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Detection of Bacterial Biofilms Thriving on the Terrestrial Plastic Waste for Degradation

by

Farzeen Malik

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

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I would like to dedicate this to my parents and teachers.

CERTIFICATE OF APPROVAL

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(Farzeen Malik)

Abstract

Plastics are significantly important across various sectors of the global economy, owing to their extensive utilization in healthcare, agriculture, construction, and daily consumer goods. It is important to adopt effective biodegradable methods to alleviate the burden of plastics on the environment. Understanding the relationship between microbes and polymers is essential to address the environmental issues caused by plastics. Plastic samples were collected from various landfills and dumpsites of Islamabad and processed to isolates the bacterial strains responsible for plastic degradation. All the samples collected were biochemically characterized using tests such as catalase and oxidase, as well as more complex examinations like , methyl red, Voges-Proskauer, Urease test , casein hydrolysis test , gelatin hydrolysis test etc. 16s RNA sequencing was performed for molecular characterization of the isolated strains. The isolated strains were further evaluated for their capability to form the biofilms independently and to degrade the plastic independently and in combinations. Scanning electron microscopy was also performed to confirm the presence of biofilms and degradation in plastics.

Microbial growth was observed only in samples 3 and 5 when cultured in nutrient broth, while the other samples did not exhibit any growth. Gram staining of samples 3.1 and 3.2 demonstrated a positive Gram-positive result, whereas samples 3.4, 5.1, 5.3, and 5.4 displayed a Gram-negative result. 16s RNA sequencing of 5 strains revealed that 3.1 (100%), 3.4 (99%) and 5.1 (92%) have sequence similarity with *Acinetobacter baumannii* (OR827196) (OR826264) and (OR826261) whereas sample 3.2 (99%) has sequence similarity with *Pseudomonas aeruginosa* (OR826267) and sample 5.3 was uncultured bacteria with sequence similarity 99% (OR826194). All samples showed significant potential for as an individual biofilm formation with sample 5.3 highest and 3.1 comparatively with lowest potential and as a consortium, 3+5 (*A. baumannii + uncultured bacterium*) showed lowest biofilm formation capacity while 1+2+3+4+5 (*A. baumannii+ P. aeruginosa + A. baumannii+ A. baumanni*i+ uncultured bacterium) showed highest biofilm formation capacity. Results of UV spectrophotometry of

consortium species at wavelength 490nm showed that Combination 2+3 (*Pseudomonas aeruginosa*+ *Acinetobacter baumannii*), 1+5 (*Acinetobacter baumannii*+ *Uncultured bacterium*) and 2+5+3+6 (*Pseudomonas aeruginosa*+ *uncultured bacterium*+ *Acinetobacter baumannii*+ unknown strain) showed lowest transmittance at 0.2nm with absorbance (2.555, 2.372, 1.734) respectively. While at wavelength 510nm, consortium 3+6 (Unknown strain + *Acinetobacter baumannii*), 4+5+1 (*Pseudomonas aeruginosa*+ Uncultured bacterium+ *Acinetobacter baumannii*) and 2+3+4 (*Pseudomonas aeruginosa*+ *Acinetobacter baumannii*+ *Acinetobacter baumannii*) showed low transmittance values 0.2nm, 0.5nm and 0.6nm respectivelty with absorbance of 1.118, 1.287 and 2.215 respectively.

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Abbreviations

APW	Agricultural Plastic Waste
ASTM	American Society for Testing Materials
BLAST	Basic Local Alignment Search Tool
BPA	Bisphenol A
BT	Billion Metric Tons
DDE	Dichlorodiphenyldichloroethylene
EDTA	Ethylenediamine Tetraacetic Acid
EG	Ethylene Glycol
EMB	Eosin-Methylene Blue
EPA	Environmental Protection Agency
EPS	Extracellular Polymeric Substance
ESDO	Environment and Social Development Organization
HDPE	High-Density Polyethylene
LCC	Leaf-Branching Compost Cutinase
LDPE	Low Density Polyethylene
MPs	Microplastics
MR	Methyl Red
MSW	Municipal Solid Waste
MT	Million Metric Tons
NCBI	National Center for Biotechnology Information.
NPs	Nanoplastics
PAEs	Phthalates
PBDE	Polybrominated Diphenyl Ether
PCBs	Polychlorinated Biphenyls

PCR	Polymerase Chain Reaction
PE	Polyethylene
PET	Polyethylene Terephthalate
POPs	Persistent Organic Pollutants
PP	Polypropylene
PPE	Personal Protection Equipment
PS	Polystyrene
PS	Polystyrene or Styrofoam
PUR	Polyurethane
PVC	Polyvinyl chloride
PVC	Polyvinyl Chloride
PW	Plastic Waste
rRNA	Ribosomal ribonucleic acid
SDS	Sodium Dodecyl Sulfate
SEM	Scanning Electron Microscopy
SUP	Single-Use Plastic
SVE	Soil Vapor Extraction
TPA	Terephthalic Acid
UNEP-WCMC	UN Environment Programme World Conservation Monitoring Center
UNICEF	United Nations Children's Fund
VP	Voges-Proskauer Test

Chapter 1

Introduction

Plastics are large, man-made organic polymers with high molecular weights, primarily sourced from hydrocarbons extracted from natural gas and crude oil. They are extensively utilized for various packaging purposes and, unfortunately, are often not recycled, resulting in their accumulation as waste [1].

Due to their simplicity in molding into a variety of forms and sizes, plastics have become more significant. They have a wide variety of characteristics, from extraordinary hardness to extreme softness, and they all have a high volume-to-weight ratio. Because of their exceptional ability to combine strength and lightness, synthetic polymers have a particular advantage that has allowed them to replace some iron-based materials with plastics that offer longevity. Plastics have also contributed significantly to composite and building materials [2].

The need for plastic is increasing along with the global population, and plastics utilization are now being found in our every systems [2]. The astonishing 311 million tons of plastic that were generated worldwide in 2014 are doubling roughly every ten years. According to projections, plastic output may triple by 2050.

There are two categories of plastics: Heat can be used to shape thermoplastic polymers like polyethylene (PE), polyethylene terephthalate (PET), polyvinyl chloride (PVC), and polystyrene (PS). Contrarily, once they are produced, thermoset polymers like polyurethane (PUR) and polyepoxides cannot be warmed and molded

[3]. The production of virgin plastic, which comes from crude oil and natural gas, is around 8.3 billion tons per year.

From 1950 to 2015, the world produced 6.3 billion tons, primary and secondary plastic waste from which 9% waste was recycled and 12% was Combusted, while the remaining 79% was unceremoniously disposed of on land. Predictions indicate that by 2050, the plastic waste in the oceans is expected to surpass the quantity of fish. [4].

Due to the presence of dangerous chemicals, using plastics puts human health at risk. When people ingest food items that have come into touch with microplastics, plastic pieces behave as persistent pollutants and raise the risk of illness in humans [4]. Risks associated with plastic food containers and bottles include those for newborns, adults, and people of all ages. Phthalates can be ingested through food and water since they are not chemically bound to polymers. Following their release into the environment, these chemicals disturb hormonal systems and cause anomalies in physiological processes in the water, land, and air.

Whales, fish, sea turtles, dolphins, and seabirds are just a few of the marine animals that are severely impacted by plastic pollution. These creatures frequently become caught up in plastic trash, drowning and becoming prey to raptors. Animals and coral reefs are severely harmed by the presence of strong nylon ghost nets. The plastic that is encasing these creatures might shatter as they develop. Every year, this plastic waste kills approximately 400,000 marine species [5].

The challenge of decomposing plastics into minuscule particles, potentially leading to the release of harmful substances, is a key focus in current environmental research on plastic degradation. Phthalates (PAEs), commonly used as plasticizers to impart specific properties like flexibility and durability to plastic products, pose a significant risk. Human exposure to PAEs primarily occurs through the consumption of food, contributing to adverse health effects such as metabolic disorders, reproductive toxicity, and disruption of the endocrine system.

Bisphenol A (BPA) is another well-known substance with endocrine-disrupting capabilities that is included in a sizable fraction of synthetic polymers and added

plasticizers. BPA is widely manufactured around the world and mostly used in the synthesis of several polymer compounds, including polycarbonate [6].

In general, plastics have minimal Poisonousness which is not absorbed by the human body easily since they are macromolecular compounds. Plastics' enhanced toxicity is generally caused by the processing of hazardous additives or by insufficient reactions during the synthesis phase [6].

In the natural world, sluggish microorganisms like bacteria, fungus, and algae control the slow degradation of plastic. Plastic is produced extensively for a variety of uses due to its affordability. The ecology as a whole is about to dramatically suffer from the effects of plastic pollution in both terrestrial and marine settings. This is mostly because of methods that are not environmentally friendly when it comes to making, using, and disposing of plastics as well as the subsequent collecting and processing. Plastic waste primarily enters the environment as a result of inadequate collection efforts, which can be attributed to a lack of manpower and logistical support, or as a result of poor post-collection management, which includes actions like open burning, improper dumping, and the insufficient management of disposal sites [7].

There are several ways that bacteria, algae, fungus, and viruses can speed up the breakdown of plastic. The majority of microorganisms include enzymes, which interact with the surface of plastic to begin the biodegradation process. These enzymes cling to plastic surfaces and start the hydrolytic enzymes from being released. Depolymerases, a class of enzymes, play an important role in the breakdown of polymers. Microorganisms are naturally capable of dissolving a broad variety of organic and inorganic compounds, including silicon, phosphorus, carbon, sulfur, and other elements present in plastics. Burning plastic raises the ambient CO2 level and causes a number of health issues. It has been reported in 2018 indicates that specific bacterial strains can utilize carbon as an energy source to facilitate the breakdown of plastics [6].

A biofilm is a stationary collection of microorganisms that adhere to a surface or polymer, enter the periplasm, and undergo phenotypic modifications. These

biofilms are produced as a result of bacteria working together to accomplish a certain goal. Another name for these quorum sensing- controlled biofilms is the phenotypic state of bacteria [8].

The phrase "quorum sensing" refers to cellular signaling and communication, which facilitates message exchange and controls gene expression. It generates autoinducers, which are crucial in controlling a range of gene and protein activities. Some genes become active while others become inactive when a particular connection threshold is met. The formation of biofilms is influenced by things like the availability of nutrients and a surface that is conducive to microbial adhesion. Microorganism communities show aggressive proliferation, a sign of their success in a favorable environment [9].

Because monomers may easily enter cells through intracellular metabolism and absorption, microorganisms break down polymers into monomers. The progressive breakdown of plastic can be sped up by things like plastic movement, mechanical forces or UV light exposure. A biofilm, also known as biofouling, or stable colony of microorganisms, invade the plastic surface. These biofilms aid in conjugation, store nutrients and offer defense against dangerous substances. Extracellular enzymes produced by microorganisms degrade synthetic polymer constituents, making them porous and starting the biodegradation process. The richness and variety of the biosphere tend to grow throughout time. Exogenous and native microorganisms compete with one another to keep this ecosystem in balance [8].

Other functions that microorganisms that degrade plastic also perform include the breakdown of xenobiotic, the reduction of antibiotic resistance, nitrogen fixation, and quorum sensing. The cleanup of plastic pollution in the environment is aided by biofilms related to plastics. Microbial biofilms and the physicochemical environment around a location control how quickly plastic degrades [10].

