vibrant role in bioleaching.

14.2.1 Mechanism of Bioleaching

The three principles involved in the metabolization of metal on minerals are: acidolysis, complexolysis, and redoxolysis. In Acidolysis, the metal solubilization using microorganisms is carried out through the process of formation of organic or inorganic acids like the production of gluconic acid by *Penicillium simplicissimum*, citric acid by *Aspergillus niger,* and sulfuric acid by *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. In these processes, protons and oxygen that combine with water and metal get detached from the surface. This process is particularly important in fungal bioleaching and is usually fast [9]. Redoxolysis is divided

into the direct and the indirect mechanism and refers to oxidation and reduction reaction. In the direct mechanism, bacteria leach the metal through redox reaction, followed by solubilization of metals by enzymatic reactions. In Complexolysis, an organic acid leaches metals through complex formation; this process occurs slowly compared to acidolysis, and solubilization of metal ions is based on the complexing capacity of a compound with which a complex is formed. Apart from organic acids, metabolites such as siderophores which is a low molecular weight chelating agent can form complex, solubilized metals such as chromium and magnesium [10].

14.2.2 Industrial Processes of Bioleaching

The industrial method consists of passing acidified water through a heap of the waste material. To improve the efficiency of extraction, the leachate is then recirculated again. The extraction procedure is done by four different methods: (i) **Dump leaching** – a process where the waste, as a dump, is treated with the leaching solution for extraction of the metal, (ii) **Heap leaching** – followed for fine grained ores. The ores are heaped in large basins, and the solvent passed for extraction; (iii) **Underground leaching** – followed in the case of abandoned mines or ore deposits in mines, where the concentration of the metal of interest is too low for conventional processes. This method is also called *in situ* leaching; (iv) **Tank leaching** – a process, where in, unlike the earlier three processes, the ores are submerged with the extractant in tanks. Though expensive, this method is quite efficient as compared to the earlier procedures mentioned [10].

14.2.3 Factors Affecting Bioleaching

Several factors such as pH, temperature, oxygen, carbon dioxide, nutrient availability, and microbial activity affect the biomining process. By altering the pH value of bioleaching environment, the bacterial growth can be adjusted to positively influence the leaching behavior and efficient solubilization. For example, the bacterial oxidation of ferrous iron and sulfide requires pH value between 2.0 and 2.5. If the value is below 2.0, *Thiobacillus ferrooxidans* gets inhibited but addition of acid will help *T. ferrooxidans* adapt to low pH [9]. The optimum temperature required for ferrous iron and sulfide oxidation by *T. ferrooxidans* is between 28 and 30 °C. A decrease in the metal extraction will occur at low temperature, but for few metals such as cobalt and nickel, bacterial solubilization occurs at low temperature. Mechanism of leaching by thermophilic bacteria occurs at peak temperature [10].

To achieve optimal growth of iron complexes, sulfur is combined with magnesium and other salts [10]. Dissolution by chemolithoautotrophs requires inorganic compounds for growth. 9K culture media consisting of magnesium and a combination of salts are the widely used culture media in industries and laboratory. A few studies show that bacterial activity and growth can be inhibited by certain inhibitors like cellobiose, etc. To ensure high activity of leaching by the bacteria, there must be an

adequate supply of oxygen needed for their growth. At laboratory scale, oxygen is used for aeration. On larger scales, supply of oxygen is tough for stack leaching [11]. Efficiency, metal extraction, and count of organisms are also affected by carbon dioxide, and it is used as energy source. It also affects leaching process and ferrous ion oxidation.

14.2.4 Advantages of Bioleaching Over Other Methods

Bioleaching is considered to be environment friendly compared to other conventional processes. Due to its flexibility, waste toxicity is reduced and also valuable resources can be recovered. Pyrometallurgy and hydrometallurgy were applied for the extraction of metals from minerals or secondary sources other than microbial leaching, and both processes are found to be efficient but still have limitations [12]. One drawback with hydrometallurgy method is that it involves usage of concentrated acids or bases, and thus, the acid waste generated has to be managed at higher downstream processing cost [11]. Similarly, due to usage of high energy in pyrometallurgy process, operating at a 1500–1700 °C is considered as inefficient and also is related to the emission of harmful gases like sulfur dioxide [13]. Both the approaches fail because of economics, account of energy, and environment, whereas in bioleaching, harmful gases are not emitted and are energy efficient.

14.2.5 Limitation of Bioleaching Over Other Methods

While bioleaching approaches have a lot of advantages, a number of drawbacks are also encountered that can be solved by applying appropriate strategies. Limitation of this process includes – (i) slow reaction rate compared to other methodology, (ii) microbes involved in process may be contaminated with toxic metals from the waste, and (iii) commercially high cost and low yield due to waste refractory properties [14]. A low metal yield is obtained as the iron hydroxides and jarosite are formed on ore surface which causes the oxidants to diffuse slowly to ore surface [15]. Therefore, if bioleaching technique is not strategically planned the process construction, material grinding cost and operating cost may make it uneconomical.

14.3 E-Waste, What Are They?

Electronic waste is also known as e-waste and refers to discarded electronic devices such as mobile phones, computers, printers, televisions, cell phones, printers, CD players, personal digital assistants (PDAs), fax machines, and many other electronic devices commonly used in homes, offices, institutions, etc. Technically, e-waste refers to discarded electronic products having primary functions with various components and circuits which are discarded when they reach end of their life due to loss of functions. E-waste is completely different from the other domestic or industrial waste as they contain precious metals and other compounds such as lead, cadmium, arsenic, mercury, polyvinyl chloride (PVC), etc. which when discarded

unprocessed, they can cause undesirable impact on human health and environment. Therefore, it is prudent to handle properly and recycle e-waste in a special way. Disposing them in incinerators or landfill creates trouble to environment, socio-economic impact due to the growing e-waste production [16].

14.3.1 E-Waste Production Scale

In India, household wastes are often found mixed with electronic wastes due to lack of knowledge on waste management and are subsequently disposed in landfills or incinerated. In 2015, 4.1 Mt. of e-waste was generated and is expected to reach 8 Mt. by 2025. Poor awareness about waste management, deprived collection mechanisms, improper processing methods, and illegal dumping among the users can cause serious threats to both human health and the environment [16]. In 2005, United States generated 80% of e-waste and exported it to India, China, and Pakistan. Only 3% of the total was recycled properly in India, while the remaining wastes were handled by workers using bare hands causing health impact. Although laws are made for appropriate recycling, due to low literacy levels and very little awareness regarding the hazards of e-waste among workers working in recycling areas, recycling is still done in an inappropriate way. In India, about 70% of e-waste is generated from top 10 states and from top 10 cities. Workers face serious health-related issues from the major e-waste dismantling areas and workshops present in India [17]. Delhi and Mumbai are the major cities involved in the e-waste dismantling unit. In 2014, India emerges as the largest producer of e-waste and ranks fifth in the world. In India, 1.7 Mt. electrical devices and electronics are discarded in 2014 and reached 2.0 Mt. by 2016.

14.3.2 Pollution Caused by E-Waste

Electronic devices contain toxic substances which cause threat to environmental and human health when disposed and also when they are transported by road and waterways, they become problematic. E-waste from chip resistors and semiconductors row head contains cadmium which can cause damage to lungs, kidney and nervous systems, lithium passes through breast milk to babies from lithium-ion batteries. Use of bare hands for the separation and arranging of e-wastes contributes to failure of respiratory system, also causes skin disorders and if burning is used for separation it produces volatilized contaminants and can cause chronic respiratory disorders [18]. It was considered that the main pathway for the entry of e-waste into humans is air through inhalation, ingestion, or skin absorption. Large amount of WEEE from several countries is discarded in landfill or incineration each year which causes environmental pollution. Computers and cathode ray tubes are the largest sources for the e-waste stream, and solid waste management for e-waste is a huge task, particularly computer wastes. The leachate generated from e-wastes during leaching process from dumping sites contaminates water bodies thereby affecting organisms living in water. Huge amount of Cu during disposal is released into the environment, and on combustion, e-wastes produces chlorofluorocarbons (CFCs) that can cause ozone depletion.

14.3.3 General Methods of E-Waste Treatment

E-waste treatment is important for safe disposal and also for recovery of metals from wastes generated. Traditional process for waste treatment includes pyrometallurgical and hydrometallurgical methods for recycling waste materials for useful applications. The three major steps in recycling of waste include: disassembly (separating out hazardous or valuable components followed by special treatment), upgrading (materials are prepared for refining process by mechanical processing), and refining (materials that are recovered in the previous step is purified using chemical processing) [18].

14.4 Role of Microbes in Bioleaching of E-Waste

Microbial agents involved in bioleaching are grouped into two types based on requirement of metabolism as chemolithotrophs and chemoorganotrophs. Chemolithotrophs also known as acidophiles survive at very low pH and are further categorized into mesophilic, moderately thermophilic, and thermophilic based on optimal growth temperatures. Chemolithotrophs utilize inorganic matter as energy source and carbon source from carbon dioxide. Carbon is used as energy source by organotrophs and chemolithotrophic autotrophs. The mode of action of these microbes in solutions is as follows: initially, microorganisms bring about oxidation and reduction reactions in the solution. This is followed by formation of acid from either organic or inorganic route and ends with the leaching of metal from the sulfide matrix [9].

14.4.1 Bacteria

Solubilization of metals is achieved by sulfur-oxidizing and iron-oxidizing organisms. Iron- or sulfur-oxidizing bacteria such as the mesophilic aerobic and chemolithotrophic microorganisms are widely used to transform metallic fractions in e-waste to water-soluble phases. The bacteria from the genus *Acidithiobacillus* have greater tolerance to heavy metals and dominate the research works done in bioleaching of e-waste. The mesophilic autotrophs such as *A. ferrooxidans, A. thiooxidans*, and *Leptospirillum ferrooxidans* have been used extensively in leaching e-waste [9]. Many other species from different genera are known to play a vital role in metal mobilizations. The widely distributed four phyla in leaching of metals include *Proteobacteria*, *Nitrospirae*, *Firmicutes*, and *Actinobacteria*. Based on optimum temperature of microbes, they are of three types: mesophiles, moderate thermophiles, and thermophiles. Organisms belonging to the domain *Euryarchaeota* and *Archaea* such as *Acidianus*, *Metallosphaera*, *Sulfurisphaera*, *Ferroplasma acidiphilum*, *Ferroplasma acidarmanus* play a vital role. Among these, a few can be grouped as oxidizer of iron and the others as sulfur oxidizers. Still another group exists which oxidizes both iron and sulfur based on their preference of substrate.

In the extraction of metal from PCBs, both *Leptospirillum* sp. and *A. ferrooxidans* play a crucial role. *Acidimicrobium ferrooxidans* and *Acidithiobacillus caldus* are active between temperatures of 25 and 55 °C. The bioleaching process undertaken by *A. ferrooxidans* occurs by direct and indirect mechanism. Lot of work has been carried out under both types, but indirect leaching is considered to be more appropriate at industrial level due to the flexibility of the process [19]. All reactions taking place under the bioleaching process occur at mild acidic conditions at ambient temperature and pressure and are found to be useful in developing eco-friendly technologies for metal extraction from waste PCBs.

14.4.2 Fungi

Although both bacteria and fungi produce organic acids, majority of the reports identify fungi as the major contributor to bioleaching process. Carbon source and energy are needed by fungi, and they produce organic acids like gluconic, citric, oxalic, etc. while growing on organic supplements [20]. Metal leaching by fungi is possible at a very low acidic pH compared to iron and sulfur bacteria, thereby reducing threat to environment. During metal mobilization, addition of organic compounds by iron-oxidizing bacteria favors better metal solubilization due to increase in concentration of ferric iron in solution form. Several fungi grow in existence of toxins at very low pH and temperature. Organic acids (oxalic, gluconic acid, citric, and malic) aid as lixiviant for base metal solubilization. They are produced by fungi such as *Aspergillus* sp. and *Penicillium* sp. Acidolysis is a process of fungal attack on mineral surfaces by producing proton which breaks the bonds followed by removal of metal ions. Other processes such as complexolysis, redoxolysis, and bioaccumulation are involved in the solubilization of metals through organic acids produced by fungi [21].

Fungi can thrive under different environmental conditions, and the filamentous soil fungi are considered to be of great interest in bioremediation. Treatment using biological methods allows the cyclization of the sediment after treatment and is considered to be economical. *Purpureocillium lilacinus* was used as a bioleaching agent which resulted in oxalates formation in the culture filtrates as confirmed through Fourier Transform Infra Red spectroscopy (FTIR) technique after treatment. To accomplish bioaccumulation involves the transportation of the metal ions that are soluble through accumulated dense elements by crossing the membrane of the cell. This might be performed by functional groups in fungal mycelium by binding to the metal ions. *Penicillium* sp. and *Aspergillus* sp. fungal strains accumulate radionuclides and metals from outside atmosphere [22].

14.4.3 Actinobacteria and Cyanogenic Organisms

Actinobacteria strains from mining areas were isolated and found to have potential to bioaccumulation. High concentration of zinc and lead, and low or moderate concentration of copper, cadmium, and chromium were identified by analyzing the residues from mining areas. It was determined that about 59 actinobacteria isolated were resistance to 50 heavy metals. Among these, 59 actinobacteria isolated,

27 belonged to *Amycolatopsis* and *Streptomyces* genera. When these heavy metals are subjected to chemical precipitation using hydrogen sulfide, they showed strong accumulation of lead [23]. To measure the availability of silicone dioxide, Al, Ca, Mg, Pb, Cu, and Zn, hibiscus was grown on waste foundry sand (WFS). *Actinomyces* sp. isolated from WFS was used to bioleach the treated sand. Hibiscus plant was grown on both treated and untreated sand for determining the presence of metals by plant and was calculated by atomic emission spectroscopy technique. Results showed that there was a reduction of metal level in WFS. This observation proves that bioleaching by *Actinomyces* sp. in WFS was adequate in the *Hibiscus rosasinensis* growth [24].

Extraction of gold and silver from gold-containing minerals using alkaline condition is known as cyanidation process. Water-soluble complex is formed by cyanide with gold which is responsible for gold extraction. Eco-friendly way for the gold extraction processes can be achieved by using biogenic cyanide referred to as alkaline bioleaching. Precious metals such as gold can be obtained from Electronic wastes such as PCBs. Gold from the metallic particles of crushed waste PCBs can be dissolved by *Chromobacterium violaceum*, a mesophilic gram-negative bacterium, and by a facultative anaerobe *C. violaceum*. It was found that combining *C. violaceum* with chemical methods or with other mechanism such as iodide, *Pseudomonas aeruginosa,* and *Pseudomonas fluorescens* can improve gold leaching efficiency by reinforcement of the cyanide generation. The efficiency was found to be 70% for gold leaching using *C. violaceum* [25]. Bio-recovery from electronic waste material was done using *Bacillus megaterium* where gold was obtained as gold cyanide complex. These bacteria were subjected to pre-treatment, mutation and allowed to grow at different pHs. The results show that mutated alkaline bacteria (*B. megaterium*) were found to be more effective than that grown in normal physiologic pH in gold biorecovery [26].

14.5 Application of Bioleaching for Recovery of Individual Metals

14.5.1 Gold

In gold mining process, metal residues are often thrown back which causes ground water pollution. This is due to the fact that the leachate contains harmful metals like cadmium and lead. Electronic scrap contains more gold content compared to that of the natural gold ores. Therefore, electronic scraps are considered to be a cheap and alternative gold source. Studies show that significant gold recovery can be obtained from bioleaching using cyanogenic bacteria. Pure cultures like *P. fluorescens* and *C. violaceum* and cyanogenic bacteria play a vital role in degrading cyanide. In cyanogenic bioleaching, adapted strains are observed to be more effective than unadapted ones. For instance, at pH 9.0, 9.5, and 10, the adapted cyanogen *C. violaceum* bioleached 18%, 22.5%, and 19% Au, respectively, compared unadapted strain which could extract only 11% Au at pH 7.0 [27, 28].

14.5.2 Silver

The supernatant from culture of *A. ferrooxidans* was used for the extraction of silver from spent battery. Technique for solubilization of metal for the development of two-stage reactor system was identified as 98% silver dissolved during bioleaching process in an indirect leaching mechanism for recovery of silver from used silver oxide-zinc button cell battery, and this can be applied for industrial application [29]. *Pseudomonas* sp. also secrete cyanide which helps in the bioleaching of elements of Ag, from ores, slag, and e-wastes [30].

14.5.3 Copper

Moderate thermophiles were used for the copper bioleaching from e-wastes using columns at laboratory scale, stirred tanks, and shake flask. Use of mesophilic strains resulted in 90% of extraction from PCBs. The findings proved that 94% and 99% of copper can be obtained using cultures of mesophilic and moderate thermophiles within six days using shake flask at temperatures of 30 and 50 °C [30, 31]. A mixed consortium of *Sulfobacillus thermosulfidooxidans* and *Leptospirillum ferriphilum* was used in spent medium process which resulted in 93.4% of Cu [32].

14.5.4 Nickel

Studies were carried out for using *A. ferrooxidans* using bioleaching method which resulted in about 96% extraction of copper from LED waste powder, and this strain was found to be effective for LED waste bioleaching. 92–97% Ni was bioleached using *A. thiooxidans* from spent petroleum catalyst [33].

14.6 Large-Scale Bioleaching of E-Waste

In year 2003, bioleaching for large-scale production for metal recovery was first established [34]. They used two successive reactors for the recovery of metal from Ni–Cd batteries where indigenous *Acidithiobacilli* was inoculated in bioreactor using series of steps from generation of sulfuric acid to thickening of slush with the help of settling tanks. Later, the effluent was passed to leaching reactor that contained the powder obtained from Ni–Cd batteries. Recovery of maximum percentage of Cd and Ni during entire process was obtained which took 50 days. Studies were carried out to determine the effect of retention time in both bioreactor and leaching reactor on recovery. Cobalt and nickel were retrieved by applying the same methodology.

14.7 Future Aspects

Biomining process is a supportable and effective approach used for extracting metals from electronic wastes. This paper is a review of previously published results

obtained from bioleaching of e-wastes and presented based on the type of e-waste, microorganism, and bioleaching method. Bioleaching process that uses acidophiles causes metal solubilization via thiosulfate or polysulfide pathway by generating sulfuric and ferric acids. Chemolithoautotrophs and heterotrophs are the two groups of microorganisms used for bioleaching of e-waste. Various groups of these microorganisms use different mechanisms to solubilize metals such as the heterotrophic fungi uses mechanisms such as acidolysis and complexolysis, redoxolysis and bioaccumulation. At present, bioleaching process is studied substantially on wastes from various wastes generated, especially from the e-wastes for extracting precious metals. However, large-scale application is very limited due to the slow rate of this bioleaching process. Therefore, several researchers have been using various methods such as addition of catalysts, prior adaptation of microorganisms, ultrasonic treatment, optimizing process parameters, to improve the efficiency of process, and the results were highly effective. The limitations and challenges faced in this approach including toxic to microbes, precipitation and variation must be described properly. Industrial development in the contemporary world causes natural resources depletion on one side, and on the other side, the amount of waste generated increases at high pace. Hence, bioleaching is the most effective and a sustainable approach to meet the upcoming needs of our future generation. Future studies must emphasis more on process used for retrieval of precious metals using bioleaching approach and also the omics approaches. This requires a deepened knowledge about the role of microorganism in this field and also toward their bioleaching ability by alteration of their genetic makeup.

List of Abbreviations

EEE	electronic and electrical equipment
WEEE	waste electronic and electrical equipment
PCB	printed circuit boards

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Part V

Bioreactors for Zero Waste

15

Photobiological Reactors for the Degradation of Harmful Compounds in Wastewaters

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15.1 Introduction

Advances in knowledge and developments in the industrialized parts of the world have come at the expense of resource base and the environment. Fortunately, environmental consciousness has also grown intensely, especially in the past few years. Several states around the world are captivating the principal in the request of new laws that control, and in many cases even ban, the use of hazardous chemicals. Contaminants are not usually known as pollutants except for those damaging the environment. Pollutant can be either natural or anthropogenic. Water biota is the largest environmental media bearing pollutants, and present-day technologies for the treatment of organic, inorganic, and microbial pollutants in water deal about the sensing or pollutant degradation with many photobiological reactors.

Based on the size or the volume of wastewater from an industry, the technique to be adopted will change. These techniques can be chemical, physical, or biological, contingent upon the method of application and the principle of operation and also the nature of the effluent. The pollution is usually quantified in terms of chemical oxygen demand (COD), biological oxygen demand (BOD), and dissolved oxygen (DO).

The categorization of techniques for the removal of the contaminants based on the need and the available technology is as follows:

Biological treatment: Aerobic digestion (oxidation) of the effluent and anaerobic waste minimization.

Chemical treatment: Direct chemical oxidation, photo-oxidation of the effluent, photocatalytic oxidation and destruction of organic compounds by sonication.

Physical (thermal) treatment: Wet air oxidation (WAD), supercritical fluid oxidation of toxic contaminant and incineration (complete combustion).

Usually, on an industrial level a combination of two or three techniques is often required to achieve better treatability mainly due to the presence of multiple contaminants. The rate at which new chemical entities are exposed and unnaturally made

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is far advanced than the rate of bio-adaptability. As a result, conservative treatment arrangements are facing problems in recent times.

Nowadays, using microalgae and other microbes in large wastewater systems has revolutionized wastewater treatment [1, 2]. Apart from the microbial-based photobiological reactors, there are other reactors that use photolytic and photochemical methods such as photo-enhanced degradation, photo fermentation, photo-activated (chemical) degradation and photocatalytic methods, membrane-based separation techniques, and nanotechnological approaches. Currently, anaerobic treatment is used for sludge management after aerobic treatment in many cases and management of sludge is shown in Figure 15.1.

Aerobic treatment stages include:

1. **Pretreatment**: Large-size solids arriving at the water treatment plant are first removed. If not disposed of effectively, these materials can lead to serious equipment failure.
2. **Primary treatment**: It involves sedimentation of solid waste within water and is done after filtering out larger contaminants within the water. Wastewater is passed through several tanks for removal of contaminants.
3. **Secondary treatment**: It is the portion of sewage treatment sequence removing dissolved and colloidal compounds measured as BOD. It is traditionally applied to the liquid portion of sewage after primary treatment.
4. **Tertiary treatment**: Depending on the quantity of the effluent obtained, its final destination, and relevant legislation, tertiary treatment may be applied to remove residual organic load and other pollutants not removed after the secondary treatment, such as nutrients, phosphorus, and nitrogen. Any combination of treatment processes can be used, whether physical, chemical, or biological.

Anaerobic treatment: Anaerobic microbes transform organic matter in the wastewater into biogas that contains large amounts of methane gas and CO_2. It is an energy-efficient process used for treating wastewater.

Different methods for the treatment of wastewater using photobiological reactors are discussed in this chapter. Studies on the use of microbes as photobiological agents for the degradation of various harmful compounds such as phenol, alkane, hexadecane, oil, etc., in wastewater and sewage have been reviewed. The chapter also discusses the different treatment methods such as photobiological, photochemical, membrane, and nanotechnology methods.

15.2 Photobiological Agents and Methods Used in PhotoBiological Reactors

15.2.1 Microbes Acting as Photobiological Agents in Various Photobiological Reactors for the Remediation of Wastewater

15.2.1.1 Olive Mill Wastewater Treatment by Immobilized Cells of *Aspergillus niger*

Apart from using as sources of phytotoxic and antimicrobial agents, the oil mill waste (OMW) can be used as raw material and fertilizer since it contains up to 11 kg of

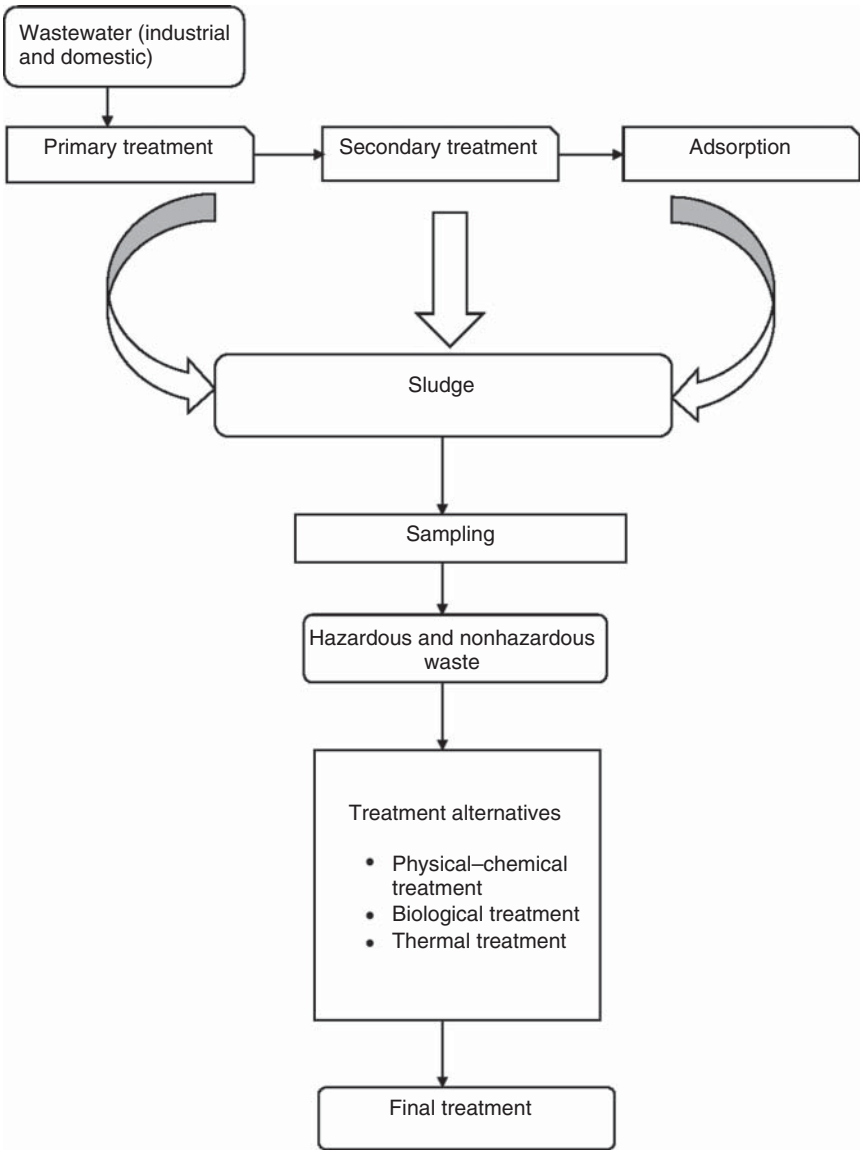

Figure 15.1 Sludge management in wastewater treatment.

K_2O, 2 kg of P_2O_5, and 0.5 kg of MgO/ton. Phenolic and toxic nature of the waste stops its extensive disposal on agricultural land. Rock phosphate (RP) added to fermentation medium helps in OMW-based fertilizer making. A study conducted using an acid-producing filamentous fungus, *Aspergillus niger* NB2, displayed advanced progress of the immobilized mycelium and substantial reduction of the total phenols when the waste material was supplemented with RP and ammonium sulfate (N). The immobilized fungus solubilized the RP with all-out level of soluble P of 0–58 g/l grasped during the fourth batch cycle of the OMW + RP treatment. The OMW + N

treatment could also be pragmatic as initial step to ease the anaerobic digestion of the waste material [3].

The OMWs are called as alpechin, and they are antimicrobial and phytotoxic because of their high organic content, mainly simple phenolic compounds. Moreover, number of compounds found in alpechin is inhibitory for microorganisms. Antimicrobial activity of alpechin can be used in many feasible applications. More appropriately tetracycline could be among alpechin components. The polyphenols from alpechin are virtuous antifungal compounds and regulate a number of fruit and vegetable diseases. Oligomers from alpechin can serve as biological response modifiers, and inquiries on polysaccharides obtained by alpechin biotransformation would be exciting to initiate. The organic acids should also be deliberated as biological response modifiers, and enzyme inhibitory assays are treasured as airing tools for the discovery of the bioactive actions of alpechin extracted compounds. All these activities can be harnessed from OMWs by filtering wastewater by using acid-producing filamentous fungus, *A. niger* NB2 and its actions are enhanced by soluble phosphate.

15.2.1.2 Isolation of Alkane-Degrading Bacteria from Petroleum Tank Wastewater

Petroleum hydrocarbons are the major environmental pollutants, and oil spills pose an excessive threat to terrestrial and marine ecosystems, and they had become main environmental challenges. When sampling was done in the petroleum reservoir, alkane-degrading bacteria were secluded with hexadecane as sole source of carbon and energy. The isolated strains were recognized by amplifying 16S rDNA and sequencing and specific primers were used for the identification of alkane hydroxylase gene. The strains that contain alkane hydroxylase gene and vital for biodegradation are *Rhodococcus jostii*, *Stenotrophomonas maltophilia*, *Achromobacter piechaudii*, *Tsukamurella tyrosinosolvens*, *Pseudomonas fluorescens*, *Rhodococcus erythropolis*, *Pseudomonas aeruginosa*. This study infers that there is a high diversity of degradative bacteria secluded from petroleum reservoir wastewater.

Growth Rate and Hexadecane Elimination by Bacterial Strains All bacterial strains were grown in hexadecane (1%) for one week with shaking (160 rpm) and hexadecane biodegradation was analyzed by gas chromatography with flame ionization detector (GC–FID). The strains, *A. piechaudii* O1 and *R. erythropolis* G2 have shown high percentage of hexadecane biodegradation (93% and 84% respectively) and growth rate. Conversely, *P. aeruginosa* strain B showed the lowest percentage of hexadecane exclusion (38%) and low growth rate among other isolated strains [4].

15.2.1.3 Development of Microbubble Aerator for Wastewater Treatment by Means of Aerobic Activated Sludge

The aerobic biochemical reactor is the most significant component in large-scale wastewater treatment plants where the oxygen supply to the microorganisms determines the overall wastewater treatment rate. Various types of microbubble distributors have been established to augment the oxygen dissolution in water, and

the oxygen absorption performance of microbubble generators was equated with typical bubble generators. To assess each bubble generator, the liquid-phase volumetric oxygen transfer coefficient, gas hold-up, and power consumption per unit liquid volume were restrained in a bubble column attached to each bubble generator. All the microbubble generators permitted the oxygen to dissolve faster than the typical aerators. To improve an industrial wastewater treatment system, a novel aeration system utilizing a spiral liquid flow-type microbubble generator was proposed, which had a highest oxygen transfer coefficient flat at a low air flow rate, but it uses high energy. Rewards such as compact size, portability, and fast oxygen dissolution rate are helpful to confirm the performance for organic wastewater treatment.

Among all gas distributors, the microbubble generators exhibited better oxygen absorption performance in contrast to the typical gas distributors. Specifically, the spiral liquid flow-type microbubble generator had a much higher oxygen transfer coefficient at a low superficial gas velocity. Although there is high power consumption, the advantage of fast gas absorption is assessed. A novel wastewater treatment system composed of a spiral liquid flow-type microbubble aerator, a draft tube, and a filtration chamber displayed a much faster oxygen dissolution rate, and if it consumes more energy than the typical ones, it is accessible either for oxygen supply into an inactive region in an aerobic sludge tank or for use in a more compact tank [5].

15.2.1.4 Wastewater Produced from an Oilfield and Incessant Treatment with an Oil-Degrading Bacterium

The species of *Bacillus* (M-12) decreased COD of the wastewater expressively, and its competence is enhanced when a nitrogen source such as $(NH_4)_2SO_4$ was added into the wastewater. Oil-in-water emulsions can be treated; however, dissolved hydrocarbons are highly toxic and hard to treat. Biological treatment is an actual and economical way. Biodegradation of crude oil by the indigenous microorganisms (bacteria, yeast, and fungi, that use crude oil as carbon source) is one of the main mechanisms by which petroleum and other hydrocarbons are eradicated from the wastewater. In contrast to pharmaceutical and food bio-transformations, they can be effectively used in the degradation of toxic compounds during wastewater treatment. Since the immobilized cells could be reused, it is cost-effective and has great potential in oil wastewater treatment [6].

15.2.1.5 Pepper Mild Mottle Virus (a Plant Pathogen) as an Apt to Enteric Virus

The pepper mild mottle virus (PMMoV) can be used to assess microbial water quality, and it acts as a useful indicator to assess wastewater treatment technologies and to quantify viral removal in full-scale systems. The PMMoV often co-occurs with pathogens of interest, and its natural high concentrations in wastewater were vital for defining the degree and mechanisms of viral reduction during full-scale treatment. Although PMMoV can be measured as an index virus for enteric viruses in areas with untreated wastewater sources, it may not relate with infectious enteric viruses in areas with better sanitation. The PMMoV detection and quantification serve as an index for enteric viruses in environmental waters, which are exposed to mixed-treated domestic wastewater. Before PMMoV is labeled as a universal

domestic wastewater tracer in environmental waters, studies are needed to confirm the absence of nonfecal sources such as food processing plant effluent and agricultural fields. Further study is required to regulate if PMMoV correlates with enteric viruses, and the concentrations of PMMoV that agree to high human health risks in environmental waters are polluted by tertiary-treated wastewater. The PMMoV is a hopeful index virus to assess the microbial quality of shellfish as well as agricultural products watered with susceptible water sources [7].

15.2.1.6 Cyanobacteria as a Bio-resource in Making of Bio-fertilizer and Biofuel from Wastewaters

Microalgae and cyanobacteria are eco-friendly and their larger cell size and effective biomass make them best and supportable solution for the problems connected to soil fertility and accessible water resources. The cyanobacteria serve as supplements to fertilizer, and micro-algal biomass yields biofuels. They act as potential candidates for lipid production due to their fast biomass production efficiency. Integration of cyanobacteria and microalgae into sewage and effluent treatment plants could be very useful for wastewater treatment. Farmers can produce bio-fertilizers and biofuels on their own by using microalgae. The test ponds and soils should be seeded with these bioagents, which are certain to be present in native habitats. These are used for sustainable agriculture and also are feedstocks for eco-friendly biofuel cohort. Microalgal and cyanobacterial arbitrated remediation could be a safe approach in the elimination of heavy metals from municipal wastewater and a squalor of toxins from the industrial wastewater. The open ponds can be cost-effective and may be used by the local farmers for the large-scale cultivation and harvesting of locally adapted microalgae. The microalgae let the recovery of phosphors and nitrogen from wastewater and sequestration of CO_2 produced during the wastewater treatment. Potential species of microalgae and cyanobacteria, which could efficiently grow in wastewater, may be exploited to fulfill the requirement of valuable bioactive compounds, bio-fertilizers, and biofuel cohort for sustainable agriculture, environment, and planet [8]. Use of cyanobacteria during the treatment of wastewater is shown in Figure 15.2.

15.2.1.7 Bio-sorption of Copper and Lead Ions by Surplus Beer Yeast

Beer yeast, which is a by-product of brewing industry, acts as an adsorbent for copper and lead ions from wastewater. This was observed in batch mode, and the adsorptive quantity was resolute to be a function of the solution pH, contact time, beer yeast concentration, salt concentration, and initial concentration of copper and lead ions. Recent studies showing the limits of Langmuir isotherm state that the maximum biosorption capacities of copper and lead ions onto beer yeast were 0.0228 and 0.0277 mmol/g at 293 K, correspondingly. The negative values of the standard free-energy change designate impulsive nature of the process. Competitive bio-sorption of two metal ions was examined in terms of sorption quantity. The amount of one metal ion adsorbed onto unit weight of biosorbents was inversely proportional to the competing metal ion concentration. The binding capacity for lead is greater than for copper, and ion exchange is perhaps one of the

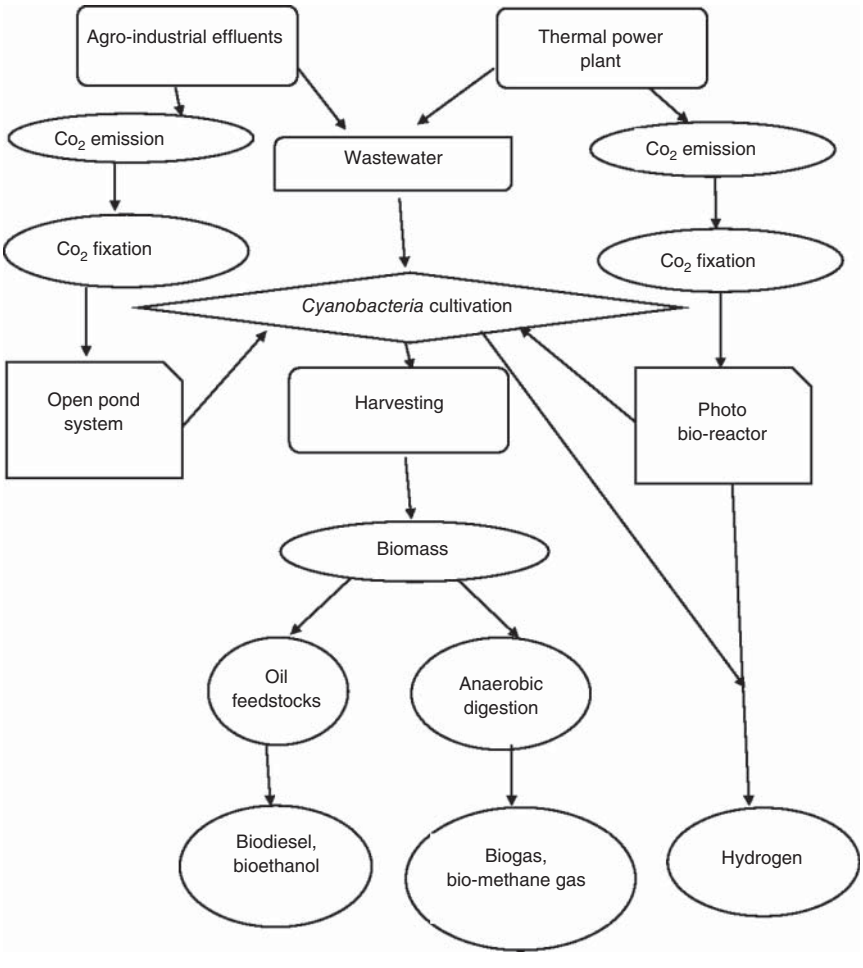

Figure 15.2 Cyanobacteria in treatment of wastewater.

main mechanisms during adsorptive process. Current studies state that beer yeast adsorbs Cu and Pb from solution. The competitive results show that the adsorptive quantity for one metal is significantly decreased due to the presence of other metal ions, but the total capacity for binding heavy metals changes a little. It was referred that ion exchange is one of the main mechanisms during adsorptive process [9].

15.2.1.8 Organization of Lipid-Based Biofuel Production with Waste Treatment Using Oleaginous Bacteria

The oleaginous bacterium was used to produce lipid-based biofuels from organic waste. The recent study was designed to isolate oleaginous bacteria capable of growing on food-processing waste to produce biofuels on a sustainable basis, and about 26 oleaginous bacteria were isolated from natural crude oils using Luria–Bertani (LB) medium under nitrogen-deficient conditions. The GC-MS analysis confirmed the capacity of 10 isolates to produce free fatty acids (FFAs), where oleic acid appeared

as the most prominent compound among the identified fatty acids. The results of aerobic wet digestion (batch mode) had shown the capacity of the strain KM15 for simultaneous lipid accumulation and waste treatment. Among different types of wastes, removal of volatile solids (VS) up to 38.5% and oxidizable organic matter removal (COD-based) up to 48.9% and accumulation of lipids up to 41.5% in 96 hours had been achieved by strain KM15.The degradation efficiency of organic matter was 30.9% and 31% for apple and orange waste after 96 hours with a lipid accumulation of 21% and 25% respectively. Mostly, *Bacillus cereus* strain KM15 was the most effective strain in the degradation of mango waste and correspondingly, the production of biolipids from waste. Recent study illustrates the concept of biorefinery for sustainable waste management and simultaneous production of lipid-based biofuels, and another study illustrates the potential of the *B. cereus* strain KM15 to produce lipids using waste as a substrate. The waste can be used as the sole source of nutrition and could be a key factor in reducing the total production cost of lipid-based bacteriological biorefineries. The use of *B. cereus* KM15 can provide a dual advantage of waste minimization and lipid accumulation in cells. The identification of FFAs also shows the biodiesel potential of lipids extracted from bacterial cells for the biorefinery concept. Simultaneous reduction of waste in terms of VS and COD removal as well as lipid production using fruit peel waste (FPW) could be exploited to produce biofuels by using bacterial strains [10].

15.2.1.9 Anaerobic Degradation of Textile Dye Bath Effluent Using *Halomonas* Species

The main objective of this study is to reduce the COD and color of the effluent containing reactive textile dye by microbial method. Anaerobic digestion has the ability to break down complex refractory organic compounds, so that they may be further degraded aerobically or completely mineralized, and this technique was applied to synthetic reactive red dye cotton textile effluent aiming at the dye degradation. Halophilic and halotolerant bacterial cultures, *Halomonas variabilis* and *Halomonas glaciei*, were used for the degradation in batch-mode under static condition. The temperature was kept constant at 30 °C in a CO_2 incubator. Maximum degradation was achieved within 144 hours of experimental run, and degradation studies were conducted by determining COD and BOD. Statistical analysis showed that the BOD and COD reduction rate was optimal in the dye concentration of 1297 mg/l within 100 hours. Recent studies have shown that the reactive dye bath effluents can be degraded using the bacterial cultures used in this degradation process, even in the presence of alkali enabling the treated water for recycling. The optimal values suggest that the effluent diluted to the optimized concentration can be degraded successfully using *H. glaciei* in the anaerobic batch reactor with a maximum COD reduction rate in 100 hours. Further studies on the anaerobically digested textile effluent should include oxidation in order to reduce COD considerably. Oxidizing the reduction products and subsequent recycling of treated wastewater will attain zero discharge [11]. Other potential microorganisms that are extensively used in dye removal are listed in Table 15.1.

Table 15.1 Various photobiological agents used in photobiological reactors with their use and prevalent examples.

Pollutant	Method of Removal
Degradation of perfluorinated compounds (PFCs) in wastewater	Electrochemical oxidation was proposed for remediation of PFCs from wastewater Four anode materials were tested – aluminum, stainless steel, Ti, Ti coated with nano-ZnO Ti coated with nano-ZnO anode exhibits an excellent removal of PFCs
Degradation of dissolved organic compounds in wastewater	In a typical visible light sensitive TiO_2 preparation by wet chemical methods, the chemical (e.g. N-doping content and states) and morphological properties (e.g. particle size, surface area) of TiO_2
Removal of colorants from wastewater	*Lactobacillus delbruckii* used for the removal of dyes. It involves the use of two commercial synthetic dyes i.e. reactive orange and black. The effect of different parameters such as pH, temperature, initial dye concentrations were studied and effectiveness of this to remove the dye solution was determined by measuring the percentage of color removal. The bacteria was able to decolorize these dyes and the optimum parameters were found to be 10 ppm, pH 6.0, and 37 °C. Uses: economical and ecofriendly
n-Hexadecane degrading	A strain of *Acinetobacter baumannii* was isolated from HC contaminated wastewater and examined for its ability to utilize hexadecane and grow as *n*-hexadecane as a sole source of carbon and energy. Disadvantage: expensive.
Removal of dyes from wastewater	The potential of microorganisms such as *Cunninghamella elegans*, *Aspergillus niger*, *Bacillus cereus*, *Chlorella* sp., and also *Citro bactor* is used in removal of dyes

15.2.1.10 Laccase Production on *Eichhornia crassipes* Biomass

The study explores the utilization of biomass of the weed species, *Eichhornia crassipes* for laccase production by using *pycnoporus sanguineus* SYBC-L1. As the sole carbon and nitrogen source, *E. crassipes* will produce laccase (7.26 U/g dry substrate). The fermentation medium for the maximum enzyme production was optimized and the laccase was then purified and characterized. The optimized culture medium contains 25.1% *E. crassipes*, 13.9% sawdust, 1.5 mM $CuSO_4$, and 40 mM gallic acid (65% moisture and initial pH 6.0), and maximum laccase activity of 32.02 U/g dry substrate was detected on ninth day, which was 4.5-fold compared with the earlier medium. The molecular mass of the purified Lac-S was 58.4 kDa, and the optimum activity of Lac-S on DMP (2,6-dimethoxyphenol) was at pH 3.0 and 70 °C. Lac-S showed not only high catalytic action at low temperature, but also good stabilities toward pH and temperature, and the residual catalytic activities

of Lac-S were 30%, 40%, and 50% at 0, 10, and 20 °C respectively. The half-lives at 50, 60, and 70 °C were 21.7, 9.7, and 1.5 hours, respectively. The results provide a compelling basis for further utilization of *E. crassipes*. Recent study confirmed that *E. crassipes*, an abundant waste in China, could be used as the sole substrate for laccase production by *P. sanguineus* SYBC-L1 in SSF. The maximum laccase production has been achieved on the agro-industrial solid substrates (*E. crassipes* and sawdust) without adding any other costly carbon or nitrogen source. The Lac-S showed high stabilities over a broad range of pH and temperature, and more notably, Lac-S was found to be not only a thermostable enzyme but also a cold-adapted enzyme [12].

15.2.1.11 Algae–Bacteria Interaction in Photo-Bioreactors

Recent work presents a simple model to describe the consortia of algae–bacteria in a photo-bioreactor. The model is influenced by the structure of activated sludge model (ASM), which includes different process rates and stoichiometric parameters, and it comprises two main biomass populations (algae and bacteria), two dissolved substrates (ammonium and nitrate), and two dissolved gases (oxygen and carbon dioxide) in the reactor. The model was calibrated with data from batch experiments performed in two lab-scale photo-bioreactors where a sensitivity analysis was done to identify the parameters to be considered for the model calibration. Results show that the maximum algal and bacterial growth rate, bacterial growth yield, and half-saturation constant for carbon were the most sensitive parameters. Recent work presents a simple model to describe the interaction between algae and bacteria in a photo-bioreactor. Inspired by the ASMs framework, the aim of the model was to predict the dynamics of the dissolved ammonium, nitrate, and oxygen concentration considering the principal reactions and components involved in the process. A sensitivity analysis was used to identify the key model parameters for calibration, where experimental data from two lab-scale photo-bioreactors was used, and the proposed model can give a good prognosis of the experimental values [13].

15.2.1.12 Photo Sequence Batch Reactor

In recent study, photo-sequencing batch reactors (PSBRs) were set up to evaluate the effect of photoperiod (i.e. 24 hours illumination and 16 hours/8 hours light–dark cycle) on NH_4^+-N removal performance of algae–bacteria consortium under long-term low-light intensity condition, and it was observed that constant NH_4^+-N removal rate (60 mg N/l/d) was achieved in both photoperiods at low light intensity (LI) (1000 lx). Longer photoperiod favored higher production of biomass. The 16 hours/8 hours light–dark cycle condition produced less biomass but showed same nitrification rate and slightly higher denitrification efficiency (30 mg/l) than the reactor that was operated at 24 hours brightness. The results of a recent study demonstrated that use of algae–bacteria consortia could be a sustainable and cost-effective method to treat NH_4^+-N-loaded wastewater, and this study also used PSBRs, which operated at low-light intensity (1000 lx), hydraulic retention time (HRT) of 2 days, and solid retention time (SRT) ranging from 5 to 13 days, to treat wastewater loaded with 130 mg N/l. A daily ammonium removal rate of 60 mg N/l/d

was achieved in both PSBRs (photoperiods of 24 hours continuous illumination and 16 hours/8 hours L/D). Longer lighting period favored the growth of biomass, which resulted in 43% more total organic carbon (TOC) production in the same photoperiod. It demonstrates that the use of algae–bacteria consortia requires less energy to treat NH_4^+-N-loaded wastewater, and this method should be further enhanced to make it more sustainable and cost-effective [14].

15.2.1.13 Detection of sul1 and sul2 Genes in Sulfonamide-Resistant Bacteria (SRB) from Sewage, Aquaculture Sources, Animal Wastes, and Hospital Wastewater

The extensive use of antibiotics has placed a lot of selective pressure on bacteria of different environments. Recent study aimed at detecting the occurrence of sulfonamide resistance genes and antibiogram of sulfonamide-resistant bacteria (SRB) isolated from sewage, aquaculture sources, animal wastes, and hospital wastewater. In this method, SRB were isolated on medium incorporated with sulfadiazine (SDZ). Antibiotic susceptibility was carried out using disc diffusion method, and detection of sul1, sul2, and sul3 genes was done by polymerase chain reaction (PCR) using specific primers. The results showed 48 SRBs, out of which 8 were from aquaculture, 16 from animal wastes, 10 from hospital wastewater, and 14 from sewage. They belong to 16 genera with *Pseudomonas* spp. and *Bacillus* spp. In the Gram-negative SRB, there was 100% resistance to ertapenem, tetracycline (77.5%), ampicillin (75%), cefpodoxime (47.5%), streptomycin (27.5%), amoxicillin-clavulanate (22.5%), ciprofloxacin (22.5%), imipenem (15%), cefotaxime (12.5%), and ceftazidime (7.5%), and in Gram-positive SRB, there was 100% resistance to ampicillin, tetracycline, and ertapenem while 50% resistance to cefpodoxime and 12.5% resistance to imipenem, and no resistance was observed to amoxicillin-clavulanate and ciprofloxacin. Most (85.4%; 41 out of 48) of the SRB were multidrug resistant (MDR). The sul1 was noticed in *P. aeruginosa* H19A (isolated from hospital wastewater), *Bacillus* sp. AQE3 (aquaculture pond), and *Leclercia* sp. S5C (sewage) while sul2 was detected in *P. aeruginosa* PG4A (animal waste) and *Klebsiella* sp. S6C (sewage). None of the SRB harbored sul3, and no co-occurrence of the sul genes was observed. The source sampled in the study is an important media for the proliferation of MDR bacteria and resistance genes and revealed a high pervasiveness of MDR SRB in hospital wastewater, sewage, animal wastes, and aquaculture wastewater. More studies should be carried out on other potential antibiotic reservoirs and environments for the discovery of novel sulfonamide resistance genes and the molecular characteristics of isolates possessing these genes [15].

15.2.1.14 Photosynthetic Bacteria as a Potential Alternative to Meet Sustainable Wastewater Treatment Requirement

Conventional activated sludge (CAS) process as the core to wastewater treatment is challenged with severe problems such as high energy consumption, sludge disposal, and inevitable greenhouse gas emission, which will posture a grave impact on the present wastewater industry. Photosynthetic bacteria (PSB) serve as an alternative to these impacts and have flexible metabolic modes and high tolerance,

which improve the removal of nutrients, heavy metals, and organic pollutants efficiently from diverse wastewaters. Recent studies have shown that PSB-based technologies are having great predictions and economic effects. The mode of PSB biodegradation processes presented a promising alternative for new wastewater treatment scheme. The varied metabolism of PSB awards them with influential adaptability, and in particular, photosynthesis under photo-anaerobic condition disrupts the carbon cycle in the CAS. The entire process decreases CO_2 and builds nutrients from wastewater, which accords with the model shift of wastewater treatment from pollution elimination to nutrient and energy recovery. Currently, PSB-based technology for wastewater treatment emphasizes on bio-transformation of nontoxic wastewater and bioresource recovery. In addition, the degradation outcome of PSB on some hazardous or refractory contaminants is considerable and the possibility of trial operations has been sufficiently demonstrated, but much of the investigation is currently limited to the laboratory or small pilot scale. Recent study shows relatively slow growth rate (five to seven days), unstable COD removal rate (30–99%), and energy input cost. Process parameter control, proper pretreatment, and posttreatment are required to meet the current water treatment discharge standards. Research in the following aspects is still at the initial stage.

- Selection of more effective strains capable to grow under wider and more extreme conditions
- In-depth mechanisms, models, and key parameters for better industrialized applications
- Development and optimization of more photo-reactors for improving biomass control and light conversion efficiency [16]

15.2.1.15 Anaerobic Fermentation for the Production of Short-Chain Fatty Acids by Acidogenic Bacteria

The nonylphenol (NP) biodegradation under anaerobic conditions is problematic. Here, anaerobic NP biodegradation by acidogenic bacteria through anaerobic fermentation of waste activated sludge (WAS) for short-chain fatty acid (SCFA) production is stated. The extreme squalor efficiency of NP (69.4%) was attained at pH 10.0 and 10 mg/l Brij 35 within eight days, which was nearly threefold of that in the control (24.6%). Examination of mechanism exposed that the bioavailability of NP, specific NP-degrading bacteria, and their useful genes were helpful for NP biodegradation with surfactant at alkaline pH. More prominently, acidogenic bacteria, the leading functional bacteria in WAS fermentation systems, were established to be complicated in NP anaerobic biodegradation by providing intermediate organic substrates and intrinsic NP-degrading aptitudes. During anaerobic fermentation of WAS for SCFAs production, NP biodegradation was enhanced at alkaline pH with surfactant addition. The acidogenic bacteria, which possess outstanding ability to degrade NP and have enough organic substrates for co-metabolism, were of great support for NP biodegradation [17].

15.2.2 Use of Photolytic and Photochemical Methods in Various Photobiological Reactors for Treatment of Wastewater

15.2.2.1 Photo-Enhanced Degradation of Contaminants of Emerging Concern in Wastewater

Emerging pollutants are posing serious global threats to the environment due to their diversity, unruly nature, and bioaccumulation. More worrying is the fact that present wastewater treatment systems do not have the volume to deal with these classes of compounds. The mission to develop new technologies to lessen the adverse effects of these pollutants has led to new research attention on photo-enhanced processes. Photo-enhanced processes, with the possibility of mineralizing environmental pollutants, currently seem to be the main feasible technologies for dealing with emerging contaminants. The process of contaminant degradation could proceed through either oxidation or reduction courses, mentioned as advanced oxidation processes (AOPs) and advanced reduction processes (ARPs) respectively. As the danger of emerging contaminants continues to increase globally, the strength of research on measuring their negative environmental impact and the examination of new and improved methods for effecting their removal continues to be on the rise. Photo-enhanced degradation processes have shown huge potential for degrading evolving contaminants. Hence, it is very important to carry out more studies for better consideration and flexibility of the process particularly for industrial-scale applications. Since, most processes still rely on UV light sources; there is the need to develop degradation systems that could use direct sunlight for degradation process. This, in a way, will recover the economic viability and environmental friendliness of these processes [18]. Various photobiological agents and methods used in photobiological reactors for treating wastewater are shown in Figure 15.3.

15.2.2.2 Pond Reactors (Photo-Fenton Process)

Currently, iron oxides have been planned as low-cost heterogeneous photo-Fenton catalysts for the concurrent disinfection and discount of micro-contaminant load from urban wastewater in channel pond reactors at near-neutral pH. The objective was not finding the best working conditions but understanding the mechanisms of iron oxide (FeOx)-driven disinfection and the insinuations presented by the matrix constituents, namely organic matter and carbonates. Another objective was to explore the possibility to apply this system after different secondary treatments in continuous flow mode. Prominent bacterial inactivation was obtained in both batch and continuous flow modes by any iron oxide used, with total inactivation (5-log reduction) in the case of hematite.

The heterogeneous photo-Fenton process was confirmed to be the heavy bacterial inactivation force in urban wastewaters. Additionally, nursing the elimination of 25 emerging contaminants in the secondary effluents was done. The operation mode (batch or continuous) was assessed and a minimum of 35% micropollutant removal was attained. This study presents the first attempt to use natural iron oxides (FeOx)

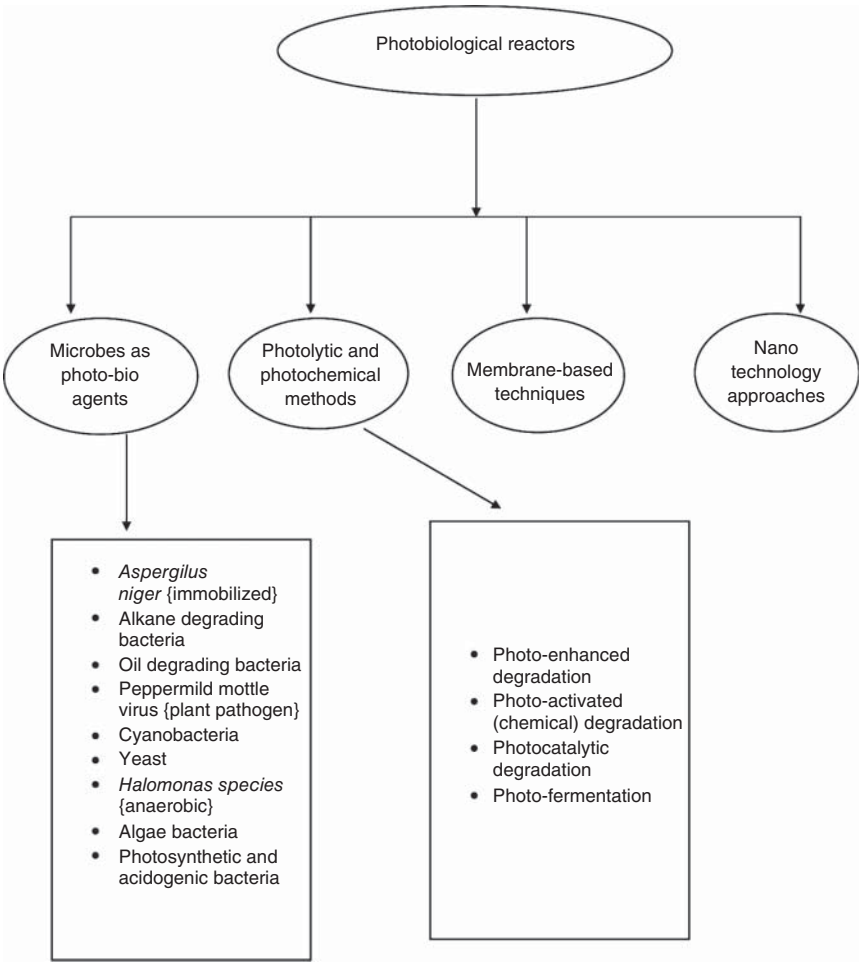

Figure 15.3 Various photobiological agents used in photobiological reactors for treating wastewater.

as photo-Fenton catalysts for the exclusion of bacteria in secondary wastes. Similar residence times and a 5-log removal of naturally occurring fecal bacteria from an activated sludge effluent were obtained. This allows designing, studying, and further expanding the use of FeOx as effective photo-Fenton catalysts, even for wastewater matrices. In order to clarify the high efficiency, the inactivation mechanism was further studied. Among the concerned processes, the removal of bacteria by adsorption process, semiconductor action mode, and the homogeneous photo-Fenton process were found to be less prevalent. The heterogeneous photo-Fenton is the key process and the heavy force of inactivation. The adsorption is not high due to the competition of dissolved organic matter (DOM) with bacteria. The carbonate content of wastewater can play a distinct role in hindering the inactivation process, while the DOM can also be a competitor for the oxidative actions.

Finally, the applied photo-Fenton configuration also permits micropollutant degradation, despite the fact that the system's operational parameters were not intended for this process. Either in batch or incessant mode, there is a 5-log bacterial reduction accompanied by a concurrent micropollutant removal (~33% in the continuous mode or ~55% with an extra addition of hydrogen peroxide), providing a high additional value during this disinfection process. Under normal working conditions, iron oxides have effectively replaced the use of iron salts, giving way to an easier and cheaper implementation of the process. Iron oxides have been proven to be fruitful in maintaining an effective photo-Fenton process. Hence, in the intended application, there will be no need for acidification of the water and subsequent neutralization, avoiding costs of acids/bases, generation of salts, and corrosion of the equipment. Furthermore, easy separation of the catalyst is possible, as iron oxide particles form residue fairly and quickly. Hence, microfiltration, magnetic separation (for ferromagnetic oxides), or simple decantation could be sufficient. Efficiency wise, the 5-log reduction of fecal bacteria enables safe discharge and efficient reuse, with balancing removal of emerging contaminants. Overall, the results of this work prove that channel pond reactors are indeed a solution with a potential for disinfection and decontamination by the photo-Fenton process at near-neutral pH, given the appropriate residence time, land use, and wastewater quantities [19].

15.2.2.3 Photochemical Approaches in the Treatment of Wastewater

Flow Reactor: (Chemical [Photo-Activated] Treatment) Recent study presents the integrated degradation of *p*-NTS (*p*-nitro toluene-*o*-sulfonic acid) by combining photochemical (Fenton) and biological flow reactors. The degradation of *p*-NTS is not possible by wastewater bacteria, and it is considered as a nonbiodegradable intermediate during the manufacture of dyes, surfactants, and brighteners.

A concomitant 20–25% decrease in the initial carbon content during the photochemical pretreatment was observed along with the abatement of the aromaticity. This study shows that the intermediates produced in the pretreatment stage are biodegradable. After pretreatment, a minimum residual (<0.2 mg/l) was attained, and this level of oxidant did not interfere with the subsequent biological degradation. The influence of the reaction parameters such as input concentration of *p*-NTS, rate of hydrogen, O_2 addition, reactor flow rate, TOC reduction rate, and BOD/COD as a function of the time of chemical pretreatment is reported. At flow rates of 0.18 l/h (~5.5 hours residence time), a photochemical degradation efficiency of 75%, a biological degradation efficiency of 52%, and an overall degradation efficiency of 88% for the coupled process were observed. The disappearance of pNTS in the photochemical reactor, the growth and degradation of the benzoquinone such as aromatic intermediate, and production of short-chain aliphatic compounds are reported as a function of pretreatment time. The increase in BOD/TOC as a function of pretreatment time has been correlated to the *p*-NTS and aliphatic recalcitrants existing in the solution.

The biological degradation was observed to be strongly dependent on the flow rate and pollutant load of the solution. These were the two main parameters affecting

the degradation in bioreactor. This study demonstrates the useful photo-Fenton pretreatment of an aromatic *p*-NTS prior to the biotreatment with a tandem reactor concept. The main parameters affecting the performance of the photo-assisted reactor have been reported in this study. In the first stage, the reactor was affected by the amount of substrate, oxidant, and the flow rate. This study concentrated on Fenton treatment under illumination since dark reactions using the Fenton reagent were shown to be slow and inefficient. The fixed biomass in the bioreactor resisted the variations of substrate concentrations and flow rate over long periods (up to two months). The reactor could be operated within this period from a low concentration (40 ppm) to a high concentration (1000 ppm) of substrate without altering the overall performance of the system. Biolite was a cheap support and allowed an adequate contact during the recirculation in the bioreactor. No appreciable increase in the biomass volume was observed during operation within a period of a week.

Toxic intermediate products did not develop during degradation in the solution as revealed by toxicity test. In spite of the initial photoreactor treatment and beneficial reduction of C in the biological second stage, no full mineralization was observed. It seems that one part of the C intermediates degrades easily, but another part that even lacking aromatic character does not undergo easy biodegradation in the second stage. Fixed biomass bioreactors take less space (up to 10 times) than more conventional reactors using suspended biomass [20].

Anaerobic Sludge Reactor (Photocatalytic Treatment) Hostile retting-pond wastewater was treated by anaerobic and photocatalytic process for phenol removal. In recent study, upflow anaerobic sludge blanket reactor was used over a period of 164 days at pH 3, 5, 7, and 9 with HRT from 35 to 20 hours. Anaerobic reactor showed COD and phenol removal of 85% and 75% respectively at pH of 7.0 and HRT of 25 hours. Research surface methodology (RSM) and regression quadratic model were used for COD removal. Photocatalytic process was industrialized by one-way analysis of variance (ANOVA). The joint anaerobic and photocatalytic treatment showed 95% and 93% COD and phenol removal respectively. The anaerobic treatment was performed in upflow anaerobic sludge blanket reactor (UASBR) at 25 hours HRT and 3, 5, 7, or 9 pH, which shows 41%, 55%, 85%, and 80% of COD removal and 56%, 60%, 77%, and 64% of phenol removal respectively. Photocatalytic process was used to treat further primary treated wastewater at pH of 7.0. Through RSM, the best treatment conditions have been found to be 3 g/l Fenton and 20 ml H_2O_2 with 30 minutes reaction time. The UASBR with photocatalytic treatment led to total COD and phenol elimination efficiencies of 95% and 93% respectively [21].

Hybrid Reactor (Dark Photo-Fermentative Hydrogen Production) The study inspected the potential of consecutive dark and photo-fermentation for wastewater treatment and concurrent bio-hydrogen production. To this end, a new shape, namely dark-photo circular puzzled reactor (DP-CBR) was obtainable, which works at ambient temperature ($21 \pm 10\,^{\circ}C$). The reactor was comprised of four identical compartments, where fluorescent tubes were connected to the last two compartments, i.e. C1–C2 (dark)

and C3–C4 (photo). The long-term impact of main operational parameters (i.e. HRT of 6, 12, and 24 hours at initial pH of 5.5 and 6.5) was measured. Maximum hydrogen yield (HY) of 0.4 l/g COD, COD removal of 82%, and organic-N removal of 95% were obtained at HRT of 24 hours and initial pH of 6.5. Increasing HRT was found to maintain the reactor efficiency at ambient temperature. Lowering initial pH to 5.5 worsened the dark treatment at C1 and C2, resulting in lower local HY and ammonification efficiency. Further, the results established that higher HY was achieved in the photo-fermentation, as the protein hydrolysis was mainly achieved in the dark fermentation. The residual free ammonia (<0.36 mg/l), under all inspected conditions, was below the inhibition limit of PSB. The microbial community analysis revealed the development of purple non-sulfur bacterial family *Rhodospirillaceae* at C3. The economic efficiency of DP-CBR was also assessed by considering the capital cost, annual costs (i.e. lightning, pumping, nutrients, and gas purification), and revenues (bio-hydrogen energy and removal of added-value). Overall, the technoeconomic assessment of DP-CBR performance highlights its feasibility (affordable removal of organics and bioenergy recovery) when commenced with gelatin-rich wastewater. The use of integrated dark photo-fermentation reactor is also sensible, since the conventional bio-methanization strategy faces the risk of ammonia inhibition when dealing with protein-rich substrates [22].

15.2.3 Membrane Bioreactor

Membrane bioreactors will have an ultrafiltration membrane module inside to separate the sludge and liquid by membranes. Management of concentrate and waste streams for membrane-based algal separation during water treatment is discussed. Frequent occurrence of harmful algal blooms (HABs) and red tides in freshwater and seawater poses serious intimidations to water treatment, energy recovery, and the application of membrane-based technologies during algal separation. Despite the high elimination efficiency of algal cells and their metabolites (e.g. organic matter and toxins) by membranes, the generation of concentrate and waste streams presents a major challenge. Currently membrane-based processes are integrated with algal separation and particular attention was given to

(i) drinking water production and desalination at low algal concentrations and
(ii) *Cyanobacteria*-laden water treatment/desalination.

The concentrate and waste streams from backwashing and membrane cleaning in each scenario are characterized, and this information facilitates a better understanding of the transport of algal cells and metabolites in membrane processes. Current strategies are (i) recycling of MF/UF(microfiltration/ultrafiltration) concentrate and beneficial use of RO (reverse osmosis) concentrate in the lower concentration scenario, (ii) decontamination of MF/UF concentrate (wastes) and pretreatment for RO feed in the high concentration and high toxicity (Hc–Htox) scenario. Hence, identification of the knowledge gap provides insights to future studies of treating wastewater [23].

15.2.4 Nanotechnology in Photobiological Reactors for the Treatment of Wastewater

15.2.4.1 Potential of Nanotechnology in the Treatment of Wastewater

Nanomaterials become crucial in water treatment plants because of their strong antibacterial activity, photocatalytic response for a broad light spectrum, highly effectual adsorbence, reusability, recyclability, and easy operation. Currently, metals and their oxides, carbon nanomaterials (CNMs such as nanocomposites, nanotubes), metal organic frameworks (MOFs), and zerovalent NPs are discussed. They possess increased surface area and more porosity, which enhances the efficiency of NPs. Magnetic nanoparticles agglomerate due to van der Waals forces. Cytotoxicity fallouts due to carbon nanotubes, quantum dots, TiO_2, silver, and gold NPs make the use of nanomaterials in wastewater treatment with greatest care. Hence, the protective and risk studies of nanomaterials have to be assessed before their application [24].

15.2.4.2 Moving Bed Biofilm Reactor

The pure bacterial culture will form a biofilm adhered to specific large surface supporters (filters fill), which are submerged and moving in the biological reactor. Paints and sunscreens contain TiO_2 NPs, which also act as catalyst in the wastewater treatment. The TiO_2 NPs will exhibit toxic effects on an anaerobic bacterium, *Macrococcus caseolyticus*, isolated from the activated sludge of a wastewater treatment. The cytotoxicity inspection was performed under both light and dark conditions, and it showed decreased feasibility on exposure to light, and it was dose-dependent. The formation of exo-polymeric substances (EPSs) is dose-dependent, and maximum EPS release was observed under UV-A. When treated with TiO_2 NPs, the accretion tendency of biofilm was more marked. The acceptance of TiO_2 NPs by the biofilm could be due to remediation of the NPs in wastewater by the organisms. The interaction of TiO_2 NPs with *M. caseolyticus* under UVA, visible light, and dark condition was observed [25].

15.3 Conclusion

Various modern emerging methods of wastewater treatment such as activated sludge, oxidation ditches, UASB, activated carbon, nanoparticles; microbial fuel cells are currently well developed. These advanced methods are of less sustainability but have high efficacy in treating wastewater. High-efficiency reactors that use various photobiological methods are pond reactors, flow reactors, anaerobic sludge reactors, hybrid reactors, membrane reactors, moving-bed biofilm reactors, and they are industrious in wastewater management.

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16

Bioreactors for the Production of Industrial Chemicals and Bioenergy Recovery from Waste

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16.1 Introduction

With the rise in human population, resource revival from waste is becoming significant for a sustainable wealth, for the preservation of the environment, and for diminishing the reliance on the limited intrinsic sources. The function of aerobic and anaerobic digestion (AD) system for the development of a bio-based circular culture was explored. The developed pathways in the AD field, such as the generation of biogas, hydrogen, and other valuable chemicals volatile fatty acids [VFAs], were considered. The possibility to recuperate beneficial components, like nitrogen through composting, was also dealt with. Stress was given on the novel models for enhanced economics and process appearance, which includes co-digestion of diverse organic solid wastes (OSWs), revival of numerous bioproducts, and integrated bioprocesses [1, 2].

Presently for OSW treatment, diverse options are reachable for translating it into valuable resources (Figure 16.1). Pure cultures are ideal for synthesis of highly pure products and refining is not needed before ultimate utilization of products (e.g. sugars). Innate diverse bacterial conglomerate is hence a smart preference for mixed solid wastes, and it facilitates reserve revival in terms of energy and high-value platform chemicals during AD. Among the biological solid waste treatment processes, biogas manufacturing has been extensively commercialized (Figure 16.2). But other products of ADs are mainly under research supervision, paying way for opening of additional scientific investigation and advancement [3].

16.1.1 Biogas Production

The AD is a well-known biological processing method appropriate for degradation of OSWs into energy and chemicals. It provides a renewable resources and fuel and thereby contributes to rounded wealth and zero waste [4]. The AD process takes place in oxygen-lacking environment and is catalyzed by innate microbial flora

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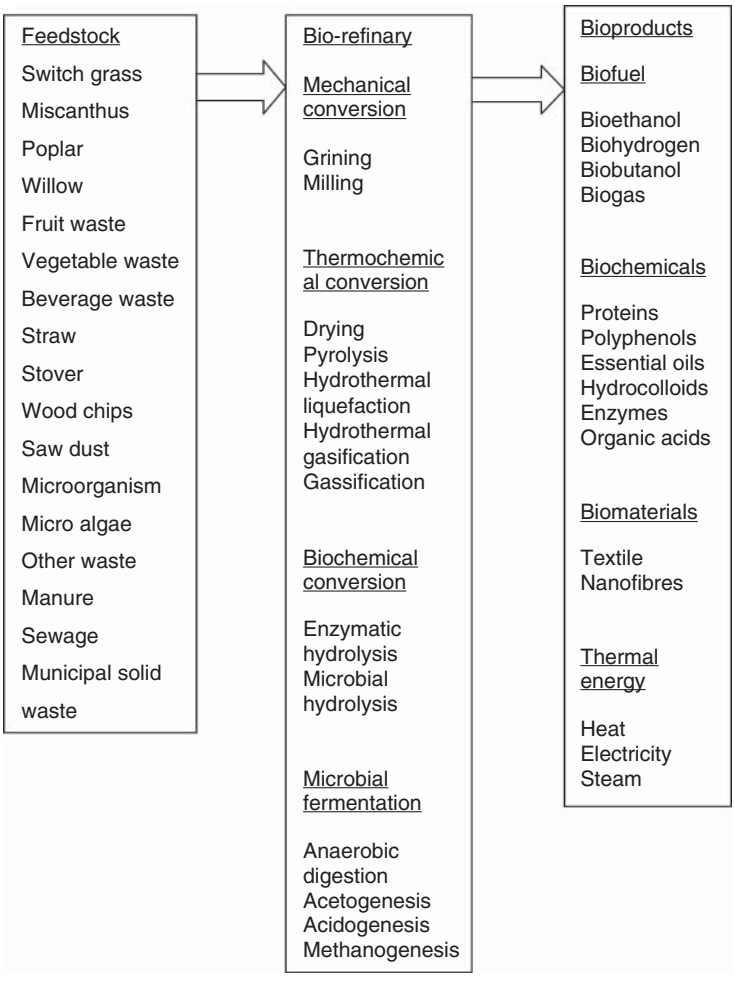

Figure 16.1 Potential feedstock, conversion technologies, and products.

in a four-stage compound procedure ensuring primarily the production of biogas. A pretreatment step that approved either biologically, chemically, or physically can go before the authentic AD procedure in order to effectively arrange substrates with complex structure such as lignocelluloses [5]. In AD practice, kinetics is determined by the nature of substrates and the physico-chemical parameters such as the pH, temperature, and hydraulic retention time (HRT). The end product generated during the splitting of the solid wastes during AD consists of monomers, and these were generated from complex substrates due to hydrolysis by the enzymes such as amylases, lipases, and proteases, and these enzymes were produced by the microbes present in the waste [6]. In the second stage, acidogenic microbes formed in the first step convert the soluble products into biomolecules, such as alcohols, VFAs, hydrogen (H_2), and carbon dioxide (CO_2). The methanogens pursue mainly the acetotrophic (aceticlastic) and Wood Ljungdahl pathways for production of CH_4

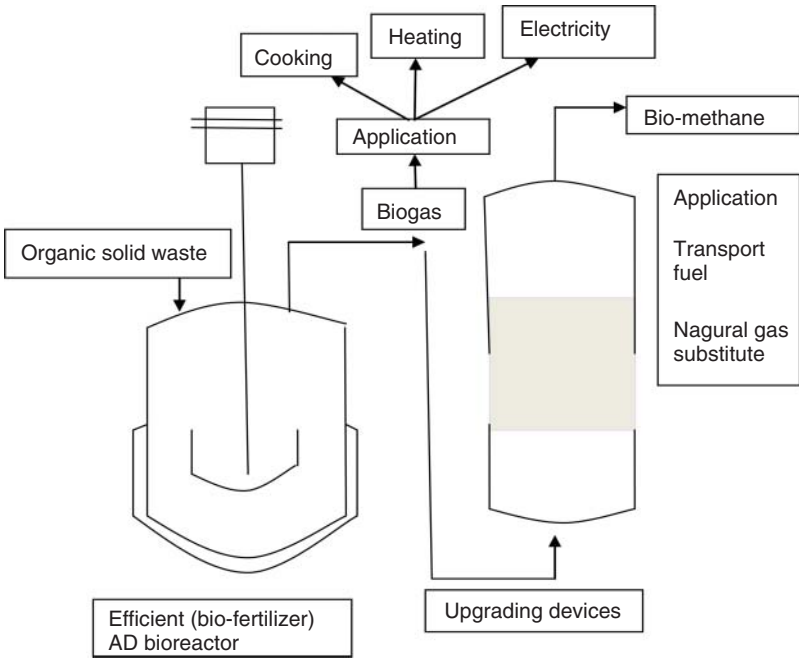

Figure 16.2 Biogas production and the potential applications.

from acetic acid and the reaction between H_2 and CO_2 with the latter being more common [6, 7]. The chemical composition of raw biogas from a typical solid waste AD facility is dependent on the process environment. Typically, it contains 50–75% CH_4, 30–50% CO_2, 0–3% N_2, ~6% H_2O, 0–1% O_2, 72–7200 ppm H_2S, 72–144 ppm NH_3, and other minor impurities [8, 9]. The raw biogas can be straight applied for making electricity and heat while upgraded gas (biomethane) can be inserted into the natural gas grid or used as vehicle fuel (Figure 16.2). Gas purifying in the early stage will exclude impurities that could spoil mechanical and electrical appliances during the use of biogas and can be accomplished by adsorbing with silica gel and activated carbon or molecular sieves. Superior techniques are used mostly for escape of CO_2 from CH_4 to rise the calorific value of the gas. These techniques are water scrubbing, pressure rollback adsorption, cryogenic technology, membrane separation, and organic scrubbing using amines such as diethanol amine, di-glycol amine, and mono-ethanol amine.

16.1.2 Biohydrogen Production

Biohydrogen manufacturing is a very smart option as an unconventional energy supply and most striking energy vector for the future. In recent times, range of biohydrogen-manufacturing pathways has been recommended to get better the main features of the practice. Nonetheless, researches are still required to conquer the residual hurdle to rational appliance such as small yields and manufacturing

rates. Allowing for sensible feature, anaerobic membrane bioreactors (AnMBRs) for biological hydrogen manufacturing is highlighted.

Among the miscellaneous biofuel choices, biohydrogen is a major future energy carrier due to its greater efficacy of transformation into utilizable power, high energy density, and lower pollutants generation [10, 11]. In recent times, diversity of technologies for hydrogen making from selected resources has been comprehensively investigated. Among these, hydrogen production from biomass considered as extremely smart choice as a less energy extensive and cheaper process. Biohydrogen can be produced by numerous biological paths like photofermentation of organic compounds and organic waste using photosynthetic bacteria, biophotolysis of water with the help of algae and cyanobacteria [12]. Some fermentative bacteria can produce hydrogen gas continuously without any light source in anaerobic wastewater treatment. Besides hydrogen, these bacteria generate other products to gratify their metabolic requirements and additional growth. These products comprise organic acids, alcohol, acetone, biodegradable plastics, and fibers [13]. Traditionally, continuous stirred tank reactors (CSTRs) have been extensively used for biohydrogen production by fermentative bacteria. The CSTRs have simple structure, ease of function, and efficient uniform mixing and operate under various circumstances of the substrate, pH, and HRT. Among bioreactor designs, merged hydrogen fermenters with AnMBR is one of the most capable solutions. Membranes in AnMBR, compared with CSTR, can put off biomass loss from the reactor, consequently allowing the long solid retention time (SRT) necessary for effectual treatment while allowing relatively short HRT. Moreover, AnMBR produces excellent quality effluent and reduces plant footstep [14, 15]. Nevertheless, membrane fouling is at a standstill the major hindrance in AnMBR applications [15].

16.2 Basic Biohydrogen-Manufacturing Technologies and their Deficiency

Biohydrogen can be generated by numerous biological paths and divided into two main categories: light-dependent and dark fermentation processes. Light-independent process is dark fermentation, whereas light-dependent processes include photofermentation and photolysis. All biohydrogen making pathways depend on either nitrogenase or hydrogenase for hydrogen evolution. These pathways gain energy either straight from light energy or via consuming photosynthetically derived carbon compounds. Among these, dark fermentation receives high scientific consideration and pilot plants have been also recognized [16].

16.2.1 Direct Biophotolysis

In this practice, biohydrogen production can be influenced by the photosynthetic ability of an organism, for example, a green algae or cyanobacterium, which captures solar energy to execute water-splitting procedure (producing O_2) and diminish ferredoxin, an electron carrier in the chloroplasts. Subsequently, electrons

are transported to hydrogenases and/or nitrogenase enzymes. Microorganisms liberate the surplus electrons using hydrogenase enzyme in anaerobic or excessive energy conditions, which convert the hydrogen ions to hydrogen gas. Molecular hydrogen production takes place, by rejoining the electrons and protons extracted from the water-splitting reactions, using chloroplast hydrogenase. Crucial issues with the coproduction of hydrogen and oxygen comprise co-culture equilibrium, photosynthetic and respiration ability ratios, concentration, and dispensation of cell biomass. In certain circumstances, a few microorganisms like algae can directly generate hydrogen. Sulfur-deficient green algae whose energy was achieved from light on anaerobic environment could sustain the hydrogenase reaction to generate biohydrogen photosynthetically.

16.2.2 Photofermentation

An anaerobic photosynthesis is carried out by a non-sulfur purple photosynthetic bacterium, which exploit captured solar energy to produce adenosine triphosphate (ATP) and high-energy electrons through overturn electron flow that diminish ferredoxin. Proton decline to hydrogen by nitrogenase and it is driven during ATP and condensed ferredoxin formation. On the contrary, cyanobacteria and/or green algae in photolysis technique and photosynthetic purple bacteria cannot gain electrons from water, and therefore, organic compounds, usually organic acids or even dihydrogen sulfide, are used as electron donors under anaerobic environment. This process can be promising in terms of inclusive alteration of substrate to H_2 and CO_2, and types of feed to the microbes.

16.2.3 Dark Fermentation

Diverse organic substrates and wastewaters can be utilized as electron donors to produce biohydrogen at higher rates and lower cost in dark fermentation as compared to other biological pathways. Carbohydrate-rich substrates can be broken down to hydrogen and other products such as acids (lactic, acetic, butyric, etc.) and alcohols (ethanol, butanol, etc.) anaerobically using diverse microbes. The oxidation state of the substrate, microbial distributions, and environmental conditions such as pH and hydrogen partial pressure can affect product distribution in this process. Dark fermentation in anaerobic environment emerges to be the most favorable among the bio-production procedure because there is no need of straight solar input and variety of waste streams can be indulged for hydrogen generation. Glucose to pyruvate formation or glycolytic pathway is generally ordinary path established in all major microorganisms. In this direction, glucose is converted into pyruvate and NAD+ to NADH (nicotinamide adenine di nucleotide) in the course of anaerobic glycolysis Eq. (16.1). The NADH, acetyl-CoA levels, and environmental conditions may influence the discarding of electrons during pyruvate-ferredoxin oxidoreductase or NADH-ferredoxin oxidoreductase and hydrogenase reactions. Therefore, NADH utilization to produce some reduced composites (such as lactate, ethanol, and butanol)

has to be done to balance the oxidation–reduction state, consequently in a lower yield of biohydrogen [13]:

$$C_6H_{12}O_6 + NAD^+ \rightarrow 2CH_3COCOOH + 2NADH + 2H^+ \quad (16.1)$$

Two directions can be established to outline molecular hydrogen production in the presence of appropriate co-enzymes, for example, either by the re-oxidation of NADH path or by formic acid disintegration pathway which could be represented by Eqs. (16.2) and (16.3):

$$NADH + H^+ + 2Fd^{2+} \rightarrow 2H^+ + NAD^+ + 2Fd^+ \quad (16.2)$$

$$2Fd^+ + 2H^+ \rightarrow 2Fd^{2+} + H_2 \quad (16.3)$$

16.3 Overview of Anaerobic Membrane Bioreactors

AnMBR technology is an excellent technology to control pollution because of its lesser carbon foot print, while generating higher effluent (permeate) qualities than conventional treatment practices. It is a combined method where membrane element is attached with an anaerobic bioreactor [13, 17]. Membranes can remove liquid from biomass and can preserve biomass efficiently in the bioreactor, thus allowing the long SRT necessary for effective treatment, while permitting action at the short HRT required for cost-effectiveness. It also gives possible benefits for the bioprocesses where product formation and separation are required concurrently in a compressed method [17]. Despite the applications, AnMBR configurations can be characterized as submerged/immersed and exterior/side stream (Figure 16.3a,b). In the previous case, membranes are immersed in the liquid state of biological reactor or sometime submerged in a different reactor. In side stream structure, liquid filtration membrane is connected to the bioreactor externally in a different unit requiring a transitional pump step. Every design has positive and negative features and, the attainable value of tetra-methyl pyrazine (TMP) is unlike and route of flow is reverted. Higher TMP in side stream plan directed to diminish the substitute area required for a particular filter through flux and enhance the claim of operation energy. On the contrary, the maintenance and changing of membranes is effortless in this configuration. Even though, submerged AnMBRs are less energy-intensive, but well-established membrane surface area is requisite to deal with high permeate fluxes [13, 16].

16.3.1 Challenges and Opportunities

16.3.1.1 Membrane Fouling and Energy Demands

Due to deposition of foulant materials, membrane fouling arise on the outside of membrane and/or inside pore matrix which is a challenge within AnMBRs because it worsens membrane permeability, thus demanding chemical cleaning which can curtail membrane life time [17, 18]. A range of diverse foulants like particulates, organics, colloids, microbes, and microbial byproducts, inorganics, and amalgamation with thereof will cause fouling. Fouling naturally influences the economy of

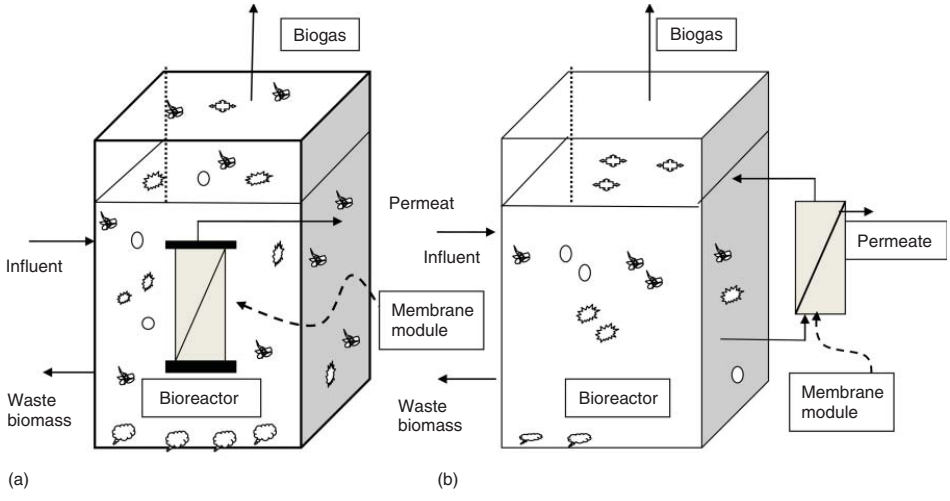

Figure 16.3 Schematic diagram of AnMBR configurations (a) submerged and (b) external (side stream).

the process (enhance the capital/working cost) and should be controlled as much as possible. The fouling is unavoidable incident and could be inhibited if mechanism and dependable factors are known. Membrane fouling is dependent on quality (Figure 16.4) associated with functional state (operational flux, temperature, HRT, SRT, pH, hydrodynamics such as shear rate on the membrane surface), membrane characteristics (hydrophilic/hydrophobic, porosity, material and composition, membrane pore size, surface charge, surface roughness, etc.), and bacterial/mixed liquor characteristics (skim milk powder [SMP]/exopolysaccharide [EPS], hydrophobicity, ionic strength, charge, populations density, species, growth phase, and biological responses, etc.) [19, 20].

16.3.1.2 Biohydrogen Generation Rate and Yield

The unfinished substrate exchange, the subsequent low yields, and production rates are major barriers which prevent scaling-up of the process. During the biohydrogen production process, inhibitory byproducts such as short-chain VFAs (acetic, butyric, propionic, and lactic acid, etc.), alcohols (butanol, ethanol, propanol, etc.), and biohydrogen-consuming microorganisms (e.g. homo acetogens, methanogens, nitrate-reducing bacteria, and sulfate-reducing bacteria) will reduce the production rates and yields [21–23]. In comparison to traditional CSTRs, AnMBRs have diverse designs, varied geometry of bioreactor, working parameters, recirculation of sludge, and sparging of gases. Gas bubbling not only helps integration and control of fouling but also can eliminate produced biohydrogen from liquid phase by increasing the rate of liquid to gaseous mass transfer, which is pleasing in fermentative hydrogen production, since hydrogenase activity can be receptive to rising hydrogen concentration in the aqueous stage. The partial pressure of hydrogen diminishes the creation of alcohols and organic acids when the hydrogen production is maximized. Therefore, it is challenging to advance hydrogen production in one-stage AnMBR.

16.4 Factors Affecting Biohydrogen Production in AnMBRs

Rates of biohydrogen generation and yields are the function of a number of parameters such as the nutrient availability, HRT, SRT, pH, temperature, and concentration of substrates.

16.4.1 Nutrients Availability

The availability of nutrients such as nitrogen, phosphate, and other inorganic trace minerals are crucial apprehension for bioreactors including AnMBRs. In order to achieve best possible microbial growth and biohydrogen generation, element substitution is required predominantly for carbohydrate-rich wastewater including other waste streams. Organic nitrogen source, mineral salt medium, and iron concentration are the key variables as they can either restrain or improve the hydrogen generation in AnMBR [21]. Biohydrogen generation from microbes can be influenced

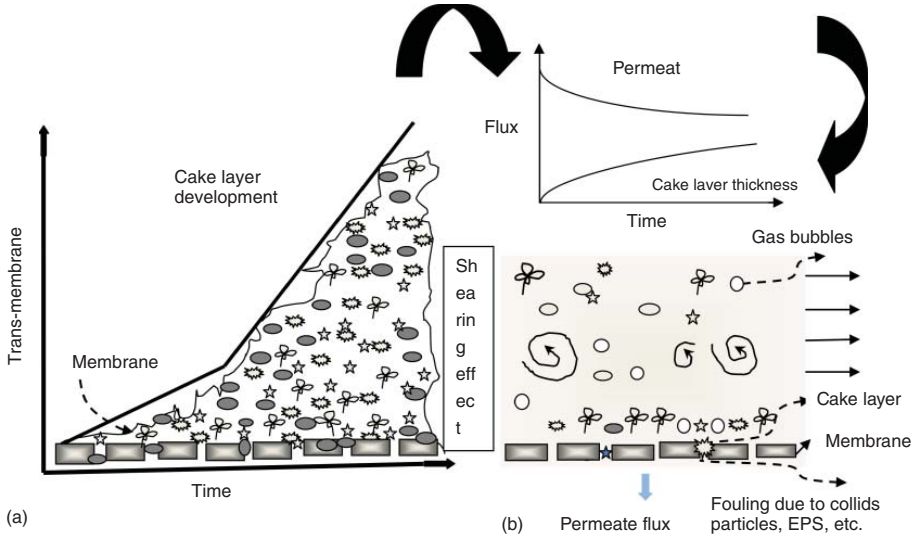

Figure 16.4 (a) Cake layer and (b) corresponding TMP development phenomenon with time in anaerobic membrane bioreactors.

by the catalytic activity of the hydrogen making enzyme. Earlier report exhibited that biohydrogen production increased 1.59 times when $FeSO_4$ concentration was increased from 2.7 to 10.9 mg/l. Another study considered the effect of trace elements and the results confirmed that Na, Zn, Mg, and Fe were essential increment to control the hydrogen production.

16.4.2 Hydraulic Retention Time (HRT) and Solid Retention Time (SRT)

Traditionally, several studies have utilized CSTRs in which HRT controls the microbial growth rate and operational behavior of the structure. Therefore, HRT must be larger than the highest growth rate of the microorganisms or else biomass washout would be a possible risk by the dilution produced via uninterrupted volumetric flow. However, membranes in AnMBR, compared to CSTR, offer solid–liquid segregation and can maintain the biomass in the system and thus approve decoupling of HRT as well as SRT. Earlier studies reported that hydrogen-manufacturing rate was enhanced in AnMBR as methanogenesis was prevented or ended by decreasing HRT. Nevertheless, the specific biohydrogen production rate stayed unchanged. Currently, it was reviewed that efficient biohydrogen generation from liquid waste streams (wastewater) would be performed by means of the best possible HRT ranges from 0.5 to 12 hours [24].

16.4.3 Design of Biohydrogen-Producing Reactor

Design of reactor and its development is a key parameter in biohydrogen manufacturing because it can change the microenvironment of the arrangement, hydrodynamic performance, ascertaining microbial population, and their contact with the substrate. The majority of studies used CSTRs as they supply perfect mass transfer and get in touch with the microorganisms and the substrate. Many researchers reported that on the whole biohydrogen manufacturing in AnMBR under steady-state procedure slightly surpass that of CSTR [25].

16.4.4 Substrate Concentration

Saleem et al. investigated the influence of concentration of substrate on biohydrogen manufacturing in side–stream anaerobic dynamic membrane bioreactor. The results confirmed that high influent chemical oxygen demand (COD) (above 30 g/l) related to high organic loading rate (OLR) favored the build up of VFAs connected to the prevention of biohydrogen generation. The steady hydrogen generation was accomplished by functioning the system at low influent concentration of COD operating in the range of 10–30 g/l and at HRT of approximately one day. An additional study was reported that steady increase in OLR from 4 to 22 g COD/l·day supported the hydrogen manufacturing after that it decreased (20%). The results disclosed that there is no widespread favorable OLR and it may vary with the exacting inoculum, substrate, and nature of the system [16].

16.4.5 Temperature and pH

Temperature is the key factor for biohydrogen generation, which potentially affects the hydrogenase activity, microbial communities, and their metabolism and a spectrum of products. It is remarkable that though hydrogen-producing microorganisms are capable to generate hydrogen at ambient temperature, hydrogen-manufacturing performance constantly advanced by rising temperature in the mesophilic system. However, hydrogen generation might be influenced by further increase in temperature beyond mesophilic range, possibly affected by the physiological characteristics of microbial culture. Temperature dependence was also verified by Chang and Lin by studying the hydrogen production efficacy of a mixed culture with temperature increasing from 15 to 34 °C [26]. In another study, temperature change strategy was applied and biohydrogen-manufacturing yield was improved by 62% as the temperature shifted from 37 to 45 °C. Likewise, metabolic pathway, cell morphology and composition, microbial population shift, and yield of biohydrogen are also powerfully influenced by the pH as it is measured as one of the important variables in ecological situation. Earlier studies assured that increase in pH below 4.5 significantly eliminates the biohydrogen from strong methanogens. Widespread literature review revealed that optimum pH value during continuous fermentative hydrogen production is between 5.2 and 6.0 using pure or mixed microbial cultures. An additional study shown that best possible pH range may differ depending on the physiological characteristics of the substrate and composition of the microbial population.

16.4.6 Seed Culture

Biohydrogen can be formed with pure or mixed cultures. Pure culture is preferred due to high selectivity and hydrogen-manufacturing ability because metabolism of microorganisms can be simply manipulated by altering growth and working environment. However, the majority of studies using pure culture were performed in batch mode and mandatory aseptic environment, thus increases overall cost. From the engineering viewpoint, mixed cultures from anaerobic sludge, municipal sewage sludge, and soil as inoculums are presumably applied in most of the studies for fermentative hydrogen production.

16.4.7 Hydrogen Partial Pressure

The hydrogen partial pressure foundation by the dissolved hydrogen concentration in the liquid part is one of the blockages in fermentative hydrogen making. Numerous techniques are now being used to conquer negative impact of hydrogen partial pressure. An enhancement in yield was observed up to 65% by sparging the system with nitrogen. A different study showed 1.5 times enhancement of biohydrogen yield when the nitrogen sparging was carried out in the system. However, the purity of biohydrogen is influenced because of dilution impact which is a most important drawback of gas purging strategy. In another study, Lee et al. applied vacuum strategy to decrease production pressure and enhance biohydrogen production rate [27].

16.5 Techniques to Improve Biohydrogen Production

In recent times, biological hydrogen manufacturing has enhanced scientific deliberation due to its possibility for infinite, low cost, and renewable source of clean energy. Among all hydrogen-manufacturing technologies, anaerobic hydrogen fermentation appeared to be most favorable since hydrogen can be generated at higher rates. In addition, a variety of wastewaters and organic wastes supplemented with carbohydrates can be treated in this process, consequently capable to generate sustainable low-cost biohydrogen with simultaneous waste minimization.

16.5.1 Reactor Design and Configuration

Biohydrogen generation could be probably enhanced through particular reactor design and configurations. Reactor design should be appropriate and should be stable for long duration of action time. Lee et al. reported that AnMBR apparently achieve comparatively enhanced volumetric hydrogen generation rates [27]. However, some studies recognized that overall hydrogen generation performance of AnMBR under steady-state operation fairly exceeds that of the CSTR. Recently, Noblecourt et al. observed usual and highest productivities of 0.75 and 2.46 l-H_2/l·hour in AnMBR, which were 44% and 51% higher, respectively, in association with control system without membrane. Latest study gave insight into blending of a stable hydrogen fermenter with incorporated membrane system [15]. The grades established that hydrogen-manufacturing rates in the rectangular AnMBR were higher (reached 0.21 ± 0.05 m^3H_2/m^3 day at 0.7 V and 0.41 ± 0.08 m^3H_2/m^3 day at 0.9 V) than that of tubular one (0.01 ± 0.01 m^3H_2/m^3 day).

16.5.2 Microbial Consortia

The use of microbial consortia as a substitute of pure culture could improve biohydrogen removal as they are prone to restrain a suite of the compulsory hydrolytic actions and are probably more vigorous against procedural differences and ecological circumstances (such as pH, temperature, growth, and nutrients). Optimized environment will increase biohydrogen yields. Microbial consortia will facilitate an economic viability because it can offer synergistic association between microorganisms and this process can utilize diverse substrates [28]. But, hydrogen-utilizing microorganisms, such as hydrogenotrophs, methanogens, and sulfur consuming ones, will also present in mixed culture. The pretreatment method is used to enrich the composition of biohydrogen producers in mixed bacterial communities and to inhibit the biohydrogen-consuming microorganisms such as homoacetogens and methanogens, which are prevalent in mixed microbial communities [29].

16.6 Environmental and Economic Assessment of BioHydrogen Production in AnMBRs

The hydrogen manufacturing mostly made from fossil fuels which contribute huge quantity of greenhouse gases. Biohydrogen manufacturing from AD could be crucial to diminish CO_2 discharge from fossil fuels. In the present situation, AnMBR is a probable budding technology extensively used in AD to translate wastewater into methane. However, methane and its combustion still widely contribute to greenhouse gas emissions and could not be worthy as a sustainable and renewable product from wastewater and other waste streams via AnMBR. Other potential pathways for the system is biohydrogen manufacturing which cause no biological damage during energy delivery (generate only H_2O as an incineration produce) and has very little ecological impact as compared to other AnMBR products, e.g. VFA and methane [14]. Further improvement in clogging control of AnMBR technology, superior flux, and supervision of greenhouse gases discharge would make AnMBR an aggressive AD procedure. The expansion of this method is still in its primary stage, and widespread theoretical and experimental work is necessary to estimate the technical, economical, and environmental implications of AnMBR technology.

16.7 Future Perspectives of Biohydrogen Production

Issues like biohydrogen production rate, yield, and membrane fouling in AnMBRs need to be addressed appropriately prior to realistic and practical implementation of the process. The considerable improvement in fouling, volumetric production rate, and yield may be required by utilizing effectual design of reactor and its configuration, suitable microbial strain, metabolic, and genetic engineering of starins. Some integrated advancements may also be possible such as two-stage procedure or the use of modified combined configuration of AnMBR and microbial fuel cell or anaerobic electrochemical membrane bioreactor.

16.8 Products Based on Solid-State Fermenter

16.8.1 Bioactive Products

Many practical advantages have been recognized to the manufacturing of biologically active secondary metabolites by solid-state fermentation (SSF). Mycotoxins, bacterial endotoxins, plant growth factors, antibiotics, immuno-suppressive drugs, alkaloids, etc., are among the main group of bioactive compounds, which have been formed through SSF. These are commonly produced on a broad range of

food grains and seeds such as wheat, oat, rice, maize, etc. Wicklow et al. reported the production of Ochratoxin A, a known mycotoxin with established toxicity to insects, from the sclerotia of the fungus *Aspergillus carbonarius* Northern Regional Research Laboratory (NRRL) 369. The sclerotia were yielded from SSF of corn kernels. Ochratoxin A accounted for the function of the methanol extract against larvae of the detritivorous beetle, *Carpophilus hemipterus* (75% reduction in feeding rate) and corn ear worm, *Helicoverpa* (50% mortality with 99% reduction in weight gain among surviving larvae) when incorporated into a pinto bean diet at levels less than those in the sclerotia. Gibberellins (GAs), which are a big family of isoprenoid plant growth hormones and most of them are bioactive growth controllers, also control the seed germination, elongation of stem, and flowering. They have been observed to be formed during SSF of the rice pathogen, *Gibberella fujikuroi* (principal bioactive composite is gibberellic acid 3). Durand et al. and Tomasini et al. compared SSF and submerged fermentation (SmF) for gibberellic acid production. The *G. fujikuroi* produced 23 mg of gibberellin per ml in 120 hours of liquid fermentation. The use of polyurethane as inert solid support resulted in very poor growth of the culture. The SSF using wheat bran produced 3 g GA per 3 kg dry substrate in 11 days. While the majority of the studies reported on a laboratory scale, several studies have been carried out on the manufacturing of various antibiotics in pilot SSF. These include penicillin, cephalosprin, tetracyclines, chlorotetracyclines, oxytetracyclines, surfactin, actinorhodin, methylenomycin, monorden, etc.

16.8.2 Enzymes

The SSF can be of unique interest in those processes where the crude fermented product may be used directly as enzyme source. Microbial enzymes will play a major role in biotransformations concerning organic solvent media. Agro-industrial residues are normally considered as the substrates for the enzyme production in SSF method (Table 16.1). Wheat bran is the key substrate and has been exploited normally in a range of processes. The SSF is mostly suitable for the production of lignocellulosic enzymes for a variety of agro-biotechnological applications. In SmF, yields of cellulase are usually about 10 g/l, and the usual fermentation expenditure in a stirred tank bioreactor is about \$200/m^3. Thus, the manufacturing cost in the crude fermentation by SmF is about \$20/kg. In SSF average production level is about 10 mg/g substrate and the normal fermentation expenditure is only about US\$ 25/mt. Thus, the unit price of SSF cellulase is just about \$0.2/kg. Similar types of results were obtained for xylanase production in laboratory scale stirred tank bioreactor [31]. More published information is obtainable on the production of enzymes of industrial importance, such as proteases, cellulases, ligninases, xylanases, pectinases, amylases, glucoamylases, etc. by SSF. Alltech (Nicholasville, KY, USA) has been recognized as a large-scale enzyme-manufacturing company. Alltech's European Bioscience Centre, in colaboration with Institut National de la Recherche Agronomique (INRA), the French government's agricultural research institution, has been implicated in the fermenter configuration for the production of phytase.

Table 16.1 Bioreactors used for production of different products.

S.no.	Source of waste	Organisms used	Products formed	Bioreactors
1.	Pearl barley	*Penicillium brevicompactum*	Mycophenolic acid	Packed-bed
2	Sugarcane bagasse and soybean bran	*Kluyveromyces marxianus*	Inulinase	Packed-bed
3	Press mud	*Kluyveromyces marxianus*	Inulinase	Packed-bed
4	Polyurethane as inert support	*A. niger*	Tannase	Packed-bed
5	Polyurethane as inert support	*A. niger*	Inulinase	Fixed-bed
6	Palm kernel cake	*A. flavus*	Enzymes	Laterally aerated moving bed
7	Wheat bran	Fungal cultures	Enzymes	Gas double-dynamic
8	Sugarcane bagasse	*A. niger*	Enzymes	Counter-current
9	Sorghum stalk	*Issatchenkia orientalis*	Ethanol	Deep-bed
10	Bagasse	*Rhizopus oryzae*	Enzymes	Tray sugarcane
11	Lemon peel pomace	*A. niger*	Pectinase	Tray with vertical columns
12	Rice	*A. oryzae*, *A. sojae*	Koji	Tray
13	Rice	*A. oryzae*	Alpha-amylase	Modified tray
14	Rice	*Trichoderma* sp.	Fungal products	Tray
15	Seaweed	*A. niger*	Fucoidanase	Rotary drum
16	Apple pomace	*A. niger*	Citric acid	Rotary drum
17	Pine wood chips and orange peel	*Trametes hirsuta*	Enzymes	Modular

Sources: Chang and Lin [26]; Méndez-Contreras et al. [30].

16.8.3 Organic Acids

Citric acid, lactic acid, fumaric acid, and oxalic acids have been known to produce in SSF. Citric acid is the generally important organic acid produced in tonnage and is comprehensively used in food and pharmaceutical industries. It is produced mainly by submerged fermentation using *Aspergillus niger* or *Candida* sp. from diverse resources of carbohydrates, such as molasses and starch-based media. However, producing by SSF using agro-industrial residues has great prospective. Citric acid production from pineapple waste in diverse bioreactors was studied, and lower yields were observed in tray and rotating drum modes. A multi-layer packed-bed bioreactor showed enhanced mass transfer significantly compared to a single-layer packed-bed controlled under comparable circumstances. Packed-bed

bioreactors confirmed higher manufacturing of citric acid compared to flask cultures. A higher citric acid yield in packed-bed column bioreactors using cassava bagasse was reported. Enhanced ventilation and heat and mass transfer effects were considered to be the cause for this [32]. Diverse mechanisms of heat elimination (conductive, convective, and evaporative) from packed-bed bioreactors in SSF for citric acid manufacturing with a lifeless carrier were evaluated. Results confirmed that the conductive heat transfer was the least efficient method (8.65%) compared to convective (26.65%) and evaporative (64.7%) heat transfer. Fungal as well as bacterial strains have been utilized for lactic acid manufacturing in SSF. Strains of *Rhizopus* and *Lactobacillus* were used. Diverse crops such as cassava and sweet sorghum and crop residues such as sugarcane bagasse, sugarcane press mud, and carrot-processing waste were utilized as substrates in these practices.

16.8.4 Biopesticides

Problems created by insects and pests to agriculture industry can be effectively met by biopesticides which are considered environment-friendly. Currently, the use of entomo pathogenic and mycoparasitic fungi for biological control of insects and pests has created growing awareness. Challenges have been taken to produce entomo pathogenic fungi in SSF. In the SSF-based process, numerous agro-industrial substrates were considered for generating spores from *Beauveria bassiana* which can be used to prevent pests in banana, sugarcane, soybean, and coffee. The *B. bassiana* was produced in SSF for use against European corn borer. The bio-insecticide was produced in a 1600-l capacity industrial reactor. The bioproduct exhibited a field effectiveness of 80%. The *Colletotrichum truncatum* is a new fungal plant pathogen, which showed assurance as a bioherbicide against the hard weed *Sesbania exaltata*. The *C. truncatum* spores were formed in SSF which use solid support such as solid perlite-corn meal-agar and vermiculite [32].

16.8.5 Aroma Compounds

Plants are the key sources of essential oils and flavors, but their exploitation depends on the factors which are not easy to control, such as weather conditions and plant diseases. An unusual route for flavor production is based on microbial biosynthesis or bioconversion. An attempt to exploit microorganisms in SmF resulted in low production, which troubled industrial use of these procedures. The SSF could be of elevated probability for this intention from agro-industrial residues such as cassava bagasse, sugarcane bagasse, coffee husk, coffee pulp, etc. Fungi from the genus *Ceratocystis* synthesize a huge variety of fruit- or flower-like fragrance (peach, pineapple, banana, citrus, and rose) based on the culture and its growth conditions. Among the species, *Ceratocystis fimbriata* has an enormous possibility for ester production. Although amaranth medium formed pineapple aroma, medium with other substrates created strong fruity odor. Aroma recovery was growth-dependent and greatest strength was identified within few hours previous to or following the highest respiratory activity. Synthesis of strong pineapple fragrance was also

done by this culture during SSF process where coffee husk was used as substrate. Production of 2,5-dimethylpyrazine (2,5-DMP) and TMP by *Bacillus natto* and *Bacillus subtilis*, respectively, on soybeans in SSF was reported [32].

16.8.6 Bio-Pigment Production

Higher cost of production of natural pigments has been solved by the use of microorganisms for the synthesis of bio-colorants due to their easy availability, higher yield, cost efficiency, and convenient downstream processing. Pigments possess anti-inflammatory, antioxidant, anticancer, and antimicrobial properties.

Kaur et al. studied bio-utilization of fruits and vegetables waste to produce β-carotene in SSF. Fruits- and vegetables-processing industries produce huge waste in the form of peels, seeds, liquid, and molasses. They can be utilized for the production of biocolors during SSF of microbial strains. *Blakeslea trispora* (+) microbial type culture collection (MTCC) 884 in SSF yielded 76% of β-carotene [33].

Sharma and Ghoshal studied optimization of carotenoid production by *Rhodotorula mucilaginosa* (MTCC-1403) using agro-industrial waste in a bioreactor [34]. They observed that bio-colorants are advantageous over synthetic colors as bio-colorants not only impart characteristic color to the food but also contain harmless bioactive antioxidant nutrients [35]. Fermentation was carried out in a 3-l bioreactor and produced more than 100 μg of carotenoids per gram dry biomass of agro-industrial waste.

16.8.7 Miscellaneous Compounds

Rotary cultures produced elevated yields of crude red and yellow pigments as compared to stationary cultures. Exo-polysaccharides, such as xanthan and succinoglycan, are the potential products of SSF. The SSF-based procedure for the manufacturing of xanthan gum using strain of *Xanthomonas campestris* was reported. The EPS was formed on spent grains, apple pomace, grape pomace, citrus peels, etc. The production of succinoglycan in SSF by *Agrobacterium tumefaciens* on various solid substrates, including agar, spent grains, ivory nut shavings, and grated carrots, impregnated with a nutrient solution was carried out and obtained an yield of 30 g/kg of soaked substrate. Polymer manufacturing in the horizontal bioreactor (HBR) was quicker, but the final yield was lower (29 g/l of permeate solution). An evaluation of SmF and SSF for the manufacturing of bacterial EPS exhibited that the latter method yielded two to four times more polymer than the former in the laboratory scale fermenter. The *Rhizopus* strains formed riboflavin, nicotinic acid, nicotinamide, and vitamin B_6 [36].

16.9 Koji Fermenters for SSF for Production of Different Chemicals

Conventionally, SSF has been prevalent in Asian countries to manufacture koji from rice to produce alcoholic beverages such as sake. Industrial koji making equipment

can be classified into three categories on the basis of ventilation configurations. First, interior ventilation where air is blown through the bed of koji (bottom up), second one is surface ventilation, where air is blown across the koji surface, and third is non-ventilated natural convection which cools the koji. Interior ventilation is by far the most common. Static flat bed-type, multistage conveyor-type, vapor exchange non-ventilated-type, drum-type, and rotary disk-type fermenters are used for koji fermentation.

16.10 Recent Research on Biofuel Manufacturing in Bioreactors Other than Biohydrogen

In the last few decades, numerous research has been done on the second-generation biofuel such as bioethanol. Current apprehension over climate change and reduction of fossil fuels are making to search alternatives for nonrenewable fossil fuels. The SSF has been investigated as an alternative tool for the generation of ethanol from agro-industrial residues. The *Zymomonas mobilis* has been measured as a proficient strain when apple pomace is used as a substrate for ethanol manufacturing in SSF. However, highest yields were achieved from *Saccharomyces cerevisiae*. It was reported that the incorporation of cellulase enzyme in the substrate enhanced ethanol yields [32]. The prospective of using a single fermenter for biomass expansion, starch hydrolysis, and ethanol manufacturing in SSF using *Schwanniomyces castellii* was reported. Numerous starchy substrates for ethanol manufacturing using *S. cerevisiae* found that rice starch and sweet sorghum gave the highest yields of ethanol.

Dogaris et al. studied landfill leachate (LL) as a sustainable source of water and nutrients for algal biofuel and bioproducts using the microalga, *Picochlorum oculatum*, in a novel scalable HBR. Pilot-scale (150 l) and commercial-scale (2000 l) HBRs that were operated outdoors in Florida using LL in batch and semi-continuous modes generated high cell density cultures (1.7×10^9 cells/ml) and reached up to 1.9 g/l of dry biomass which is appropriate for biofuel manufacturing [37].

Li et al. reported *n*-butanol production from *Clostridium tyrobutyricum* in the presence of hydrolysates of lignocellulosic biomass in a fibrous-bed bioreactor. Acetone–butanol–ethanol fermentation suffers from high substrate cost and low butanol yield. In this study, engineered *C. tyrobutyricum* immobilized in a fibrous-bed bioreactor was used for butanol production from glucose and xylose present in the acid pretreated and enzymatic hydrolysates of low-cost lignocellulosic biomass including corn fiber, cotton stalk, soybean hull, and sugarcane bagasse. A techno-economic analysis showed that *n*-butanol could be produced from lignocellulosic biomass using this novel fermentation process at ~$2.5/gal [38].

Xue et al. studied cellulase production, lignocellulose saccharification, and bioethanol fermentation in a modified gas lift bioreactor using *A. niger* mycelia immobilized within the reactor in wire meshes, and *S. cerevisiae* cells immobilized in resin beads. During four repeated batch fermentations, cellulase activities were more than 6.28 U/ml and bioethanol production was over 45.9 g/l after 48 hours.

The factual bioethanol conversion efficiency was 86.8% [39]. Mahboubi et al. made to remediate some issues associated with hydrolysis and fermentation, by integrating immersed membrane bioreactors (iMBRs) into lignocellulosic bioethanol production process. In this regard, double-staged continuous saccharification filtration and co-fermentation filtration of wheat straw slurry were conducted using iMBRs at filtration fluxes up to 51 l/m^2 h (liter per meter square hour [LMH]) [40].

Process effectiveness for the second-generation ethanol production depends mostly on the type of lignocellulosic raw material. Thus, the optimization for each step concerned in olive tree pruning biomass valorization was studied: (i) alkaline pretreatment of the original feedstock, (ii) diluted acid hydrolysis of pretreated solids, and (iii) fermentation of the hemicellulosic hydrolyzates for ethanol production by *Scheffersomyces stipitis*. The recommended alkaline pretreatment conditions were 30 minutes, 90 °C, and 0.5% w/v NaOH, with losses of 88.3% of acetyl groups from starting biomass, but only 6.9% of D-xylose. A significant improvement in ethanol production was observed in treated hemicellulose liquor (20.4 g/dm^3, $Y_{P/S} = 0.20$ g/g, and $Q_P = 0.21$ g/dm^3 h) [41].

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Part VI

Waste2Energy with Biotechnology: Feasibilities and Challenges

17

Utilization of Microbial Potential for Bioethanol Production from Lignocellulosic Waste

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17.1 Introduction

The growing human population and globalization have led to an increase in the energy demand and consequently resulted in a rampant decline in the world's reservoir of non-renewable energy sources such as petroleum-based fuels. It has also brought in the issues of environmental pollution and climatic changes. This has inspired the efforts on exploring alternative fuels which are considered clean, renewable, and environmentally sustainable [1]. Among the various biofuels, ethanol (CH_3CH_2OH) is considered most efficient and economical biofuel in the current world market. Bioethanol, popularly termed "Fuel of the future" by Henry Ford, holds several advantages over other energy sources. Bioethanol is produced from naturally available and inexhaustible sources such as agricultural products and non-food raw materials such as straw, sweet sorghum, or bamboo in contrast to perishable fossil-derived products. Bioethanol has less toxicity compared to available alcoholic fuels. The end products of partial oxidation of ethanol (e.g. acetic acid and acetaldehyde) are less poisonous and nearly carbon neutral, thus reducing carbon dioxide (CO_2) emissions and associated climate change. Hence, it can be treated as a safe and eco-friendly alternative to conventional fuels [2]. The low octane and centane numbers compared to gasoline make ethanol burn inefficiently by ignition compression and render it immiscible with diesel fuel. Thus, it finds wide application in spark ignition internal combustion (IC) engines [3].

17.1.1 Bioethanol from Different Feed Stocks

Bioethanol is a liquid biofuel which can be produced by utilization of a wide variety of feedstocks via various conversion mechanisms. Feed stocks or available biomass can be categorized into three major groups or generations depending on their source

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and its conversion technology. First-generation feedstock involves the practice of fermentation of sugar-based substrates. In tropical countries like India, Brazil, and Colombia, sugarcane is used as a substrate, while use of corn is prevalent in other parts of the world [4]. This generation of feed stock competes directly with available food resources making it costly and unavailable for bioethanol production. This gave rise to utilization of second-generation feedstock or non-edible lignocellulosic biomass (LCB) which are crop residues such as corn stalks and wheat straw or woody biomass and whole plant biomass such as hardwood, soft wood, and grass [5]. Its wide availability and cost-effectiveness make it a suitable renewable source for biofuel production. The third-generation feedstock comprises microalgal biomass and is still an unpopular and less studied substrate [3].

17.1.2 Sources of Lignocellulosic Biomass

The yield of LCB obtained globally in a year is around 1.3 billion tons, making it the most abundantly available bioresource [6]. It can be obtained from three different sources: primary, secondary, and tertiary (Figure 17.1). Primary source includes either crops or key products such as sugarcane, secondary source comprises residues of production processes such as rice straw, bagasse, and husks, while tertiary source involves end products or refuge such as organic fraction of municipal solid waste (MSW), sewage treatment sludge, and wood trimmings [7]. The commercial application of these substrates in biofuel production is dependent on their availability, ease of transportation, cost of processing, treatment, and final yield of bioethanol [3, 7].

17.1.3 Structure and Composition of Lignocellulose

Lignocellulose is a major component of the cell wall in plants, consisting of a backbone that comprises cellulose $(C_6H_{10}O_5)_n$ mixed with hemicellulose $(C_5H_8O_4)_m$,

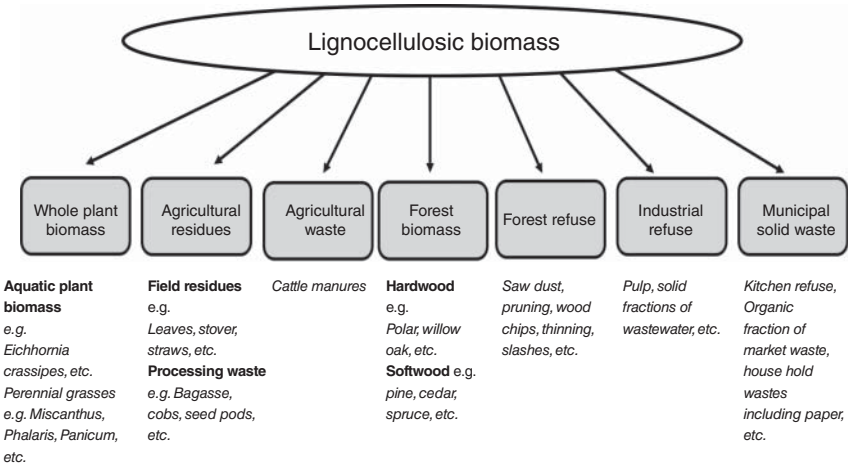

Figure 17.1 The sources of different lignocellulosic biomass.

along with lignin ($[C_9H_{10}O_3(OCH_3)_{0.9-1.7}]_x$), and an insignificant quantity of proteins, ash, pectin, and other compounds [8]. Studies so far have revealed that cellulose, hemicellulose, and lignin contents account to 30–60%, 20–40%, and 15–25% of dry weight of LCB, respectively [9]. Cellulose is the major structural subunit of LCB. It is a linear polysaccharide with β-(1, 4)-glycosidic bonds linking individual subunits of D-glucose [10]. It is soluble in water when the pH is drastically low or high but easily miscible with *N*-methylmorpholine-N-oxide (NMMO) as well as ionic liquids (ILs) [11]. Cellulose is biocompatible, structurally stable, hydrophilic, possesses reactive hydroxyl group which makes it suitable for the manufacture of fibers, films, composites, fuels, and high value chemicals [12]. Hemicellulose is the second constituent of LCB, composed of various polysaccharides, some of which include arabinoxylan, galactomannan, glucomannan, glucuronoxylan, xylan, and xyloglucan. These polysaccharides are present in the form of short chains, linked by β-(1,4)- or β-(1,3) glycosidic bonds [13]. Cellulose and hemicellulose exhibit decreased levels of polymerization and are non-crystalline in nature; therefore, they can easily degrade to monosaccharides and are considered commercially important [14]. Lignin acts as a protecting barrier by covalently bonding to other subunits of LCB which enhances its recalcitrance. The complex three-dimensional structure shows cross-linked polymers of phenyl propane that are bound to each other by carbon–carbon (5–5, β–β) and aryl-ether bonds (β-O-4, α-O-4). The polymers are known to be altered when the methoxyl groups located on the aromatic rings are substituted. For example, the three key units of lignin are guaiacyl (G), *p*-hydroxyphenyl (H), and syringyl (S) [15].

17.1.4 Challenges in Bioethanol Production from LCB

Ethanol which is derived from LCB is one of the most preferred fuel candidates in the present world. Not only this, but also biomass obtained from ethanol can act as a precursor to the different materials that are currently obtained from sources that are unsustainable. But, the drawback of this novel concept is that the treatment cost for ethanol is higher which hinders the process to be commercially replicable and profitable. Several technologies are coming up to give rise to high product yields with overall low cost [1]. Bioethanol is one of the renewable sources along with eco-friendly characteristics which is a promising alternative to fossil fuels. Although practically ethanol is created from edible sources, LCB has attracted a lot of attention lately. In any case, the transformation efficiency of the biomass varies enormously concerning the origin furthermore, nature of LCB, essentially because of the variety in lignocellulosic composition. The two polysaccharides in LCB, cellulose and hemicellulose, are firmly connected to lignin and make a lignocellulosic network that is exceptionally vigorous and difficult to depolymerize. To introduce LCBs into commercial ethanol creation, ongoing exploration endeavors have been dedicated to the techno-monetary upgrades of the general change process [2].

The main objective of the book chapter is to study the potential role of different microorganisms and efficiency in enhancing bioethanol production from LCB as a substrate.

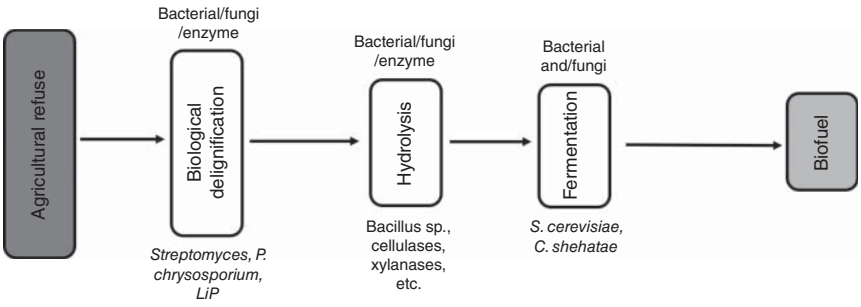

Figure 17.2 Overview of bioethanol production from lignocellulosic biomass.

17.2 Processing of Lignocellulosic Biomass to Ethanol

Pretreatment of LCB is the primary step in bioethanol production protocol. It is considered to be the basic step which largely affects edibility of cellulose and that unequivocally affects downstream expenses including detoxification, catalyst stacking, squander treatment requests, and different factors (Figure 17.2). Pretreatment establishes for over 40% of the all-out handling cost. The cellulose in LCB is secured by lignin and hemicelluloses. Subsequently, it decreases the available surface area accessible for enzyme-mediated saccharification. Pretreatment is necessary to change the LCB to naturally visible and minute size. Appropriate pretreatment might expand the convergence of fermentable sugars post-enzymatic saccharification in this way increasing the general procedure efficiency. A perfect pretreatment process is essential to enhance the hydrolysis of LCB [5]. The various pretreatment methods of LCB include mechanical pretreatment, physico-chemical pretreatment, chemical pretreatment, and biological pretreatment. Mechanical pretreatment reduces cellulose crystallinity, is easy to handle, reduces degree of polymerization, and increases surface area. But unfortunately, it has no high energy input nor removes any lignin. Summary of commonly used pretreatment methods are listed in Table 17.1. Physico-chemical pretreatment increases pore volume, improves enzyme accessibility, removes hemicellulose, and reduces particle size. But, it contributes to less lignin removal, it has high energy demands, and it decomposes sugars.

Chemical pretreatment has high reaction rates, it removes hemicellulose more efficiently, increases surface area, and creates an alteration in lignin structure. Little lignin removal, requirement of neutralization process, inhibitors formation, and requirement of disposal of neutralization salts are some of the drawbacks of chemical pretreatment. To fill up the voids of all these pretreatments of LCB, a new method of biological pretreatment has come up. It can degrade lignin successfully, has less formation of inhibitors, has low energy and capital cost demands, no chemical reagents are required, and it can reduce the polymerization degree of cellulose and hemicellulose in mild environmental conditions. It just has a few disadvantages like slow rate of delignification, longer residence timings, and loss of carbohydrates as they are metabolized by the microbes [3]. Plenty of LCB namely

Table 17.1 Summary of commonly used pretreatment methods with their advantages and limitations.

Sl. No.	Pretreatment	Nature	Intermediate operation	Advantages	Disadvantages	References
1.	Mechanical	Physical	Temperature greater than 300 °C with sheer mixing	Amorphous and crystalline cellulose matrix disruption	High energy consumption	[1]
2.	Microwave	Physical	Microwave irradiation (optimized according to substrate)	Easy operation, high heat generation in rapidly	High energy consumption, significant when used with alkali treatment	[2]
3.	Sonication	Physical	10–100 kHz ultrasound (power and duration should be optimized to meet desired pretreatment effect)	Formation of cavitation bubbles that ruptures cellulose and hemicelluloses rapidly.	Sensitive process. Deviation in power or duration of treatment can adversely affect the substrate	[3]
4.	Alkaline	Chemical	Sodium hydroxide, potassium hydroxide, ammonia	Lignin and hemicellulose degradation with increased surface accessibility	Frequent washing required, foul odor and salt formation	[4, 5]
5.	Acid	Chemical	Nitric acid, sulfuric acid, dicarboxylic acid	Hemicellulose solvation, modifies lignin, cellulose swelling, and inexpensive	Expensive, hazardous and corrosive, production of inhibitors	[6]
6.	Ozonolysis	Chemical	Ozone with low moisture biomass and particle size between 1 and 200 nm	Degrades lignin, no toxin inhibitors production	Highly sensitive and expensive	[7]
7.	Organosolv	Chemical	Aqueous organic solvent like alcohol, ethylene glycol, acetone with specific temperature and pressure	Depolymerization of lignin and hemicellulose	Solvent recycling and draining required, formation of inhibitors, expensive	[8]
8.	Sulfite pretreatment to overcome recalcitrance of lignocellulose	Physico-chemical	Calcium or magnesium sulfite with disk miller	Remove hemicellulose, dissolve, and lignin sulfonation	Sugar degradation, large volume of water required post treatment	[9]

(Continued)

Table 17.1 (Continued)

Sl. No.	Pretreatment	Nature	Intermediate operation	Advantages	Disadvantages	References
9.	Ammonium fiber explosion	Physico-chemical	Liquid ammonia at high pressure and temperature	Swelling and phase change in cellulose crystallinity, modification of lignin to increase water holding capacity and degradability	Ammonia could be recovered, no inhibitor formation	[10]
10.	Whole cell or enzyme mediated	Biological	White-rot fungi, Brown-rot fungi, bacteria, and archaea	Energy efficient, lignin and hemicellulose degradation, economically sound	Lower hydrolysis rate	[2]

sugarcane bagasse rice straw, bamboo, wheat straw, cotton stalk, and sugarcane tops are a few of the largely available agro-wastes. Most of these by-products are used for the production of bioethanol [4].

Pretreatment of bioethanol generally uses bacterial strains or their enzymes. This strategy is attracting consideration because of its capacity to work in the moderately shorter response time, not only that, but also it needs low sustenance prerequisite for the enzymatic responses [6, 8]. A few microorganisms, for example, *Clostridium* sp., *Cellulomonas* sp., *Bacillus* sp., *Thermomonospora* sp., *Streptomyces* sp. and so forth., and a few parasites such as *Phanerochaete chrysosporium*, *Trichoderma reesei*, *Trichoderma viride*, *Aspergillus niger* are generally utilized in natural pretreatment process [7, 9]. By utilizing the sugars, cellulose and hemicellulose degrading microorganisms typically hydrolyze the complex molecules to monomeric sugars [10]. Most significant benefits of natural strategies incorporate no compound reusing after pretreatment, lower downstream handling charges, least inhibitor arrangement, straightforward working, and lower vitality utilization [11]. In any case, the amazingly low pace of hydrolysis is the principle hindrance in creating bioprocessing (BP) strategies [12]. Furthermore, microscopic organisms can create hydrolytic and oxidative catalysts which can break down unbending structures of LCBs. Cellulase is the enzyme that is primarily added, and then β-glucosidase and xylanase are added [13]. In light of the past investigations, enzyme-mediated pretreatment improves the methane content more than 100%; consequently, the viability of enzymatic pretreatment is dictated by various elements [14].

Fungal pretreatment requires higher brooding time, while enzymatic and bacterial pretreatments need a couple of hours to end. Contagious pretreatment diminishes the unmanageability of structure of LCBs [15]. For instance, *Trametes versicolor* has been utilized for BP of grain harvests, for example, wheat, rye, and grain before

Table 17.2 Different yeasts used for sustainable bio ethanol production along with their natural environments.

Sl. No.	Yeast	Natural environments	Details	References
1.	*Candida* spp. *Cyniclomyces* spp. *Pityrosporum* spp.	Animals	Yeasts can be pathogenic as well as non-pathogenic to animals and are generally found to adhere to the intestinal walls of the host.	[19, 20]
2.	*Ashbya* spp. *Nematospora* spp.	Plants	Habitat includes the interface between nutrients of plants and the septic habitat.	[19, 20]
3.	*Rhodotorula* spp. *Debaryomyces* spp.	Water	Inhabitants of fresh as well as estuarine waters.	[20]
4.	*Lipomyces* spp. *Schwanniomyces* spp.	Soil	Survives in aerobic soil.	[21]
5.	*Debaryomyces* spp. *Zygosaccharomyces* spp.	Extreme environmental conditions	The yeast cells are carried by air currents and dropped on the surface of the soil.	[20]
6.	*Cryptococcus* spp. *Rhodotorula* spp. *Sporobolomyces* spp.	Atmosphere	Yeasts which are halotolerant are inhabitants of salty areas. Osmophilic species are found on glaciers.	[15]

anaerobic co-processing of cow fertilizer which enhanced the cellulose degradation up to 80% [16, 17]. *Saccharomyces cerevisiae* is one of the basic microorganisms utilized in ethanol creation since it is equipped for delivering higher amounts of ethanol, higher resistance of ethanol, and capability of maturing wide scope of sugars. There are quite a few problems in yeast-mediated fermentation, for example, higher temperature, higher ethanol fixation, and the capacity to ferment pentose sugars. Yeasts have the ability to straightforwardly turn basic sugars into ethanol. The normal forms included in ethanol creation are pretreatment, hydrolysis, and fermentation. Bioethanol while fermentation relies upon a few factors, for example, temperature, sugar fixation, pH, aging time, fomentation rate, and size of the inoculum. The efficiency of the bioethanol being produced can be improved by immobilization of the yeast cells [18]. The natural environments of yeasts are highlighted in Tables 17.2 and 17.3 consists of the biological pretreatment techniques for LCB and corresponding benefits.

17.3 Biological Pretreatment

The hemicellulose and cellulose parts of LCB have tremendous ability to act as feedstock in bioethanol production. This is restricted due to the complex structure of LCB where lignin act as a barrier for the hydrolysis, saccharification, and fermentation

Table 17.3 Biological pretreatment techniques for lignocellulosic biomass and corresponding benefits.

Sl. No.	Biomass	Microorganisms	Effects	References
1.	Corn stalks	*Irpex lacteus*	Hydrolysis yield is 82%	[22]
2.	Corn stover	Fungal consortium	Removal of lignin by 43.8%	[23]
3.	Corn stover	*Ceriporiopsis subvermispora*	Two to three-times increase was observed in reducing sugar yield	[24]
4.	Bamboo culms	*Punctualaria* sp. TUFC20056	Removal of lignin by 50%	[25]
5.	Plant biomass	Fungal consortium	Total termination of use of hazardous reagents	[26]
6.	Straw	Fungal consortium	Seven times increment in hydrolysis	[27]
7.	Wheat straw	*Ceriporiopsis subvermispora*	Minimum cellulose loss	[28, 29]
8.	Eucalyptus grandis saw dust	*Pleurotusostreatus*/ *Pleurotus pulmonarius*	Twenty times increment in hydrolysis	[30]

steps during production process. To overcome this hurdle, breakdown of the lignin is essential to alter the LCB backbone and allow further bioprocessing of the feedstock [31]. The process of biological pretreatment of LCB prior to its enzymatic saccharification holds promise as an eco-friendly and cost-effective method. This avoidance of the formation of inhibitory substances and subsequent removal of antimicrobial agents present in the substrate without significant energy consumption renders the technique immensely beneficial, as compared to the various pretreatment processes conventionally applied in industries [20, 32, 33].

17.3.1 Potential Microorganisms Involved in Lignin Degradation

17.3.1.1 Lignin Degrading Fungi

Various studies on filamentous fungi have revealed that white- and brown-rot fungi can degrade LCB effectively and are widely employed in the pretreatment process. White-rot fungi are known to actively participate in the breakdown of cellulose, hemicellulose, and lignin. Brown-rot fungi on the other hand are restricted to the cellulose and hemicellulose fractions with minimal effect on lignin. These fungi are known to be the potential degraders of bermuda grass, beech wood, bamboo culms, oak wood, and red pine [34–36] as highlighted in Table 17.4. White-rot fungi are frequently used for lignolytic pretreatment as whole cell microorganisms. These fungi possess an intricate lignolytic system that secretes extracellular enzymes that effectively metabolize lignin.

Table 17.4 Fungi and bacteria participating in biological pretreatment of LCB.

Sl. No.	Microorganism	Category	Substrate	Incubation (d)	Lignin degradation (%)	References
1.	*Ceriporia lacerata*	White-rot fungi	Red pine	56	13	[37]
2.	*Ceriporiopsis subvermispora*	White-rot fungi	Corn stover	42	39.2	[37]
3.	*Echinodontium taxodii 2538*	White-rot fungi	Bamboo culms	28	24	[37]
4.	*Irpex lacteus*	White-rot fungi	Corn stalks	11.48	15	[35]
5.	*Pleurotus ostreatus*	White-rot fungi	Beech wood	120	56.5	[38]
6.	*Phaenerochaete chrysosporium*	White-rot fungi	Cotton stalks	30	40	[38]
7.	*Phlebia* sp. MG-60	White-rot fungi	Oak wood	56	40.6	[39]
8.	*Stereum hirusutum*	Brown-rot fungi	Red pine	56	13	[37]
9.	*Trametes versicolor* spp.	White-rot fungi	Bamboo culms	28	9–24	[37]
10.	*Acinetobacter* spp.	Actinobacteria	Poplar wood	30	47–57	[40]
11.	*Pseudomonas* spp.	Proteobacteria	Poplar wood	30	40–52	[38]
12.	*Pseudomonas* spp.	Proteobacteria	Kraft lignin	52	39	[38]
13.	*Streptomyces badius*	Actinobacteria	Inulin lignin	35	3–4	[37]
14.	*Streptomyces cyaneus*	Actinobacteria	Barley straw	21	29–52	[31]
15.	*Streptomyces virifosporus*	Actinobacteria	Induline lignin	35	3–4	[37]
16.	*Thermomonospora mesophila*	Actinobacteria	Barley straw	21	36–48	[37]
17.	*Xanthomonas* spp.	Proteobacteria	Poplar wood	30	39–48	[39]

Phanerochaete chrysosporium is the most established prototype for studying the lignin degrading properties of white-rot fungi. Shi et al. [38] evaluated the activity of *P. chrysosporium* on cotton stalks to illustrate the efficacy of microbial pretreatment to promote hydrolysis and fermentation facilitating bioethanol production under two culture conditions: submerged cultivation and solid-state cultivation using untreated stalk as control. It showed that about 28% of lignin was removed from the substrate along with a significant amount of cellulose after 14 days of pretreatment. This shifted the focus on fungal pretreatment using various other fungal strains such as *Ceriporiopsis subvermispora*, *Cyathus stercolerus*, *Pleurotus ostreaus*, *T. versicolor*, *Phlebia subserialis*, *Sternum hirsutum*, *Gloeophyllum trabeum*, and *Echindodontium taxodii* [39] to promote selective lignin degradation. However, the carbon–carbon bonds present within large lignin polymers pose a challenge to lignin degradation.

17.3.1.2 Lignin-Degrading Bacteria

Bacteria play a major role in nutrient cycling and plant biomass degradation in the terrestrial ecosystem. Many bacterial species belonging to the group of Actinobacteria, Proteobacter, and firmicutes play an essential role in lignin degradation in feedstock. *Streptomycetes*, a genus in the Actinobacteria group, are most common and largest contributors in the biological treatment process. Over 500 species of *Streptomycetes*, mainly *Streptomycetes viridosporus*, *Streptomycetes flavirens*, and *Streptomycetes Cyaneus*, have been reported to actively participate in the degradation of synthetic lignin, kraft lignin, aromatic dyes, and plastic with decrease up to 52% lignin content with 21 days of treatment in some cases. Studies on *Psudomonas* spp. and *Thermospora* spp. have shown similar results using popular wood and barley straw, respectively [39]. Bacterial strains often act selectively on different lignocellulosic substrates but have greater potential for pretreatment owing faster growth rate as illustrated in Table 17.4.

17.3.2 Mechanism Involved in Delignification

White-rot fungi are capable of disintegrating complex carbon–carbon bonds and mineralize lignin by depolymerizing enzymes. Various experiments conducted to study the action of fungi on ^{14}C-labeled lignin measure the $^{14}CO_2$ generated after its disintegration. Pyrolysis gas chromatography–mass spectroscopy (GC–MS) analysis revealed potential change in the ratio between *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignin units by action of the fungal-derived lignolytic enzymes [31]. It was reported that 10 U of fungal peroxidase per mg of straw reduced the quantity of phenolic H units compared to 31% in control to 3% in the treated straw, the G units from 40% to 4%, and completely eliminated the minute quantity of phenolic S present in the treated straw. This revealed that the sensitivity of lignin units to fungal degradation is in the following order: S > G > H.

The mechanisms involved in the delignification of the lignocellulosic substrate by fungi can be broadly classified into two groups: hydrolytic and oxidative types. Oxidative mechanisms involve action of free radicals of reactive oxygen species, primarily hydroxyl ions generated as a result of iron reacting with hydrogen peroxide, from fungal enzymes like aryl-alcohol oxidase, glyoxaline oxidase, and pyranose-2 oxidase on lignin, breaking it into low molecular weight products. Oxidative mechanism is also achieved by hydrolytic breakdown of hydrogen peroxide mediated by manganese peroxidase (MnP) and laccases which leads to oxidation of Mn^{2+} to Mn^{3+}. Hydrolytic type mechanism involves hydrolytic enzymes that aid in breakage of the glycosidic linkage in the complex lignocellulose structure.

17.3.3 Enzymes Involved Biological Pretreatment

Microorganisms are commercially important due to their versatility in enzyme production. Complex and multiple enzyme production systems permit them a higher level of delignifying capacity. The following sections deal with different enzymes and mechanisms of their action involved in the pretreatment process.

17.3.3.1 Lignin Peroxidase

Lignin peroxidases (LiPs) (EC 1.11.1.4) were discovered upon culturing the white-rot fungus *P. chrysosporium* in medium deficient in nitrogen. These are glycosylated enzymes made up of about 360 amino acids with molecular mass ranging from 38 to 50 kDa coupled with two calcium ions and one heme group. Extraction of LiP has been reported from different strains of white- and brown-rot fungi such as *Phlebia flavido-alba*, and *T. versicolor*, *Aspergillus* species and bacterial strains of *Streptomyces*, *Acinetobacter*, and *Calcaceticus*. LiPs are present in the peripheral region of the cell where they assist delignification occurring in the outer side by engaging in substrate interaction at heme edge and glutamine 146 site and subsequently leading to hydrolysis of the compound. This depolymerization is a H_2O_2-dependent process involving multiple steps including formation of oxo-ferryl intermediate followed by electron reduction of the intermediates and non-enzymatic hydrolysis of the radical generated previously.

17.3.3.2 Manganese Peroxidase

Manganese peroxidases (MnPs) (EC 1.11.1.13) primarily oxidize the phenolic rings of lignin in a manganese-dependent reaction. The reaction yields phenolic compounds such as 2,6-dimethyloxyphenol syringol, 3-ethylthiazoline-6-sulfonate, and guaiacol, as well as alcohol and other non-phenolic compounds. The enzyme itself is composed of 350 amino acids with a molecular weight of 40 kDa. It is commercially extracted from *P. chrysosporium*, *Ceriporiopsis* sp., *Schizosporium* sp., *Lentinula edodes*, *Dichomatius squalens*, and *T. versicolor*. The production of MnPs greatly varies between microbial strains and species, nutritional source, and presence of the aromatic moiety. The enzyme oxidizes Mn^{2+} to Mn^{3+}, which then reacts with phenol rings, resulting in phenoxy radicals that degrade the compounds.

17.3.3.3 Laccases

Laccases (Lac) (EC 1.10.3.2) are benzenediol oxygen oxidoreductases. Chemically, the enzyme is a glycoprotein with multiple copper (Cu) catalytic cores, with a molecular weight between 60 and 80 kDa. The presence of Lac is widely studied in both fungi and bacteria. *Pycnoporus* sp., *Myceliopthora* sp., *Trametes* sp., *Pleurotus* sp., *Bacillus* sp., *Haloferax* sp., and *Streptomyces* sp. are few industrially used microorganisms for the production of Lac. This enzyme facilitates oxidation of phenolic compounds, where oxygen serves as an electron acceptor. The Cu atoms are arranged in three different ways leading to the emergence of diverse groups: blue copper core or type 1, normal Cu core or type 2, and binuclear copper core or type 3. This organized structure participates in lignin degradation involving a series of steps: (i) lignin oxidation by reduction of copper, (ii) electron released in step 1 is transferred to two groups of Cu atom, and (iii) oxygen is reduced to water at the core of type 3 and type 2 Cu [33]. Laccases can potentially oxidize heterogeneous substrates as well as aromatic diamines, polyphenols, and methoxy-substituted compounds by creating a split between Cα–Cβ and alkyl–aryl bonds [8].

17.3.3.4 Versatile Peroxidase (VP)

Versatile peroxidase (VP) (EC 1.11.1.16) possesses catalytic activities of both MnP and LiP. Hence, effectively oxidize both phenolic and non-phenolic aromatic compounds. Like MnP, it is able to oxidize Mn^{2+} and have high-redox potential. VP is used in conjunction with other microbial peroxidases including MnP and LiP to degrade non-phenolic aromatic compounds, such as the breakdown of veratyl alcohol or veratryl glycerol β-guaiacyl ether to veratraldehyde, and the decomposition of *p*-dimethoxybenzene to *p*-benzoquinone. The enzyme is found in different strains of *Bjerkandera* sp. and *Pleurotus* sp. and mediates oxidation of variety of substrates including substituted phenols, plant peroxidase hydroquinone's, and the bulky, recalcitrant lignin, directly without redox mediators, the property makes it a suitable catalyst for pretreatment processes.

17.4 Enzymatic Hydrolysis

Cellulose along with hemicellulose within the LCBs is converted to fermentable sugars through a procedure known as hydrolysis [41]. In view of the pretreatment strategy, hemicellulose might either be totally hydrolyzed into monomeric sugars and changed into ethanol in due course of fermentation, or it could be changed into oligosaccharides on experience of inadequate depolymerization furthermore, require hydrolysis before exposure to fermentation. The predominant molecules in hemicelluloses are xylose found in hardwoods and mannose found in softwoods and alongside a limited quantity of arabinose and galactose [40]. Indeed, in spite of the fact that a little segment of cellulose may likewise be changed over into glucose, a large portion of this mass still stays unreacted [42].

Enzymatic hydrolysis incorporates the preparation procedures that helps in conversion of the starch molecules into monomeric sugars. Cellulases at pH of 4.5 and temperature of 50° are generally required. A few proteins like swollenin become vital toward the release of the cellulosic fibrils from the cellulose molecule in a non-hydrolytic manner, without acting upon the β-(1,4) glycosidic bonds. The cellulose conglomerations are therefore scattered by the swollenin, resulting a higher accessibility of cellulases to the cellulose chains [31].

The enzymatic hydrolysis is dependent on how accessible cellulose is to the cellulase enzyme, as well as the viability of cellulose. A significant body of research pointed toward a concrete association between the adsorption of the compound and the speed of hydrolysis. The expulsion of xylan, with respect to the evacuation of lignin, affects the activity of cellulases on cellulose. Although xylan or lignin evacuation improves saccharification rate, the xylan expulsion legitimately affects glucan chain reaction. Henceforth, expulsion of xylan is better than expulsion of lignin. Expulsion of xylan helps in decreased compound restraint by xylo-oligomers just as decreased necessities of frill enzymes. Cellulose is the most abundant polymer of glucose. Its specific structure favors the polymer chains to be firmly stuffed that are insoluble and impervious to depolymerization [20]. The other segment of LCB incorporates hemicelluloses, an extended polymer of glucose subbed along

with arabinose, xylose, galactose, fucose, mannose, glucose, or glucuronic acid [32]. Hemicellulose and the cellulose microfibrils fashion the hydrogen bonds, providing the auxiliary spine to the plant cell divider [33].

17.4.1 Hydrolysis of Polysaccharides

17.4.1.1 Cellulose and Hemicellulose Degrading Enzymes and Mechanisms

Cellulases catalyze the breakdown of β-(1,4) linkages within cellulose. Conversion of cellulose to glucose requires three separate catalysts – cellobiohydrolase, endoglucanase, and β-glucosidase. While the endoglucanases hydrolyze the β-(1,4) glycosidic linkages present in the cellulose chain, cellobiose is converted to glucose by the β-glucosidase, leaving the cellobiohydrolase to remove the individual cellobiose units from the terminal end of the chain [36]. Cellulases have a sugar-attaching region which is associated with the enzymatic area by an adaptable linker. All these modules play a vital role in associating the enzymes with cellulose, thereby enhancing the enzymatic activity of cellulase [38].

Hemicellulose being a branched polymer is made up of a mixture of monomeric units of sugar and glucose. The most prevailant hemicelluloses are xylan, which is made up of pentose units, such as xylose. Enzymes called xylanases catalyze the hydrolysis of xylan. For the complete hydrolysis of xylan, the activity of different xylanases, each with different specifications and activities, is essential [31]. Softwood hemicelluloses are essentially made out of arabinogalactans, glucomannans, xyloglucans, and arabinoglucuronoxylans; hard woods are mostly made out of xylans and glucomannans [39].

Microorganisms used for the commercial production of xylanase on a large scale include *Bacillus* sp., *A. niger*, *Humicola insolens*, and *T. reesei*.

17.5 Fermentation

17.5.1 Microorganisms Involved in Fermentation

The most well-known and broadly utilized microorganism for bioethanol formation is a yeast (*S. cerevisiae*), appropriate for LCB fermentation. It can effectively ferment hexa carbon sugars, be that as it may, barely pentoses because of the absence of proteins that transform xylose to xylulose. The basic bacterial species utilized for bioethanol formation is *Zymomonas mobilis*. Some thermophilic anaerobic microscopic organisms, for example, *Clostridium thermohydrosulfuricum*, *Thermoanaerobacter ethanolicus*, *Thermoanaerobium brockii*, *Thermoanaerobacter mathranii*, and *Clostridium thermosaccharolyticum*, have been examined for bioethanol production. Despite the fact that most microscopic organisms have a wide substrate run, ethanol is once in a while the single item of their digestion that makes downstream processing challenging of ethanol product recovery.

17.5.2 Fermentation Process

Post–pretreatment and hydrolysis of LCBs, basic monomeric sugars are delivered because of depolymerization of the individual components cellulose and hemicellulose that are then fermented by important microorganisms. The general procedure is alluded to as fermentation. Ethanol maturation should be possible either by solid state fermentation or submerged fermentation. Water is a significant fluid in submerged fermentation that is utilized to make fermentation. Dampening of LCB is done in a solid-state fermentation. The most widely recognized commercial process of ethanol formation is submerged fermentation. A novel innovation concentrated to increment xylose utilization by *Pichia stipitis* gave an increment in bioethanol yield by 20–51%. Fermentation is carried out in different modes namely batch, fed–batch, and continuous fermentation. It depends on kinetic characteristics of microorganisms causing fermentation and different types of feedstocks. Batch culture is primarily carried out in contained culture environment by inoculating fermenting microorganisms into a primary fermentation media having calculated amounts of nutrients and is set to ferment till the total depletion of the nutrients. Batch culture is fermentation at its simplest where no supplementation is given after inoculation apart from acid or alkali for maintenance of pH. Microorganisms have been found to worn in high substrate concentration initially in batch mode of fermentation. It also yields higher amount of bioethanol as a product.

Fed-batch systems are broadly utilized to make ethanol for commercial purpose. In fed-batch mode, the microorganism is on low substrate feeding but it yields higher amount of bioethanol. Fed-batch cultures are known to obtain higher yield with respect to batch cultures for the generation of microbial metabolites. Primary benefit of fed-batch over batch mode of fermentation is its potential to obtain maximum viable cell concentration, allow product accumulation, and prolong culture lifetime. The rate of feed flow limits the yield portions of fed-batch modes. Critical process variables like pH, temperature, and dissolved oxygen at definite levels maintain the optimum operation of fed-batch systems. Different kinds of bioreactors are suitable for continuous fermentation like plug flow reactors or stirred tank reactors. It gives a higher product yield, highest productivities being obtained at lower dilutions. The feed contains the nutrients for microbial growth, culture medium, etc. which is pumped into a vessel continuously which has agitation system installed to ensure equal distribution of nutrients.

17.5.3 Product Recovery of Bioethanol Post Fermentation

Distillation and distillation combined with adsorption are the two techniques by which bioethanol is recovered from the fermentation broth [42]. The first step for ethanol discovery is distillation of the fermentation broth to separate ethanol from the broth water to obtain a concentration of 95%, and the residual liquid which contains residual lignin, hemicellulose, unreacted cellulose and ash, organisms,

Table 17.5 List of microorganisms, biomass, and ethanol yielded.

Sl. No.	Microorganism	Bioethanol yield (%)	Biomass	References
1.	*Pichia stipitis* NRRL Y-7124 *Pichia stipitis*	0.35 g/g yield 0.41 g/g yield	Wheat straw	[20, 33]
2.	Genetically modified organism, *E. coli* KO11	91.50% yield, 3.15% (w/v) ethanol titer	Bagasse	[20]
3.	*Candida shehatae* NCL-3501 *Saccharomyces cerevisiae* ATCC 26603 *Pichia stipites* NRRL Y-7124	0.45 and 0.5 g/g of sugar taken up, production from auto hydrolysate by free and immobilized cells in 48 h. 0.37 and 0.47 g/g of sugar taken up, production from acid hydrolysate by free and immobilized cells in 48 h. Ethanol production was 4 g/l Ethanol production was 6 g/l	Rice straw	[25]

enzyme, and other components are sent for wastewater treatment. Different organisms tend to yield different quantities of ethanol with respect to fermentation of specific substrates. The ethanol yields by microorganisms from diverse substrates are listed in Table 17.5.

17.6 Conclusion and Future Prospects

Many researchers have independently reported the potential role of different fungi and bacteria in the bioprocessing of LCB to bioethanol. However, their large-scale implementation in industries is limited because of high cultivation time and selective performance of microbes to substrates. Recent advancement in biotechnology has opened doors for application of genetically engineered microbial strain to manipulate their biosynthetic pathways for rapid production of enzymes in short span of time. These enzymes can also be tailored to produce effective results increasing the yield. Microbes can also be engineered to overcome the toxic and inhibitory substances produced during the fermentation process by activating their survival mechanisms through altered stress response genes, membrane proteins, and heat shock proteins. Efforts can be made to study the interactions between potential microbes and their use as a consortium in depolymerization and saccharification process. The recalcitrant nature of LCB for hydrolysis, release of inhibitory substances, and long resident time has negatively impacted the bioethanol production. However, with proper selection of microbes and optimized techniques, application of microbes in bioethanol production can be commercialized.

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18

Advancements in Bio-hydrogen Production from Waste Biomass

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18.1 Introduction

Fossil fuel-driven energy is prevailing for decades and has led to enormous industrial and economic development across the globe. But, the energy demands with the growing population of the entire world have led to a fast depletion of fossil resources, resulting in an energy crisis in the coming years. Moreover, fossil-based energy is nonrenewable and has adverse effects on climate change due to the emission of toxic greenhouse gases [1]. In recent decades, renewable energy has gained much attention with enormous efforts for the transition from fossil fuels to alternative sources of energy. It has further motivated the scientific community and the policymakers to investigate, develop, and promote new technologies based on renewable energy. In recent years, bioethanol and biodiesel have received substantial interest as an alternative energy source due to their similar characteristics to conventional liquid fuels, which makes them compatible with the existing engine configurations. Hydrogen is another promising alternative clean fuel with high energy and zero waste as it produces only water as the by-product in combustion. The energy content of hydrogen is 141.9 MJ/kg, which is around three times higher than other conventional fuels (gasoline 47 MJ/kg and diesel 45 MJ/kg). Therefore, H_2-fueled vehicles have a promising future due to zero pollution and high efficiency. As a result, the H_2 fuel has made a place at the top in the race of green fuels discovery.

Currently, the commercial production of H_2 gas is being performed by both chemical and biochemical methods. Around 95% of the world's H_2 is either produced by a catalyst-based chemical route from biomass or fossil fuels or by electrolysis of water producing hydrogen and oxygen [2]. The catalytic route of H_2 production involves

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Table 18.1 Comparison of the catalytic and biological route of hydrogen production.

S. No.	Catalytic route (steam reforming)	Biological route (fermentation)
1.	It is an endothermic reaction and requires high temperature (800–1000 K) and pressure (10–30 bar). The control of this high temperature is a difficult task, and it adds to the operational cost and capital cost of the reactor	It can be operated at ambient temperature and pressure (upto maximum 40 °C)
2.	The impurities present in feedstock (water, ash, or lignin) may affect the chemical reaction and lead to the decrease in the overall cost of the process, as the refining stage of would be eliminated	The impurities present in feedstock do not have any adverse effect on hydrogen production. Some impurities such as methanol, fatty acids, and salt are reported to be beneficial for the growth of microorganisms
3.	The cost for reactor design and catalyst is higher, making the process less economical	The zero cost of biological catalyst and modest reactor design makes it an efficient process
4.	The selectivity of the steam reforming process is low due to the side-products such as methane, which hinder the production as well as the purity of hydrogen	The side-products of the fermentative route are butyric and acetic acids, which are a part of the pathway and are further utilized to form butanol and ethanol, which are also alternative fuels
5.	The process also deals with the formation of coke/carbon during the process. This carbon/coke acts as a poison and clogs the pores of the catalyst and hence deactivates the catalyst, thus affecting the process as well as the yield and purity of hydrogen	No such coke is formed in this process

Source: Adapted from Sarma [3].

various processes such as gasification or partial oxidation, supercritical water, thermal, and steam reforming of hydrocarbons. But, these catalytic routes of H_2 production are fossil fuel-based and have limitations, which have paved the way toward the search of biological routes of H_2 production. Table 18.1 depicts the advantages of the biological route over the catalytic conversion of H_2 production [3]. Biological routes are based on microbial pathways that have the ability to metabolize various carbon sources through a series of enzymatic reactions to produce biohydrogen and other value-added products. These microbial entities act as cell factories containing various pathways specific for the production of targeted products. A wide variety of renewable feedstock serves as the source of carbon for the growth of these microbial factories that can be channelized toward the production of biohydrogen. It includes feedstock such as biomass, wastewater, food waste, microalgae biomass, etc.

This chapter outlines various routes of biological H_2 production, substrates, and utilization of various biomasses as feedstocks for biohydrogen production. It also provides information on different process parameters affecting fermentative H_2 and strategies to enhance its production.

18.2 Routes of Production

18.2.1 Biophotolysis

This route of biological hydrogen production is predominant in photosynthetic organisms such as green algae and cyanobacteria, which utilizes enzymes as catalysts to split water in the presence of sunlight. Two specific hydrogenases catalyze these reactions, viz. Fe–Fe *hydrogenase* in green algae and *nitrogenase* in cyanobacteria. Based on the mechanism of H_2 evolution, biophotolysis is further categorized as direct and indirect biophotolysis. During direct photolysis, the electrons generated by water splitting in the presence of light are transferred to photo-system II (PS II), PS I, and finally to the ferredoxin (Fd) as shown in Figure 18.1a. Reduced Fd acts as an electron carrier and reduces *hydrogenase* to produce molecular H_2 [4]. Indirect photolysis is characterized by the conversion of light to biochemical energy, which is stored in cells in the form of carbohydrates, which is later used for H_2 production. *Nitrogenase* enzyme present in the cyanobacteria catalyzes the H_2 production reaction simultaneously with the reduction of N_2 to ammonia.

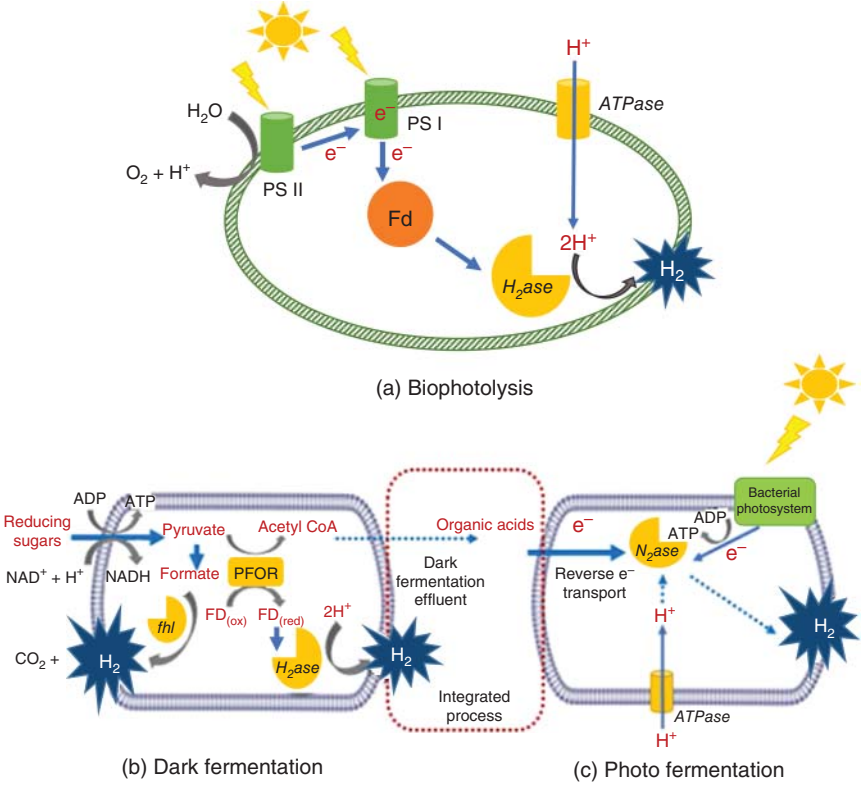

Figure 18.1 Mechanism of biological routes of hydrogen production. (a) Biophotolysis; (b) Dark fermentation; (c) Photo-fermentation. Fd: Ferredoxin; H_2ase: hydrogenase; PS: photosystem; PFOR: pyruvate ferredoxinoxidoreductase; N_2ase: nitrogenase.

18.2.2 Dark Fermentation

The dark fermentation is advantageous over light-dependent routes in terms of a high rate of hydrogen production, utilization of various organic substrates, and the presence of a diverse microbial catalyst. It is a complex process carried out in an anaerobic environment manifested by a series of enzymatic reactions. The key enzymes that drive this process in diverse groups of bacteria are mostly Fe only and Fe–Fe *hydrogenases*. Both facultative and obligate anaerobes produce H_2 through dark fermentation, but the mechanism driving the H_2 producing pathway is different. Both types of anaerobes consume organic carbon redirecting carbon flow toward pyruvate. In facultative anaerobes, pyruvate is transformed to acetyl CoA and formate catalyzed by pyruvate formate lyase followed by reduction of formate by formate hydrogen lyase to produce H_2. On the other hand, obligate anaerobes transform pyruvate to acetyl-CoA and carbon dioxide, catalyzed by pyruvate ferredoxin oxidoreductase enzyme as depicted in Figure 18.1b [5]. Solventogenesis and acidogenesis are two major phases of product formation during anaerobic fermentation, and H_2 is a primary product of acidogenesis resulting in the formation of volatile fatty acids as a by-product. The yield of H_2 varies based on the type of acids produced during the phase. Acetate as the by-product releases maximum H_2 as 4 mol of H_2/mol of acetate, while the butyrate pathway generates 2 mol of H_2/mol of glucose [6].

18.2.3 Photo-Fermentation

The photo-fermentation route of H_2 production is distinguished by higher hydrogen yield, the use of organic acids as substrates, and sunlight as a source of energy. During the photo-fermentation, organic acids such as acetic acid and succinic acid are metabolized by photosynthetic bacteria to produce nicotinamide adenine di nucleotide (NADH), which reduces nitrogenase via reverse electron transport [7]. The reduced nitrogenase, in turn, uses photosynthetically produced adenosine triphosphate (ATP) to reduce the protons to molecular H_2, as shown in Figure 18.1c.

$$2H^+ + 2e^- + 4ATP \rightarrow H_2 + 4ADP + 4Pi \tag{18.1}$$

Although this process has some distinct advantages, it shows poor light conversion efficiency due to the high energy (ATP) requirements of the nitrogenase enzyme. Moreover, photo-fermentative bacteria cannot directly feed on sugar hydrolysates; instead, they feed on volatile fatty acids, which are major by-products of dark fermentation. Thus, an integrative process of dark and photo-fermentation can be used to produce a generous amount of H_2 from waste biomass.