The body of existing research points to microbial-mediated degradation as a more acceptable and ecologically responsible method. This technique focuses on the bacterial enzymes' capacity for breakdown with the goal of achieving effective and environmentally benign biodegradation [10].

The outcome and impact of plastics in the environment depend on the nature and intensity of the degradation process, yet our current understanding of environmental plastic degradation is limited. In order to pinpoint vital microorganisms with efficient biodegradation abilities, it is crucial to explore the capacities of bacteria and the interplay between bacterial enzymes as well as plastics. Comprehensive research is needed, taking into account variations based on the chemical composition of plastics and the specific enzymes possessed by bacteria. The study also highlights shortcomings in existing microbial breakdown methods and proposes potential solutions. Special attention is given to comprehending the processes of biodegradation, the efficacy of microbe-derived enzymes, and the role of preprocessing in facilitating the biodegradation process [11].

1.1 Problem Statement

Research into the microbial colonization of plastic waste has been well-documented in marine environments, but studies concerning terrestrial waste are infrequent and largely centered around the isolation of microorganisms capable of degrading plastic. A thorough evaluation of bacterial communities inhabiting terrestrial plastic waste and their involvement in plastic degradation remains relatively underexplored.

1.2 Gap Analysis

Studies on terrestrial plastic wastes are limited. With respect to biofilm farm bacterial communities Biofilms identification and isolation are still under study.

1.3 Aim

To access the composition of bacterial communities thriving as a biofilm on the plastic surfaces of terrestrial waste.

1.4 Objectives

1. To recover the bacterial strains from terrestrial plastic wastes samples collected from Waste disposal sites.

2. To perform isolation and molecular characterization of cultivable bacteria recovered from plastic.

3. To evaluate the biofilm forming potential of recovered strains.

4. To access the plastic degradation capability of molecularly characterized bacterial strains.

1.5 Impact on Society

The planned study will center on evaluating the bacterial compositions responsible for biofilm formation, a key factor in plastic degradation within terrestrial waste environments. These findings will enhance our comprehension of the microbiological and ecological aspects of the impact of terrestrial plastic waste, as well as inform its management within natural environmental settings. This research is crucial given the ongoing and enduring threat posed by plastic degradation to the environment.

1.6 Scope of Study

Global plastic pollution affects microbiomes in a variety of ways, including direct effects from hazardous leachates on microbial populations and their functioning as well as indirect effects from the presence of plastic on host organisms and ecosystems. In the breakdown of plastic, several microbial species, genes, and enzymes are involved. This study may be used as a basis for assessing plastic changes related to certain bacterial groups, quantifying mass loss, identifying degradation by products, identifying microbial strains and enzymes responsible for degradation,

and locating related genes. The scope of plastic breakdown may also be accelerated and expanded by altering the bacterial populations responsible for biofilm formation.

1.7 Research questions

Q Are there any biofilms present on plastic recovered from waste degradation sites?

Q What type of bacteria are involved in biofilm formation responsible for plastic degradation?

Chapter 2

Literature Review

2.1 Types of Plastics

The prolonged and molecular main chain that is generated by affiliation of massive number of repeating units typically thousands or in some cases, hundreds of thousands is known as the backbone of a polymer. It is possible for the backbone to include only carbon atoms or additional elements, most frequently oxygen or nitrogen with sporadic silicon or sulphur atoms [12].

The form of polymer backbone are used to classify plastics into different kinds of polyesters, silicons, halogenated plastics for example PVC. The synthetic method is also used to classify plastics for example polycondensation, cross linking 19 and having definite physical properties, resistance heat and glass transition temperature [13].

The two different kinds of plastics are divided into 7 categories: Low Density Polyethylene (LDPE), High-Density Polyethylene (HDPE), Polypropylene (PP), Polyvinyl Chloride (PVC), Polystyrene or Styrofoam (PS) and Polycarbonate. To control the adverse effects of plastics, different kind of methods are composed which consist of chemical, biological and physical treatments [14].

Low-density polyethylene (LDPE) and high-density polyethylene (HDPE) are produced through the addition polymerization of ethylene utilizing organometallic

catalysts. In LDPE, the presence of a significant degree of long and short chain branching prevents the polymer molecules from arranging themselves into a crystalline structure. Conversely, HDPE features polymer molecules that are 500,000 to 1,000,000 carbon units with minimal branching. The higher proportion of crystalline structures in HDPE contributes to its strength and opacity, distinguishing it from the more transparent and less robust LDPE [15].

Recycling is essential because almost 90% of the plastic produced come from virgin fossil feedstock. On average plastic are produced with 6% of global oil consumption. A large portion of greenhouse gas emission are caused by plastic production process [16].

A significant amount of plastic production each year is attributed to throwaway products, which are typically discarded within one year of their manufacture. Polyethylene (PE) divided into HDPE and LDPE and polypropylene (PP) are the most common families of polymer used in industry. Polyvinyl chloride (PVC) and polystyrene (PS) form most of the polymers used in industry. PP (Polypropylene) are the least recyclable plastic polymers despite their widespread use.

This variety of plastic materials, combined with their exceptional capabilities, resulted in an unregulated increase in production, resulting in massive amounts of plastic trash [17].

2.2 Production and Consumption of Plastic

As a result of rising plastic consumption, 6.3 billion tons of plastic are produced per year. The plastics sector is still growing, which results in more plastic being produced and ensuing environmental problems. With 245 million tons of plastic produced annually globally in 2008, plastic is a serious pollutant of the environment. In Europe, plastic packaging alone accounts for 40% of consumption, while integration of plastic into electrical devices, consumer products, agricultural applications, and building materials accounts for 20%, 22%, 3%, and 6% of usage, respectively. In 2015, the regions with the highest rates of plastic production were

Asia, China, Europe, and North America. By destroying their habitats and clogging the gills of fish and other aquatic animals, this pollution also harms marine life [18]. A serious hazard of increased carbon emissions in the environment is posed by the volume and prevalence of plastic, which is growing. The carbon cycle is being disturbed by this increase in carbon levels, which also poses a hazard to the food chain. Microplastics (MPs) may also be biologically broken down by a variety of organisms, including bacteria, fungus, viruses, and microalgae. These biological techniques for microplastics breakdown are eco- friendly strategies [19].

Since 1964, the amount of plastic produced has increased twenty-fold, reaching 311 million tons in 2014. According to projections, its production should treble nearly every ten years [20].

Pakistan's plastics sector has made considerable strides and achieved great success. The fourth-largest import category now is plastic materials, which significantly contributes to the national coffers in a number of ways. This industry has outpaced other industrial sectors in growth, averaging about 15% yearly increase. With the exception of occurrences like those following 9/11 and hostilities with India, the nation has seen an increase in per capita plastic usage during the previous 15 years [21].

Pakistan now consumes 2.7 kg of domestic plastic per person, which is less than the global average. Nevertheless, Pakistan is Southeast Asia's second-largest domestic market, only behind India. By 2007, it is anticipated that Pakistan's population of 175 million will consume 4.0 kilograms of plastic per person due to population growth of 2.2% annually, increased foreign and domestic investments, and government policies encouraging the use of polypropylene woven bags for industrial packaging.

Customs tariffs on raw materials for plastic were unacceptably high at over 110% in the early 1990s, but these rates were gradually decreased to the present level of 20% [22].

Plastic items are widely used in modern life and have become necessary. However, due to the exponential rise in plastic manufacture, there is now an excessive

amount of plastic trash, which is more than society can adequately handle. More than 454 million tons of plastic made from fossil fuels were produced worldwide in 2018 compared to 2 million tons in 1950 [23].

The output of plastic is expected to double by 2025 and quadruple by 2050 from the 9.7 billion tons produced between 1950 and 1980 [24]. According to Geyer (2020), each year, 343 million tons of plastic garbage are produced. East Asia and the Pacific account for 57 million tons of this garbage, while Europe and Central Asia contribute 45 million tons each. North America accounts for 35 million tons of this waste [25].

2.3 Plastic Source

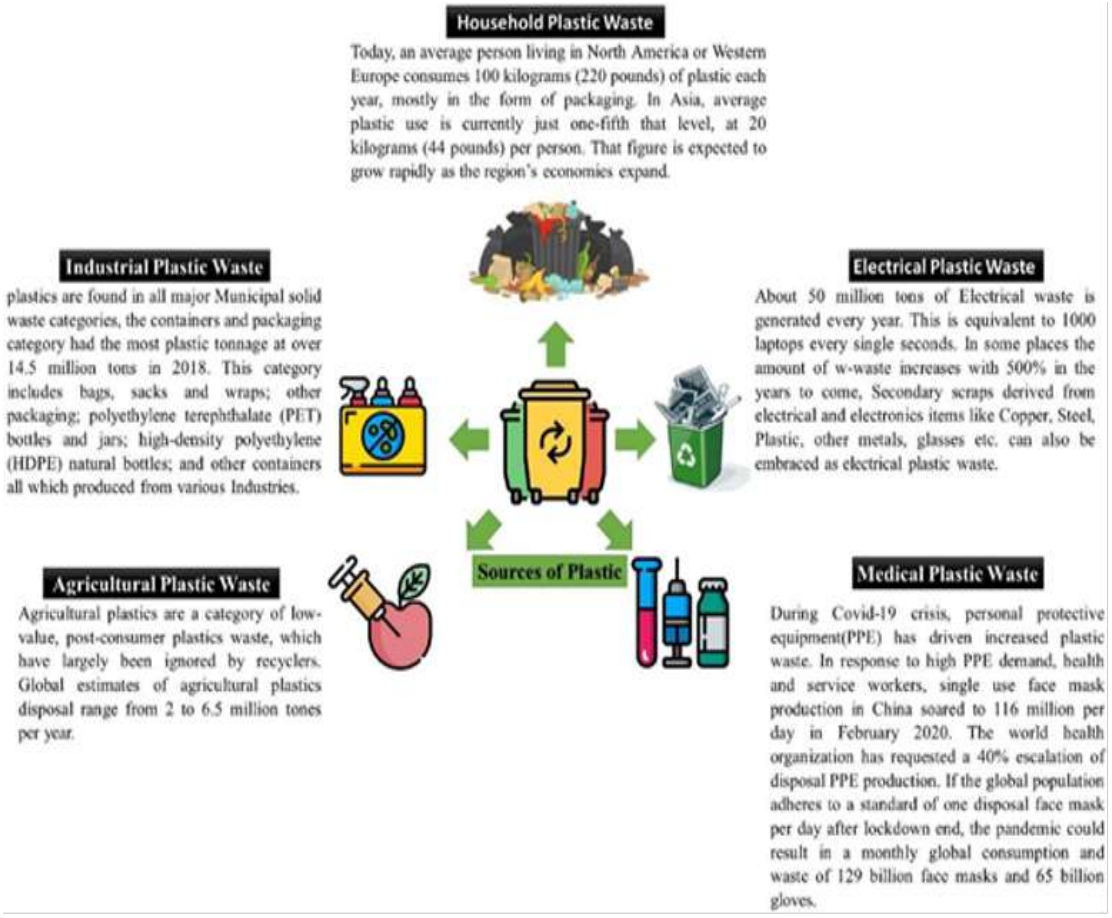

FIGURE 2.1: Sources of plastic wastes [26]

2.3.1 Household Wastes

Residential waste comprises discardable items. This category encompasses items like shopping bags, packaging from various food products, printed materials, diapers, organic waste from both crops and animals, among others. Household waste encompasses both hazardous and non-hazardous types. Non-hazardous waste include food scraps, paper, bottles, while hazardous waste includes items like plastics, dry cell batteries, electronic waste, and medical waste. Hazardous waste is characterized by toxic organic chemicals, elevated levels of heavy metals, substances depleting the ozone layer, and the potential for explosiveness or flammability. Proper collection and disposal procedures tailored to hazardous waste are imperative for ensuring safety and environmental protection [27].