18.3 Biomass as Feedstock for Biohydrogen

Biomass has recently received much attention as a suitable feedstock for waste to energy conversion, and it can be categorized as:

(i) Agricultural waste includes both agricultural food crop residues and animal wastes.
(ii) Forest residues which encompass all terrestrial trees/shrubs and aquatic plants, wood, and logging residues.
(iii) Municipal waste involves both household and industrial waste and sewage sludge.

All these categorized biomasses are mainly composed of three elements, i.e. cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are polymers of C6 and C5 sugars, respectively. Various researchers have evaluated the direct conversion of raw lignocellulosic biomass to hydrogen using microbial fermentation to avoid cost-intensive pretreatment processes. However, this resulted in very low yield and efficiency, mainly due to the inaccessibility of biocatalyst to sugars embedded inside the lignin and cellulose content of the biomass. It has, in turn, streamlined research toward the application of pretreatment methods for efficient sugar release from lignocellulosic biomass. The pretreatment methods are preferable over lignocellulosic biomass since it improves hydrolysis, solubilization, and recovery of high sugar yields for fermentative H_2 production. Many research groups have investigated various forms of pretreatment, which again depends on the type of biomass used for energy conversion [8]. Several techniques that include mechanical, chemical, enzymatic, thermo-chemical, and thermal processes are used to enhance the solubilization of organic matter. The lignin-rich biomass requires harsh treatment, which includes a combination of a few of these pretreatment techniques for breaking the recalcitrant polysaccharides into small sugar molecules that can be easily metabolized by microorganisms for their growth and hydrogen production. Mechanical grinding and sieving are essential steps of pretreatment of lignocellulosic agricultural and forest residues to produce uniform particle size and increase the surface area for fermentation. This step is followed by a chemical or thermo-chemical pretreatment method using alkali, acids, oxidizing agents, solvents, ionic liquids, and sometimes combined with thermal treatment at different temperatures. Cornstalk waste, which is a major component of agricultural waste, has been used to produce hydrogen after pretreatment with alkali and acid, followed by heat treatment [9]. This resulted in maximum cumulative H_2 yield ranging from 57 to 150 ml-H_2/g volatile solid (VS), which was many-fold higher than the initial value, thus supporting the efficiency of pretreatment methods.

Similar to lignocellulosic biomass, municipal solid waste (MSW) also requires pretreatment for biological hydrogen production. The composition of MSW varies from place to place, and it is the nature and composition of MSW, which significantly affects hydrogen production yields. Numerous studies have been conducted by researchers to evaluate the effect of composition and sources of MSW on hydrogen yield, which concluded that MSW rich in simple carbohydrates or sugars such as food waste and vegetable waste give high hydrogen yields [10]. It is further corroborated that MSW rich in fat and protein act as low-grade feedstock for H_2 production.

18.4 Factors Affecting Biohydrogen

18.4.1 Influence of pH

The pH is a major contributing factor of fermentative hydrogen production as it affects the growth and enzyme activities of hydrogen-producing microbes. Hydrogen is a growth-associated product and is produced during acidogenesis phase, which is marked by the co-production of volatile fatty acids. The pH range of 4–7 is the most preferable for biohydrogen production. However, the optimum values depend upon substrate and inoculum used. Hydrogen-producing microbes generally require acidic pH for their growth and metabolism as the hydrogenase enzyme has higher catalytic activity in a pH range of 5–6. Most of the studies on biohydrogen are carried out in batch mode, where only the initial pH is monitored and optimized. However, the optimum value obtained for each study varies with other parameters used in the fermentation process. For example, the optimal pH for H_2 production reported by using organic waste as inoculum such as compost, anaerobic sludge, municipal sewage, and activated sludge is in the range of 4.5–6.5. Whereas cracked cereals or anaerobic digested sludge is used as inoculum, the reported optimum pH for H_2 production is 8 and 9, respectively [11]. It is also noted that the use of pure cultures such as *Enterobacter cloacae* IIT-BT 08, *Clostridium butyricum* CGS5, *Thermoanaerobacteriumthermosaccharolyticum* PSU-2 as inoculum for H_2 production requires slightly optimum pH of the fermentation medium in the range of 5.5–6.0. In contrast, the use of mixed cultures as inoculum favored an optimal pH of 7 [12]. This is due to the diversity of H_2-producing microbes present in the mixed cultures, which requires different optimal pH for their growth and metabolism; hence, neutral pH is favored for optimum growth of all microbes.

18.4.2 System Temperature

Temperature is another crucial factor that influences fermentative hydrogen production. As the enzyme kinetics depends on the system temperature, an optimum range is essential for higher activities of the enzymes that catalyze the metabolic pathways of H_2-producing bacteria. The optimum range of temperature for H_2 production varies again with the type of inoculum and substrate used for fermentation. The temperature range of 35–40 °C is mostly favorable for microbial H_2 production. The decrease in temperature below 35 °C favors the production of other metabolites such as ethanol, acetic acid, butyric acid, and propionic acid due to metabolic pathway shift, thus favoring the production of co-products of the pathway. However, the optimum temperature range of mesophilic bacteria and thermophilic bacteria was higher and varied between 40 and 60 °C. Batch mode of H_2 production by a thermophilic bacteria, *Thermoanaerobacteriumthermosaccharolyticum* PSU-2, produced 2.53 mol/mol hexose at 60 °C [13]. Yokoyama et al. (2007) also reported 60 °C as the optimum temperature for H_2 production by using cow dung as inoculum, which resulted in a maximum H_2 yield of 743 ml/kg cow dung [14].

18.4.3 Inoculum

Inoculum refers to the microbial catalyst used for fermentative hydrogen production. Size and type of inoculum are the two factors affecting biological H_2 productivity. H_2 production by dark fermentation is driven by both pure and mixed cultures of strict and facultative anaerobic bacteria belonging to family *Enterobacteriaceae* or *Clostridiaceae*. Obligate anaerobes are highly sensitive to atmospheric oxygen and mostly belong to *Clostridium* sps. such as *Clostridium pasteurianum*, *Clostridium acetobutylicum*, *Clostridium butyricum*, *Clostridium thermocellum*, *Clostridium tyrobutyricum*, *Clostridium paraputrificum*. *Obligate anaerobes* are highly efficient for H_2 production compared to facultative anaerobes, which includes *Enterobacter* sps., *Escherichia coli*, *Bacillus* sps. etc. This is mainly due to the existence of broad diversity in enzymes catalyzing the hydrogen-producing pathway in both types of anaerobes. Several authors have investigated the capacity of numerous pure bacterial strains to produce H_2 by using various substrates. Table 18.2 represents various studies on H_2 production by pure cultures with the strain *Clostridium bifermentans* demonstrating highly efficient H_2 yields [15–31]. The yield of 3.29 mol-H_2/mol-glucose has been reported, which is near to the equivalent of the theoretical yield of 4 mol-H_2/mol-glucose [15]. The strains were isolated from landfill leachate sludge and have been reported to favor butyrate-associated H_2 production pathway. On the other hand, another genus of *Clostridium* species *C. acetobutylicum* produces H_2 with a maximum yield of 2 mol-H_2/mol-glucose [20]. Thus, it could be corroborated from these observations that the maximum yield of H_2 varies from strain to strain and widely depends on the sources of isolation, type of substrate utilized, and operating conditions favorable for a particular microbial strain.

Mixed culture comprises naturally developed consortia of microbes, which feeds on various organic wastes or pure carbon sources to produce H_2 as the major metabolic product. Table 18.2 summarizes the various sources of mixed culture which are enriched to produce H_2. Anaerobic sludge, cow dung slurry, sewage sludge, dairy sludge, municipal sewage sludge, and compost are commonly used natural sources of mixed culture for fermentative H_2 production. Mixed culture as inoculum for H_2 production is advantageous over pure culture as it can feed on complex carbon sources like cellulose, food waste hydrolysate, dairy wastewater, corn starch, and other organic wastes. Moreover, H_2 generation from organic wastes using natural consortia is more cost-effective and feasible for industrialization, thereby resulting in energy production along with waste reduction. Depending on the source, mixed cultures may contain both H_2-producing and H_2-consuming bacteria along with methanogens. Therefore, various pretreatment methods are recommended for deactivating the H_2-consuming bacteria and enrich the H_2-producing spore-forming bacteria. Heat shock, acid/base treatment, and freeze–thaw are the most prevalent pretreatment methods applied to enrich H_2-producing bacteria in mixed culture. The range of temperature, duration of heat shock or freeze–thaw cycles, and concentration of acid/base varies and requires optimization to produce maximum fermentative hydrogen.

Table 18.2 Pure and mixed cultures for biological hydrogen production.

Microbial strain	Substrate	Maximum H_2 yield	References
Pure culture			
Clostridium bifermentans WYM	Glucose	3.29 mol/mol glucose	[15]
Clostridium pasteurianum MTCC 116	Crude glycerol	0.627 mol/mol glycerol	[16]
Clostridium pasteurianum CH4	Sucrose	2.07 mol/mol hexose	[17]
Clostridium butyricum CGS5	Xylose	0.73 mol/mol xylose	[17]
Escherichia coli	Glucose	2.0 mol/mol glucose	[18]
Clostridium thermocellum 27405	Cellulosic biomass	2.3 mol/mol glucose	[19]
Clostridium acetobutylicum	Glucose	2.0 mol/mol glucose	[20]
Enterobacter aerogenes	Starch	1.09 mol/mol glucose	[21]
Clostridium paraputrificum M-21	Chitinous wastes	2.2 mol/mol substrate	[22]
Enterobacter cloacae IIT-BT 08	Sucrose	6 mol/mol sucrose	[23]
Mixed culture			
Activated sludge	Alkaline pretreated-sludge	15.6 ml/g volatile suspended solids	[24]
Thickened sludge	Sludge/ryegrass	60 ml/g volatile solids	[25]
H_2-producing sludge	Food waste hydrolysate	85.6 ml/g food waste	[26]
Mixed microflora	Sucrose	3.31 mol/mol sucrose	[27]
Bacillus cereus A1 and *Brevundimonas naejangsanensis* B1	Corn starch	1.94 mol/mol substrate	[28]
Digested sludge	Glucose	3.1 mol/mol glucose	[29]
Cattle manure sludge	Glucose	1.0 mol/mol glucose	[30]
Digested wastewater sludge	Sucrose	6.12 mol/mol sucrose	[31]

18.4.4 Substrates

Like other biofuels, biohydrogen generated by the biological route is also highly influenced by the type of substrate, availability, and cost of substrate. Among all fermentation routes, dark fermentation has received enormous popularity due to its ability to utilize a wide range of feedstock. Hydrogen-producing microbes can metabolize both simple carbons like reducing sugars (glucose/xylose) obtained by pretreatment and saccharification of lignocellulosic biomass and complex substrates like starch, sucrose, and wastewater through dark fermentation. Hydrogen is clean energy, and its production from waste further makes this process an economically attractive concept for large-scale production. Table 18.2 lists various substrates (simple and complex) used by different microbes for hydrogen production. Several studies have demonstrated that it is essential to determine the optimum range of a substrate for fermentative H_2 production, as increasing substrate concentration beyond the optimum range may inhibit the growth of H_2-producing bacteria, which would eventually result in a decrease in H_2 yield. This is because the active sites of enzymes catalyzing H_2 production are saturated with a particular concentration of substrate, beyond which may result in enzyme inhibition or substrate inhibition kinetics.

18.4.5 Type of Reactor

The yield and production rate of H_2 by biological routes highly depend on the vessel used for fermentation. In order to attain the required rate of production, it is essential to operate under controlled ambient conditions, which can be achieved by using an automated bioreactor. Apart from this, reactor configuration and mode of operation also play an essential role in maximizing the production rates. Biohydrogen reactors can be operated under the following modes of operations, viz. batch, fed-batch, and continuous.

18.4.5.1 Batch Mode

The batch process is the most widely used mode of operation for biohydrogen production from various feedstocks operated under a wide range of operating conditions. In the batch process, strain cultivation is carried out in closed vessels, which offers many advantages such as simple configuration, ease in monitoring substrate utilization, and effect of physical factors on H_2 production. Several studies have been reported on batch fermentation for H_2 production by different species of *Escherichia*, *Clostridium*, *Enterobacter*, *Archaea*, *Rhodopseudomonas,* and *Rhodobacter*. Under batch operation, *E. cloacae* DM 11 and *Caldicellulosiruptor owensensis* have reported the highest hydrogen yield ($Y_{H_2/S}$) of 3.9 and 4.0 mol-H_2/mol-substrate, respectively. *Clostridium* is the most studied organism for biohydrogen production via dark fermentation, showing a hydrogen evolution rate of 27 mmol/l/h [32, 33]. Batch fermentation with other species of *Clostridium* reported a maximum H_2 yield of 3.35 and 2.3 mol-H_2/mol-glucose [34]. Under a similar mode of operation, a maximum hydrogen yield of 6.63 mol-H_2/mol-sucrose was achieved by using *Rhodobacter*

by photo-fermentation [35]. Although the literature includes a large number of studies on the batch mode of H_2 production, but it has some disadvantages over other modes of operations. The culture conditions are highly unstable due to the periodic removal of the sample. Moreover, due to the closed system, the removal of extracellular products is not possible, which results in product inhibition by liquid and gaseous metabolites.

18.4.5.2 Continuous Mode

The continuous mode of operation is often favored for biohydrogen production from various strains. Chemostat is characterized by a constant supply of culture medium or substrate to the reactor, which maintains the microbes in the exponential growth phase for prolonged periods and regular removal of product streams. A continuous stirred tank reactor (CSTR) is widely used for both photo and dark fermentation. *Rhodopseudomonas* and *Clostridium* are cultivated in CSTR for photo- and dark-fermentation, respectively [36]. Kim and Lee (2010) have used a microbial consortium in CSTR to obtain 2.3 mol-H_2/mol-glucose, whereas 3.4 mol-H_2/mol-glucose was obtained by using anaerobic sludge [37, 38]. The chemostat mode of fermentation is also used to enhance the yield of biohydrogen from 5.8 to 11.61 mol-H_2/mol-sucrose by varying the pH of the media and cultivation temperature. To further increase the biohydrogen production using CSTR, the use of biofilms or immobilized organisms has been reported previously [39].

18.4.5.3 Fed Batch

The fed-batch mode of fermentation involves the periodic addition of substrate to the reactor, followed by the removal of the product only after completion of a reaction cycle. This mode of fermentation ensures that there is no saturation or substrate inhibition. The literature survey suggested that only a few reports on fed-batch dark fermentative hydrogen production as compared to other modes of H_2 production. This is mainly due to the massive formation of organic acids, alcohols, and other metabolites in the reactor, which lowers the kinetics of the process and also inhibits the growth of the microorganism. The fed-batch fermentation has reported up to 3.1 mol-H_2/mol-glucose using microbial consortium and a yield of 2.15 mol-H_2/mol-substrate using a recombinant strain of *Clostridium* [32]. The potential of the fed-batch process for efficient production of fermentative H_2 needs further detailed investigations in terms of quantity and quality.

18.5 Strategies to Enhance Microbial Hydrogen Production

The earlier studies on the improvement of biohydrogen production have suggested several approaches, such as statistical optimization of process parameters and fermentation medium, reconstruction of a metabolic network by metabolic flux analysis (MFA), genetic engineering of microorganisms, and improved fermentation kinetics by application of ultrasound. This section brings forward the strategies in detail and describes their potential to enhance microbial H_2 production.

18.5.1 Integrative Process

The earlier sections have highlighted various processes involved in biohydrogen production, which varies with the type of microbes involved. Microbial H_2 production has several challenges over chemical processes, and yield or production rate is one such major hurdle of the process. An integrative approach to generate a single-stage hybrid system or two-stage system for H_2 production has recently gained much attention intending to overcome the limitations of single operated processes shown in Figure 18.1b,c. The dark fermentation process is characterized by a massive accumulation of organic acids, which leads to an inhibitory effect on H_2-producing enzymes and the growth of microbes. These acid-rich effluents are high in carbon content and can act as a substrate for further energy recovery through a two-stage process. The dark fermentation effluents generated in the first stage is processed by the second stage and can be used for methanogenesis for methane production or H_2 production through photo-fermentation, microbial electrolysis cells (MECs) for H_2, MFCs for bioelectricity, bioplastic production, and heterotrophic algae cultivation for lipids [40–44]. These integrated processes are involved in the efficient valorization of waste effluents for additional energy production or other value-added products. This makes the integrative approach more economically feasible and practically applicable to industrial scales.

18.5.2 Medium and Process Optimization

Fermentative hydrogen production is influenced by several factors that have been discussed in detail in Sections 18.4 of this chapter. The composition of the fermentation medium is very crucial for the activity of enzymes catalyzing H_2 production, like pyruvate ferredoxin oxidoreductase, hydrogenase, formate hydrogen lyase, and pyruvate formate lyase. The fermentation medium is a source of nutrients essential for the growth and metabolism of the microorganisms, which includes sources of carbon, nitrogen, metal ions, and other trace elements. However, an optimum range of these nutrients is necessary for efficient strain cultivation and production of metabolites because a higher or lower range may lower the fermentation kinetics, thereby reducing the product yield. Similarly, an optimum range of operating conditions is also necessary to be maintained throughout the fermentation process, which includes temperature, pH of the medium, substrate concentration, and inoculum. Optimization of both the process parameters and medium components is, therefore, also essential for biological H_2 production. Several studies have investigated the effect of these factors on the yield of H_2 production and estimated the optimum range of factors required for maximum production [16]. The co-factors and the enzymes of all microbes are mostly active in an optimum pH and temperature range; therefore, parameter optimization is the key technique to obtain maximum yields. Different experimental design methods like central composite design (CCD), Box–Behnken (BB) design, full factorial design, Plackett–Burman design, Taguchi design, and one-variable-at-a time (OVAT) design can be used to evaluate the optimum range and the effects of various parameters. Response

Surface Methodology (RSM) has been reported in several optimization studies for H_2 production as it is a standard method to assess the individual and interactive effects of the variables with minimum error. It can be inferred from the literature that for H_2 production, the major processes for optimization have been done using Plackett–Burman design followed by Central Composite Design/Box–Behnken design. Plackett–Burman applies a first-order polynomial model for studying the effects of different parameters based on experimental results. It is a two-level fractional factorial design used to select the main parameters for further analysis. CCD and Box–Behnken (BB) designs are the second-order polynomial model used for estimating the relationship between the major factors and the response and obtaining optimum values. Contour or surface plots can be used to display this second-order polynomial. The significant factors can be determined by using an analysis of variance (ANOVA) of the model.

18.5.3 Metabolic Flux Analysis

MFA plays a vital role in the genetic engineering of microbes as it provides prior information on the effects of targeted genetic modification on microbial growth or target production. It provides an *in-silico*-based evaluation of intracellular fluxes within a metabolic pathway, either to boost the product yield or to analyze the effects of genetic engineering [45]. It thus helps to elucidate the central metabolic pathway by considering the rates of consumption and production of metabolites within a biological network.

Metabolic fluxes are quantified by two model-based approaches ^{13}C MFA and Flux Balance Analysis (FBA). Both methods use thermodynamic, stoichiometric, and experimental constraints to obtain a range of feasible intracellular fluxes within a metabolic system followed by determining the flux distributions across the provided space to optimize the objective function. However, these methods differ in the type of objective function optimized.

Similarly, MFA approach is also necessary to overcome the limitations of lower H_2 yield by the biological route. An extensive investigation and understanding of the biohydrogen pathway in microorganisms are essential, which may provide insights to reconstruct the existing metabolic network toward the maximization of H_2 yield. The intracellular consumption and production rates of metabolites (fluxes) could be analyzed by solving the mass balances of metabolites. MFA approach has been widely used in most research to maximize the production of various products such as acetate [46], lysine [47], and ethanol [48]. Literature reports many articles on MFA of H_2 production by *C. butyricum* W5, *Clostridium thermosuccinogenes*, and *C. acetobutylicum* [49]. It was well presented by Oh et al. (2008) that H_2 production can be maximized to 8.7 mol-H_2/mol-glucose if glucose flux is redirected toward the pentose phosphate pathway in *Citrobacter amalonaticus* Y19 [50]. Cheng et al. (2013) reported MFA application of *C. tyrobutyricum*, which revealed that hydraulic retention time (HRT) significantly affects the flux toward H_2 [51]. A similar report exists on MFA application in *C. butyricum* W5, which suggested that pH has a significant impact and initial glucose has less effect on H_2 production.

18.5.4 Application of Ultrasonication

The literature survey showed that there are rare studies on the enhancement of biohydrogen production by the application of ultrasonication. The majority of these studies have focused on the application of ultrasonication in other pretreatment processes like biomass/substrate and inoculum pre-treatment [52]. An increase of 38% H_2 production has been reported with the application of ultrasonication over non-sonicated palm oil mill effluent (POME) pretreatment [53]. For fermentative production of biohydrogen, the application of ultrasonication has been reported only twice. Taguchi method was applied to optimize the ultrasonic intensity and the time of exposure [54]. It was concluded that ultrasonication affects the H_2 production rate and efficiency significantly, triggering 19.11% enhancement in production efficiency under optimal conditions. Other similar reports showed a marked increase in the yield of biohydrogen by 40% and 50% in the consumption rate of glycerol on application of controlled sonication cycles during fermentation [55].

18.5.5 Strain Development

Microbial strain development by metabolic engineering or genetic modification is a promising tool for improving the yield of fermentative H_2 yield by enhancing substrate consumption rates or blocking the production of by-products of the pathway. Metabolic engineering approaches can overcome the limitations related to lower yield either by deletion of competitive pathways or by over-expression of the genes specific to H_2 production. With the help of MFA, researchers have elucidated the role of essential genes involved in the biohydrogen metabolic pathway and other competitive pathways. In recent years, various investigators have reported several ways to increase the yield of H_2 by application of genetic engineering techniques such as over-expression of heterologous or homologous genes, knockout of competitive pathways, and reconstruction of the metabolic pathway, thereby channeling the carbon flow solely toward molecular H_2 production [56]. Also, several genetic engineering approaches have been successfully attempted on the hydrogen production pathway of *E. coli* to boost biohydrogen production by over-expression of hydrogenase3, formate hydrogen lyase, and hydrogenase gene in *C. paraputrificum* [57, 58]. Recent studies on the development of genetic engineering toolkits for efficient H_2 producers such as *Enterobacter* sps. and *Clostridium* sps. have motivated many researchers to explore them for enhanced H_2 production [33, 58, 59]. Sarma et al. (2019) have reported 1.5 times enhancement in H_2 yield compared to the wild-type strain of *C. pasteurianum* by over-expression of hydrogenase and glycerol uptake enzymes [33].

It can be inferred from Table 18.3 that various metabolic engineering strategies have been applied to different H_2 producing bacteria to improve yield and production rates [32, 33, 56, 58, 60–63]. The studies suggested *hydA* as the key gene involved for H_2 production in *Clostridium perfringens*. The deletion of *hydA* gene blocked the H_2 gas production completely in the organism. A comparative study on *C. butyricum* and *C. acetobutylicum* reported higher specific activity of hydrogenase in *C. butyricum,* and negligible hydrogenase activity was reported for *C. acetobutylicum* [60]. It was also reported that lactate and succinate inhibit H_2 production.

Table 18.3 Various genetic engineering approaches used to enhance the biohydrogen production.

Microorganism	Genetic approach	Target gene	Yield (mol-H_2/mol-substrate)	References
Clostridium pasteurianum	Over expression	*hydA* *dhaD1* *dhaK*	1.11 0.93 (0.7 for wild type)	[33]
Clostridium acetobutylicum DSM 792	Over expression	*thl* promoter	1.77 (1.79 for wild type)	[60]
Clostridium acetobutylicum DSM 792	Over expression	*hyd*A	1.81 (1.79 for wild type)	[60]
Enterobacter aerogenes ATCC 13408	Over expression	*hyd*A	2.31 (1.18 for wild type)	[61]
Enterobacter aerogenes IAM1183 Ea	Over expression	*fdh*F	1.16 (0.96 for wild type)	[62]
Enterobacter aerogenes IAM1183 Ea	Over expression	*fhl*A	1.09 (0.96 for wild type)	[62]
Clostridium tyrobutyricum	Knockout	*Ack*	2.16 (1.44 for wild type)	[32]
E. coli W3110 (SR15 mutant)	Knockout	*ldh*A + *frd*BC	1.82 (1.08 for wild type)	[56]
E. coli W3110 (SR14 mutant)	Knockout and over expression	*ldh*A + *frd*BC and *fhl*A	1.87	[56]
Clostridium paraputrificum M-21	Overexpression	*hyd*A	2.4 (1.4 for wild type)	[58]
Enterobacter cloacae IIT BT-08 (A3 mutant)	Knockout	Alcohol dehydrogenase + butadienol dehydrogenase	1.65 (2.16 for wild type)	[63]
Enterobacter cloacae IIT BT-08 (DM11 mutant)	Knockout	Alcohol dehydrogenase + butadienol dehydrogenase + acid-blocking	3.4 (2.16 for wild type)	[63]

Source: Adapted from Sarma [3].

Therefore, deletion of the genes *ldhA*, *frdBC* encoding lactate dehydrogenase and fumarate reductase, respectively, resulted in twofold increments in the molecular H_2 production. The combined approach of over-expression of formate hydrogen lyase activator protein (*fhlA*) and deletion of *frdBC*, *ldhA* caused a further increase in H_2 yield [56]. The lower yield of biological H_2 is due to the negative impact of the organic acids produced by competitive pathways. Disruption of these competitive

pathways producing acids and alcohol redirected flux solely toward H_2 production, thereby resulting in a 1.5-fold increase in hydrogen yield.

18.6 Future Perspectives and Conclusion

Biohydrogen has the enormous potential to develop into a sustainable green and clean energy globally. The major advantage of biohydrogen over other biofuels that projects it as a clean future fuel is that it produces only water as a side-product during energy conversion via combustion or fuel cells. In the last 10 years, numerous researchers and economists have widely explored various aspects of biohydrogen as fuel [64]. Literature reports various articles which emphasized on various factors and technologies to increase the yield of biohydrogen. The conversion of various wastes to energy by H_2 producing microbes is another approach to make the biological H_2 production economical [64]. Optimization of process parameters, medium components, substrate concentration, inoculums enrichment, pathway engineering, bioreactor design, and bioprocess optimization is some of the essential attributes that are being considered to maximize biohydrogen production. Apart from these strategies, there are other critical traits in the process that must be equally studied. Due to environmental stress, microbes mutate at a higher rate, and therefore screening of noble strains for biohydrogen production is very much essential. Development of the genetic toolkit for these non-model microbes is another challenge that needs to be overcome fast with developing modern genome editing tools such as CRISPR Cas9, TALEN, zinc finger nuclease [64]. Moreover, to get insights into the mutations and the key genes to be targeted for increasing the biohydrogen yield, the construction of a genome-scale metabolic model and MFA is necessary [55]. A balanced combination of all these approaches is required to develop a sustainable, economic, and competitive process for biohydrogen production on a large scale, which can meet the current demands of the world energy requirements.

This book chapter has described and assessed every aspect involved during the biological production of H_2. Initially, it has elaborated on the routes or pathways adapted by various microbes for biohydrogen production, and it could be concluded that dark fermentation is the more efficient route of biological production reporting higher yields. It then emphasizes various substrates, including natural organic matter and waste that provides an attractive solution for fermentative H_2 production. The development of efficient bioprocess technology, whether batch, continuous, fed-batch, or two-stage process, is the essential feature to be considered for efficient and large scale production of H_2 via biological route. Various strategies to maximize biohydrogen production have also been assessed critically, providing insights into future research on biohydrogen.

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19

Reaping of Bio-Energy from Waste Using Microbial Fuel Cell Technology

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19.1 Introduction

Microbial fuel cell (MFC) is a bio-electrochemical system that involves conversion of chemical energy into electrical energy with the help of useful microbes. MFC is not highly efficient with respect to electricity generation. According to M.C. Potter, microbes convert the available chemical energy into electrical energy such as power and current [1]. In 1960, MFC became popular from the waste of anthropomorphic. During 1970s, microorganisms were used as catalysts to treat wastewater in fuel cells and MFCs, and these were examined in 1991. On the other hand, enhanced electricity production was providing chance for their commercialization and implementation. Complex substrates and different carbon sources expounded in wastewater were directly converted into electricity in a sustainable power generation technology (MFC) [2]. The components of MFC include electrodes (anode and cathode), membrane (proton exchange membrane [PEM]), microorganisms, energy recording meter, and electrolyte. The Chief components of MFC such as membrane, electrode, and microorganism can considerably influence the cost and performance of MFC [3].

A good numbers of publications regarding MFC are elevating rapidly by virtue of the drawbacks of conventional wastewater treatment and energy production. In the extinct years, MFC had a new source of bio-energy production. It has been widely reviewed in different aspects. They are configurations and designs, microbial communities, electrode materials, electrode surface area changes, potential and real field environmental relevance [4]. MFC is widely used for wastewater treatment and energy production, because the system has more advantages than existing technology. The first merit of MFC is its ability to directly convert substrate into electricity that enables high conversion efficiency. Second, it can exhibit safe and reasonable good performance. Third, it does not require any gas treatment and free from CO_2 generation. Fourth, it operates efficiently in ambient temperature, and it is insensitive to the operational environment. Finally, MFC has the potential

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for pervasive uses in location which lacks electrical infrastructures. This review discusses the notion of MFC, details of microorganisms, electrodes, practical environmental applications, challenges, and future perspectives of exploration in detail.

19.1.1 Effects of Industrial Wastes on Environment

Contamination of water:
- Underutilization of solid waste from tanneries can pollute nearby water sources.
- Tannery fleshes are buried into the land, and it contaminates the groundwater highly due to the presence of calcium salts and sodium sulfide [5].

Contamination of air:
- Burning of cane to speed harvest causes air pollution and increase erosion.
- Bagasse is commonly used as fuel in boilers. It produces fly ash, which escapes into the atmosphere and can affect the population leading to irritation in eyes, nose, throat, and lungs. It has also got the potential to damage crops.

Contamination of soil:
- Heavy infield transport machinery is most commonly associated with soil compaction problems.
- Soil compaction decreases porosity and water infiltration rate, thereby restricting the rooting ability of the crop.
- Conventional tillage commonly promotes erosion by exposing soil aggregation to rainfall and also drastically changes the soil structure.
- Most of the farmers still use the flood irrigation pattern which can cause huge wastage of water, electricity. It results in salinization of the soil which is another important cause of lower productivity.
- Untreated tannery solid waste is toxic to the environment and leads to soil contamination [6].

Impact on field level:
- Erosion is a significant issue in areas under sugarcane or beet cultivation. Erosion rates in tropical agro-ecosystems are usually greater than the rate of soil formation.
- Cane harvesting can cause a significant removal of soil from the roots. Declining soil quality is associated with cane and beet production, due to soil compaction, loss of organic matter, salinization, and acidification. About 10–30% of the total beet harvest weight is soil.

Solid waste management:
- The bagasse when used as fuel in boilers, release particulate matter such as nitrogen oxide and sulfur. If pollution control equipment is not installed, fly ash will escape to the atmosphere and can affect people with a number of health related problems [7].

19.1.1.1 MFC as Energy Source

Industrial, commercial, and agriculture activities are generating massive volume of wastewater every year. To treat this wastewater, huge amount of energy is

Table 19.1 Various designs of MFC and its power densities.

Type of MFC	Fuel	Power density (mW/m^2)
Single chamber	Glucose	766
Single chamber	Domestic wastewater	464
Two chamber	Glucose	860
Two chamber	Acetate	480
Up flow	Sucrose	560
Single chamber	Complex substrate	600
Single chamber	Glucose	355.5
Two chamber H type	Acetate	13
Two chamber H type	Glucose	33.4
Two chamber	Glucose	40.3
Single chamber	Sewage sludge	6000
2-Chamber air cathode MFC	Glucose	283
Two chamber	Marine sediment (acetate)	14
Two chamber	Lactate	52
Two chamber	Ethanol	36
Two chamber H type	Lactose	17.2

required for the treatment process. In conventional wastewater treatment, sludge activation process is largely utilized. Large quantity of sludge produced during the MFC process, increases the plant operation cost, maintenance, and initial establishment process. So, it becomes difficult to operate the wastewater treatment plant in an effective manner. In addition, the waste sludge contains large amount of organic matter and energy. Energy can be converted into electricity by using microorganisms which is a known technique for years. Table 19.1 shows the various MFC designs and their power densities. The cost-effectiveness and sustainable environment can be taken from squandering water. Moreover, the energy generated and converted into electrical power might be used for effluent treatment plant along with water treatment. MFC is a low power consumption process and can reduce the expense of water treatment. Finally, MFC is a good and reasonable technology for reaping energy from organic waste matter compared to other energy harvesters [8].

19.1.1.2 Theory of Microbial Fuel Cell

Basic principle behind the MFC is conversion of organic matters into bio-energy with the help of microbes present in substrate. It incorporates anode and cathode coupled by an external electrical circuit, divided by a membrane shown in Figure 19.1. At anode compartment, the substrate is oxidized by microorganism and produce H^+ and e^- ions [9]. The anode compartment is an anaerobic section (i.e. absence of oxygen) because oxygen should be far from the anode region, and it acts as electron acceptor. The cathode chamber is an aerobic section (i.e. presence of

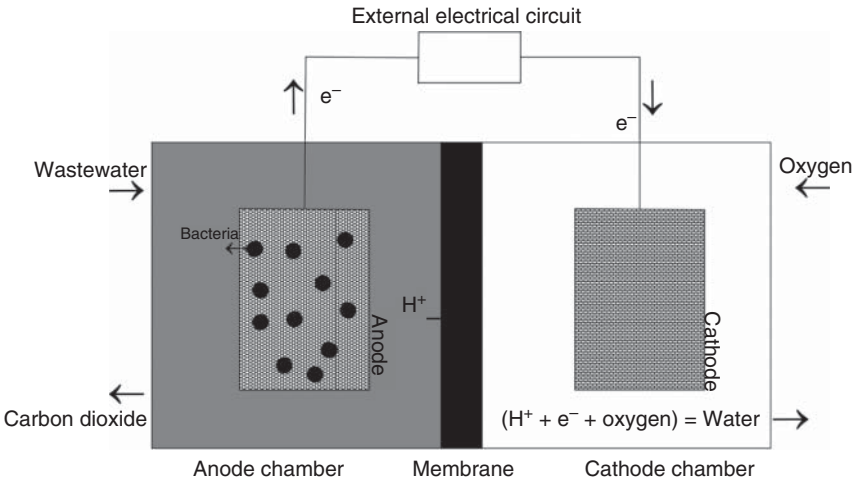

Figure 19.1 Schematic representation of a MFC for bioelectricity production.

oxygen) because oxygen reduction process takes place and it acts as electron donor [10]. In the anode compartment, the degraded organic matters produce protons and electrons by metabolic activity of microorganisms. The generated electrons are transferred from the anode to cathode through external electrical circuit. Protons are passed via membrane to reach cathode and forms water. The MFC is a dual-chambered bioreactor having anode and cathode compartments which is separated by a proton switch membrane. The membrane (PEM) permits the transport of protons from the anode region to the cathode region, by maintaining pH and electro-neutrality. The MFC performance is affected by separators because of its ohmic resistance. The fundamentals of MFC systems are governed in research for scrutinizing the performance of new electrode ingredient. General dual part mode is divided by membrane [11]. The dual-chamber design of MFC would be difficult for nonstop treatment of commercial plants.

The active parts of MFC might be included in simple designs, and effective cost materials will offer more perspective for harnessing the energy from wastewater [12]. Naveen Kumar et al. states that in a single chamber MFC design consist of anode placed at the nethermost and cathode placed at the chief as shown in Figure 19.2 and Table 19.2. This setup reveals that the performance and efficiency can vary according to the type of MFC.

19.2 Microbial Fuel Cell Components and Process

19.2.1 Mechanism Behind MFC

Microbes oxidizing the substrate in the anode chamber produce electrons by metabolic actions. Main electron transfer mechanism is by direct electron transfer (DET) and mediated electron transfer (MET) [13]. Electrons are directly transferred

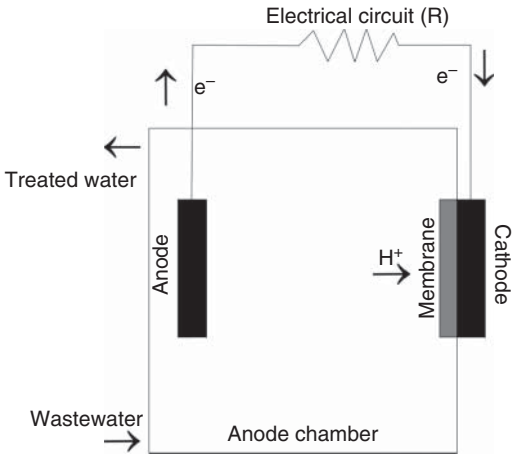

Figure 19.2 Schematic representation of single chambered microbial fuel cell.

Table 19.2 Microbes used in MFC.

Microorganisms	Note
Electricity-producing bacteria with mediator	
Klebsiella pneumoniae	HNQ as mediator
Proteus mirabilis	Thionin as mediator
Gluconobacter oxydans	Mediator (HNQ, resazurin, or thionine) needed
Desulfovibrio desulfuricans	Sulfate/sulfide as mediator
Streptococcus lactis	Ferric chelate complex as mediators
Proteus mirabilis	Thionin as mediator
Escherichia coli	Mediators such as methylene blue needed
Actinobacillus succinogenes	Neutral red or thionin as electron mediator
Electricity-producing bacteria without mediator	
Desulfuromonas acetoxidans	Deltaproteobacteria identified from a sediment MFC
Geobacter sulfurreducens	Generated current without poised electrode
Aeromonas hydrophila	Deltaproteobacteria
Pichia anomala	Current generation by yeast (kingdom Fungi)
Acidiphilium sp. 3.2 Sup5 power	Production at low pH
Thermincola sp. strain JR	Phylum Firmicutes
Desulfobulbus propionicus	Deltaproteobacteria

to the MFC in DET method. In MET, electrons are transferred by electrochemical method, which is possibly metabolized by the microbes. The anaerobic bacteria should act as a biocatalyst in MFC when acting as electron acceptors, by using the way of facultative respiration species [14]. Recently, plenty of research reports related to electrochemically active bacteria (EAB) are emerging. In MFC, several microorganisms produce small amount of energy. With the presence of a mediator, the electron transfer from the bacterial shell to anode shell is made easy. But, many type of microorganisms can employ electron transfer directly from substrate to electrode surface without mediator [15].

19.2.1.1 Electrode Materials in MFC

Electrode material is one of the unique performance in MFC for adhesion of microorganism, electrochemical efficiency, and electron transfer. If the researchers choose to use cost effective materials, the MFC will yield high electricity and possible commercialization of MFC along with the environmental applications. However, the electrode materials should have properties such as stability, durability, electrical conductivity, surface area, porosity, cost effective, and ease of access for effective operation of MFC.

Anode Material Anode materials should have the following qualities such as (i) good electrical conductivity, (ii) low resistance, (iii) chemical stability and anticorrosion, (iv) high surface area, (v) robust biocompatibility, (vi) suitable mechanical strength, and (vii) toughness. Most of the MFC studies are presented with carbon electrode material. Carbon material-based anodes are in several varieties such as carbon cloth, carbon paper, graphite rod, carbon felt, and graphite fiber brush [16]. The simplest carbon anode used in MFC is graphite rod, in the interest of their excellent electrical conductivity and low cost. Initially, materials made of carbon were used in hydrogen fuel cell. Later, these materials were utilized in MFC for better performance while decrease electrode distance and internal resistance. The performance of few non-corrosive metals is assessed, and it does not produce more efficiency than carbon materials.

Cathode Material The vital aspect of MFC is to control electricity generation. Electrode support, catalyst, and air diffusion layer form the cathode in a typical MFC. Generally, in MFC, the anode electrode materials can also be used as cathode electrode material. However, the possible cathode material must have the properties of good electric conductivity, high mechanical strength, and efficient catalytic nature [17]. Commonly, MFC will be operated in Neutral state pH and ambient temperature conditions. At this phase, the oxygen reduction rate is very low. So, it limits the performance of MFC. For vigorous reactions at cathode chamber in MFC, the carbonaceous materials must be revised with additional catalyst. In most of the MFC operations, platinum is placed as a chief element because it plays a major role in maintaining the survival of cathode catalyst which has high efficiency of oxygen reduction rate. By using expensive cathode materials as catalyst in MFC, restricts the commercialization of MFC technology. While using low quality water in MFC

Pt, catalysts become more responsible for fouling. Numerous research attempts have been examined to minimize the cost of cathode catalysts by using effective or cheap material. An attempt has been examined for cathode catalyst material, which is made of metal porphyrines and pthalocyanines supported on Ketjenblack carbon. The investigation explains the rate of oxygen reduction in MFC along with catalytic activity. Since, the transition metal of macro cyclic catalysts is cheap and can be fruitfully applied to practical applications of MFC.

19.2.1.2 Proton Exchange Membrane

Membrane is one of the most important parts of MFC. It is used to split up protons from the anode to cathode chamber. The foremost objective of membrane is (i) dividing the chamber; (ii) transfer the H^+; (iii) to reduce oxygen diffusion in anode chamber; (iv) increase the efficiency of electricity production; and (v) maintain longtime operation terms. A majority of MFC operations uses nafion as membrane because of its high proton conductivity. The difficulty in using nafion membrane as MFCs is that they can cause contamination and more deluxe. Tainted can decrease the proton transport from electroplate to the photo electronic and increase internal resistance of MFC which decreases power output.

The membrane causes potential internal resistance that leads to minimization of power production. Plenty of investigations were carried out in the past and discovered an alternative for nafion membrane as salt bridge [18], porcelain septum, interpolymer cation exchange membrane [19], microporous filter, physical barriers, and sulfonated polyether ether ketone (SPEEK) [20]. The abovementioned are different types of membranes for proton transfer systems. The membrane while performing in MFC will be permeable to chemicals, substrates which are present in the system. In the current scenario, the membrane market is persistently increasing and needs more research or studies for the performance of membrane and longtime stability [21].

19.3 Application of Microbial Fuel Cell to the Social Relevance

MFC is a promising technology for the following fields in our society and helps to make sustainable development of environment.

19.3.1 Electricity Generation

Through catalytic action of microorganisms, MFC can convert the chemical energy into electrical energy. The research on MFCs tilted as bioelectricity production has taken away bountiful wastes since 1988. A comparison between dual chambered and single chambered fuel cells showed that the electricity produced in the last one is high for the same value of voltage. Four cells were connected into one block and tested with plain graphite electrodes. MFCs are the promising devices that can produce electricity by anaerobic fermentation of organic or inorganic matter from

easily metabolized biomass to complex wastewater using microbes as biocatalysts. The application of platinum group metal-free catalysts as air breathing cathode of the MFC helps to activate the sludge, in addition to acetate for the source of carbon energy [22]. A maximum power density of 1.3 W/m^2 (54 W/m^3) is obtained with iron aminoantipyrine catalyst. It is the highly reported type of MFC which is capable of continuous operation in wastewater and shows constancy and enhancement in longtime operation [23].

19.3.1.1 Bio Hydrogen

MFC can be easily modified to produce hydrogen instead of electricity. The protons and the electrons produced by the metabolism of microbes in MFC are thermodynamically unfavorable. It applies an external potential to amplify the cathode potential in a MFC circuit and thus overcome the thermo dynamic barrier. Protons and electrons produced by the anolyte reaction are combined at the cathode chamber to form hydrogen [24]. The external potential for an MFC theoretically requires 100 mV, much lower than the 1110 mV required for direct electrolysis of water at neutral pH. This may be due to the fact that some energy comes from the biomass oxidation process in the anodic chamber. In bio hydrogen production using MFC, oxygen is no longer needed in the cathodic chamber. Thus, oxygen leak to the anodic chamber is no longer an issue in the improved MFC. The main advantage is hydrogen that can be accumulated and stored for the future usage. Therefore, MFC provide a renewable hydrogen source which can contribute to the overall hydrogen demand in a hydrogen economy [25].

19.3.2 Wastewater Treatment

An important application of MFC is treating domestic as well as industrial wastewater. Urban wastewater has a massive amount of organic compounds that can fuel MFC. During the wastewater treatment process, electric power generated potentially bisects the electricity needed in a conventional treatment process. A hybrid technique incorporating both electrophiles and anodophilies is especially suitable for wastewater treatment because organics can be biodegraded by a variety of organic substances [26]. MFC using certain microbes has a special ability to remove sulfides in wastewater treatment. During wastewater treatment, MFC can improve the growth of bio-electrochemically active microorganisms. Continuous flow and single-compartment MFC and membrane-less MFC are favorable for wastewater treatment due to scale-up concerns. Sanitary waste, food industrial wastewater, swine wastewater, and corn Stover are great biomass sources for MFC as they are rich in organic matters. Up to 80% of the chemical oxygen demand (COD) can be removed in some cases and columbic efficiency as high as 80% has been reported [27].

19.3.3 Biosensor

Another potential application of the MFC technology is to use it as a sensor for pollutant analysis and *in situ* process monitoring and control, apart from the

aforementioned applications. The correlation between the columbic yield of MFCs and the strength of wastewater make MFC's possible to serve biological oxygen demand (BOD). A number of works showed a good linear relationship between the columbic yield and the strength of wastewater in a quite wide BOD concentration range [28]. MFC-type of BOD sensors is advantageous over other types of BOD sensor because they have excellent operational stability, good reproducibility, and accuracy. An MFC-type BOD sensor constructed with microbes and enriched with MFC can be kept operational for over five years without extra maintenance.

19.4 Conclusion and Future Perspectives

This chapter concludes that MFC is one of the cost-effective wastewater treatment process along with electricity production, without incorporating any costly component such as electrode and membrane. The existence of MFC technology has surfeit of applications in the day-to-day lives as it is environmental friendly, eco-friendly, and more importantly it is a green technology. There are more challenges left over for the complete utilization of MFC, to find ways to make it cost effective, and to fabricate the innovative MFC bioreactors for industrial effluent treatment. Identification of new microorganisms that can directly transfer electrons to or from an electrode is essential to treat contaminated effluent with generation of electricity. There is a broad scope for design and development of these reactors as the power density is too low for consumption in various industrial applications. Besides, the organism may be genetically altered in order to form high reducing microbial strains with a wide range of MFC applications. Future research studies are necessary to minimize the internal resistance and corrosion-related problems in MFC. MFCs can also have utilization in army applications in order to power up remote surveillance and communication gears for use in unmanned applications. Further, potential research on optimization of electricity production from the two chambered MFC is also necessary. With further improvements and optimization, it could be possible to increase power generation and researchers are working to enhance it for the scale up and commercial applications. Also, MFC as a continuous reactor may also be studied. Research toward the conditions to be maintained in the reactor, aerated condition in order to produce more electricity may be needed. Thus, the combination of wastewater treatment along with electricity production might help in compensating the cost of wastewater treatment. However, the MFC technology is still in initial stage and needs special attention in future research attempts.

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20

Application of Sustainable Micro-Algal Species in the Production of Bioenergy for Environmental Sustainability

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20.1 Introduction

Biomass is one of the most promising resources available to satisfy our increasing energy demand. Biomass is renewable and is an organic material which can be used to synthesize heat, electricity, and transportation fuels. Biomass feedstocks are resources that can be used directly as fuel or can also be transferred to energy products to satisfy various domestic and industrial needs. There are various biomass feedstocks including agricultural crop residues, algae, forest and wood residues, municipal and urban organic waste, food waste, etc. Agricultural residues like stalks and leaves can be effectively used for bioenergy production without interfering with food production. These may generate additional income to farmers. Forest biomass feedstocks including branches, tree parts, etc., are used for the production of bioenergy without affecting forest ecosystem as well as its essential functions.

Feedstocks of algae constitute productive organisms like microalgae, macroalgae, and cyanobacteria. They utilize sunlight and nutrients to produce biomass which can be transferred to biofuels. Based on the type of strain, algae may grow on fresh, saline, or brackish water. They also grow in treated industrial, municipal, agricultural, or aquaculture wastewater, etc. Bioenergy can also be produced from sorted municipal wastes including commercial and residential garbages like papers, plastics, leather, rubber, textiles, food wastes, and textiles. Use of sorted municipal wastes for bioenergy production significantly reduces residential and commercial wastes.

20.1.1 Classification of Biofuels

Biofuels that have emerged adding to/replacing fossil fuels are brought under three categories. The biofuels are classified as shown in Figure 20.1. Usual biofuels obtained from natural resources and use vegetable, creature waste, and landfill gas. Besides, other biofuels are fuel woods intended for the most part for cooking, warming, block furnace, or power creation. The biofuels produced from edible plant

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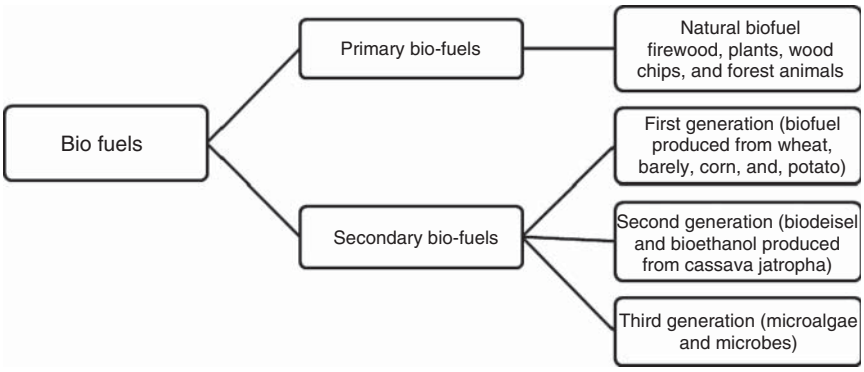

Figure 20.1 Classification of biofuels.

parts (potatoes, grains, and oilseeds, etc.) fall under the category of "first-generation fuels," which are also known as "conventional biofuels"; second generation of biofuels are the fuels generated from nonedible plants and its components; lastly, third generation of biofuels are produced from microalgae.

First- and second-generation biofuel resources include certain restrictions that pose novel disputes on land usage for biomass resource production which would contribute to the food crisis. Hence, the third-generation biofuels are considered as the most appropriate option, concerning economic and environmental sustainability [1].

20.1.2 Microalgae and Bioenergy

The chapter gives an elaborate view of the highly promising bioenergy source – microalgae. Algal biomass can be converted into biofuels like cellulosic ethanol and biodiesel, for transportation. These fuels are functionally equivalent to petroleum fuels and form the most appropriate alternative for conventional fuels. Microalgae have got an extensive application and widely used as a feedstock for biofuel creation. They can produce a valuable quantity of polysaccharides as well as triacylglycerides. Microalgae additionally produce proteins which can be utilized as a source of food [2]. The possibility for microalgae as an unlimited and practical feedstock for biofuel manufacture has motivated a focus in the biorefinery. The modern advancement of microalgae to deliver biofuel and bioproducts has expanded significantly in the course of the most recent couple of decades [3]. Some of the microalgae species used in the research for biomass generation are chlorophyceae, euglenophyceae, prasinophyceae, haptophyceae, eustigmatophyceae, bacillariophyceae, cyclotella cryptic, cyanobacteria, arthrospira (spirulina) platensis, etc [4].

Biofuel is a highly flexible bioenergy resource which can be turned positive and downward rapidly to meet the fluctuating energy requirement. It forms a big encouragement for dependent renewable technologies like wind and solar energies. To keep the energy supply clean and ethical, the bioenergy should meet the following requirements:

- The origin should be from waste or sustainable sources;
- Produced using land sustainably thereby considering habitats and biodiversities;
- Do not disrupt/impact food production;
- High-efficient biofuel generators;
- Negligible impact on air quality.

Biofuels are the most anticipated results of logical exploration. The petroleum products are being depleted, and contamination is expanding internationally. Green growth biofuels are one of the promising choices [5]. The fast-developing populace of the world constantly builds the worldwide interest for fuel vitality, and the serious utilization of fossil overall prompts its consumption and will bring them near the purpose of weariness because of impractical and non-sustainable nature [6, 7].

20.2 Cultivation and Processing of Microalgae

Many pathways for generating biofuels distribute certain general aspects irrespective of the biomass feedstock being used, as shown in Figure 20.2. The pathway for algal biofuel production includes a sequence of processes from algae cultivation to collection and harvest, and finally to fuel conversion. Certain crops are being used as biofuel feedstock because of their capability to generate oil and carbohydrates which can be converted into fuels using microorganism. Some of those crops are soybean, jatropha, etc., which are harvested and their oils are separated for further processing. Dedicated energy crops for biofuel production include poplar, switch grass, and miscanthus; they can grow with low inputs of nutrients and have the ability to store carbon in the soil. The lignocellulosic biomass is then converted biologically, chemically, or thermochemically into liquid fuels. Algal biofuel systems exist similar to the above-mentioned feedstock types.

Different pathways are available for cultivating and processing algae to fuels and their products. The pathways are depicted in Table 20.1 [7]. These pathways illustrate the requirement of resources and impacts associated with each method. The

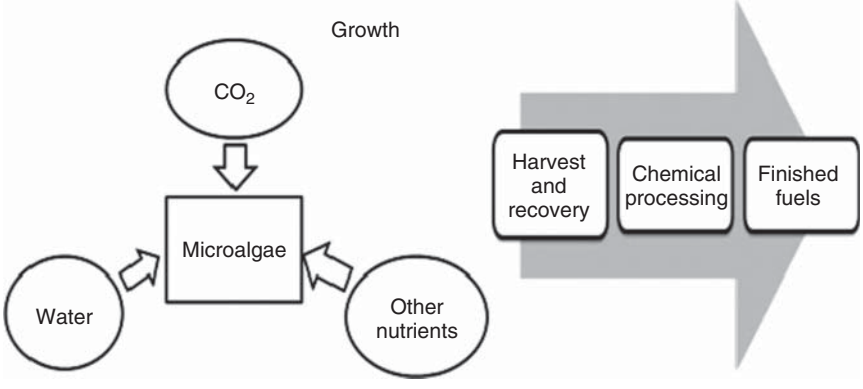

Figure 20.2 Biofuel production pathway.

Table 20.1 Cultivation and processing of microalgae.

1	Cultivation	Species selection	Cyanaobateria
			Unicellular microalgae
			Multicellular microalgae
		Cultivation system	Open terrestrial
			Closed terrestrial
			Open offshore
			Closed offshore
		Water	Fresh water
			Salt water
		Growth mode	Phototrophic
			Mixotrophic
2	Harvesting	Intermediate constituents	Hydrocarbons and alcohols
			Lipids
			Carbohydrates
			Proteins
			Consolidated biomass
3	Processing	Conversion processes	Biochemical conversion
			Thermochemical conversion
			Direct synthesis
			Anaerobic digestion
4	Products	Fuels/products	Alcohols and chemicals
			Esterified biodiesel, biogas/hydrogen
			Animal feed and other products

different methods can be compared based on techno-economic feasibilities. There are yet other methodologies in which species are grown at a maximum carbon fixation rate that retains large fractions of the fixed carbon in their final products. A few such technologies are as follows:

- Algal biomass fermentation to yield hydrocarbons or alcohols;
- Conventional catalysis process;
- Synthesis gas fermentation;
- Hydrous pyrolysis;
- Gasification and syngas translation to alkanes, aromatics, and alcohols;
- Anaerobic digestion to methane.

The above-mentioned pathways are not generally used but can be placed into practice. The feedstocks should not contain high water content. The central of

water expulsion from the item is basic in any conversations about enormous scope fuel creation. The solar drying process may be used for water removal but has a serious concern of large land requirement for drying which thus makes it unfit for large-scale processes [8].

20.2.1 Cultivation of Microalgae

It is very important to isolate and cultivate the microalgal species for biofuel production. Cells are segregated and grown in appropriate surroundings. The method of cell culture consists of various steps for a long-lasting period. The culture conditions should be similar to that of the natural environment. The cultivation of microalgae is done in the following sequence.

20.2.1.1 Isolation of Cell Cultures

The sample collection is the initial procedure followed by isolation of the required cell. The growth of these green algae requires simple elements like light, water, carbon source, temperature, and fewer macronutrients. These conditions are more prevalent in coastal and oceanic sites and some from rock pools or even shorelines. These samples are collected from these sites. Samples from these sites are collected in separate bottles consisting of a cylindrical tube with stoppers at the ends; it may be fitted with a closing device activated by a messenger. The microalgae samples that are collected need to be handled gently with care. These have to be transferred rapidly because the damaged and dry cells cannot produce viable cultures. The collection details including date, location, date, depth of sample from where they are collected, water temperature, etc., must be recorded for references. The collected samples may then be transported in small plastic water bottles. Before the bottle is filled, it may be rinsed with the sample water to avoid the bubbles. The bottle containing samples must be well insulated.

20.2.1.2 Single-Cell Isolation

The micropipette is used to isolate the single cell. It is done to remove a particular cell from the water sample without damage and put the same into a germ-free droplet of growth medium. The sterile droplet consists of the target cell. Optimum culture medium for algal growth has to be established as the newly isolated strain will be difficult for maintenance. The isolates can be put into several culture media and their growths are monitored. Algal growth is monitored regularly under microscopic examination. If the culture begins to grow, it has to be relocated to a glass flask or culture vessel. On establishing the culture, it may be grown in huge volumes. Other isolation methods are also used by different researchers. They include the use of agar, centrifugation, flow cytometry, dilution techniques, and photo axis.

20.2.2 Techniques

20.2.2.1 Filtration

Filtration is the most appropriate method for sterilization of the samples of smaller quantities and heat-sensitive elements like vitamins. Membrane filters may be used

to remove contaminating microorganisms like bacteria. Autoclavable and single-use filtration systems are also available.

20.2.2.2 Autoclaving

Autoclaving is done to sterilize the equipment and media using high-pressure saturated steam at a higher temperature of 121 °C. Commercial autoclaves that are generally used are both large and small, for small quantities, cookers may be used. For liquids' autoclaving, maintain the liquid level in the containers in such a way to prevent excess buildup of pressure. Precipitate formation should be avoided in the liquid because they may aggregate the nutrients, organics, and metals in the form of particulates. Fewer scientists have also considered it as a positive attribute because the precipitates may slowly release the nutrients. Autoclaving can be done in Teflon bottles to reduce the development of precipitates in the saltwater. Distilled water can also be added to avoid precipitation. Autoclaving glass/plastic wares at higher pressure and temperatures may cause the release of certain substances from the walls of the vessels. Pyrex bottles will release metals that are used for its manufacture. Polycarbonate bottles will release plastic stabilizers during the process of autoclaving. It is advised to use a small quantity of deionized water in plastic containers to confine the dissolved plasticizers.

20.2.2.3 Dry Heat

Dry heat is also used for sterilizing glassware like tubes, beakers, and glass pipettes, etc. This glassware has to be cleaned and dried. The vessel openings should be layered with aluminum foil to protect the sterility. The glass vessels are placed in an oven at a temperature approximately equal to 160 °C at least for two hours.

20.2.2.4 Pasteurization

It is the most effective method of sterilizing seawater and the growth media. The process of sterilization by this method is time-consuming. This is applicable for sterilizing liquids that cannot be autoclaved to temperatures greater than 100 °C. The process includes heating of liquid to a certain temperature for a particular time interval and then cooling it immediately to remove it from the heating source. The entire process is carried out in a water bath, fitted with provisions for temperature regulation. The major disadvantage associated with this process is that it is carried out for a longer duration. Other methods of sterilization are microwaving and using of UV radiations. For the UV treatment to be effective, the water must be placed in a quartz glass tube.

20.2.3 Culture Conditions

20.2.3.1 Temperature

Temperature reflects the conditions from which the microalgae are isolated. Temperature should not be above 30 °C because high temperatures are fatal for marine algae. Following are the isolation temperatures that need to be implemented:

- Polar isolates: <10 °C
- Temperate isolates: 10–25 °C
- Tropical isolates: >20 °C

20.2.3.2 Lighting

Cell cultures are placed in an area with a huge window along with supplementary lighting. Natural lighting may also be provided. The most commonly used system involves usage of the white fluorescent bulb which can give the required flux density needed for the cell growth. Recently, light-emitting diodes (LEDs) are also being used. Continuous lighting may also damage the algal cells and it even kills some algae. The light and dark regimes may be varied between 12 : 12 and 16 : 8. It is strictly recommended to avoid direct sunlight.

20.2.3.3 Culture Media

There are two different culture media, defined and undefined. In the case of defined media, high-quality deionized water is used along with the nutrients which are needed to make the formulation. Undefined media is of seawater base, which can be natural or artificial and is added with nutrients. It is very important to prepare the media with good quality seawater. The quality of seawater can be improved by aging to allow for bacterial degradation. Sometimes, brands of synthetic seawater may also be utilized. Pre-filtering of seawater may also be done to remove larger particles. Nitrogen (N_2) and phosphorous (P) are the major nutrients of all culture media. Nitrate or ammonium may also be used. Organic nutrients are also added. Amino acids and inorganic orthophosphate may be used for additional nitrogen and phosphorous, respectively. Certain algal groups such as diatoms and chrysophytes require silica. Certain metals such as iron, copper, zinc, cobalt, manganese, and nickel are also added in trace amounts. Vitamins consisting of thiamin (B1), biotin (H), and cyanocobalamin (B12) are also used. Historically, the soil enrichment extract was also used for the growth of many algal cultures. Sieved sandy loam soil is most commonly used. There are many variations in different culture media preparation.

20.2.3.4 pH

The control of pH in culture is very significant because some algae will grow merely with certain pH values. Seawater has a pH of 8. Seawater has its own buffering capacity and hence it is easy to keep the pH of such media. The pH of seawater can be decreased before autoclaving in order to compensate the subsequent increase in pH.

20.2.3.5 Aeration

Aeration is not suggested for many processes, as it may become a physical disturbance for the growing cells. In certain situations involving heterotrophic dinoflagellates, aeration is very much essential.

20.2.4 Culture Methods

20.2.4.1 Batch Culture

In a complete culture medium, algal inoculums are placed in a fixed amount and are incubated in an appropriate environment for promoting its growth. When algal cultures are grown in limited volumes, resources are finite and are used up completely. The pattern of growth involves lag phase, exponential phase, and stationary phase.

20.2.4.2 Continuous Culture

Continuous cultures have infinite resources. Hence, algae are retained at a fixed point in the growth curve by the periodical adding of new culture. Fresh culture is being added regularly at the rate which is proportional to the growth rate of the alga in the medium. And since it is a continuous process, the same quantity of culture is also taken away regularly.

20.2.5 Harvesting Cultures

There are several procedures including separation of algae, drying the cells, and further processing, in order to get the required product. Techniques used for harvesting depend on the grown-up category of algae. Most commonly used harvesting procedures are flocculation, centrifugation, and filtration. For harvesting large quantities of culture, continuous flow of centrifugation is used [9].

20.2.6 Bioenergy Production Process from Microalgae

Microalgae can be used to generate energy in various manners. A few algal species can produce hydrogen gas as indicated.

20.2.6.1 Production Processes

The creation of algae in more quantity has been dependent on the test for a long time. The attention on using green growth was concerning significant things like fluids or proteins [10, 11] for food. Cadge created quantities per region are equivalent to current worth, green growth creation for biofuel, and this has become a significant issue [10]. Two unique methods for green growth creation are encouraged and examined during research; open lake reactors (OPR) and shut photo-bioreactors (PBR). OPRs are available to the condition the bowls are frequently formal like raceways, up to 3000 cm long and 150 cm wide. In numerous reactors, an oar wheel is applied to give the progression of medium. Points of interest of these reactors are the moderately low speculation cost and simply taking care of. The drawback is the ecological impact presents a genuine weakness. Creation rate may be approximately 10–25 g dry issue (PM) of biomass growth every day per m^2 [11, 12]. The hindrances and insecure creation in OPR top many examination organization changes to shut down PBR. Likewise in the PBRs, the green growth medium is siphoned through a shut framework to give sun-based protection for growth of a few structures that are available, for example, vertical-requested-level acrylic glass tubes, plastic sacks, or flat plate reactors [11].

20.2.6.2 Biomass Production from Marine Water Algae

Naturally Occurring With respect to kelp, essential biomass age can be used normally sprouting of marine microalgae in pungent needs or lakes. These are instances of chances with the gathering of microalgae that are congested in these zones. This is like floating kelp. The counterfeit eutrophication of water by human exercises creates nearby adjustment of the biological systems. It may end in enormous

anticipated sprouts of miniaturized scale green growth. The green growth natural issue will experience microbial corruption, fundamentally diminishing oxygen level in the water. During some green growth of sprouts, the oxygen level can fall underneath accompanying adequate cutoff points for the remainder of the neighborhood biological system. Therefore, wide mortality of different creatures is being watched. Marine microalgae populaces are commanded by phytoplankton in suspension in seawater. Anyways, frames which connect to a substrate additionally exist. This is known as biofouling when the colonized surfaces are artificial things, for example, pontoons or wharfs.

PreTreatment Gathering produces a slurry material with 2–7% of algal focus, and the following stage is dewatering to get 15–25% fixation. This is generally accomplished by squeezing or centrifugation. Fixation by warming is conceivable to diminish water content; however, the working cost is high except if modest warming is accessible (geothermal). A significant issue in biomass treatment is the protection of synthetic quality. In the wake of reaping, synthetic compounds in the biomass might be subject not exclusively to corruption incited by the procedure itself, but additionally by inward protein movement in the microalgae. For example, lipase chemical is known to hydrolyze cell lipids substance to free unsaturated fats after passing the cell. This response is sufficiently quick to decrease the piece of lipid content, reasonable for creation. After the pressure time frame, the whole-cell digestion will be committed to re-establishing the underlying condition of the cell before stress. In acquiring biofuel from microalgae, this just applies to lipid development. Maturation courses use the entire algal biomass developed utilizing standard non-stress development strategies.

Downstream Processing Downstream procedures are dismissed in numerous algal ventures and there are a wide number of choices accessible and available to process the biomass (dewatering, concentrating, and drying). A noteworthy piece of biofuel improvement activities ought to be gear trying both from an operational and efficient perspective.

Economic Issues and Life Cycle Assessment The high efficiency and important structure of green growth have demonstrated incredible potential in making an essential commitment to the overall fuel advertise with quick development rate. Green growth is outfitted with another preferred position, which is a better return of wanted segments than the first and second ages feedstock for biofuel creation. Regardless of the yield and supportability of biofuel, it is created from green growth biomass. The financial concentration on the monetary, perspectives to look for the chance of commercializing green growth as the feedstock of biofuel on the modern scale [12]. Green growth biofuel, as a trade for nonrenewable energy source, is immature presently with the mechanical advances we have today. Like the second era of biofuel, green growth biofuel still needs to conquer a few more significant boundaries, for example, lessening the vitality input, decreasing the carbon impression, and improving the monetary possibility.

Development of green growth includes the reaping process and shielding the development framework from the sullying of wild green growth. They may bring about expanded creation costs, anyway, the lignocellulosic bioethanol process additionally has the issues of exorbitant and vitality escalated pretreatment process just as costly protein used for the hydrolysis of lignocellulosic materials [12]. The procedure financial matters can be improved impressively using the algal buildup through biorefinery innovation along with the creation of numerous green growth-based items using biorefinery that forms green growth biofuel. It can be monetarily serious to lignocellulosic bioethanol.

Life cycle assessment (LCA)) has become critical in assessing the natural effects brought about by the procedure and the enhancing of the stage change from biomass to biofuel. The net vitality utilization is shifted relying upon the development technique. It shows development by reusing carbon for CO_2 obsession. Similar vitality LCA is decided through a variety of development framework (open framework and shut framework) and sort of reactors that utilized the estimation of net vitality proportion (NER) > 1. This emphasizes a point that the vitality of microbial biomass created by OP is higher than the vitality devoured by the framework. Operation is found to have lower ozone harming substance emanation than PBR, since a piece of the green growth biomass may experience anaerobic absorption.

20.2.7 Large-Scale Production and Processing of Microalgae

Microalgae are cultivated for producing renewable bioenergy. There are various routes of bioenergy production from microalgae that are presently available. They also have properties which makes them the appropriate sources of eco-friendly fuels [13]. The oil is first extracted from the algae using a series of techniques like using simple oil expeller, microwave-assisted, ultrasonication-assisted, supercritical extraction, wet extraction, etc. The oil is then converted to biofuel using different processes.

20.2.7.1 Biomethane Production by Anaerobic Digestion

Anaerobic digestion (AD) process converts microalgae into biofuel. It is a biochemical process that converts organic compounds into biogas, also methane through the synergistic action of microorganisms under anaerobic conditions. Biogas is composed of methane (around 50–70%), CO_2, fractional amounts of ammonia, H_2S, and volatile organic compounds. AD is a mature technology that treats organic waste streams. Owing to its simple usage and environmental benefits, it is widely practiced. It is suitable for both dry and wet organic feedstocks. In the case of wet feedstocks, it has to be dewatered before processing. The process does not require any pretreatment. AD has high economic and environmental benefits, since the process effluents are captured for the repeated use of carbon dioxide, phosphorus, and ammonia. The following are the steps involved in the AD process:

- **Hydrolysis**: the breakdown of macromolecules such as carbohydrates and lipids into sugars, amino acids, and fatty acids.

- **Acidogenesis and acetogenesis**: the molecules which are hydrolyzed are converted to acetate, CO_2, and H_2.
- **Methanogenesis**: production of methane from acetate, carbon dioxide, and hydrogen.

Efforts are currently being undertaken to develop inexpensive biomass feedstock, maximize energy production, and minimize associated environmental risks [14].

20.2.7.2 Liquid Oil Production by Thermal Liquefaction Process

Thermal liquefaction (HTL) or hydrous pyrolysis is the process of transformation of algal biomass into liquid fuels in the presence of water. In this process, in a closed reactor, the wet biomass is converted into biofuel. The change in reaction conditions affects the reaction progress. Since subcritical water has good heat transfer and mass transferability, the HTL process is independent of the heating rates and biomass particles size. Depending on the type of feedstock, processing conditions, and presence of a catalyst, the product yield is obtained using relevant physiochemical properties. HTL of the feedstock containing high protein content results in the formation of the product with high nitrogen content and aromatic content. It is essential to upgrade the HTL biocrude for it to be necessary for transportation grade. HTL also produces gaseous products which contain malodours. They have to be reduced before being discharged into the environment [15].

HTL is highly expensive than pyrolysis and gasification. It has also been observed that the energy balance of hydrothermal liquefaction is unfavorable in case of water content, thus exceeding 90% of the biomass. Based on the type of microalgae, around 23–49% of the original dry mass is recovered as bio-oil. The bio-oil recovered contains up to 75% of energy of the initial biomass feedstock. The inorganic nutrients obtained in the hydrothermal liquefaction process can be used as crop fertilizer [16].

20.2.7.3 Transesterification Process

This is the most common method of production of biodiesel. In the presence of a catalyst, oil is converted into methyl ester in the consecutive reactions between alcohol and vegetable oils. In this process, the oil which is extracted from the algae is preheated to 60 °C. It is then added to a mixture of methanol and sodium hydroxide in suitable proportions. The transesterification reaction occurs in the reactor. Once the products are formed, the products are allowed to settle. Initially, the glycerine will be removed; the desired product biodiesel is then filtered and washed [17].

20.2.7.4 Nano-Catalyzed Transesterification Process

Initially, for the production of biodiesel from microalgae, algal strains are selected because each strain has varying lipid composition. In conventional methods, biodiesel is produced using homogeneous and heterogeneous, also enzymatic catalysts such as potassium hydroxide, sodium hydroxide, zeolites, and lipase. Recent studies involve the use of nanocatalysts for the generation of biodiesel using microalgae by extracting algae oil and converting into biofuel. The process involves a biocompatible, mesoporous nanoparticle that absorbs hydrophobic molecules.

These nanoparticles extract oil from livelihood algae without killing them. The nanoparticles draw oil by carefully entrapping the lipid molecules produced by the selected algal strain between the cell membrane and the cell wall. The sponge-like mesoporous nanoparticles are used without disturbing cell membrane. The oil thus produced within the rigid space is absorbed into the pores of the sponge-like structure of the catalyst. To enable the transesterification of the entrapped lipids, oxides of calcium and strontium are fed into the pore structure of the nanoparticles. Recently, nanoporous carbons are also used as adsorbents for biofuel separation. [18]

20.2.7.5 Biohydrogen Production by Photobiological Process

Microalgae which are photosynthetic use solar energy to generate hydrogen gas from water. Among various species of microalgae, *Chlamydomonas reinhardtiii* is considered for producing H_2. The catalysts photosystem II (PSII) and Fe–Fe hydrogenase are used in this process. The biochemical reaction occurs among H_2O and H_2 in the presence of the light energy. Biophotolysis is divided into two categories: direct and indirect biophotolysis. Direct biophotolysis is the PSII-dependent pathway, in which there is a direct link of water-splitting activity to H_2 evolution. Indirect biophotolysis is the PSII-independent pathway. Concentration is required for it to be promoted as a technology for enhancing great environmental benefits. There is a wide opportunity for research in this area mainly about technological and economical barriers concerning this process. [19]

20.3 Genetic Engineering for the Improvement of Microalgae

Genetic manipulation is a cost-effective strategy for moving forward the algal-based production. Microalgae genetic manipulation provides various advantages to the biotech industry. Selected algal species will increase productivity with novel cultivation techniques. Harvesting and downstream processing can be greatly enhanced. This process reduces energy inputs, increases stability, and minimizes extraction costs. The focus of microalgae strains research is to develop single microalgae "super-strain" to improve the profitability of the production process, and profitability, identification, and isolation of the algal strains for commercialization of bioproducts.

Genetic engineering process facilitates the manipulation of the genome of the microalgae. *C. reinhardtii* was the first microalgae to have its genome sequenced. Evolution of newer sequencing technologies facilitated the genome sequencing of various strains. To insert foreign DNA into microalgae, various protocols have been developed. Some of them are biolistic, electroporation, and agrobacterium-mediated transformations. These protocols are widely used in plant applications and are adopted because of similar characteristics of their cell wall. Natural strains cause less environmental harm which is not in the case of genetically modified microalgae, depending on the type of manipulation involved. The microbial cultivation of genetically modified microalgae can be done in closed or open ponds. Open ponds

are used mainly for wastewater treatment or for feed production because they are cheaper to set up and run. They also offer limited control over CO_2 conditions, light, and temperature. Closely packed bed reactors provide improved culture stability and densities of biomass. There is a lower chance of contamination. They require high capital and operating expenditure. The harvesting of microalgae is one of the most challenging aspects of the biofuel production process when applied at an industrial scale because of the high operational costs. Selection of harvesting method depends on the strain, culturing conditions, as well as the intended use for the biomass and the derived products [10, 12, 20].

20.4 Conclusion and Challenges in Commercializing Microalgae

In the prevailing market circumstances, commercial biodiesel is not generated from the microalgae. The major challenge in producing biodiesel from microalgae is the reproducibility of the results that have been obtained in the lab-scale studies. It was inferred from the laboratory research that the oil production rate is directly proportional to the microalgal growth rate and also oil content present in the biomass. Hence, the microalgae with good oil production capacities may be preferred for producing biodiesel. But producing biomass is highly expensive when compared to growing crops. The temperature has to be maintained within the range 20–30 °C. The most important economic challenge for microalgae producers is to identify low-cost oil extraction and harvesting methods. It was also inferred that the utilization of fatter algae with around 60% oil content in comparison to lower oil content algae can reduce upto half of the size and footprint of algae biofuels production systems. It will also reduce the capital and operating costs involved in the entire process. A cost-effective and simple method can provide an enhanced scope for commercialization. Usage of a nonlethal extraction process called milking can indeed keep away from a sequence of processes such as harvesting and extraction.

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Part VII

Emerging Technologies (Nano Biotechnology) for Zero Waste

21

Nanomaterials and Biopolymers for the Remediation of Polluted Sites

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21.1 Introduction

Increasing globalization and industrialization have profound impact on the environment. Environmental pollution together with global warming is a serious day-to-day problem faced by the developing and the developed nations worldwide. Extensive use of anthropogenic materials contaminates the natural ecosystem which in turn damages the environment in long run by several means such as loss in biodiversity, introducing heavy metals and other organic recalcitrant compounds [1]. Air, water, soil, and solid waste pollution due to the anthropogenic sources contribute a major share to the overall imbalance of the ecosystem.

Major part of the biosphere which is contaminated with anthropogenic substances is soil and water. Rapid industrialization and urbanization with growing human population, various industrial, municipal, and agricultural sources have augmented the pollution of soil, groundwater, and surface water.

Water pollution has become a very serious crisis in recent years. Water is mainly contaminated with microorganisms like bacteria, fungi, viruses, chlorinated hydrocarbon, heavy metals, chlorine compound like trichloroethene (TCE), polychlorinated biphenyls (PCBs) or mixtures of anthropogenic organic chlorinated compounds (He and F), dyes, mutagens, detergents, and pesticides which are highly reactive and water soluble in nature [2]. These compounds are carcinogenic and are naturally non-degradable, so it persists in water and soil for longer time and remediation of these compounds remains challenging. The presence of microorganisms in water is also an important issue for drinking water production. Even very low counts of pathogenic microbes in drinking water can lead to severe water-borne disease.

Soil is a dynamic ecosystem which provides a support to the plant's life. It consists of organic matter, minerals, and various organisms. Numerous microorganisms dwell luxuriously in the soil. Soil is defined as "the part of the solid earth

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that has been altered by the loosening of the earth, humus formation, chemical decomposition, and by the transportation of humidification and chemical decomposition products" [3]. The modern agricultural practices are heavily dependent on the application of chemical pesticides, inorganic fertilizers, and growth regulators which has not only raised the agriculture production but has also resulted in depletion of natural resource, environmental deterioration, and loss of crop diversity [4]. Owing to their exceptional ability to efficiently adsorb as well as high surface area, biopolymeric nanocomposites are considered as an excellent support materials of metallic photocatalysis for the removal of contaminants from the polluted sites. The present chapter envisages about application of various nanomaterials such as metal/metal oxides and biopolymeric nanocomposites for effective remediation of water and soil.

21.2 Water Remediation

21.2.1 Application of Nanotechnology for Water Disinfection and Textile Dye Degradation

The availability of potable water is a serious problem in rural areas of developing countries. The economic, social, and environmental impacts of poor water supply and sanitation have posed a lot of implications on the health and safety of the people, especially children, elderly, and poor closely associated with the accessibility of adequate, safe, and affordable water supplies. Hence, there is an increasing demand for providing potable water to people in both the developed and developing countries which can be addressed by the development of innovative new technologies and materials [5].

The textile industry represents a major threat to the environment due to release of dye effluents into the surrounding water bodies due to consumption of large quantities of water at their different steps of dyeing and finishing process. Due to the presence of —N=N— bond, synthetic textile dyes often become recalcitrant and carcinogenic in nature. These synthetic dyes also consists of complex aromatic structures which cannot be easily degraded. Most of the synthetic dyes have been intentionally designed to resist aerobic microbial degradation and are converted to toxic or carcinogenic compounds [6].

The traditional techniques deployed for the removal of dyestuff are the application of biological, adsorption, and coagulation. Each of the method has its own advantages as well as bottlenecks.

In recent years, emergence of nanotechnology has been the subject of extensive research and can provide us ways to purify air, water, and soil using engineered nanoparticles as catalysts. Nanotechnology is defined as the "deliberate manipulation of matter at a scale of 1–100 nm." This process involving deliberate manipulation of matter size scales of less than 100 nm offers the possibility of an efficient removal of pollutants, mutagens, and microorganisms pertaining to the area of water purification, air purification, and soil remediation. Furthermore, the utilization of

light and ultrasound waves to activate such nanoparticles opens up new avenue to design green oxidation technologies for environmental remediation. Amalgamation of nanotechnology with biotechnology has significantly expanded the application domain of nanomaterials in numerous fields. Several metal and metal oxide-based, carbon-based materials, nano-sized polymers, and biocomposites nanomaterials [7] are being developed for wide applications. Metal and metal oxide-based nanoparticles include silver, gold, aluminum, copper, silica, iron, zinc, zinc oxide, titanium dioxide, and cerium oxide. These materials are generally found useful in environmental remediation, wastewater treatment, water purification, food processing, drug delivery, packaging, and smart sensor development [8].

The photocatalyst-mediated oxidants are strong enough to degrade and inactivate most organic pollutants and pathogenic microorganisms by mere contact. All these features are particularly attractive for installations in remote and rural areas where electricity requirement is a prohibitive handicap for competing technologies.

Photocatalysis is one of the thrust areas of research and has witnessed a transformation over the past two decades with remarkable advancements being made in the synthesis of novel materials and nano-structures, and the design of efficient processes for the degradation of pollutants and the generation of energy. Currently, lot of research is going on in the biosynthesis of nanoparticles using microorganisms which has emerged as rapidly developing research area in green nanotechnology worldwide, with various biological entities being employed in synthesis of nanoparticles constantly forming an impute alternative for conventional chemical and physical methods [9].

Nanoparticles like semiconductors, zero-valence-based metal, and some bimetallic type, etc., are typically used for the treatment of environmental pollutants such as chlorpyrifos, azo dyes, organochlorine pesticides, nitroaromatics, etc. [10]. Numerous reports are available in the literature which illustrates the effectual removal of these pollutants from wastewater using TiO_2-based nanotubes [11]. However, the most common and significant metal oxides employed as nano-photocatalysts are silicon dioxide (SiO_2), zinc oxide (ZnO), titanium dioxide (TiO_2), aluminum oxide (Al_2O_3), etc. [12]. Among them, TiO_2 is one of the excellent photocatalysts compared to all existing material due to its unique properties such as toxic-free, low cost, and chemically stable and due to its availability on earth [13]. Photocatalytic properties of TiO_2 have been exploited in several environmental applications to remove contaminants from both air and water [14]. Extensive studies on TiO_2 nanoparticles-mediated oxidative and reductive transformation of organic and inorganic species present as contaminants in air and water has led to the development of several products such as self-cleaning glasses, disinfectant tiles, and filters for air purification for day-to-day operations. Commercialization of such products has established the early successes of nano systems for environmental applications.

ZnO is a multifunctional material which is widely used in several applications due to its eco-friendly and diverse properties [15]. Nano-sized ZnO is observed to be an excellent material for optoelectronics, nanosensors as well as antibacterial applications due to some of its exceptional properties such as morphology, surface properties, crystal defects, and size tailoring properties.

21.2.2 Nanobiopolymers for Water Disinfection and Textile Dye Degradation

Biopolymers are exceptional materials which are unique in their composition and possess various physiological properties. Biopolymeric nanomaterials can be formed by impregnating metals to biopolymers. These materials form a molecular capsule through intramolecular hydrogen bonding. Incorporation of metals/metal oxides inside starch/chitosan molecules forms polymeric nanocomposites. Chitosan is an excellent biomaterial which has found several useful applications in the field of nanotechnology due to its wide compatibility [16]. Sorption and impregnation are the two main techniques used for the incorporation of nanomaterials inside biopolymers [17]. Polymeric nanomaterials are nothing but the solid colloidal particles ranging from 10 nm to 1 μm. The inorganic counterpart of the composites has been accountable for their photocatalytic activity and organic counterpart, i.e. polymers such as pectin, cellulose, guar gum, polyaniline, and polyacrylamide, etc., act as adsorbent for hazardous organic dyes. The polymeric material acts as a support and is responsible for increasing the surface area by acting as a backbone for the attachment to inorganic part. Polymer-based nanocomposites have attracted several researchers over other composite materials due to their characteristic properties such as cost-effectiveness, easy processability, renewable nature, and high-volume applications [18]. Due to the presence of high surface area nanoparticles in a polymer matrix, polymer nanocomposites possess highly tunable adsorption behavior.

This optimized adsorption behavior of polymeric nanocomposites makes them suitable for different applications such as chemical sensor, water purification, drug delivery, and fuel cell technology. Polymeric nanocomposites have been extensively used for the removal of various toxic metal ions, dyes, and microorganism from water bodies (Figure 21.1).

Biopolymers include starch, alginate, chitosan, dextran, and chitin which are generally present in various organisms such as plants, algae, fungi, bacteria, and animals. Chitosan, starch, dextran, and cellulose are the polysaccharides which are commonly used as support for nanomaterials and are derived from plants and microbes [19].

Owing to its exceptional ability to efficiently adsorb as well as high surface area, chitosan is considered as an excellent support material for metallic photocatalysis. Chitosan material helps in reducing the number of intermediates produced during photocatalytic reactions. Also, chitosan allows quick and trouble-free recovery of the photocatalyst, which can be recycled with or without any regeneration [20].

Chitosan is one of the most abundantly available, natural, environmentally benign, nontoxic, biodegradable, low-cost, and biocompatible biopolymer. Chitosan-based nanocomposites as adsorbents have significant benefits such as attractive surface area, chemical accessibility, ease of functionalization, and absence of internal diffusion [21]. Many researchers all over the world are endeavoring the application of various chitosan-based materials for removal of heavy metals as well as dye degradation. Amino and hydroxyl groups present in chitosan

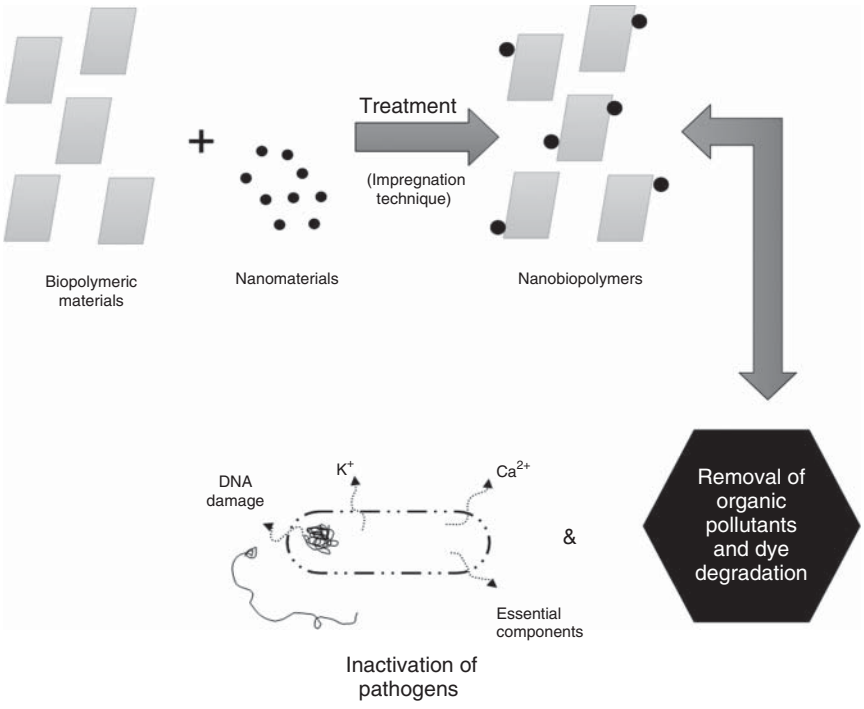

Figure 21.1 Application of polymeric nanocomposites for the removal of various toxic metal ions, dyes, and microorganism from polluted sites.

material act as the active sites; hence, it can be used as an adsorbent for the removal of heavy metals and dye molecules [22].

Mansur and Mansur [23] have reported the development of nano-photocatalyst (chitosan-based quantum dots and ZnS) for effective removal of methylene blue dye. Biopolymeric nanocomposites comprising of TiO_2/Ag hybrid incorporated in carboxymethyl cellulase and gelatin have illustrated improved photocatalytic activity toward benzene and NH_3 present in the chemical structure of organic pollutants [24]. Sathiyavimal et al. [25] have reported the preparation of chitosan-copper oxide (CS-CuO) nanocomposites via green route using *Psidium guajava* plant leaf extract and its ability in effective removal of industrial dye as well as killing bacteria. Amphoteric chitosan-TiO_2 bionanocomposites exhibited excellent catalytic activity in the degradation of malachite green under visible light [26].

Kora and Rastogi [27] have reported a facile, one pot synthesis of palladium nanoparticles via green route using glucurono arabino-galactan polymer, gum olibanum for the removal of anthropogenic dyestuff, and this material can be even extended for the removal of other toxic, mutagenic, and microorganisms. Similarly, nanobiopolymer comprising of gelatin-Zr(IV) phosphate nanocomposite prepared via sol–gel method was found effective in killing of *Escherichia coli* as well as in the removal of fast green (89.91%) and methylene blue (87.81%) dyes within five hours. Elfeky et al. [28] have developed a multifunctional cellulose nanocrystal/metal

oxide hybrid comprising of zinc and copper oxide via acid hydrolysis and sol–gel method for the effective removal of bacteria and degradation of Rose Bengal dye. Green synthesis of pure zinc oxide nanoparticles using quince seed mucilage as stabilizing agent for the photocatalytic degradation of methylene blue has been reported recently.

Gum polysaccharides are natural polysaccharides which are abundantly available in nature [29]. In spite of its structural diversity and excellent property, Gum Arabic cannot be used in its native form due to certain limitations such as uncontrolled rate of hydration and contamination by microbes, drop in viscosity upon storage, and thickening. In order to overcome these limitations, in most of the cases, it is modified with different vinyl monomers. Application of Gum Arabic-grafted polyacrylamide (GA-cl-PAM) hydrogel as a self-template for the *in situ* synthesis of zinc oxide nanoparticles as a potential adsorbent for the degradation of malachite green dye is well documented.

Hydrogels developed using Gum Arabic are considered as an efficient adsorbent material for the treatment of contaminated water [30]. Gum olibanum, a natural oleoresin released from the bark of *Boswellia serrata* (a native tree of India), has gained considerable attention (Burseraceae family) in recent times. Many scientists have exploited this novel material as support for the development of nanobiopolymer for environmental remediation.

Among the biopolymers used, gelatin is one of the excellent materials ever used to make films. Gelatin is a biopolymer derived from collagen. Gelatin, derived from collagen, is hydrophilic in nature, and the strength of the gel is essentially dependent on its concentration. Films developed using gelatin possess favorable optical, mechanical, and protective properties against gas, oxygen, and odor at low relative humidity. Modification of cyclodextrin (oligosaccharide produced from enzymatic conversion of starch) with nano-TiO_2 was found to be effective for the treatment of wastewater. Likewise, ZnO/carbon black grafted in cellulose acetate has been used to treat azo dyes such as Congo red, methyl orange, and methylene blue. MnO_2/cellulose nanoparticles of size lesser than 100 nm was effective in degrading 90% of indigo carmine within 25 minutes duration under optimum conditions. A novel bionanocomposite comprising of a blend of chitosan–guar gum incorporated with silver nanoparticle was synthesized using palm shell extract for catalytic degradation of individual and binary mixture of dyes as well as in the reduction of 4-nitrophenol to 4-aminophenol [16].

21.3 Soil Remediation

Industrial operations such as metallurgic operations, discharges of smelter slags, coal, bottom fly ash, and mining activities also contribute to the soil pollution by releasing effluents to the soil that includes chlorinated compounds, polycyclic aromatic hydrocarbon (PAH), toxic heavy metals, and radionuclides. Unlike organic contaminants, heavy metals do not degrade, becoming a persistent threat to the terrestrial environment. These released heavy metals not only pollute soil, sediments,

surface, and groundwater, but they also accumulate in living organisms thereby disrupting the food chain. Disposal of coating materials to prevent rust, dry cell batteries, brass, bronze alloys, and certain other substances also contribute to the soil pollution thus affecting the quality of soil.

These contaminants hide in saturated and unsaturated layer of the soil which is underlying between the ground surface and groundwater level. Consequently, these sites can have a high concentration of organic contaminants in soil layers in addition to plausible groundwater contamination. They can even depict harmful effect on the flora and fauna of affected habitats through uptake and accumulation in food chains, and in some instances, serious health problems or genetic disorders in humans are also observed.

Soil remediation is generally intended at removal of hydrocarbons (petroleum), heavy metals, pesticides, cyanides, volatiles, creosotes (carbonaceous products released during the distillation of several types of tars), and semi-volatiles. Conventional methods that are chiefly being employed in remediation processes are bioremediation, thermal soil remediation, air sparging, electrokinetic remediation, phytoremediation, and soil washing [31]. However, these existing methods have certain bottlenecks/disadvantages such as laborious and time consuming in such cases, and immediate remediation is quite difficult.

21.3.1 Application of Nanotechnology for Soil Remediation

Application of nanotechnologies for environmental remediation has received significant attention from the scientific community, specifically its use for remediating heavy metal contaminated soil. Recently, nanoremediation is also being used for the treatment of hazardous waste sites. It was Gillham, in 1996, who for the first time investigated and presented the idea of utilizing zero-valent iron nanoparticles (nZVIs) in the permeable barrier for the effective decontamination of water-halogenated pollutants. Extensive studies have been carried out and lot of literature is available pertaining to the application of nanotechnologies to remediate the contaminated soils [32].

According to the literature, the nanoparticles have the ability to adsorb and facilitate degradation of pollutants through various mechanisms, such as redox reactions, surface processes, adsorption, ion exchange, surface complexation, and electrostatic interaction. Shi et al. [33] have analyzed nZVIs and zero-valence iron nanoparticles impregnated on a matrix of bentonite (B-nZVI), in the effectual elimination of chromium(VI) present in water and soil contaminated with heavy metals.

In a particular study, iron nanoparticles impregnated biocarbon depicted a positive influence on the growth of cabbage and mustard plant grown in chromium(VI)-contaminated soil compared to untreated plants.

Similarly, SiO_2 nanoparticles coated with a lipid derivative of choline have been extensively used in the bioremediation of PAHs. Other nanomaterials that have been used are iron sulfide stabilized with carboxymethylcellulose for immobilizing mercury in the polluted soils.

Trujillo and Reyes [34] described the efficiency of nZVI for the remediation of aqueous solutions contaminated with ibuprofen in soils. Likewise, Olson et al. [35] conducted studies on polluted soil with bivalent metal nanoparticle (Fe and Mg) in order to reduce the concentration of PCBs in soils. Lot of efforts are being made to make use of nano-N fertilizers for controlling the loss of nutrients. A high-performance nanoformulation was developed using clay–sodium polyacrylate–polyacramide for binding nitrogenous fertilizer [36].

Engineered nanomaterials used in soil remediation are nanoscale calcium peroxide, nanoscale zero-valent iron, nanoscale metal oxides, and other nanoparticles, such as carbon nanotubes, bionanoparticles, polymeric nanoparticles, etc. They are mainly used in the removal of aromatic and heavy metal contaminants. However, in-depth study regarding the impact of these engineered nanomaterials on the surrounding environment and their mobility is very much necessary. Calcium peroxide nanoparticles have recently been applied to remediate soil contaminated with oil the most widely exploited nanoremediation technique is the application of nZVI in groundwater and soil remediation [37]. Other classes of nanomaterial which are being used in the process of soil remediation are carbon nanoparticles, polymeric nanoparticles (nanowire of polypyrrole, polyaniline, and poly(3,4-ethylenedioxythiophene), dendrimers poly(amidoamine) [PAMAM]), nanocomposites, and bionanoparticles (virus, plasmids, proteins, etc.) [38]. Allophane is a nanoscale hydrous aluminosilicate and an effective sorbent of copper. Yuan have demonstrated the removal of Cu from soil using a natural nanomaterial as an adsorbent material, allophane under laboratory scale [39].

Nanoparticles are seen to be the potential entities for the remediation of soil. Nanoparticles are much more efficient and highly selective for the removal of heavy metal contaminants. Iron-based nanoparticles are being widely used for the heavy metal removal from soil as they possess very strong adsorption property and excellent reducing property. The small size of the nanomaterials increases their mobility and deliverability in soil, and thus the heavy metals are stabilized or converted to less toxic species in soil. Nanotechnology has become a reliable means to remediate heavy-metal-contaminated soil. Most of the studies are conducted in laboratory scale, and therefore, much effort should be made to improvise in bringing it to the field scale.

Nanotechnology as an upcoming technology has shown great potential in various fields such as solar, electronics, optics, and pharmaceuticals. Nanomaterial-mediated environmental remediation offers great alternative in cleaning up of large-scale contaminated sites at low cost. Several metal/metal oxides and biopolymeric materials can be successfully employed for a variety of environmental remediation. Selection of appropriate nanomaterial for the mitigation of polluted area requires in-depth knowledge. Although several publications and patents have been reported pertaining to the application of nanomaterials/nanobiopolymeric composites developed via green route for the removal of contaminants from nature; deeper understanding along with extensive research regarding the ecological effect of the same is very much essential.

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22

Biofunctionalized Nanomaterials for Sensing and Bioremediation of Pollutants

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22.1 Introduction

Nanomaterial has its dimension in the range of 1–100 nm. Inherent characteristics of nanoparticles like small size, high surface area to volume ratio, and distinctive physicochemical properties possessed by some elements like surface plasmon resonance and conductivity are widely researched and are progressively being applied. With ease to use a bottom-up or top-down approach for nanoparticle synthesis, researchers can tune its properties as per requirement. Top-down approaches of synthesis include lithography, physical, chemical, ultrasonic, and printing techniques. In contrast, bottom-up methods include layer-by-layer self-assembly, molecular self-assembly, direct assembly, coating and growth, and colloidal aggregation. Characterization of nanoparticles before and after functionalization is of paramount importance. The average particle size and distribution in a medium can be determined by the dynamic light scattering (DLS) method.

In contrast, potential difference parameters of nanoparticles in a medium conducting charge can be accessed by ζ (zeta) potential. A UV–visible spectrophotometer can determine the optical absorption parameters of nanoparticles. Morphological characterization of nanomaterial can be done by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM).

Specific vital parameters are needed to be evaluated while developing an efficient bioremediation technique. Reproducibility, cost-effectiveness, ease of production, efficiency, and recovery time are critically monitored for biofunctionalized nanomaterial-based bioremediation. Nanoparticles are commonly biofunctionalized by nucleic acid, antibody, polymer, surfactant, protein, peptide, or enzyme. The interaction between nanoparticle and bio-part is mediated by covalent bonds, non-covalent bonds, encapsulation, or adsorption. The nanoparticle's biofunctionalization is a sensitive method, and retaining maximum efficiency, preventing cross-linking, and stability are the crucial parameters that are carefully monitored during the production process. With the ability to reduce xenobiotics

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and organic pollutants by chemical reduction or catalysis, biofunctionalized nanoparticles can be used in solid waste remediation, wastewater remediation, and even in combating air pollution. As compared to the microbial degradation process, which is relatively slower, biofunctionalized nanoparticles are used for bioremediation, including the removal of intransigent organic compounds such as tetrachloroethylene (TCE) and polyvinyl biphenyls (PCBs) is rapid and reliable. Magnetite nanoparticles are widely used for bioremediation because of its ease of preparation, economic production cost, and reusability. Magnetite nanoparticle biofunctionalized with *Rhodococcus erythyropolis* LSSE8-1 and *Pseudomonas delafieldii* showed enhanced bio-desulfurization activity, and these particles can be mobilized and recovered easily using a magnet. Magnetic nanoparticles functionalized with the IgG antibody had been demonstrated successfully for the removal of coliforms from polluted water. In this technique, coliforms, attached nanoparticles, were separated under a magnetic field.

There are various methods for sensing pollutants in trace amounts. However, the majority of widely accepted conventional methods require sampling and *ex situ* lab-based analysis. These methods require decent expertise and time and are sometimes not very accurate when a dynamic parameter is observed. In-field application of sensors for sensing pollutants is of paramount importance. Biofunctionalized nanoparticle-based sensors can overcome these problems. Novel enzyme and antibody functionalized nanoparticles are highly specific and precise and produce results quickly. These sensors can be used for both *in situ* and *ex situ* sensing applications. A wide variety of bioconjugated nanoparticle-based sensors are now able to sense pollutants of distinct nature, including organic, inorganic, and microbes. 11-mercaptoundecanoic acid and chitosan-functionalized gold nanoparticles are used for sensing heavy metals like lead, cadmium, and mercury. Gold nanoparticles, along with IgG antibody and protein, were successfully investigated for the detection of *Staphylococcus aureus* and *Staphylococcus saprophyticus*. Antibody-conjugated quantum dots are used for the detection of a variety of microorganisms. Many sensitive sensors are developed by targeting spore biomarker binding enzymes, which can detect spore concentration in a very minute amount.

The biofunctionalized nanoparticle is a quantum leap for environmental bioremediation technology. High efficiency and reliability make this technology promising. However, with continuous evolution, concern like the toxicity of engineered nanoparticles is continually being reduced. Recent advances in remediation techniques now allow recovery of nanoparticle from the field of action, which reduces any chances of toxicity. After the introduction of the "green production of nanoparticles" by plants, the dependency of harmful chemicals and capping agents will decrease in the future. In this chapter, we will see how biofunctionalized nanoparticles are helping us in the remediation of various pollution-related challenges for a cleaner, better, and greener future.

22.2 Synthesis and Surface Modification Strategies for Nanoparticles

Nanoparticles can be synthesized using either the top-down or bottom-up method. There has been a massive development in the field of nanoparticle synthesis in recent years. As per the requirement, nanoparticles can be synthesized in sophisticated machines, or we can use the biosynthesis route in which microbes fabricate the synthesis of nanoparticles. The top-down method is a destructive nanoparticle synthesis mode in which material chunks are broken down into nano-range. The top-down approach includes mechanical milling, chemical etching, LASER ablation, sputtering, exfoliation, lithography, electro-explosion, and arc discharge. The bottom-up synthesis method is a constructive way of producing high-quality nanoparticles. The bottom-up approach includes biosynthesis, atomic condensation, DNA scaffolding, LASER pyrolysis, microemulsion, melting mix, ultrasonication, sol–gel synthesis, plasma spraying, microwave synthesis, and electrospinning techniques.

The application of nanomaterials for bioremediation requires controlled dispersion and interaction with other molecules near their vicinity, which can be controlled by functionalization and surface modification. Surface modification significantly impacts distribution, assembly, and stability in a colloidal solution. While using nanoparticles for sensing application, specificity and selectivity features are also influenced by surface modification. By customizing the surface properties, attributes like electrical conductivity and corrosivity in a medium, hydrophilic, and hydrophobic nature of nanoparticles can be modified. Biofunctionalization of protein, peptide, and enzyme requires careful selection of nanoparticles with a hydrophobic or hydrophilic surface. Hydrophobic surface modification of nanoparticle is achieved by incorporating hydrophobic molecules like trioctylphosphine oxide, oleylamine, tetraoctylammonium bromide, triphenylphosphine, oleic acid, or dodecanthiol. Similarly, hydrophilic surface modification is mediated by hydrophilic molecules like mercaptosuccinic acid, mercaptoacetic acid, mercaptopropionic acid, bis-sulfonated triphenylphosphine, mercaptoundecanoic acid, dihydrolipoic acid, polyethylene glycol, mercaptosuccinic acid, or aminenated polyethylene glycol.

22.3 Binding Techniques for Biofunctionalization of Nanoparticles

The functionalization of biomolecules with nanoparticle is mediated by an interaction like covalent, non-covalent, encapsulation, and adsorption. The type of binding techniques critically influences the applicability, usability, functionality, and stability of biofunctionalized nanoparticle for remediation purposes. Properties of biomolecules like shape, size, hydrophobicity, hydrophilicity, and type functional group are examined thoroughly before functionalization.

22.3.1 Covalent Functionalization

Covalent biofunctionalization enables the binding of various biomolecules to nanoparticles through covalent bonds. Covalent attachment of nanoparticles and biomolecule prevents random reaction between interacting molecules, minimizes steric interference, provides stability from environmental hindrances, and supports biorthogonality and reversibility. Generally, the covalent linkage is mediated by an active group of nanoparticle and biomaterial; the subsidiary functional groups like carboxyl, amine, and thiol form covalent attachment by ester, amide, and disulfide linkage, respectively. Plentiful studies were done to determine the appropriate functional group for both a particular nanoparticle and a biomolecule to be conjugated. Chemical selectivity and their conjugation-related findings by Massey and Algar [1], covalent binding using carbodiimide coupling by Kamra et al. [2], "click" chemistry-based functionalization by Poonthiyil et al. [3], "SpyTag" and "SpyCatcher" by Reddington and Howarth [4], and supramolecular interaction of nanoparticles and molecules by Steed and Gale [5] are some of the great demonstrations in understanding vital insight of covalent binding of molecules with nanoparticles. The most popular approaches of covalent functionalization of enzymes, DNA, RNA, small ligands, proteins, peptides, oligonucleotides, and different nanoparticles are made using glutaraldehyde, organofunctional alkoxysilanes, *N*-hydroxysuccinimide, and 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide chemistry.

22.3.2 Non-Covalent Functionalization

Non-covalent interactions are formed by π–π interaction, van der Waals forces, electron sharing ligand system, hydrogen bonding, or enfolding of polymers. While the strength of non-covalent forces is lower than that of covalent bonding, the broad application's resulting impact is comparable. The non-covalent binding provides reversibility and kinetic freedom to binding molecules and some level of resistance toward the minute disturbance. These interactions are ideal for developing various sensors as they are very responsive to change in physical or chemical stimuli. Non-covalent interaction does not disturb the sp^2 carbon network like covalent functionalization. This non-covalent binding property helps in gaining novel usability without affecting the inherent property of nanoparticles and biomolecules. As compared to other binding techniques, non-covalent interaction can generate biofunctionalized nanoparticles with enhanced catalytic efficiency, bioavailability, sensing capability, dispersion efficiency, and biocompatibility. Different bio-nano composite has been reported, which have been fabricated through non-covalent interaction involving polylactic acid and various forms of nanofillers. Some successful examples of the research based on non-covalent functionalization of nanoparticles include non-covalently attached protein nanoparticles using avidin–biotin assembly by Aubin-Tam and Hamad-Schifferli [6], binding of redox enzyme–protein complex using non-covalent binding by Diaz, Care, and Sunna [7],

supramolecular assembly using host–guest interaction by Ma and Zhao [8], binding of carbohydrate–protein–nanoparticle by Penadés, Davis, and Seeberger [9], binding of nanoparticles to biomolecules using hydrophobic–hydrophilic interaction by Chen and Jiang [10], binding using "dock and lock" mechanism by Gong et al. [11], DNA nanoparticle functionalization by Seeman [12], and self-assembly of high-affinity protein to nanoparticles by Gurunatha et al. [13].

22.3.3 Encapsulation

Nanoencapsulation is a method of entrapment of nanoparticles, which can be in any form, i.e. solid, liquid, or gas, inside another shell or matrix made up of different materials. The particles entrapped in a shell are also referred to as core/active nanoparticle, which have the conjugated active ingredients. The outer shell provides selective interaction of nanoparticles with the environment of the application. It can be fabricated in such a way that the shell remains throughout the application, or there is a breakdown of the outer shell in response to a stimulus like pH/temperature change or any enzymatic activity. Several types of material could be used for shell fabrication. Enclosing matrices are chosen on the basis of essential properties required for the desired use. Some protein-encapsulating matrices include albumin, gelatine, lecithin, and legumin. Polysaccharide-based matrices include starch, alginates, chitosan, dextrin, and gums. There are numerous more examples of matrices used for the encapsulation of nanoparticles like liposomes, biopolymers, micelles, metal/polymeric/emulsion nanoparticles, dendrimers, organogels, or various other kinds of functionalized/non-functionalized nanoparticles. Nanoencapsulation can be done by chemical, physicochemical, or physico-mechanical technique. The chemical technique follows synthesis through nucleation and growth, incorporating the building blocks. Encapsulation involving suspension, emulsion, precipitation, sol–gel, and polymerization are some of the methods used in chemical encapsulation of nanomaterials. Compared to other techniques, the chemical technique provides uniform size, high purity, and good chemical homogeneity. The physicochemical encapsulation technique is based on both physical and chemical synthesis procedures. Physicochemical encapsulation includes phase inversion nanoencapsulation, coacervation and phase separation, inclusion complexes, solid lipid nanoparticles, layer-by-layer deposition, and controlled encapsulation. The physico-mechanical process of encapsulation exploits the physical properties of nanoparticles and mechanical instrumentation for the entrapment of nanoparticles. Some techniques for physico-mechanical encapsulation include spray drying, electro-encapsulation, and solvent extraction/evaporation. Enhanced pollution-degrading capabilities were seen in a comparative study where alginate polymer-encapsulated nano-zero-valent iron (nZVI) was used. The native technique was able to remove 43–56% of the pollutant, while 50–75% polycyclic aromatic hydrocarbons (PAHs) removal was seen using encapsulated nZVI. Encapsulation also helps in protecting enzyme-conjugated nanoparticles from protease attack.

22.3.4 Adsorption

In this technique of biofunctionalization, nanoparticles hold biomaterial(s) as a thin film on the outer surface. Adsorption of any molecule onto the outer surface of a nanoparticle is also known as "corona" formation. Adsorption mostly relies on the surface charge of both nanomaterial and biomaterial, and these parameters are sensitive to pH. Generally used biomaterials for adsorption-based functionalization include lipids, carbohydrates, DNA, proteins, dendrimers, small micelles, liposomes, and biologically or synthetically manufactured polymer. However, the exact mechanism of surface protein adsorption remains elusive. Surface modification enhances the adsorption of biomolecules to nanoparticles.

22.4 Commonly Functionalized Biomaterials and Their Role in Remediation

The role of biofunctionalized nanomaterials for environmental applications has been a critical focus of progressive research with a particular emphasis on reducing pollution and the elimination of diverse pollutants and xenobiotics from solid waste, water, and air. Integration of biomaterials of immense potential and nanoparticles of remarkable properties makes biofunctionalized nanoparticles a promising tool to be used in the environmental application. Commonly employed biomaterials for nanoparticle conjugation and pollution remediation include biopolymers, biosurfactants, nucleic acid, enzymes, proteins, and polypeptides (Table 22.1).

22.4.1 Biopolymers

Polymeric molecules having a bio-origin containing small monomeric units which are held together by the covalent bond are called biopolymers. Polymers derived using synthetic chemistry using resins, proteins, amino acid, fats, sugars, and oil of biological origin are also called biopolymers. The primary factor which promotes the use of biopolymer over fossil-based or synthetic polymer is their inherent property of biodegradability. Biopolymer doesn't cause harmful byproduct upon degradation, which makes them a sustainable component for bioremediation. "Polyol method" is often used for the synthesis of polymer nanomaterial in which the metal component is reduced and dissolved at high temperature by different alcohol-based solvents. The use of alcohol as a reducing agent is not problematic as the produced byproducts are generally less harmful organic materials. Lipophilic and oleophilic active particles created by lipophilic plasma polymerization are widely used for water refinement. Nanoparticles-conjugated biopolymers are also used for the degradation of a wide variety of pollutants, including dyes and heavy metals, and can be used for soil and water remediation.

Rosin amidoxime-conjugated magnetic nanoparticles are used for the removal of crude oil-based PAH from water bodies. The synthesis mechanism for the

Table 22.1 Various biofunctionalized nanomaterials used for bioremediation.

Functionalized material[a)]	Nanoparticle (NP)	Pollutant	References
Polymer			
Rosin amidoxime	Magnetic NP	Polycyclic aromatic hydrocarbons	[14]
Chitosan	Magnetic NP	Metallic	[15]
Polystyrene-block-polyacrylic acid	Carbon NP	Crude and refined petroleum	[16]
1,7-Octadiene	Silica NP	Aromatic hydrocarbon	[17]
Polyvinylpyrrolidone	Magnetic NP	Aromatic hydrocarbon	[17]
Cell wall of *Alcanivorax borkumensis*	Altered polyelectrolyte and magnetic NP	Oil spills	[18]
Chitosan/glass	TiO_2	Methyl orange	[19]
Natural rubber	TiO_2	Reactive red 4	[20]
Cellulose acetate	TiO_2	Methylene blue	[21, 22]
Chitosan	Iron	Arsenic	[23]
Surfactant			
Hexadecyltrimethylammonium chloride (HDTMA-Cl), and *N*-cetylpyridinium bromide (CPB)	Zeolite	Aromatic hydrocarbon	[24]
Cetyltrimethylammonium bromide (CTAB)	Silica and magnetic NP	Polycyclic aromatic hydrocarbons	[25]
Hydroxypropyl-β-cyclodextrin or native β-cyclodextrin	Mesoporous silica NP	Polycyclic aromatic hydrocarbons	[26]
Nucleic acid			
Double-stranded RNA	Nanosheet protectants	Fungicides	[27, 28]
DNA	Magnetic NP	Understanding horizontal gene transfer effects on bacteria involved in remediation	[29]
Proteins and peptides			
Sodium tetrachloropalladate(II)	Pd nanocrystals	Transition metal	[30]
Hemoglobin	Silica	Polycyclic aromatic hydrocarbons	[31]

(Continued)

Table 22.1 (Continued)

Functionalized material[a)]	Nanoparticle (NP)	Pollutant	References
Collagen	Supermagnetic	Oil	[32]
Soy protein	Zero-valent iron	Multiple oil-based pollutants	[33]
Pb-specific metalloprotein	Calcium alginate	Pb(II)	[34]
Enzymes			
Laccase	Chitosan	Chlorophenol, dyes	[35]
Phosphohydrolase	Lipid-coated NP	Ethyl-paraoxon	[36]
Metalloenzyme	Magnetic NP	Paraoxon	[37]
Peroxidase	Fe_3O_4	Azo dyes	[38]

a) Biological origin or their conjugation to nanoparticles is mediated via bio-synthesized linker/adapter/stabilizer/nanoparticles.

preparation of rosin amidoxime-conjugated nanoparticles is simple, precise, and economical and carried out at 45 °C by the co-precipitation method. The final product is filtered, washed, and dried at 30 °C. These nanoparticles can easily be separated after application using an external magnetic field [14]. Chitosan has shown promising results in water treatment application as flocculant. It degrades at a prolonged rate and leaves no harmful residues to the environment. Chitosan can also neutralize metallic pollution in water through the formation of chelates. In chelation, metal particles get attached to several areas on the polymeric chain and form a cage-like framework, which is then separated easily from the solution [15]. Polystyrene-block-polyacrylic acid conjugated with carbon nanoparticles provides enhanced hydrophobic interaction and has a high level of polymerization. These types of nanoparticles can be used to treat crude (with an efficiency of 80%) as well as refined (with an efficiency of 91%) form of petroleum [16]. 1,7-Octadiene-conjugated silica nanoparticles synthesized by plasma polymerization and radiofrequency-assisted reactor have been successfully tested for the removal of aromatic hydrocarbons. In the initial test with motor oil, 1,7-octadiene-conjugated silica nanoparticles have registered 99.5% adsorption efficacy in 10 minutes of exposure time. Polyvinylpyrrolidone-conjugated magnetic nanoparticles also showed comparable results when tested on aromatic hydrocarbon-based pollution [17]. The cell wall of bacteria can also be used as a polymer for remediation purposes. The cell wall of *Alcanivorax borkumensis* can be doped with altered polyelectrolyte–magnetic nanoparticles and can be used for decomposing oil-based pollutants [18]. Azo dye wastes are one of the most prominent types of pollutants from textile industries. Discharge of these dyestuffs to water bodies leads to a sudden change in the vital physicochemical parameters like salinity, biological/chemical oxygen demand, pH, temperature, and salinity. It also possesses a threat to aquatic life forms. Azo dyes have an anthropogenic origin,

with only a few types of having oxide azo, which occur in nature. Photodegradation of methyl orange was successfully demonstrated by Zainal et al. using combined TiO_2-chitosan/glass under illuminated visible light [19]. The modified double-layer system made by chitosan functionalized on the glass then, TiO_2, and epoxidized natural rubber is used for the removal of "Reactive red 4" dye under illumination [20]. Cellulose-based biopolymer doped with nanoparticles is widely used for fabricating filter membranes and adsorption of various kinds of pollutants. A hybrid film synthesized from flexible cellulose acetate coated with TiO_2 is able to reduce Methylene blue dye [21, 22]. Biopolymers having an affinity for metal binding utilize elastin-like polypeptide made of single or double hexahistidine groups. These configurable biopolymers preserve the feature of the elastin group even when they undergo a phase transition at high temperature. Dynamic aggregation can be achieved by adjusting the biopolymer's length at varying temperatures. Cadmium ions present in water attach to the biopolymer strongly due to the presence of histidine group in polymer. Recovery of polymeric biomaterial can be ensured by doping them with magnetic nanoparticles. Iron-doped chitosan fabricated by electrospinning was successfully demonstrated by Min et al. for effective arsenic filtration [23]. Similarly, numerous biopolymers can be tuned with variety of nanoparticles for environmental remediation as the combination of biology with chemistry has immense potential and never-ending possibilities.

22.4.2 Surfactants

Surfactants are the compounds that lower the surface tension between the liquid and liquid/gas/solid. Surfactants are used in bionanotechnology for tuning the surface properties and providing stability to nanoparticles. Surfactant as stabilizer prevents agglomeration of nanoparticles by combined electrostatic and steric forces. Surfactant-coated nanoparticles are generally used to clean up hydrocarbon-based pollutants from the environment. The surfactant-conjugated nanoparticles and their self-assembly help in combating various pollutants by interaction like π–π stacking, charge-based binding, or hydrophobic effect. The zeolite nanoparticles were modified with a cationic surfactant like hexadecyltrimethylammonium chloride (HDTMA-Cl), and *N*-cetylpyridinium bromide (CPB) can be used to separate various components of aromatic compounds occurring in petroleum-like xylene, toluene, benzene, and ethylbenzene [24]. Silica and magnetic nanoparticles like Fe_3O_4 are often pore-functionalized with cetyltrimethylammonium bromide (CTAB) for the removal of PAH from water bodies [25]. Mesoporous silica nanoparticles fabricated with hydroxypropyl-β-cyclodextrin or native β-cyclodextrin and condensed tetraethyl orthosilicate, catalyzed with the help of acid and alkali, can also be used for the removal of different PAH-based pollutants [26]. Many microorganisms like *Ustilagomaydis*, *Pseudomonas aeruginosa*, *Rhodococcus erythropolis*, *Candida bombicola*, *Bacillus subtilis*, *Bacillus licheniformis*, *Acinetobacter calcoaceticus*, and *Microbacterium* produce biosurfactants like cellobiose lipids, rhamnolipids, trehalose lipids, sophoro lipids, surafactin, lichenysin, emulsan glycolipopeptide, and microbactan glycolipopeptide, respectively. Many of these

biosurfactant-producing microorganisms were identified to produce nanoparticles of different sizes. Biosurfactants produced by these microorganisms are economically significant and can be used in bioremediation as an emulsifier, antimicrobial agent, and oil recovery agent. However, the functionalization of these biosurfactants to nanoparticles and the study of their impact and efficiency in remediation are yet to be explored.

22.4.3 Nucleic Acid

The combination of nucleic acid with nanoparticles has been widely explored for environmental application. Gold and silver nanoparticles conjugated with DNA had been investigated for bioremediation of various hydrocarbon-based pollutants, but the cost-effectiveness, large size of DNA/RNA, and sequestration over metallic nanoparticle remain a significant problem, and thus they have limited applicability for environmental remediation. Recent advances in the field of biotechnology had let to the development of many double-stranded RNA-based fungicides. These fungicides are more effective than conventional biochemical counterparts and leave no harmful residues in the environment after use. In RNA-based fungicide preparation, the double-stranded RNA is synthesized to hybridize with the messenger RNA of fungal species. This RNA preparation is then sprayed on the area of application, which leads to the silencing of vital housekeeping genes in fungi and, eventually, the death of fungal species. However, the life span of naked RNA is significantly less. To counter this issue, nanosheet protectants are used. These protectants enhance the life of naked RNA and ensure extended biocidal action [27, 28]. Magnetic nanoparticle-labeled DNA has been used to study the horizontal gene transfer events in bacteria used in soil remediation. This technique provides better insight into various bioreaction mechanisms and their role in bioremediation. The recovery of these organisms having magnetic nanoparticles-labeled DNA can be easily achieved under the external magnetic field [29]. The development of micro/nanomachines for the removal of various heavy metals from water bodies has also been demonstrated. These machines contain a self-propelled tube having conjugated DNA that can bind selectively to a particular heavy metal.

22.4.4 Proteins and Peptides

Protein and peptide are made up of small amino acid chains linked by a peptide bond. Protein and peptide-conjugated nanoparticles are widely used for environmental remediation purposes. Protein-conjugated nanoparticles are efficient, precise, and specific in their action but are sensitive to pH and temperature. However, their efficacy and speed of action make them an appropriate candidate for sustainable bioremediation. Protein and peptide are also used as stabilizing/binding agents of various nanoparticles, which are used in bioremediation.

Sodium tetrachloropalladate(II) can bind to palladium and reduce it by increasing the proportion of tryptophan progressively, which is an example of peptide-based remediation of transition metal [30]. Many protein-conjugated nanoparticles have

been tested for their applicability for PAH remediation. Oxidizer-catalyzed reaction leads to the covalent attachment of PAH to protein biomolecules. Iron-containing hemoglobin protein having four protein chains (two α and two β) encapsulated with silica nanoparticles have been tested for PAH removal from wastewater. This protein conjugate was tested *ex situ* and recorded a hydrocarbon removal efficiency of 82% from PAH-polluted water at pH 5 [31]. Citric acid-conjugated magnetic nanoparticles react with collagen to form superparamagnetic iron oxide-based nanoparticles with excellent oil-absorbing capability. These superparamagnetic oxides functionalized nanoparticles are functional and stable even at 87 °C and can absorb oil twice its weight [32]. Zero-valent iron conjugated with soy protein has been patented for its promising application in water and soil remediation [33]. With the advancement of genetic engineering technologies, we can now exploit various recombinant proteins for the bioremediation of multiple pollutants. One such example of the recombinant fusion protein is Pb-specific metalloprotein "PbrD" which is cross-linked to nanoparticles like calcium alginate for the removal of Pb(II) from water. The uptake rate of Pb by Pb-specific metalloprotein cross-linked calcium alginate was found to be 8.82 mg/g at 100 mg/l concentration in the initial screening. This promising result shows its applicability in recovering Pb from acid mine drainage and industrial effluents [34].

22.4.5 Enzymes

Enzyme-immobilized nanoparticles have the benefit of prolonged operational flexibility, fast recovery, ease of reuse, lower associated expenses, and high efficiency. These advantages are driving the attention of researchers for developing techniques for bioremediation of environmental pollution. Enzyme characteristics and dynamics are susceptible to pH, temperature, nature of medium/environment of action, type of attachment with nanoparticles or matrix, and distance of core nanoparticle with the enzyme. These parameters critically drive the catalytic efficiency of the functionalized enzyme. Conjugating enzyme on the outer surface of the nanoparticle, where the nanoparticle is at the core, provides the particle enhancement/dual functionality and generally referred to as enzyme-nanoparticle corona. Enzyme-conjugated nanoparticles prevent agglomeration and provide better bioavailability. The main enzymes used for bioremediation include peroxidase, oxygenase, and laccase. These enzymes are the member of the oxidoreductase family and have an enzyme commission (EC) number 1. They catalyze a reaction by transferring an electron from donor to acceptor, and the contaminants are oxidized to a less harmful form. Monooxygenase enzymes are used for remediation of various aromatic and aliphatic compounds, and they catalyze many by dehalogenation, denitrification, ammonification, desulfurization, and hydroxylation reaction. Various examples of enzyme-based nanoparticles for environmental applications are discussed below.

Laccase is a highly potent catalytic agent for the bioremediation of contaminants from the textile and petrochemical industries. Organic pollutants like chlorophenol, dyes, and various paper wastes can be remediated using laccase. *Trametes versicolor*-derived laccase conjugated into chitosan-encapsulated magnetic

nanoparticle is used for the reduction of dyestuff and hydrocarbons from polluted water. These nanoparticles are synthesized by reverse-phase surface adsorption, and they retain their activity even after repeated use [35]. Ginet et al. [36] have successfully developed an easy to sieve, stable, and reusable biocatalyst to degrade ethyl-paraoxon pesticide using engineered lipid-coated nanomagnetic particle conjugated with phosphohydrolases. *In vivo* immobilization of phosphotriesterase derived from bacteria was studied for its role in soil and water remediation. Magnetic nanoparticle-conjugated metalloenzyme can be produced in a single step and was able to hydrolyze harmful pesticides like paraoxon. Paraoxon pesticides are lethal to human due to its neurotoxic nature [37]. Peroxidase enzymes have an essential role in removing phenolic compounds like azo dyes, a prominent pollutant found in textile industry waste. Fe_3O_4-conjugated peroxidase enzyme altered with the help of glutaraldehyde co-precipitation was able to reduce the azo dyes present in wastewater to a considerable amount [38].

22.5 Biofunctionalized Nanoparticle-Based Sensors for Environmental Application

Biofunctionalized nanoparticle-based sensors have a biomolecule-conjugated nanoparticle which interacts with the analyte, and a signal transducer generates the signal (Figure 22.1). The generated signal may be directly visible or processed and quantified by other electronic components. Nanoparticle-based biosensors generally provide a quick result when compared to conventional technologies like gas/liquid chromatography. However, various kinds of biomaterials are used in combination with nanoparticles for the development of sensors, but the majority of them have enzymes or antibodies as a primary bioelement. Sensors based on the quantification of electrochemical properties are generally based on the redox reaction. In general, biosensors can be of two types, i.e. colorimetric or electrochemical.

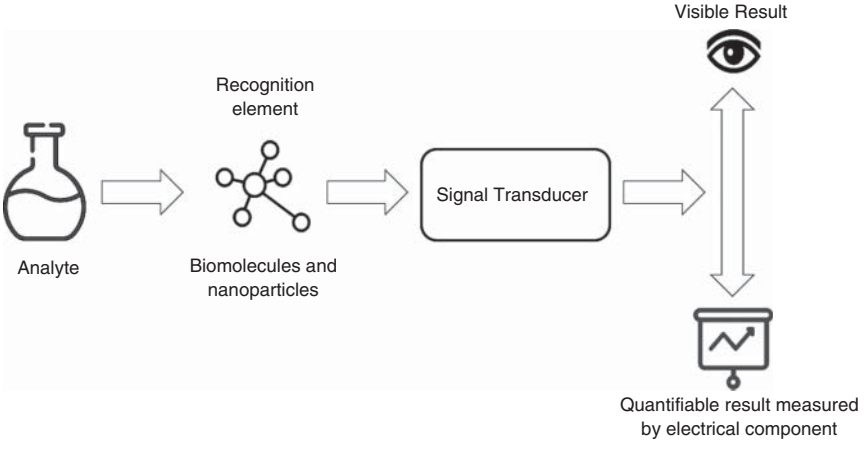

Figure 22.1 Components of biofunctionalized nanosensor.

Colorimetric sensors are the group of optical sensors that quantify/detect color in response to external stimuli. Electrochemical biosensors use electrical and chemical parameters and are further divided into conductometric, potentiometric, or amperometric biosensors. The potentiometric biosensor reads the potential difference between the analyte and reference probe in a medium. Conductometric biosensor measures the flow of current through a medium when the analyte undergoes a reaction with bioconjugated nanoparticles. In amperometric sensing, the current is measured across two electrodes as a function of time during a redox reaction between biomolecule and medium containing analyte. Various biofunctionalized nanoparticles have been developed which can detect inorganic/organic compounds, heavy metals, pesticides, herbicides, coliforms, and xenobiotics in soil and water.

An amperometric sensor fabricated by multiwalled carbon nanotubes conjugated with mushroom tyrosinase is developed to sense bisphenol A in plastic products. Another amperometric biosensor designed from liposome bioreactor and chitosan nanocomposite along with mushroom tyrosinase was successfully tested for sensing the presence of phenolic compounds. A biosensor fabricated from urease enzyme and ZnO nanoparticles was developed by Eghbali et al. and can detect urea in water [39]. A core–shell magnetic iron functionalized with acetylcholinesterase is demonstrated for the sensing of organophosphorus pesticide. This biosensor was able to retain its activity even after prolonged use in initial trial. Heavy metal pollution is the most persistent problem across the globe. It affects both soil and water bodies. A colorimetric sensing assay developed by Liu and Lu uses the Au nanoparticle cross-linked DNAzyme. In the presence of water contaminated with Pb^{2+}, the cleavage of cross-link was seen, which trigger a color change in the medium of action [40]. Various pathogen and coliform recognition sensors have also been developed using nanoparticles and antibodies. Engineered/polyclonal/monoclonal antibody-conjugated nanoparticles have been tested for their capability to sense the presence of viruses, bacteria, spore, toxins, and xenobiotics.

22.6 Limitation of Biofunctionalized Nanoparticles for Environmental Application

Nanoparticle-based remediation innovations have gained a lot of attention in recent years. The inherent properties of nanoparticles and biofunctionalized biomaterials make them an exceptional tool to be used in maintaining the well-being of our environment. These extraordinary capabilities of nanoparticles support their use but also attract the researcher's attention to the novel toxicity caused by them. With widespread use, nanoscale materials can find their way to air, water, and soil. Nanoparticles can affect the food chain and, ultimately, the heath of animals and humans. Once reactive nanoparticles find their way to the living organism, they lead to the production of reactive oxygen species, which can later affect DNA, proteins, and cellular membranes. Inhalation of nanoparticles with people affected with asthma can cause long-term lung disorders. In a study conducted in a mouse model, exposure to carbon nanotubes has shown the development of granuloma

in the intestine. Metal oxide toxicity study in rats exposed to TiO_2 and SiO_2 also showed lung tissue toxicity. Animal model experiments also revealed that metal oxide nanoparticles like TiO_2 could penetrate the skin and cause dermal toxicity, affect vital organs, deplete collagen, generate free radical, and ultimately cause oxidative stress. In the aquatic environment, nanoparticles can enter through the gills of aquatic organisms and affect their metabolic pathways' vital functioning. Scientists have also found a delay in embryo hatching and a high rate of mortality.

Furthermore, some limitations of using biofunctionalized nanoparticles include pH, temperature, and other environmental factors that demarcate a boundary for bioconjugate nanoparticles to be used within a specific range of parameters that do not affect the functionality of the biomolecule in use.

22.7 Future Perspective

With the recent advancement of bioinformatics, there are plentiful resources to discover vital parameters and protocols for bioconjugation of biomaterials to nanoparticles. Magnetic nanoparticles have also reduced the concern for the recovery of nanoparticles used in water remediation. However, there is a colossal scope persisting in bioinformatics that can accurately predict the life cycle of nanoparticles based on their nature. The development of simulation software to get an insight into probable toxicity will help researchers understand the aftereffects if nanoparticles make their way to the living system. There is a rapid progression in this field, and with recent innovation, bionanotechnology will be more reliable, sustainable, and cost-effective for environmental applications.

22.8 Conclusion

The use of bionanotechnology for remediation of pollutants is the most noteworthy revolution in the twenty-first century. This domain fulfills the two crucial requirements for in-field bioremediation, i.e. sensing and degradation of contaminants. Growing urbanization and industrialization has created a heavy load on current pollution control strategies. The growing interest in the field of biomolecule-conjugated nanoparticles has shown promising results through cutting-edge innovations. Biomolecules being extremely precise and useful in bioremediation when incorporated with nanoparticle get enriched with better support, more efficiency to reach medium of action, efficacy, and sustainability. Progression in innovation has significantly reduced the production cost for many nanoparticles. Various open-source softwares with simulation functionality had led to enhancement in understanding the dynamics of binding and can give us vital information regarding the fabrication of bioconjugate nanoparticles for environmental use. With the ease of producing nanoparticles by the bottom-up or top-down approach, we can produce desirable surface properties parallelly during synthesis.

Nanoparticle can also be folded into two-dimensional (tubes, fibers, and wires) and three-dimensional (quantum dot, shell, ring, and microcapsule) structures. Conjugation of biomolecule provides bio-nano system immense potential for bioremediation of vast number of pollutants. With excellent physiochemical properties of nanoparticles along with biomolecules, we can access the ever-changing dynamics of soil and water bodies in real time. Bio-nano sensors are compact and sensitive, produce a quick result, and do not require sophisticated laboratory conditions. The capability of bio-nano sensor to be integrated with electronic devices makes data logging easier than ever.

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23

Biogeneration of Valuable Nanomaterials from Food and Other Wastes

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23.1 Introduction

Annually, millions of tons of agricultural and food wastes are produced throughout the world, which are disposed into environment directly or after the treatment. Agricultural waste consisting of corncob, rice husk, rice straw, oil palm empty fruit bunch, sugarcane bagasse, and wheat straw weighs approximately 2 billion tons worldwide. These wastages have deep impact on economy, and direct disposure of wastes without treatment leads to severe environmental pollution. During fruits and vegetable processing, wastages are generated due to operations like, cleaning, processing, cooking, packaging, etc. Agriculture wastes are the by-products of agricultural activities, and agricultural wastes mainly come from crop residues like residual stalks, straws, leaves, roots, husks, shells, etc. Animal wastes also contribute to agricultural waste which can be used as manures. Food and agricultural wastes are freely available almost throughout the year [1, 2].

According to Food and Agriculture Organization (FAO), there have been three times increase in agriculture productivity because of increase in agricultural land, technological developments, and rapid human population growth [3]. Hence, generation of food and agriculture waste is unavoidable, but it can be minimized by the utilization of proper agricultural practices, better postharvest management schemes, and understanding the consumer's requirement. With the evolution of knowledge and technical development, humans realized that food and agro-waste could be better utilized for the production of various products of industrial importance, i.e. organic acids, protein-rich feeds, aroma compounds, bioactive secondary metabolites, pigments, etc. Nanotechnology is an upcoming domain and its advancement has empowered the users a wide variety of applications. Plenty of research and review work has already been reported on various applications of different nanomaterials. However, few works have been reported the utilization

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of food and agricultural wastes for nanomaterials synthesis. The present chapter throws light on synthesis of nanomaterials using different agriculture and food wastes [4–6].

23.2 Green Synthesis of Nanomaterials by Using Food and Agricultural Waste

Hussain et al. [7] reviewed about nanoparticles production by classical methods which are expensive, use toxic solvents, generate harmful wastes, and produce imperfect surface structures. These methods pollute the environment and affect the human health. Besides those methods, green synthesis method can be adopted for nanomaterial synthesis, which does not use harmful and expensive chemicals and use natural resources like plant leaves and fruit materials for the synthesis of nanoparticles [7]. Food and agricultural wastes can be utilized for the generation of number of metal and metal oxide nanoparticles. This biogenic method of nanomaterial synthesis is considered as a better alternative, since this synthesis method is environmental friendly and follows a green chemistry-based route. Agricultural and food wastes contain number of important biomolecules like alkaloids, amino acids, enzymes, phenolics, flavanoids, proteins, polysaccharides, saponins, tannins, and other valuable phytochemicals. Hence, these wastes can act as bio-reducing agents for the synthesis of nanomaterials, and the wastes may also find their applications in the stabilization of nanomaterial synthesis system [8, 9]. Ghosh et al. [9] reviewed nanoparticle synthesis from the waste by a bottom-up approach. Nanomaterials produced using the agricultural and food wastes are cost-effective, easily available, and eco-friendly and utilize the waste to create valuable products. Various metal and metal oxide nanoparticles synthesized using food and agro-wastes were found to be interesting for waste utilization and they are shown in the Table 23.1 with suitable precursor material and type of waste utilized. However, many other important metal and metal oxide nanoparticles can also be synthesized using agriculture waste, i.e. grapheme oxide, magnesium oxide, copper oxide, etc. [9].

This green synthesis of nanomaterials is a new emerging domain, but limited studies were found particularly for nanomaterial synthesis using food and agro-wastes. As green synthesis method utilizes wastes, this method is economical and is helpful as it promises to utilize waste for value-added nanoparticle synthesis.

23.3 Synthesis of Bionanoparticles from Food and Agricultural Waste

Agricultural and food wastes are organic in nature, which could be used for synthesis of bionanomaterials. Biopolymers like xylan, cellulose, protein, starch, and chitosan have been applied for the generation of bionanoparticles [24]. Different types of bionanomaterials synthesized from the agriculture and food waste would be discussed in this section.

Table 23.1 Biogeneration of nanomaterials using agro-waste.

Nanomaterials type	Precursors	Type of waste used	References
Silver nanoparticles	Silver nitrate	Grape seed extract	[10]
Silver nanoparticles	Silver nitrate	*Annona squamosa* peel extract	[11]
Silver nanoxylan	Silver nitrate	Xylan from waste corn cob wastes	[12]
Silver nanoconjugates	Silver nitrate	*Punica granatum* (pomegranate) peel's polyphenols	[13]
Gold nanoparticles	Gold chloride trihydrate	Mango peel extract	[14]
Gold nanoparticles	Auric chloride	Aqueous extract of nonedible onion peels	[15]
Gold nanoparticles	Gold chloride	Aqueous extract of outer most waste green watermelon skin	[16]
Platinum nanoparticles	Chloroplatimic acid	Sugarcane bagasse extract	[17]
Palladium nanoparticles	Palladium acetate	Aqueous extract of *Annona squamosa* peel	[18]
Palladium nanoparticles	Palladium chloride	Aqueous extract of water melon rind	[19]
Palladium nanoparticles	Palladium chloride	Banana peel extract	[20]
Palladium nanoparticles	Palladium acetate	Papaya peel extract	[21]
Iron nanoparticles	Ferrous sulfate heptahydrate	Aqueous leaf extract of mango, curry, neem, and champa	[22]
Iron nanoparticles	Ferric chloride	*Citrus maxima*'s peel extract	[23]

23.3.1 Cellulose Nanomaterials

Synthesis of nanocellulose can be done from different sources of agricultural waste, containing lignocellulosic materials, by chemical, physical, and microbial methods. Lignocellulose is nonedible agricultural waste residue and it is abundantly available resource in the nature. Lignocellulose is made up of two carbohydrates, cellulose and hemicellulose. Besides, there are presence of noncarbohydrate compounds, phenolic polymers and lignin. Lignin is responsible for binding cellulosic fibers for strengthening the plant cell walls. Separation of cellulose, hemicellulose, and lignin is a big challenge because of highly crystalline cellulosic structure is attached in the polymer matrix of lignin and hemicelluloses. This separating resistance is called recalcitrance, which can be overcome by chemical pretreatments, like acid hydrolysis, alkaline hydrolysis, oxidation agent, and ionic liquids. Separation of lignin

Table 23.2 Cellulose-based bionanomaterials.

Bionanomaterial type	Type of waste used	Method	References
Cellulose nanocrystals (CNCs)	Sugarcane bagasse	Acid hydrolysis	[31]
	Potato peel waste	Acid hydrolysis	[32]
	Pineapple leaves	Acid hydrolysis	[33]
Cellulose nanofibers (CNFs)	Oil palm (*Elaeis guineensis*) tree waste	Mechanical grinding of cellulose	[34]
	Sugar beet pulp waste from sugar industry	High-shear homogenizer	[35]

from carbohydrates (cellulose and hemicellulose) is achievable by pretreatment. Nanocellulosic material synthesis using mild treatment is gaining more popularity. Top-down synthesis of nanocellulose can be achieved by mechanical forces, i.e. cryo-crushing, grinding, high-pressure homogenization, etc., which are quite energy-intensive operations. Chemical methods like oxidation, acid hydrolysis, etc., may be combined with mechanical treatments for increasing size reduction efficiency with reduced energy consumption. Nanocellulosic materials can be of two types, i.e. cellulose nanocrystals (CNCs), and cellulose nanofibres (CNFs) [25–27]. Principal method of CNC isolation from cellulose fibers is by acid hydrolysis [28]. Synthesis of CNFs was done by research group at University of Toronto, which was reported in the year 2007. The synthesis of CNFs was done by combining a number of treatments including chemical treatment, mechanical refining, homogenization, and crushing of hydrated materials in liquid nitrogen [29]. Both CNC and CNF can be produced from the plant cell walls. The CNCs are needle-like nanostructures, which are produced by strong acid hydrolysis of natural organic materials like bleached wood pulp, cotton, etc. On the other hand, CNFs are long flexible fiber networks, which are synthesized by homogenization (high pressure), enzymatic hydrolysis, or by mechanical action [30]. Synthesis of cellulose nanomaterials from food and agricultural wastes has been summarized in the Table 23.2.

Nanocellulose synthesis is quite promising method for utilization of agricultural waste for development of higher value products of economical interest.

23.3.2 Protein Nanoparticles

Peng et al. (2017) reported about utilization of rice bran waste for the synthesis of rice bran albumin-chitosan nanoparticles, which were found to have application in hydrophobic active agent delivery [36]. In another study, protein nanoparticles were prepared from chicken feather waste by reduction and ultrasound treatment [37]. Zein is extracted using polar solvents like ethanol or isopropanol, from corn mill waste, i.e. gluten meal. Basically, zein is the water-insoluble protein found in corn that has been generally recognized as safe (GRAS) by Food and Drug Administration (FDA). Zein is soluble in polar solvents and insoluble in water, but when

added to water, forms zein nanoparticles dispersed in water. Zein nanoparticles find their applications in nanocomposites for packaging materials, functional or bioactive compound encapsulation, drug delivery, etc. [38].

Besides above-mentioned nanomaterials, other nanomaterials can also be synthesized from food and agricultural waste. Ngu et al. (2016) synthesized carbon nanoparticles from waste rice husk, where bottom-up approach was used, by carbonization of rice husk using sulfuric acid. Developed carbon nanoparticles were used for sensing applications. In another study, food wastes were used for the synthesis of carbon nanodots [39, 40].

23.4 Conclusion

Food and agricultural wastes are generated across the world need to be utilized for valuable products manufacturing by their transformation either physically, chemically, or biologically. Re-utilization of these wastes has several advantages, including a renewable source of biomolecules and bioactive compounds. The beneficial potential of developed products needs to be evaluated by assessing the effects on the economy and changes caused in consumer's life. Utilization of agricultural and food wastes would be helpful to prevent environmental pollution caused by the degradation of the wastes. Various methods of biogenic synthesis of nanomaterials using agricultural and food waste would find wide applications in biomedical, environmental, electronics, energy storage and nutrition sectors. Food and agricultural biowastes are available worldwide and inexpensive, and they are under-exploited source, which could be utilized for the synthesis of nanomaterials. As this particular domain of nanotechnology is considered to be in its early stage, more investigations need to be carried out to assess the safety of the final deliverable products for final acceptability and approval.

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24

Biosynthesis of Nanoparticles Using Agriculture and Horticulture Waste

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24.1 Introduction

Nanotechnology plays an important role and includes materials, technologies, and processes that are adopted to enhance the rate of production and create products that are in demand for everyday use. The study of nanotechnology comprises material that is extremely small in size ranging between 1 and 100 nm. Emerging and unique properties of nanomaterial such as optical, magnetic, and electrical have the probable prospects of impacts in the area of medicine, electronics, and other fields of applications [1]. Most of the synthesized nanomaterials exhibit different properties and effects when compared to the similar material in a macroscale, as they have high surface-area-to-volume ratio. The development of nanoscience will change and develop next-generation materials that are durable, lighter, and stronger than the materials used today in different fields of applications. Physical, chemical, and mechanical methods are widely used for the synthesis of nanoparticles; however, the process is not economic and adheres the use of toxic chemicals. Thus, there is a need for an eco-friendly and cost-effective biological route for the synthesis of nanoparticles to overcome any toxicity towards human health and the environment as well. Hence, the biosynthesis of nanoparticles is gaining importance in the current nanoscience research. Biosynthesis of nanoparticles refers to use of living systems such as microorganism and plant material for the synthesis of nanoparticles through reduction mechanisms. One such option is to channelize underutilized agricultural and horticultural wastes into biosynthesis of nanoparticles, as it is observed that relatively few research articles are surveyed and evaluated.

The magnitude of organic waste generated worldwide by agricultural and horticultural activity is exceptionally large and offers potential renewable sources of bioactive compounds and biomolecules. The availability of resources has created a unique opportunity to develop new methods of waste management and recycling strategies.

Biotechnology for Zero Waste: Emerging Waste Management Techniques, First Edition.
Edited by Chaudhery Mustansar Hussain and Ravi Kumar Kadeppagari.

Biosynthesis of nanoparticles using agro-wastes such as *Cocos nucifera* coir [2], corn cob [3], fruit seeds and peels [4], wheat bran [5], rice bran [6], and nut shells [7] is reported in the literature. Plant-based resources are rich in tannins, steroids, saponins, flavonoids, alkaloids, and other nutritional compounds [8]. These compounds are responsible for reduction of metal ions into metal nanoparticles. They are also present in various parts of plants such as stems, leaves, roots, barks, flowers, shoots, and seeds. Plant extracts having these compounds are responsible for reduction of metal ions and are capped on the surface of nanoparticles. Biosynthesis of metal and metal oxide nanoparticles of required size and morphology has the potential importance due to the size-dependent properties for various applications.

24.2 Agricultural and Horticultural Waste

Agriculture waste generated from various farming activities comprises of basic functional moieties. These can be explored for the biosynthesis of nanoparticles. The agricultural waste includes rice (straw, husk), corn (leaves, stalks, cobs, husks), coconut (coir, pith, leaf), sugarcane (bagasse, leaf), banana (leaves, trunk, peels), pineapple (pulp, core, plant, peel, crown), coffee pulp, mango (pulp, peel, rejects), tobacco (reject leaves, midribs), abaca leaves, cocoa (core, pods), cassava (washings, peel, leaves, trunk), peanut (reject beans, pods, plant), mung bean (reject beans, pods, plant), areca nut (leaf, coir, shell), husk of rice, ragi, corn, cereals.

According to the International Society of Horticultural Sciences (ISHS), horticulture includes the olericulture, cultivation of medicinal and aromatic plants, growing fruits, floriculture, and arboriculture. Horticulture activity generates waste materials such as stem, leaf, flower, peel, seeds, shells, pods of flowering plants, rejected flowers, dried leaves, and vegetables. These wastes contain bioactive compounds such as tocopherols, sterols, carotenoids, anthocyanins, flavonoids, cinnamic acids, and phenolic acids [9]. Hence, horticulture waste has the potential to be channelized for biosynthesis of nanoparticles.

24.3 Biosynthesis of Nanoparticle

To synthesize nanoparticles, dry form is more suitable, since it can be stored for a long period of time. General steps involved in processing of waste, preparation of metal salt solution, and biosynthesis of nanoparticles are highlighted with a practical approach and examples (Figure 24.1).

24.3.1 Processing of Agriculture and Horticulture Waste

Agriculture and horticulture waste will either be in wet or dry form. Prior to using it for nanoparticle synthesis, processing is an essential step. For long-term storage, drying, powdering, and sieving are considered to be a suitable option. For the immediate use of wet waste material, removing surface water is recommended. During the

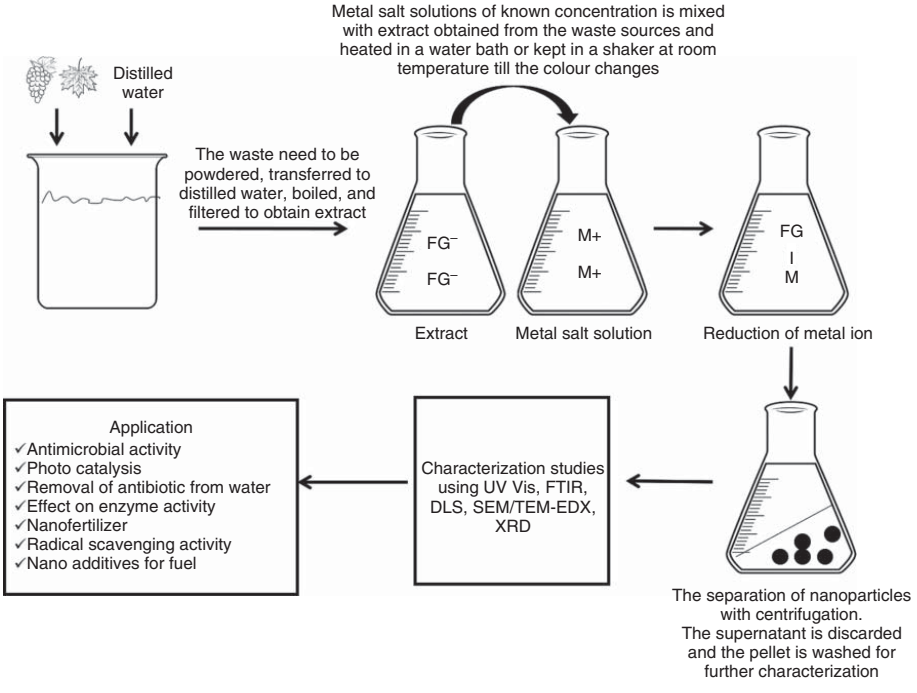

Figure 24.1 Overview of biosynthesis of nanoparticles, characterization, and application. FG^- indicates functional group (e.g. OH^-), M^+ indicates metal ion (e.g. Ag^+).

Table 24.1 Waste material used in the biosynthesis of nanoparticles.

Waste material	Specification	Operation	Storage	References
Cocoa pod shell	Has high moisture	Material need to be cut to small pieces, dried under sunlight, powdered and sieved	At 4 °C	[10]
Leaves	Washing and drying	Can be powdered or made to small pieces	Room temperature in airtight containers	[11]
Flowers	Washing and drying	Diced into pieces and drying under appropriate sunlight	Stored in airtight containers	[11]
Fruit peel	Washing and drying	Cut into small pieces and either shade dried or sunlight	Stored in airtight containers	[12]

preparation of the extract, the waste needs to be powdered, transferred to distilled water, boiled, and filtered. Table 24.1 specifies the waste material that can be used in the biosynthesis of nanoparticles and its various features necessary to be followed when used for any experimental purpose. General procedure of preparing metal salt solution, extract from waste material, and storage is mentioned in Table 24.2.

24.3.2 Synthesis of Nanoparticles

Metal salt solutions of known concentration are mixed with extract obtained from the waste sources and heated in a water bath or kept in a shaker at room temperature till the color changes. During the heating process, the metal ions and its respective functional moieties will have interaction in a short period of time. Also a change in color is an indicator of nanoparticle synthesis. For the synthesis of nanoparticles at room temperature, metal salt solution should be mixed with the extract and is kept under vortex in an orbital shaker for a long period of time. This enhances the interaction between metal ions and functional moieties present in the solution triggering reduction of metal ions. Periodically, surface plasmon resonance (SPR) is recorded using UV spectrophotometer that is essential in monitoring the SPR values. Vortex of the extract with salt solution leads to the reduction of metal ions. As the SPR value increases, the reduction reaction of ions will reduce accordingly indicating the completion of the process.

24.3.3 Separation of Nanoparticles

The separation of nanoparticles is followed with centrifugation with a suitable rpm maintained at a fixed temperature. The supernatant is discarded, and the pellet is

Table 24.2 Procedure for preparation of metal salt solutions, extract and storage.

	Procedure	Storage
Metal salt solution	Prepared by dissolving the required metal Ag, Au, Cu, ZnO, Ag in 0.1, 0.5, 1 mM of their metal salts in distilled water	Prepared solution to be retained in dark condition to avoid photodegradation.
Cocoa pod shell	1 g in 100 ml distilled water, boiled and filtered using Whatman paper	Filtrate to be stored at 4 °C.
Leaves	Required quantity of leaves are boiled in distilled water. Extract cooled and filtered using Whatman paper.	Stored at 4 °C prior to the synthesis of the desired nanoparticle.
Flower	1 g of dried flowers are added to 100 ml of deionized water and boiled in water bath for 10 min.	The extract is cooled to room temperature prior to synthesis of nanoparticles [13].
Fruit peel	10 g of processed peel is transferred to 250 ml of double distilled water. Further heated at 60 °C for 30 min	Extract cooled to room temperature, filtered and stored at 4 °C [12].

washed several times to remove the excess extract and unreduced ions present in the respective salt solution. Further, the pellets are used for characterization of the biosynthesized nanoparticle.

24.4 Characterization of Biosynthesized Nanoparticles

Characterization protocol is an important tool in determining the size, shape, composition, and functional groups capped on the biosynthesized nanoparticles. The following are the specific instrumental approach used in the study of characterization:

24.4.1 UV Spectrophotometer

Colloidal solutions of biosynthesized nanoparticles exhibit SPR under ultraviolet–visible spectrum. SPR is the total oscillation prevailed by surface electrons for the range of incident wavelengths of light. When the size of a nanoparticle is smaller than the wavelength of incident radiation, SPR is generated. At a specific wavelength, maximum oscillation is recorded where SPR peak corresponds to the unique characteristic feature of the synthesized nanoparticles at corresponding wavelength of light. SPR can be determined by using a UV spectrophotometer. Nanoparticles such as silver and gold exhibit characteristic SPR peak in the visible region (400–700 nm).

24.4.2 Fourier-Transform Infrared Spectroscopy (FTIR)

The extract obtained from the agriculture and horticulture waste contains functional groups taking part in the reduction process and capped on the surface of synthesized nanoparticles. In order to identify these functional groups present in the relevant extract and on the surface of nanoparticles, Fourier-transform infrared spectroscopy (FTIR) analysis is carried out. The mid infrared spectrum is divided into four regions in the absorption verses wave number data. The wave number 2500–4000 cm^{-1} corresponds to the single bond region, 2000–2500 cm^{-1} to triple bond region, 1500–2000 cm^{-1} to double region, and 600–1500 cm^{-1} to finger print region, respectively [14].

24.4.3 Dynamic Light Scattering (DLS) and Zeta Potential

Size distribution of the nanoparticles in colloidal suspension is measured based on its Brownian motion using dynamic light scattering (DLS) technique. The measurements are recorded by maintaining uniform temperature. The technique is noninvasive in nature. DLS measures the hydrodynamic diameter of the particle and relates to diffused light particles scattered in the carrier fluid. The fluctuations in scattered light intensity are measured with respect to time. Fluctuations in intensity arise due to the random Brownian motion of the nanoparticle. Larger particles diffuse more slowly than small particles, wherein particle size can be related to the measured fluctuation in light scattering intensity.

The stability of the colloidal nanoparticles is decided based on the zeta potential measurement. The colloidal particles suspended in the solution are electrically charged due to the dipolar characteristics and ionic attributes. This leads to net electric charges at the surface of nanoparticles that causes the accumulation of counter ions (opposite charges) around them to form an electrical double layer. The ion with a set of counter ions forms a fixed part of the double layer. Under the applied electric field, the particles are attracted toward electrodes depending on their polarity. The potential at which the fixed part of the double layer along with a part of the mobile layer move toward an electrode is termed as zeta potential. The particle in solution with large positive or negative zeta values will repel each other. However, with low zeta values, there is no force to prevent the nanoparticles from coagulation. Greater the zeta value, greater will be the stability, wherein the threshold zeta potential value is ± 30 mV.

24.4.4 Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) with Energy-Dispersive X-ray (EDX)

To determine the shape and size of the nanoparticles, scanning electron microscope (SEM) and transmission electron microscope (TEM) are considered to be most suitable. Both methods adopt different principles of working. The resolution of SEM and TEM is less than 5 and 1 nm, respectively. Generally, SEM is suitable for the analysis. TEM is recommended prior to proceeding with a critical step in research.

To confirm the composition of the nanoparticle, energy dispersive X-ray (EDX) is suitable. EDX is coupled with SEM and TEM devices. The X-ray emitted is the unique characteristic for an individual element. The EDX spectrum provides presence of different elements in the sample. Purity of the synthesized nanoparticles can be determined based on the composition.

24.4.5 X-ray Diffraction (XRD)

The X-ray diffraction technique is basically used to determine the crystal structure and lattice parameters of the given material. The crystallinity and amorphous nature of the material can be identified through X-ray diffraction (XRD) patterns. The sharp peak corresponds well to crystalline material and amorphous materials do not exhibit sharp peaks.

24.5 Applications of Biosynthesized Nanoparticles

24.5.1 Antimicrobial Activity

Most of the metal nanoparticles are reported to exhibit antimicrobial activity. Nanoparticles synthesized using agriculture and horticulture material can be evaluated for the antimicrobial activity using either disc diffusion or perforated well method. For representing the same, the gram positive and negative bacteria are cultured separately. These cultured bacteria can be used for performing the inhibition activity as described below:

a. To evaluate the antibacterial activity using disc diffusion method, a bacterial lawn is cultured in the petriplate. A clean sterilized paper disc dipped in colloidal solution of nanoparticle is taken and is placed in the bacterial lawn along with the control disc. A zone of inhibition is achieved after preferably 24–48 hours.
b. In well diffusion method, previously grown bacterial culture plate is taken. Appropriate wells are punched in the petriplates, and the colloidal solution of nanoparticle at required concentration is added. The respective zone of inhibition is evaluated after 24–48 hours.

24.5.2 Photocatalysis

Nanoparticles are reported to be a promising tool for the dye degradation application. Photocatalysis can be carried out by mixing the required concentration of any dye considered in the experimental protocol along with the biosynthesized nanoparticles under natural sunlight condition for any fixed duration of time. In continuation with the said protocol, the absorbance of the dye can be measured at different intervals of time using UV visible spectrophotometer at its characteristic wavelength. As the photocatalysis progresses further, the resultant absorbance of the solution gets reduced. Thus, the percentage of photocatalysis can be calculated in comparison with control samples (without nanoparticles). Accordingly, photocatalysis under

sunlight can be carried out in two differential modes known as static and dynamic. Also photocatalysis is performed using reactors by providing visible light sources.

24.5.3 Removal of Antibiotic from Water

Biosynthesized nanoparticles can be used in effluent treatment plants effectively. In this application, the antibiotic contaminant present in the water can be removed using adsorption mechanisms using nanoparticles. The effluent water is mixed with nanoparticles that is kept under agitation for a specified duration of time at room temperature. Samples are withdrawn at a fixed interval of time to assess the remaining antibiotic present in the effluent. These samples can be analyzed using various analytical methods like liquid chromatography mass spectrometry (LCMS), high performance liquid chromatography (HPLC). Few reports have suggested the use of iron oxide nanoparticles in the removal of antibiotics [15].

24.5.4 Effect on Enzyme Activity

To assess the effect of nanoparticles on enzyme activity, enzyme should be preincubated with nanoparticles and further incubated with its suitable substrate. Substrate concentration should be fixed; however, concentration of nanoparticles has to be varied in order to assess the enzyme activity. The outcome of this respective assay will be beneficial in food processing industries. Thus, these nanoparticles serve as potential activators or inhibitors of the enzyme activity. The effect of these nanoparticles can be estimated with respect to the control sample (without nanoparticles). The kinetic parameters such as Maximum velocity (V_{max}) and Michaelis constant (K_m) can be calculated using Michaelis Menten equation for the respective enzyme activity.

24.5.5 Nanofertilizer

Biosynthesized nanoparticles have the relevant potential to be used as nano fertilizer in agricultural sectors, wherein plants require micronutrients for their growth and development for achieving quality as well good yield of crops. In order to investigate the effect of nanoparticles on the plant growth, seeds of suitable plants should be cultivated. Nanoparticles of different concentrations need to be mixed with soil during sowing of seeds either in culture pots or in suitable plant trays. Control sample (without nanoparticle) can be used as reference to determine the effect of nanoparticle on plant growth. Plants harvested after a suitable duration have to be evaluated for height, number of leaves, leaf area, root length, and number of flowers.

24.5.6 Radical Scavenging Activity

Most of the nanoparticles exhibit radical scavenging activity. The DPPH (2,2-diphenyl-2-picrylhydrazyl) assay is preferred to determine the antioxidant

capabilities of nanoparticles. With respect to control samples, the percentage of antioxidant activity can be calculated as follows:

$$\text{Antioxidant activity}\,(\%) = ([\text{Control absorbance} - \text{sample absorbance}]/\text{Control absorbance}) \times 100$$

Absorbance is measured using a UV visible spectrophotometer at 517 nm.

24.5.7 Nano Additives for Fuel

Nanoparticles can be blended with diesel or biodiesel to investigate the engine performance without engine modification. In engine characteristic investigations, usage of carbon nanoparticles blended with biodiesel enhanced the break thermal efficiency and a substantial reduction in emission of harmful pollutants [16]. Biosynthesized nanoparticles also can be explored for its additive property by blending with diesel or biodiesel. Biosynthesized colloidal nanoparticle has to be separated using centrifugation method. The specified quantity of nanoparticle can be blended with fuel by using sonication and engine performance can be investigated.

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25

Nanobiotechnology – A Green Solution

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25.1 Introduction

Environmental pollution, whether it's land, air, or water, is increasing at an alarming rate and also the global consumption of finite resources. Random disposal of waste in limited landfills is not only potentially hazardous for the mankind and the planet but sometimes untreated valuable wastes are also being discarded. "Recycle and reuse" is the basis of sustainability. Proper waste disposal protects the environment, society, as well as persons involved in disposal works [1]. The global production of municipal solid waste (MSW) in 2012 as reported by World Bank accounted to 1.3 billion tonnes and is expected to double by 2025 to 2.2 billion tonnes pa. United States alone generated 250 million tonnes of MSW in 2012, of which the material content is estimated to be around 136 million tonnes [1, 2]. Discarding enormous quantity of waste in a limited landfill causes a great wastage of the intrinsic energy value and also a loss in valuable material content that could have been recycled. Before discussing on "zero waste economy" and the associated technologies that are applicable for its attainment; some important facts on "*waste*" are highlighted. Waste has a value and is produced in all classes of the society. If waste send to landfills is recycled and reused properly, it can serve as a source of other useful raw materials. Irrespective of social class, period, and time, all human society produce *waste*; in other words, *waste* is an unavoidable outcome of the human society. Waste being the result of human activities, its generation is always not inevitable nor should the amount of waste necessarily continue to rise [2]. Waste generation may be from daily consumption of households, food wastes, goods and materials in households, waste generated in wide range of industries, e-wastes, nuclear wastes, and so on. Huge amount of wastes are generated during the major festivals and ceremonies. Although different waste management strategies are being trumpeted, but societal hierarchy marks "working with waste" a low status occupation that is to be done by unskilled, migrants laborers; waste is considered as socially contaminating. However, with the passage of time, waste is always not "rubbish" but can be recycled and repurposed thus providing a sustainable basis [2]. Rapid industrializations, accelerating globalizations, affluence are literally exhausting the nonrenewable natural resources and

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simultaneously adding to environmental pollution. The ideology of "*zero waste*" for the sustainability of the planet is needed to be implemented both at micro and macro level, i.e. starting from individuals, families, communities, industrial, and business organizations to local municipal and governmental levels. Attainment of zero waste is a long-term goal. It is necessary to adopt new technological solutions that will foster the development of sustainable units, promote resource conservation, enhance energy efficiency, aim at zero-carbon transport, and reduce environmental pollutants. The other facet for the attainment of "zero waste economy" demands the development of technologies that will be infinitely sustainable, promote recycling of natural resources, will be solar powered, zero emissions, and will bio-mimic the nature's inherent "cyclic design" [3]. Attainment of "zero waste economy" follows some strategic plan of action. First step is "*refuse*," i.e. avoidance of waste generation on first hand; this can be achieved by use of reusable products; second step is "*reduce*" which can be attained by implanting efficient processes and technologies that will convert maximum of raw materials to useful products and *reuse* the material products. The concept of third step "*reuse*" is based on the reuse of "waste" generated in one processes or industry as a raw material in another process or industry. "*Recycling*" of waste of all kinds is the most vital step for attaining "zero waste." Recycling companies play a vital role in this regard. "*Recovery*" is another vital strategy for waste reduction [1–3].