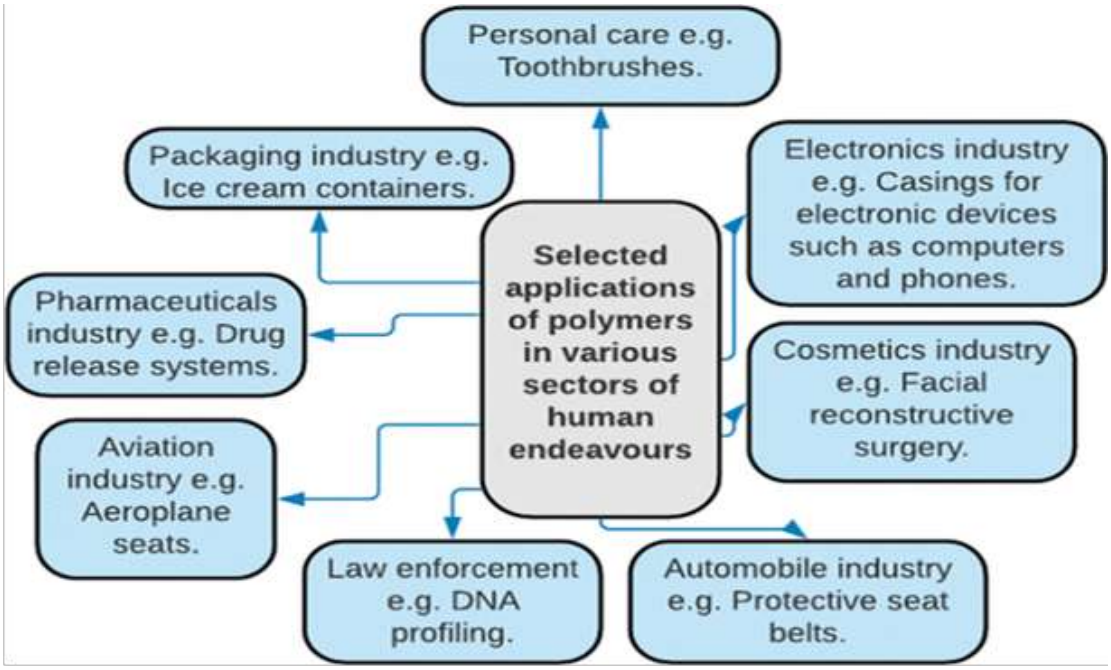

FIGURE 2.2: Polymers significantly contribute to human advancement, playing a substantial role in nearly every sector of the economy [28]

2.3.2 Industries

Industrial plastic waste, stemming from extensive manufacturing, processing, and packaging industries, encompasses materials from diverse sources like electrical

and electronics; automotive; packaging companies, small and medium-sized businesses, and many others. A notable advantage of industrial plastic waste is its often substantial volume and relatively clean, contaminant-free nature. To ensure both efficient utilization and minimal environmental impact, it is crucial to establish precise regulations and laws governing the proper disposal and recycling of industrial plastic waste [28].

Municipal solid waste (MSW), in contrast, is a heterogeneous mixture of hazardous, recyclable, and degradable elements. Repalletization and remolding are simple and efficient techniques for treating homogenous plastic waste, as opposed to disposal or combustion with ordinary MSW. However, reclamation becomes difficult when plastics in MSW are heterogeneous and comprise mixed resins since those resins demand different processing temperatures and pressures [29].

2.3.3 Agriculture

Plastics have become indispensable in modern agriculture, playing a crucial role in various aspects. They are extensively utilized in irrigation systems, crop protection, post-harvest procedures, and the production of packaging for seeds and fertilizers. Additionally, a substantial quantity of plastic is employed in constructing nets and covers designed to safeguard crops from adverse weather conditions, insects, and wildlife. The widespread application of plastics in agriculture contributes to the generation of a significant volume of agricultural plastic waste (APW) annually, corresponding to the projected global consumption of 6.5 million tons of agricultural plastics each year [30].

The rising volume of agricultural plastic trash provides a burden, but it also offers a chance because it can be properly collected and treated. Nevertheless, the rate of recycling for agricultural plastic waste is still rather low, and it differs greatly from one nation to the next and depends on the accessibility of local recycling facilities. Unfortunately, a large amount of agricultural plastic trash is finally disposed of by burying in the ground, burning carelessly in fields, or just being dumped in

agricultural regions, with the majority of it ending up in Waste disposal sites or being dumped close to rivers and canals [31].

2.3.4 Medical Wastes

Since January of 2020, UNICEF has provided more than 200 million medical masks, considerably increasing the amount of MPW during the COVID-19 epidemic. It's important to note that during the crisis, Hubei Province, where the epidemic first appeared, had an astounding 370% increase in the output of MPW, with a sizable amount of this waste consisting of plastics. China generated almost 207,000 tons of medical waste from January 20 to March 31 of that year [32].

The usage of single-use plastic (SUP), which includes goods like medical suits, Protective hand covering, protective masks, Disinfectant containers, disposable plastic bags, food and pharmaceutical product containers, Diagnostic kits and more, increased significantly as a result of the COVID- 19 epidemic. Unprecedented growth in the use of SUPs has led to the discharge of millions of these pandemic-related products into the environment, posing serious environmental dangers.

Facemask disposal is a serious environmental concern since they can take over 400 years to degrade and may release microplastics while they do so [33]. Therefore, it is projected that the CO2 emissions from the manufacture, administration, and transportation of these facemasks in nations like India, the United States, Brazil, Nigeria, the United Kingdom, and others range between 22.4 and 53.6 tons. According to *Graulich et al.* 2021, these nations generate 1.6 million tons of facemasks per day [34].

Regarding the consumption and disposal of COVID-related plastics, the situation is the same in Bangladesh. As per the information provided by ESDO, a total of 14,500 tons of plastic waste (PW) were discarded within a month, namely March to April 2020, with Dhaka City alone accounting for 3,076 tons of this amount.

Discarded hand gloves made up a sizeable amount of plastic waste (PW) in terms of tons, totaling 5,877 tons from 1,216 million gloves, 3,039 tons of which were

made of plastic. Additionally, worn surgical masks generated 900 tons of garbage to the waste stream and 1,592 tons of PW [35].

With an estimated 1.56 billion facemasks ending up in the water, aquatic habitats have been severely damaged. About 107,212 pieces of personal protection equipment (PPE) have been found by community scientists in rivers and on beaches. A number of bird species have been discovered nesting among this trash, which is noteworthy. Unfortunately, some of these birds have perished as a result [36].

2.4 Plastic Pollution

Pollution is a worldwide issue that presents a persistent challenge with characteristics that are not bound by national borders, span various institutions, and require solutions that transcend disciplinary boundaries. Pollution originates both naturally, such as through intrusion of saltwater into freshwater sources and the emission of harmful gases during volcanic eruptions, along with human activities, referred to as anthropogenic activities. These activities initiated by humans include the utilization of the environment and its resources, as well as the introduction of substances or energy into the environment that deviate from its natural composition. The introduction of such anthropogenic elements has the potential to disrupt and jeopardize the delicate and interconnected systems of the Earth, setting off a chain reaction known as a "domino effect."[37]. An other definition of pollution is an unnatural disturbance brought about by the introduction of materials or energy into the ecosystem. This incursion may change or deteriorate a system's or environment's natural state, which raises the possibility that the system will diverge from its initial parameters and operational capabilities. For instance, when water alters the fundamental properties and functions of commercial petroleum products, such as petrol, in motor engines, it might be regarded as pollution [38]. It follows that these artificial disruptions frequently cause chemical processes that result in the change or transmutation of substances from one form to another, whether irreversible or reversible. Thus, pollution has the potential

to affect matter dynamics and habitats, which in turn can affect the properties of both living and nonliving materials.

Pollution has serious negative effects, especially in light of the current environmental issues. For example, studies show that even a small reduction in air quality due to pollution can cause major disruptions to bee behavior, endangering bees' essential roles in the ecosystem and threatening food security [39]. A noteworthy correlation has been found by other research to exist between birth abnormalities and community exposure to chemicals associated with environmental pollutants [39]. According to a recent investigation, environmental contamination is the reason why nursing women' breast milk quality is declining. According to the study, contaminants like polychlorinated biphenyls (PCBs) can disrupt and change a mother's milk's natural composition, which could have negative health effects on nursing infants. Allergies, endocrine problems, and impaired neurodevelopment are a few of these outcomes. To highlight the existential threat that pollution poses, a global health assessment found that health issues resulting from pollution are responsible for more than 20% of fatalities worldwide. The living and nonliving elements of the environment are both impacted by pollution, which has an effect on almost every aspect of our lives. For example, three decades of satellite data show the dire effects of environmental pollution-caused global warming. As a consequence, Greenland's ice sheets have significantly shrunk, which has raised sea levels worldwide [40].

Due to its broad and difficult-to-address influence on organisms and nonliving structures, pollution from plastic poses a large and urgent worldwide concern, resulting in environmental pressure. Under this context, the term "plastic pollution" refers to the entry or penetration of plastic materials (polymeric structures) into habitats, whether through direct input or degrading processes. This includes macro-, micro-, and nano plastic detritus. The habitats where these components are not normally present are negatively impacted by their intrusion. Plastic pollution transcends national borders and legal authorities, just like greenhouse gases, persistent substances, and other polluting substances do. It can travel across water bodies, scatter through the atmosphere, and be carried to far-off places by

human activity [41].

Plastic pollution has been a problem for almost fifty years, and it is clear that the damage it poses is not going away. In order to effectively address and mitigate this issue, all parties must make concrete promises and restart their complete effort. An similar volume of water might be replaced by the significant amount of plastic that is finding its way into the world's aquatic habitats. This might result in a decrease in aquatic ecosystems, raising the possibility of flooding and accelerating global warming. As a result, these incidents have a number of unfavourable effects, such as endangering public safety, resulting in property damage, and putting a heavy burden on government budgets, hospital facilities, and the insurance sector. This demonstrates the wider effects of plastic pollution [42].

2.4.1 Land Pollution

Before harming and contaminating the aquatic environment, plastic waste and plastic contamination of products can hurt and pollute the environment on land. There are not as many statistics on the entire quantity of plastic garbage on land as there are regarding plastic debris in marine habitats, even though sources on land account for around 80% of the plastic waste found in the ocean. Plastic additives like stabilizers, plasticizers, harmful colorants, both soil and water can become contaminated by heavy metals that seep into the environment.. When plastics are disposed of on land or in Waste disposal sites, they undergo abiotic and biotic degradation, further contributing to the contamination [43]. Five years after they were sprayed, sewage effluent and soils may still include plastics made of synthetic polymers. Chlorinated plastics can emit toxic substances into the earth's crust, which may subsequently leach into the local marine system or underground water and harm the natural environment. Methane, a dangerous greenhouse gas that has a major impact on global warming, is released when plastics break down under the action of microbes [44]. Plastic waste is often transported to a secondary transfer station where it is sorted after being collected from homes, offices, enterprises, and industries. Depending on the infrastructure and technology available, the plastics

are either recycled or repurposed after this sorting process. If the requisite infrastructure and tools aren't available, plastic garbage might also be burnt outside or dumped in open areas or Waste disposal sites. When plastics are incorporated into the soil, they may change its porosity and binding qualities, which may have an effect on soil aggregation and water transport. Microplastics (MPs), a particular kind of plastic trash, can interact with different soil constituents. Additionally, plastic pollution prevents the development of helpful bacteria and earthworms in the soil, which ultimately results in soil sterility [45]. The presence of plastics as stressors in ecosystems has a negative effect on soil health and changes its physical characteristics, which complicates how other pollutants behave in the soil environment.