In the twenty-first century, biotechnology and nanotechnology have emerged as a versatile promising technology with multidimensional applications in medicine, biomaterials, electronics, catalysis, etc. Nanotechnology or nanotech deals with the development of materials, devices, and structures at atomic and molecular scale of 1–100 nm. Richard Feynman in 1959 for the first time mentioned about the technology. The word "nanotechnology" was coined for the first time by Norio Taniguchi in 1974. Biotechnology deals mostly with biological subjects, their metabolic and physiological processes. The essence of the two technologies are merged in the multidisciplinary domain of *nanobiotechnology* that aims in bringing the science of the almost incomprehensibly small device closer and closer to reality. The outcomes of the domain are vast enough to influence all branches of science and technology in future [1, 4].

Nanotechnology aims to develop high-performance products at nanoscale using the available tools and techniques with a bottom-up approach. The objective of nanotechnology is to develop eco-friendly products via eco-friendly techniques. Thus, nanotechnology is a "clean and green" technology. A technology is designated as "green" when it is eco-friendly and helps in the conservation of natural resources. Alternatively, the aim of a "green technology" is to avoid hazardous processes and contaminants, reduce environmental pollutants, and thus waste reduction [5]. Nanobiotechnology is a combination of engineering, nanotechnology, molecular biology, and biotechnology. Nanobiotechnology aims to develop, modify, and improve its utility for improved biotechnological applications. Any green techniques eliminate the use of hazardous and expensive chemicals, avoid energy expenditure, and develop safe and environmentally benign products and by-products [6, 7].

Nanotechnology and nanobiotechnology are having immense applications in medicine, drug delivery, surgery from the biological side and nanochips, nanobiosensors, nanorobots being the outcomes in engineering domain; this chapter focuses on the "*greener and cleaner*" aspects of the technologies, their versatile applications, and their role in achieving "zero waste economy."

25.2 Nanotechnology and Nanobiotechnology – The Green Processes and Technologies

The main goal underlying "green technology" is to conserve natural nonrenewable resources; provide a sustainable basis so that present need do not compromise the requirements of the future generations; devise alternative technologies that are cost effective, eco-friendly, utilize renewable energy sources, e.g. sunlight, energy from tidal waves, wind, water current in contrast to fossil fuel, e.g. oil, gas, coal. The aim of the technology is also to develop products and by-products that can be reused and recycled and overall improve the quality of human life and society. However to justify the word "green," the main aim of the technology is to reduce waste or achieve "zero waste economy." Thus, green technology (GT) is also known as environmental technology or clean technology. GT encompasses different domains, viz. *green chemistry* that focuses on the development of products by processes that minimize the use and generation of hazardous substances; *Green nanotechnology* aims to develop eco-friendly products at nano scale, minimize use of starting materials, and reduce waste generation. Again the concept of *green building* that involves sustainable design in raising buildings by utilizing water, energy, and material resources, the basic concept that extends much beyond the walls of the buildings and focuses on the impacts on human health, society, and environment throughout the lifespan of the building. *Green building* concept aims to protect biodiversity, ecosystems, reduce waste production, conserve natural resources, minimize the strain on local infrastructure, and boost the overall quality of life [5, 8].

If nanoparticles be considered as the building blocks of nanotechnology, they can be synthesized by different physical, chemical, biological, and hybrid methods by either top down or bottom up approaches. The top-down approach begins with microsystems and miniaturizes them, whereas the bottom-up approach starts at atomic or molecular level and then proceeds for build-up procedures by different physical and chemical processes. In "top-down" approach, there is much waste generation and thus "bottom-up" approaches are gaining priority in developing nanostructured materials. Such materials can be one-dimensional (1-D), e.g. nanofilms and nanocoatings, two-dimensional (2D), e.g. nanotubes and nanorods, three dimensional (3D), e.g. fullerenes and nanoparticles [5, 7, 9]. Physical methods associated with nanomanufacturing include *arc discharge method*, *electron beam lithography*, *mechanical grinding*, *milling*, *spray pyrolysis*, *ion implantation*, *vapor phase synthesis*; chemical methods include *electrochemical method*, *pyrolysis*, *microemulsion method*, *coprecipitation method*, *phytochemical method*, *sonochemical method*, *sol–gel process*, *solvothermal synthesis*. Manufacturing at nanoscale

needs strict maintenance of purity; repetition of preprocessing, processing, and post processing of steps in a single batch operation, yet the yield being low; use of toxic and hazardous chemicals, use or generation of greenhouse gases; generation of wastes associated with top-down techniques are some of the associated problems with the conventional procedures of the technology. Waste reduction can be achieved by adopting eco-friendly synthesis procedures of a biological nature. Here comes the application of *green nanobiotechnology* that applies biological routes involving microorganisms, viruses, plant, and their products like proteins and lipids with the aid of different biotechnological tools. The bio-based synthesis of nanoparticles utilizes mostly a bottom-up approach where due considerations are given in the selection of solvent medium, a "green" reducing agent, i.e. it should be eco-friendly and a nontoxic capping agent for the stabilization of the nanoparticles. Since the method makes use of plant extracts, industrial and agricultural wastes, enzymes and biomolecules, microorganisms, e.g. bacteria, fungi, algae, seaweeds, etc. Thus, the technology is not only "green" but also contributes significantly to waste reduction by reuse of wastes in many cases [5, 7, 9].

25.2.1 Green Chemistry

Though green nanotechnology and nanobiotechnology are the major focus of discussion in this chapter; however, the significant role of "*green chemistry*" in "zero waste economy" also needs to be highlighted. The 12 principles of green chemistry, as postulated by Paul Anastas and John Warner in 1990s (Table 25.1), emphasized on reduction or rather elimination of use of toxic solvents, avoidance of synthetic procedures, and analytical techniques generating hazardous byproducts. Green chemistry aims to attain a "green and clean earth" and synonymously also known as "clean chemistry," "eco-friendly chemistry," "environmentally benign chemistry," "sustainable chemistry," "atom economy," "e-chemistry," etc. The green chemical approach is not only eco-friendly and holistic in nature but aims to synthesize or utilize the products in an eco-friendly manner and finally reduce waste production. The subject encompasses a broad domain of major branches of chemical sciences, viz. organic and inorganic synthesis, material science, polymer chemistry, nanochemistry, supramolecular chemistry, and so on [10–14]. The 12 principles of green chemistry (Table 25.1) stresses on waste prevention rather than its further treatment or clean-up; use of renewable starting materials with minimal energy expenditure, and reducing the use of auxillary substances and chemically synthesizing nontoxic, nonhazardous, environmentally benign substances. [10–14]. Here it is to be mentioned that green chemistry is totally different from environmental chemistry as the later emphasizes on pollution and the methods of prevention. But green chemistry focuses on the elimination of pollution from the beginnings. If "prevention is better than cure." green chemistry is the preventive pathway, and environmental chemistry is the curative pathway [10–14].

Samantha Tang, Richard Bourne, Richard Smith, and Martyn Poliakoff suggested a condensed 24 Principles of Green Chemistry and Green Engineering, with the mnemonic "IMPROVEMENTS PRODUCTIVELY." Here the word

Table 25.1 Twelve principles of green chemistry.

Number	Principle	Description of principle
1	Prevention	Prevention of waste generation is better rather than to treat it after its generation
2	Atom economy	Developing synthetic schemes so that the final product incorporates as much of the reagents used during the process as possible. This will help to minimize waste generation
3	Safer chemical synthesis	Designing of synthetic methods to generate products that are environmentally benign
4	Safer chemical design	Selection and designing of chemicals should be such that they obviously fulfill their functions but must be ecofriendly and non toxic
5	Use of safer solvents and auxiliaries	Use of other solvents or additional reagents are to be avoided wherever possible; when the use of auxillary reagents is necessary then care is to be taken that they are non-hazardous
6	Design for energy efficiency	Energy requirement is a vital issue while running chemical processes from the point of economy and environment; for minimizing energy expenditure synthetic schemes should be designed to conduct at ambient room temperature and pressure
7	Use of renewable raw materials	If technically feasible, renewable raw materials should be preferred over non-renewable
8	Reduction of derivatives	Unwanted derivatization processes should be avoided so as to minimize additional use of reagents and waste generation
9	Catalysis	Catalytic reagents are to be preferred over stoichiometric reagents so as to minimize waste generation
10	Degradation products design	Chemicals should be designed so that after their use they completely degrade to harmless products and do not persist in the environment
11	Real time analysis for pollution prevention	Analytical methods should be monitored in real time to avoid the formation of hazardous substances
12	Inherently safer chemistry for accident prevention	Choice of substances and their forms in chemical processes should be such that it minimizes the risk of potential hazards of chemical accidents, chances of explosions, fire break outs, etc.

Source: Anastas and Warner [10].

"IMPROVEMENTS" can be spelled out as, I stands for "inherently non-hazardous and safe," M – "minimize material diversity," P – "prevention instead of treatment," R – "renewable materials and energy inputs," O – "output let design," V – "very simple," E – "efficient use of mass, energy, space, and time," M – "meet the need,"

E – "easy to separate by design," N – "networks for exchange of local mass and energy," T – "test the lifecycle of the design," and S – "sustainability throughout product lifecycle." The word "PRODUCTIVELY" can be described as where P stands for "prevents waste," R – "renewable materials," O – "omit derivatization steps," D – "degradable chemical products," C – "catalytic reagents," T – "temperature and pressure ambient," I – "in process monitoring," V – "very few auxillary substances," E – "E-factor, maximize feed in product," L – "low toxicity of chemical products," Y – "yes, it's safe." While devising any synthetic scheme, the main aim is to maximize yield and purity. However, "atom economy" as mentioned in the "principles of green chemistry" states the economic use of atoms, i.e. utilize maximum number of atoms of the reactants so as to minimize waste generation [15].

25.2.1.1 Advantages and Challenges

GTs are now being adopted in different levels of the society, industries, and corporations due to the versatility of the technology. International organizations such as Intel, Dell, Cisco, Nokia and Indian companies such as Wipro, Tata metallics, Reliance, etc. are focusing on the adoption of "green procedures" and development of "green products" [5, 7–9]. Considering the advantageous aspects, GTs will protect the planet, provide a sustainable basis by use, reuse, recycle of natural resources thus preventing their exhaustion; reduce waste production that will impart cleanliness and economic benefits in certain cases. The products, by-products, and processes will not be detrimental for the planet [16].

As discussed earlier, nanotechnology and nanobiotechnology have been justified as GTs. "*Going green*" is a broad term that refers to implementation of clean-up procedures and technologies, e.g. the use of bioreactors, bio-enzymes, biofiltrations, bioremediations, electrocoagulation, nanotechnology in sewage treatment and waste management, and ultimately produce a green and clean environment. Applications of GTs in agriculture, food processing, and pharmaceutical will help in the elimination of toxic components in food products, food packaging processes, there will be reduction in food and agricultural wastes, drug synthesis schemes will follow the principle of "atom economy," avoid use of hazardous chemicals; all products, by products and processes will be eco-friendly. Both end users and the planet will be benefitted from safe, ecofriendly products and clean atmosphere. Use of biofiltration will help to get huge amount of potable water without any detrimental effect on the environment. Nowadays, new "zero power systems" are being developed using renewable energy processes and zero emissions. GTs are being extensively applied in automobiles to produce "green machines" like the hydrogen vehicles, fuel cells packed with carbon nanotubes to store hydrogen, and increase the reactivity. Different parts of a car, viz. tyres, chassis, and wind screens, are being manufactured by applying GTs, thus developing the "green cars." Extensive researches are being done in the domain of "green nanoelectronics" by developing biodegradable electronic devices performing biological functions to attain sustainability and e-waste management [5, 8, 16].

However, since GTs are novel, implementations in every phases are obvious to face the challenges of lack of in-depth basic and engineering research and proper

guidelines. In contrast to conventional procedures, GTs are expensive since it takes into account the environmental issues, which are mostly not considered in other conventional technologies. Wide implementations of GTs in industrial scale are often hindered due to lack of efficient engineers and scientists in the domain as well as trained personnel, lack of prior information, and data sources. There is a constant need for updating the protocols in contrast to conventional procedures regarding toxicology and other analytical methods; this often creates a problem due to unavailability of prior data store. GTs and procedures often face the barriers of regulatory authorities to a greater extent than the conventional technologies. Furthermore, additional financial investments are necessary for setting up relevant infrastructure, training of the personnel, etc. Often there is lack of skilled personnel and other human resources. Lack of alternative procedures, alternative sources of raw materials, reagents and chemicals, uncertainty in performance rates are other challenges that need to be addressed. Furthermore, uncertainty remains regarding end users acceptability of the novel green products in comparison to existing conventional products [5, 14, 16–18].

Still, overcoming the constraints, implementations of GTs will obviously escalate the product quality to qualify the rigid product specifications for foreign export. Companies implementing GTs will be much more well equipped and advanced in technology than others and thus justify different regulatory issues. There will be reduction in input costs as the technology will utilize natural renewable resources, energy conservation, focus on recycle and reuse of by products and waste products, and finally protect the biodiversity, the burning issue of the day [8].

25.3 The Versatile Role of Nanotechnology and Nanobiotechnology

Nanotechnology and nanobiotechnology in the light of "green innovation" have shown versatile multiple applications. Rapid industrializations, meeting the growing need of human demands starting from basic needs to luxury items, great advancements in health care, necessitated automation in every field. In nanotechnology, precise tailoring is done at atomic, molecular, and supramolecular level to develop safe, clean, and smart products. Green nanotechnology is slowly being recognized as a "general purpose technology" finding applications both in industrial and different societal levels [16].

The potential applications of nanotechnology and nanobiotechnology are discussed under the following broad headings:

25.3.1 Agriculture, Potable Water, and Food Processing

Development of framework for sustainable agriculture, food processing, and food engineering is the need of the hour in order to secure food and nutrition for the future. Soil conservation, integrated pest management, cover crops, rotational grazing, agro-forestry are some of the essential requirements in sustainable

agriculture. Nanotechnology plays a crucial role here through precise farming by using nanopesticides, nanoherbicides, nanosensors, etc. Nanocarriers, i.e. nanocapsules and nanoparticles (e.g. silica nanoparticles, polymeric nanocapsules), are designed for the controlled delivery of pesticides, plant growth factors, etc. Nanoparticle-mediated gene or DNA transfer in plants helps to develop insect-resistant varieties. Use of nanoparticles in agriculture comes under "precision farming." Nanofertilizers, i.e. nanoencapsulated micronutrients, are necessary for plant growth, and biofertilizers are found to increase crop yield and quality to a much greater extent than conventional fertilizers. Nanoemulsions, nanocages, and nano containers are some of the formulations for the delivery of pesticides. Carbon nanotubes with high penetrating power can fasten the germination of seeds as studied with tomato seedlings. Specifically, engineered nanosensors are used for *in situ* and real-time monitoring of crop diseases, and growth rate, nutrient deficiency, and environmental conditions help to detect traces of pesticides and herbicides in crops, vegetables, and fruits, etc. Graphene, a nanomaterial, can detect pathogen in wastewater and help to purify it as drinking water, thus finding a potential application in aquaculture [16, 19, 20].

Water is one of the most precious natural resources. However, fresh usable water is only 3% of the world's supply, and about 70% of the fresh water is essential for agriculture. Nanotechnical approaches help in the detection of contaminants at molecular level, provide cost-effective decentralized water purification system, with efficient nanofiltration system helps in recycling rainwater to drinking water or seawater to drinking water. Thus, with the aid of green nanotechnology, huge amount of drinking water can be produced using solar and wind energy, the renewable sources of energy in a cost effective, sustainable way. Nanotechnology and nanobiotechnology have a great potential in wastewater treatment and thus waste management [16, 20].

Nanotechnology has a vast application in food industry and has given rise to the domain "*food nanotechnology*." Food nanotechnology finds applications in food processing, preservation, value addition, and packaging. The food matrix is a complex system with several nanosized elements whose self-assembly further gives rise to higher structural units. Food quality and safety is a matter of great concern. Food nanotechnology and nanobiotechnology focus on enhanced efficacy and bioavailability, increased stability and shelf life, value addition, and improved organoleptic acceptability of the food items. Food nanotechnology is applied in two forms: as "nano inside," i.e. in food additives, and "nano outside," i.e. in food packagings. The two approaches, "top down" and "bottom up," do find applications in food nanotechnology. Physical processing of food materials like grinding and milling comes under "top-down approach." On the other hand, the concepts of self-organization, self-assembly derived from biology are considered as "bottom-up approaches" in food nanotechnology. Nanotechnology has led to the development of *nanocomposites* and *nanoemulsions* having substantial applications in food industry. *Nanocomposites* made up of polymers in combination with nanoparticles help to keep food fresh for long, avoid microbial spoilage, act as gas barriers, and help to detect leakage of CO_2 from carbonated beverage bottles. Nanocomposites of SnO_2 help to detect oxygen leakage in packagings. *Nanoclays* are a variety of nanocomposites broadly

categorized into intercalated nanocomposites and exfoliated nanocomposites. Aegis, Imperm, and Durethan are some marketed nanoclays. Aegis acts as oxygen scavengers and improves the barrier properties of the clay to retain carbon dioxide in the beverages. Durethan, made up of polyamide, provides stiffness to the paperboard containers for fruit juices. Imperm made up of nylon and nanoclay is meant for oxygen scavenging. Nanocor, a nano clay-based polymer, also acts as gas barrier and is used in plastic beer bottles. Nanobiocomposites made up of starch, cellulose, poly lactic acid, polyhydroxybutyrate, polybutylene succinate as efficient layering materials used in food packagings [16, 19–25]. *Nanoparticles* are found to improve the flow property, stability, bioefficacy, color of the food items. Silicate, titanium oxide, zinc oxide nanoparticles provide barrier to oxygen in food packagings. Silver nanoparticles (AgNPs) are found to extend the shelf life of fruits and vegetables by absorbing and degrading ethylene. Biopolymeric nanoparticles made of chitosan, alginate are used for the delivery of value-added additives (e.g. vitamins, minerals, phytochemicals) and thus find wide use in functional foods. *Nanoemulsions* formulated either by high-energy approach or low-energy approach find applications in food processing, in developing nutraceutical products and functional foods, salad dressings, flavored beverages with higher stability, shelf life, and efficacy. Nanoemulsions are much preferred than conventional emulsions owing to their high stability, and they do not bring any alterations in food products. They are very suitable in delivering essential phytochemicals with enhanced bio efficacy. Casein micelles are very suitable for delivering hydrophobic nutraceuticals. Self-assembled nanoemulsions help in retaining flavored compounds in functional foods from degrading actions of enzymes, temperature, oxidation, hydrolysis, etc. [16, 19–25].

Nanosensors play a crucial role in food quality control. These sensors help in detecting any changes in color and any off odors due to food spoilage and growth of microorganisms. Some examples of nanosensors include array biosensors, electronic nose, nanotest strips, nanocantilevers, and many more. Electronic nose and electronic tongue sensors are used to detect the changes in organoleptic acceptability due to food spoilage. Electrochemical sensors are useful for detecting adulterants in food items. Nanosensors used in food packaging industries are provided with time–temperature integrator and gas detector. Nanobiosensors are helpful for the detection of viruses and bacteria. Biomimetic sensors developed using protein and biomimetic membranes are helpful in detecting the pathogens. Same work is done by the surface plasmon-coupled emission biosensor [16, 19–25]. Some of the developed nanoceuticals, food items, and supplements include carotenoid nanoparticles that are easily water dispersible and can be added to fruit juices that will provide improved bioavailability; micelles in nano size range is a delivery medium for different vitamins, minerals, phytochemicals in a canola oil base; some of the nanoceutical products include nanocages, nanoclusters, a patented product "nanodrop system" that can effectively deliver vitamins, minerals, and other value added additives in different nutraceutical products with improved efficacy, bioavailability, and stability. Nanoparticles, nanoclays, and nanocoatings are finding great use in food packagings. Packaging can be categorized as "improved," "active," and "intelligent." Nanoclay particulates, nanoparticles play a crucial

role here. "Intelligent nanocoatings" developed can help to indicate presence of contaminations during the storage of food items. Active packaging contains specific molecules that create a passive barrier to oxidative, photolytic, hydrolytic deteoriations and also release antioxidants and antimicrobials thus ensuring dual stability [16, 19–25].

25.3.2 Health, Medicine, Drug Delivery, and Pharmaceuticals

Green nanotechnology provides a cost-effective, rapid technology in drug delivery, diagnostics, new drug development strategies, etc. Nanotechnologies provide options for advanced medical treatment like repairing of DNA and cellular damage, customized drug delivery. Green nanotechnology provides the basis of sustainable health treatment. This has given rise to "nanomedicine" that employs the science and technology of "nano" in the field of biomedicine. Nanosensors are used for the purpose of medical diagnosis. Pharmaceutics and green nanotechnology combined hands to develop "nanorobots." Patients can ingest these programmed nanorobots in drinks and beverages. These nanorobots can perform sophisticated, most delicate surgeries not leaving any scar marks as like the conventional surgical procedure. Nanorobots also find great applications in cosmetic surgeries and can aid in gene manipulation altering the physical appearance of individuals as per personalized demands. Nanoparticle-based drug delivery has developed a range of formulations, e.g. nanoemulsions, micelles, liposomes, dendrimers, niosomes, and solid lipid nanoparticles, polymeric nanoparticles play a crucial role in sustained release drug delivery systems [16, 26–31].

Although the immense potency of phytomedicine is evidence-based, the pharmaceutical companies are still hesitant to invest capital in screening of novel chemical entities of natural origin. But plant secondary metabolites have exhibited a wide range of pharmacological actions (antioxidant, anti-inflammatory, antidiabetic, antimicrobial, etc.) as well as preventive and curative potentials against several communicable, noncommunicable, infectious diseases, and life-threatening diseases like cancer. The abovementioned nanoparticle-based formulations find extensive uses in phytoformulation research. Pharmaceutical companies have their own research wings focused on nano-based drug delivery systems. Different encapsulation technologies are being applied in formulating the nano scale devices for delivery of therapeutic entities and other chemical components. Metallic nanoparticles, e.g. AgNPs encapsulating different herbal bioactives and synthetic drugs are available. AgNPs have potential antimicrobial effects as silver ions are toxic to microbes. *In vitro* cell culture study results have shown that AgNP have potential cytotoxic effects against leukemia cells and a number of other cancerous cell lines, viz. hepatic carcinoma cell lines, squamous cell lung carcinoma cells, human alveolar cell line, melanoma cell line, etc. [20, 27, 29, 30]. Gold nanoparticles (AuNPs) are used not only for the delivery of therapeutics but also in genetic engineering, as biosensors, delivery of antibacterials, in hyperthermia therapy, etc. Herbal bioactive-loaded nanoparticles have been formulated where either the standardized plant extract have been encapsulated or isolated plant secondary

metabolites in quantifiable outputs. Some of the herbal drug-loaded nanoparticles include curcuminoids loaded solid lipid nanoparticles prepared by micro-emulsion technique with anticancer and antioxidant effect; artemisinin-loaded nanocapsules prepared by self-assembly method having anticancer potentials; glycyrrhizic acid loaded nanoparticles with antihypertensive and anti-inflammatory effect prepared by rota evaporated film ultrasonication method; berberine-loaded nanoparticles with anticancer effects formulated by ionic gelation procedure; camptothecin nanoparticles and taxel-loaded nanoparticles both with anticancer effects are prepared by dialysis method and emulsion solvent evaporation method, respectively, and many more. Coming to the synthesis of synthetic drug molecules, *green synthetic procedures* are being adopted by pharmaceutical industries. Established synthetic procedures for the synthesis of drugs such as Quinapril (used to treat hypertension and congestive heart failure), celecoxib (anti-inflammatory agent), sildenafil citrate (oral treatment for erectile dysfunction), etc. have been replaced with the use of greener solvents and thus eco-friendly procedures [20, 27, 29, 30].

25.3.3 Automobile, Aircraft, Space Travel

Rapid industrializations and tremendous development in science and technology necessitated frequent movement from place to place in different parts of the world. The ever-curious mind of man prompted him to explore the space. Space exploration is required to know the condition of our planet, learn about new resources that will support the survival, growth and prosperity of the human kind, and also for satisfying the curiosity of human mind. Safety, reliability, and highest level of performance are required for space travel. Green nanotechnical approaches can provide materials that are much safer, efficient, capable of self-repairing, and of low weight. In addition, it can also influence space exploration with smart costumes, propulsion fuels, and other life support systems [16].

25.3.4 Sustainable Energy, Building Technology

Technological advancements, changes in lifestyle, industrial development obviously direct toward increased energy expenditure. Increased demand for energy is likely to exhaust the fossil fuels in coming future. The situations are further complicated due to natural calamities, global warming, and increased concentration in greenhouse gases. Green nanotechnology can help in energy conservation by developing solar powered cells, hydrogen storage, fuel cells, power grid, etc. The main focus of green nanotechnology is to utilize maximally the renewable solar and wind energy and is in progress in developing highly efficient solar powered cells [16]. Green nanotechnology is also likely to open a new paradigm in construction technology where nanomodification of cement, tailoring of construction materials at nanoscale will provide with highly efficient, durable, ultrahigh strength yet extremely light weight materials that will not only increase the speed and durability but the challenges also lies in improving energy efficiency and heat control of the buildings [16].

25.3.5 Society and Education

The global economy is dependent largely on the natural resources and may be renewable or nonrenewable. However, climatic changes, natural disasters such as floods, draught, landslides, depletion of natural resources, put typical challenges in front of agriculture, food security, human health, and sustainability. But for implementation of GT in different spheres, it is essential to incorporate the "subject" in higher education level. To establish this sustainable technology, it is necessary to acquire in depth the knowledge, skills, techniques associated with the subject along with a deep research insight. Furthermore, it is also needed to incorporate the moral ethics and virtues associated with the subject. Green concept, green jobs, green management are already in high demands. Computer and information technology are already considered as GTs. Many industrial sectors are being fully automated. Along with the knowledge and skills associated with the technologies considered as "green" subjects such as organic farming, green medical services, green transport, and tourism comes under the domain [16].

25.4 Nanotechnologies in Waste Reduction and Management

The main focus of this chapter is the role of nanotechnology and thus nanobiotechnology in waste management. Proper waste disposal is a global problem. Waste management typically refers to solid wastes landfills, major sources of which are household waste, commercial wastes from industries, streets, hotels, restaurants, medical wastes from hospitals and clinics, etc. Microbes act as scavengers and breakdown wet organic waste to manure. Conventional methods of solid waste disposal or wastewater treatment are either adding further pollutants to environment or imply high investment cost, poor performance, etc. Since nanotechnology and nanobiotechnology deals at atomic level, they have shown great promise in contrast to conventional methods for achieving zero waste economy. They are the emerging technologies in environment protection industry [32–35].

Nanoscale structures, thus nanomaterials have morphological characteristics, absorption ability, and power of catalysis greatly different from those at macro scale. In the "*bottom up approach*," nanomaterials and nanostructures are made from individual atoms or molecules linked together by chemical bonds to form more complex structures. In "*top down approach*," synthesis of nanostructures is made of larger entities without configuration control at atomic level. "*Treatment and remediation*," "*sensing and detection*," and "*pollution prevention*" are the different areas, where nanostructures the outcomes of nanotechnology and nanobiotechnology are finding successful outcomes in wastewater treatment [32].

Nanotechnical and nanobiotechnical approaches find both *in situ* and *ex situ* applications in waste management. In *in situ* applications, metallic nanoparticles with zero valency find their uses by providing a permeable reactive barrier, creating a reactive zone on the subsurface of the contaminant. Also in *ex situ* waste management nanomaterials find their use, where exploitation is made use of properties

such as adsorption, membrane filtration, separation, and photocatalysis. Natural products like activated carbons, kaolins, peat, clays, zeolite, aluminosilicates have high adsorption power. Some nanoparticles destroy the contaminants (oxidation in presence of nanocatalysts), separation and isolation of contaminants (nanofiltration). Metallic nanoparticles with zero valency, nanocomposites, carbon nanotubes are finding great promise in wastewater remediation. Carbon-based nanomaterials, e.g. multiwalled carbon nanotubes that have much higher metal-ion sorption power than activated charcoal or cylindrical membranes with tiny pores capable enough to filter out microorganisms, zero valent metallic nanoparticles of iron, aluminum, nickel, and zinc are finding great potentials in wastewater pollution prevention and treatment [32–35]. Membrane filtration plays an important role in water purification process. Nanofiltration is a high-pressure membrane treatment process unlike reverse osmosis it requires much lower pressure drive (7–14 bar) and thus is an energy efficient procedure. Wastewater after nanofiltration is found to be suitable for reuse, and it found to meet the stringent quality requirements. A wide range of membranes, viz. nanostructured ceramic membrane, organic–inorganic membrane, biologically inspired membranes, thin film composite membrane, carbon nanotube-polymer, zeolite-polymer, aquaporin-polymer are used for the purpose of nanofiltration [32]. Metallic nanoparticles are good catalysts in oxidative reactions; these nanocatalysts can be used for oxidation of organic–inorganic pollutants in wastewater by advanced oxidation processes. Photocatalytic reactions absorb the photon and create highly reactive radicals that can react with the molecules of pollutants and break them down. Though practical application of photocatalysis is challenged by proper optimization of catalysts and efficient separation of nanocatalysts but as the method utilizes the renewable solar energy and being an open air process, calls for the development of efficient and costeffective procedures for wastewater treatment [32, 34]. Remediation of wastewater using nanotechnology is an important strategy in waste management. The nanostructures or nanoparticles, specifically used for the purpose of remediation of contaminated ground water must be highly reactive toward the contaminants, have an appropriate life span with proper mobility in porous materials and negligible harmful effects. Nano scale zero valent iron (nZVI) finds potential application in remediation of wastewater. The popularity of nZVI in waste management is owing to its high reactivity toward the contaminants, eco-friendly nature, low production cost, capability of surface modifications, and selective specificity toward certain contaminants and their stabilization [32, 34]. For the purpose of solid waste disposal, it is also necessary to assess the "life cycle" of the products. But proper sorting and recycling may be expensive and tedious. Nanotechnology offers the solutions, where nanomaterials can be used as "nanotags." Such barcodes are easily detectable by infrared spectroscopy or Raman fluorescence and will help in easy tracking of products throughout their lifecycle [1].

Nanobiotechnology exploits the techniques of nanotechnology to study the biological systems, whereas bionanotechnology utilizes natural or biomimetic systems to fabricate classic nanostructures with versatile applications. The outcomes of bionanotechnology are the self-assembled nanostructures, biomolecular nanostructures,

and biopolymeric materials, whereas quantum dots, nanotemplates, cell on a chip are the outcomes of nanobiotechnology. Chitosan, the deacetylated form of chitin, derived from crustacean's shells, is a unique biocompatible, biodegradable natural polymer finding extensive applications in nanobiotechnology researches. Zero-dimensional carbon-based nanomaterials with chitosan, e.g. chitosan with carbon dots, chitosan with quantum dots, different biomarkers and biosensors signify the role of nanobiotechnology in "green research," and waste management [35]. Agricultural wastes generated in huge amounts in different farming processes and their irresponsible disposal contribute significantly to environmental pollutions. Proper recycling and reuse of wastes and conversion of wastes to "value-added products" are essential for a sustainable future. Eggshell is an agrowaste and being considered useless and discarded elsewhere. But this eggshell has a potential source of producing hydroxyapatite, a major component of bone and teeth. By eco-friendly process eggshell can be transformed to hydroxyapatite and nano hydroxyapatite having applications in bone repair and tissue regeneration. Such nanobiotechnology-based research will help in the development of biomaterials applicable in regenerative medicine, surgery, tissue engineering from discarded wastes and aids significantly in waste management [36]. Nanobiotechnology also aids in the green synthesis of metallic nanoparticles using biological entities, e.g. microorganisms. Plants and microorganisms have the ability to accumulate and absorb metallic ions from the surrounding and thus contribute significantly in reducing environmental pollution hazards. Such ability of microorganisms find applications in bioremediation and bioleaching. Synthesizing nanoparticles utilizing plants and microorganisms is a "green procedure." Nanobiotechnology exploits the inherent ability of the microorganisms and their biochemical processes, i.e. enzymatic activities to transform inorganic metallic ions to metal nanoparticles. Microorganisms have the ability to interact with the environment and their lipid-based membranes enable them to take part in different redox reactions and in bioconversion process. Depending on the degree of biochemical processing capabilities, bacteria, viruses, algae, fungi, and actinomycetes find significant roles in the synthesis of nanoparticles. However, synthesis of plant-based nanoparticles using either plant extracts or isolated plant bioactives is considered to be much cost effective and convenient rather than use of microorganisms since the use of microbes requires well-maintained culture preparation and isolation techniques. Microorganisms such as actinomycetes, *Chlorella vulgaris*, *Escherichia coli*, *Pseudomonas aeruginosa*, etc. find applications in the synthesis of metallic nanoparticles of gold, silver, cadmium, etc. [37].

In the context of discussion, mention is to be made of a new branch of bioscience, "*Bioeconomy*" that encompasses multidisciplinary technological knowledge from different branches of engineering, chemistry, biology, computer science, etc. *Bioeconomy* focuses on biological value of materials including organic waste. It refers to eco-friendly production, cost-effectivity, energy conservation, and conversion of biomass to a range of products in food, medicine, and other industrial products for future sustainability, social well-being, and a green planet. The broad-based *enabling technology*, the "*bionanotechnology*" and its nanoscale versatile products,

has attracted research fund investments and created interests amongst manufacturers and producers in this innovative sector. Thus, bioeconomy along with nanobiotechnology, bionanotechnology, nanotechnology accounts to zero waste economy in a circular fashion and is a sustainable solution for future food security, conservation of natural resources, and thus biodiversity [38].

25.5 Conclusion

Though nanotechnology and nanobiotechnology are really "green solutions" to achieve "zero waste economy" but simultaneously the bioaccumulation of nanowastes, nanowaste toxicity, proper recycling, and reuse of nanowaste are to be given due considerations [39]. Still, for the sake of future sustainability of the planet and thus humanity, GTs (referring here nanotechnology and nanobiotechnology) need to be embraced despite several hurdles in overall implementation. Along with the implementation of technologies, public awareness and knowledge are necessary at all societal levels for proper waste disposal and to make their household, workplace, society, and this planet a green sustainable place to live in.

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26

Novel Biotechnological Approaches for Removal of Emerging Contaminants

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26.1 Introduction

In general, contaminants are the waste substances deposited into the water source or onto land which affects the soil, environment, and water sources. Contaminants are of various forms such as liquid, solid, and gases. All the three forms of contaminants should be monitored and treated periodically. It is the prime duty of all the industries to treat the waste appropriately before channeling into the environment. Emerging contaminants (ECs) are the substances those cause no pollution when deposited in minor quantities. The increased use of various products and disposal of the same into the environment causes serious hazards. It is the prime duty of biotechnologists to educate the society about the ECs and need of treatment for those contaminants. ECs are unregulated and they are not included in the regular monitoring system for waste deposition and treatment. They include wide range of substances like personal care products (PCPs), pesticides, pharmaceuticals, plasticizers, nourishment-added substances, wood additives, clothing cleansers, surfactants, disinfectants, fire retardants, etc. The ECs need to be monitored, analyzed, and treated to protect the environment. Water is the major place for the deposition of the ECs.

Treatment of EC is one of the major step to protect the environment and water sources in order to save the lives from the hazards. Various methods include chemical, physicochemical, and biological for treatment of ECs. When compared to all, biological approach is more efficient and harmless to environment.

26.2 Classification of Emerging Contaminants

ECs might be appearing in the environment from long ago, but appeared as pollutants recently. They cause not only pollution in the environment but also affect

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human health [1]. Microfibers, microplastics, pharmaceutical products, PCPs, inorganic metals in foods and water, perfluorinated compounds, and disinfection byproducts are some of the major ECs [2].

26.2.1 Microfibers and Microplastics

Microplastics and microfibers are the major contaminants in the ocean basins. Around 9 million tons of microfiber is produced and used annually. Barrow et al. done a detailed study on the microfibers and their environmental impact in open ocean, coastal region, and understudied ocean region and reported that 57% of contaminants were synthetic, 12% are semi-synthetic, and 31% are non-synthetic. They have analyzed the samples using micro-Fourier transform infrared (μFT-IR) method [3]. Plastic-based microfibers cause major hazards to the environment, aquaculture, and humans. Polychlorinated biphenyls (PCBs) are the main forms of microfiber contaminants. Improper handling of these microfibers leads to the mixing of the contaminants with groundwater which will affect the drinking water quality.

26.2.2 Pharmaceutical Contaminants

Pharmaceutical contaminants include the hospital waste or biomedical waste such as discarded needles, scalpels, lancets, blood, microbiological cultures, and other devices capable of penetrating the skin. When compared to other contaminants, these are very dangerous and infectious. Some of these wastes are also radioactive in nature. The used cotton, needles, blood samples, and cultures for various analyses are to be treated and disposed carefully. The pharmaceutical industry also place a major role in the production of these contaminants, and the wastewater from these industries must be monitored and treated before letting into the environment [4, 5]. The pathogenic samples, cultures used for testing and analysis, and kits used for the biomedical sample collection must be treated and disposed using a standard procedure and the process should be monitored regularly.

26.2.3 Personal Care Products and Its Contaminants

One of the leading industries in the world is PCPs. About 5500 million tons of PCPs are being manufactured and used. Nowadays, both natural- and chemical-based PCPs are being used. In the formulation of PCPs, chemicals such as nitro and polycyclic musks (fragrances), methylbenzylidene camphor (UV blockers), parabens, and isothiazolin derivatives (preservatives) are included for chemical and biological stabilization of the product. These ECs are not yet studied, but the bioaccumulation has been reported. The major source of these ECs is the municipal waste water. The proper treatment is the only solution for the protection of the environment and human beings. The PCPs contribute around 15% of the total contaminants worldwide. There is rapid rise in the level of contamination due to rapid rise in the use [6].

26.2.4 Inorganic Metals in Foods and Water

Contamination of water and food materials by inorganic metals and compounds is the one of the major issues all over the world. Even trace of some of these metals will affect the health of the individuals. Common inorganic metals that found in groundwater and foods are lead (Pb), arsenic (As), and mercury (Hg). Millions of people worldwide suffer due to arsenic (As) toxicity, particularly the oxyanions arsenate (As(V)) and arsenite (As(III)) are more toxic than organic forms. Metals like As, Pb, and Hg are carcinogenic and toxic in nature. They are non-biodegradable and spread in the land and waters [7].

26.2.5 Perfluorinated Compounds

Perfluorinated compounds (PFCs)are major combination of perfluorooctane sulfonate (PFOS) and perfluoro octanoic acid (PFOA) which are mainly used in the production of fire-fighting foams, metal spray, detergent products, lubricants, inks, varnishes, coating formulations (for walls, furniture, carpets, and food packaging materials), waxes, water and oil repellents for leather, paper, and textiles. The PFCs are considered as bioaccumulative and hazardous to the humans and other living organisms due to their high heat and light tolerance and highly indigestible nature. Various samples are being collected and analyzed for the PFC contaminants. The obtained results show that they were found in surface water, sea, wildlife water sources, drinking water, human serum, and even breast milk. The PFC is identified as carcinogen and it is one of the major sources of cancer causing agents in humans and animals [8].

26.2.6 Disinfection Byproducts

Disinfection products are the chemical substances used on various surfaces and in water sources to remove the microorganisms. However, disinfectant byproducts (DBPs) are hazardous to the humans and other living organisms. When chlorine is used in the disinfectant product, it reacts with air and water and creates an odor which will cause breathing problems when inhaled in excess amounts. More than 600 DBPs have been discovered, which include iodinated trihalomethanes (THMs), aldehydes, ketones, halomethanes, hydroxy acids, carboxylic acids, alcohols, keto acids, esters, and even nitrosamines (NDMA). The DBPs are carcinogenic and may lead to other health risks like infertility, fetal loss, long gestational duration and poor fetal growth, and fetal anomalies [8].

26.3 Various Sources of ECs

26.3.1 Deposition of Solid and Liquid Waste on Land

Land pollution is caused due to the deposition of solid or liquid waste materials on the land. When this is followed for long term, it may penetrate underground in a manner that can contaminate the soil and groundwater, threaten public health,

and leads to many environmental issues. These solid wastes may contain hazardous solids, semi-solids, and greenhouse gases which may be flammable at times. Landfills are also one of the major reasons for global warming and environmental imbalance. The waste includes both biodegradable and non-biodegradable substances. The biodegradable substance will decompose and does not cause hazardous effects. However, the non-biodegradable substances will cause serious hazards, such as land pollution and air pollution, and contaminate the groundwater sources if not disposed off appropriately.

26.3.2 Deposition of Solid and Liquid Waste into the Water Sources

Most of the water bodies are being contaminated by discarding the industrial solid waste and wastewater into them. The waste includes all types of PCP, biomedical waste, textile waste, DBP, pesticides, biofibers, bioplastic, paper and pulp industry waste, and other chemical and domestic waste. All these wastes are deposited in huge quantity into the water sources, such as lake, pond, river, seas, and oceans. These wastes will affect the chemical and biological oxygen demand, which leads to insufficient level of oxygen to the aquatic plants and animals leading to their death.

26.4 Need of Removal of ECs

The removal of these contaminants is very vital, because

- ➢ They have the potential to induce a large range of acute and long-term effects on the human health and ecosystems;
- ➢ Studies showed that high-level exposure of these contaminants will cause endocrine disruption, immunotoxicity, neurological disorders, and cancer in humans and animals. Some of these contaminants are identified as known carcinogens by United States Environment Protection Agency (EPA);
- ➢ Many of these contaminants are used and released into the environment even in very low quantities will cause chronic toxicity, and the development of bacterial pathogenic resistance;
- ➢ High level exposure of these contaminants will increase the risk of birth defects, kidney, and liver damage.

26.5 Methods of Treatment of EC

The EC can be treated and managed using various methods. The major methods of treatment of EC are physical, chemical, physiochemical, and biotechnological methods. Biotechnological approach is a recent and innovative.

26.5.1 Physical Methods

Physical method of treatment of contaminants is done by simple filtration or sedimentation of the wastewater in the large tanks. By this method, only the undesirable

solid particle and heavy metals can be removed. Other chemicals and hazardous materials cannot be treated in this method.

26.5.2 Chemical Methods

After the physical treatment, waste will be treated with chemical means to remove the undesirable contaminants. The use of chemicals for the treatment of waste may also create byproducts that are hazardous to the environment and the living organisms. Chemical digesters are used for the digestion of the solid waste to digest the harmful chemicals from the waste.

26.5.3 Biotechnological Approach

Recent and efficient approach for the waste treatment is biotechnological one. The biotechnological approaches include biodigestion, enzymatic treatment, biofiltration, and bioremediation. These methods involve microorganisms and algae for the degradation of the waste material and they play a very significant role in the decomposition and removal of the hazardous materials. It is the novel and advanced method for the biodegradation.

26.6 Biotechnological Approaches for the Removal of ECs

26.6.1 Digestion by Membrane Bioreactor

Membrane bioreactors are used for the removal of contaminants from sludge. They were used in the treatment of wastewater that contains micro-propellants and pathogenic microbes. The substances like diltiazem, estrone, progesterone, and acetaminophen were completely removed by this method. The activated sludge treatment for the compounds such as bayrepel acid, diclofenac, and diethyltoluamide (DEET) showed the removal efficiency of 50–100% [2].

26.6.2 Enzymatic Treatment

Enzymatic treatment of the waste is done using two common types of enzymes, laccases or peroxidases. These enzymes are very good biocatalysts in the conversion of the toxic organic components into nontoxic or less toxic products. These treatments are mostly considered as tertiary ones. Laccases catalyze the conversion of the alcoholic compounds, such as bisphenol A, triclosan, and nonylphenol [2]. A study shows that laccase is more efficient in the removal of Orange 2 (72.8%) and Acid Orange 6 (45.3%). Many studies have reported the effective and extensive use of peroxidases in the oxidation of a vast variety of ECs, including azo dyes, nonsteroidal anti-inflammatory drugs (NSAIDs), hormones, antibiotics, and pesticides. Enzymatic treatment was efficient in decolorizing Bromophenol Blue (98%), while heterocyclic dyes, Methylene Blue, and Toluidine Blue O were least decolorized (only 10%) (Table 26.1).

Table 26.1 Enzymatic treatment of EC and its efficiency.

Enzyme	Contaminant	Removal efficiency (%)
Laccase from *Trametes pubescens*	Bisphenol A	>99
Laccase from *Myceliophthora thermophila*	Morphine	100
Laccase from *Trametes versicolor*	Orange 2	72.8
	Acid Orange 6	45.3
Laccase from *Fomes fomentarius*	Remazol Brilliant Blue R	100
Laccase from *Myceliophthora thermophila*	Estrogens	100
	Diclofenac	100
	Naproxen	100
Peroxidase from *Pleurotus ostreatus*	Bromophenol Blue	98
	Methylene Blue	10
	Toluidine Blue O	10
Bacterial peroxidases	Bisphenol A	100

26.6.3 Biofiltration

Biofiltration is used for the removal of PCP and pharmaceutical products during wastewater treatment. This method is more efficient in the removal of estradiol derivatives when compared to ibuprofen and triclosan which are partially removed (Table 26.2) [2].

26.6.4 Bioremediation

Bioremediation is a method of removal of contaminants from the water and other polluted sites. It involves the use of living organisms, such as microbes and plants, to reduce/degrade, eliminate, and transform contaminants present in soils, sediments, and water. This method has gained wider acceptance in the recent years because of its potential to remove various organic and inorganic contaminants from various sites of the environment. Bioremediation provides an effective treatment of inorganic and organic contaminants under *in situ* and *ex situ* conditions by natural means. Potential of microbes and plants have been exploited to achieve maximum removal of inorganic and organic contaminants. The genetic engineering strategies have been employed to improve the efficacy of this technique for achieving complete degradation of contaminants. Bioremediation follows biosorption and bioaccumulation methods for the removal of the hazardous substances from the waste.

During biosorption process, the contaminants are absorbed on the cellular surface of the sorbents depending on the rate of kinetic energy and composition.

Table 26.2 Efficiency of removal of EC using biofiltration.

S. No.	Emerging contaminant	Removal efficiency (%)
1	Cashmeran	68
2	Ibuprofen	86
3	Benzothiazole, 2-(methylthio)	66
4	Tributyl phosphate	22
5	Methyl dihydrojasmonate	97
6	Tri(2-chloroethyl)phosphate	2
7	Diazone	8
8	Caffeine	49
9	Galaxolide	89
10	Tonalide	90
11	Terbutryn	94
12	Carbamazepine	5
13	Naproxen	72
14	Oxybenzone	89
15	Triclosan	87
16	Ketoprofen	99
17	Diclofenac	93

Biosorption is easy and cost-efficient and can be used for the environmental cleaning. This process can be used for reducing heavy metal pollution from industries and agriculture. Most common biosorbents used for the sorption include bacteria (*Bacillus subtilis* and *Magnetospirillum gryphiswaldense*), fungi (*Rhizopus arrhizus*), yeast (*Saccharomyces cerevisiae*), algae (*Chaetomorpha linum*), and marine microalgae (seaweed). There are other bioremediation methods, and some of them have been discussed below.

26.6.4.1 Bioaugmentation

Bioaugmentation is the process of adding cultured microorganisms into the subsurface of the contaminants for biodegrading specific soil and groundwater contaminants. Two factors that affect the use of bioaugmentation: (i) non-indigenous cultures rarely compete well enough with an indigenous population to develop and sustain and (ii) most soils with long-term exposure to biodegradable waste have indigenous microorganisms which act as effective degraders if the land treatment is well managed.

26.6.4.2 Bioreactors

The bioreactors are used in the treatment of EC when the amount of waste is small. They can be used to treat slurries or liquids. Slurry or aqueous reactors are used

for the treatment of contaminated soil. Bioremediation in the reactors involves the processing of contaminated solid material (soil, sediment, and sludge) or water through an engineered containment system. A slurry bioreactor will have containment vessel to create three-phase mixing condition to increase the bioremediation rate of soil-bound and water-soluble pollutants. In general, the rate and extent of biodegradation of EC is greater in a bioreactor system than other system because the contaminated environment is more manageable and hence more controllable and predictable. The contaminated soil requires pretreatment using excavation; alternatively, the contaminant can be stripped from the soil via soil washing or physical extraction like vacuum extraction before being placed in a bioreactor. Bioreactors have been used to treat soil and other materials contaminated with petroleum residues.

26.6.4.3 Biostimulation

Biostimulation involves the modification of the environment to stimulate the existing microorganisms for the remediation of the EC. This can be done by adding various forms of rate-limiting nutrients. This can also be done using electron acceptors, such as phosphorus, nitrogen, oxygen, or carbon.

26.6.4.4 Bioventing

The process of passing oxygen through the contaminated medium stimulates the microbial growth and activity. Bioventing is the most common *in situ* treatment and involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing uses low airflow rates and provides only the required amount of oxygen that is necessary for the biodegradation while minimizing the volatilization and release of contaminants into the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface. In many soils, effective oxygen diffusion for desirable rates of bioremediation extends to a range of only a few centimeters (30 cm), although depths of 60 cm and greater have been effectively treated in some cases.

26.6.4.5 Composting

Compositing is an aerobic and thermophilic process, and EC will be mixed with a bulking agent here. Composting may be performed using static piles, aerated piles, or continuously fed bioreactors. Here, EC will be combined with nonhazardous organic amendments, such as manure or agricultural wastes. The presence of these organic materials supports the development of microbial population and elevates temperature which is characteristic of composting. Typical compost temperature ranges from 55 to 65 °C. The increased temperatures result from the heat produced by microorganisms during the degradation of the organic material present in the waste. Composting of EC is done by following the basic steps. The ECs are excavated, screened, and transported to a composting pad (with a temporary structure to provide containment and protection from weather extremes). Amendments (straw, alfalfa, manure, agricultural wastes, and wood chips) are used as a supplemental carbon sources. Soil and amendments are layered into long piles, known as

windrows. The windrow can be thoroughly mixed by turning with a commercially available turning machine. Moisture, pH, temperature, and explosive concentration are monitored. After the completion of the composting, the windrows would be disassembled and the compost will be taken to the final disposal area.

26.6.4.6 Land Farming/Land Treatment

Land farming is a simple bioremediation technique in which EC containing waste or soil will be spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous biodegradative microorganisms and to facilitate their aerobic degradation of ECs. This practice is limited to the treatment of superficial 10–35 cm of soil. Since land farming has the potential to reduce monitoring and maintenance costs, and clean-up liabilities, it has received much attention as a disposal alternative. Spilled oil and wood-preserving wastes have been bioremediated by land farming treatments.

26.6.4.7 Biopiling

Biopiles are hybrid of land farming and composting. Essentially, engineered cells are constructed as aerated composted piles. Adding compost to contaminated soil enhances the bioremediation. Compost enhances the oxidation of the aromatic contaminants of the soil into ketones and quinones, which will eventually disappear. This method is used for the treatment of surface of EC like petroleum hydrocarbons. It is a refined version of land farming that tends to control physical loss of the EC by leaching and volatilization. Biopiles provide a good environment for indigenous aerobic and anaerobic microorganisms [9].

26.6.5 Phytoremediation

Phytoremediation involves the use of plants and their associated microbes to accumulate, detoxify, or stabilize EC. It is an environment-friendly and sustainable means of remediating contaminated soil and water. It has been an important aspect of constructed wetlands, which is used to detoxify large volumes of wastewater with dilute concentrations of EC successfully, including petroleum, hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals, and radio nuclides. The most important requirement for this method is the use of fast-growing, high-biomass plants those are capable of uptaking and accumulating large amounts of toxic metals. Biotechnology makes it possible to isolate such microbes and enrich the soil so as to enhance phytoremediation by respective plants. The scientific studies on genetics, physiology, and biochemistry of plant tolerance to inorganic and organic contaminants have dramatically increased which will be important for improving the phytoremediation techniques. These techniques for restoring soil at specific level in determined site will depend on the chemical nature and concentration of the pollutant and also on the physicochemical and biological characteristics of the soil. However, there are still some barriers for the adoption of phytoremediation which will impair the successful application of this remediation technique. This technique is subdivided into number of phytoprocesses depending on the characteristic used by the plants to remediate a polluted site.

26.6.5.1 Phytoextraction and Phytoaccumulation

Phytoextraction and phytoaccumulation are probably the best known phytoremediation processes for EC with inorganics. In these processes, plants will remove metals from the soil and accumulate in their parts which will be harvested subsequently. Metal-rich plant materials obtained by these methods can be appropriately disposed or incinerated, or depending on the economical value of the metal and its concentration in plant tissues, they can be phytomined and recovered from the plant ashes [10–12]. In some cases, if the element extracted from the plant material is a valuable nutrient for plants, the harvested metal-containing biomass can be used as a source of fertilizer in deficient areas [13].

26.6.5.2 Phytostabilization

This method uses metal-tolerant plants and its associated microorganisms to reduce pollutant mobility and bioavailability by means of its immobilization technique in the root zone. This process prevents leaching of metals to groundwater reservoirs and reduces the possible risks of offsite contamination through erosion.

26.6.5.3 Phytovolatilization

Phytovolatilization is a method of reduction of EC using plant-mediated processes that favor the volatilization of pollutants. This process is possible only for some particular organic compounds and mercury, in which after plant uptake, pollutant is released in a volatile form.

26.6.5.4 Phytofiltration

Phytofiltration is the process of using plant roots to absorb and adsorb pollutants from waters. This process is mainly used for removing metals and inorganics from industrial and water treatment effluents. When seedlings could achieve this process, the technique is called blastofiltration.

26.6.5.5 Phytodegradation

Phytodegradation is the degradation of pollutants by plant–microbe systems during which enzymatic activities can facilitate organic biodegradation. Phytoscrubbing is the process of removal of atmospheric pollutants by plants, as in the case of organics such as benzene that can be efficiently removed from air by *Dracena sanderiana* plants. Another recent example of phytoscrubbing is from plants of Ericaceae family which were screened for the removal of gaseous pollutants.

26.7 Conclusion

ECs will pose major problem in the environment, since there are no standard procedures for monitoring or the treatment of these contaminants. For other contaminants, there are standard operating procedures and several laws to monitor, treat, and also to control the emission. The ECs will be formed due to the rapid growth of population and urbanization which intern will lead to the higher usage of sources of ECs. It is our prime duty to make the society aware of the health hazards

and environmental impacts of ECs. It is also our duty to protect the environment and living organisms from ECs. A watershed-scale approach has to be developed for comprehensive solutions at source, transfer, and fate levels. Control at source can be a cost-effective solution for reducing EC loads in water bodies.

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Part VIII

Economics and Commercialization of Zero Waste Biotechnologies

27

Bioconversion of Waste to Wealth as Circular Bioeconomy Approach

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27.1 Introduction

The aim of sustainability in consumption and production is to ensure efficient and sustainable utilization of resources, energy, and infrastructure. This in turn brings about sustainable development that aims at reducing costs on economy, environment, and society while enhancing economic competitiveness and decreasing poverty. Consumption of natural resources during the year 2017 globally has registered a 254% jump as compared a similar global consumption in 1970. This rate has been on an exponential growth since 2000 [1].

27.1.1 Circular Economy

Nature is the creator of all matter in an effective manner. Every single material produced by nature, after its usage, is also effectively disposed by nature. However, humans, during the course of advancement and development, have failed to understand and appreciate the basic laws of nature and are paying a heavy price for it. Ozone layer depletion and pollution are the direct results of humans not understanding the laws of nature. To overcome this problem, and to develop a sustainable, economical, and eco-friendly method of waste disposal/recycling, the concept of "Circular economy," was proposed in the late 1970s. In contrast to "linear economy," which is based on creating, utilizing, and ultimately destroying the waste, "circular economy" considers that everything can be regenerated and reused. Therefore, creation is actually a cyclic step before waste regeneration and not actually the starting point [1].

The most widely acknowledged definition/concept is that by Ellen MacArthur Foundation and is represented by the "butterfly diagram." This concept is based on the "Cradle to cradle™," set by the German chemist, Michael Braungart, along with American architect, Bill McDonough. This concept considers circular economy (CE) to comprise of two cycles – (i) the biological cycle and (ii) the technical cycle.

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The cycles revolve around two candidates – (i) the consumer for biological cycle and (ii) the user for the technical cycle. This philosophy considers all the materials that go into any industrial or commercial process as nutrients. The concept sees nature's way of recycling and refurbish (biological metabolism) as the blueprint for an efficient flow of industrial materials (technical metabolism). This concept analyses methods for an effective usage of all technical "nutrients" for maximizing the positive impact and minimizing the negative impacts, both technically and economically.

27.1.2 Bioeconomy

Paralleling the development of concept and application of circular economy, the European Commission, in 2012, put forth the concept of "bioeconomy." Accordingly, bioeconomy is defined as "production of renewable biological resources and their conversion into food, feed, bio-based products and bioenergy." This encompasses agriculture, forestry, fisheries, as also food, and pulp and paper production, and a part of the chemical, biotechnological, and energy industries [2].

The goals of this approach include (i) developing food which is healthy, safe, and also nutritious, (ii) healthy animal feeds obtained through an effective utilization of resources, (iii) development of new food supplements, (iv) providing chemicals and polymers with new properties and functionalities, and (v) replacement of fossil energy with bioenergy and biofuels. Other goals include development of novel agricultural and marine practices that are sustainable and efficient, improved bioprocessing and biorefinery concepts, novel eco-friendly process technologies, and making the atmosphere cleaner by exploring and popularizing the usage of biofuels which have low greenhouse gas emissions (GHG). Of particular interest and importance is the impetus given to new concepts in biorefining that favor products that have the highest value addition and resource efficiency [3]. This is suggested as an alternative to the conventional methods in biorefining that are aimed to producing only biofuels.

The other noteworthy aspect of bioeconomy is its insistence on the concept of "cascading use of biomass." This concept is defined as a system that extends the total biomass available, through effective utilization of the so-called "used" materials (residues and recycled materials).

27.1.3 Circular Bioeconomy

Circular bioeconomy (CBE) integrates the concepts of the circular economy and bioeconomy and aims to improve resource and environmental efficiency, reduce trace gases (GHGs), reduce carbon demand, and value waste. It points to similarities and differences between the "circular economy" and the "ecological economy." The global economy seeks to improve resource utilization and waste recycling to reduce more fuel emissions while processing and extracting processes. Bioeconomy features transcend rounded economic objectives that include product or service areas. Bioeconomy is largely driven by a growing cycle by including in the global economy a large number of biological processes and waste from livestock, forestry, fisheries, food and animal feed, and natural waste from production. Biomass wastes

generated from agriculture, forestry, fisheries, aquaculture, animal feed, and biological processes are used for a variety of applications such as aquaculture and chemical processing. In addition, decay products can re-enter the nutrient and biological cycle. Extraction of oleochemicals through recycling of vegetable and/or animal fats and recycling of bioplastics also could be foreseen as possible outputs of CBE [4, 5].

27.2 Biovalorization of Organic Waste

In scientific literature, food loss and waste are classified as products for human consumption that are subsequently discharged, lost, degraded, contaminated, and ultimately is a source of pollution. Food content discharge occurs along the entire food supply chain and includes all sectors of waste management right from its collection to disposal [6]. The issue of sustainable waste management is an important challenge that faces our society today. Solutions to this pressing problem should achieve socioeconomic and environmental benefits. Conversion of food waste into a source of renewable energy has transformed the food sector to a potentially feasible carbon economy [7]. Toward achieving the aims of CBE, two of the options available for biovalorization include (i) extraction of bioactives and economically important compounds from the wastes generated and (ii) utilization of the wastes to generate biofuels that are alternatives to the existing fossil fuels.

27.2.1 Extraction of Bioactives

Food wastes contain a combination of carbohydrates, lipids, and proteins. Depending on the source of these wastes, and their chemical nature, these can be used as the starting material for the production of different bioactive compounds. Some of the wastes and their potential reuse include

a. **Fruit and vegetable processing wastes**: These wastes could be used for (i) extraction of polymers like pectin, cellulose, etc., from fruit and vegetable processing wastes [8] and (ii) for the production of natural colorants, including anthocyanins, flavonoids, etc. [9, 10].
b. **Dairy wastes**: These wastes can be used for the production of a variety of compounds, including antioxidants, antimicrobials, etc. [11].
c. **Meat and poultry processing wastes**: These wastes can be used for the extraction of collagen, gelatin, polypeptides, etc. [9].
d. **Sea food wastes**: These can be used for protein isolates and hydrolysates [9].
e. **Dairy industry wastes**: These can be used for the isolation of casein, lactose, and whey protein isolates [9].

27.2.2 Bioenergy Production

The components of wastes, i.e. carbohydrates, proteins, and lipids, can be considered as potential sources of different biofuels. The biofuels can be produced using bioprocesses or thermochemical processes depending on the composition of the food waste [12, 13].

Anaerobic digestion (AD) systems convert organic wastes to methane (CH_4) and carbon dioxide CO_2 by utilizing the acid-forming and methane-forming bacterial biomass. Comparisons of phase one and two anaerobic diets based on methane and hydrogen recovery showed two-phase approach to achieve a complete hydrogen output and a 20% higher energy gain because of increased methane production as compared to hydrogen-related production [13]. Studies on life cycle assessment performed to determine the environmental impacts of replacing traditional digestive feedstocks with anaerobic show that these replacements reduce gas pollution, can control the loss of nutrients in water sources, and can reduce the effects of eutrophication [14].

The use of organic wastes for biofuels production was studied as an alternate approach to using fossil fuels. Some of the key barriers identified include high production costs and high energy consumption. The report also suggests a novel idea to improve biodiesel production from waste oil using a combined reactor [15]. The current trends in research are focused on improving the sustainability and economic feasibility of the concept of consolidated bioprocessing and on the fourth generation [16].

27.3 Bioeconomy Waste Production and Management

Reuse includes the segregation, classification, promotion, and repair of discarded material from a solid waste stream. Today the modification, remanufacture, and recycling of these items to be used as a product feedstock for brand new items are gaining much popularity. Circular economy (CE) is achieved through the 3R system (Resources, Recycling, and Recovery), which leads to use of sustainable resources and eventually leading to enhanced economic development. CE has recently gained much attention from the industrial economy through the loop economy, with a focus on measures such as waste prevention, resource efficiency, and job creation [17, 18]. Municipal solid waste (MSW) has great potential toward renewable energy generation, by linking concepts of waste management and recycling [19].

The recycling practice is executed in residential, commercial, and industrial markets. The streams of MSW found in these locations can be classified as:

1. **Residential solid waste**: solid waste produced from either single or multifamily living arrangements. The recyclables that are prevalent in this stream include paper, plastics, metals, food scraps, and individual hardware.
2. **Commercial strong waste**: strong waste generated by organizations, workplaces, stores, markets, organizations, government, and other business institutions. Some of the wastes with potential recyclability include paper, plastic, metals, food, yard trimmings, wood, materials, and electronic gadgets.
3. **Industrial strong waste**: strong waste created from non-process lines, delivering, and plant workplaces.

The biorefinery concept could become an important part of the development of a circular economy. Integration of multidisciplinary approaches to biomass/waste

operations could help develop attractive intermediates and final products. A critical aspect of a bioprocess strategy is the financial viability associated with the natural resilience of low carbon emissions [20]. Organic fraction of municipal solid waste (OFMSW) can be used effectively as an anaerobic digestion feeder and a preferred source for biogas production. The effectiveness of this structure is strongly dependent on the location and time of separation. The assembly of food supply and consumer welfare (FSCW) is based on the primary crude substance considerations. Typically, OFMSW mainly contains a large number of organics including carbohydrates, proteins, fats/oils, and minerals. OFMSW includes cooking and kitchen waste from restaurants, food waste, cafes, markets, and family food waste that are normally highly damp and generally biodegradable [20]. Recyclables are collected through many approaches that may include (i) single-stream separation (path separation of coexisting recyclables), (ii) separate path for the recyclables which are isolated, (iii) path separation of blended MSW multi-method separation, (iv) drop-off and systems for repurchase, (v) deposit mandates and legislations, and (vi) industrial and modern segregation driven through public interest generator. Reuse of material from waste streams should be supplemented by actions such as (i) legislation/compulsory projects, (ii) voluntary projects by private and public organizations or institutions, and (iii) reward or incentives-based policies.

Based on the nature and composition of the waste stream, a variety of items can be generated or created. Through different methods based on physical, chemical, or biochemical processes, high-value materials from different streams of OFMSW can be produced [21]. Bioactive compounds, including polyphenols, carotenoids, nutrients, cell fortification, flavonoids, fibers, and gelatin, are generally used as additives in the food industry and active compounds in the manufacture of various pharmaceutical products. Citrus, apple pomace, and berries are a rich source of phenolic compounds. Citrus strips, apple pomace, sugar beet, watermelon, and sunflower heads are a source of gelatin that is used as gelling, thickening, and balancing agent in the food processing industries. Stripping of biocolors from pastry shops has also been proposed. Research was conducted on bread kitchen waste which was converted into hydrolyzate and tested biocolorants development by staining using parasitic stain of *Monascus purpureus*. Common shades derived from organic sources can be used in food and meat application. The regular color hues increase from 55% in 2015 to 60% and is expected to increase though advertisements by 2026. Agricultural waste, animal waste, and food processing are used in the production of biosurfactants as soon as possible. Sophorolipids, rhamnolipids, and surfactin contain a variety of mechanical properties, including glues, moisturizing flocculating and frothing specialists, de-emulsifiers, and penetrators.

In addition, biosurfactants are also acts as a pesticide for bioremediation. Bioethanol assembly is a well-known process for valorization of FSCW by microbial bioconversions. Studies have been done on family food product waste, waste from restaurant, and also skin/peels of fruits and vegetables on their potential to produce ethanol from MSW schemes. Some researchers have also described the valorization of FSCW to create wealth from waste in the form of synthetic materials, intermediate materials, biofuel precursors, and biodegradable [22].

27.4 Concerns About Managing Food Waste in Achieving Circular Bioeconomy Policies

Feedstocks, obtained from house-hold organic wastes, are regarded as secondary waste and these are used to increase the load of biomass for subsequent conversion to biofuel or production of bioactives [23–25]. However, it should be noted that the extent of contribution of these secondary feedstocks to circular bioeconomy is debatable. Hence, greater fillip is needed to create interest in developing technologies for such products and to encourage investments [24]. Information pertaining to the availability and nature of wastes should be reliable and transparent. This will indicate a positive step toward environmental protection. Consumption of bio-based assets and substitutes should have substantial equivalence with the fossil fuels that they are used to replace. Life cycle analysis evaluation (LCA), life cycle cost (LCC), and social life cycle assessment (s-LCA) should be performed to determine the level of sustenance that a particular project can achieve. This is very important considering its economic and social characteristics [26]. Such life cycle studies highlight the benefits of bio-based products to policy-makers and to the society. This is of prime importance to ensure the success of sustainable circular models with support from the people at large.

A strengths weaknesses opportunities and threats (SWOT) review conducted on the key barriers and benefits associated with the case identified the following strengths. The strengths of the event include improved agriculture and sustainable food production with an improved method for value addition to wastes, increasing jobs that are eco-friendly as also profitable, improving technologies for producing eco-compatible chemicals and bioenergy, and identifying strong bio-based plastic. The opportunities identified were job creation and economic process in the green sector. The EU commission in 2018 implemented bioeconomic communication and bioeconomy strategy implementation to encourage industrial decision-making. While the event benefits both the community and the industry, the general public is concerned about certain weaknesses and threats.

As this concept is still in its infant stage, the current technologies are not economically viable. This is turn has reduced the demand for this novel approach making this approach financially unviable. Supply of biomass feedstock needs technical, economic, and seasonal factors. Laws and policies need to be properly implemented at various stages to deduce the wastage perceived at various stages in the food supply chain. Lower refund policies on food supply chains will apply. Lack of guidelines on sustainable bio-based products and biofuels could reduce confidence in the sector. Detailed reports on the information regarding such products along with the technology involved safety of the processes and products should be made available in the public domain. This will improve the awareness among the people and thus increase the sustainability of such technologies and products. Finally, the profitability of biofuel products is greatly influenced by the cost of oil from fossil sources. Hence, there is an increased need to develop technologies and products whose costs can compete with those of fossil-based fuels.

Some of the points which need due consideration and solution are as follows. (i) The amount and volume of waste varies greatly, depending on the effectiveness of the product collection system. It is therefore important to improve the uniformity of the waste collection and homogeneity of waste. (ii) Waste resources include a good range from distributors to hospitals, leading to lower collaboration. (iii) As waste decomposes rapidly, there are problems with timely transfer and temporary storage facilities [23, 24]. (iv) The market for organic goods is dependent on enforcement policies, which require strong legal support and significant public participation. Compared to conventional products, public opinion may be concerned about the increasing demand for bio-based products, as they are more expensive [27, 28]. (v) The technology for such plants, in its infant stage, is still not economically viable.

27.5 Economics of Bioeconomy

The future or rather the success of bioeconomy is dependent on how economically viable the approaches/ideas are put forth. This in turn depends on the following factors: (i) advancements and the degree of integration between the fields of biological sciences and information technology, (ii) validation of alternatives to fossil fuels and their economic feasibility, and (iii) changes that are likely to take place in terms of the organizational structure of an industry and among industries and also integration of industries with varying outputs [29, 30].

27.6 Entrepreneurship in Bioeconomy

Entrepreneurship can be defined as the ability to ascertain, manage, and maintain a business venture together with its potential risks to form profits. Any situation that fosters uncertainty also fosters the entrepreneurial skills. The current business setup is still wrought with uncertainties that can fail any entrepreneurship venture. Support from government agencies is hence important to encourage entrepreneurs, and policies drafted to ensure their sustenance during times of economic failures [31].

Though opportunities in bioeconomy are high, it is important to assess the viability and profitability of these entrepreneurial opportunities. Assessment of entrepreneurship opportunities should include questions such as:

1. What are the values that are more sought for by customers?
2. Do opportunities meet competence and experience?
3. Would the customers be willing to pay more?
4. Is there an opportunity for growth in the market?
5. Will the risk and potential be balanced out?

This is an important part of entrepreneurship as this is an innovative process and assessment is extremely crucial in bioeconomy. Both businessmen and customers should collaborate to produce a realistic business model [31].

27.6.1 Current Trends in Bioeconomy

1. Biodiplomacy
   This concept pertains to the approach of diplomacy among countries to include a concerted and sincere effort to tackle global warming and other climate changes. This includes a global approach toward bioconservation that also ensures a secure human livelihood [32].
2. Identifying targets for an internationally agreeable sustainable development goals and linking them to bioeconomy, for monitoring progress [33].
3. Developing an educational framework for a better understanding and propagation of the concept of CBE [34].

27.7 Conclusion

CBE is a concept that ensures an effective utilization of natural resources, while trying to develop technologies that are eco-friendly. These technologies need to be economically feasible and also need to get acceptance from the public at large. This concept, currently at its nascent stage, is bound to be the future solution for environmental protection and sustenance of natural resources.

List of Abbreviations

CBE	Circular bioeconomy
CE	Circular economy
FSCW	Food supply chain waste
LCA	Life cycle analysis
LCC	Life cycle cost
MSW	Municipal solid waste
OFSMW	Organic fraction of municipal solid waste

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28

Bioconversion of Food Waste to Wealth – Circular Bioeconomy Approach

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28.1 Introduction

Around 1.3 billion tons of food is wasted globally and is equal to one-third of edible part of the food produced for human consumption. Also, it was estimated that if the one-fourth of the wasted food is saved, it could feed 870 million malnourished people. Food wastes generated in developed countries from household, catering, retail, and manufacturing industries cost around US$ 680 billion and US$ 310 billion in developing countries. Global population is increased to 1 billion within next decade and it is expected to reach 9.1 billion by 2050 [1]. This growing world population will demand more food, and this will make excess pressure on food supply chain, thus becoming a challenge for global food security and supply. Food security will become a key issue when people lack access to safe and nutritious food which may be caused due to food unavailability, insufficient purchase power, and inappropriate distribution [2]. Increased food production without sustainable waste management strategies will result in increased greenhouse gas emissions due to additional land use and increased waste production. Food waste being generated throughout the food supply chain (Figure 28.1), which includes production, pre- and post-harvest handling, processing and value addition, storage and distribution, retailing, preparation, cooking, and serving food, is termed as food supply chain waste [3].

According to FAO (2015), in developing countries, 80–90% of the food waste is generated during pre- and post-harvesting and processing stages of the supply chain. On the other hand, in developed countries, more than 40% of the food is wasted in retail and consumption stages. Pfaltzgraff et al. [4] highlighted the agro-industrial residues generated around the globe and the examples include olive mill residues, waste vegetable oil, tomato pomace, wheat straw, whey, brewer's spent grain, pea pods, egg shells, spent coffee grounds, potato peels, sugarcane bagasse, grape pomace, corn residue, orange peel, cocoa pods, cashew shell nut liquid, rice husk,

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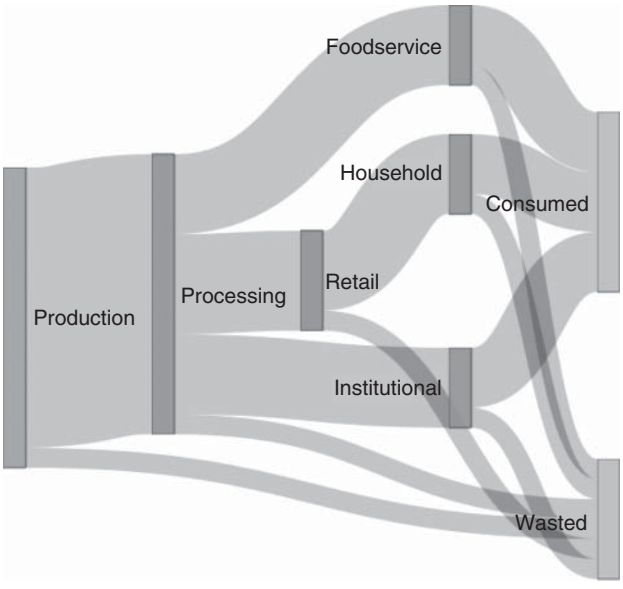

Figure 28.1 Simplified model for generation of food waste through the food supply chain. Source: Read et al. [3].

apple pomace, and rapeseed meal. Conventional agro-food waste disposal methods such as landfilling, composting, and incineration cannot reduce the adverse impact it has on environmental and social issues. The perspectives on economic and societal issues add pressure to the food industries to achieve zero waste. Adequate measures and integrated strategies need to be focused on minimization of food waste and valorization of unavoidable food waste [5]. Novel waste valorization technologies such as thermal, chemical, and bioconversion methods add marketable value to the food waste. Transformation of food waste into valuable products can reduce the production cost and environmental pollution [5, 6]. Agricultural and food processing waste are excellent renewable feed stock for bioconversion of high-value bioproducts such as enzymes, pigments, organic acids, bioactive compounds, and biodegradable plastics. Research on food waste management over the past decades mainly focuses the operational conditions on waste treatment and disposal methods to produce energy and bioproducts. In addition to this, there is a need for interdisciplinary collaboration among the scientists, economists, and policy makers to search new solution for food waste valorization in an attempt to address the emerging bioeconomy.

28.2 Circular Bioeconomy

Circular economy aims to conserve the value of products, materials, and resources for a long period by enhancing the efficiency of resource utilization and reducing waste generation. Circular economy increases the efficiency of resource utilization by transforming the industry byproduct into resource for second industry, thereby

reducing the impact of industry in the environment, and this alters the linear utilization of resources into closed loop. Adopting novel technologies and socioeconomic restructuring is essential to promote the circular economy and closing the loop of resources [7]. In food system, reducing the disposal of waste and finding appropriate solution to manage the remaining waste are the important strategies to implement circular economy. Development in research in the last decades has highlighted several options by conversion of food waste and byproducts into bioenergy or valuable raw material. The principles of circular economy are complementary to bioeconomy and it focuses on the establishment of integrated sustainable approaches for resource utilization. Bioeconomy encompasses transformation of biomass and biowaste into wide range of bioproducts and biofuels. Bioeconomy demands renewable biomaterials which include plant materials, animal, and microbial constituents which have the potential to produce bio-based products [7].

Consumption of natural resources has been increasing globally and the extensive utilization of fossil fuels causes negative environmental impact that urged the development of biofuels and biomaterials through sustainable feedstocks. Food wastes are valuable bio-based resources, which are primary alternative to fossil fuels. Bio-based renewable resources are important for sustainable economic growth and environment preservation. Biomass obtained from food waste is found to be a promising renewable resource. Through sustainable biorefinery approaches, food and agricultural processing wastes can be converted into valuable bioproducts that has driven the circular economy. Biorefineries can enable the recognition of circular bioeconomy by allowing the valorization of multiple products [8, 9].

Circular bioeconomy is the intersection of bioeconomy and circular economy which lists the common concepts such as efficient utilization of renewable resources, reduction of greenhouse gas emission, reduction of the use of fossil fuel, and valorization of waste. Wastes are important components of circular bioeconomy, where reuse, recycle, and remanufacture can be attainable through different conversion technologies and pathways. Food waste-based biorefinery concepts are the most promising approach for effective conversion of biomass into valuable products such as bioethanol, biopolymers, bioplastics, biogas, biochip, syngas, bio-oil, and biochar. On the basis of conversion route, biorefineries are categorized in to thermochemical and biochemical refineries [8]. Pyrolysis and gasification are the thermochemical refining processes which break down the biomass into cellulose, hemicellulose, and lignin, and these intermediates are further processed into wide variety of marketable products. Chemicals and biological components like microorganisms and enzymes are involved in biochemical refining process which break down the biomass into numerous compounds through enzymatic/chemical hydrolysis, fermentation, and digestion process [8]. Integrated biorefinery is preferable for efficient use of biomass, including waste generated from different conversion pathways and convert into valuable bioproduct streams. Integration of biology, food science, biochemistry, biochemical engineering, and biotechnology has the potential to bridge the gap between valorization strategies and biorefinery concept for producing marketable products from renewable feedstock. High concentrated volume, preferably homogeneous composition (e.g. tomato pomace,

spent brewer's grain, wheat straw, rice husk, and citrus peel) of food supply chain waste is required for production of marketable products with cost-effectiveness of the process. Major components present in the citrus fruit peel are pectin, flavonoid, and cellulose [4].

28.3 Food Waste Management Current Practices

Food waste is a global issue, according to FAO, every year one-third of the food produced for human consumption is wasted globally and it is approximately amounting to 3.3 billion tons of CO_2 equivalent greenhouse gas released into the atmosphere and up to US$ 750 billion direct annual economic loss. There is a continuous debate on reduction of food waste for sustainable society and environment. Reducing the food waste and food loss are the key issues associated with sustainable development. Food sustainability index is defined as the ability of country's food system to be maintained without depletion or exhaustion of its natural assets or compromises to its population's health without compromising future generation's access to food [10]. Food waste is defined as "the amount of food wasted in food service chains, with 'food' referring to edible products for human consumption" [11]. Food waste is also defined as "end products of various food processing industries that have not been used or recycled for other purposes and they are the non-product flows of raw materials whose economic value is less than the cost of collection, recovery and reuse; therefore, discarded as waste" [12]. Generation of huge volume of organic waste from meat, fruit and vegetable, and dairy processing industries is a major issue worldwide. This abundant volume of waste generated throughout the food supply chain has significant potential for the production of novel value-added materials, biofuels, and chemicals, as an alternate approach to the conventional practices such as animal feed, composting, landfilling, and incineration. Wastes generated in each process of the supply chain are organic in nature and are characterized by associated chemical and biological oxygen demand, fluctuating chemical composition, and pH due to seasonal variations, rapid bacterial contamination, high moisture content, and high accumulation rate [4]. Advanced valorization practices are essential to overcome significant issues associated with conventional food waste management system including (i) reducing landfill options, (ii) increased greenhouse gas emissions, (iii) groundwater pollution through leaching of inorganic matter and (iv) low efficiency [12]. Heterogeneous composition (protein, carbohydrate, and fat), fluctuating volumes, high water content, and low calorific value are the challenges related to the development of large-scale industrial waste management practices. Waste valorization is the process of transforming the waste into wide range of valuable products and has a great potential to provide economic and environmental benefits [13]. The viability of extracting valuable compounds from food waste and assessment of potential uses of such compounds need to be explored.