2.4.2 Water Pollution

Floating plastic waste can serve as a quick colonization site for sea animals, and its prolonged presence on the ocean's surface may impede the spread and growth of associated species. Given their widespread presence in seabed and coastal environments, as well as the small-size, plastics are readily accessible to a diverse range of marine organisms. Plastic possesses the ability to attract and retain pollutants-found in saltwater, originating from various sources within the marine ecosystem. These pollutants comprise biological contaminants like nonylphenol, PCBs, dichlorodiphenyldichloroethylene (DDE), phenanthrene, among others, and exhibit a higher tendency to accumulate on plastic waste compared to the surrounding ocean. The ingestion or entanglement of plastic waste by over 260 marine species, including, fish, turtles and seabirds, invertebrates, & mammals, can impede their ability to move, feed, heal, reproduce, and survive. The significant increase in plastic production, coupled with low recycling rates and inadequate waste management, results in its release into aquatic environments, creating new ecological niches for microbial communities and the development of a "plastisphere" [45].

Possibly, our comprehension of the environmental buildup of plastic initially focused on aquatic debris and has significantly broadened in recent decades [46].

In the subsequent decade from 2011 to 2020, there was a rapid and notable increase in the number of research articles. Most research efforts in the earlier phase, which ran from 2000 to 2006, were focused on measuring marine debris and investigating the effects of plastic pollution on aquatic life [47]. However, in the last several years, there has been an increasing amount of data showing that plastics are accumulating in various settings, including air and terrestrial regions [48, 49].

Most research efforts in the earlier phase, which ran from 2000 to 2006, were focused on measuring marine debris and investigating the effects of plastic pollution on aquatic life [47]. However, in the last several years, there has been an increasing amount of data showing that plastics are accumulating in various settings, including air and terrestrial regions [48, 49].

Once introduced into the marine environment, plastic demonstrates extensive transport facilitated by its buoyant and durable characteristics. For instance, compared to the outer near-shore seas, the abundance of macroplastics and microplastics has been found to be four and fifteen times higher, respectively, in coastal turbulence zones formed by breaking waves [50]. This observation suggests that the physical characteristics of the plastic particles, such as surface area, buoyancy, and density, affect the accumulation of plastic. Denser particles are more likely to experience vertical transfer, whereas lower density particles prefer to stay in surface water. In the central Gotland basin, for example, it has been recorded that 5 mm polyoxymethylene particles with a density of 1.6 g cm-3 sink through the water column of about 250 m in less than 18 hours [51].

The process known as "biofouling," which is typified by microorganisms quickly colonising submerged plastic surfaces, can improve plastics' horizontal dispersion as well as sedimentation [52]. Low-density microplastics may sink as a result of an overall increase in density brought on by microbial growth in the form of biofilm [53]. Furthermore, the development of complex microbial biofilms facilitates the adhesion of suspended dense materials such as marine snow and iron hydroxides, hence augmenting the total mass and hastening the sinking of microplastics [54].

Riverine routes play a major role in the movement of plastics from land to marine habitats. *Napper et al.* estimate that the Ganges and the combined flows of the Brahmaputra and Meghna rivers release one to three billion microplastics every day into the Bay of Bengal, which is the northeastern part of the Indian Ocean. The concentration of microplastics increases as one moves from the source to the sea. This study represents a crucial initial step in understanding the contribution of major rivers to oceanic microplastics levels.

Urban surface runoff serves as a major source of plastic in rivers. For example, it has been observed that tyre and road debris carried by pollutants from cities account for roughly 42 percent of micro plastics in European streams [55]. Moreover, it is estimated that urban rainwater runoff, including sewage overflow, is responsible for 62% of the plastic particles in the Baltic Sea's waters [56]. The number of microplastics that are reportedly carried to the ocean by rivers is estimated differently than the quantity of micro plastics that accumulate at the ocean's surface. This disparity gave rise to the idea of a "missing" plastic sink in the ocean [57].

2.4.3 Air Pollution

Due to their light weight & low density, plastics have the potential to be transported by wind. As a result, the atmosphere has been recognized as a significant pathway for the regional and even global-scale transportation of suspended particulates, with microfibers from clothing suspected to be substantial contributors [58]. Research has been done on the rates at which micro plastics, mainly fibers, are deposited in the atmosphere in both urban and remote locations. The results range from ten m-2 the d-1 in Gdynia, the nation of Poland, to the year 771 m-2 d-1 in Central London in the United Kingdom, and from twelve a-2 d-1 in Mount Sinai Derek, Iran, to a total of m-2 d-1 in the French Pyrenees [59].

Furthermore, although plastics are frequently carried from land into aquatic ecosystems, they can also linger on land, migrate from water to land, or even enter the atmosphere. The pollution of terrestrial habitats, notably soils used for agriculture, by plastic has only recently garnered attention but forms a considerable

amount of the total plastic emitted into the natural world [60, 61]. Sludge left over from wastewater treatment facilities is another way that plastics can get into the terrestrial environment [62]. Waste sludge can be burned, dumped at a landfill, or used to generate electricity. But in some nations, as much as eighty percent of wastewater from cities sludge can be utilized in agriculture, and in such bio solids, there is a significant amount of plastic (between 4,200 and 15,800 particles per kilograms) [63].

2.5 Threats to Living Organism & Human Health

Plastic pollution poses risks to organisms through physical harm, chemical damage, and biological threats [64]. Two common forms of physical harm to organisms are entanglement and the ingestion of plastic debris. Entanglement can be lethal for mammals, causing harm through strangulation, sepsis, or starvation resulting from neck entanglement. For instance, some seabirds have been fatally ensnared by fishing gear. Not only can eating plastic waste kill larger animals by impaction of the stomach or obstruction of the digestive tract, but it can also have long-term effects that could build up throughout the food chain [65]

It has been established that a number of compounds associated with plastics are detrimental to living things, causing hormonal disruption, oxidative damage, malignancy, and accumulating throughout the food chain [66]. For instance, chemicals found in plastics, including phthalates and bisphenol A, can have negative effects on living things and build up via the food chain. Additionally, compared to saltwater, these related pollutants are frequently transported into creatures more easily, which can directly cause toxicity or accumulate in species before moving up the food chain. These results suggest that organisms come into contact with tiny plastics more frequently, which can have an impact on their chemistry and biology also, there is currently limited knowledge on these matters, underscoring the need for extensive research to comprehensively understand the adverse effects of plastics on organisms [67].

Moreover, recent studies have estimated that adults in America ingest tens of thousands of micro plastics annually. The main pathways for plastics to enter the human body involve ingestion through daily dietary intake inhalation of air containing plastics, and skin contact. Although some potential negative consequences have been suggested, the specific hazardous effects of plastic on human health are still unknown. For instance, human cells are susceptible to the harmful effects of nano plastics, which include oxidative stress and inflammation [68].

According to a 2011 study, plastics enter the marine ecosystem through various ocean-related activities such as fishing, aquaculture, and the operation of commercial ships and ferries. Unlike plastics originating from land-based sources, managing marine plastics presents a challenge as they drift unpredictably in the open sea. About 98% of these aquatic plastics come from land-based activities, with the remaining 2% coming from activities that take place in the water. Additionally, the terms "Microplastics" (MPs) and "Nanoplastics" (NPs) are used to classify these polymers. The presence of harmful chemicals and the accumulation of micro- and nanoplastic waste can lead to various issues, including harm to living organisms [69].

2.6 Plastic Waste and Its Management

Plastic garbage is thought to have been produced in an astounding 6.30 billion metric tons (BT) between 1950 and 2015. Based on research conducted by *Liang et al.* in 2021, a mere 9% of this waste was subjected to recycling, with over 80% being disposed of in Waste disposal sites or entering the ocean. According to Aryan analysis in 2019, the usage of plastic materials increased significantly between 2011 and 2015, rising from about 2 million metric tons (MT) to 320 million MT [70].

Additionally, according to a 2017 report by *Hermabessiere et al.,* global plastic production has doubled over the past two decades, reaching approximately 335 million metric tons, and a prediction by UNEP-WCMC in 2018 anticipates an increase to over 600 million metric tons by 2030. The increase in the prevalence

of plastic is linked to the buildup of plastic debris, which has negative impacts on both the environment and human health and takes thousands of years to break down. Despite being an essential part of daily life due to its versatility, plastics have become a major constituent in municipal solid waste (MSW) and endure as a hazardous material. However, the widespread presence of plastic, particularly single-use plastic, in MSW also indicates insufficient waste management and a lack of enforcement of rules and regulations [71].

Petrochemical processes and fossil fuel resources play a crucial role in the production of plastic. As highlighted by *Nielsen et al.* in 2020, approximately 99% of the raw materials utilized in plastic production are derived from fossil fuels, constituting 8–9% of global oil and gas consumption. Various recycling methods are available, including mechanical recycling, energy recovery, and chemical recycling. Recycling becomes a more viable and sustainable option when the environmental, social, and economic impacts of producing a polymer outweigh those of mechanically recycling the material. Mechanical recycling, a conventional method, involves collecting plastic waste, cleaning it, melting it, and converting it into raw materials for manufacturing new items from the waste. In contrast, chemical recycling is a process that transforms polymeric waste into materials suitable for producing new goods or generating energy through waste-to-energy conversion. Energy recovery encompasses diverse procedures, including gasification, pyrolysis, and incineration [72].

2.7 Plastic Waste Management on Land

Being non-biodegradable, the plastics are difficult to reintroduce into the environment's carbon cycle, resulting in their end-of-life cycle on land or in the water, as stated by *Luo et al.* in 2000. According to *Miskolczi et al.* in 2006, a variety of techniques are used to dispose of industrial and municipal garbage, including burning, landfilling, chemical recovery, among others. According to *Delattre et al.* in 2001, efficient management of plastic trash is a critical component of waste management with substantial consequences for the environment and the economy. In

the early 2000s, recycling made up just approximately 10% of the plastic garbage that was disposed of, with landfilling making up the majority (65-70%) and incineration making up the remaining (20-25%) [72]

Plastics recycling and incineration were quite minimal before 1980. According to study conducted in 2017 by *Geyer et al.,* non-fiber plastics were the primary target of recycling programs. About 287 million metric tons (MT) of plastic garbage were produced overall, and this number has risen over time. According to predictions based on Geyer research, by the year 2050, the total amount of plastic garbage generated might be close to 24,712 million MT. Earlier years saw relatively little recycling of plastics. However, recycling plastic has become more significant over time, particularly in response to the rising problem of plastic trash buildup [73].

2.7.1 Landfilling

Municipal solid waste (MSW) contains a sizeable number of plastics, and many of them are simply dumped in Waste disposal sites without any further processing. According to *Garforth et al.* in 2004, this practice has developed into a serious environmental danger as a result of factors including regulatory demands, inadequate infrastructure maintenance, greenhouse gas emissions, and the low biodegradability of frequently used polymers. The Environmental Protection Agency (EPA) has enhanced both federal and state regulations pertaining to landfill disposal in an effort to address the challenges associated with hazardous waste management. These regulations promote responsible practices such as the proper utilization of landfill beds, conducting groundwater testing, and implementing post-landfill maintenance. However, a significant challenge arises in the allocation of suitable landfill space, primarily due to the high volume-to-weight ratio of plastic waste [74].

2.7.2 Mechanical Reprocessing

Mechanical recycling is turning the discarded thermoplastics into new items that are either the same sort of product or one that is comparable. According to La Mantia's 2002 description, it is a type of recycling that starts with the separation of reversible plastics meant for recycling. Waste homogeneous thermoplastics are

frequently repurposed into identically-precise new goods. In the beginning, petrochemical businesses receive natural resources like salt, crude oil, and natural gas that have been removed from the environment and use them to manufacture polymers. Before being given to customers, the polymers are then modified in terms of color, thickness, and size. These objects are seen as garbage once the primary product life cycle is through. These items are transported to recyclers after being sorted, where they make an attempt to recycle them and create either same or alternative products. A continuous loop is created when this recycled material is given back to customers [75].

It is important to know that recycling operations might not be economically feasible due to the need for substantial energy input in the reformation process, advanced technology for sorting, cleaning, transportation, and processing, along with additional expenses to ensure that all these processes are conducted in an environmentally responsible manner [76].

2.7.3 Biological Recycling

FIGURE 2.3: Mechanical recycling loop of plastic products [77]

The usage of biodegradable plastics has been the subject of much study in an effort to address the rising need for organic recovery. It is crucial to build a strong biological infrastructure and the required technical know-how in order to

meet future requirements. Additionally, techniques like aerobic composting, which involve aerobic bacteria and fungus that use polymers for energy and reproduction, can be used to recycle biopolymers. Additionally, biomass is created via biological recycling from organic carbon sources. Biomass has an energy potential and aids in environmental preservation. If there are any difficulties during this process with the decomposition of these polymers, they are recognized and isolated for treatment as non-degradable sections [78].

2.7.4 Thermal Recycling or Incineration

Depending on the specific properties of the resin, as also indicated by *Verma et al.* Although the operational costs, including energy and labor expenses, are relatively high in thermal recycling, the convenience of the reprocessing process and the ability to use mixed plastics as feed stock materials have contributed to the widespread popularity of thermal recycling over other recycling methods [79].

2.7.5 Chemical Recycling

The many polymer types utilized in the creation of plastics are related with specific production parameters even though the name "plastic" is general. It is impossible to recycle several polymers at the same temperature throughout the recycling process since each polymer has a unique melting point. Consequently, it is crucial for efficient recycling to classify polymers by type and take into account the anticipated ultimate products. The advantage of chemical recycling over mechanical recycling is that it provides a better way to cope with additives and inadvertently added compounds in plastics .According to studies, chemical recycling is in line with the concepts of sustainable development since it lays a major emphasis on energy recovery [80]. By reviving the deteriorated polymer structure to coincide with the precise uses and attributes required for the end products, the chemical recycling process is able to manage mixed and contaminated polymers.

- Solvent-based purification involves breaking down plastic waste into its polymer components.
- Chemical depolymerization is a process that reverses plastic waste into its monomers through a chemical reaction.
- Thermal depolymerization, which includes gasification and pyrolysis, is a method closely associated with chemical recycling. This process breaks down polymers into monomers and converts them into hydrocarbons [81]

2.8 Plastic Management in the Ocean

2.8.1 Floating Marine Debris

The most efficient way to move this material over substantial distances is through large-scale open ocean transport, which has been used since 1970 .According to Van Sebille, in addition to the processes already mentioned, researchers have also looked into a number of other processes that can move debris, including mesoscale and submesoscale currents, Stokes drift, internal tides, transportation influenced by direct wind force, Langmuir circulation, and the effects of ice formation, melting, and drift. Additionally, sea cleaning boat services have been used in the fight against marine debris, notably in the Balearic Islands, where they have successfully removed floating marine trash from the coast. Additionally, Compa noted that spatial thermal monitoring has demonstrated the diverse distribution of floating plastic garbage. Japan has also been involved in research using an innovative method known as the spectral angle mapper, which proves effective [82].

As reported by Aoyama in 2018, a method known as the spectral angle mapper has been used in studies in Japan and has proven to be successful when dealing with marine debris of significant size and area [83].

Trawling, which uses nets and is appropriate for deep, soft seafloors, and driving surveys, which are conducted in regions with rocky seafloors and use scuba or snorkel divers, are the two main techniques for removing marine trash. Numerous

shoreline cleaning initiatives are organized to gather marine waste; however, these efforts are deemed insufficient. The technology known as the floating debris containment boom has its origins in Korea, has been developed to prevent river and water canal waste from entering the ocean [84].

2.9 Innovative Techniques Employed in the Management of Plastic Waste

Incineration, dumping, and landfill disposal are examples of traditional ways for managing non-biodegradable plastic trash; they are not sustainable solutions. As a result, continuing research projects are looking into cutting-edge methods to deal with the significant buildup of plastic garbage. Degradation and recycling are the two main approaches under investigation [85].

2.9.1 Hydrocracking

Long hydrocarbons chains are broken down into smaller molecules, such as kerosene & gasoline, by a process called hydrocracking, often referred to as hydrogenation. According to Al-Salem description, this transition happens by the addition of hydrogen when catalysts are present and under high pressure. Hydrocracking technique has a number of benefits over pyrolysis [86].

Additionally, by lowering the production of aromatic heteroatoms, hydrocracking plastic waste can dramatically lower the concentration of heteroatoms and improve the quality of the end products [87].

2.9.2 Gasification

Gasification, a thermolysis process, offers a viable alternative to hydrocracking and pyrolysis. In this approach, feed materials undergo a conversion process, producing syngas through interaction with light hydrocarbons like CO_2, water,

and methane. This transformation takes place at elevated temperatures in the presence of oxygen [84].

As stated by Al-Salem, diverse gasification techniques, including the Battelle method, Texaco gasification, the Akzo process, and others, are employed in industrial applications. The Texaco gasification process comprises two primary elements: the liquefaction stage and the entrained bed gasifier. During the liquefaction phase, plastics undergo thermolysis, breaking down into various particles [85].

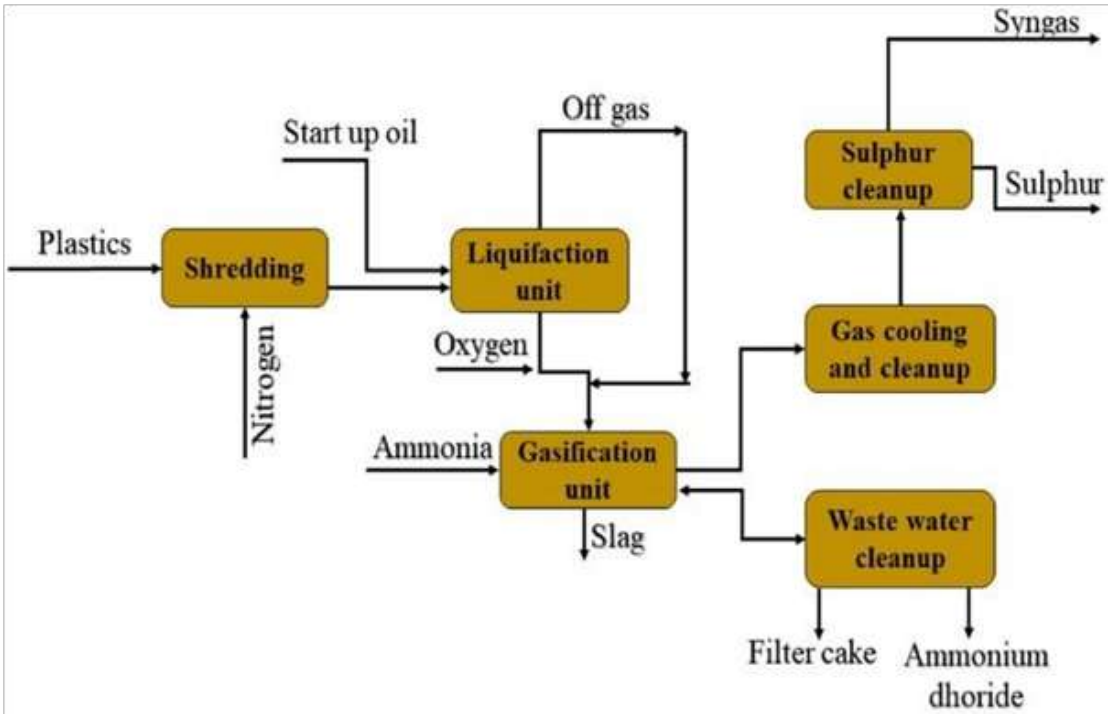

FIGURE 2.4: Diagram of Texaco gasification process [86]

2.9.3 Chemolysis

Another approach for resource recovery is chemolysis, which is often referred to as depolymerization or solvolysis. Individual polymers are chemically broken down into monomers during this process at temperatures between 80 and 280 degrees Celsius. The chemolysis resource recovery method frequently uses a variety of unsaturated resins and polyesters as feedstock, including substances like polyesters,

polyethylene terephthalate, polyamides, polycarbonate, polyurethane, and others [87].

Recycling monomers from mixed plastics may be difficult since the kind of plastic has a considerable impact on the efficacy of the chemolysis process.

Because of this, the chemolysis process use is somewhat restricted, and it is not thought of as the main method for recovering plastic trash. However, due to its chemical reaction routes, certain nations and respectable businesses do adopt this method. Glycolysis, methanolysis, hydrolysis, and alcoholysis are a few of the chemical reaction pathways that make up chemolysis [87].

2.9.4 Alcoholysis

Alcoholysis refers to the unique process of depolymerizing a plastic polymer in the presence of alcohol to produce monomers. Polyurethane can degrade chemically thanks to alcoholysis. This reaction results in the production of polyhydroxy alcohols and carbamate fragments. It's significant to observe that there is no emission of carbon dioxide. Converted monomers from polyurethane can be recycled by mixing various ratios of new polyhydroxy alcohols and isocyanates [88].

2.9.5 Hydrolysis

Plastic polymers are dissolved in a solution of water by a process called hydrolysis, whether it is acidic, alkaline or the neutral. To produce the same kind of foam, raw and fresh ingredients can be combined with the hydrolyzed monomers. This method of using monomer materials increases economic gains and pollution reduction efforts [89].

2.9.6 Methanolysis

Methanolysis is the word used to describe depolymerization that occurs when methanol is present. Dimethyl terephthalate and ethylene glycol monomers are

produced when PET is subjected to methanolysis. These monomers are essential for the production of synthetic resin-type materials and epoxy resins. This depolymerization process normally takes place between 270 and 300 degrees Celsius and between 0.1 and 15 megapascals of pressure [90].

2.9.7 Glycolysis

Glycolysis, which involves breaking down polymers in the presence of glycol, is considered a universal method for material recovery. This approach is extensively utilized in the commercial recycling of PET, with notable companies such as DuPont and Dow employing glycolysis for PET material recovery. Glycolysis can also yield specialized oligomeric products, including dihydroxy chemicals [91].