Life cycle assessment is a powerful tool to quantify and to identify the pros and cons of different food waste management system and its environmental impacts. Most life cycle assessment on food products mainly focused on farm level. Food

waste become the important topic for research on resource utilization, environmental sustainability, and food security. Different types of solid and liquid waste generated throughout the food chain are categorized as avoidable which may be edible and unavoidable which may be inedible. The generation of edible food waste can be reduced or avoided by taking all precautionary measures from harvest, production, distribution, and consumption stages along the supply chain through better-quality management and process optimization. The main components of unavoidable food wastes are protein, carbohydrates, fat, and inorganic components which can be converted into energy and bioproducts by implementing suitable chemical and biological processes. Some of the proposed strategies for valorization of food waste are extraction of nutrients and bioingredients, production of chemicals and biofuels, conversion into animal feed, composting, anaerobic digestion, incineration, and landfill. Sustainability of these waste management strategies and its impact on environment depends on greenhouse gas emission and carbon footprint [14]. Food wastes are identified as bioresource, and for sustainability, the global drivers involved in bio-based economy which enhances the bioresource utilization via biorefineries.

28.4 Techniques for Bioconversion of Food Waste Toward Circular Bioeconomy Approach

In recent years, many research efforts have been made in the efficient processing and utilization of food waste for the production of high-value bioproducts. On one hand, food waste represents a substantial ecological burden and on the other hand, these wastes are rich in carbohydrates, proteins, and lipids. Thus, they hold a significant potential for biotransformation into a variety of high-value compounds. A feasible bioconversion of food waste into valuable products not only has economic advantages but also diminishes the troubles arising due to food waste decomposition in the landfills and surrounding environment. Circular bioeconomy-based sustainable development is now becoming a global agenda for strengthening the connection between economy, society, and the environment. The current approaches for bioconversion of food waste management are discussed below:

28.4.1 Anaerobic Digestion

Considering the adverse effect of conventional waste treatment methods (landfilling, composting, and incineration), anaerobic digestion is a promising cost-effective technology to produce renewable energy from food waste [15]. The food wastes obtained from different sources are highly varied with the moisture content (74–90%), volatile solids (VSs) to total solids ratio (80–97%), and carbon to nitrogen ratio (14.7–36.4%). High moisture content food waste is found to be a suitable substrate for anaerobic digestion due to good biodegradability potential and higher percentage of methane yield compared with gasification and combustion [16]. In developed countries, 95% of the food wastes generated in production and processing

stages are converted into animal feed, chemicals, and fuels, resulting low disposal rate of up to 5%. However, waste generated in retailing stage and consumer end has lower recycling rate due to various reasons such as logistics, poor traceability, health, and safety issues. Anaerobic digestion is the preferable method for treating and recycling impure and low-quality food waste generated from homes, restaurants, schools, and hospital cafeterias [15]. Low-quality and contaminated food waste can be treated by anaerobic digestion to produce methane for energy. Among all types of food wastes, restaurant and household food wastes have high methane potential due to high level of lipids and easily digestible carbohydrates. Moreover, recovering bioenergy from the food waste through anaerobic digestion involves less cost.

In anaerobic digestion, 40–60% of food waste solids are degraded and energy recovered in the form of biogas (methane 60–70%, carbon dioxide 30–40%, traces of hydrogen, hydrogen sulfide, and other gases), and the remaining nutrient-rich solid residue can be used for land application or require further disposal [15, 17]. The digested solid residue can be used as a biofertilizer. However, if the heavy metal contents in the solid residue exceed the limit, it need to be further disposed by incineration or landfill. Anaerobic digestion involves following four phases: (i) hydrolysis, (ii) acidogenesis, (iii) acetogenesis, and (iv) methanogenesis. Hydrolysis is the first step in anaerobic digestion process. In this phase, complex organic matters in the food wastes such as proteins, carbohydrates, and lipids are broken down into amino acids, simple monomers, and fatty acids by extracellular enzymes of hydrolytic bacteria, i.e. protease, amylase, cellulase, and lipase. During the second stage called acidogenesis phase, fermentative bacteria decomposes the monomers into volatile fatty acids including lactic acid, pyruvic acid, acetic acid, and formic acid. Then in the acetogenesis phase, lactic and pyruvic acids are digested into acetic acid and hydrogen. Then in the last stage called methanogenesis also known as gas production phase, hydrogen and acetic acids are transformed into methane and carbon dioxide [18]. In methanogenesis phase, pH of the substrate influences the volume of methane gas production. Higher pH disintegrates the carbon dioxide in the biomass and this enhances the methane concentration in biogas. The generated biogas rich in methane content has higher energy value. Biogas can be utilized as a fuel for internal combustion engines to generate electricity. Compressed biogas can be a petroleum gas alternate for vehicle fuel.

Extensive research has been conducted on anaerobic digestion of food waste management on few decades. Ahamed et al. [19] compared the incineration, anaerobic digestion, and conversion of food waste to biodiesel system. Anaerobic digestion is most preferable if implemented in local environment when applicable. On the basis of cost analysis, in case the oil content is greater than 5%, food waste-to-energy biodiesel system is preferred and anaerobic digestion otherwise. In general view, food waste-to-energy biodiesel can be chosen over incineration. Kim et al. [20] developed modified three-stage anaerobic fermentation system which consists of semi-anaerobic hydrolysis/acidogenesis, anaerobic acidogenesis, and anaerobic methanogenesis. This three-stage reactor system showed higher methane yield by increasing the rate of hydrolysis, acidogenesis, and methanogenesis without affecting the pH of the substrate. Pineapple processing waste and pineapple on-farm

waste were found to be a promising feed in the production of biogas. Anaerobic digestion of fish processing waste decreases the problems associated with landfill and incineration. Higher concentration of lipids and insoluble proteins in fish processing waste inhibits the anaerobic digestion and is recommended to combine with other substrates.

28.4.1.1 Factors Influencing Anaerobic Digestion

Factors that affect the design and performance of anaerobic digestion process are substrate characteristics which include moisture content, volatile solid content, nutrient content, particle size, and biodegradability [17]. Biodegradability of the substrate is indicated by methane yield. Even though anaerobic digestion is successfully employed in animal manure, agricultural and industrial waste treatment challenges associated with the implementation of anaerobic digestion in food waste management are volatile fatty acid accumulation, foaming, low buffer capacity, and process instability. Lack of process control and optimization leads to generation of harmful intermediate substances which reduce the system stability and methane yield. Carbohydrates in the food waste are easily digested at the early stages of anaerobic digestion process, resulting in a considerable pH drop, which often leads to digester instability and system failure. To prevent this, anaerobic digestion system is to be performed on limited organic load with enough buffering capacity [21].

To improve the digestion efficiency and system performance, food waste can be co-digested with animal manure, sewage sludge, or lignocellulosic biomass. Simultaneous digestion of two substrates has the advantage of balancing C/N (carbon/nitrogen) ratio, diluting the inhibitors, and enhancing methane production [22]. C/N ratio is an important factor for nutrient balance and stability of microorganisms involved in anaerobic digestion. Animal manure is found to be a suitable substrate for co-digestion due to its high buffering capacity. Furthermore, co-digestion of food waste with animal manure can provide an excellent environment for anaerobic microbes. Co-digestion of cow manure with food processing industry waste (7 : 3 wt/wt, wet basis) at thermophilic temperature enhances the methane production more than twofold [23]. Solid-state anaerobic fermentation of spent hay (high C/N ratio) co-digested with soybean processing waste (low C/N ratio) in the ratio of 25 : 75 enhances the methane production [22]. Higher alkalinity and huge quantity of active microorganisms present in the sewage sludge also recommended as a co-substrate; however, if the light and heavy metal ions in the sewage sludge are too high, it may inhibit the digestion process. Co-digestion of microalgae with food waste improved the digestion and maximized the methane production [21]. Co-digestion of rendering waste with potato pulp improved the methane yield by inhibiting the accumulation of digestion intermediate products such as volatile fatty acids, long-chain fatty acids, and free ammonia [24].

Foaming is another complex phenomenon. When it occurs, the produced biogas gets dispersed in the liquid slurry and it increases the volume of digestate which in turn reduces the volume of digester. The following reasons are responsible for foaming: (i) higher levels of surface-active agents such as proteins, fatty acids, detergents, and other compounds present in the food waste; (ii) generation of surface-active

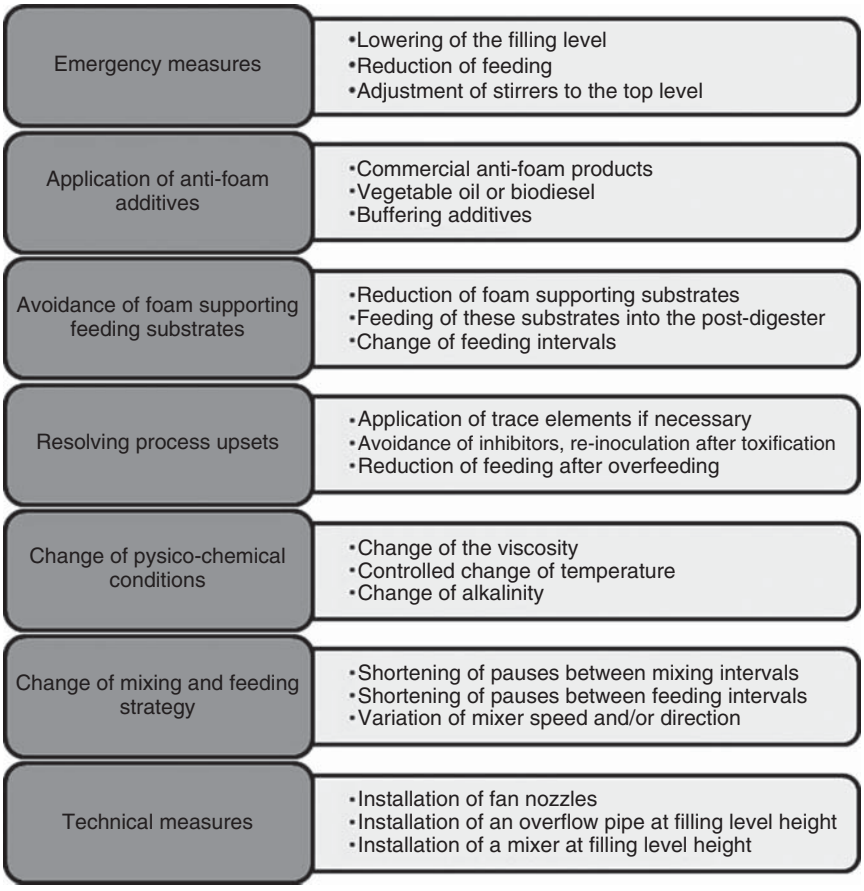

Figure 28.2 Strategies applied to reduce the foaming in 327 biogas plants . Source: Lindorfer and Demmig [26].

agents by microorganisms due to improper digester operations; and (iii) high loading of organic dry matter [25, 26]. Unexpected decrease in pH and increase in temperature can also cause foaming due to sudden release of large volume of dissolved CO_2 gas [27]. Foaming problems in digesters can be avoided by (Figure 28.2) controlling the loading rate of foam generating substrates, changing the physicochemical conditions, and application of anti-foaming agents.

Agro- and food processing wastes are grouped into seven categories based on chemical characteristics including energy crops, byproducts of lignocellulosic, herbaceous, vegetable and fruit crops, livestock effluents, and miscellaneous food processing byproducts [13]. Energy crops such as millet, barley, sorghum, maize, and triticale are justified by high methane yields (250–350 l CH_4/kg total volatile solids) due to high hydrolysis constant rates (0.15 d^{-1}) which indicate the good degradation potential. Food wastes are the most abundant waste in urban area and are characterized by their methane yield (250–350 l CH_4/kg total volatile solids) [13]. This will overcome the disadvantage of mono-digestion of food waste by increased

alkalinity and balanced micronutrients [15]. Characteristics of the same type of food waste obtained from different sources are highly variable due to differences in sources, handling and processing methods, eating habits, culture, climate, and seasons. Most of the food waste are acidic in nature, which will negatively impact the anaerobic digestion process. Thus, in order to increase the efficacy, different anaerobic digestion processes have to be designed for specific kind of food waste [15]. Methane potential of food waste is comparatively higher than the other substrates used for anaerobic digestion such as biomass, animal manure, sewage sludge due to the presence of proteins, carbohydrates, and lipids. Chemical composition of the food waste is linked with methane yield. Methane production potential of food wastes rich in fats and lipids is comparatively higher (1.014 m^3/kg VS) than that of proteins (0.74 m^3/kg VS) and carbohydrates (0.37 m^3/kg VS) [15, 28]. Physical and biological pretreatments are adopted to accelerate the hydrolysis. Physical pretreatment includes mechanical and heat treatment. Mechanical pretreatment and grinding reduce the particle size of the substrate and release the cell compounds which proliferate the anaerobic bacteria thereby enhancing the anaerobic process. To promote the hydrolysis of the substrate, biological pretreatments like inoculating microorganisms and enzymes are carried out.

28.4.2 Microbial Fermentation

Microbial fermentation is the suitable approach to convert the food waste into valuable bioproducts. Selection of fermentation method for bioconversion of food waste is highly dependent on type of feedstock. Solid-state fermentation is suitable for solid substrate, whereas the submerged fermentation is used for liquid substrates. The most common bioproducts produced through solid-state fermentation are hydrolytic enzymes such as cellulase and hemicellulase, and mostly these carbohydrases are associated with biofuel production. The other bioproducts obtained from solid-state fermentation are antibiotics from fig residues, aromas from sugarcane bagasse and sugar beet molasses, biopesticides from brewer's spent grain, biofuels and bioplastics from food and agro-industry waste, and biosurfactants from sugar beet molasses, soybean oil refinery waste, and palm oil refinery waste. Among these, biosurfactant is considered as a potential alternative to chemical surfactants due to their lower toxicity and biodegradability and has many applications in agriculture and cosmetics industry.

Several species of *Trichoderma* are used as inoculum for cellulase production, and the yield is induced by cellulose content of the waste substrate. Material homogeneity is important for higher yield of bioproducts and development of consistent and continuous operation of solid-state fermentation at large scale. This method of fermentation utilizes low energy and water; thus, it is eco-friendly to produce concentrated bioproducts. Submerged fermentation usually implemented for production of enzymes at industrial scale level due to simplicity in process control, low processing cost, and high throughput [29]. Value-added products such as biofuels, enzymes, animal feeds, bio-pulp, compost, biofertilizer, biopesticide, and secondary metabolites can be obtained by bioconversion of lignocellulose by

solid-state fermentation method [30]. Several kinds of pretreatments including mechanical milling, steam explosion, acid treatment, and organic solvents are reported to increase the yield of bioethanol by enhancing the hydrolysis and microbial fermentation of sugars into chemical substances. The yeast *Saccharomyces cerevisiae* is mostly used for production of bioethanol due to its high yield and tolerance to accumulation of inhibitory compounds during industrial fermentation [31]. As compared with bioethanol, biobutanol has more energy content, and chemical components like butyl acetate and acrylate can be obtained as co-products. However, butanol concentration above 13–20 g/l will have inhibitory effect on microbial growth and to avoid this, the produced butanol is removed from the broth during fermentation. Adopting liquid–liquid extraction, adsorption, gas stripping, or butanol tolerant strains may overcome the limitations.

The main constituents of the food waste are carbohydrates, proteins, and lipids which can be anaerobically fermented by association of hydrolytic, acetogenic, hydrogen producing, and acetate forming microbes to produce methane, hydrogen, and volatile fatty acids. Hydrolysis is the first step in anaerobic fermentation followed by acidogenesis, acetogenesis, dehydrogenation, and methanogenesis [32]. Volatile fatty acids are intermediate products recovered during acidogenesis and are widely used in food, pharma, textile, leather, and plastic industries. Optimization of acidogenic metabolic pathway is important for efficient recovery of volatile fatty acids and their derivatives. During hydrolysis treatment, sugars like glucose, fructose, galactose, and ribose are mostly extracted, and the composition of sugars vary with the food waste substrate composition.

Hydrogen is regarded as the most promising renewable source of energy mainly due to its high energy content (energy yield of hydrogen is 122 kJ/g which is 2.75 times higher than that of fossil fuel) [33]. Generally, biological hydrogen production can be divided into two categories: photosynthesis and dark fermentation [29]. Dark fermentation is seemed to be a more feasible biotechnology for hydrogen production than the photosynthesis due to less energy consumption and no light limitation [34]. Dark fermentation method is now being widely researched globally by scientists in an attempt to produce hydrogen from food waste more efficiently as this method requires only less chemicals and low energy in its application when compared to other processes. As this method depends on food waste as the raw material, when implemented globally, this can successfully decrease the issues arising with respect to food waste management. Although currently there are still researches going on regarding this process for hydrogen production to establish a clear knowledge for global implementation, the idea of this process is clear and has been limited only in a laboratory scale. However, low hydrogen production rate and high cost are the dominant obstacles for large-scale dark fermentative hydrogen production [35].

Food wastes are cheap carbon and nitrogen source for microbial fermentation to produce numerous bioproducts including enzymes, proteins, antioxidants, and pigments. Bioconversion of food waste into valuable bioproducts can reduce the environmental pollution by eliminating the waste. Lactic acid and ethanol are the common end products in food waste fermentation [36]. Proteins and starch are the two main components essentially present in the food waste that are suitable economic source for the production of biofuels. However, nutrients stored in food

waste are in the form of macromolecules (such as starch and protein) which have to be broken down into utilizable forms (glucose and free amino nitrogen) before utilized by microorganisms for fermentative hydrogen production [37]. Although some reported pretreatments were able to convert the macromolecules into utilizable forms, various inhibitory products (such as furfural) for fermentative hydrogen production could also be produced [38]. Enzymatic hydrolysis could release the nutrients (glucose and free amino nitrogen) from food waste with advantage of high hydrolysis speed which would be a promising way. However, there is little information about fermentative hydrogen production from enzymatic hydrolysis of food waste.

Biotechnological processes such as one-stage H_2 fermentation, two-stage $H_2 + CH_4$ fermentation, combining dark fermentation with anaerobic digestion, and photofermentation with anaerobic digestion are for the production of hydrogen. Production of H_2 from food waste depends on co-substrate such as animal manure and sewage sludge, pH, temperature, and pretreatment. Food waste rich in carbohydrate is most suitable for biohydrogen production than proteins and lipids [39]. This hydrogen-producing bioprocess at the initial stage usually accompanied with acidification. Hydrogen is generated from the food waste by the microflora *Clostridium* and *Enterobacter*. To increase H_2 production, pretreatment of food waste by heat is necessary to suppress lactic acid bacteria. Food waste co-digested with olive husk improved the yield of biohydrogen [40].

Lactic acid production from food waste is biodegradable and it is widely used in food, pharma, cosmetic, and textile industries. *Lactobacillus pentosus* is used to produce lactic acid from wheat bran by fermentation. To control pH and inhibit the accumulation of lactate, neutralizing agents such as calcium carbonate, sodium carbonate, and sodium hydroxide are added to fermentation medium [41]. Lactic acid is produced by hydrolysis and acidogenesis, which are first two steps of fermentation. To increase the lactic acid yield, the operating conditions such as inoculum temperature, pH, and organic loading rate need to be optimized. The hydrolysis and acidification process enhanced at pH 4–5 for the food waste as a substrate. Temperature influences the substrate conversion rate and microbial activity thereby lactic acid production [42, 43]. Fruit and vegetable waste, mango peel waste, and potato peel are found to be potential substrate for lactic acid fermentation. Thermal pretreatment of sole food waste substrate for fermentation greatly reduces the fermentation time and enhances the lactic acid yield [44]. The use of mixed cultures tolerates the variability of complex food waste due to metabolic flexibility. The complex food wastes are efficiently converted into useful bioproducts from intermediate feed stock chemicals such as short-chain carboxylates produced by hydrolysis and fermentation with undefined mixed cultures under anaerobic conditions. Propionate, lactate, acetate, and *n*-butyrate are the intermediate chemicals obtained from carboxylate platform.

28.4.3 Enzymatic Treatment

European Union has established Directive 2008/98/EC, the hierarchy related to reducing food waste. Accordingly, food waste must be valorized through (i) the collection of biowaste, (ii) biowaste treatment, and (iii) the generation of

environmentally safe product. We have successfully developed procedure to compost the food waste into fertilizers and incinerate the food waste for the generation of energy (heat). However, we should also account for the harmful issues associated with the incineration like CO_2 emission, air pollution, and generation of dioxins during their combustion [45].

Food industries generate numerous byproducts and waste streams which are rich in lipids, carbohydrates, and proteins, and these waste streams generate negative impact on the environment. These compounds can be potentially transformed into revenue streams like biofuels, animal feeds, nutraceutical ingredients, etc. Most industries rely on the chemical reactions (esterification) for value-added products, like esterification of oil with alcohol to produce biodiesel, esterification of sugar to produce surfactants, esterification of starch for biodegradable plastics, etc. However, most industries practice chemical process for valorizing the food waste involving chemical catalyst and energy, which results in another byproducts [46].

Traditional chemical reactions with catalyst can be replaced with biocatalysts (enzymes) and generate the revenue without high energy input. Enzymes (also called as biocatalysts) are water-soluble proteins which can enhance chemical reactions like chemical catalyst with outstanding specificity, high regio- and stero-selectivity, with "green, eco-friendly" label. Enzymatic treatment is a process of digesting the food waste and converting the food waste nutrients (carbohydrates, proteins, and fat) into smaller and more digestible nutrients like amino acids, free sugars, and fatty acids [47]. These enzymatic treatments are considered as a "green" method due to the absence of harmful chemicals and possibility of achieving value-added products without any degradation or damage. For instance, enzymatic treatment of defatted rice bran yielded glucose, amino acids, and peptides without any damage [48].

On the other hand, application of food waste for the conversion of bioenergies has attracted more attention as they are renewable and clean energy. Researchers successfully reported the production of biomethane through anaerobic digestion of food waste and sewage treatment plant sludge (co-digestion technique), transesterification of cooking oil to produce biodiesel, and bioethanol from fermentation of carbohydrate-rich food waste. Though researchers succeeded in the production of bioenergy from food waste, the conversion ratio falls between 40% and 70% only with significant energy input up to 184.4 kWh/t food waste [49, 50]. The main reason for the reduction in conversion rate is due to the non-accessible portion of organic matter for microbial utilization for bioenergy production. It is possible to enhance the production efficiency by prior hydrolysis of food waste through enzymatic pretreatment. Figure 28.3 shows the different production techniques and the corresponding enzymatic pretreatment for the production of bioenergy from food waste.

Similar to food waste, sludge from the sewage treatment plant is another environmental challenge due to the prediction of billions tons of sewage and wastewater sludge every year. The presence of valuable resources like organic matter, energy, and nutrients can make a suitable combination with food waste for an excellent substrate for the anaerobic digestion. Yin et al. [51] investigated the production of biomethane for the collected food waste from university canteen and sewage

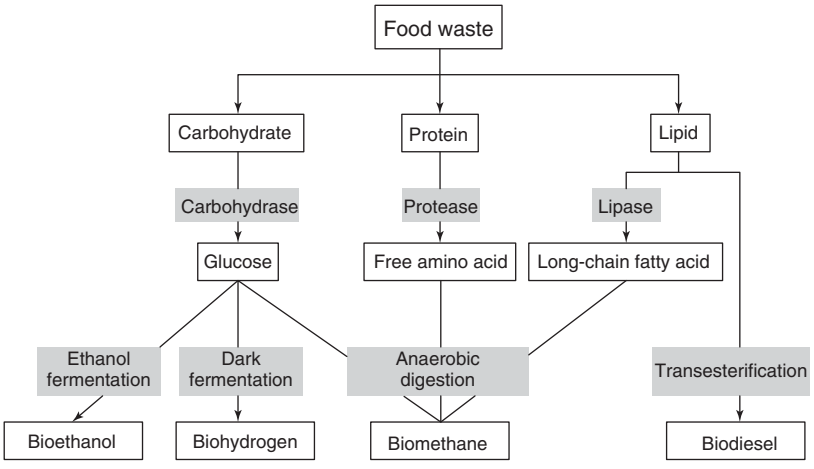

Figure 28.3 Bioenergy recovery through enzymatic pretreatment process . Source: Zou et al. [50].

sludge from municipal treatment plant. Initially, authors hydrolyzed the sludge and food waste by fungal mass (enzymatic treatment). Fungal mash is a potential wide spectrum carbohydrase which can hydrolyze the complex organic matters into simple glucose structure through pullulanases, xylanases, cellulases, hemicellulases, α-glucosidases, β-amylases, and β-glucanases. Enzymatic pretreatment of sludge and food waste yielded 2.5 times higher in biomethane than the substrates without enzymatic pretreatment. In a recent study, Taheri et al. [52] introduced another pretreatment step before enzymatic hydrolysis of food waste. Authors claimed such pretreatment induces the structural changes of biomass, maximizes the generation of glucose, improves bioconversion rate, and minimizes the requirement of chemicals and energy input. Authors used six different pretreatment methods including:

- **Hydrothermolysis:** Autoclave the food waste sample for one hour at 100–120 kPa;
- **Sonolysis:** Food waste in water is exposed to ultrasonication for one hour at 20 kHz and 225 W;
- **Electrochemical oxidation with boron-doped diamond (BBD) anode:** Electrochemical pretreatment with BBD anode and stainless-steel cathode for one hour;
- **Electrochemical oxidation with graphite anode:** Electrochemical pretreatment for one hour with graphite anode and stainless-steel cathode;
- **Sono-electrochemical oxidation:** Combining ultrasonication and electrochemical oxidation;
- **Solid–liquid fat extraction:** Fat content in the food waste is leached out by Soxhlet extraction.

Then, the pretreated samples were hydrolyzed with amylase which breaks down the starch into glucose and cellulase to break down cellulose into glucose. Pretreatment enhances the residual content of carbohydrates from 9.34% to 13.06% for starch

and from 10.14% to 18.10% for cellulose. Solid–liquid fat extraction pretreatment technique provided high sugar yield and high degradation of starch and cellulose by enzymatic hydrolysis.

Utilizing food waste as animal feed is a successful alternative technique to landfill. Food waste was given as pig feed many decades, but sometimes the presence of meat in food waste and non-heat-treated food waste could end up with foot-and-mouth disease and African swine fever. In 2001, the outbreak of foot-and-mouth disease caused a crisis in British agriculture and farm, by slaughtering more than 6 million animals, costing 8 billion pounds to the public and private sector. Enzymatic digestion helps in digesting the food waste into pasteurized feed with more digestible nutrients like free sugars, amino acids, and fatty acids. Pandey et al. [53] converted food waste into organic soil amendments (OSAs) by three stages starting from (i) enzymatic digestion, (ii) pasteurization, and (iii) acidification. The developed OSA can be used as an effective fertilizer (chemical and pathogen-free), and authors revealed 25% increase in growth rate for the strawberry plant. Further, the byproduct of this process can be potentially used as feed for pork or chicken. Authors succeeded in converting food waste to OSA with a non-detectable level of pathogens (*Escherichia coli* O157: H7, *Salmonella* LT2, and *Listeria monocytogenes*). Later, Jinno et al. [47] took a step further by feeding the enzymatic digested food waste to the growing pigs and compared with the control diet (based on corn and soybean meal). There was no significant difference in body weights between the control and enzymatic digested food waste. For instance, a pig weighed between 32 and –33.6 kg on day 1 was grown to 108.15 kg (control diet) and 98.77 kg (for enzymatic digested food waste). One can observe that animal feed from enzyme-treated food waste should be able to provide necessary nutrients and can be effectively used as a substitute for corn or soy compositions in their diet.

28.4.3.1 Enzyme Immobilization Technology

The application of enzymes in valorizing food waste has numerous advantageous over the conventional chemical process. However, it is important to note the major issues in maintaining the stability and activity of enzymes due to the non-favorable environment for enzymes like non-neural pH and higher temperature. Enzyme immobilization technology is tailored to improve the enzyme catalytic features like activity, selectivity, and resistance to inhibitors [54]. Immobilizing enzymes on a solid support or cross-linking via enzyme–enzyme will improve the performance and stability and also enable the reuse of enzymes. This enzyme immobilization technique is in use from 1960s, and the past six decades of research and industrial practice has abandoned the tedious trial-and-error approach and brought the rational approach for designing immobilized enzymes [55]. Several immobilization strategies can be used like entrapment, adsorption, covalent binding, ionic binding or metal-linked immobilization, and the selection of immobilization technique is depending on the physicochemical characteristics of enzyme, support material, and substrate matrix. Further, the selection of support material should possess high physical stability (mechanical strength), chemical stability, and biological stability during processing, inert on immobilized enzyme and the target analyte, and adequate functional groups for effective binding of enzymes and achieve high loading capacity and biodegradability.

28.5 Conclusion

Food waste management through circular bioeconomy approach creates an opportunity to promote bio-based industries to produce bioproducts/biochemicals and utilizes resources in a more effective way. The integrated biorefinery approach still need process optimization, efficient recovery/separation of the products, and scaling up for the utilization of large volume of foods waste. Suitable technical, economical, and scientific plan in multidisciplinary approach can help to develop a sustainable food waste management through biorefinery concept in order to address the objectives of circular bioeconomy and bridge the gap between waste remediation and product recovery.

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29

Zero-Waste Biorefineries for Circular Economy

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29.1 Introduction

The world has warmed 1 °C, since the emergence of the Industrial Revolution, and is expected to reach a temperature of 1.5 °C in coming years. This temperature rise can set humanity on ventilators, and the world has only 12 years to shift its economic reliance from fossil fuel [1]. The primary attempt of climate science is to nullify and balance the ongoing emission of heat-trapping gases. The process like carbon sequestration in the forest, soil, or geological area is the most popular choice among the rest. A combined increase in development and consumer demand for a variety of food characteristics leads to the rapid growth of globally integrated "bioeconomy," which made bioeconomy the next great wave of the economy [2]. Our national bioeconomy strategies follow more of a sustainable approach. An immediate shift toward renewable resources is the primary approach that can prevent both the depletion of resources and climate change. To suppress climatic change, the relationship between carbon sequestration and emission is important. According to the Intergovernmental Panel on Climate Change (IPCC) report on climate change, BECCS (bioenergy with carbon capture and storage) was the key technology to reach targets of low carbon dioxide atmospheric concentration [3]. But currently, the scenario is showing limited opportunity for the technology to reduce net emission from the current level.

Therefore, for CO_2 removal and storage, various technologies came into action. Biochar, carbon sequestration using marine algae, CO_2 air capture, biomass burial, and direct air capture (DAC) are a few of the widely adopted technologies [4, 5]. Nowadays, the residues of biorefineries such as biologically degradable waste are the possible source of high-quality end products. These final products can overtake both technical and nontechnical barriers but are still underrated and are not being

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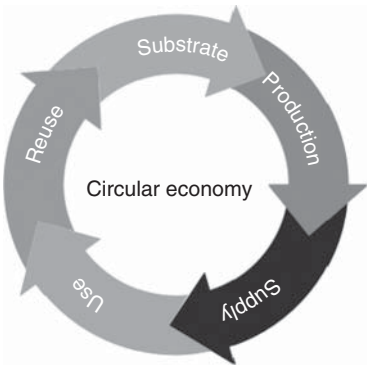

Figure 29.1 Schematic representation of the process involved in circular economy concept.

used to its fullest ability. Therefore, this write-up follows a holistic approach that can target atmospheric CO_2 removal and storage by utilizing biologically degradable waste. This creates a win-win sustainable solution from all ends. (Figure 29.1) [6].

29.2 Bioenergy, Bioeconomy, and Biorefineries

Growing bioeconomy manages to provide required elements for the overall global sustainability transition which includes biological processes and bio-based product dependency [7]. The overall agenda of the bioeconomy is to simply substitute fossil fuel economy with sustainable bioeconomy and simultaneously mitigate out the dependency of countries on the natural economy [8]. The compatibility of sustainable bioeconomy with a circular economy aims to use across all its different uses with minimal waste and optimal valorization of biomass. The two closely related priorities of bioeconomy are biofuel and biorefineries. Biorefineries being the fundamental technology can diminish reliance on petroleum-based refineries. However, bio-based products and bioenergy are the principal products of bioeconomy [9]. Bio-based transport fuels can be categorized into first-generation, second-generation, and third-generation biofuels. Among all, third generation is at the initial growth phase of the development (Table 29.1) [6].

Biorefinery being at the early R&D phase facilitates biomass conversion to produce fuel, power, heat, and therefore, maximizes the value derived from biomass feedstock (Figure 29.2) [17]. The high value-added product increase profitability and high-value fuels achieve energy demand at low power production. This reduces greenhouse gas (GHG) emissions from the traditional power plant facilities [18]. Bioenergy can be considered as carbon neutral, as during combustion released carbon dioxide is assumed to be compensated by the CO_2 which is absorbed by the tree during its growth [19]. However, the sustainability of using wood as an energy source is still questioned due to the long regeneration cycle of forest biomass [19]. There might be competition in bioenergy with the food sector directly. Therefore, maximizing resource efficiency is our ultimate agenda till 2050. This could be done by the utilization of wood biomass to decrease fossil fuel and energy potential to be utilized at the end of the cascaded life cycle (Table 29.2) [23].

Table 29.1 Sources, processes, and product recovery of waste biorefineries-based circular economy.

S. No.	Proposed model	Wastewater source	Methodology	Transitional substrate	Product recovery	References
1.	*Photosynthetic model*	Municipal wastewater and flue gas	Open pond	Biomass	Biofertilizer, methane, bioethanol, biohydrogen	[10]
2.		Fishery industry wastewater	Microbial degradation	Hydrolyzed substrate	Fish feed [11]	[[12]]
3.		Industrial wastewater and flue gas	Photosynthetic system	Algal biomass	Biogas and biofertilizer	[13]
4.		Brewery wastewater	Photosynthesis	Algal biomass	Biofertilizer and cattle feed	[13]
5.		Natural and wastewater	Open and closed system cultivation	Algal biomass	Biogas and liquid biofuels	[14]
6.	*Carbon sequestration model*	Paper pulp industry waste	Microbial fermentation through *Lactobacillus* and *Escherichia coli*	Sugars	Lactic acid	[14]
7.		Crude glycerol waste from biodiesel industry	Microbial degradation	Polyhydroxy alkenoates	Biodegradable plastic	[15]
8.		Paper pulp industry waste	Microbial fermentation	Bioethanol	Polyethylene	[16]
9.		Domestic wastewater	Microbial degradation	Polyhydroxy alkenoates	Biodegradable plastic	[12]
10.		Paper pulp mill sludge	Polyhydroxy alkenoates	Polyhydroxy alkenoates and volatile fatty acids	Biodegradable plastic	[12]

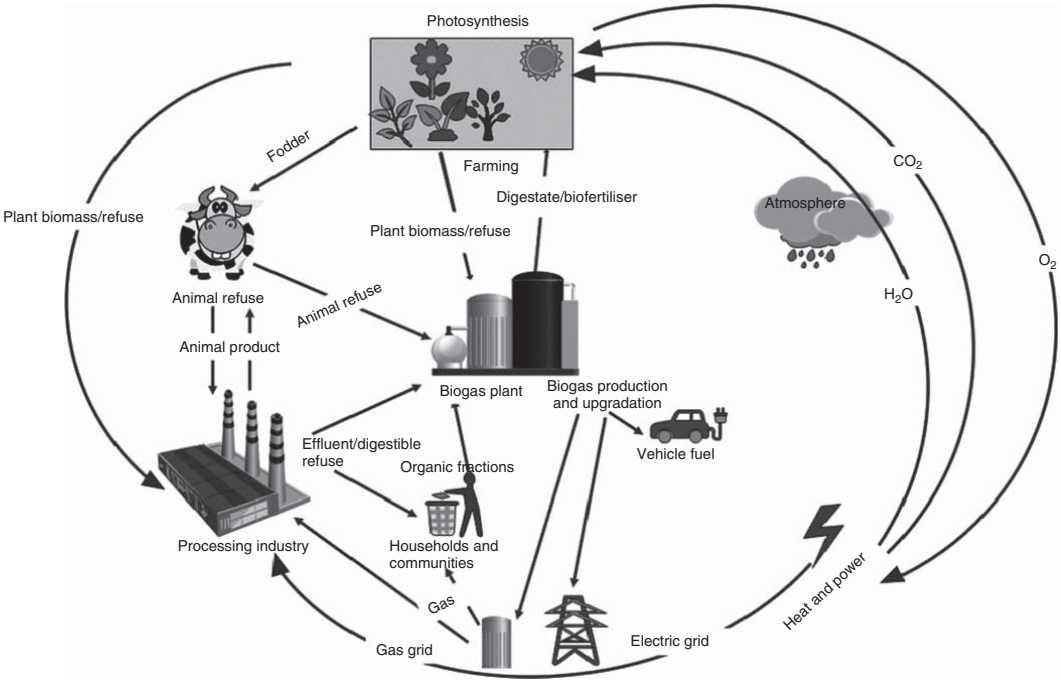

Figure 29.2 Schematic representation of process involved in life cycle of biodegradable refuse (waste) from start to end considering biorefinery concept.

Table 29.2 Biorefinery types and their sustainable assessment for circular economy process.

S. No.	Biorefinery	Sustainable assessment	Process	Remarks	References
1.	Wastewater	Production cost, environment, and health hazard	Life cycle analysis and economic feasibility	Utilization of waste for energy production system with nutrient recovery and circular economy	[10, 12]
2.	Glycerol	Investment cost, global warming, and environmental pollutant	Technical and economic feasibility	Cost analysis on the input materials and circular economy evaluation from recovered product	[13, 14]
3.	Kitchen refuse	Energy consumption, release of nutrients, and health hazard	Life cycle analysis and economic feasibility	Process integration with aquatic biorefinery for improved utilization and circular economy over linear ones	[15, 16]
4.	Lignocellulose (Sugar industry)	Eutrophication, acidification, GHG emission, aquatic toxicity, terrestrial toxicity, photooxidation, and human toxicity	Life cycle analysis	Strategies involved in value addition and waste utilization for closing energy loop and circular economy	[20–22]

29.3 Bioeconomic Strategies Around the World

The two driving forces of bioeconomy are reduction in CO_2 footprint and feedstock conversion from limited carbon resources to a sustainable one. These forces have a dramatic impact on global stock production, trades, industrial process, and infrastructure and therefore require disruptive technologies to come into action [24]. Currently, there is an increasing demand for the bio-feedstock by the growing companies which drives the purchase of land and value in biomass-producing areas. However, societal acceptance, agreeing legal framework, industrial processes, and research

along with industrial investment in this environment are extremely complex issues [25]. Therefore, strategies need to be built to coordinate align bioeconomy's global infrastructure, industries, and supply, specific to the nation's concern.

Countries like Malaysia and Brazil are known for renewable biomass abundance; however, lack of enough integrated processing industries is of major concern [26]. The United States and Canada have strong feedstock, chemical industries, and highly developed fuel. However, developed nations like Germany depend on feedstock import. Many countries already accepted and implemented the national bioeconomy [26].

29.3.1 Malaysia

Malaysia in 2011 builds a national biomass strategy that makes it the number one exporter of palm oil. Strategies like efficient harvesting, collection process, and product transportation are formed to mobilize palm oil biomass. For the utilization of biomass and energy purpose application, strategies were implemented by Biotech-Corp organization [27].

29.3.2 Brazil

Brazil is the number one sugar producer and the nation of the arable land reservoir in 2007 published a biotechnology development policy. Its concept was stoned by the National Biotechnology Center and the competition Forum in biotechnology for the application of available waste and resources for energy applications [28].

29.3.3 United States

The United States is the exporter of wheat, corn, and soy. Bioeconomic concern leads to address climatic change and focus to end dependency on foreign oil for fuel exchange. In 2007, to address bioenergy crops of the coming generation, degradation of biomass, and production of microbe-mediated biofuel, the three bioenergy research centers (BRCs) have been formed [5, 29].

29.3.4 Canada

Being the exporter of plant-based oil, cereals, and wood, in 2009, to bring advancement in Alberta's bioindustry sector, biorefining conversions network (BCN) was implemented by Alberta as part of the *Alberta Innovates* system. For improving feedstock logistics, reduction in GHG, biomass energy, biopolymers, and green fluids, remediation of land, and abandonment services, Alberta Innovates was created in May 2011 [29, 30].

29.3.5 Germany

Germany produces a substantial amount of biomass of up to 2.3 million ha which covers 86% for energy and the rest of 16% for industrial use. Even being strong in

industry and science, Germany fails to the supply-based industrial establishments [31, 32]. An article was published by the BMBF in 2010 which considered national research strategy bioeconomy 2030 as the future vision with a program budget of €2.4 billion [33, 34].

29.3.6 European Union

The bioeconomy of Europe is responsible to produce 17% of European Union (EU) gross domestic product (GDP) which is about €2000 billion and has 21.5 million employees working [33]. To bring biorefineries under the framework of its 7th EU program, in 2011, a joint European biorefinery vision was developed by the Star-COLIBRI project. By 2030, different roadmaps were built to target bio-based products, supply chain, the supply of versatile biomass, and growth of the bio-based sector [35]. The European Commission (EC) stated in 2002 that life science and biotechnology are the most reliable technologies with high contribution potential in the achievement of Lisbon agenda objectives [36].

From the consultation, three main conclusions were drawn. First being the optimistic response on the bioeconomy, over 60% of respondents believed to count a greater number of positives like reduction in waste, GHG emission, and pollution by 2020 to 2010 period [37]. Second, the respondents who pointed out the associated risk with bioeconomy such as overexploitation of natural resources and further lead to effect food security are of major concern. The third section of the respondent shows a significant concern over the competition with the United States and Asia which might hinder Europe's bioeconomy as a single identity [38].

29.3.7 Scenario of Bioeconomy in India

India holds a potential feedstock in terms of biogenic waste for constructing a bio-based economy and one of the valuable supplements for petroleum feedstock if used properly. With a yearly development rate of nearly 20%, by 2025, the Indian bioeconomy has a potential to establish a more than 100-billion-dollar market [39]. Economic and technological development leads to an increase in wastewater content hence ended up causing enormous environmental impact. Therefore, India's present strategy is all about shifting toward renewable resources and eliminating carbon emission. National biofuel policy was one of the dominant action requirements for the target regarding renewable fuel and replacing 20% of petroleum fuel consumption in 2009 [39].

29.4 Challenging Factors and Impact on Bioeconomy

While studying bioeconomy all the factors which drive and hurdle the system in both positive and negative terms, a contrasting approach needs to be followed. The approach should be based on visions and ideas comparison between present and future. [40]. A diverse set of forces are involved to run bioeconomy smoothly around

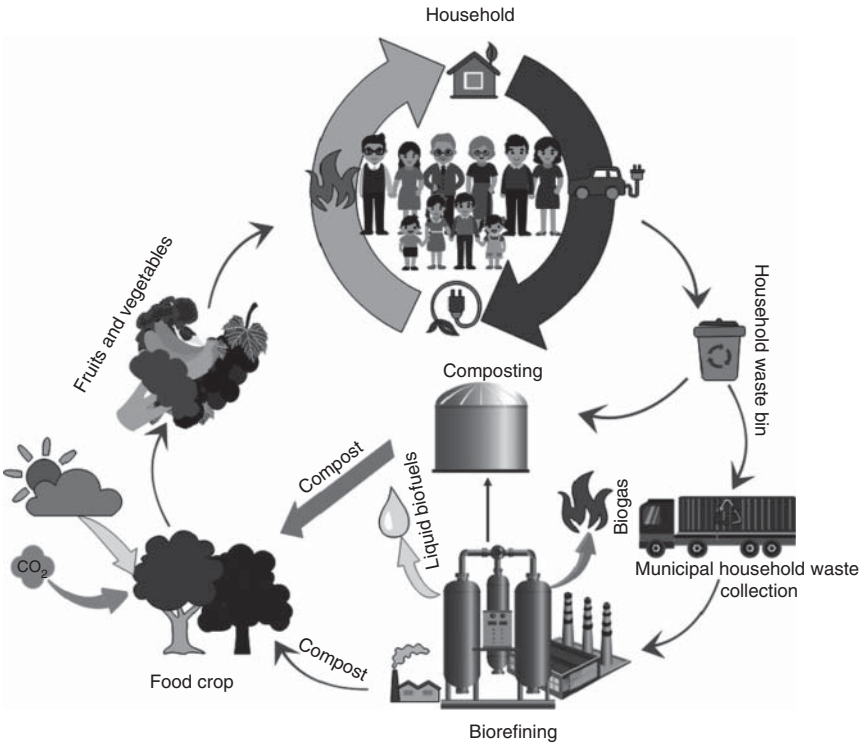

Figure 29.3 The schematic representation of zero-waste management concept.

the globe. Besides, regulatory factors as human resources, intellectual property, regulatory condition, and social acceptance are stimulating factors. The principal element which can dissolve the complications of the economy is the sustainable resource demand which can bypass various challenges. There is a connection between the risk and opportunities factor of bioenergy with that of bioeconomy. The full utilization of waste resource from thermal and power application to composting and application as biomanure to achieve zero-waste concept and circular economy is shown in Figure 29.3. For the generation of energy from the biomass, the ratio of the number of benefits with that of the negative impact of bioenergy production can be encountered by biodiversity, food security, and water quality areas [41]. The dependency of these impacts for the actualization is largely on the design and bioenergy system implementation. How much reduction in GHG emission is going to happen is dependent on another parameter being technology and resource management of the feedstock and land used [42]. Bioenergy can be considered as a test study for bioeconomy, especially in achieving sustainability goals. However, the impact of bioenergy systems is recruiting complications by growing international trades and increasing competition for biomass resources [42]. Policies established by the EU played an important role in the birth of bio-based fuel. In 2008 as per the climate and energy policy package, by 2020 in the overall EU

for consumption and transportation, various targets were set to achieve renewable energy of up to 20% and renewable fuel including biofuel of up to 10% [42].

To eradicate first-generation biofuel impact, second- and third-generation biofuel came into action. EC established biofuel binding sustainability criteria in 2009 within the renewable energy directive (RED). This says for new biofuel production area with high biodiversity and carbon stock should not be exploited and in comparison, with fossil fuel, the increment of biofuel usage should be done. The aim is to reduce the emission of GHG by 35% which further increased up to 50% and 60% by 2017 and 2018, respectively [43].

29.5 Effect of Increased CO_2 Concentration, Sequestration, and Circular Economy

Over the centuries, humans used all possible ways to use fossil fuel, which lead to increase CO_2 concentration tremendously without understanding the grave consequence of such use. The upsurge CO_2 level has become the prime cause of terrible climatic changes. If proper measures are not taken over anthropogenic activities, then there is an increase in quantities of CO_2 from 28.8 Gt in 2007 to 40.3 Gt by 2030, and 50 Gt by the end of 2050 can be seen [32]. For the infrastructural and economic growth, global energy demand acts as a driving force to use these biological methods for sequestration. Hence, another biological method like biorefinery should be explored to reduce carbon footprint. Petroleum biorefineries' basic concept has been already established. However, the approach is to develop CO_2 biorefinery to obtain a diverse bio-based product that offers a sustainable, eco-friendly, and renewable environment [32]. This paper highlighted few biological CO_2 sequestrations methods using the biorefinery approach which target both how to sequester CO_2 and how to reduce it.

29.6 Carbon Sequestration in India

Between the period of 2015 and 2017, India covers a total land area of about 708 273 km^2. World Bank recently reported out 60.4% of the total country area is covered by the agricultural sector. The number shows that a large part of Indian land can be used for carbon sequestration and GHG mitigation if proper management practices are followed [44]. However, measures need to take for controlling the transmission of carbon as currently India contributes 7% of the total emission of GHG globally and becomes the fourth biggest emitter worldwide. India being a country of huge land has a great potential to use the land in agroforestry practices for carbon sequestration and can sequest 66–228 MgC/ha of carbon. But various environmental factors come into consideration such as in tropics, from the top 20 cm of the soil, only 70 mg/ha of carbon can be sequest by the agroforestry system [45]. Depending on different agroforestry systems, carbon sequestration can occur

both belowground and aboveground. For plywood and fuelwood manufacturing, *Populus* and *Eucalyptus* are growing as a trend in Haryana and Punjab.

29.7 Methods for CO_2 Capture

DAC is a physicochemical method, in which CO_2 get directly captured and stored. This involves sequestration of more CO_2 per acre of land as compared to sequestration by trees in the same area of land. Streams of pure CO_2 produced from air capture have significant roles in various chemical industries and storage facilities [46]. Methods like post-combustion, precombustion, and oxyfuel combustion have been categorized which depends on the site of capture. After combustion, CO_2 is produced from flue gas and needs to be captured by the post-combustion system [45].

29.7.1 Scenario 1. Photosynthetic Bacterial Model for CO_2 Sequestration

Adenosine triphosphate (ATP) is the energy source for photosynthetic bacteria, which give biomass, biofuel, and bioproducts after converting from CO_2 as an end product. Photosynthetic bacteria (PB) followed a very unique and complex pathway to fix CO_2 via type I (Fe–S type in sulfur bacteria) reaction centers. Both photoautotrophs and chemoheterotrophs play a central role by glyceraldehyde-3-phosphate (G3P) [44, 45]. It serves as a primary intermediate in pathways like homolactic, solventogenic, and ethanolic fermentation by chemoheterotrophic organisms. For CO_2 sequestration, *cyanobacteria* from the photosynthetic phyla are considered to be the key player. For H_2 production, both enzymes, hydrogenase and nitrogenase, are used by photosynthetic organisms [45, 46]. Using light energy, organic compounds can produce hydrogen by anoxygenic photosynthetic bacteria.

29.7.2 Scenario 2. Biochar Model for CO_2 Sequestration

Crop residues also contain plant nutrients such as carbon, nitrogen, phosphorus, potassium, calcium, and magnesium. These nutrients are added to the soil by fertilizers. The crop residues are harvested every year and microbes also decompose these plant residues to maintain the soil organic carbon levels [47]. Charcoal being incredible adsorbent can enhance the capability of the soil to absorb nutrients and other agricultural chemicals. As charcoal is relatively low in density, it decreases the density of high-clay soils. It increases the capability of sandy soil to hold water and nutrients. Biomass pyrolysis is one of the methods to produce biochar by thermochemical decomposition of biomass under 300–700 °C of temperature with oxygen-limiting conditions. Biomass pyrolysis and restrain of its products like biochar and bio-oil could result in a viable solution to agricultural and forest slags. Biochar amendment to the soil could result in improved fertility of the soil, and it can also mitigate the GHG emission of the soils which will ultimately alleviate climate change.

29.7.3 Scenario 3. Biofuels

Various studies and efforts have been done to replace fossil fuel with biofuels (ethanol and biodiesel). Although CO_2 is again recovered from the carbon present in biofuel, it directly increases the atmospheric CO_2 concentration and acts as an anthropogenic release of carbon from fossil reserves [48]. Over the past few years, it is so evident to get substituent of fossil fuel which thus offers to redeem the negative effect caused by the enhanced atmospheric CO_2 concentration. Another method is the valorization of food waste for the production of biodiesel, biofuel, and biogas. It has set milestones for the eradication of fossil-based products, especially fruits, processed refined flour-based food products, and vegetable wastes [49, 50].

29.7.4 Biological-Based Methods to Capture CO_2

Biological methods solve over-dependency on energy and provide a high number of bioproducts.

29.7.4.1 Photosynthetic Model

Photosynthesis is a primary source of C sequestration. Photosynthetic organisms can be used as light-capturing tools and CO_2 can be used for the generation of a varied range of products like fuel, chemicals, and material.

Marine Algae for CO_2 Sequestration Macroalgal primary products can be used to sink a considerable amount of CO_2 and thus end up as a key performer in C sequestration of GHG emissions [48]. Industrial Revolution holds an account of 48% of emissions with the sea being the potential carbon fixer for anthropogenic CO_2 emissions [48, 49].

Marine Productivity and Capacity for Carbon Reduction Ocean photosynthesis is responsible for the generation of the planet's 50% of the total primary productivity of 54–59 PgC/year. Different types of marine macrophytes have a different rate of photosynthesis and productivity in which seaweeds and seagrasses found in the coastal regions can account for ~1 PgC/year [51, 52]. Kelps *Microcystis* and *Laminaria* are the potential contributors to the biological reduction of the CO_2 cycle due to maximum photosynthetic activities it fixes a huge amount of carbon produced annually worldwide.

29.7.4.2 Substrate in Biorefinery and Carbon Management

Case 1. Algal Biorefinery Today, annually from both wild and cultivated source, around 7.5–8 million tonnes of wet weight seaweeds are harvested. Around the world, China produces 5 million tonnes (wet weight) and becomes the largest seaweed producer in which *Laminaria japonica* is the major contributor. Besides, 800 000 tonnes from Korea with a 50% contribution from *Undaria pinnatifida* and 600 000 tonnes from Japan with 75% cultivation from *Porphyra* sp. come annually [52]. From FAO details, a wide variety of macroalgae species are cultivated, but

only five genera of seaweeds are responsible for annual bulk production which are brown algae such as *Laminaria* [53]. Cultivation of other seaweed species like *Kappaphycus alvarezii* and *Eucheuma denticulatum* (carrageenophytes) as well as *Gracilaria* species (agarophytes) is done by countries such as Philippines, Indonesia, Tanzania, and India [54]. Cultivation site, type of season, and methodology used during cultivation are the governing factors for the growth rate of various species. For example, *K. alvarezii* growth rate varies daily between 3% and 12%, and that of *Gracilaria* spp.

Out of 221 species, for food purposes, 145 species are used and phycocolloid production can be done using 110 species. However, macroalgae are known for nutrition and economy builders for some countries [55]. The most important global contribution of algae is the conversion of algal carbon to biofuel in terms of CO_2 sequestration. Dependency on fossil fuel for transportation and chemical feedstock purpose can be reduced by both macro- and microalgae. Lipid content in both algal types can be converted into fuel like ethanol and chemical feedstocks (Figure 29.4) [56]. Another development in which red algal pulp can be used as an alternative to the tree for pulp production and hence deforestation can be controlled. Thus, conservation of ecological damage by converting fossil fuel into atmospheric CO_2 and conservation of terrestrial forest which play an important role in the carbon cycle can be done by bringing algal-based fuel and algal-based pulp [57]. There has been a change in macroalgal distribution and diversity along with photosynthetic and physiological performances with the change in climatic conditions. This can directly contribute to CO_2 sequestration. Under present-day CO_2 levels, some species are already CO_2 saturated, and no further performance increment can be seen in the future.

Case 2. Biomethanation Since the 1950s, the oldest, cheapest, and exploited technology of India is the production of biogas from organic waste. Until 2013, about 45 lakhs of domestic-type biogas have been installed by the Indian Government with an estimation potential of 36.85%. India being a huge country holds around 1.5 billion of the population and about 70% of energy comes from fossil fuel of the total energy consumption by the country. Around 53% of energy import increment is expected by the end of the year 2030. To avoid food versus fuel conflict in 2008, Indian biofuel policy was made. The aim is to focus on nonfood feedstocks for bio-based energy/fuel rather than exploiting limited natural resources by importing fossil feedstock and refined petroleum products. From the same context, between 2002 and 2010, increment in renewable energy share from 2% (11 628 MW) to 11% (18 155 MW) is seen in the Indian market. Presently, biomass is the source responsible for providing 32% of primary energy [58]. Biofuel is another new sustainable dimension that can open doors for waste management and energy generation. Biofuel has lower GHG emission as compared to fossil fuel and therefore becomes the priority for bio-based economy development.

According to the reports published by the Ministry of New and Renewable Energy (MNRE), waste as a resource can never be diminished. Waste to electricity conversion accounts for 1700 MW from municipal solid waste (MSW), 225 MW from Department of Science and Technology (DST), and 1300 MW from the industrial

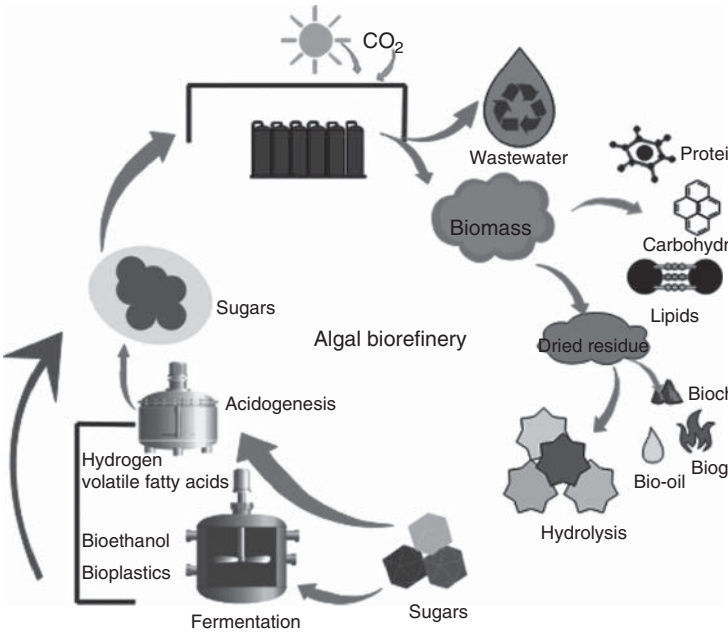

Figure 29.4 Schematic representation of algae-based biorefinery.

waste of the resources. The project named "SAHYOG" was started by joined funding of the Department of Biotechnology (DBT), Ministry of Science and Technology, India, and the European Commission. The EU listed the availability of excess biomass and biowaste in India and in context with a biobased economy that can be used as a feedstock for the production of biofuel.

Case 3. Composting Recycling of organic matter by decomposition leads to the formation of soil conditioner-compost, with the help of most traditional and age-old process, i.e. composting. Composting addresses the most critical issues of waste management by diverting reliance on landfills and reclaiming values that can provide agriculture products of high quality and yield by transforming waste into organic fertilizers [59]. To eliminate the use of fossil-based fertilizer and bring agriculture fertilizers into the market, composting can come into play. Indian Government starts giving subsidies to promote organic agriculture practices and they formulated their policies to blend along with chemical fertilizers. This practice of organic farming is flourishing day by day as it does not disturb the environmental balance by working and composting together to get a good yield in return. [60]. There are several opportunities in the Indian market which can further enhance organic product demand to grow on an industrial scale and utilize MSW as feedstock.

29.8 Conclusion and Future Approach

The CO_2 has always been identified as the havoc for climatic conditions and is considered as repulsive feedstock for biocatalysis. However, elevated technologies and

methods have been set to achieve several goals. Reduction and utilization of CO_2 is the prime example of the technology. This can be done by bringing bio-based products (biofuel and fertilizers) into the market to set a valuable economy. This serves to provide the required energy demand for sustainable renewable futures which can change the mindset of CO_2 as a solution rather than as a problem. The waste management practices via circular economy using closed energy loops in biorefinery concepts may help in the reduction of GHG emissions and climate change mitigation policies worldwide. Despite the impressive outputs provided by the discussed methods, there are some inevitable hurdles because of the complexity of the process and socioeconomic acceptance. Soil management practices like crop rotation, cover crops, judicious use of fertilizers, and growing deep-rooted crops could also make agricultural soil a sink for carbon, which could both protect and enhance the soil quality in the currently available situation to overcome the increasing carbon load in the environment.

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Feasibility and Economics of Biobutanol from Lignocellulosic and Starchy Residues

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30.1 Introduction

An alarming rise in population and modernization has relentlessly increased energy demand worldwide. The major energy consuming thrust areas include industrialization, transportation, and agricultural sectors. Fossil fuels are key sources to bear the burden of the entire world energy demand. Currently, about 80% of the worldwide energy is fulfilled by fossil fuel among which 58% is taken up by the transportation sector [1]. The continuous depletion of oil deposits, ever increasing fuel consumption and environmental pollution has stimulated mankind to develop an alternative approach from the sustainable and renewable source of energy. One of the promising alternatives to the fossil fuel is biofuel that is renewable, biodegradable, domestically grown source, safer, and cheaper [2]. Biofuels has many advantages over fossil fuels, such as, negligible sulfur, ash content, less emission of greenhouse gases and are eco-friendly than their petroleum-based competitors [3]. Biofuels produced from various biomass sources are abundant and renewable in nature. Currently, one such biofuel to gain attention worldwide is biobutanol produced from lignocellulosic and starchy feedstock, like agricultural and forest residues.

In recent years, conversion of waste biomass to produce bioenergy such as biogas, biofuel and valuable chemicals are gaining importance. It is estimated that 146 billion metric tons/year of available biomass in the world is not utilized and discarded as waste or incinerated [4]. The waste lignocellulose and starchy biomass reduces the soil fertility and cause environmental pollution. Inadequacy of these waste disposal techniques in the twenty-first century has become a global issue for many developing as well as for developed countries. The ever increasing generation of lignocellulose and starchy wastes due to industrial and agricultural activities has relentlessly increased the problem of waste management and disposal. Many developing countries are tackling this problem by switching to zero waste concept by channeling these wastes into biobutanol which is the main center of bio-economy.

Biotechnology for Zero Waste: Emerging Waste Management Techniques, First Edition.
Edited by Chaudhery Mustansar Hussain and Ravi Kumar Kadeppagari.

Biobutanol is an alcohol, usually produced by fermentation via the "acetone–butanol–ethanol (ABE)" process employing several genera of bacteria, generally *Clostridia* sp. [5]. Present, agricultural products such as cane sugar, molasses, corn, and cassava are used as prospective feedstock for butanol production worldwide [6]. However, most of the products mentioned are considered as food wherein the supply is insufficient for the production of biobutanol thus leading to food versus fuel crisis. From an environmental perspective, food crops for biofuel synthesis are not economically sustainable. Therefore, lignocellulosic biomass of agricultural and forest waste is feasible and is environmentally sustainable resource for production of butanol. The conversion of biomass into biobutanol involves an initial pretreatment process to expose carbohydrate present in the biomass. Pretreatment process being one of the key sources for the conversion of lignocellulosic sources to butanol has a great potential in improving the economy and efficiency of butanol production. Pre-treated biomass undergoes microbial degradation to produce biobutanol. The selection of cost effective substrate and process condition optimization increases the butanol yield from lignocellulosic residues, thus decreasing the cost of butanol production and making it economically more competitive.

30.2 Opportunities and Future of Zero Waste Biobutanol

Biobutanol is a colorless alcoholic biofuel comprised of a four carbon structure with the chemical formula C_4H_9OH (molecular weight: 74.12) having boiling point of 117 °C. Biobutanol appears to be the prospective substitute for petroleum derived gasoline fuels owing to elevated calorific value, low volatility, less corrosiveness and reduced moisture affinity than bioethanol [7, 8]. The first industrial scale biobutanol production began in 1916 during World War I due to high demand of acetone to produce cordite for British war industry [9]. The large portion of butanol produced worldwide is used in industry for the synthesis of methacrylate esters and acrylate (Figure 30.1). Other main derivatives such as glycol ethers and butyl acetate are primarily used as an industrial chemical and solvent for surface coating, paints,

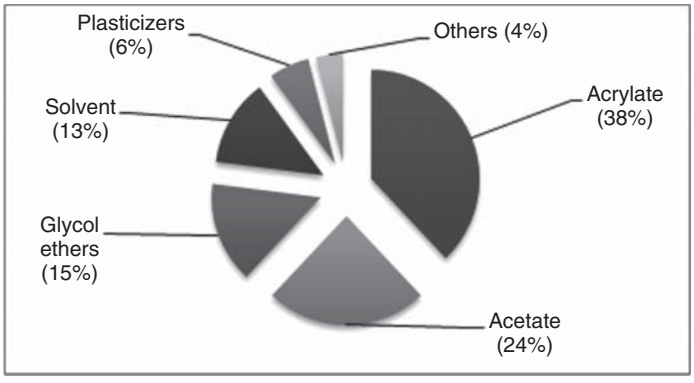

Figure 30.1 World butanol utilization. Source: Guzman [1].

printing inks, sealants, adhesives [10]. Butanol is also used as plasticizers, chemical intermediate for hydraulic fluids and detergent formulations [11]. The industrial utilization of butanol for the synthesis of variety of solvents and chemical intermediate is shown in Figure 30.1.

The physiochemical characteristics of biobutanol are identical to petroleum derived gasoline; therefore biobutanol can be used as a substitution for petroleum derived gasoline with 10% lesser energy content [12]. At the beginning of twenty-first century biobutanol gained importance as an excellent sustainable biofuel derived from the plant materials such as starch and lignocellulose thus increasing the consumption of biobutanol in the transportation and aviation industry. As per the International Organization of Motor Vehicle Manufacturers, the total sales of passenger and commercial vehicles in 2016 were increased by 3% in 2017. The increase in sales of vehicles leads to greater consumption of motor fuels, which in turn drives the market for bio-butanol as a renewable transport fuel. The Biofuel Advisory Council of European Union (BACE) aimed that the butanol usage in transportation sector has to increase by 25% by 2030 [3]. The Energy Independence and Security Act (EISA) of USA predicted that use of renewable fuel in transportation sector will grow to 36 billion gallon in 2022. Today several countries have started imposing ban on internal combustion engine run on fossil fuel. At the end of 2018 London, Paris, Mexico City, and Athens declared banning of the diesel cars and vans by 2025. The Government of India (GOI) has also reconfirmed their plans to go full ballistic for renewable fuel by 2030 [13]. This aforementioned initiation by these countries will increase the future demand for butanol in the market.

Biobutanol production through fermentation suffers great disadvantages such as cost of production, low product yield, sluggish fermentation and inhibition caused by end product, further making it difficult to run ABE fermentation in commercial scale. The decrease in quality and quantity of molasses due to improved sugar processing technology has also hampered the butanol production by fermentation [6]. To overcome these fermentation difficulties several studies have been laid out to improve the butanol yield and productivity for more economical ABE production process. The production cost is also reduced by selecting less expensive and free feedstock and exploring cost efficient processing method for ABE fermentation [14].

The widespread applications of biobutanol in transportation and industrial sector have seen significant growth in the global biobutanol market. As per the global market report, the worldwide annual production volume of biobutanol is 5 billion liter and is expected to cross 22 billion liter by 2022 [15]. Butanol currently holds an annual market of more than US$ 6 billion and is expected to reach US$ 18 billion by 2020 due to growing fuel needs. In recent years in Asia Pacific region, the countries like China, Japan, and India are the largest consumer of biobutanol due to the growing industrialization and transportation sector.

30.3 Generation of Lignocellulosic and Starchy Wastes

Lignocellulose and starch are the largest abundant natural resources underutilized material in the earth. Lignocellulose waste consists of cellulose, hemicellulose, and

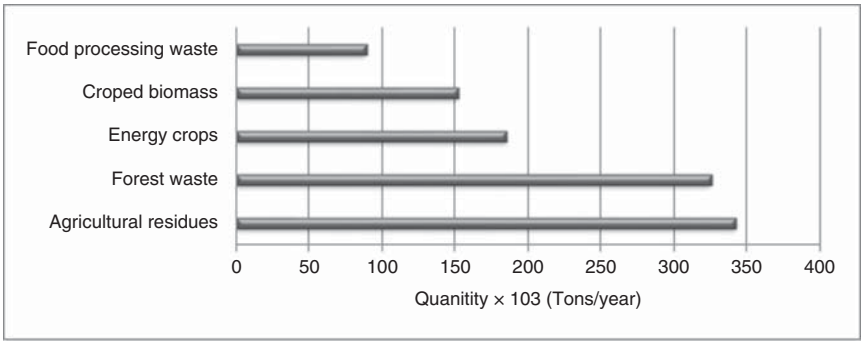

Figure 30.2 Worldwide availability of lignocellulosic feedstock.

lignin at various compositions. Lignocellulose and starchy waste generated from various industrial activities from food, paper and pulp, timber processing and forestry waste creates a serious waste management problem. Surplus of biomass from these sectors can be diverted for biobutanol production which will benefit the combination of waste management and energy generation to fulfill the current energy demand.

30.3.1 Types and Sources of Waste Generation

Few of the commonly used lignocellulosic feedstock and its availability for biofuel conversion are highlighted in Figure 30.2. China produces 830 million tons of lignocellulosic biomass per year from bagasse, straw, rice husk, and wood residue. According to statistics in a year 9.8% global energy is derived from biomass, where 70% of biomass is used for traditional purpose such as domestic heating and combustion [16]. As per the report made by United Nations Environment Programme (UNEP 2015), worldwide generation of agricultural biomass amounts to 140 billion tons. Among these 415.5 million tons of agro wastes are generated in India. The shares of various lignocellulosic sources from agricultural and non-agricultural commodities for the global biofuel production have strongly increased since 2019. The biomass generated from the agricultural activities contributes major lignocellulosic resources (342 million tons/year) for biofuel production followed by forest waste (hardwood and soft wood) with a capacity of 325 tons per year [17]. From an economic perspective, forest waste is probably a good choice of feedstock for the production of biobutanol (Figure 30.2).

The lignocellulosic waste generated from agro and forest wastes are proved to be cheaper than other sources. One of the lignocellulosic feedstock generated from agro waste are cocoa pod biomass. It is reported that the chocolate industry generates about 3.5 million tons/year of cocoa pods and is disposed off as waste [18]. The forest waste such as Kans grass, growing on the barren land and on the banks of river as weeds are generally disposed off by incinerating. The waste biomass generated from industrial activities also can be diverted toward producing biofuel [19]. In twenty-first century, biodiesel production from non-edible seeds has gained importance resulting in generation of large quantities of seed cake as a by-product from biodiesel industries. The above mentioned lignocellulosic feedstocks are inexpensive

and possess rich quantity of carbohydrates thus, showing the potential of being converted into biofuel.

The lignocellulosic feedstock for biobutanol production is categorized into waste biomass, virgin biomass and energy crops. All natural terrestrial plants like grasses, bushes, and trees are virgin biomass. Waste biomass is generated as a low quality by-product from various industrial and agricultural sectors which includes, rice straw, corn straw, wheat straw, pineapple peel, palm kernel, etc. [20]. Energy crops like Elephant grass (*Pennisetum purpureum*), switch grass (*Panicum virgatum*), poplar tree (*Populus*), carrot grass (*Parthenium hysterophorus*) and sugarcane (*Saccharum officinarum*) have high lignocellulosic content [21]. The major portion of lignocellulosic resources available for biofuel production is generated from the agricultural activities. Some of the lignocellulosic resources already investigated for biofuel production are wheat straw, corn stalk, oil palm biomass, rice straw, and sugarcane bagasse. The energy crops such as phragmites, switch grass, and king grass have been also explored for biobutanol production [21].

Starch residues generated from agro-industrial activities shows a greater potential for being converted into biobutanol economically. It was estimated that 4×10^7 tons/year of starch waste is generated worldwide from agricultural activities. Starch waste biomass generated from agricultural activities provides a compelling advantage for biobutanol production since this biomass is readily available, inexpensive and can be easily hydrolyzed into fermentable sugars. Biobutanol production from starchy resources is often cost-effective due to lower pretreatment costs and having a renewable fuel from waste greatly lowers waste treatment and disposal costs.

30.3.2 Composition of Lignocellulose and Starchy Residues

The structural composition of lignocellulose is a key factor in biochemical conversion of biomass into biofuel and can have significant influence on biofuel productivity and cost of production. The composition analysis of lignocellulosic feedstock reported by several studies revealed that the ratios of various constituents present in the lignocellulose vary depending upon the plant type, age of the plant, growth stage, and geographical location. The variability in feedstock composition affects the process economics and conversion yield of biobutanol production; therefore a reliable and effective method of biomass analysis is essential.

The efficiency of biomass to biofuel conversion is decided by estimating the lignin and carbohydrate content present in the lignocellulosic materials by sulfuric acid hydrolysis method. A review by Sluiter et al. [22] reveals the history of compositional analysis of biomass based on sulfuric acid approach. For large-scale application, the standard wet method of chemical analysis of lignocellulosic feed stock is not feasible as it suffers from the drawback such as labor intensive and time consuming process. Hou et al. [23] proposed an integrated method to analyze the chemical composition of feedstock by multivariate calibration model. This method combines the traditional chemical analysis with spectrophotometer. The study suggested that near infrared (NIR) spectrophotometer analysis is able to provide rapid quantitative

Table 30.1 Structural composition of lignocellulosic residues (dry basis).

Lignocellulosic residues	% Lignin	% Cellulose	% Hemicellulose
Paper	0–15	85–99	0
Newspaper	18–30	40–55	25–40
Waste papers from chemical pulps	5–10	60–70	10–20
Grasses	10–30	25–40	35–50
Switch grass	12.0	45	31.4
Coastal Bermuda grass	6.4	25	35.7
Wheat straw	15	30	50
Nut shells	30–40	25–30	25–30
Corn cobs	15	45	35
Cotton seed hairs	0	80–95	5–20
Hardwoods stems	18–25	40–55	24–40

Source: Sun and Cheng [26].

estimation of biomass properties. Recent progress done in the wet chemical method offers high throughput in process to large samples in reduced time [24].

Recent research is carried out on wet chemical method to process large scale biomass in lesser time. However, this method still suffers a drawback such as cost of instrument, limited biomass estimation, requirement of pre-conditioning, etc. Thus, time saving, low cost, and reliable method is required for biomass estimation. The analytical problem encountered in wet chemical method is overcome by the use of infrared spectroscopy (IRS) for qualitative and quantitative estimation of biomass [25]. The biomass estimation by IRS technology is fast and precise, ease in sample preparation and many biomass constituents being analyzed at the same time. The cost of sample estimation using this technique is about US$ 10 per sample and US$ 17 when compared to wet chemical method. The structural composition of most common agricultural and forest biomass are listed in Table 30.1 [26].

A lignocellulosic residue with rich cellulose and hemicellulose content and lower lignin is favorable for biobutanol production. However, this single criterion cannot be the deciding factor for a good substrate for ABE fermentation. The other factors such as biomass yield rate per hectare of land, transportation cost and ease in hydrolysis with minimal generation of inhibitor also influence the fermentation performance. A case study performed by Swana et al. [27] showed that corn stover is the best substrate for ABE fermentation followed by switchgrass.

30.4 Value Added Products from Lignocellulose and Starchy Residues

A wide verity of value added chemical products can be derived from lignocellulose and starchy residues such as aromatic compounds, 5-hydroxymethyl-furfural

(HMF), citric acid and lysine. Some of the organic acids such as acetic acid, butyric acid, lactic acid, oxalic acid, itaconic acid, succinic acid, and propionic acid are the value added products produced from the lignocellulose and starchy residues. Apart from these valuable chemical compounds, this feedstock can also be used for producing renewable fuels such as biogas, bioethanol, and biobutanol.

30.4.1 Feasibility of Biobutanol Production from Lignocellulose and Starchy Residues

The butanol production cost depends on the price of feedstock and its availability. An economic analysis reports that 70% of the butanol production cost is associated with the naturally available feedstock [21]. In 1950 corn and molasses were used as a substrate for butanol fermentation but these substrates being expensive brought high competence with the food production leading to increased cost of crops and food. This problem today is overcome by using inexpensive and sustainable sources such as lignocellulosic residues.

Some of the lignocellulosic resources already investigated for biobutanol production are listed in Table 30.2. Other than these feedstock some of the lignocellulose waste generated from agricultural activities such as wheat straw, corn stalk, oil palm biomass, rice straw, sugarcane bagasse and energy crops such as switch grass, phragmites and king grass have also been tested for biobutanol production [5].

30.4.2 Pretreatment

Pretreatment of lignocellulose is required to expose the cellulose and hemicellulose for hydrolysis to get fermentable sugars. This step is crucial as yield of butanol and its economy depends on the success of this pretreatment step. Many efforts have

Table 30.2 ABE and butanol yield comparison from different substrates.

Hydrolysis method	Substrate	ABE (g/l)	Butanol yield (g/g)[a)]	ABE yield (g/g)[b)]	References
H_2SO_4 (3.05%, 64.02 min, 121 °C) LPG	Cocoa pod	5.27	0.19	0.30	[28]
H_2SO_4 (1%, 60 min, 121 °C)	Apple pomace	10.70	0.22	0.34	[29]
H_2SO_4 (1%, 15 min, 121 °C)	Rice straw	20.05	0.34	0.58	[30]
Enzyme hydrolysis	Oil palm biomass	6.44	0.11	0.19	[31]
Direct fermentation	Date fruit	14.5	0.48	0.63	[32]
H_2SO_4 (0.75%, 45 min, 121 °C)	Wheat bran	11.80	0.41	0.54	[33]
None	Paper sludge	18.00	0.14	0.30	[34]

a) g of butanol/g of total sugar.
b) g of ABE/g of total sugar.

been made by the researchers in recent year for the bioconversion of lignocellulosic biomasses into biobutanol in an economic way. One of the major cost intensive steps in biobutanol production involves cost of feedstock and biomass pretreatment. The former can be tackled by selecting feedstock which is waste biomass generated from industrial and agricultural activities. As reported in literature the worldwide availability of plant biomass reaches 200 billion tons/year, of which lignocellulosic resource constitute 90% of world biomass [35].

Extensive research is carried out to reduce the cost of pretreatment and achieve high degree of hydrolysis. Pretreatment protocols necessitate the efficient utilization of biomass for the conversion to fuel, wherein lignocellulose biomass is broken down into its monomeric sugars. The microorganism used in ABE fermentation utilizes the released sugar producing biobutanol. The main aim of the pretreatment is to disrupt the crystalline structure of the cellulose by breaking the lignin barrier and ease acids or alkali to access and hydrolyze the cellulose and hemicellulose. Pretreatment for lignocellulosic residues is carried out in mild condition to reduce the formation of fermentation inhibitory compounds. Formation of inhibitory compounds in turn makes the process more expensive by incorporating detoxification step to increase the efficiency and yield of butanol.

Physical, chemical, or biological pre-treatments are thoroughly explored process for butanol production from lignocellulosic biomass. However, the methods of pretreatment and optimum pretreatment condition are decided by the type and nature of lignocellulose biomass. The major factors affecting the degree of hydrolysis are cellulose crystallinity, available surface area and composition of lignin and hemicelluloses [36]. Different pretreatment processes have been investigated for lignocellulose biomass, which includes physical pretreatment (grinding, milling), chemical pretreatment (dilute alkali, acid, organic solvents, etc.), physico-chemical pretreatment (steam explosion, autohydrolysis, hydrothermolysis) and biological pretreatment. Although, there are many pretreatment steps discussed in the literature, the success of any pretreatment method depends upon energy requirement, consumption of chemicals and formation of fermentation inhibitors. Therefore, exploration of new feedstock for biofuel production demands a systematic study of the pretreatment method and conditions that are required.

The process of ABE fermentation is described in Figure 30.3. After pretreatment, the biomass releases fermentable sugars which are used as a substrate for ABE fermentation by *Clostridia* sp. For example *Clostridium acetobutylicum*, *Clostridium saccharobutylicum*, *Clostridium beijerinckii*, etc. [37] undergoes metabolic shift producing a significant amount of acetic acid and butyric acid thus producing acetone, butanol, and ethanol respectively. *Clostridia* sp. has the vast ability to ferment variety of sugar monomers such as fructose, glucose, starch, dextrins, mannose, lactose, and sucrose. It also partially ferment sugars such as xylose, galactose, mannitol, raffinose, and arabinose [38]. The production of ABE by *Clostridia* species follows an intracellular pathway. The products of this intracellular pathway are categorized into (i) organic acids (butyric acid, acetic acid, and lactic acid), (ii) solvents (acetone, butanol, and ethanol), and (iii) gases (CO_2 and H_2). The first stage of ABE fermentation begins with exponential growth phase called acidogenic phase wherein, each

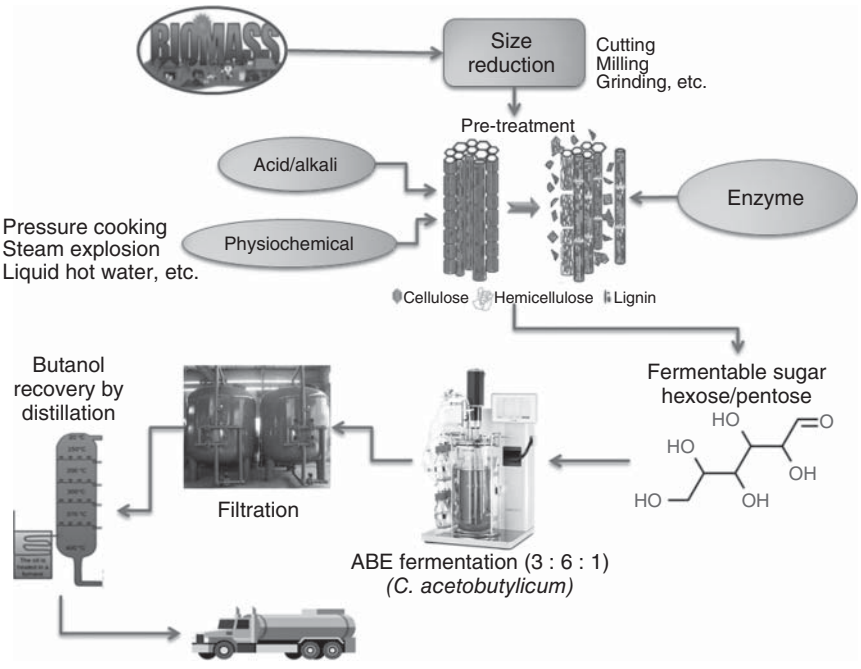

Figure 30.3 Biobutanol process descriptions.

mole of glucose is converted into either 1 mol of butyric acid or 2 mol of acetic acid via acidogenesis. In the stationary phase products of acidogenesis are shifted into solventogenic phase. In this phase, bacteria form spore and acid are transformed into solvents. The acetic acid is converted into acetone and ethanol, while butyric acid is converted into butanol. At the end of fermentation the ABE solvents like acetone, butanol, and ethanol are formed in the proportion of 3 : 6 : 1. The solvent from fermentation broth can be recovered by gas stripping, pervaporation, adsorption, extraction through reverse osmosis, etc.