2.10 Biodegradation of Plastic

The creation of a microbial biofilm, known as the plastisphere, on the plastic surface is the first step in the complex process of plastic biodegradation, the development of biodegradation begins with the establishment of the biofilm. Exopolysaccharides are secreted by microorganisms, which improve their adherence to the plastic surface and aid in the polymer's breakdown into simpler forms. As a result of the breakdown, oligomers, dimers, and monomers are created .Enzymes are key in weakening the carbon backbone of polymers and causing their fragmentation. Mineralization, the penultimate stage of plastic biodegradation before the end product is liberated. An innovative plastic made from naturally renewable plant-based resources was created by BioLogiQ, Inc., a firm that manufactures bioplastic resin. The plastic is guaranteed to decompose after 28 days. When biopolymer is combined with polybutylene adipate terephthalate, the biodegradable plastic also breaks down in aquatic conditions. According to test data, bioplastic degrades 97% faster in ocean water than in freshwater after a year [92].

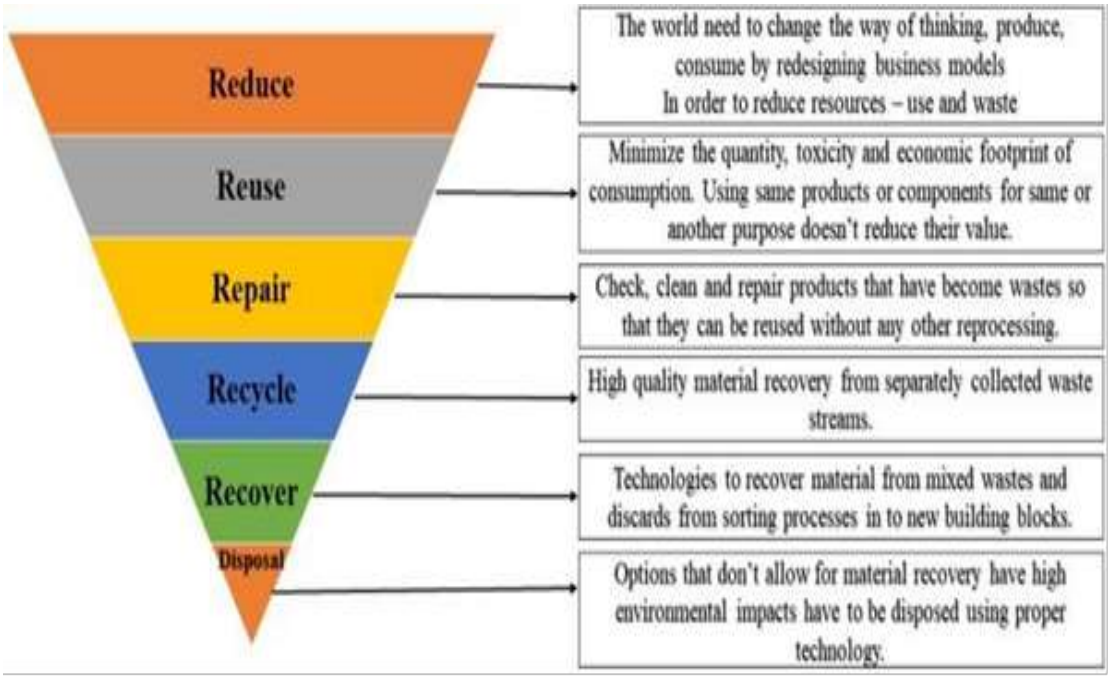

FIGURE 2.5: Six level pyramids to reduce plastic wastes [92]

The six level pyramid in Figure 2.5 depicts the relative importance of several waste management techniques, such as reduce, reuse, repair, recycle, recover, and disposal. The most important aspect of these measures is lowering our use of unneeded plastic go odds. Reusing plastic products appears as the most advantageous approach, even though a full ban on plastic is not a workable answer, especially in light of the numerous small and medium-sized chemical enterprises that employ millions of people globally [93].

Unfortunately, a lot of plastic is still either wasted, where it may rot for hundreds of years, or it is recycled, which requires energy to turn into new items .At this point, we need to reconsider and modify our culture of disposability in favor of the idea that practically anything we acquire may serve a new function. Items that can't be recycled or used again should be given to the appropriate authorities. An alternate strategy is to promote waste-reduction initiatives and behavioral modifications. Such mental changes can have a positive psychological impact as well as larger-scale economic worth. We may advance efforts to eliminate plastic pollution and make it easier for the creation of efficient regulations by attaining a more favorable cost- benefit analysis of behavioral changes and support [94].

2.10.1 Bio-augmentation

Exogenous microorganisms are inserted into a natural or genetically altered environment using the bioaugmentation process to either reduce pollution or improve the inherent capabilities of already present microorganisms. In this approach, enzymes are used and produced by microorganisms through degradative metabolic pathways to reduce pollution. Practical applications of bioaugmentation include the cleanup of contaminated water and the treatment of sewage waste. For instance, it can assist in converting chlorinated ethenes- contaminated water into less hazardous substances like chlorine and ethylene. Forestry, agriculture, soil bioremediation, wastewater management, and treatment are some industries where this approach is used [95].

The two primary categories of bioplastics are bio-based and biodegradable plastics. Low greenhouse gas emissions may be achieved by producing biodegradable plastics from renewable feedstocks. They are environmentally beneficial products. The fact that biodegradable plastics produce relatively little carbon dioxide when they break down highlights the significance of incorporating biodegradable plastic manufacture into routine activities [96].

Bio-based and biodegradable plastics are the two types of bioplastics. Greenhouse gas emissions may be reduced and biodegradable polymers can be produced using renewable feedstocks. The fact that bioplastic breakdown produces relatively little carbon dioxide emissions highlights the necessity of producing biodegradable plastic on a regular basis [97].

2.10.2 Bioventing

A useful and economically sound bioremediation method is bioventing. In order to supply oxygen and nutrients to areas that are deficient in oxygen, this technique concentrates on the regulated flow of air. The purpose is to promote the growth of local microorganisms, improving their capacity to carry out bioremediation tasks.

The use of various microbes varies depending on the job at hand. In a very short amount of time, harmful bacteria may be efficiently eliminated by bioventing [98].

Due to its nonintrusive nature and low interruption during both the installation and operating stages, bioventing is especially ideal for regions adjacent to buildings and frequently utilized places. This method works well for dealing with problems involving petroleum distillates like toluene and non-chlorinated solvents [99].

2.10.3 Bio-slurping

Vacuum pumping and bioventing principles are combined in the technique known as bioslurping. The recovery of substances like non-aqueous phase liquids, gases, and other light chemicals is the main goal of this bioremediation technology. Dealing with organic chemicals that are completely or partially volatile calls for the use of bioslurping. It works by making it easier for oxygen to get to the areas that need to be treated [100].

One drawback of this approach is that soil moisture levels can affect air permeability, slowing oxygen transport and, as a result, lowering microbial activity. Low-permeability soils may not be amenable to bioslurping remediation. However, since it reduces the amount of groundwater used, there are fewer costs associated with storage, treatment, and disposal [101].

2.10.4 Bio-sparging

While bioventing and biosparging have certain commonalities, they also have some differences. In bio-sparging, oxygen is added to the soil's lower surface's saturated zone to encourage the upward flow of volatile organic components. This seeks to promote the development of advantageous microorganisms at polluted locations. This technique's efficacy depends on the soil's permeability and the microorganisms' capacity for degradation [102].

Biosparging primarily promotes biodegradation in contrast to bioventing and soil vapor extraction (SVE), which are closely related procedures reliant on high airflow rates for pollutant volatilization. This technique has been widely used to clean up polluted aquifers [103].

2.10.5 Phytoremediation

By utilizing a variety of plant interactions in polluted environments, including physical, biochemical, biological, chemical, and microbiological activities, this strategy aims to lessen the negative effects of pollutants. In contrast, treatments including degradation, rhizoremediation, stabilization, and volatilization are generally used to handle organic contaminants such hydrocarbons and chlorinated chemicals. It's interesting to note that some plant species, including alfalfa and willow, may mineralize organic contaminants. Any phytoremediation technique's effectiveness mostly depends on increasing the ability of native plants that grow naturally in polluted places to clean up the area, either by strengthening them with endogenous or exogenous plant material. Because some precious metals may concentrate inside particular plant species and be collected after the remediation process, utilizing plants to clean up contaminated environments has a number of advantages [104].

2.10.6 Biological Degradation-Aided Microorganism

It has been demonstrated that microorganisms, particularly fungal-species & bacterial, are efficient in accelerating the decomposition of plastics. Numerous biological and environmental elements in the environment have an impact on the process's performance. In addition to the specific characteristics of the plastic polymer, and crystallization, these variables also include pH levels, exposure to light, temperature, moisture, the presence of surfactants related to the life sciences, the existence of bacteria, enzymatic agents, and the presence of surfactants related to life sciences [105].

Individual bacterial strains or improved bacterial colonies have been shown to drastically reduce the quantity of produced plastic particles when paired with photocatalytic technology, with reductions of up to 50% or more. Utilizing photocatalytic technology shows remarkable results in the deterioration [106].

2.11 Biofilms

A biofilm is a stationary community of microorganisms that develops as a result of cells' permanent adhesion to a surface. Cells in these communities interact and communicate with one another while being encased in a matrix of polymeric materials. When compared to gene transcription and growth rates, biofilms display unique behavioral traits [107].

According to research, biofilms can increase the adsorption of plastics, acting as transporters and lengthening the persistence of plastics and related pollutants in the environment. Among the prominent characteristics governed by quorum sensing is biofilm development. Polysaccharides, proteins, lipids, and nucleic acids are just a few of the biomolecules that make up these biofilms, which encase the bacterial cells inside the biofilm structure. This matrix offers defense against a variety of stressors, including immune cell assaults and contact with antimicrobial chemicals [108].

Biofilms are plentiful and simple to reach in natural settings. Plastics break down into safe byproducts like water and carbon dioxide, which are good for the environment.

Extracellular polymeric substance (EPS), which surrounds bacterial cells in biofilms, stops antimicrobial agents from penetrating the cells. Around 90% of the bulk of the biofilm is made up of EPS, which helps to create its three-dimensional structure. Some substances, including lipopolysaccharides, teichoic acids, and capsule polysaccharides, may at first interact with phages in a reversible way, but cell wall components are necessary for long-term attachment. Phage penetration into biofilm cells may be hampered by the presence of matrix components. [11].

Furthermore, the study contends that diffusion becomes more difficult as biofilm density rises, with both phage form and density having an impact on phage penetration. It may be possible to identify the processes by which bacteria and target cells participate in gene expression through a complicated chain of occurrences known as molecular cross-talk with the advent of upcoming technologies like DNA microarray chips [109].

Antimicrobial drugs cannot penetrate the bacterial cells within biofilms because of the extracellular polymeric substance (EPS) that surrounds them. About 90% of the bulk of the biofilm is made up of EPS, which gives it a three-dimensional structure [11].

While some substances, like as lipopolysaccharides, teichoic acids, and capsule polysaccharides, may at first interact with phages in a reversible way, cell wall components are necessary for long- term attachment. According to a research on the dispersion characteristics of phages, the presence of these matrix components may make it difficult for phages to enter biofilm cells, just like it makes it difficult for antibiotics to penetrate [110].

According to the study, phage penetration depends on both phage shape and biofilm density, and diffusion becomes more difficult as biofilm density increases. It may be able to appreciate how bacterial and cells being targeted coordinate the expression of genes through the intricate series of events known as chemical cross-talk because of improvements in science e.g. DNA array chips [111].