30.4.3 Economics of Biobutanol Production

The economic production of biobutanol depends on the mode of processing, separation technique and quality of the feed stock. The fixed capital investment is determined by the first two factors and the total production cost is controlled by the third factor. There are many studies on economic analysis of ABE fermentation of corn, molasses, wheat straw, and whey permeate [39]. Utilization of these food crops for butanol production is not feasible. Though there are many reports on butanol production from various feedstocks, industries still experience many challenges which needs to be resolved for economical production. There are several bottlenecks restricting the commercial use of feedstock, such as continuous supply of feedstock, selection of non-food crop, low cost processing, and high carbohydrate content.

The fermentation of biobutanol is possible in economic way, when cheap, low grade feedstocks are processed on relatively small industrial scale. In butanol industry cost of feedstock and its processing techniques plays a dominant role in cost of production. It was estimated that feedstock amounts 65% of the total annual production cost. Extensive study is still needed to make large-scale ABE fermentation feasible. More efforts are required to suppress inhibitors formed during fermentation in a large scale. The different method of pretreatment may differ based on the variety of feedstock, therefore choice of pretreatment technique which is economical, fast and efficient need to be explored for new feedstock in order to make an attractive resource for biobutanol production. Though there are several literature on production of biobutanol using various feedstocks, there still needs an improvement in research regarding the upstream and downstream process to reduce the toxicity level of end product and enhance the production yield to commercial scale [5].

The butanol production cost is calculated by considering the three factors such as fixed cost, variable operating cost and by product formed during the process. The fixed cost includes reactors, separation/purification equipment, maintenance, depreciation, insurance, labor, etc. The variable operating expenses depends on the cost of feedstock, chemicals, and utilities. These factors can be related to each other using the formula given below.

$$\text{Butanol Production cost} = (\text{Fixed cost} + \text{variable cost} - \text{byproduct credit})$$

Kumar et al. [40] conducted economic analysis of ABE fermentation with lignocellulosic and starchy feedstock in 10 000 tons/year butanol. It was reported that the production cost of butanol from glucose and sago were high as US$ 5.32 and US$ 3.87 per kg of butanol. The data were compared with cheaper lignocellulosic feedstock such as corn stover, bagasse, sugarcane, barley, and wheat straw reported lowest production cost between US$ 0.59–0.75 per kg of butanol. Therefore the availability of feedstock, high residue yield rate and ease in cultivation also directly influence the economic production of biobutanol. The overall annual production cost of biobutanol yield is increased by 6% due to the utilization of lignocelluloses involving enzymatic hydrolysis of cellulose and hemicellulose when compared to any starchy residual sources. The production cost for lignocellulose and starchy residues are calculated by Kumar et al. [40] is listed in Table 30.3.

A comparative economic analysis of butanol production from corn and glycerol is reported by Qureshi and Singh [1]. The economic comparison is represented in Table 30.4. The cost of butanol from the Table 30.4 is high for starchy feedstock due to high processing cost, whereas butanol produced from glycerol is cheaper than other feedstock. The glycerol feedstock does not require any costly processing techniques such as pretreatment thus reduction in overall operating cost. The cost of lignocellulose feedstock is less compared to starchy and glycerol but process involves pretreatment at high temperature and costly enzymatic hydrolysis.

The process cost of hydrolysis for lignocellulose can be reduced by adapting new technology for pretreatment technique such as microwave and irradiation techniques. The pretreatment of lignocellulose using induction and liquefied petroleum gas (LPG) assisting heating was explored for biobutanol production. The

Table 30.3 Butanol production cost from lignocellulose and starchy residues.

Feed stock	Production cost (US$/kg)
Glucose	5.33
Corn	1.3
Sago	3.87
Sugarcane	0.62
Barley straw	0.75
Wheat straw	0.69
Corn stover	0.59
Switch grass	0.63

Table 30.4 Economics of butanol production from corn and glycerol.

	Corn	Glycerol	Lignocellulose
Plant capacity	115×10^6 kg/year	115×10^6 kg/year	122.26×10^6 kg year
Amount of feedstock	432.33×10^6 kg/year	287.5×10^6 kg/year	450×10^6 kg year
Acetone	34.50×10^6 kg/year	—	36.68×10^6 kg year
Butanol	69.0×10^6 kg/year	89.8×10^6 kg/year	73.36×10^6 kg year
Ethanol	11.50×10^6 kg/year	3.5×10^6 kg/year	12.23×10^6 kg year
1,3-propanediol	—	21.71×10^6 kg/year	—
ABE yield	0.38 g/g	0.4 g/g	0.38 g/g
Plant cost	US$ 65.24×10^6	US$ 48.53×10^6	US$ 63.9×10^6
Cost of feedstock	US$ 73.5×10^6/year	US$ 115×10^6/year	US$ 29.93×10^6
Selling cost of butanol	US$ 1.31/kg	US$ 0.23/kg	US$ 1.10/kg

study has shown that utilization of induction technique in pretreatment utilizes less energy and cost compared to conventional heating [28]. Channelizing the effluent acid and use of ionic liquid in the pretreatment process reduces the need for costly enzymes thus reducing the overall production cost and equipment cost.

The economy of the ABE process can be improved by integrating the process with product recovery. Qureshi and Singh [1] have combined the ABE model with continuous product recovery to research the model economy. The total capital investment was US$ 1.2 million with total production cost reaching up to US$ 1.47 million/year for 1.94 million liter of butanol production with break-even of US$ 0.74/l. It was well observed that the break-even for this process was reduced to US$ 0.44/l by expanding the plant capacity to handle 6000 m^3/day of feedstock. This concludes that the cost of feedstock and plant scale directly affects the overall economy of biobutanol production.

An extensive research on enhancing the process economy by studying the operational parameter affecting the ABE fermentation was reported widely. However, usage of new feedstock and development of stress tolerance microbe alter the ABE operational condition. Maintaining the fermentation parameter inside the reactor due to formation of inhibitory compounds is one of the challenges in ABE fermentation. Research is still needed to explore the development of kinetic model to study fermentation behavior under transient state for different feedstock and microbes to increase productivity and to overcome the challenges involved with reactor design for commercial scale production.

30.5 Conclusion

In the twenty-first century mankind is facing the problem of waste disposal and energy demand due to its growing megacities and industries. The lignocellulose and starchy residues are one of the waste by-products generated from these industries. Thus, production of biobutanol from lignocellulose and starchy waste residues is found to be feasible because of the presence of high carbohydrate content present in it. Biobutanol is one of the promising advanced renewable fuel which has the potential to meet the current energy demand. The use of lignocellulose and starchy waste residues generated from agro-industrial activities to produce biobutanol could reduce the production cost, making it more economical.

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31

Critical Issues That Can Underpin the Drive for Sustainable Anaerobic Biorefinery

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31.1 Introduction

In the quest for renewable energy sources (RES), next-generation biofuels seem to provide energy security and ecological relief. The debate for the ecological and cost-efficient character of biofuels has raged for several years the entrepreneurial communities and governmental parties. This calls into question whether large-scale production of next-generation biofuels renders a sustainable solution for renewable heat, electricity, and transport fuel production. The consensus of policymakers to elucidate biofuels' future role is resonant, and the disjunction between technological practicalities and law can accelerate the bioenergy deployment. The vast amount of organic waste and its abatement are a major challenge most countries are facing [1]. Worldwide political pressures increased because of the consumption of energy derived from fossil fuel reserves on the climate. These pressures induced the Paris climate agreement, which shifted the focus of the industrial energy sector toward energy derived from RES instead of energy derived from fossil reserves such as crude oil, coal, and natural gas [2].

Technology for the production of biogas, anaerobic digestion (AD), is usable in a broad range of applications, such as electricity production, heating and transportation, and storage due to the flexibility of the energy carrier biogas [3]. The versatile use of biogas embroiders the AD landscape and enhances its bioenergy practicalities. In addition, AD produces nutrient-rich digestate that is recycled to farmlands to act as organic fertilizer and diminish negative environmental impacts such as odors and eutrophication of freshwater systems, which could occur if the disposal of such nutrients is not accurately controlled and managed [4].

Biowaste is most of the time a significant and sometimes the sole component in different waste streams from diverse sources such as agricultural residues (crop residues, straws, etc.), industrial wastes (paper mill discards, sawdust, etc.), forestry wastes (grass, wood, etc.), domestic wastes (municipal paper, etc.), and municipal solid wastes or agro-industrial waste [5]. Biogas is the metabolic product of the decomposition of a wide spectrum of organic waste (e.g. manure, crop residues,

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distiller's waste, and glycerol). To date, biogas production is mainly affiliated with the abatement of sewage sludge from municipal wastewater treatment plants. Biogas as an energy carrier can play an essential role in the bioeconomy.

In comparison with fossil fuels, biogas production by AD can diminish greenhouse gas (GHG) emission by using regionally available resources, and in contrast with other bioenergy production techniques with GHG emission reduction benefit, biogas production by AD is the least energy-consuming process [6]. In addition, evaluations show that biogas production offers significant advantages over other bioenergy production techniques because AD is a more energy-efficient and environmentally friendly technology in terms of recovering energy and decreasing the amount of organic waste [7, 8]. Moreover, current research activities aim to improve AD efficiency, which indicates the growing economic potential of biogas production in the coming decades over yet established bioenergy production techniques [9]. This study grapples with several (bio)technological issues of AD and discusses subtle perspectives of the biogas-based green economy by an updated consolidated literature brief review.

31.2 Biogas – An Energy Vector

Although energy industry promulgates the gaseous fossil fuels over biogas, its combustion for power and heat recovery is widely used in residential biogas plants. Table 31.1 shows the properties of biogas compared with other gaseous fuels. The biogas utilization consists of a mundane spectrum of industrialized applications. The most common applications are electricity generation and heat recovery in the combined heat and power (CHP) plants [12]. Electrical and thermal conversion efficiencies of the CHP unit are around 40% and 50%, respectively. The physicochemical properties of biogas affect the choice of technology used for cleanup and combustion; therefore, knowledge of these properties is useful to optimize the end use of biogas [13]. To date, almost all the biogas produced worldwide is used for heat and electricity production. Several countries contemplate biogas as a solution

Table 31.1 General properties of gaseous fuels [10, 11].

	Low calorific value (MJ/m^3)	Specific gravity	Boiling point (°C)	Ignition temperature (°C)	Flammability limits on air (% v/v)
Biogas	23.1	0.80	—	650	8–18
Carbon dioxide	—	1.52	−78.5	—	—
Methane	39.8	0.55	−161.4	590	5–15
Natural gas	38.7	0.65	−258.7	628	—
Ethane	60.8	1.048	−88.1	515	3–12
Propane	88.4	1.52	−43.4	470	2–9

Source: Park et al. [10].

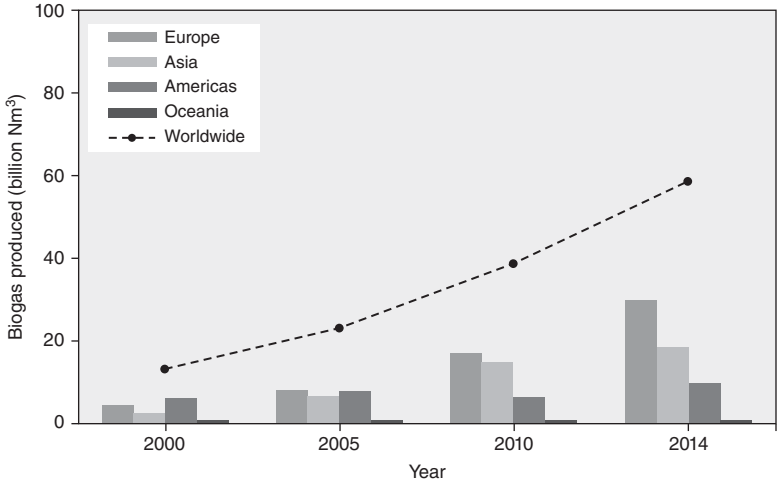

Figure 31.1 Biogas production in continents and worldwide.. Source: Modified from WBA [15].

for a sustainable eco-mobility with Sweden being the leading country using biogas as vehicle fuel [14].

The wide range of biogas applications in the mobility and power sector creates a basis for potential customers. Business leaders ideate biowaste as a nascent energy source inasmuch orientating to biomethane is beneficial for the bioenergy industry and may lead to a salubrious economy (Figure 31.1).

Aligned economic and environmental considerations may reinforce the waste markets. Bolstering efforts to embrace waste treatment practices is conceivable; however, contemplating efficient techniques to abate wastes is stimulus and requisite to transit the economy into a sustainable path [16]. Broadly speaking, mitigation of GHGs, secure supply of energy and commodities, and pursuit to ameliorate economies in rural areas are contaminant drivers to assure the leapfrogging to a green gas economy.

The fickleness of the incumbent business and the uncertainty of the project profitability hinder the boosting of renewable fuels (Figure 31.2). Censure on the biogas market has been ascribed to the absence of a standardized framework. Albeit the economic reforms are a pivotal impetus for the biogas industry economy, it may efface the precariousness derived from biofuel projects [16]. However, the incessant imminence of technological findings and improvements in the production of other gaseous fuels might erode its competitiveness.

31.3 Anaerobic Biorefinery Approach

The wobbling price of biofuels, elevated power demand, and fossil fuel depletion are subtle reasons for the transition into the biogas economy. Biogas has become a point of contention for the biofuel industry that argues the mediocre AD efficiency is

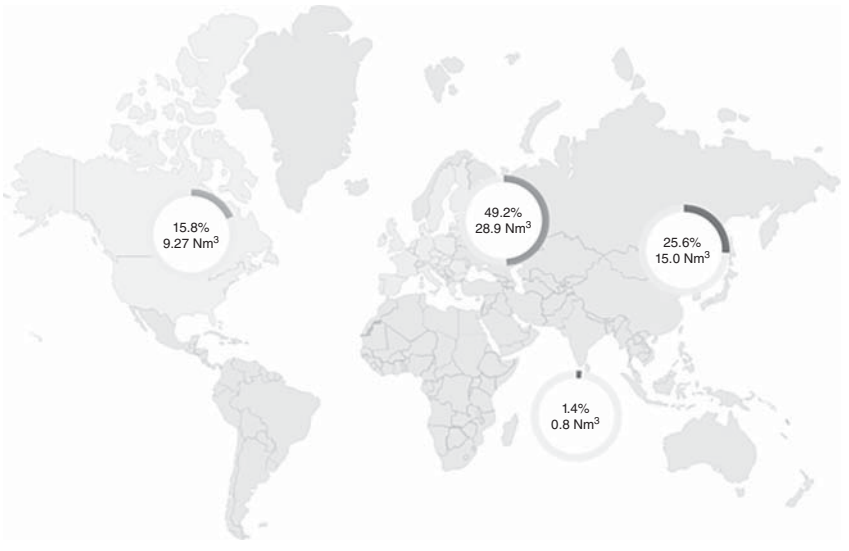

Figure 31.2 Biogas production in North America, Europe, China, and India. All values in billion Nm^3 in 2014. Source: Based on WBA [15].

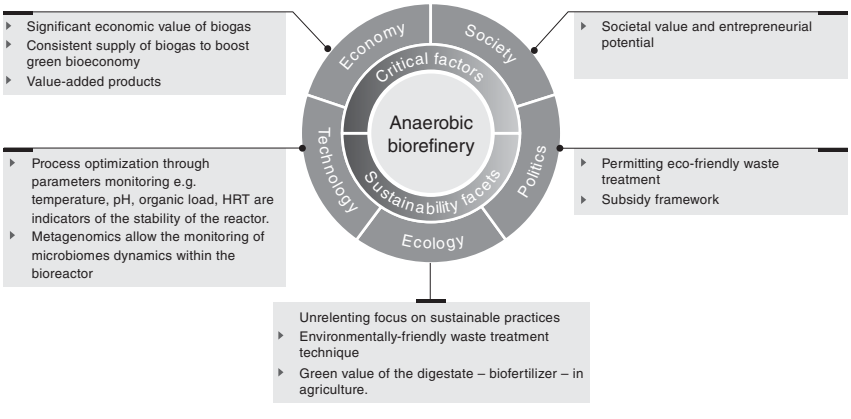

Figure 31.3 Aspects for a sustainable anaerobic biorefinery.

an economic constraint. The anaerobic biorefinery is a promising concept, in which the anaerobic reactor/digester acts as a centerpiece for bioconversion of feedstocks (substrates) into diverse high-value products and intermediates (Figure 31.3). AD technology has several inherent merits such as remediation of highly putrescible organic wastes at a smaller environmental footprint, capturing GHGs, and at the same time valorizing organic wastes into high-value products/chemicals and intermediates.

Diverse organic materials ranging from industrial wastewaters to municipal and farm wastes could be used as feedstocks in an anaerobic biorefinery to produce biogas with concomitant generation of digestate (i.e. solid residue and liquid effluent).

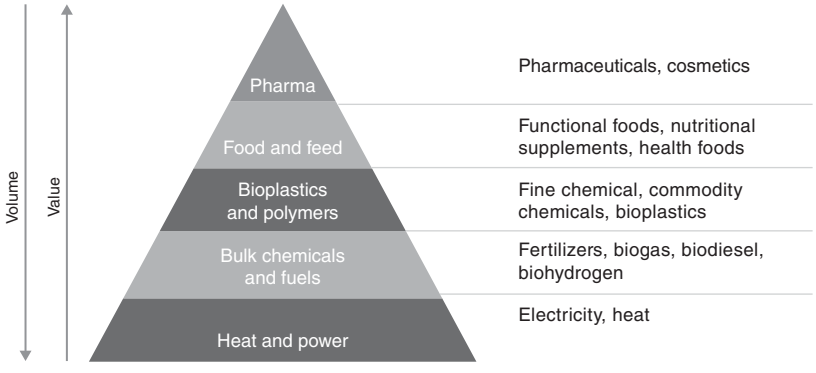

Figure 31.4 Valorization process in biorefineries.

The valorization of raw material into products is a well-established procedure in the current fossil fuel processing industry (Figure 31.4). Along with the same principle, biorefinery incorporates biomass as input material leading to a wide variety of bioproducts and bioenergy. The biosolids can be applied to crops and the liquid used for fertigation. The digestate can be further processed to a plethora of biobased products and chemicals. However, an alternative approach is to use nutrients in the liquid fraction for the cultivation of protein- and lipid-rich algae.

31.4 Technological Trends and Challenges in the Anaerobic Biorefinery

31.4.1 Pretreatment

Currently, biorefineries are attempting to produce additional bioenergy by integrating AD from organic waste because of the importance of future direction. Lignocellulosic feedstocks have high methane potential in anaerobic digestion. However, it is not widely applied due to its complex, recalcitrance structure that decreases the stability of the AD process. Research efforts on the biowaste treatment increased during the last decade, and several reports were published recently, aiming at challenges and opportunities during pretreatment [9].

The complex structure of the feedstock is a major challenge, which can be overcome by pretreatment. Pretreatment can enhance the digestibility of high-content lignocellulose feedstock by increasing the substrate porosity, reducing cellulose crystallinity, increasing the surface area for enzymes to attack, and solubilizing cellulose, hemicellulose, and/or lignin. The conversion of cellulose and hemicellulose into more accessible substrates for the extracellular enzymes improves the AD of lignocellulosic feedstocks [17]. Various methods for pretreatment including chemical, physical, biological, and physicochemical processes have been suggested to improve the hydrolysis of cellulose and hemicellulose in biomass [18]. A selection of these methods together with their advantages and disadvantages are presented in Figure 31.5.

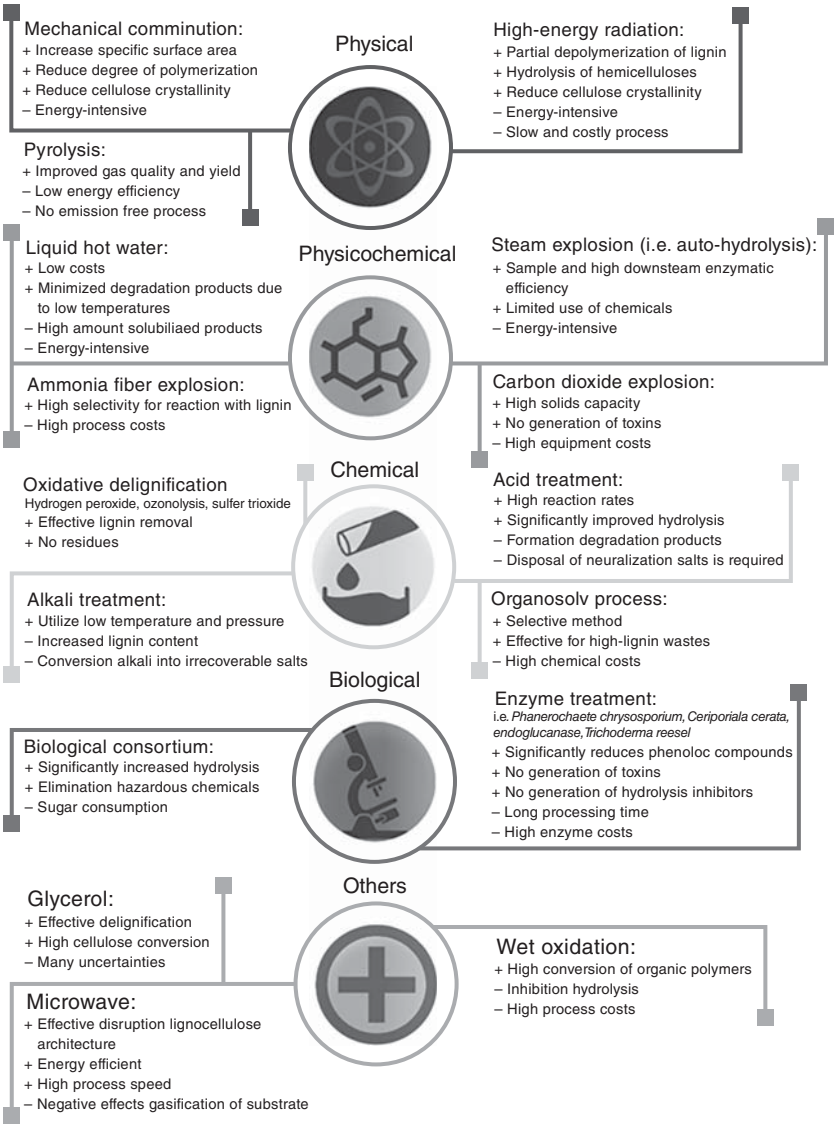

Figure 31.5 Pros and cons of lignocellulosic waste pretreatment methods.. Source: Zhang et al. [19]; Calabro et al. [20]; Chaturvedi and Verma [21]; Hou et al. [22]; Lemões et al. [23]; Rosero-Henao et al. [24].

The efficacy of each pretreatment technique is considerably dependent on biomass composition and properties such as cellulose crystallinity, lignin fraction and structure, acetylation degree of hemicelluloses. A cost-effective pretreatment for lignocellulosic biomass must meet the following requirements: (i) enhance the ability to produce sugars, (ii) avoid the loss of cellulose and hemicelluloses, and (iii) minimize the production of inhibitors. However, an optimal pretreatment

technique for lignocellulosic waste is not available yet, so various methods are often combined to optimize biological conversion into biogas.

The advances in biochemical reactor engineering mainly focus on process integration and intensification to increase overall energy production and substrate decomposition, reduce the number of required process steps, and decrease the required reactor volume. Up to now, biogas production by anaerobic digestion, upgrading to the quality of natural gas, and its necessary compression to be injected into the national gas grid are three separate procedures. Nowadays, a technique based on high pressure favors the upgraded biogas production reaching 95% of methane in biogas [25].

The Role of High Pressure in Anaerobic Digestion

Pressure changes affect the performance of anaerobic digestion and the solubility and release of the gaseous end products. Several studies examined the impact of elevated pressure on biogas quality. According to Henry's Law, at a given temperature, an increment of the total pressure increases the partial pressure and consequently the solubility of CO_2. The equilibrium of CO_2 and HCO_{3-} in the liquid form is affected, and thereupon the pH and buffering capacity of the digester influence the biogas composition [26]. CO_2 is sparingly soluble in water, and its solubility depends on the partial pressure of the individual species according to Henry's law:

$$C_{CO_2} = y_{CO_2} * P_T / H_{CO_2} \tag{31.1}$$

where C_{CO_2} is the liquid-phase concentration of CO_2, y_{CO_2} is the gas-phase mole fraction of CO_2, P_T is the total pressure, and H_{CO_2} is Henry's law constant for CO_2.

Recently, a novel process condition based on elevated pressure (up to 100 bar) within the digester reached a methane composition of up to 95%. The goal of high-pressure digestion is to combine biogas production and purification into a single process in such a way that the natural gas network accepts this produced and purified biomethane. At the gas–liquid interface, the concentration of each gas is in equilibrium, and its diffusivity is affected by any total pressure change. The gas-to-liquid transfer rate is related to the diffusion, which is driven by the concentration difference.

As mentioned, biogas mainly consists of CH_4 and CO_2. The solubility of the two gases significantly differs under pressure. The CO_2 is dissolved much more readily in water, therefore increasing the methane content in the biogas [27]. During the degradation of organic matter, microorganism produces gases in the liquid. The gases escape when the liquid is fully saturated and enter the gas phase. Lindeboom et al. showed that pressure up to 20 bar increased the methane yield suggesting that the high-pressure auto-generative AD more efficiently degrades the substrate [28]. If more CO_2 dissolves in the water under high pressure, the biogas contains less than 5% CO_2. Merkle et al. (2017) studied the anaerobic digestion up to 100 bar using grass and maize silage hydrolysate as substrate [29]. The results showed significantly higher methane yield; however, more research is required to determine the pressure dependence of the microbial processes. However, the use of multistage

and high-pressure approaches can promote and accelerate the future use of lignocellulosic feedstocks for biogas production.

31.4.2 Multistage AD Process

Studies to evaluate and improve different reactor configurations (e.g. single- or multistage reactors) optimize the AD performance concerning improved methane yield, organic loading rate (OLR), and process stability [30]. For example, AD performed at two separate conditions produces more methane from organic compounds than a single-stage process. The so-called two-stage AD involves two separate reactors for hydrolysis + acidogenesis and acetogenesis + methanogenesis. The main reason for the two-stage AD is the prevention of inhibition of microorganisms at low pH. The first stage usually operates at an acidic pH of around 5.5–6.5 and utilizes a relatively short hydraulic retention time (HRT) of 10 days for acid fermentation. Thus, the first stage allows a relatively high OLR with a low pH value due to fast acid accumulation and organic compound degradation. The benefits of the first-stage reactor are better pH control and stability, increased volatile solids (VS) reduction, and better pathogen removal. The second stage is usually maintained at a pH between 6 and 8 and utilizes a longer HRT of 20–30 days to facilitate the growth of slow-growing methanogens [31].

Considering that the optimal pH for hydrolysis is lower compared with the optimal pH of acidogenesis, Zhang et al. constructed a three-stage AD system in which hydrolysis, acidogenesis, and acetogenesis + methanogenesis operate separately. The three reactors should be vertically connected, resulting in a small footprint in such a way that materials flow from one reactor into the next reactor by gravity [32]. Based on several studies, most two-stage AD systems reported a 10–20% increase in biogas production compared with single-stage AD [30]. The constructed three-stage AD system reached more than 50% increase in biogas production and nearly triplicated VS removal compared with single-stage AD [32].

The concept of multistage AD systems improved since technologists built the first setups 20 years ago. However, the costs of such systems are a significant drawback for economical applications [30]. Furthermore, a multistage AD system is of much higher technical complexity compared with a single-stage AD system, and robust automatization and process control algorithms are necessary to develop an efficient process in the long run.

31.4.3 Dynamics of Methanogenic Communities

Researchers focus not only on the classification of microbial communities at a particular time spot but also on the community shift between different classes of methanogens along with different conditions [33]. Although extensive mapping of the metabolomics networks and their interdependence are difficult, several methane-synthesizing pathways exist in methanogens [34]. The methanogenic pathways that convert methanol, CO_2, or acetate in anaerobic digesters are:

$$CH_3COOH \xrightarrow{\text{acetotrophic methanogens}} CH_4 + CO_2 \tag{31.2}$$

$$4H_2 + CO_2 \xrightarrow{\text{hydrogenotrophic methanogens}} CH_4 + 2H_2O \tag{31.3}$$

$$4\,CH_3OH \xrightarrow{\text{methylotrophic methanogens}} 3\,CH_4 + CO_2 + 2\,H_2O \tag{31.4}$$

$$CH_3OH + H_2 \xrightarrow{\text{methylotrophic methanogens}} CH_4 + H_2O \tag{31.5}$$

The reason for the shift of acetate consumers to hydrogen consumers is not known. Many studies suggest that a syntrophic relationship between acetate-consuming and hydrogen-consuming methanogens is present. Although acetoclastic *Methanosaeta* species are the most dominant organisms for biogas production, the stability of the reactor correlates better to the presence of hydrogenotrophic methanogens [35]. Quantitative real-time PCR experiments showed that a shift in the archaeal communities is associated with changes in the chemical composition of the reactor [36]. Delbes et al. monitored the metabolome dynamics of several archaeal species throughout an anaerobic digester crisis period [37]. They detected a high activity and a substantiation of acetoclastic methanogens in the digesters, which followed the acetate degradation pattern.

The resistance of methanogenic species in suboptimal conditions is an indicator of efficient operation. Goux et al. reported that in a failed AD reactor a negative correlation between *Methanosaeta* sp. and total volatile fatty acids (VFAs) content in the reactors exists [38]. They proposed the addition of hydrogenotrophic *Methanoculleus* sp. to restore the performance of the anaerobic digester. This species tolerates acidosis and promotes process recovery. Adaptation of *Methanoculleus* sp. has also been examined in a membrane reactor treating swine manure and a hybrid bioreactor exposed to OLR changes [39]. Studies conducted elsewhere found that *Methanosarcina* sp. dominated methanogenic populations in two-stage anaerobic digestion to alleviate VFAs accumulation [40]. The second reactor showed an elevated number of methanogens making up a likely scenario for enhanced methane production. *Methanosarcinaceae* consume the remaining intermediates from hydrolysis. They might improve their growth and resistance in digesters with a lower pH [41, 42]. The acclimation procedure increased the *Methanosarcina* population helping to reduce acetate and ammonia loads [41, 43].

The temperature of the digester is an important parameter for the methanogen activity, and the feasibility of hydrogenotrophic methanogens dominance was examined in thermophilic conditions [44]. *Methanothermobacter thermautotrophicus* can form >90% of the methanogenic community in a hyperthermophilic digester [45]. Another study treating synthetic wastewater and glucose to examine the effects of high temperature (65–80 °C) on the methanogenic distribution and the AD efficiency showed a similar behavior of methanogens in the upper-temperature levels [46]. Tuana et al. applied biological approaches (denaturing gradient gel electrophoresis (DGGE), clone library, and pyrosequencing technique) for the identification of archaeal sequences in a thermophilic digester that belonged to the order *Methanobacteriales* instead of *Methanomicrobiales* in previous studies [47].

Psychrophilic anaerobic digestion (<20 °C) has also been studied to determine the dynamic of methanogenic species under low temperature and revealed that there

was no specific shift toward psychrophilic microorganisms [48, 49]. McHough et al. studied the biological treatment of VFAs and sucrose-based wastewaters in continuous digestion at 16–37 °C for 300 days where a proliferation of *Methanocorpusculum parvum* sp. was detected [50]. Similarly, Leclerc et al. detected the presence of *M. parvum* sp. in a mesophilic lab-scale anaerobic digester treating sludge and glucose for 100 days [51]. The separation at the archaeal genus levels within the reactors treating different carbon sources is most likely related to differences in the process parameter [52]. Sundberg et al. conducted an archaeal DNA sequence analysis from full-scale biogas reactors treating various combinations of wastes from slaughterhouses, restaurants, and households [53]. The development and distribution of microorganisms that degrade cellulosic materials were modeled based on isotopic data from batch experiments and showed that the majority of the Archaea fell within the hydrogenotrophic genus *Methanobacterium* [54].

31.5 Perspectives Toward the Revitalization of the Anaerobic Biorefineries

31.5.1 Reciprocity Between Research, Industry, and Government

Recent research efforts indicate the potency of biogas production from biowaste. However, the process typically has technical challenges that originate from a poor understanding of the ideal reactor operation. Innovation becomes more expensive due to the complexity of AD and the increased risk that is involved in investment in new AD technologies [55]. These constraints, affecting the improvement of the AD, should be overcome by active collaboration between the research institutes, the biogas industry, and the observing government [56]. Universities often work together with R&D departments of biogas companies to mature AD technology. Subsequently, governments facilitate the implementation of biomethane in the transportation fuel markets and meeting their interests. Ultimately, the improved innovative concepts in AD persuade governmental institutes to supply additional subsidies for further technology development by research institutes and industries.

The resemblance in stakes of research, industry, and the government is the understanding of AD science and technology and the evaluation of the impact of economic, ecological, and technical barriers. The approaches to improve unitary stakes, however, differ very much per stakeholder. For example, design engineers in the industry usually apply a problem-solving approach, whereas research engineers tend to start with the science-related empirical approach. However, the combination of both methods in research engineering and design engineering would be more favorable since engineers require research to define design quality and decisions, and researchers have to design their experiments. The combination will thus lead to much more effectivity and efficacy in the research and design process. The crossovers between research engineering (academia) and design engineering (industry) are often misunderstood. Figure 31.6 presents a joint incentive cycle between academia, government, and industry that should overcome misunderstanding between stakeholders.

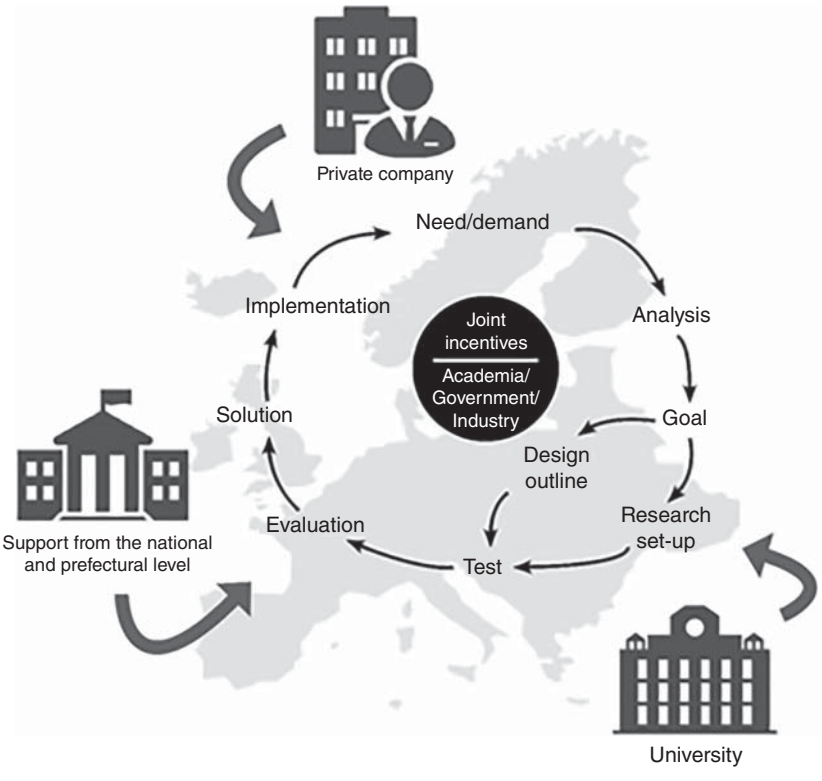

Figure 31.6 The relation between academia, industry, and government.

First off, the researchers connect to the outside world (holism) that needs and demands innovation. Secondly, the research design starts with identifying opportunities and problems in a real application environment, comprised of the analysis part. Consequently, the unitary goal can be formulated and the R&D department will test their positioned strategy from the design outline and research setup (reductionism). Test criteria usually evolve from the goal and experimental design. The evaluation (validity) phase assesses the application and acceptance of the research findings in a commercialized context. Ultimately, technologists provide and implement a solution to the need/demand of the society and meet the input requirements of stakeholders to their full extent.

31.5.2 Transition to the Biogas-based Green Economy

The transition to a biogas-based green economy requires nothing short of a revolution, transforming the society based on consumption into one based on sustainability and low carbon energy. Despite technological challenges and the pressure of global competition, European Union (EU) members can lower their GHGs in line with the European gas decree. Biogas-based economical trajectory combines innovations and knowledge across the full breadth of the biogas cluster to reframe the sustainability

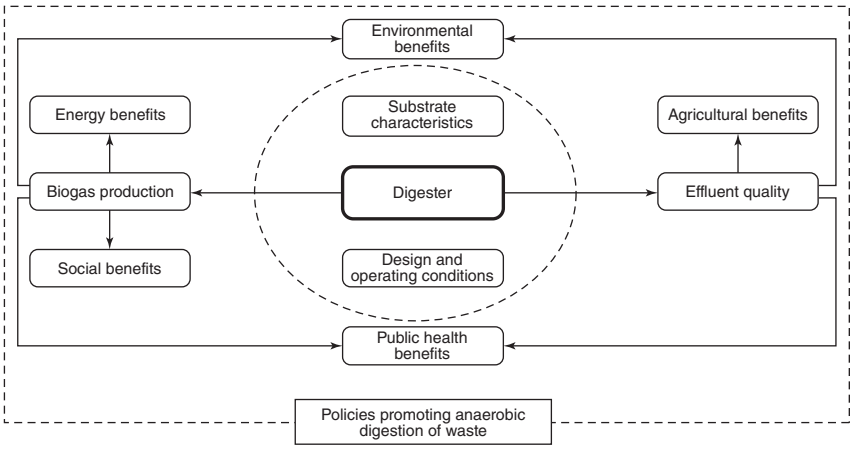

Figure 31.7 Multi-beneficial system context of the AD.

transition, in close cooperation with the gas sector [57]. The potential for technological improvements on the economic horizon is so significant and so comprehensive that it could redefine the biogas industry and help foster the integration of green power into the national energy system.

AD technology has been commercially demonstrated within Europe and is a crucial factor for European development as biogas provides the potency for sustainable bioenergy production. However, biogas economy is related to several technical factors such as waste availability, AD efficiency (digester performance), and end-product properties [58]. Interrelationships between the two major performance influencers of an anaerobic digester, design and operating conditions and substrate characteristics, and their influence on the effluent quality and biogas production induce various benefits in the fields of agriculture, energy, environment, public health, social, and political, which are depicted in Figure 31.7.

There is also a wide variety of biowaste with low cost and high availability that can be converted into biogas. The scenario of biogas production from biowaste types can save space for composting waste and provide clean energy, thus mitigating GHG emissions and waste. Substrate properties are important for the digester type selection, the quality and quantity of the biogas yield, and hence the project costs [59].

However, biogas-based engines are not yet developed enough to deal with the technical issues of biogas use. Aside, the nontechnical barriers vary significantly across each country. Poor or directly restrictive national legislative framework, not suitable to support the implementation and operation of biogas projects, lack of economic incentives (such as higher electricity tariffs, tax exemptions, etc.) hinder the development of biogas projects [60]. A transversal approach for policy integration is recommended to ensure that all relevant concerns are considered. European policy aims to trigger the incentives of the member countries on the direction of green mobility by establishing criteria for sustainable gaseous biofuels, such as the feed-in tariffs in Germany, the obligation certification for energy renewability in the United Kingdom, and the tax policy in Sweden [61].

According to the recently published European Biogas Association Biogas Report, [62], there are already more than 17 000 biogas plants in Europe and this number is continuing to grow making biogas-based electricity a high share of the electric power in Europe. Most of the AD plants in Europe process food crops, which are rich in glucose, xylose, mannose, arabinose, proteins, and lipids (e.g. maize, sugar beet, etc.), as feedstocks. However, the EU biofuel policy does not encourage their further use due to the negative impacts of crop-based biofuels on food production and land use [63]. Accordingly, lignocellulosic feedstocks, which are not involved in food production, provide more sustainable substrates for AD [64]. The biogas sector also has a pivotal role to play in repositioning the EU to thrive in a low-carbon future, and this kind of technical knowledge has to be shared at the European level. The implementation of biogas projects in many regions of Europe is required to spur the green economy.

31.6 Conclusion

Although the AD technology has been operationally demonstrated, there are knowledge gaps in the interaction between the microbial species and the operational parameters. A deeper understanding of the trophic species activities will allow the improvement of the AD technology as research studies have shown that anaerobic digestion performance is reliant on the dynamics of methanogens. The complex and widespread range of trophic species remains an issue of biotechnological applications. In addition, biogas projects are complex and require the involvement of many stakeholders as well as a supportive legislative and economic frame. Biogas production is a green technology, providing quantifiable benefits for the agricultural and energy sector, and a competitive solution in GHG reduction. Thus, sustainable engineering and biology have introduced novel concepts that can improve the AD performance and therefore promote new practices on environmental and economic development.

Conflict of Interest

The authors declare no conflict of interest.

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32

Microbiology of Biogas Production from Food Waste: Current Status, Challenges, and Future Needs

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32.1 Introduction

There is a growing energy scarcity and rising environmental concern owing to rapid urbanization. Hence, biofuels are gaining increasing global importance. Biofuels, including compressed biogas (CBG), are obtained from renewable biomass resources such as agricultural residue, municipal solid waste (MSW), distillery spent wash, sugarcane press mud, sewage treatment plant waste, cattle dung, food waste, food processing industry waste, forestry residues, etc. This ensures pursuit of a higher degree of national energy security in an eco-friendly and sustainable manner by complementing conventional energy resources and plummeting dependence on imported fossil fuels to meet the energy requirements of India's urban and massive rural population.

Advanced biofuels are fuels that can be produced from lignocellulosic feedstocks, non-food crops, or industrial waste and residue streams, which generate low CO_2 and greenhouse gas (GHG) emissions and are non-competing with food crops at terrestrial level. Fuels such as second-generation (2G) ethanol, algae-based third-generation (3G) biofuels, bio-compressed natural gas (bio-CNG), biomethanol, dimethyl ether (DME) derived from biomethanol, biohydrogen, drop-in fuels with MSW as the feedstock material can be entitled as "advanced biofuels."

The manifold advantages of generating CBG from food waste and MSW on commercial scale include – smart waste management, extra income source for farmers promoting rural economy and employment, upholding national responsibilities in accomplishing climate change goals, and saving on import of natural gas and crude oil.

The National Policy on Biofuels 2018 of India accentuates the active promotion of advanced biofuels, including bio-CNG. The goal of the policy is to enable

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availability of biofuels in the market via development of new technologies for conversion of waste to biofuels, thus creating a suitable environment for biofuels and its integration with conventional fuels. Under this policy, the Government of India (GoI) had launched the GOBAR-DHAN (Galvanizing Organic Bio-Agri Resources) scheme promoting conversion of cattle dung and solid waste from farms to bio-CNG and compost. This program intends to benefit households in identified villages with the help of gram panchayats. The Ministry of New and Renewable Energy (MNRE) has notified a central financial assistance of 4 crore/4800 kg of CBG generated from 12 000 m^3 of biogas/day with a maximum of Rs. 10 crore/project [1].

The estimated potential of CBG production in India from several sources is about 62 million tonnes/annum. The CBG network can be integrated with the city gas distribution (CGD) network by injection of the CBG into CGD pipelines for efficient distribution thus ensuring enhanced access to a quality and affordable fuel [2].

32.2 Fundamentals for Accomplishing National Biofuel Policy

Today's waste can be converted to tomorrow's energy. A large portion of the biomass produced can be converted into biogas which goes a long way in making a country self-reliant, help combat global warming, and ease climate changes.

The key drive of any national biofuel policy is to ensure accessibility of biofuels from indigenous feedstock. This can be achieved by creating a national biomass repository by conducting a biomass assessment across the nation. Research and development in major areas of feedstock production, newer feedstocks, and biofuels processing for various end-use applications need to be focused. Identification of locations with excess accessible biomass and generation of feedstock such as energy grasses and fast-growing crops by employing unutilized lands will play a critical role in encouraging industrial set up.

Surveys undertaken in India have anticipated a yield of 3000 crore liters of ethanol annually with surplus biomass availability to the tune of 120–160 MMT annually. Bio-CNG, which is one of the major by-products in 2G ethanol biorefineries and transport fuel, needs to be brought under offtake assurance by the public sector gas marketing companies. There is an estimated annual generation of 62 MMT of MSW in India, which has tremendous potential to produce drop-in fuels and generate power including refuse-derived fuel, biogas/electricity, and compost for agriculture [1].

It is essential to demonstrate on commercial scale, the existing technologies for transformation of waste biomass into drop-in fuels, bio-CNG, and biohydrogen. Such technologies would be modeled to meet energy demands in rural areas and address the environmental issues. Establishments of such plants for the production of advanced fuels need to be promoted with financial incentives such as GAP funding, subsidies, grant for biofuels, and offtake assurance. Prospects of generating carbon credits for the savings on CO_2 emissions on the account of biofuel feedstock generation and use of biofuels, in pure or blended form, may be explored.

It is essential to reinforce the current R&D centers and establish a network between the research organization, institutions, and industry for significant

application. Government needs to promote participation of the Industry in R&D and technology development including transfer of know-how to the Industry. Encouraging international collaboration for advanced biofuel research and capacity building is the need of the hour. Skill development to ensure availability of trained and skilled manpower is essential to meet the new demands of the biofuel industry. Manufacturing of equipment that are compatible with biofuels needs to be accomplished. It is vital for the leading companies to participate in distribution and marketing of biofuels certifying quality standards, consumer awareness about blending percentages, warranty requirements, etc. The pricing of biofuels can be encouraged with incentives. Coordination between states and urban local bodies (ULB) is necessary to track the availability of MSW feedstock for biofuels including urban areas.

32.3 Significances of Anaerobic Microbiology in Biogas Process

Anaerobically transforming the agro-industrial waste, agro-residuces, and both terrestrial and aquatic weeds into biogas and bio-compost results in sustainable energy security to a large population of a nation. There exists vast knowledge and experience regarding animal waste-fed biogas plants. However, low availability of animal waste can enable accessibility of biogas to only about 12–17 million homes in India. Hence, there is a need to explore agro-residues and agro-industrial residues as resources for the production of biogas. The inadequate scientific knowledge and technologies for the production of biogas from plant-based feedstock are the limiting criteria. This can be accomplished with better understanding of fundamentals of anaerobic microbiology, biochemical, and physico-chemical concepts of the entire process resulting in sustainable energy production. With growing population, there is a surge in generation of food waste. Disposal of food waste by means of landfilling, incineration, and composting results in the emission of GHG. Hence, anaerobic digestion (AD) of food waste by means of co-digestion with animal dung resulting in the production of biogas and by-products such as CO_2 and nutrient-rich manure is a sustainable management approach [3, 4].

32.4 Microbiology and Physico-Chemical Process in AD

The conversion of organic components in biomass feedstock by consortia of bacteria in the absence of oxygen to methane (biogas), carbon dioxide, and anaerobic compost takes place during anaerobic digestion. It is imperative to analyze the complex microbial biochemistry involved in anaerobic digestion to harness enhanced production of biogas. The production of biogas in anaerobic digestion is carried out by the following four key steps: (i) hydrolysis, (ii) acidogenesis, (iii) acetogenesis, and (iv) methanogenesis.

32.4.1 Hydrolysis and Acidogenesis

In the hydrolytic process, bacterial fermentation breaks down the plant-based biomass feedstock, rich in macromolecules such as carbohydrates (pectins,

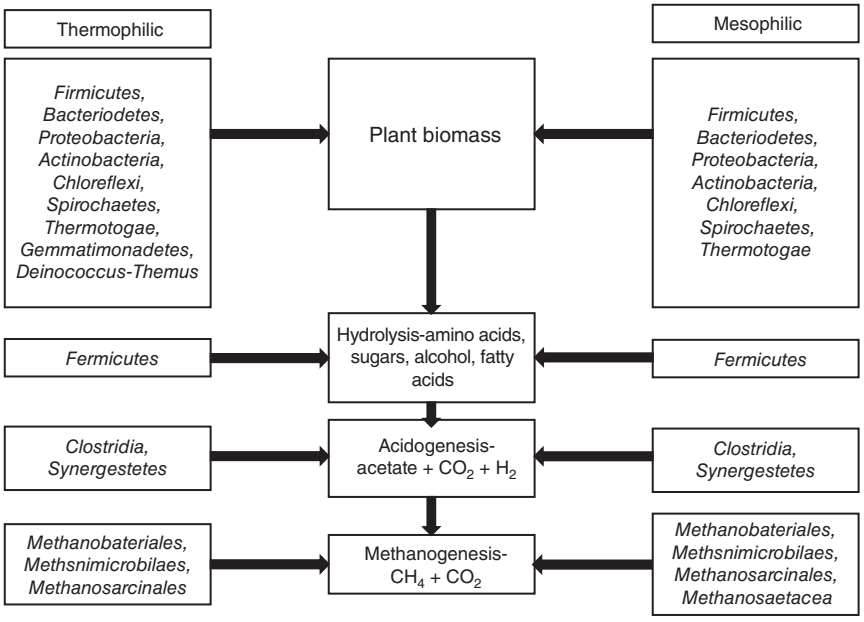

Figure 32.1 Microorganisms in anaerobic digestion of plant biomass under mesophilic and thermophilic condition.

hemicellulose, and cellulose), proteins and fats to sugars, amino acids, and long-chain fatty acids (LCFA) along with various other end products. Lipolytic enzymes produced by *Clostridia* and *Micrococci* convert lipids to LCFAs which is further degraded by ß-oxidation to produce acetyl CoA. The proteases produced by *Clostridium*, *Bacteroides*, *Fusobacterium*, *Butyrivibrio*, *Streptococcus*, and *Selenomonas* hydrolyze proteins to amino acids. *Campylobacter*, *Peptococcus*, *Clostridium*, *Bacteroides*, and *Selenomonas* degrade the amino acids to acetate, propionate, and ammonia.

The plant cell wall polysaccharide present in the biomass feedstock is hydrolyzed by enzymes produced by hydrolytic bacteria of *Firmicutes* and *Proteobacteria* as indicated in Figure 32.1. Hexose metabolism in anaerobic bacteria results in pyruvate and Nicotinamide adenine dinucleotide (NADH) via Emden-Meyerhof-Parnas pathway (EMP). Further fermentation of pyruvate generates lactate, propionate, acetate, and ethanol. However, sugars and amino acids also undergo acidogenic fermentation to produce low-concentration acetate and H_2. Hydrolyzing bacteria are the significant microbiome in degradation of plant-based feedstock [3] (Table 32.1).

32.4.2 Acetogenesis

During the process of biogas production, hydrogen-producing acetogenic bacteria are capable of producing acetate and H_2 from higher fatty acids which is a high free-energy-consuming process. H_2 generated during acetogenesis is known to inhibit the isolation and growth of acetogenic bacteria. *Syntrophobacter wolinii* and *Syntrophomonas wolfei* are, respectively, the propionate and butyrate decomposing

Table 32.1 Action of microbial enzymes on feedstock polysaccharides.

Substrate	Enzyme produced	Product
Cellulose	*endo*-l,4-Glucanases *exo*-l,4-Glucanases cellobiase ß-Glucosidase	Glucose
Starch	α-Amylases ß-Amylases Amyloglucosidases Debranching enzyme Maltase	Glucose
Pectin	Pectinase	Galacturonic acids
Xylans	α-*endo*-Xylanase α-Xylosidase	Xylose
Fats	Lipase	Fatty acids, glycerol
Proteins	Protease	Amino acids

bacteria cultured. These limitations can be overcome by co-culturing the acetogenic bacteria with H_2-consuming bacteria, such as methanogens and sulfate-reducing bacteria. In addition, there is breakdown of lactate and ethanol to acetate and H_2 by *Clostridium formicoaceticum* during acetogenesis [3].

32.4.3 Methanogenesis and the Essential Microbial Consortia

During methanogenesis, acetate, hydrogen, and carbon dioxide are utilized by methanogenic bacteria to generate methane. Methanogens can be classified as hydrogenotrophic, acetotrophic, and methylotrophic methanogens. Hydrogenotrophic methanogenesis (*Methanothermobacter thermautotrophicus*, *Methanosarcina thermophila*, and *Methanoculleus* sp.) involves utilization of H_2 and CO_2 for the production of methane as indicated in the following chemical reaction.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

Hydrogenotrophic methanogenesis are leading in energy crop digestion. Some of the bacteria belonging to this class are capable of consuming formate for the production of methane.

Acetotrophic methanogenesis is a primary pathway associated with methane generation from acetate by a few strains such as *Methanosaeta* spp. and *Methanosarcina* spp. as shown in the following chemical reaction.

$$CH_3COOH \rightarrow CO_2 + CH_4$$

Methylotrophic methanogenesis which involves the production of methane from the substrate methanol, a less common pathway

$$CH_3OH + H_2 \rightarrow CH_4 + H_2O$$

Anaerobic digestion with animal feedstock generally results in 60% methane and 40% carbon dioxide. As acetate and hydrogen form substrate for various other chemical reactions, the biogas yield in an anaerobic digestion is influenced by digester conditions, microbial consortia, and the substrate [3].

The decomposition in the digester halts when the concentration of methanogenic *Archaea* drops below the threshold value of the total consortia population associated with low biogas and accumulation of volatile fatty acids (VFA), respectively. Rod-like or coccoid hydrogenotrophic methanogens, *Methanosarcina* species are favored in thermophilic condition. Methanogenic *archaea* are weakest in food wastes, distillery wastes, etc. [3, 5].

About 70–75% methane is generated from acetate produced from the feedstock and the rest from hydrogen and CO_2. High hydrogen concentration can inhibit acetogenesis resulting in accumulation of fatty acids [6].

32.5 Pretreatment

Generally, pretreatment is given to feedstock rich in lignocellulose or keratinase as it causes complex floc structures of microbial biomass. Hence, degradation of such waste can be achieved by physical (heat, pressure), chemical (acid, base, ozonation), and biological (coculturing, enzyme addition) pretreatments. Such pretreatments facilitate biomass porosity-enhanced surface area for microbial degradation. But, certain pretreatments can result in the production of inhibitors such as furfural, vanillin, and other phenolic compounds [5].

32.6 Variations in Anaerobic Digestion

Hydrolysis of plant-based feedstock is delayed owing to the organization of complex cellulosic biomass. Recalcitrant lignin is known to be detached by alkali treatment. Beet silage-fed digester in mesophilic phase and continuous operation revealed bacterial consortia of *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Chloroflexi* in different percentages with increasing days of operation. The same reactor under thermophilic conditions had *Clostridia*, *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* in varying concentration with increasing days of operation. A continuously stirred tank reactor (CSTR) fed with straw was dominated by *Clostridia*, *Bacteroidetes*, *Acidobacteria*, *Deltaproteobacteria*, while the digester with animal waste feedstock recorded prevalence of other species, like *Ruminococcus* and *Cellulomonas*.

Clostridium thermocellum and *Clostridium stercorarium* were the prime microflora responsible for digestion of cellulose and hemicellulose, respectively, in maize silage in thermophilic phase resulting in ethanol, lactate, acetate, butyrate, and other short-chain products in addition to CO_2 and H_2 gas. Hydrogenotrophic methanogen, *Methanothermobacter thermautotrophicus* can be co-cultured with *Tepidanaerobacter* which hydrolysis lactate. The predominant microflora in grass

silage digester liquid during the first 30 days of digestion was *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Firmicutes*, *Actinobacteria*, and *Chloroflexi*. The leaf biomass feedstock floats due to the adhering biogas bubbles resulting in drying, ununiform distribution of bacteria and decreased decomposition rate which can be overcome in plug-flow-like digesters. The plant feedstock in an anaerobic digester undergoes degradation of pectin by *Proteobacteria* first followed by hemicellulose and cellulose degradation by *Firmicutes*. The initial VFA flux created during anaerobic digestion of biomass feedstock limits the enhanced conversion of VFA and thereby colonization by methanogens which needs to be prevented by innovative technologies [3].

32.7 Factors Influencing Biogas Production

The physico-chemical aspects of significance in the microbial process of biogas production are pH, temperature, feedstock composition, and microbiological factors such as inoculum, phages, etc.

32.7.1 Temperature

Both mesophilic and thermophilic temperatures prevail during anaerobic digestion. The microbial enzymes are significantly influenced by minor temperature fluctuation resulting in activation or denaturation of the enzyme. Gradual start up procedures and precautions to prevent heat loss in the mesophilic reactor are the limiting aspects. This can be overcome by extending the residence time to facilitate the maximum feedstock decomposition and use of solar enabled digester to warm the water used for mixing animal-based food stock [3].

A slow increase or decrease in temperature (±1 °C/day) is suggested to be ideal for the microbial adaptation. In thermophilic condition, it is noted that the shift is higher toward ammonia generation which has an inhibitory effect on biogas production [5].

Under thermophilic conditions, the methane production rate is about 25–50% higher than psychrophilic conditions, while the net sludge growth is about 50% lower. Thermophilic bacteria are more sensitive to temperature shocks than mesophilic bacteria. Temperature changes greater than 2° will reduce methane former activity, while acids are still forming. This results in losing the buffering capacity and possibly incapacitating the reactor. The best bacterial activity will occur in reactors operating at a constant temperature somewhere between 36 and 40 °C. Once the best temperature for the individual reactor is found, based on the highest gas production and ability to hold the pH near 7.0, that temperature should be held within ±1 °C [6].

32.7.2 pH

Fluctuation in feedstock can significantly influence the activity of microbial enzyme activity. The optimum pH range for the functioning of methanogenic bacteria was

found to be 6.7–7.4. Fruit industry waste and certain urban solid waste feedstocks are known to initiate high VFA flux with drop in pH which has an inhibitory effect on methanogenic bacteria [3]. The methane workers are inhibited when the pH falls below 4.2 in reactor operation. The amount of volatile acids produced and the alkalinity in the reactor control the pH of the liquid undergoing anaerobic digestion. If a large amount of readily digestible organic matter were added suddenly, excess amounts of acids would be produced and will lower the pH. When this occurs, the methane formers slow down; they cannot keep up with the acid formers, and volatile acids accumulate in the reactor. The pH values in anaerobic reactors are mainly the result of the presence of weak acids (carbon dioxide, VFA, hydrogen sulfide) and weak bases (ammonia), which buffer the wastewater [6].

32.7.3 VFA

Acetic, propionic, and butyric acids are the common VFA produced in the initial stages of biogas process in an AD. VFA levels above 6 g/l of acetic acid equivalent are known to decrease the functioning of methanogenic microflora. Beyond this level, the long-chain VFA was found to be detrimental to several bacteria and thereby limiting essential process in the anaerobic digester. Application of pretreatment methods was found to control VFA accumulation [3]. *Methanosarcina* was more efficient at higher concentration of VFA than *Methanothrix* which was important for granulation in upflow anaerobic sludge blanket (UASB) system. In a healthy reactor, the volatile acids of the digesting reactor content usually run in the range of 2500–3500 mg/l expressed as acetic acid [6, 7].

32.7.4 Microbial Consortia in AD

The structure of the microbial community varies in different biogas digesters, depending on parameters such as type of substrate, operational conditions, etc. However, abundance microorganism is considered to improve stability and to decrease the risk of disturbances of biogas process. Generally, the bacteria dominate the microbial community, whereas the methanogenic bacteria only represent a few percentages of the total microbial flora as shown in Figure 32.2 [8].

Generally, hydrolysis of the agro-residue and feedstock with minimal processing is the rate-limiting step in the AD process. The growth of acidogens was faster than methanogens. The methanogens dominating during short solid retention time (SRT) and long SRT were *Methanosarcina* and *Methanothrix*, respectively. The dry biomass feedstock has low key microbial inoculum as compared to the animal-based feedstock essential for the generation of biogas [3].

The microbial consortia in chicken droppings inoculum included *Campylobacter* spp., *Bacillus cereus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Rhizopus* spp., and *Aspergillus* spp., *Yersinia enterocolitica*, *Clostridium*, *Methanosarcina*, *Methanobacterium*, *Aspergillus*, and *Penicillium* species. AD in fabricated laboratory-scale biodigester with feedstock of chicken dropping, vegetable waste, animal waste, and fruit waste in the ratio of 1 : 1 : 1 : 1 generated

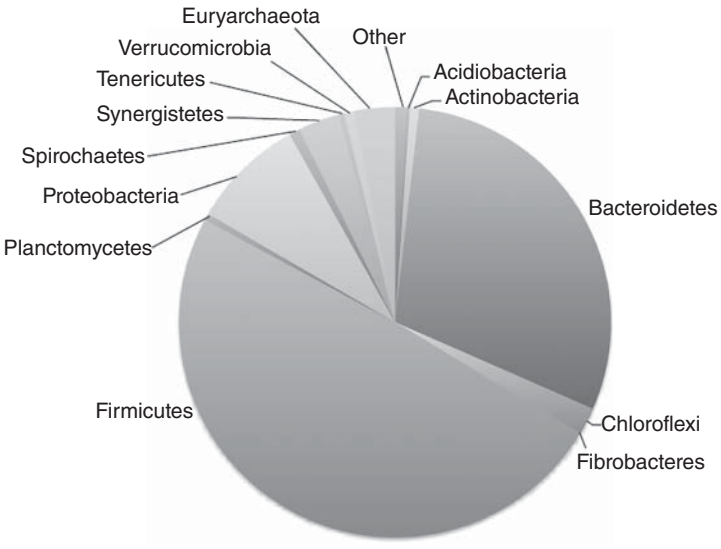

Figure 32.2 Microbial community in a typical biogas digestion system treating Sewage sludge, lignocellulosic biomass, and household food wastes.

highest volume of biogas (21 cm^3). This enhanced yield could be attributed to the availability of adequate moisture and nutritive content from fruit waste that is necessary for the metabolic processes of the microorganisms. It is reported that co-digestion of cattle dung feedstock in combination with cheese whey enhanced the production of biogas. Use of rumen fluid as inoculum in co-digestion of cattle manure with food waste increased the biogas yield by 24–47% over the control [9].

Enhanced production of biogas from lignocellulosic material can be brought about by phylum *Neocallimastigomycota*, an anaerobic fungus commonly present in ruminants [5].

32.7.5 Recirculation of Leachate

In a solid-state-stratified bed (SSB) reactor developed by Centre for Sustainable Technologies (CST), recirculation of leachate of the digester fed with dry biomass feedstock is necessary as the microbial consortia is low in the newly fed biomass. This facilitates initiation of hydrolysis and acidogenesis of the freshly fed feedstock and circulates the accumulated VFA to lower portion of the digester for transformation into biogas [3].

32.7.6 Ammonia

Protein digestion in an AD results in the generation of ammonia which could be inhibitory to methanogens when liberated in high concentration. *Methanosarcina barkeri*, *Methanobacterium thermoautotrophicum*, *Methanobacterium formicicum*, and *Methanospirillum hungatei* were the most sensitive. With favorable ambient

temperature in India, the concentration of ammonia and VFA can be comfortably handled in the biogas process [3]. Concentrations of ammonia between 1500 and 3000 mg/l can be inhibitory if the reactor pH is greater than 7.4 [6].

32.7.7 Feedstock Composition

32.7.7.1 Protein-Rich Substrate

The unionized ammonia produced during the digestion of protein is toxic to the microflora when released in high concentration. Hence, ammonia-tolerant microflora is suggested for digester that is responsible for methane generation from acetate via syntrophic acetate oxidation (SAO). Bacteria such as *Syntrophaceticus*, *Thermacetogenium*, *Tepidanaerobacter acetatoxydans*, *Clostridium*, and *Pseudothermotoga* can bring about SAO. Protein-rich food waste feed to digester is known to increase the population of bacteria of the families Caldicoprobacteraceae, Porphyromonadaceae, Lactobacillaceae, and Actinomycetaceae.

32.7.7.2 Lipid-Rich Substrate

Lipid-rich feedstock in the digester results in glycerol and LCFA. It is known to be one of the microbial inhibitors during AD. Biogas generation for pulse feeding oleate is recorded by hydrogenotrophic *Methanoculleus* and *Methanobrevibacter*. Mesophilic co-digestion of lipid-based feedstock can be met with bacteria, *Syntrophomonas*.

32.7.7.3 Carbohydrate-Rich Substrate

Carbohydrate-rich feedstock results in high C/N ratio for microbial physiology, low hydrolysis of lignocellulosic material, and fast acidogenesis from easily hydrolyzed carbohydrates. Metagenomic studies have revealed the role CAZymes (Carbohydrate-Active Enzymes) from microbiome acclimatized to lignocellulosic feedstock [5].

Co-digestion of food waste with cabbage and cauliflower leaves and stalks at C/N ratio 45 resulted in high biodegradability, a methane yield of 475 ml_{STP} CH_4/g VS, and an organic loading rate (OLR) of 0.06 kg of VS/m^3 h. An increasing C/N ratio in AD of dairy manure resulted in decreased methane production. Maximum methane production per unit loading rate was recorded when the C/N ratio of the feedstock was 25 [10]. Laboratory-scale studies in co-digestion of mixed fruit and vegetable waste and cow dung as substrates resulted in enhanced methane yield of 112.9 l in a semi-continuous stir tank over a period of 40 days [4].

32.7.8 Trace Element Supplementation

Acetogenic and methanogenic bacteria require trace elements such as iron, nickel, cobalt, tungsten, and molybdenum for methane generation. Low availability of these trace elements in feedstock from food waste and slaughter house waste may result in low yield of methane. The sulfide produced during protein digestion may form complexes with metals and result in decreased bioavailability of essential

trace elements to the microbial activity. Supplementation of trace elements has demonstrated promising effect on methanogenic population [5].

32.7.9 Environment/Alkalinity

A favorable environment for the microbiome in a digester can be maintained by proper mixing, preventing overloading, excess water, and temperature in the digester. The optimal volatile acid (VA)/Alkalinity (ALK) relationship ratio is 0.4, thereby resulting in good buffering capacity. The digester becomes sour when volatile acids increase and alkalinity decreases. Shift in VA/ALK ratio from 0.5 to 0.8 is associated with drop in pH (less than 6.7). Under this condition, the percentage of CO_2 increases with the inability of the digester gas to burn. Addition of bicarbonates to the digester is recommended to increase the alkalinity. The optimal AD results in 25–35% CO_2 and 65–70% methane by volume [11].

The process stability depends largely on the reactor's ability to resist a change in pH. This is commonly known as its buffering capacity measured as alkalinity. Buffers are essential in the reactors. During the digestion process, the methane workers also produce some buffering material, such as bicarbonates, carbonate, and ammonia, which goes into the solution. The amount of buffer produced at this stage is usually enough to balance the acid produced by the acid workers so that the pH will remain at a constant level. Reactors need to add a caustic material such as lime, soda ash, caustic soda, or agricultural ammonia to raise the alkalinity. The most important buffer system in anaerobic wastewater treatment is the bicarbonate buffer system. Another important buffer system is ammonia: the presence of ammonia (NH_3) shifts the pH to higher values, which will decrease the toxicity of H_2S and VFA, but the toxicity of ammonia increases with increasing pH. Therefore, a low concentration is beneficial (as nutrient and as buffer), but high concentration can be harmful [6]. The environment must be kept within extremely narrow ranges. The optimum conditions are (Table 32.2):

Anaerobic conditions	No oxygen (air)
Temperature	$36 \pm 2\,^{\circ}C$
PH	7.0–7.2
No toxic material	
VFA/Alkalinity	At least 0.50

32.7.10 Toxicity

The sensitivity for toxic compounds by anaerobic bacteria is always mentioned as the major drawback of anaerobic. The toxic compounds can be classified as –inorganic, natural organic, man-made organic (-antibiotic), and antibiotic toxins. Ammonium, sulfur, and high salt concentration are some of the examples of inorganic toxins. Natural organic compounds cause toxicity to AD microbiota by polarity and hydrogen

Table 32.2 Direction of process occurring in a bioreactor and simultaneously indicating possible process problems.

Indicator	Trend of graph	pH	CH_4	CO_2	Alkalinity	Volume
pH	Down	—	Down	—	—	—
CH_4%	Down	Down	—	Up	Down	Up
CO_2%	Up	—	Down	—	—	—
Alkalinity	Down	Down	—	—	—	—
Volatile acids	Up	—	Down	—	—	—

bonding. The furfurals produced during caramelization of sugarcane are found to be toxic to methanogenic bacteria. The bacteria can tolerate between 50 and 100 mg/l of soluble sulfide with little effect [6].

32.8 Application of Metagenomics

Anaerobic digestion is associated with intricate microbial diversity. Isolation of certain microflora in biogas digester through cultural techniques cannot be accomplished owing to the presence of non-culturable microbes. Hence, molecular characterization and metagenomic tools can aid in the insight of anaerobic digester microbiome. Such advanced techniques can identify the pathway and microbiome needed for transformation of feedstock to biogas. High-throughput sequencing data along with bioinformatics analysis play a crucial role in characterization of microbial metagenome. This in combination with artificial neural network is anticipated in a big-data-based precision fermentation with enhanced biogas yield [12].

Molecular biology techniques applied to explore the relationship between microbial diversity and biogas generation included DNA extraction and quantification, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), analysis of DGGE profiles, PCR amplification for illumina next-generation sequencing (NGS), and its data analysis. The prevalent phyla were *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria*. Fruit waste being rich in carbohydrates hydrolyzed rapidly with acid formation and generates CO_2 as it lacks the microflora essential for methanogenesis. The DGGE and NGS data revealed the presence of *Actinobacteria* in cow dung. The genus *Syntrophomonas* capable of hydrolyzing long-chain organic acids to acetate and propionate was dominant in co-digestion. *Firmicutes* in cow dung and food waste plays a key role in degradation of cellulosic material. The PCR-DGGE results indicated maximum microbial diversity in co-digestion as compared with mono-digestion of cow dung and food waste with predominance of methanogens (*Methanosaeta* and *Methanosarcina*). The functionality of microflora in AD is imperative for enhanced generation of biogas from the feedstock [4] (Table 32.3).

Table 32.3 Metagenomics insights into microbial ecology during anaerobic digestion of diverse feedstock.

Feedstock	Metagenomics tool	Microbial community	AD condition	References
Food waste	High-throughput 16S rRNA gene sequencing Shotgun metagenomics sequencing	*Coprothermobacter proteolyticus*, Genus *Thermacetogenium*, *Dictyoglomus thermophilum*, *Thermodesulfovibrio yellowstonii*, *Syntrophomonas wolfei*, *Anaerobaculum* spp.	60 °C	[13]
Cow dung, mixed fruit and vegetable waste	PCR-DGGE High-throughput sequence analyses	*Methanosarcina*, Phylum *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, Class *Bacteroidia*, *Clostridia*, Genus *Syntrophomonas*, *Methanobrevibacter*, *Methanobacterium*	Co-digestion Semi-continuous stir tank reactors, 30 °C	[4]
Municipal sludge Industrial wastewater	DNA Extraction and Illumina Sequencing	*Methanothermobacter*, *Methanospirillum*, *Methanobrevibacter*, *Thermococcus*	—	[14]
Food waste Dairy cow manure	16S rRNA gene sequencing	*Chloroflexi*, *Methanosaeta*, *Methanothermobacter*, *Thermotogae*, *Firmicutes,* and *Synergistetes* *Methanobacterium* and *Methanosaeta Methanothermobacter*, *Thermotogae*	Mesophilic digestion Thermophilic digestion Mesophilic co-digestion Thermophilic co-digestion	[15]
Organic fraction MSW, maize silage and cow dung waste, mixed waste, brewery waste water	Metaproteomics	*Bacillales*, *Enterobacteriales*, *Bacteriodales*, *Clostridiales*, *Rhizobiales*, *Thermoanaerobacteriales*, *Methanobacteriales*, *Methanosarcinales*, and *Methanococcales*	UASB fermenter, Mesophilic and Thermophilic temperature	[16]
Cattle manure	High-throughput shotgun sequencing Novel binning strategy	*Phylum Firmicutes*, *Proteobacteria* and *Bacteroidetes* *Eubacteriaceae*, *Syntrophomonadaceae*, Class *Clostridia*, Genus *Methanoculleus*	CSTR at 54 °C	[17]

32.9 Conclusions and Future Needs

The bioconversion of food waste to biogas is known for many decades, but the acceptability is still limited, due to lack of reliability and robustness in the anaerobic digestion process. This is mainly due to limited and poor understanding of microbiology of anaerobic digestion, which is one of the most important aspects to facilitate the proper operation and control of the anaerobic digestion process. Further, there is also undesirable-unavoidable issues like variation in food waste composition due to seasonal, geographic, and temporal variations along with slower degradation rates, lower biogas gas yield (<100 g/kg biomass/day), and higher footprint area (<3 kg/m^3/day). Further, huge operational and maintenance problems have been continuously observed and reported in biogas plants. Hence, there is a need of performance evaluation of the existing operational plants of multiple configurations (Plug Flow Reactor/mixed) with reference to various parameters such as linkages b/w input waste characteristics, reactor configurations, and microbial communities on the system output. Microbial ecology and microbial interactions in anaerobic digestion need to be rigorously assessed using both conventional and emerging tools like metagenomics. Developing insights into kinetics of digestion of multi-substrates, inhibition levels and interactions, and understanding the rate-limiting steps and evolving strategies to overcome the same is also the need of the hour. The development of a suitable mathematical model to describe the functional relationship between input and output variables of the bioreactor system is also desirable for better process control. The stoichiometric and kinetics analysis of biogas formation via development of a suitable mathematical model to describe the functional relationship between input (substrate, microbial consortia, detention time, temperature, pH, hydrodynamic, etc.) and output variables (biogas yield, quality) of bioreactor system along with transient analysis of the system with response to various perturbations will also be needed to establish the stable operation and control of anaerobic digestion systems.

List of Abbreviations

2G	second generation
3G	third generation
AD	anaerobic digestion
ALK	alkalinity
CBG	compressed biogas
CDG	city gas distribution
CST	centre for sustainable technologies
CSTR	continuously stirred tank reactor
DME	dimethyl ether
EMP	Emden-Meyerhof-Parnas pathway
GHG	green house gas

GoI	government of India
LCFA	long-chain fatty acid
MMT	million metric ton
MNRE	ministry of new and renewable energy
MSW	municipal solid waste
NADH	nicotinamide adenine dinucleotide
NGS	next-generation sequencing
ORL	organic loading time
PCR-DGGE	polymerase chain reaction-denaturing gradient gel electrophoresis
SAO	syntrophic acetate oxidation
SRT	solid retention time
SSB	solid-state stratified bed reactor
UASB	up flow anaerobic sludge blanket system
ULB	urban local bodies
VA	volatile acids
VFA	volatile fatty acids

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Part IX

Green and Sustainable future (Zero Waste and Zero Emissions)

33

Valorization of Waste Cooking Oil into Biodiesel, Biolubricants, and Other Products

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33.1 Introduction

Reuse of effluents from an industry as affluent for another is always more economical and contributes to circular economy. Throughout the world, cooking methods tend to utilize immense amount of vegetable oil. The used vegetable oil (UVO) can be reprocessed and recycled for different industrial purposes. When obtained from biological origin, diesel and lubricants are found to be renewable, biodegradable with lower combustion rate and emit limited greenhouse gas (GHG). Vegetable oil has been used for lubrication of machines, equipments, and devices since long time [1]. Vegetable oil has unique physiochemical properties which include higher viscosity indices and improved lubrication property and it is much more durable and economical in nature due to lower friction among the machine parts and higher flash points [2].

Waste cooking oil (WCO) is generated from vegetable oils namely coconut, sunflower, soyabean, palm, cottonseed, rapeseed, and olive oil [3]. There are around 350 oil-bearing crops, and high-oleic vegetable oils (HOVOs) are more suitable for the production of biolubricant [1]. Yet, the use of virgin vegetable oils (VVOs) as lubricant feedstock is seen to create certain controversial issues, which include lubricant versus food/feed competition, the emission produced due to GHG, and the change in land use. These concerns can be addressed using WCOs which are usually two to three times lower in price than the VVO and can effectively decrease biolubricant cost [4].

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Vegetable oils primarily consist of triacylglycerols (TAGs, 92–98%), polar lipids (phospholipids and galactolipids), monoacylglycerols, diacylglycerols, and trivial quantity of free fatty acids and polyisoprenoids [5]. During the frying of vegetable oil (160–200 °C), numerous physical and chemical reactions occur and toxic compounds are formed through oxidation reactions, hydrolysis, and polymerization of TAGs [6]. Physical parameters like viscosity, color, and surface tension of the oil will also change after the process of deep frying. Also the food that is fried releases water into the oil. Once the frying process takes place in an open air environment, the structure of vegetable oil will be changed by oxidation reaction and produces hydrogen peroxide which will be further oxidized into toxic products like 4-hydroxy-2-alkenals [7].

While producing biodiesel and biofuel from vegetable oil, various things should be considered like cultivating immense amount of oilseed crops. The compounds present in WCOs are found to be mutagenic, carcinogenic, neurotoxic, and hepotoxic [8]. However, the WCOs can be wisely used, so that it will be not only economical but also good from the ecological point of view. Reprocessing and reutilizing will be important for saving the environment. Biodiesel has the potential to supplement or even to replace the fossil oils. But, the cost of production for biodiesel is always higher than that of petroleum diesel, since the biodiesel production is from edible vegetable oils which are expensive [9]. The feedstock of biodiesel is impacted by not only current land usage but also previous land usage pattern. The requirements of both the quantity and quality of biofertilizers for the production of these oil-bearing crops depend variably on different geographical locations. These various factors eventually sum up to stand as a barrier in the way of using vegetable oils for biodiesel or lubricant production. Hence, utilizing WCOs will be advantageous.

33.2 Treatment

After treatment and reprocessing, WCO can be conveniently used for various industrial purposes like production of biofuels and biolubricants, as additives for asphalts, and can also be utilized as animal feed [10–12]. The currently used protocol for the production of biodiesel is alkali-catalyzed transesterification of triglycerides, and the obtained biodiesel will have low molecular weight alcohol and this process is operated in batch mode. This process is preferable due to its efficiency and lower corrosive nature than the acid catalyzed transesterification. Acid catalyzed transesterification is a faster reaction and occurs in the presence of lower amount of catalyst. But, alkaline catalysts are most preferred, for example NaOH, KOH, $NaOCH_3$, etc. The alkali-catalyzed process also has a few hindrances like glycerol separation, sensitivity to the impurities of lipid feedstock, large reaction time, and complex biodiesel purification steps [13].

33.2.1 Chemical Treatment

The UVO is subjected to water treatment while the waste water is cleared. The utilized degummed oil is pushed through a pressure pipeline and subjected to a

filtration process. The WCO is separated based on its boiling point. The raw material is subjected to a few common processes like subjecting to an unpolished filtration to remove solid contaminants and later heating under vacuum. At a constant reduced pressure, distilled crude oil is purified from the volatile fraction which results in refined oil. The quality of the final product of the recycled oil depends not only on the quality of the WCO but also on the special configuration of the machines.

33.2.2 Microbiological and Biotechnological Treatment

Utilized vegetable oils have the potential to be used as low-cost and copious substrates for the microbial growth and metabolite production. This process will help to reduce the production costs of valuable compounds and also becomes environment-friendly. According to various studies, it is seen that oxygen has a vital role in the biosynthesis of microbial lipids from oils and fats. Meanwhile, if a water-immiscible substrate is used, oils can coat the gas–liquid interface with their layer and reduce the oxygen mass transfer to the culture medium [14].

33.3 Evaluation of Waste Cooking Oil and Valorized Cooking Oil

Increasing demand and huge consumption of biofuels and the lesser availability of raw materials for their manufacturing are motivating factors for the exploration of substitute methods and raw materials. Utilized vegetable oil which is produced as effluent in food chains and outlets worldwide in huge quantities need to be managed skillfully. Biodiesel which is also known as "green fuel" is a perfect alternative for petroleum as discussed earlier. Although on survey it was seen that the physical and chemical properties of waste vegetable oil varies from fresh edible vegetable oil in that waste oil exhibits a higher free fatty acids and moisture content than fresh vegetable oil owing to the thermolytic, oxidative and hydrolytic reactions occurred during frying. Different polymerized triglycerides are found in the WCO [15].

World's largest chain of fast-food restaurants, hotels, food suppliers, and food industries serves around 68 million clients daily in 119 countries across 35 000 outlets. Around 90% of the WCO produced is utilized as after use for various industries. The WCOs from the food outlets are collected, recycled, and put back into distributor's trucks to fuel their distributions. Utilized vegetable oil is warehoused at the restaurants in a particularly designed ampule and then utilized for delivery trucks to power the distribution fleets and are also sold to other companies to reduce their transportation impacts [16]. The post-processing techniques are essential which point toward the steps after the transesterification reactions which are performed to purify the biodiesel in accordance with the existing rules and regulations. Even though the final product depends a lot on the kind of feedstock utilized, researchers have also concluded that the functioning temperature is a foremost prevailing factor in the descent of the characteristics of fuel produced [17].