Both availability of nutrients and presence of surface is necessary for bacterial growth are prerequisites for the start of biofilm formation. Accumulation of microbial communities, embedded in a multiplex matrix, and divided by a system of water channels make up biofilms. These are environments in which bacteria naturally interrelate with one another by secondary metabolites, genetic and signaling molecules. According to the formation of biofilms on living surfaces is vital process that sets off a chain reaction in both bacteria and host cells [112].

The presence of nutrients and a surface that is conducive to bacterial growth are necessary for biofilm development. Microbial colonies are embedded in a matrix

to form complex structures called biofilms, which also have water channels flowing through them. Bacteria naturally interact in these settings via secondary metabolites, genetic signaling molecules, and other mechanisms. On living surfaces, the development of biofilms is a crucial step that initiates interactions between bacteria and host cells [113].

According to current beliefs, biofilm production starts when a single bacterium attaches to a surface. The development of microcolonies, cellular communication via quorum sensing signals, and maturation of these microcolonies into structured biofilms come next. Shear flow can increase cellular density and reduce biofilm thickness, whereas pouring liquid over the biofilm can improve matrix synthesis. Initial connection to the substrate, adhesion, colonization, generation of polysaccharides, biofilm maturity, and diffusion are the major phases of biofilm development.

Members of these closely-knit microbial communities in biofilms have extraordinary resilience to common antibiotics, hydrodynamic shear pressures, and biocides when compared to free-floating planktonic bacteria [114].

A hydrogel is a three-dimensional network of hydrophilic polymers that includes a large quantity of water and maintains its shape by chemically or physically cross-linking polymer molecules. A biofilm is similar to a hydrogel. A healthy and useful microbial population emerges as a result of biofilm production. Due to their close closeness inside the biofilm structure and the protection it provides from external dangers, the bacteria within a biofilm can exchange nutrients effectively. Typically, biofilms initiate when a planktonic bacterium adheres to a surface [115].

2.12 Enzymes Participating in the Biodegradation of Plastics & Microplastics

Numerous enzymes derived from fungus and bacteria have exhibited the capability to degrade various polymers, including PE, PET, and PP. The repertoire of enzymes involved in this process includes cutinases, esterases, lipases, laccases,

peroxidases, proteases, and ureases. Studies have demonstrated that fungal cellulase systems can depolymerize cellulose directly from solid polymeric substrates, ultimately converting it into monomeric components. For example, the hydrolysis of cellobiose into glucose is one of the outcomes of this process [116].

The principal microorganisms responsible for degrading polyethylene materials are bacteria and fungus, with fungi like *F. oxysporum* and *Aspergillus fumigatus* dominating the scene.

Particularly, it has been discovered that *F. oxysporum* strains have a high tolerance to high concentrations of PE while cultivating non-degradable plastic waste PE, which results in powerful oxidation effects and discernible alterations [117]. The low density polyethylene (LDPE) degradation method used by Aspergillus fumigatus results in the present experimental efficiency shown in the fungal breakdown of LDPE [118].

Ideonella sakaiensis 201-F and other highly effective degrading bacteria, as well as bacterial enzymes like hydrolase and keratinase, are key players in the breakdown of certain hydrolyzable polymers and PET. Typically, a mixture of PETase and MHETase enzymes is in charge of degrading PET. Esterases, lipases, keratinases, and other hydrolytic enzymes were often used in earlier studies for PET breakdown.

However, PETase is the name given to a particular enzyme that has been shown to have the ability to hydrolyze PET. PET is hydrolyzed by PETase into its monomers, ethylene glycol, throughout this process [119].

In the process of PET depolymerization within the *I. sakaiensis* strain, TPA and EG are released, and this process requires the synergistic action of PETase and MHETase. The noteworthy potential of both enzymes in degrading PET has garnered considerable interest in mitigating plastic waste.

PETase plays a crucial role in destabilizing PET molecules due to its esterase activity, catalyzing the ester bond. Subsequently, MHETase converts MHET into TPA (terephthalic acid) and EG (ethylene glycol) [120].

2.13 Enzymes for Biodegradation Produced by Organisms Thriving in Extreme Environments

Extreme environmental microbes like halophiles and psychrophiles have demonstrated the ability to breakdown plastics successfully. Bacteria that may degrade synthetic plastics reside in these extreme conditions, which include thermophilic, alkaliphilic, halophilic, and psychrophilic habitats. Finding more effective plastic-degrading bacteria is important because of the peculiar features of these settings [121].

Extreme environmental circumstances are commonly encountered in areas affected by plastic pollution. The extended stability of the enzymes generated by thermophilic and halophilic bacteria makes them particularly useful for room temperature without suffering a major loss of enzymatic activity. It offers enormous potential to investigate thermophilic and extremophilic microbiomes as sources of microbes and enzymes that may break down plastic [122].

The greatest rates of PET depolymerization were seen at 65 °C when the enzyme leaf-branching compost cutinase (LCC), renowned for its extraordinary thermal durability, was tested. Thermophiles have demonstrated a significant capacity for polymer degradation at high temperatures, acting as efficient catalysts for the destruction of high-temperature plastics. Multiple enzymes with increased activity can be produced by these bacteria, which eventually increases the substrate's availability and solubility. Notably, the ability to breakdown PE was found for the first time in *Chelatococcus sp.* [123].

Pre-treatment plays a vital role in enhancing the biodegradability of plastic waste. This process involves recycling or retrieving microplastics from wastewater to mitigate the toxicity of additives, prevent the attachment of persistent organic pollutants (POPs), and facilitate the depolymerization of polymer molecules. To promote bacterial adhesion and breakdown, the polymer needs to undergo chemical or biological oxidation processes, ultimately increasing its Water-attractiveness.

Various pre-treatment methods have been identified to enhance the effectiveness of biodegradation [124].

Nevertheless, it is crucial to consider the unique properties of plastics to prevent potential polymer deterioration. In contrast to traditional plastics, biodegradable polymers are more conducive to pretreatment, thanks to their favorable physico-chemical characteristics. Plastics can change their geometrical and structural characteristics by undergoing a variety of physical and chemical processes. In these treatments, molecular weight is decreased, chemical connections are broken, surface fissures are created, and functional groups are enhanced. Evaluating the impact of different pretreatments on the biodegradation of plastics and microplastics is essential for improving the degradation percentage [125].

Polyethylene (PE) may be made more easily metabolizable by microorganisms by pretreatment techniques including pyrolysis. The effectiveness of the enzymes responsible for PET degradation is optimized by temperature-based pretreatment, which enhances PET breakdown. The most efficient pretreatment strategy for PET breakdown is thermal treatment. Both the structure of the PET and the ambient temperature have an impact on the biodegradation activity of enzymes, in particular PETase [126].

The enzymatic activity of bacterial enzymes reaches its max at ideal temperatures, resulting in excellent catalysis, while the flexibility of PET molecules declines with decreasing ambient temperatures. Additionally, research results suggest that some bacteria have a higher biodegradability of plastics that have been heated, leading to a noticeable breakdown of long- chain polymer molecules. For instance, *Klebsiella pneumoniae* CH001 efficiently degraded thermally processed high-density polyethylene (HDPE), with heat pretreatment considerably lowering HDPE's tensile strength [127].

Accelerating the degradation of polyethylene (PE) can be achieved through the use of biodegradable chemicals, photo-initiators, or co-polymerization. In recent years, photocatalysis has emerged as a rapidly advancing environmentally friendly strategy. Notably, various simple iron salts, with ferrous chloride being particularly

effective as a photocatalyst, have demonstrated efficient catalysis in the oxidative cleavage of bonds under 400 nm LED irradiation in the presence of pure oxygen or air. Catalysis has also proven effective in breaking C-C bonds in aromatic hydrocarbons like isopropylbenzene and cyclohexylbenzene, converting them to benzoic acid within the catalytic system. This technique has shown the capability to break down commercial high molecular weight polystyrene (PS) samples [128].

The enzymatic surface modification of polymer fibers entails the modification of surface functional groups in plastic fibers, resulting in enhancements in wettability, color fastness, dyeing capabilities, and resistance to pilling. Ultimately, this modification improves the Water-attractiveness of the polymers [129].

To boost the water-attracting properties of textiles, enzymes like lipase from *Candida antarctica* and keratinase from *Aspergillus oryzae* are employed. The utilization of whole-cell enzymes in the biodegradation approach is regarded as a potentially superior strategy for plastics with C-C backbones. A multidisciplinary approach has been implemented, especially in the biocatalysis of PET monomers using whole-cell catalysis, aiming to produce a biopolymer that can seamlessly integrate into a biological cycle. Whole-cell catalysis improves the interaction between enzymes and the highly crystalline PET substrate, simultaneously reducing the hydrophobicity of plastics. The process involves adsorption and hydrolysis as the two key phases. Experimental results reveal that a PET film lost over 60% of its weight during a 14-day incubation at 60^{o}°C. Given these findings, whole-cell catalysis emerges as a promising addition to pretreatment catalysts [130].

Several pretreatment methods, encompassing factors such as temperature, enzymatic pretreatment, photo-treatment, and various additives, have shown the potential to expedite the biodegradation of plastics and microplastics [131].

Biofilms, which involve the immobilization of microbial cells producing various enzymes crucial for bioremediation processes, play a crucial role in wastewater bioremediation. Biofilms are preferred over planktonic cells in wastewater treatment due to their ability to facilitate effective gene transfer among their members [132].

Chapter 3

Methodology

3.1 Material and Methods

3.1.1 Methodology Chart

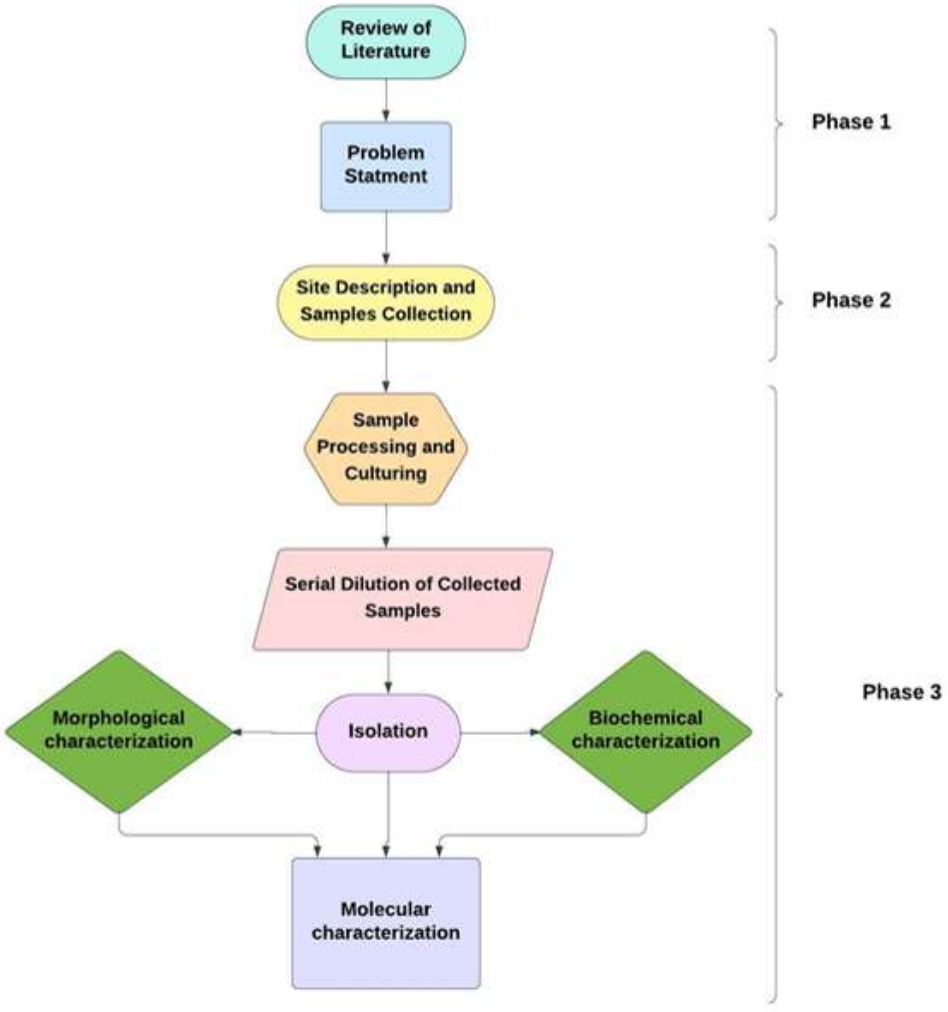

FIGURE 3.1: Flow Chart shows Major Steps of Methodology

3.1.2 List of Equipment

ABI-PRISM BigDye Terminator version 3.1 Cycle Sequence Kit, Mini-Elute TM PC Purification Kit, Ice Box (5 litre), Autoclave, Incubators, Vortex, Scanning Electron Microscopy, the Shakers, pH Metre, Centrifuge, Measuring Balance, Laminar Flow, Thermometer, and Nucleotide Sequencer.