33.4 Versatile Products as an Outcome of Valorized Waste Cooking Oil

33.4.1 Biosurfactants and Liquid Detergents

Biosurfactants are compounds exhibiting surface activity and properties of reduction in interfacial tension. Biosurfactants possess a hydrophilic and hydrophobic group imparting them with amphiphilic character and governing their orientation activity at the solid–liquid, liquid–liquid, or liquid–gas interfaces. They are commonly obtained from biological sources. Owing to their surface-active properties, they have been used in versatile domains of waste treatment protocols comprising of elimination of heavy metals and removal of oil spills from natural land and aquatic resources. Their properties of foam generation, emulsion stabilization, and high wetting will help in their waste treatment functions [18].

Surfactants can be produced by chemical or synthetic means. Owing to the complex procedures involved in the manufacturing of chemical surfactants and their complex remediation measures, various biological means have been adopted to produce eco-friendly surfactants. One of such methods is the use of microbial organisms belonging to *Candida*, *Rhodococcus*, *Bacillus*, and *Pseudomonas* genera to produce biosurfactants in varied controlled environmental conditions. These biosurfactants exhibit higher cleansing action of high specificity with added benefits of biodegradability and ease of remediation [19].

The WCO- and agriculture-derived wastes have been found to be commonly explored sources for biosurfactant generation using microbiological methods. Different microbial strains utilized for biosurfactant production using WCO as the substrate include *Pseudomonas aeruginosa*, *Pseudozyma aphidis*, *Candida lipolytica*, and *Bacillus*. In order to optimize the quality and yield of biosurfactant production, several optimization strategies utilizing models like Plackett–Burman design and Taguchi have been adopted [20].

Biosurfactant production trials involving vegetable oils as carbon sources for fermentation have indicated that they prove to be most economical substrates for generating biosurfactants utilizing the aforementioned microbial species. The desirable microbial strains effective in catalyzing the conversion of oil to biosurfactant can be screened using different advanced techniques. The screening of six microbial strains for the generation of biosurfactant from coconut oil was found to indicate that the *P. aeruginosa* D will yield maximum amount of rhamno lipid homologs, mainly the *mono rhamnolipids* and *di rhamnolipids* [21].

There are several means to utilize the WCO. Among those, one of the effective means of remediation of WCO is its utilization to produce liquid soaps or detergents. Surface-active agents containing suitable functional groups in combination with amines have been used for the conversion of cooking oil into chemical functionalities with cleansing activity and detergency. Furthermore, the rich fatty acid contents of WCO have been explored as raw materials for biodetergent generation using alkaline hydrolysis treatment [22].

33.4.2 Green Chemical Lubricants

The presence of fatty acids and oleochemicals in WCO forms a rich source for generation of versatile green chemicals. The WCO can be converted into a series of industrially viable chemicals and lubricants exhibiting biodegradable and environment-friendly properties. Several sophisticated extraction techniques have been designed to utilize the different types of WCOs in order to generate value-added chemicals [23].

Lubricant comprises of base oil which imparts it with desirable property of lubricity, viscosity, and biodegradability. On the basis of base oil composition, lubricants may be classified into classes like synthetic lubricants, mineral lubricants, and biolubricants. Mineral lubricants exhibit properties like low viscosity and anticorrosion properties, but they are non-biodegradable in nature [24]. Hence, there arises a need to identify resources to synthesize biolubricants from natural sources with comparable lubrication properties, but suitable biodegradation profile.

33.4.3 Biodiesel Production

Several biological sources like fats and vegetable oils have been explored as feedstock for biodiesel generation. Biodiesel shows distinct advantage over petroleum diesels in terms of biodegradability. Biodiesels contribute to extensive reduction in toxic emissions and emission of pollutant particulate matters. The use of biodiesel provides the highest reduction in pollution issues without compromising on heat of combustion values. Vegetable oils can be used as sources for biodiesel production after effective modifications in composition using different processing strategies [25]. Some vegetable oil properties like high viscosity, and variable composition of fatty acids, make direct use of vegetable oil as biodiesel inappropriate. Some chemical transformative steps like transesterification aid to lower the viscosity of vegetable oils making them more convenient to use as sources of biodiesel [26].

33.4.4 Microbial Lipids

Microbial oils are mainly comprised of TAGs and neutral lipids. Microbial oils are enriched with essential fatty acids mainly polyunsaturated fatty acids belonging to omega-3 and omega-6 class. Also, known as single cell oils (SCOs), microbial oils can be used as a good source of food supplements. Microbial lipids are produced and stocked as intracellular components in lipid body structures during the growth phase of microbial species like *Yarrowia lipolytica*, *Pseudomonas*, and *Bacillus* [27]. The WCO has been extensively explored as a feedstock for the metabolism by microbial strains for effective conversion of the lipidic contents of oil into organic acids and enzymes with versatile industrial utilizations and applications. The mechanism of lipid production in each type of microbial strain may vary depending on the inherent metabolic processes in the microbial strains. In the model strains of yeast like *Y. lipolytica*, the intracellular production of lipids is mediated by hydrolytic activity of lipase enzymes on the oil infused in yeast media [28].

The lipid composition may vary from unsaturated fatty acids to saturated fatty acids depending on the stage of microbial growth. The lipid content production can be enhanced using different methods such as metabolic engineering, supplementation of medium with emulsifiers, surfactants, phosphorus, and magnesium ions. Furthermore, dispersed waste vegetable oil in water can be used as raw material for emulsion preparation which can be utilized by microbial consortium of *Trichosporon gracile* (fungus) and bacteria to generate versatile microbial lipids [29].

33.4.5 Vitamins and Nutraceuticals

Carotenes play an important role in physiological functions. Studies on carotene have demonstrated that carotene finds wide applications in strengthening immunity and osteoporosis and exhibits anticancer activities. Chemically, carotenes are isoprene derivatives, mainly tetraterpenoids. Commercially, carotene can be produced by microbial processing of hydrolyzed mung bean waste flour, grape juice, cheese, and molasses. Different strains of yeast, bacteria, and fungi can be used for carotene production. Vegetable oils such as corn oil, sunflower oil, coconut oil, and olive oil can also be used as feedstock material for microbial carotene synthesis. However, the high manufacturing costs involved in carotene production using vegetable oils make this process less feasible for commercial applications. The abundance of WCO and the feasibility of using WCO for carotene production make it a prospective feedstock material. The WCO comprising soyabean oil, cottonseed oil, and corn oil can be used as a raw substrate material for metabolic action of *Blakeslea trispora* (mold) for getting carotene as a by-product [30].

Different vitamins play an important role in the physiological development of human body. Currently, research is being conducted on synthesizing these vitamins at pilot scale in order to fulfill the demands for supplementation. Vitamin B_{12}, one of the vitamins of B complex group, can be synthesized with the help of certain microorganisms. Synthesis of Vitamin B_{12} by fermentation is costly, produced in small amounts, and difficult to separate. Synthesis of food grade vitamin B_{12} in the fermentation of microbial strains like *Pseudomonas* and *Propionibacterium* is accompanied by the production of chemicals like acetic acid and propionic acid which accumulate and inhibit the cell growth thus adversely affecting the production of vitamin B_{12}. To overcome the cost involved in vitamin B_{12} synthesis, cheap feedstocks like sucrose, whey, molasses, and waste sunflower cooking oil can be used in the fermentation of *Propionibacterium freudenreichii* [31].

33.4.6 Biopolymer Synthesis

Plastics as synthetic polymers mainly comprise of polyethylene units. The commonly used plastics for packaging and storage comprise of low-density polyethylene (LDPE) and high-density polyethylene (HDPE) . The mechanical strength of plastics can be modulated by altering the content of polyethylene. Polyethylene is mainly obtained from petroleum which is a nonrenewable source. Because, petroleum is not able to degrade and the sources are limited, there arises a need for renewable

natural sources for plastic synthesis. Polyethylene obtained from natural sources is known as bio-based polyethylene and less contributes to environmental pollution owing to degradable nature. Relatively, lower greenhouse emissions have been observed by the use of bio-based plastics [32].

One of the ways for producing plastics with biodegradable properties is mixing natural biodegradable monomeric units with plastic precursors such as polyethylene. Presence of biorenewable materials in raw substrates can aid to alter the desirable extremes of processing conditions for plastic synthesis. Processing temperature of injection molding is one such condition which can be lowered by the addition of biorenewable monomeric units thus aiding to lower the energy consumption. Waste vegetable oil can be used to generate hydroxylated biorenewable biopolymer using appropriate catalyst systems and processing conditions. Furthermore, WCO like waste soyabean cooking oil can be processed by the addition of suitable chemicals such as maleic anhydride for the synthesis of new polymers with desirable properties [33].

33.4.7 Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHAs) are polymers produced by certain microorganisms when subjected to stress conditions like nutrient deficiency. PHAs are aliphatic esters with high molecular weight and excellent biodegradation profile and exhibit use as bioplastics. PHAs can be metabolized by microbes using enzymes like lipases and esterases. Based on the number of monomers units and chain length, PHAs are classified into short-chain length polyhydroxyalkanoates (SCL-PHAs) and medium-chain length polyhydroxyalkanoates (MCL-PHAs). Palm oil and other carbon sources have been used by microbial species to produce PHAs [34].

The feedstock containing combination of xylose, glucose, and fatty acid can be utilized by *Escherichia coli* for the production of PHAs. Industrial synthesis of PHAs involves the use of costly raw materials. The WCOs like palm oil, corn oil, soybean oil, sunflower oil, and coconut oil with their high saturated and unsaturated fatty acid content serve as excellent carbon sources for the synthesis of PHAs. Microbial strains of *Pseudomonas*, *Bacillus*, *Klebsiella pneumoniae*, and *Cupriavidus necator* are commonly used for PHA synthesis from WCO. The PHAs are produced as intracellular granules and exhibit versatile uses in diverse fields like food industry, tissue engineering, etc. As raw material substrate for PHA synthesis, the WCO is found to be a prospective starting material as compared to sugars in terms of commercial viability. The yield of PHAs is affected by the source of cooking oil and can be enhanced by genetic manipulations. High yield of PHAs can be obtained using WCOs with saturated fatty acids like palmitic acid [35].

33.4.8 Feedstock for Microbial Processes

Some of the desirable properties for fermentable raw materials to be used as input for the generation of industrially viable products are the percent content of carbon and nitrogen, ease of processing and disinfection, economical viability, easy availability,

and complete convertability into desirable endproducts. The abundance of WCO and the chemical composition makes it a preferred feedstock for fermentative breakdown by selected microbial strains. The conversion of WCO into intermediates and by-products is mediated by enzymatic hydrolysis preferably by enzymes like lipases. Microbial strains of *Aspergillus*, *Candida* (*C. lipolytica*, *C. bombicala,* and *C. utilis*), and yeast (*Y. lipolytica*) have been commonly utilized for the enzymatic hydrolysis of oils to obtain value-added products like biosurfactants, fatty acids, and enzymes [36].

33.4.9 Bioasphalt

Bituminous asphalt binder is produced from crude oil. The generation of bituminous asphalt binder is costly, and the decreasing supply of crude oil has led to the need for the synthesis of other binders as replacement for traditional asphalt binder. Asphalt binder produced using green sustainable technology is known as bioasphalt. The supplementation of WCO to asphalt binder produces bioasphalt binder with altered properties of viscosity, and softening. Increasing the amount of WCO is helpful in lowering the viscosity and enhancing the penetration property of asphalt binder [37].

33.4.10 Bioplasticizers

Plasticizers have been frequently employed in polymer industries as an important part of synthesis reaction. However, the use of versatile plasticizers is restricted owing to their accumulation in the environments due to low biodegradability profile. This is mainly true for plasticizers like phthalate esters. Recently, bioplasticizers or plasticizers produced from natural sources are being widely explored. Several raw material sources for generating bio-based plasticizers are being scrutinized. Different vegetable cooking oils like castor oil, soyabean oil, etc., have been reacted and derivatized to yield acetylated, methyl-epoxylated, and amyl-epoxylated oil derivatives. However, the high costs of the oils have restricted their use. Hence, the epoxidation of WCO has been attempted to overcome the issue of environmental accumulation and contribution to pollution [38].

33.4.11 Biosolvent

The chemical composition of WCO imparts it with solvent properties for certain organic molecules. The property of certain classes of WCOs to solubilize volatile hazardous organic compounds and hydrocarbons can be exploited by designing effective bioreactors enriched with processed WCO and capable of entrapping and degrading hazardous volatile molecules, emission fogs, and hydrocarbons using suitable microbial strains [39].

33.5 Conclusion

One side, the growing global burden of environmental pollution caused by abundant production of WCO and on the other side natural oil resources depletion can be

tackled utilizing advanced scientific strategies. The rich fatty acid content of WCO makes it amenable to be a feedstock for various biotechnological processes which can yield advanced bio-based substances like biosurfactants, biofuels, biopolymers, and microbial lipids. Use of biosurfactants, biopolymers, and other such derived products help to lower the pollutive nature of synthetic chemicals making the process environmental-friendly. The ease of availability of WCO also helps to lower the high costs incurred in producing biodegradable bio-based fuels and chemicals thus making the process commercially viable. Thus, smart utilization of WCO can aid to lower the burden of waste in environment and provide industrially viable bio-based chemicals with advanced properties and versatile advantages.

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Agri and Food Waste Valorization Through the Production of Biochemicals and Packaging Materials

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34.1 Introduction

The worldwide shift from a linear economy model to a circular one during the production and consumption of various commodities has placed greater emphasis on recycle and reuse, in a bid to ensure that human development in all walks of life can learn and follow the principles of nature. Traditional agriculture and livelihood relied on the local use of agri-food wastes (AFWs), in enriching the fertility of soil and used solar energy to dry agricultural wastes to derive energy from them, as household fuel. These infact can be considered as the earliest efforts of mankind in valorization, i.e. converting waste to valuable resources. Modern agricultural practices with increased land holdings, centralized handling and processing of raw produce have brought with it the problem of AFW that now needs to be managed in a more efficient way as compared to the days of traditional agriculture.

The problem in itself and the economic implications of these AFW has been realized, researched and debated only since the last decade or so, especially with reference to international efforts in addressing the problem. The FAO estimates, about a third of the world's food supply being wasted, was an eye-opener [1] which was later corroborated by FUSIONS, a European Union Research Project which pegged the per capita food wastage at a whooping173 kg/person/year [2]. Apart from the socioeconomic impacts, this wastage is also associated with environmental costs as well, with AFW presenting global environmental problems. Annual smogs seen while burning crop stubbles contribute significantly to carbon dioxide emissions. This practice of burning agricultural wastes is equally common in the developed [3] as well as the developing world. A significant amount (88%) of the carbon dioxide produced in the course of worldwide agricultural activities comes from crop residues and the rest 12% from the process of burning [4]. Apart from carbon dioxide, the emission of other greenhouse gases like methane and nitrous oxide has also been traced partly to AFW [5].

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A notable difference is that in developing and not-so-rich countries, wastage is common at the preliminary stages of handling like production, transport, storage, and processing due to the lack of proper infrastructure and technology, whereas in medium and rich countries, food is wasted at the final stages, i.e. at distribution and household levels, nevertheless contributing in almost equal proportion to the total world food wastage. The causes of wastage vary across countries and sectors. Although industrial level agri-food wastage is less, it offers a convenient model to work out strategies to address the problem, since the sources and types of wastage are homogenous and predictable [6]. Further, more often than not, handling these wastes where they are generated makes perfect economic sense instead of transporting to a centralized place [7].

34.2 Importance

The three strong reasons why valorization of AFW is important can be summarized as:

- The ever-increasing population is putting undue pressure on our agriculture and farming systems to address food shortages. Hence, preventing food wastages is imperative [8].
- Food wastes also mean the wastage of precious resources used at all stages of production [9] which could be at times as high as 23% [10] in some countries.
- Food wastes also lead to environmental concerns by generating greenhouse gasses [11] because of the high organic content of the residues and effluents as they undergo uncontrolled decomposition.
- Thus, valorization is equally important both for the industry and the environment. This also makes the agri-food sector more responsible, sustainable and profitable.

Agri-food wastage can be classified as avoidable, probably avoidable and unavoidable waste [12]. These can be utilized as substrates for recovery of biochemicals, bioplastics, or for the production of renewable or recoverable energy. These come under valorization (the recovery of beneficial compounds, products, or energy). AFWs can also end up as landfills which will be classified as non-valorizable means [13].

In order to address the problem of food waste, it is important to prioritize solutions according to a suggested waste hierarchy [14] with prevention, reuse, recycle, recover and disposal being given preference in that order. Imbert [15] has analyzed each of these in an elaborate manner.

34.3 Worldwide Initiatives

Researchers have been working on the problem of food processing with respect to the environment since the 1990s [16]. However, concerted institutional effort toward valorization of wastes is a relatively new development.

One of the earliest worldwide initiatives can be traced to AWARENET, the Agro-Food Wastes Minimisation and Reduction Network, targeting the primary

sectors of meat, fish, dairy, wine and vegetables at the European level [17]. Three main issues that were supposed to be addressed by this initiative were regulatory issues, technological aspects, market potential and requirements of agro-food industrial wastes to propose a global R&D strategy.

REFRESH is a European Union H2020 funded research project involving 26 partners from 12 European countries and China with the objective of significantly reducing the food wastage at all steps in the agri-food processing chain and also maximizing the valorization to beneficial products [18].

SIVEQ short for *Sistema Integrato per la Valorizzazionedelle Eccedenze Alimentarinel Quartiere*, "Integrated system for the valorization of surplus food in the district" in English, is a data acquisition platform with a web interface which aims for the redistribution of food before it turns unfit for human consumption [19] using a systematic approach of IoT and Big Data Analysis [20].

A collaborative project called "New Advances in the Integrated Management of Food Processing Waste in India and Europe" (NAMASTE-EU) between India and European Union (European Commission, 2010–2013) which is worth €1.5 M has proven to be effective. The project in co-ordination between Department of Biotechnology, Government of India and seven partners from six European countries including three companies is focusing on valorization of citrus byproducts, mango, pomegranate, rice bran and wheat bran processing.

Many worldwide initiatives have brought the importance of agri-food valorization into limelight.

34.4 Composition-Based Solutions and Approaches

Majority of AFWs contain starch, cellulose, hemicelluloses, pectin, soluble sugars such as glucose, fructose and sucrose, proteins, lipids [21], lignin and antioxidants [6]. The valorization approach and application largely depend on the initial chemical constituents of AFW's. Fatty acid methyl esters are the common biochemicals recovered from wastes rich in lipids. Extraction of organic acids from volatile fatty acids [22] is also possible. High cellulosic wastes or sugars have the potential for conversion into ethanol, butanol, acetone, etc., via fermentation and bioactive peptides can be recovered from high-protein wastes.

The current chapter will delve into different approaches of agri-food valorization with applications in biochemicals and bioplastics. Biological solutions involving production of biochemicals, enzymes, food/feed supplements, thermochemical and microbial solutions of generating energy have also been dealt. Cursory reference has also been made with respect to applications in food, feed supplements, cosmetics, food pharmaceutical industries and also for fuels and energy employing thermochemical and microbial methods.

34.5 Biochemicals

Biochemicals are plant-derived chemicals having commercial value and are increasingly gaining importance in human, animal health and nutrition being protective

antioxidant and antimicrobial in nature. The recovery of such biochemicals from AFW assumes primary priority as this is a high-value product.

AFWs have the potential to be a valuable source of biomolecules [23] like fatty acids and triacylglycerides, carboxylic acids (acetic, glycolic, oxalic, 3-hydroxypropionic, fumaric, succinic, asperic, malic, butyric, levulinic, itaconic, glutamic, adipic, citric and gluconic acids), olefins (ethylene and unsaturated fatty acids), alcohols (ethanol, glycerol, propane diols, 1,2,4-butane triol, 2,3-butane diol, 1-butanol and sorbitols), enzymes (protease, lipase, cellulose, phytase, amylase, lignisase and xylanase), L-glutaminase, citric acid, lactic acid, gallic acid and gibberellic acid and other chemicals such as sucrose, furfural, acetone, lysine, antibiotics, polyhydroxyalkanoates (PHA), poly-γ-glutamate and aromas [5].

This enormous and diverse variety of potential biochemicals has led to the evolution of the biorefinery concept which uses these AFWs to generate chemicals of commercial importance. Central to the theme of biorefinery concept is the sequential, integrated and multifunctional processes used to exploit the AFW thereby generating commercial intermediates and end products ultimately achieving zero waste. It is also imperative that such a concept should be economically feasible and environmentally sustainable with a lower carbon footprint [24].

34.5.1 Functional Phytochemicals

Functional phytochemicals have some beneficial aspects for human health and nutrition and can have antioxidants, antimicrobials, antivirals and antiplatelet properties, cytotoxicity, anti-inflammatory compounds, etc.

Some of the wastes having the potential for extraction of these types of phytochemicals are waste peels of Chinese yam (*Dioscorea batatas*) rich in phenanthrenes [25], the extracts from mango byproducts (peel, husk seed and seed mango) rich in monogalloyl compounds, tetra- and penta-galloylglucose, ellagic acid, mangiferin and benzophenones such as maclurin derivatives and iriflophenone glucoside [26], bioactive peptides from fish waste [27], wine-making wastes for their antioxidant potential [28], etc. Antioxidants and bioactive compounds can also be extracted from citrus, pomegranate and lemon peel wastes [29–31]. Antioxidants and functional phytochemicals are increasingly being sought after for their various therapeutic and functional values and have a large potential.

34.5.2 Industrial-Relevant Biochemicals

Many industrially relevant biochemicals have also been produced from AFW. Pectin has been extracted from apple pomace, citrus peel, sugar beet, sunflower heads and watermelon [32, 33] which have gelling, thickening and stabilizing properties in foods. Production of biosurfactants from orange peel [34] dairy and food processing wastes [35] has been attempted employing solid fermentation which has a range of industrial applications as in adhesives, flocculating, wetting, foaming agents, de-emulsifiers, penetrants and also have applications in bioremediation. The use of

AFW in the treatment of polluted water bodies has been dealt with extensively in the book chapter of Abosede [36].

The role of microorganisms toward valorization of AFW is noteworthy. Microbial solutions for AFW valorization have distinct advantages like being environmental-friendly, reliable and cost-effective as compared to other approaches. Biocolorants have been produced by the fermentation of bakery waste using the fungal strain *Monascus purpureus* [37]. Agri-food residues have also been used as substrates for the production of biopesticides from *Bacillus thuringiensis* (Bt) [38, 39]. Xylitol is another biochemical produced by many microorganisms by the fermentation of xylose present in hemicellulosic hydrolysates derived from AFW [40]. Commercial production of ethanol and tartaric acid is possible from wine lees [41, 42]. Organic fraction of municipal solid wastes like household food wastes, food waste from cafeteria, citrus waste, grape pomace, potato peel waste, pineapple and banana peel have also been used successfully to produce ethanol [43]. Attempts for recovery of other bioactive compounds like phenolic compounds [44] squalene [45] and also to use wine lee as supplement in lactic fermentations are also known [46]. Fermentations have also been used to produce succinic, citric, lactic acids, butanol and poly-3-hydroxybutyrate (P3HB) from food waste [47].

Microbial oils have been produced through the valorization of kitchen waste from households which in turn can be used for the production of biodiesel and various oleochemicals such as biolubricants and wax esters [48], while bacterial cellulose has been produced from pineapple pomace, tomato wastes and coconut water using *Acetobacter xylinum* [49] which have applications in medical, textiles, cosmetics and food sectors.

34.5.3 Enzymes

AFWs are also important sources of enzymes. Enzymes are a key ingredient ensuring the success of the biorefinery concept. Conventional production of enzymes is complex but when produced on AFW employing solid-state fermentation can prove to be economical [50–55]. Kitchen and domestic wastes have been used as substrates to produce a wide variety of enzymes like glucoamylase, pectinolytic enzymes, lipase, cellulose, glucoamylases and proteases via solid-state fermentation involving fungal strains [43, 56]. However, studies and technological interventions to make the process more economically viable are needed.

34.5.4 Foods/Feeds/Supplements

Some of the AFWs generated by industries have the potential to be directly used in human foods. Brewers spent grain, a byproduct of the brewing industry rich in arabinoxylan, lignin, cellulose [57], proteins [58], can be directly used to increase the fiber, protein and calorific contents of bread and baked snacks [59]. This is also known to improve the technological and nutritive properties of breads. Such type of supplementation should also consider the synergetic and antagonistic effects of phenolic compounds derived from the incorporated wastes and bread ingredients as

noted in the case of onion [60]. Certain seeds of leguminous plants can also be used as protein supplements [61] during enrichment of foods.

Production of prebiotics employing spoilage fungus from AFW is another area which is equally important and this aspect has been dealt elsewhere and readers are directed to these research publications [62].

34.6 Biofuels

Under the biorefinery concept, the terminal use of AFW should be for either composting or energy production after all the bioactive compounds have been recovered from the wastes and after the cellulose, hemicelluloses and pectin have been utilized in polymer composites.

Thermochemical solutions involve the conversion of AFW into liquid hydrocarbons by the application of heat. This valorizable means usually tend to convert AFW into biofuel through the pyrolysis of agri-food residues into three fractions such as bio-oils, biochar and biogas [63, 64], fast pyrolysis [65], hydrothermal liquefaction [66], gasification [67], followed by Fischer–Tropsch synthesis [68].

Microorganisms are also used to convert AFW into biofuel. Microorganisms acting on AFW can be either in an aerobic or anaerobic environment to degrade organic wastes. When it is aerobic it is called composting and generates fertilizers, while under anaerobic conditions it generates both fertilizers (digestate) and biogas which is primarily 60–70% methane and 30–40% carbon dioxide. The development of microbial fuel cells producing hydrogen in an anaerobic digestion process has been attempted [69]. El Mekawy et al. [70] have explored food and agricultural wastes as substrates for bioelectrochemical system achieving both waste treatment and energy recovery.

34.7 Packaging Materials and Bioplastics

Packaging is a combination of technology and art to pack a commodity for safe storage, convenient transport, and enhanced trade. Plastics form an essential part of packaging in different forms and accounts for >30% of global plastic consumption [71]. Plastics are mainly made from petroleum-based polymers and exist in the environment for hundreds of years. It is a challenge to recycle the plastics due to large differences in their varieties. Thus, a major part (80%) goes into landfills. It is not a surprise that a small share (~3%) ends up in the ocean also threatening life in water body. A serious concern from the above activities and increasing consumer awareness prompted the packaging industry to seek bio-based alternatives such as bioplastics that involve the use of renewable resources and reduce the dependency on fossil fuels. Today, valorization of agriculture and food waste in packaging is an upcoming research area and is gaining the attention of researchers worldwide.

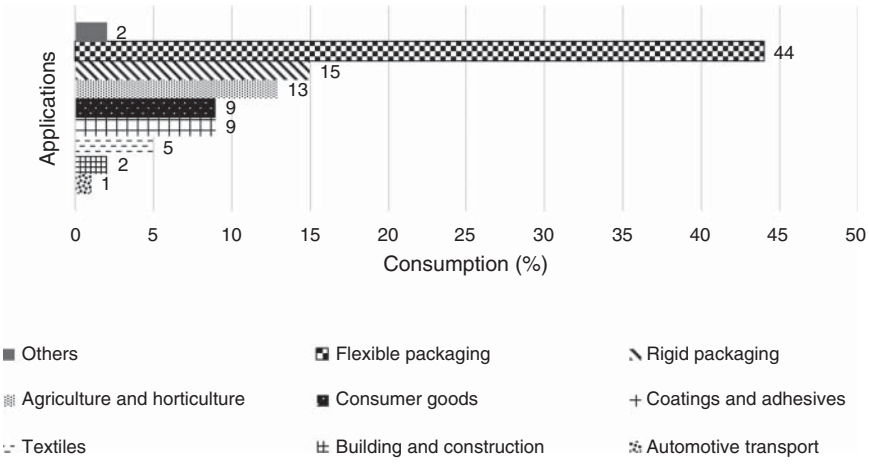

Figure 34.1 Global production capacities of bioplastics in 2017 (by market segment). Source: European Bioplastics (2017). http://www.bio-based.eu/markets and http://www.european-bioplastics.org/market.

34.7.1 Scope and Features

The packaging materials made from bioplastics account for 60% usage of bioplastic applications. However, it is important to note that not all bioplastics are biodegradable (decomposition by biological means). A significant amount of research has been done in the last decade over the development of bio-packaging materials (BPMs) with improved functionalities such as enhanced barrier, mechanical and low diffusion properties as compared to conventional packaging materials.

Bioplastics from AFW are mainly obtained by the process of fermentation of waste. The resultant products are further modulated by adding a plasticizer, natural fiber/extracts, chemical reaction with the polyol acids, etc. The polylactic acid and PHA are the main examples of biodegradable packaging materials synthesized by the process of fermentation of AFW. Table 34.1 lists some of the works carried out in utilizing AFW for bioplastics production. An excellent review on the production of bioplastics from organic acids derived from the organic fraction of the municipal solid wastes is that of Vea et al. [99]. Here, they mention the technologies followed by about seven researchers in producing PHA along with their valorization efficiencies.

34.7.2 Polylactic Acid (PLA)

Lactic acid, an organic acid, is obtained from the fermentation of sugar and starch present in several AFWs of corn crop, sugar beet, tapioca, sugarcane, etc. The polymerization of lactic acid wherein molecules combine to form lactide rings and their further linking results in the formation of continuous chain of poly lactic acid (PLA). It is a biocompatible compound and has high modulus strength similar to

Table 34.1 Agri-food wastes (AFWs) used for synthesis of biopolymers and its composites.

Bioplastic/composite	AFW	References
PLA	Starch	[72]
	Spent coffee grounds (SCGs), coffee silver skin and cotton waste	[73]
	Kitchen waste	[74]
	Fish meal wastes	[75]
	Paper sludge	[76]
PLA monomer L-lactic acid	Pear pomace and ricotta cheese whey (RCW)	[77]
	Food waste/bakery waste	[78]
	Kitchen residue	[79]
PHA (P3HB)	Molasses from sucrose/sugar beet	[80–82]
	Whey	[83]
	Waste frying oil	[84]
Chitosan film composite	Incorporated with carotenoids of *Bactris gasipaes* fruit waste	[85]
Polysaccharide-based biopolymer	Starch from mango seed	[86]
	Pectin from tree tomato	[87]
Polyol, polyester	Corn stover	[88]
PLA composite	Egg shells and mussel shells	[89]
	Hemp	[90]
	Wood	[91]
	Kenaf and rice husk	[92]
	Rice straw	[93]
	Jute	[94, 95]
	Abaca	[96]
	Flax	[97]
	Bamboo	[98]

synthetic plastic such as polystyrene. Due to its biocompatibility and non-toxicity, the US Food and Drug Administration (FDA) gave it generally recognized as safe (GRAS) status. This brought a great deal of revolution in the packaging industry. The PLA films were transparent and biodegradable. Its application was realized for short-lived foods [100]. The brittle and low barrier properties restricted its use for moisture sensitive, long-shelf life and frozen baked goods [101]. Further, its thermal instability was also a concern. The first high-barrier PLA-based flexible film for long shelf life foods was produced by Nature Works and Metalvuoto [102].

34.7.3 Polyhydroxyalkanoates (PHAs)

The term PHAs is used to describe various aliphatic polyesters, such as poly-3-hydroxybutyrate (P3HB), poly(3-hydroxy butyrate-*co*-3-hydroxyvalerate (PHBV), poly-4-hydroxyvalerate (PHV), polyhydroxy hexanoate (PHH) and polyhydroxy octanoate (PHO) naturally produced by bacterial fermentation of AFW carbohydrates. The PHA granules are recovered by disrupting the cells of natural molecules [103]. The bacteria (*Cupriavidus nector*, *Methylobacterium rhodesianum*, *or Bacillus megaterium*) produce polyhydroxybutyrate (PHB) under nutrient-deficient conditions. It is considered that the bacteria use the product as energy storage molecule and metabolize in the absence of any other sources. The microbial synthesis of PHB involves condensation between two molecules of acetyl-CoA to form acetoacetyl-CoA that is further reduced to hydroxybutyryl-CoA. The later compound acts as a monomer for polymerization to form P3HB [104].

The heat resistance and gas barrier properties of PHA/P3HB are similar to polyvinyl chloride and polyethylene terephthalate (PET) thermoplastics. However, the stiff and thermally unstable nature of BPM *vis a vis* synthetic plastics pose limitation to their widespread use. The incorporation of another PHA monomer 3-hydroxyvalerate (HV) into the P3HB polymer to synthesize the copolymer PHBV improved P3HB properties to a certain extent [105]. The high production cost of PHA is another challenge and is the impetus for the development of cost-effective process using AFW.

34.7.4 Reinforcement in Bioplastic Properties

The modulation in bioplastic properties was envisaged to enhance its packaging applications in food sector [106]. The approaches involved the addition of plasticizer such as glycerol, natural fiber such as almond/walnut shell [107], hull of soyabean [108], laminating with paper, bilayer of PLA and P3HB, copolymerization, composite formation, etc. The plasticization involves the use of glycerol and other food grade polymers in bioplastics. The cross-linking and compatibility between PLA and poly(glycerol succinate-*co*-maleate) (PGSMA) is known to improve the toughness of BPM [109]. The high tensile strength and elongation of pectin cellulose (obtained from orange waste) thin film was achieved using glycerol as plasticizer and maleic anhydride as compatibilizer [110].

34.7.4.1 Natural Extract

The addition of natural extracts from plant parts in the BPM not only improves the gas barrier property but also extends the shelf life of food by preventing it from spoilage. The olive leaf and propolis extract were incorporated in PLA matrix with the aim to release antimicrobial/antioxidant agents in the bio-based food packaging systems [111]. Del Nobile et al. [112] incorporated lemon extract in packaging based on PLA, polycaprolactone (PCL) and LDPE. Mesquita et al. [85] extracted carotenoids from the *Bactris gasipaes* fruit waste and incorporated into chitosan film to enhance its mechanical property and antioxidant activity.

34.7.4.2 Copolymerization

It is a process of synthesizing new compounds (copolymers) using different types of monomers. The copolymers are also called as biopolymers. PLA was copolymerized with glycolic acid, caprolactone, [113] and D,L-lactide to synthesize poly(lactic-*co*-glycolic acid) (PLGA), PCL, PLA and poly(ethylene glycol) (PEG) copolymers [114], respectively. The work indicated that the rates of drug release and biodegradation of copolymer could be tailored by adjusting polymer composition. The material was suggested to have medical applications as biodegradable sutures. Bigg [115] copolymerized PLA with PET to produce flexible, tough and clear fibers/films.

34.7.4.3 Green Composites

Green composites are fabricated using bioplastics and the fibers extracted from natural resources. The natural fibers can be divided into three categories: plant fibers (jute, coir, husk, bamboo, palm leaf, etc.), animal fibers (silk, wool and hair) and mineral fiber (asbestos). The green composites are the best example to fulfill the concept of valorization of AFW for food packaging as the fibers required for composites formation are mostly derived from unutilized or discarded materials. The reason behind imparting reinforcement to bioplastic by fiber is the presence of unidirectional cellulose microfibril in the matrix of lignin and hemicellulose [116]. The natural fibers improve the mechanical properties and provide several environmental benign characters to composites. These fibers are easily available, can be recycled, decomposed without any toxic emission and keep the composite lightweight because of their low-density property. Purkayastha et al. [117] developed fluorescent carbonaceous nanoparticles (FCDs) from the "end-of-pipe" (spent material) of oilseed-press cake. The protein was extracted from the oilseed-press cake and the environmental-friendly method was used to convert the remaining fibrous material into composites. Banana fibers and stem wastes have been used to increase the water absorption capacity and improve the impact strength of epoxy materials and polyvinyl composites [118] and hence can be thought of to have polymer-reinforcing capacities. Hammajam et al. [119] studied the importance of chemical treatment on the natural fiber (millet fiber) to form green composites and its degradability in the municipal soil. The alkalization of millet fiber enhanced its mechanical property and reduces the rate of degradation. Wahit et al. [120] reviewed, in detail, the use of natural fibers such as ramie, hemp, bagasse, rice husk, palm, wood and flax in the preparation of PLA and PCL composites to enhance their mechanical properties.

In today's time of nanoscience and technology, the composite matrix has also been incorporated with nanoparticles (<100 nm) to drastically improve its mechanical property, toughness, electrical and thermal conductivity [121]. There are several film-making techniques where modifications were done to make nanocomposites like injection molding [122]. Extrusion was followed by injection molding [123], melt compounding followed by compression molding [99], direct melting and solidification [124], solution casting after gelatinization [125] and one-step *in situ* intercalative solution polymerization [126]. The *in situ* polymerization method is mostly preferred because of facile processing of material and better performance of product.

In this method, monomer(s) and nanofillers are dispersed in the same container to prepare homogenous solution that is followed by polymerization reaction [127]. The nanofillers are modified by incorporating functional groups to enhance its interaction with the polymer matrix.

34.8 Green Valorization

The extraction of either biochemicals or bioplastics can be seen broadly whether they are green environmental-friendly technologies or conventional technologies. These have also been called as conventional or emerging [128] and thermal or non-thermal [129, 130]. Green valorization refers to technologies which follow the six principles put forward by Chemat et al. [131] of environmental-friendly extraction of natural products from wastes taking into consideration the economics as well as effectiveness of the extraction process.

Carciochi et al. [132] and Panzella et al. [133] have done a detailed review on the existing technologies for the green extraction of valuable bioactive compounds from AFW. Various green extraction strategies like microwave-assisted extraction, ultrasound-assisted extraction, supercritical fluid extraction, deep eutectic solvent extraction, enzyme-assisted extraction, pulsed electric field technologies, pressurized liquid extraction and instant controlled pressure drop have been dealt in great detail. Green valorization technique alone will achieve the concept of zero waste and sustainability of processes, since it is important for any valorization technique to be environmental-friendly providing solutions to integrate into existing processes rather than posing fresh challenges.

34.9 Conclusion

The post-COVID world is likely to see food shortages and hunger and the poorest are going to bear the brunt of this reality. Predicted poverty number is set to rise and the accessibility to good food is likely to take a beating. Under this scenario, it is next to crime to waste foods. Prevention and valorization of AFW is here to stay with the biorefinery concept likely to make innovative strides in extracting biochemicals, bioplastics and bioenergy from this wastage. Sequential extraction techniques enable systematic removal of commercially valuable compounds with final wastes being used in energy production thereby reaching to zero-waste situations. Such an effort also makes economic sense to the agri-food handlers, processors to lessen environmental impacts, help municipal workers in solid waste management, legislators fulfill social obligations making development and progress sustainable. Support from government legislations across the world is imminent for the success of the initiative. A starting point would be the brilliant European initiative [134] compiling a compositional database with an intention to identify important wastes, valorization approaches, exploitation, suitability and market potential. Such a database and waste-handling strategy need to be compiled by individual industries and effectively implemented to ensure environmental and social obligations.

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35

Edible Coatings and Films from Agricultural and Marine Food Wastes

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35.1 Introduction

Utilization of petroleum-based polymers like plastics (polyesters, polyolefins, and so on) has been increased progressively as a packaging material, because of their accessibility in huge amounts easily and ideal useful attributes such as good mechanical strength and great barrier properties. But, they lack good water vapor transmission rate [1]. Being non-biodegradable, these are causing environmental pollution. Among all the commercial sectors, food manufacturers need a lot of packaging material and that too it should be food grade and should improve the product shelf life and stability [2]. In recent times, conventional plastic is replaced with packaging films, which are biodegradable and compatible with the environment [3]. Along with this, consumer interest in nutrition, health, and the long shelf life will contribute to the development of edible coatings and films.

A thin layer that is applied as a coating on the surface of the food is called edible coating and when it got placed in between the food and the surrounding environment is called edible film [4]. The chemical composition of both the film and coating is same, the only difference is the thickness. A special attention devoted to the chemicals isolated from the food processing industrial wastes, and plastics obtained from these resources can be used as an alternative to the petroleum-based plastic packaging materials [5].

Fruits and vegetables are the part of the human's daily diet as these are rich in antioxidants, nutrients, and fibers. The edible portions of fruits and vegetables are consumed, whereas the seeds and peels are thrown out as the food waste. The wastes obtained during postharvest, distribution and processing stages are called agri-wastes [6]. Marine-based processing industries produce a lot of animal wastes

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like shells, skins, and muscle wastes. Among all the sea living varieties, crustaceans contribute a lot of waste by-products, nearly 75% of its total weight [7]. Huge mountain of agri- and marine-based food-related wastage has been generated by industries in the process of production of valuable products. It was discarded as a waste, even though most part of it has the potential to be utilized in some other manner [7]. Agricultural and marine waste can be used for recovering biopolymers which can be used for making biodegradable packaging.

Biopolymers like hydrocolloids (proteins, carbohydrates, and lipids) are obtained from agri-biowastes such as husk, fruit peels, seeds and stalks, grain wastes, and so on [2]. Some of these wastes are rich in bioactive compounds which are beneficial for many purposes. So, the scientific research on the utilization of bio-based material is on the top of interest. The antimicrobial and antioxidant properties will influence the quality and shelf life of the food items. The marine-based by-products include chitosan and gelatin [8]. The best compound that can be extracted from the sea waste is chitosan which can be obtained by deacetylation of chitin. It can be utilized as biopolymer and has antimicrobial and antioxidant properties, and it can be used as the bioactive film for food preservation [9]. However, the main motive of edible packaging is to isolate food from the surrounding environment, limiting exposure to spoilage variables (microbial, mechanical damage, and physical), thus expanding the food shelf life [3]. These edible films also provide antimicrobial and antioxidation protection [2]. The global market size of commercial edible film and coating valued at US $697 million in 2016 and expected to reach US $1097 million by the end of 2023 [10]. Plasticizers are used to reduce the film's brittleness and improve the flexibility and pliability [1]. The functional properties of edible packaging material vary depending on the nature of the raw material used as biopolymer. Protein- and carbohydrate-based film shows good mechanical properties but lacks the moisture barrier due to their hydrophilic nature [6]. The inferior properties of mechanical barrier can be enhanced by adding reinforcing agents such as fibers and cross-linking agents [11]. The degree of biodegradable kinetics depends on the nature of the biopolymer and filler used to improve the properties. Application and utilization of food by-products for the production of polymers and bioactive compounds will provide a new way of developing packaging material which will be feasible alternative to the plastic polymers and also offers an advantage of reduction of food waste composts and safeguard the environment [5].

35.2 Sources of Food Waste

The materials used in the film/coating preparation of food material can be obtained from renewable resources extracted from the agricultural wastes which mainly include the fruit, vegetable, grain, and marine wastes, and their by-products. It should be Generally Recognized As Safe (GRAS) to consider as an eatable [12] and should be utilized as per the directions and guidelines of the Food and Drug Administration (FDA), United States of America and other relevant regulatory bodies's guidelines. The main components in the formation of edible coatings and films are biopolymers and additive.

Nearly, 14% of the total food produced globally gets wasted during postharvest processing stage before it reaches the retail market. According to the European Commission in the directive, 2008/98/EC, the waste definition that was established in the general food law and the waste framework directive, respectively, states that food waste refers to food appropriate for human consumption being discarded, whether or not after it is kept beyond its expiry date or left to spoil [13].

The plant origin components from the agricultural waste such as pomace, pulp, and seeds applied in different food system due to their nutritional value, bioactivity, viscosity, other functional properties and, potential applications. Industrially, the wastage/by-products of the grains contain protein isolates, concentrate and oil seed protein and, meal which are evaluated and used for edible film production [14]. The waste produced from the processing of shrimp has large potential as long as its use can reduce ecological effect and increase the revenue of shrimp production [15].

In order to utilize and preserve the quality of the compounds obtained from food waste/by-products which have different components from different mixtures, one need to evolve with technologies which utilize extraction, refining, purification, and so on, depending on the compound [5]. This chapter will focus on the two food waste groups, which are considered for the recovery of film/coating compounds. They are of agricultural and animal sea food origin. Again, they are having different subcategories such as fruit and vegetable residue (apple, blueberry, carrot, sweet lime, potato, banana, and so on), grain wastage (soy, corn, hazelnut, wheat, rice, sunflower, and rapeseed), and marine wastage (crabs, fish, squids, cray fish, shrimps, and sea urchins) which are considered as a source for edible film/coating development [2, 6, 9].

35.3 Film/Coating Made from Agri-Food Waste

35.3.1 Biopolymers from Fruits and Vegetables Waste

The fruits, vegetables, and grain processing industries discard a large number of by-products such as peels, stalks, seeds, oil extraction, and refining wastes depending on the need and the processing technologies. The by-products mainly include a high measure of hydrocarbons, proteins, and bioactive components having antimicrobial and antioxidation performance [16]. The hydrocarbon compounds are sugar, cellulose, hemicellulose, and lignin [5]. Sugarcane bagasse is a lignocellulosic material, which can be further modified into polyethylene and vinyl chloride for styrene-based biopolymer production [17]. The fruit juice industrial wastages mainly include pomace and the biomass rich in pectin, phytochemicals, and dietary fibers [3]. These compounds can be valorized by utilizing as a structural matrix in the development of edible film/coating formation.

The agri-waste is the good source of starch and can be used for the development of edible film. Potato is the most commonly available resource. Such starches are further modified chemically or physically to develop the biopolymer into thermally resistant synthetic plastic polymer [17]. The films made from plant-originated compounds may represent an inherent problem in exhibiting the poor water vapor

barrier and mechanical properties due to their polar (hydrophilic) nature. So, there is a need to focus on the development of new strategies for enhancing the characteristic properties of biopolymers to meet the food packaging requirements, in particular, high water vapor barrier and mechanical properties. These strategies might involve the chemical modification, incorporation of firming agents or other additives, and utilization of nanoparticles that provide the functionalities which were lacking in the original film [9].

Fruit pomace mainly consists of cellulose and pectin. These are the primary polysaccharides present in the cell wall, which determine the integrity and rigidity of the tissue. The composition ranges vary from fruit to fruit and also on the fruit varieties. Agricultural and food industrial by-products mainly constitute fruit pomaces from apple, sugar beet pulp, citrus, and so on, which are rich in pectin and antioxidant substances. The only drawback of fruit pomace is low solubility [12]. Utilization of the fruit peel in the film development showed a good characteristic property as the peel contains a good composition of nutrients like carbohydrates and protein and also rich in pectin. The industrial extraction for pectin mainly utilizes apple pomace or citrus peel. Pectin-based film shows a good mechanical property. Banana flour films rich in pectin formed with slight yellow appearance and transparency. The film showed a good mechanical and oxygen barrier properties and sealability [18].

Borah et al. [19] developed a composite film by varying the concentration of potato peel and sweet lime pomace, with an ultrasound treatment. On studying their characteristic properties, the results shown that the film made in the ratio of 0.5 : 1 have given the best result and a good quality of bread on storage of five days.

In the development of composite film, strong interfacial action needed among the components was used. A study was done by Briones et al. [20] on utilizing the liquefied agricultural residues such as corn stover, corncob, vine shoot, and blueberry tree pruning for making films. These are rich in lignocellulose and incorporated in to the starch-glycerol film. This film showed a good compatibility and exhibited the better thermal stability and dynamic mechanical properties than the control starch film. In a study done by Andrade et al. [6], the author developed an edible film using the fruit and vegetable residual flour (FVR), without the plasticizer addition. The flour mainly includes residue from passion fruit, carrot, watermelon, lettuce, taro, cucumber, rocket, selecta orange, courgetti, mint, and spinach. And the film is made with two proportions, i.e. with 10% and 8% of the flour and the 8% sample contains 2% of potato peel. Incorporation of potato peel resulted in enhanced tensile strength. The film containing the high carbohydrate content showed good flexibility than the starch-based edible film.

35.3.2 Biopolymers from Grain Wastage

Cereal wastages from rice, wheat, rye, barely, and corn are the good sources for proteins. The wastage is obtained after processing, polishing, washing, grinding, or refining of the grains. Recent studies have shown the development of edible film and coating by the application of protein from the pulse wastage which provided an

improved mechanical and barrier properties than the carbohydrate- and lipid-based edible films [5].

Lignocellulosic material of corn stover was used to produce polyols, which form biopolymer. The polyols by cross-linking with cyclic acid, anhydrides, and polyurethane foams lead to the formation of biodegradable polyester [17]. Soy protein, zein, and gluten extracted from the food residues or wastes are commonly used for the development of edible films. Defatted soybean meal-based edible film has limited application due to its low moisture barrier and mechanical properties.

Guo et al. [21] developed a composite film using wheat gluten and corn zein and studied the effect of different factors. The results showed reduced water vapor permeability due to the zein hydrophobicity, and with the increase in glycerol content the barrier property values increased and provided a good mechanical property. Starch and its derivatives are utilized as biopolymers in the edible film development due to their low cost and availability. The structure, size, chemical composition, and shape of film vary depending on the sources. Starch consists of two polysaccharides, namely amylose and amylopectin. Amylose is mainly responsible for film formation [5]. Starch from corn, having high amylose content, shows an excellent film character due to its strong gelation and good gas barrier properties.

The by-product after oil extraction from the oil seeds like olive, flaxseed, rapeseed, coconut, cotton, sunflower, peanut, and sesame is called as meal or cake, which is rich in protein and carbohydrates. Extracted compounds from these by-products or a whole by-product material can be utilized for the edible biopolymer packaging material. An edible film based on the defatted mustard meal, a by-product from the defatted biofuel industry, was developed [22]. This film showed antimicrobial activity against the *Listeria monocytogenes* without adding any external antimicrobial additives and a good physical property. The study demonstrated that the film has the potential to be used as the film and coating material for food. The edible films made from the agri-waste residues are shown in Table 35.1.

35.3.3 Bioactive Compounds from Plant Residues

The plant-originated waste residues are used for the extraction of the bioactive compounds such as phenolics, terpenoids, and flavonoids having antioxidant and antimicrobial characters. Dietary fibers are also extracted from plant residues [9]. The sources of these compounds are citrus peel, pomegranate peel, seed extracts, grape skin, and tomato pulp. A study was conducted [16] on the development of active biodegradable packaging film by utilizing the industrial wastes, dietary fibers from blueberry juice processing, and gelatin capsule waste containing glycerol as a plasticizer. This film showed prolonged shelf life of food. In another study [19], incorporation of clove oil resulted in an antimicrobial character after wrapping on the bread. In another study [27], a gelatin-based nanocomposite film was developed by incorporating black rice anthocyanins, and it showed a slight decrease in mechanical properties but an excellent UV light barrier and antioxidant nature. So, the plant extracts containing high antioxidant and antimicrobial compounds will be useful for the development of active food packaging.

Table 35.1 Edible films made from agri-waste residues.

Biopolymer source	Effect	References
Fruit and vegetable residue		
Blueberry pomace (dietary fiber and ethanolic extract)	Improved UV barrier properties, provided antioxidant properties to the gelatin-based film against sunflower oil oxidation	[16]
Blueberry pomace and red grape skin	Provided active atmosphere to the chitosan and carboxymethylcellulose films, increased oxygen permeability property	[12]
Banana peel flour	Low tensile strength, good barrier properties depending on the starch content	[23]
Potato peel and sweet lime pomace	Improved mechanical and barrier properties	[19]
Sweet potato starch and lemon waste pectin	Incorporation with TiO_2 increased the tensile strength and elongation provided; UV protection capacity	[11]
Selecta orange, passion fruit, watermelon, lettuce, courgetti, carrot, spinach, mint, taro, cucumber, and rocket and potato peel	Provided the high solubility film, addition of potato film residue improved the tensile strength with accepted color values	[6]
Grain waste		
Defatted mustard meal	Antimicrobial properties against *L. monocytogenes*	[22]
Soybean residue	Addition of citric acid improved the mechanical and barrier properties and hydroscopic surfaces	[24]
Isolated hazelnut meal (protein)	The film provided with bioactive nature, lightest colored with the good gelation properties of proteins	[14]
Rapeseed protein	Film showed antioxidation effect against its oil oxidation	[25]
Rice flour	The water vapor permeability showed two times greater than the starch-based film. Film with glycerol showed bad water barrier property and with sorbitol showed good water resistance	[26]

35.4 Film/Coating Materials from Marine Biowaste

In a global marine food production of 79.3 million tons, the sea food industry waste is estimated to be 39.6 million tons. The wastes from the marine food processing industries mainly include bones, skin, cut offs, shell, viscera, and heads which are utilized as a fertilizer or dumped in to sea causing serious environmental impacts. Fish waste

containing nitrates and phosphates can devastate the aquatic life by reducing the oxygen levels and increasing the algal blooms [28]. Reutilization of these wastes by turning into valuable compounds can provide many benefits and opportunity to assist the environment.

35.4.1 Fish Processing By-products

Many fish processing industries generate a lot of wastes, nearly 50–80% of its total production. Fish skin, scales, fins, and bones contain a huge amount of collagen that serves as a structural protein. Collagen is used as emulsifying, stabilizing, and gelling agent in food products. The frequent utilization of collagen and its derived products made a demand for newer sources. It can be used for the gelatin production by means of denaturation. Its biodegradability and fibril-forming nature made the collagen as a biopolymer in the edible film production [28]. The only drawback of utilizing the marine collagen is that it has inferior rheological properties, so that it has a very limited application [29]. Utilization of fish gelatin results in good film-forming properties and is an alternative to the synthetic packaging [30].

The composite film made with fish gelatin and a food grade chitosan is applied as an aliphatic aroma compound barrier in food packaging [8]. It showed a less extraction of aroma compounds from the film due to their interaction with the amino group of the gelatin which leads to covalent bond formation [31]. There are few limitations for applying fish gelatin-based film, such as high-water solubility and water vapor transmission rate. This can be controlled by adding any other compatible hydrophobic component to the film-forming solution [30].

A film was made with protein concentrate extracted from Grouper fish (*Epinephelus marginatus*) and plasticizers (sorbitol and glycerol). The mechanical and barrier properties were evaluated and represented as the alternative utilization for food material packaging [32]. The processing of tuna fish produces a large amount of food waste having skin, bones, and fins. These can be valorized using proteins, gelatins, collagen, and biofuel [5]. In one study [33], a biocomposite film made up of cold fish skin gelatin and chitosan (incorporated with essential oil of *Origanum vulgare* L.) was developed. Addition of essential oil directly resulted in the reduction of its barrier, mechanical properties and transparency, but it showed a positive result by inhibiting the Gram-positive bacteria (*Staphylococcus aureus* and *L. monocytogenes*), so it is more suitable for antibacterial applications [8].

35.4.2 Crustacean By-Products

The processing of the sea foods, namely, crabs, shrimps, and lobsters, produce a huge range of crustacean waste material which majorly includes chitin, proteins, and carotenoids. Among these, chitin is extracted from the shell (exoskeleton) waste of shrimp and is the most abundant polymer in the world [5]. Utilization of this chitin and value addition will provide a way in the reduction of waste. Chitin is a heteropolymer composed of (1-4)-linked-*N*-acetyl-β-D-glucosamine made in a linear and complex form [17]. Because of its structure, chitin is insoluble and not directly

used for further processing. Alkaline *N*-deacetylation of chitin will lead to chitosan and is composed of (1-4)-linked 2-amino-2-deoxy-β-D-glucose monomers [7, 8]. It is a cation polysaccharide with high molecular weight and also reported to have antimicrobial activities. It is advantageous to use chitosan in the film-forming due to its nontoxic and biocompatible nature [34].

Chitosan has good film-forming properties and it can be used for food preservation. To be used as a film, chitosan needs to be solubilized into gel by the solvent dissolution. Crude chitosan being only acid soluble and cannot be used for film making, as pH plays a main role on its biocompatibility [35]. The main advantage of using chitosan-based film is that it has antimicrobial activity. In one study [15], fish waste was evaluated for chitosan generation. The author developed a chitosan coating for commercial application to improve the quality of lettuce for 15 days at 4 °C. It also resulted in the reduction of microbial count, preservation of the chlorophyll phenolic compounds, and antioxidant activity during the refrigerated storage period. The chitosan utilization during the postharvest storage opened the possibility of valuing the fish industrial waste. Sea urchin spines with chitosan gel were used for the biodegradable film preparation. This provides a good hydrophobicity properties and a smooth surface [8, 36].

Squid is the most abundant species in the aquaculture. Squid processing wastes include its skin, viscera, tentacles, and muscle traces. Nearly, 200–400 g/kg of its total weight produces the by-products. During skinning of the mantle, a large amount of muscle waste is generated which is high in protein content. Valorization of these components may lead to their commercialization. In another study [37], a film based on the myofibrillar protein concentrate was developed. The edible films made from the marine biowaste were shown in Table 35.2.

35.5 Film/Coating Formation Methods

All the components must be mixed properly in a solvent, such as water or other solvent mixtures with a low-speed stirrer to obtain the homogenous layer. Adjusting the pH, heating, degassing, and deforming of the solution is required to remove the air micro-bubbles which after the suspension cause mechanical failure [3]. The most commonly used film-forming methods are wet process or solvent casting and dry extrusion process. However, for coating process dipping, spraying, and brushing are used.

35.5.1 Solvent Casting

Dispersions of the edible materials are spread on appropriate base and then let it to dry. This results in solvent evaporation leaving the polymer macromolecules to align themselves to a cohesive free-standing layer (film). Careful controlling of drying rate and environmental conditions is needed because of their high effects on film thickness and structural characteristics. Usage of infrared drying can fasten the drying process. Removal of the film without tearing is done by peeling it off from

Table 35.2 Edible films made from marine biowaste.

Sea waste	Effect	References
Tuna fish skin gelatin	Developed the film with antioxidant properties by incorporating Soloyo Grande ecotypes	[29]
Fish skin (*Centrolophus niger*) collagen	The extracted collagen used in chitosan film formation; incorporation of pomegranate peel showed excellent antibacterial properties	[28]
Fish bone (gelatin)	Significantly different mechanical properties than the mammalian gelatin film	[30]
Fish gelatin	A good barrier property to the chitosan-gelatin based film, i.e. gas and aroma	[31]
Fish gelatin (dried *Alaska pollock*)	Edible film incorporated with rosewood oil and used for the quality enhancement of grapes	[38]
Grouper fish skin (protein concentrate)	Provided good mechanical properties after the addition of calcium salts	[32]
Fish chitosan	The chitosan film obtained resulted in the reduction of the microbial count, improved the quality of processed lettuce on storage and extended its nutritional value than the commercial chitosan film.	[8, 15]
Shrimp muscle protein (*Litopenaeus vannamei*)	Lower pH (2)-based film having higher mechanical properties than the one with pH of 11. This provided a potential application for fish preservation.	[39]
Shrimp shell (protein)	Developed a film with the addition of chitosan and incorporation of plant-origin-active compounds showed a feasible active packaging	[40]
Jumbo squid myofibrillar protein concentrate	The effect of acidic and alkaline solubilization on the characteristic properties was studied	[37]

one edge of the base, by maintaining the moisture content of 5–8% [1]. Fruits- and vegetables-based edible films are mainly done through casting because of the thermosensitivity of the components and biopolymers themselves [3].

35.5.2 Extrusion

The other mode of processing is the dry thermoplastic extrusion process, which is economically effective, with higher throughput than the wet solvent casting. It mostly depends on the thermal properties of the film biopolymer. The main

drawback of using the extrusion process is that it cannot form a proper film, as the biopolymers do not have a fixed melting point and they experience decaying on heat applications [1].

35.5.3 Dipping Method

Membranous film formation takes place over the food surface when it got dipped into a coating solution. Then it will be removed and air-dried further, and this method was mainly used on fruits, vegetables, and meat products [1]. The dipping method is not suitable for fresh-cut fruits as it is difficult to form an adhesion of the coating on the hydrophilic surface. To overcome this, a multilayer technique called layer-by-layer electrodeposition is applied. Here, two or more layers are bonded physically or chemically to each other [4].

35.5.4 Spraying Method

It is a conventional method generally used for a low viscosity coating with a high-pressure range of 60–80 psi for better coverage [1] and also offers thickness control and uniform coating. A spray can generate a coating through combined hydrophilic and hydrophobic interactions, and using an emulsion, directly forms layer before atomization, or a bilayer formation occurs after two pulverizations. The classical spray system can produce a spray with a drop size distribution of up to 20 μm. However, the polymeric coating formation by spraying method can be affected by other factors like drying rate, temperature, and drying method [4].

35.5.5 Spreading Method

This is otherwise called as brushing and comprises of controlled spreading on the material surface and drying by the hot air. The brushing method is found to be more effective than dipping or wrapping in terms of moisture loss [4].

35.6 Conclusion

Large amount of food wastes are generated (peel, pomace, husk, seed, and animal waste) and this causes very serious issues in the food resources management and also has a negative environmental impact. Increased interest in environmental conservation and valorization of food wastes has encouraged the development of new products like edible film from food wastes of plant and animal origin. Researchers have put a lot of efforts in order to optimize and facilitate food wastes into edible film and coating formation. However, still lot more research need to be done.

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36

Valorization of By-Products of Milk Fat Processing

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36.1 Introduction

The dairy industry is vital sector of the food processing industry converting raw milk to extended shelf life and safe-to-consume products. Some of the common elements of the product profile of dairy processing include packaged fluid milk (pasteurized/sterilized/flavored variants), cream, butter, fermented dairy products such as yoghurt, curd, and fermented beverages, cheese, dairy powders, ice cream, etc. Many countries and regions also have specific products that are locally popular, and the dairy industry often processes and markets these products as a means of product diversification and value addition. For example, the Indian subcontinent region is well known for the different types of indigenous sweets prepared using heat-desiccated milk solids (*khoa*) and acid-coagulated milk solids (*channa)* and products such as clarified milk fat (*ghee*), sweetened fermented milk coagulum (*shrikhand*), acid-coagulated cottage cheese (*paneer*), etc.

During the processing of milk to the various products handled by the dairy industry, a large number of by-products are generated. Cheese processing industry generates a large volume of whey, which used to be a concern in terms of the pollution potential and its treatment and disposal. However, it is now recognized as a great nutritional resource and value proposition for the industry. Similarly, milk fat processing also generates a significant amount of by-products. Butter manufacture yields buttermilk as a by-product, while the heat clarification process for ghee results in sediment popularly called as ghee residue. These by-products could be valorized either by utilizing these as ingredients in other process lines or by fractionating the by-product to extract valuable principals. This could, in turn, benefit the industry by increasing the profitability through processing gains as well as reducing the environmental foot print of the dairy industry.

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36.2 Processing of Milk Fat and Its By-Products

Raw milk received in the dairy plant is often standardized in terms of its fat and solids not fat (SnF) content, to meet statutory guidelines. This requires the separation of cream from the received milk, and recombining the cream and skim milk in pre-calculated proportions, resulting in a stock of cream with the dairy. The cream is either directly or after a period under cold storage channeled to the process line of other value-added products such as ice cream, butter, butter oil, ghee, etc. In process of conversion of cream to butter or ghee, two major and potentially valuable by-products, namely butter milk and ghee residue, respectively, are generated.

36.3 Valorization of Buttermilk

The churning of cream (oil-in-water emulsion) leads to formation of butter (water-in-oil emulsion) through a process of controlled phase reversal. The aqueous solution that is removed amid the process of cream churning (destabilization) during butter preparation is referred to as butter milk. This aqueous solution is known to contain the components of the cream, which majorly include water-soluble components such as minerals, milk proteins and lactose and are more importantly fragments of milk fat globule membrane (MFGM).

It is well established that the MFGM is a thin (~15 nm) layer and is built of a complex mixture of polar lipids (constituting 90% of dry weight) and proteins [1]. The polar lipids present in MFGM are primarily glycerophospholipids consisting of phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), etc. A second group is composed of sphingolipids; among which sphingomyelin (SM) is the most abundant.

The composition of sweet (from fresh cream), sour (from aged cream) dried buttermilk, in terms of moisture, fat, proteins, lactose, total minerals, respectively, are 2.8% and 4.8%, 5.3% and 5.7%, 34.3% and 37.6%, 50.0% and 38.8%, and 7.6% and 5.7% [2]. Since buttermilk contains nutritive elements such as proteins, minerals, phospholipids, etc., this by-product is a valuable commodity in terms of its nutritional and functional attributes. Buttermilk, being a by-product of fat processing, is low in fat and calories and hence an ideal beverage for persons having obesity-related disorders. It is also a good source of vitamin B12, thereby helping in cell repair due to an active functional role in the synthesis of amino acids and fatty acids. Regular consumption of buttermilk is known to protect against ailments such as heart diseases, cancer, diabetes, etc. [3]. It also alleviates stress and anemia and plays an important role in promoting nerve cell growth [4]. The addition of buttermilk, both in its fluid and powdered form, to foods with special function, offers benefits such as improved antioxidant potential and notable buffering effect.

Buttermilk is known to help to prevent colon cancer and helps in reduction of cholesterol levels [5]. Even at minute amounts, sphingolipids are highly bioactive compounds making it beneficial. Regular consumption of buttermilk could provide

a steady supply of taurine and, in turn, ensure longevity and delay aging [6]. The bioactive principals of buttermilk proteins are known to exert functional characteristics, such as antibacterial effect including some pathogens. These proteins are also known to be beneficial in autoimmune conditions e.g. autism and multiple sclerosis. Buttermilk is an alternate option for lactose-intolerant people who are not able to consume milk; it also acts as a digestive aid. The low-fat buttermilk is known to have around 28% calcium. Hence, consumption of 500 ml of buttermilk would be able to fulfill the calcium requirement by the human body, which requires 1000 mg of calcium per day [7].

36.3.1 Buttermilk as an Ingredient in Food and Dairy Products

Buttermilk, due to its composition and functional attributes, instead of being drained as a waste stream of cream/butter processing, can be effectively valorized as an ingredient in other process lines within the dairy and food industry. A brief description of the various options in this regard is as follows.

36.3.1.1 Market Milk

Sweet cream buttermilk (SCBM) can be mixed with whole milk for supplementing the volume of fluid milk in a processing plant. It can also be mixed with skim milk for the purpose of drying. The addition of buttermilk does not affect the shelf life or heat stability when used for toning milk such as buffalo milk. In case of such attempts, it has been found to be palatable, improve the viscosity, and also reduce the curd tension [8].

36.3.1.2 Dahi

SCBM can be incorporated into whole milk for preparation of curd. Such supplementation of the fermentation medium may result in softer-bodied curd. This is attributed to the altered electrical charge on the casein micelles during the process of churning and due to the availability of other MFGM materials, phospholipids, and free fat in buttermilk [9]. In such an eventuality, the body and texture of dahi prepared from SCBM can be improved with the addition of 1–2% of skim milk powder (SMP).

36.3.1.3 Yoghurt

Thick body and texture of yoghurt are obtained when the solids content of the milk is increased to 14–16 g/100 g [10]. One of the approaches to achieve this level of solids is concentration of milk by boiling to 2/3rd of its original volume. Another method practiced by the industry is addition of SMP in the yoghurt milk. An alternate approach for this could be using buttermilk powder instead of SMP. Low-fat yoghurts prepared by incorporating buttermilk powder (at about 4.8%) exhibited smooth body and texture [11]. Ahmed and Razig [10] prepared acceptable quality supplemented yoghurt milk by using buttermilk, fresh milk, and SMP.

36.3.1.4 Cheeses

It is possible to prepare low-fat cheeses by mixing buttermilk and skim milk and using this mix as the cheese milk. When buttermilk is added to the low-fat cheddar cheese, it can improve the texture, since buttermilk has high water-holding capacity due to the phospholipids content. Mistry et al. [12] reported the use of ultrafiltered sweet buttermilk (up to 5%) to supplement cheese milk for making cheddar cheese with reduced fat. This resulted in softened body, increased water retention of the curd, high moisture content and poorer curd fusion, and higher cheese yield. Joshi and Thakar [13] prepared cheddar cheese by blending SCBM and buffalo milk. This resulted in improved firmness of cheese. Further, this cheddar cheese could be used for preparation of processed cheese. Poduval and co-workers [14] used ultrafiltered sweet buttermilk and homogenized cream for preparation of reduced-fat mozzarella cheese. The cheese, thus made with 5% ultrafiltered buttermilk, had a softer body and texture with open and spongy protein matrix.

36.3.1.5 Indian Traditional Dairy Products

The use of buttermilk in the preparation of various indigenous dairy products has also been widely reported. Buffalo milk for preparation of acid coagulated milk solids, also known as *channa*, was partially supplemented with SCBM. The addition may enhance the body and texture with no effect on color, appearance and flavor. Supplementation of the milk for preparation of *paneer* (an Indian cheese) did not cause any adverse effect on the textural or sensory properties [15]. Similar approaches were also successfully reported in preparing acceptable milk sweets such as *basundi* [16], *sandesh* [17], and *shrikhand* [18].

36.3.1.6 Buttermilk Ice Cream

The use of buttermilk as a base for the preparation of ice cream was explored by Szkolnicka et al. [19]. Different types of buttermilk such as SCBM and cultured buttermilk, with and without the addition of emulsifiers and stabilizers, were tried. Calculated quantities of the ingredients were taken so as to obtain a composition of fat of 10% and sucrose 12%, milk SnF 12% in the mix. The study concluded that SCBM was suitable for usage as a raw material in ice cream manufacture.

36.3.1.7 Dairy-Based Beverages

Traditionally, buttermilk in itself is consumed as a popular dairy drink in many countries. In the Indian subcontinent, buttermilk obtained during the churning of butter, also known as *chhach*, is consumed as a thirst quencher, after dilution with water. The beverage is often flavored in various ways, e.g. with the addition of salt, mint, cumin, ground chillies, fresh ginger, garlic, curry leaves, etc.

Buttermilk has been blended with fruit juices to formulate various dairy-based beverages. Fruits such as mango, orange, banana have been blended with buttermilk (62–85%) to prepare nutritious and flavorful beverages [20]. There are also reports of its use in formulating a carbonated fruit flavored beverage, using various filtration techniques such as pre-filtration, ultra filtration and use of fruits such as orange, pineapple, and mango [21].

36.3.1.8 Probiotic Drinks

Buttermilk cultured with suitable probiotic strains has successfully demonstrated the utility and health benefits with a significant decrease in low density cholesterol levels in the serum and liver, with concurrent increase in high-density lipoproteins in animal systems [22]. In another investigation, buttermilk supplemented with *Lactobacillus reuteri* (1%) did not show any change in composition and sensory attributes [23]. Thus, there is ample scope for exploring the use of other probiotic strains for the preparation of probiotic buttermilk with potential health attributes.

36.3.1.9 Dried Buttermilk

Dried buttermilk can be prepared from the sweet, sour, and high-acid butter milk. It provides desirable flavor, helps in incorporation of air into the product, and aids in development of browning during baking. Furthermore, buttermilk powder has techno-functional abilities such as water binding, emulsification, and improving viscosity of lightly whipped products such as ice cream, pudding, sauces, beverages, and chocolates.

Both low-acid and high-acid versions of SCBM in powder form have been reported. The basic difference is that, for the latter, the pasteurized/concentrated SCBM is pre-inoculated with 1–5% *Lactobacillus bulgaricus* with agitation, to enhance the acidity (equivalent to 10–12% in the dry product) of the product. Both spray and drum drying can be used to dry the powder to <5% moisture content. The drying process has to be carefully monitored to avoid excessive browning and maintain a high solubility index in the powder. Other than food applications, the powder can also be used as an ingredient in poultry or animal feed formulations.

36.3.2 Buttermilk as Encapsulating Agent

The components of buttermilk such as phospholipids, casein colloids, whey protein, and other minor peptides make it an excellent encapsulating material, since each of these components possesses good emulsification ability. Hence, buttermilk has been evaluated as a shell constituent for the microencapsulation of certain oils such as omega-3 fatty acids rich oils. Augustin et al. [24] reported that whole buttermilk powder was a better encapsulating agent than high-heat SMP when used as an encapsulating medium for omega-3 fatty acids (fish oil). This was attributed to the enhanced pH values and changes during the heat treatment of buttermilk.

The shelf life viability of *Lactobacillus rhamnosus* GG (LGG), one of the most documented probiotics, was preserved by a special encapsulation approach using regular buttermilk proteins (RBMPs). Airbrushing technique was used to prepare LGG-loaded alginate microcapsules, which were then coated with a layer of RBMP [25]. These microcapsules were found to be stable under lyophilization, while maintaining the probiotic count even after a month of storage. The developed microcapsules were also stable under simulated gastrointestinal conditions, thereby exhibiting the protective role of RBMP on LGG.

36.3.3 Buttermilk as a Source of Phospholipids

Phospholipids are the polar lipids that are essential for emulsification of fat in milk matrix. MFGM contains phospholipids along with glycol protein, cholesterol, free fatty acids, glycolipids, total and partial glycerides. During churning of cream for butter manufacture, the mass is separated into aqueous phase called buttermilk and oily phase called butterfat. The process involves mechanical destabilization, causing globule coalescence leading to formation of solid phase. Different washing procedures are used during butter manufacture and bring out various degrees of damage to MFGM affecting the lipid profile of the buttermilk. The glycerophospholipids present in buttermilk could be classified as phosphatidylethanolamine (PE – 39%), phosphatidylcholine (PC – 24%), phosphatidylserine (PS – 8%), and phosphatidylinositol (PI – 9%) [26]. Another important component of the phospholipids profile of buttermilk is SM. It exerts a significant biological role due to its effect on regulating the cell growth and development and its role in controlling aging and aging-related disease and cell apoptosis [27]. Additionally, buttermilk is a good source of 9-*O*-acetyl-GD_3, a ganglioside having vital bioactive role [28]. This molecule has the potential to modulate the production of specific antibodies and is being explored as indicative molecule for detection of melanoma. Thus, buttermilk has a unique lipid profile making it a store house of many valuable molecules, which when extracted and purified can be exploited for its economic and functional importance.

36.4 Valorization of Ghee Residue

Ghee residue is the by-product obtained as the solid retentate during the production of the ghee. It is primarily composed of the light brown or darker brown sediment of charred or burnt particles during the heat clarification of butter. Irrespective of the method of ghee preparation (country/*desi*, creamery butter, direct cream, and continuous butter making method), the heating process induces physicochemical variations in the SnF fraction. The solid matrix thus produced settles down as a residue, trapping within it the caramelized lactose, denatured milk protein, phospholipids, minerals, moisture, and some of the flavoring compounds such as lactones, carbonyls, and free fatty acids, etc.

The yield and composition of ghee residue depends on the type of milk as well as the method of ghee preparation. Moisture content of ghee residue prepared from various methods and bovine milk may range from 5% to 14%, while the fat content may usually range from 60% to 80%. Protein content in the ghee residue is observed to be between 20% and 35%, while the by-product may have <5% of mineral content [29]. Among its various constituents, the fat and protein profiles are the most valuable components in terms of scope for economic valorization.

The easily absorbable short and medium-chain fatty acids present in ghee residue make it beneficial for disease treatment [30]. The amino acid profile of ghee residue is known to influence immune function, antioxidant activity, inhibit apoptosis, anti-inflammation property, prevent autoimmune neuro-inflammation. Thus, ghee

residue as a source of nutrients can be tapped to address the protein energy malnutrition (PEM) among the undernourished section of the society. Ghee residue also has significant amount of various antioxidants, namely phospholipids, free sulfhydryls, retinol, α-tocopherol, amino acids, and proteins, dependent on the temperature of ghee clarification. Ghee residue that is obtained from the ghee prepared at low temperature around 110 °C showed lesser development of peroxides, whereas ghee residue from creamery butter prepared at 150 °C was found to have more antioxidants.

36.4.1 Utilization of Ghee Residue for Value-Added Products

The composition in terms of nutritive and functional attributes of ghee residue presents ample scope for this by-product to be economically utilized by the Industry. However, ghee residue tends to harden during storage. Therefore, sometimes a preprocessing protocol may be followed to regain the softness and the smoothness of its texture [31]. The process includes breaking of the big lumps and pulverization of the hardened residue by passing it through the 40 mesh sieve. In addition to the physical process, chemical processes including boiling of the residue (tied in a muslin cloth) and heat treatment in the presence of agents such as vinegar, sodium bi carbonate, etc. are also suggested. Pressing of the ghee residue to a cake of reduced fat content may enhance its keeping quality.

36.4.2 Ghee Residue as an Ingredient in Dairy and Food Industry

Ghee residue is a valuable by-product of milk fat processing that can be used as an ingredient for preparation of various food products. The unique attributes of ghee residue due to its fat and protein profile, energy content, browning, and flavor compounds have been suitably exploited for the preparation of various product categories as discussed below.

36.4.2.1 Baked Products

Ghee residue can be incorporated in bakery products such as cookies and sponge cakes as a substitute for hydrogenated fat. Incorporation of ghee residue was found to improve the "healthiness appeal" of the product and enhanced the spread factor and sensory attributes in cookies [32]. Thus baked products with improved color, taste, appearance, flavor, and overall acceptability can be formulated using ghee residue.

36.4.2.2 Chocolate and Confectionery

A chocolate analogue was prepared using ghee residue, sugar, cocoa powder, and SMP. The product was highly acceptable in terms of sensory attributes and was found to be stable even after three months of storage [33]. Ghee-residue-incorporated confectioneries were prepared by Ananthakumar et al. [34] by blending with sugar, dry coconut powder, SMP, sugar syrup, liquid glucose, and also orange peel powder. The products were found to be highly acceptable in terms of sensory attributes.

36.4.2.3 Ghee-Residue-Based Flavor Enhancer

Ghee residue is a rich source of flavoring compounds characteristically associated with ghee flavor. Hence, limited quantity of ghee residue may be used as a flavor enhancer while preparing ghee from unripened cream.

36.4.2.4 Indian Traditional Sweetmeat

The rich flavor and grainy texture of ghee residue may be used to improve the acceptability of Indian sweetmeats prepared by heat desiccation of milk solids. For example, *Burfi*, a popular traditional Indian dairy product, was prepared by the incorporation of ghee residue (which was treated with 0.5% sodium bicarbonate for half an hour) and blended with khoa and chocolate powder [35]. The product had a pleasant flavor due to the ghee residue, and it recorded excellent consumer acceptability.

36.4.3 Ghee Residue as Animal Feed

Due to the rich nutrient profile of ghee residue, especially in terms of the crude protein content, one of the common avenues to which this valuable by-product has been currently valorized is in the form of animal feed supplement. Ghee residue can be incorporated as a supplement in the diet of the animal/bird/fish feed as a replacer of other common plant feed sources such as cereals (maize, rice), oil seed cake (soybean/mustard/coconut meal), and vegetable oils. Since it is an animal source protein, the caution to be exercised in this attempt is with regard to the cholesterol content of the bird meat and other/derived products.

Arumugam et al. [36] discussed the use of ghee residue as a nontoxic poultry feed. However, a problem of higher liver weight was observed, possibly due to the higher content of saturated fatty acids. It was suggested that it could be balanced using methionine supplementation. Singh et al. [37] showed that ghee residue (up to 20%) can be incorporated in the fish feed for *Labeo rohita*. The economical use of ghee residue to supplement piggery feed is also discussed by Selvamani et al. [38].

36.4.4 Ghee Residue as Source of Phospholipids

Ghee residue constitutes <10% of phospholipids, and it is highly influenced by the method of preparation of ghee. During the preparation of ghee, only a minor fraction of phospholipids from the milk fat is released to the ghee, while most fractions of this important component are retained in the ghee residue due to its polar nature. The phospholipid fraction of ghee residue is inversely related to the intensity and duration of period of heating during ghee clarification. In fact, more intense the heat treatment, greater is the transfer of phospholipids from the residue to ghee phase. This effect is well reflected in the varying amounts of phospholipids in the ghee residue obtained by creamery butter method (17.39%), *desi* butter method (4.95%), and direct cream approach (1.57%). In any case, these levels are definitely much greater than in ghee itself, where the value typically ranges between 0.004% and –0.08% [39, 40].

Actually, the phospholipid content of ghee residue has a functional role in its shelf stability, since it acts interactively with other reducing agents in ghee residue to exert antioxidative effects. In addition, phospholipids are good emulsifiers, and its content in ghee residue, therefore, can be beneficial during the preparation of certain products where emulsification of the different phases becomes desirable. Now that there is an understanding on the beneficial role of phospholipids in terms of its health benefits and its functionality as an emulsifier, there is an interest in extraction, quantification, and purification of phospholipids sourced from ghee residue. Thus, there is ample scope to utilize ghee residue as a source for the extraction of crude phospholipids and standardize suitable methodology for the purification and fractionation of this component to enhance the value of ghee residue.

36.5 Conclusion

The two major by-products of milk fat processing operations, namely buttermilk and ghee residue, have ample scope to be utilized economically by the dairy and food industry. Both products are rich sources of nutrients and have unique functionality in terms of health benefits and technological attributes. Therefore, it is in the interest of all stakeholders, including the processing industry, consumers, and policymakers, to dissuade the thought of these by-products as waste streams of processing and encourage the valorization of these products to realize their full potential.

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