3.1.3 List of Apparatus

Falcon Tubes (15 ml and 50 ml), Sterile Polythene Bags (5 inch and 122 inch), Beakers (100 ml and 250 ml). Spatula, Petri Dishes (90 mm), Spirit Lamp, Conical Flask (250 ml, 500 ml, and 1000 ml), Eppendorf Tubes (1.5 ml), PCR Tubes (5 μl), Micropipette (0.5-10 μl, 5-50 l, 100-1000 pl, and 0.5 - 5 ml), Dropper, Gloves, Mortar, Pestle, Butter Paper, and Inoculation Loop.

3.1.4 List of Chemicals

Nutrient Agar (g/litre), Nutrient Broth (g/litre), PPE Powder, Tri-Na-Citrate, Gram lodine Solution, Crystal Violet, Acetone/Ethanol, Distilled Water, Hydrogen Peroxide, Methyl Red / Voges-Proskauer (MRVP), Kovacs Oxidase Reagent, Urease Agar, Simmons-Citrate Agar, Indole, Amylase , Decolorizer, Safranin, Peptone (2mg), NaCl (1g).

3.2 Site Description and Samples Collection

With the aid of a sterilized spatula, samples of plastic were taken from landfills and dumpsites of I8 and Westridge Islamabad (5 samples from each site) and were kept in polythene bags. A unique ID was assigned to each sample. Aseptic methods were used while sampling. All samples were brought right away to the lab for evaluation.

FIGURE 3.2: Map of sector I-8 Islamabad

FIGURE 3.3: Plastic samples labelled and collected in plastic bags from I-8 dumping site

3.2.1 Sample Processing and Culturing

Plastic samples after processing were cut carefully into square pieces 12 × 12 cm. These pieces were inoculated in nutrient broth and incubated for 24 to 48 hours at 37^{o}C.

Serial dilution were prepared from the nutrient broth with bacterial growth for five bacterial samples (10^1, 10^2, and so forth). Using sterile pipettes, defined volumes (1 mL) of each bacterial sample was transferred into their respective tubes, adding the necessary sterile diluent (e.g., 9 mL) for the desired dilution and ensuring thorough mixing. These steps were replicated for the remaining four bacterial samples, establishing separate dilution series for each. Each sample was poured into three petri plates with nutrient agar PPE powder for replication and then placed in incubator for 4 days at 37^{o}C. After 4 days there was visible growth of

colonies on nutrient media. The petri plates with prominent growth results were selected for further colony morphology.

3.2.2 Gram Staining Method

To obtain a more profound insight into bacterial colony morphology, a gram staining procedure was executed. The samples underwent systematic preparation for the identification of their gram stain and cell morphology. A small droplet of the bacterial sample was placed on a slide and heat-fixed by passing it through the flame of a Bunsen burner three times. The primary stain, Crystal violet, was administered to the slide using a dropper and allowed to stand for 1 minute. Removing excess stain was carried out by rinsing the slide with water for no more than 5 seconds. Gram's iodine was subsequently applied to fix the crystal violet to the cell wall, followed by a brief rinse with acetone for approximately 3 seconds and a gentle rinse with water. Gram-negative cells lost their color, while gram-positive cells retained a violet or blue hue. The counterstain, safranin, was implemented and left for 1 minute before a careful rinse with water lasting not more than 5 seconds. The slide was then scrutinized using a compound microscope.

3.3 Biochemical Tests

3.3.1 Catalyze Test

Bacteria are recognized for producing the enzyme catalase, which aids in converting hydrogen peroxide into water and molecular oxygen. The assessment of this catalase activity can be conducted using either the tube or slide method (*Facklam & Elliott, 1995*). In this procedure, a small amount of the bacterial colony was transferred to a clean, dry glass slide using a loop or sterile wooden stick to ensure visibility of the colony. Following this, a drop of 3% hydrogen peroxide (H_2O_2) was administered to the slide and thoroughly mixed. A positive result was

indicated by the rapid release of oxygen, manifested as bubbling, occurring within 5-10 seconds.

3.3.2 Oxidase Test

A piece of filter paper was soaked with a small quantity of a recently prepared 1% reagent solution. A sterilized loop was used to pick a well-isolated colony from a bacterial plate, and this colony was subsequently transferred onto the treated filter paper. A positive reaction was identified by a vibrant deep-purple color appearing within 5-10 seconds. A delayed positive reaction was characterized by coloration occurring between 10-60 seconds, whereas a negative reaction indicated either the absence of coloration or coloration happening after 60 seconds.

3.3.3 Motility Test

All the isolated strains underwent a motility test in a semi-solid agar medium composed of 200 milliliters of distilled water, 0.4 grams of agar, 2 milligrams of peptone, and 1 gram of NaCl. Using a sterile inoculated needle, a suspicious colony was extracted from an 18-24-hour culture and introduced into the motility agar medium by delicately piercing the needle 3-4 cm into the medium and then withdrawing it to leave a visible line of inoculum. The tubes were subsequently incubated aerobically at 35-37^{o}C for 18-24 hours. Non-motile organisms displayed a singular line of growth that did not deviate from the original inoculum stab, while motile organisms formed a diffuse growth zone around the inoculum stab.

3.3.4 Voges-Proskauer (VP) Test

Prior to inoculation, let the medium reach room temperature. Incorporate the organisms into the medium gradually after an 18–24 hour pure culture. For twenty-four hours, ferment aerobically at a temperature of 37 degrees Celsius. Once this incubation time has passed, pour 2 millilitres of the broth into a sterile test tube.

Give the leftover broth another 24 hours in the incubator. To aerate, add six drops of five percent alphanaphthol and carefully mix. Then, to aerate, add two drops of forty percent potassium hydroxide and thoroughly mix. After 30 minutes, shake the tube violently and look for a pink-red colour at the surface.

3.3.5 Methyl Red (MR) Test

The MR-VP was first allowed to come to room temperature. Next, an undiluted culture of organisms was added to test tubes with MR-VP broth. The broth mixture was left to incubate at 35 degrees Celsius for a minimum of 48 hours following inoculation. Five teaspoons of the methyl red detection solution were applied to each test tube after the incubation period. Next, each tube's subsequent colour shift was observed.

3.3.6 Urease Test

Around 24.52 grams of the dehydrated medium were dissolved in 950 ml of distilled water in a beaker. The solution was heated until it reached boiling point to ensure complete dissolution of the medium. Following this, the prepared suspension underwent sterilization through autoclaving at 15 lbs pressure and 121^{o}C for 15 minutes. A sterile 40% urea solution was added and thoroughly mixed in a 50 ml beaker. The medium was dispensed into tubes, and an inoculating loop was used to inoculate the broth with a loopful of a well-isolated colony. The tubes were incubated at 35 to 37^{o}C with loosened caps, and observations were made for the development of a pink color.

3.3.7 Casein Hydrolysis Test

The required quantity of SM agar media powder was measured and mixed in the appropriate volume of water within a conical flask. The media were brought to a boil to ensure the complete dissolution of all components and agar in water.

Subsequently, the media underwent autoclaving at 121^{o}C and 15 lbs pressure for 15 minutes. After autoclaving, it was cooled to approximately 40 – 45^{o}C before being poured into a sterile petri plate. The media solidified completely at room temperature. Culture inoculation was carried out on the plate, either in a straight line or a zig-zag pattern. The plate was then incubated at either 25^{o}C or 37^{o}C. Milk agar plate cultures were examined for the presence or absence of a clear area, or zone of proteolysis, surrounding the growth of each bacterial test organism.

3.3.8 MacConkey Agar Plate

Dissolved 49.53 grams of dehydrated medium in 1000 ml distilled water and then heated it to a boil to ensure full dissolution of the medium. Sterilization was accomplished by autoclaving at 121^{o}C for 15 minutes. After cooling, the mixture was poured into sterile Petri plates. Inoculum from the agar plate was then applied. Lactose-fermenting strains were noted to develop as red or pink colonies, surrounded by a zone of acid-precipitated bile on the agar plate.

3.3.9 Eosin-Methylene Blue (EMB) Agar Test

The dehydrated media were dissolved in 1000 ml distilled water, and the resulting suspension was mixed and boiled to ensure the complete dissolution of the medium. After sterilization, the cooled media were poured into sterile Petri plates. A pure culture was then inoculated onto the agar plate, with subsequent examination focused on observing the colonial morphology.

3.4 Molecular Characterization of Isolated Bacteria

3.4.1 DNA Extraction Method

Using the conventional CTAB approach, the DNA process of extraction from pure specimens was started. To create colonies of bacteria in pellet form, 1.5 ml of nutritional broth was first collected in labelled Eppendorf tubes and centrifuged

at a speed of 7000 rpm for 5 minutes. Following centrifugation, the pellet was again suspended in 500 millilitres of lysis buffer, and the supernatant was properly disposed away. The lysis buffer, prepared freshly and pre-warmed at 60-65^{o}C, was composed of 500 μl of 100 mM Tris-HCl, 200 μl of 20 mM EDTA, 1160 μl of 1.4 M NaCl, and 0.1 g of 2% CTAB, mixed with 3000 μl of sterile distilled water to yield 5 ml of lysis buffer. Following the addition of lysis buffer, the pellet was thoroughly mixed either by vortex or pipetting. Subsequently, 20 μl of Proteinase K, 40 μl of 10% SDS, and 6-8 μl of β-Mercaptoethanol were incorporated into the mixture.

3.4.2 Gel Electrophoresis

To observe the amplified product of the 16S rRNA gene, gel electrophoresis was performed. 1% agarose gel was created in 1X TBE buffer with ethidium bromide (5 μl/100 ml). The PCR products, along with a marker (Lambda Hind-III), were applied to the gel. Following this, the gel was examined under UV light, and a photograph was captured using a mobile camera.

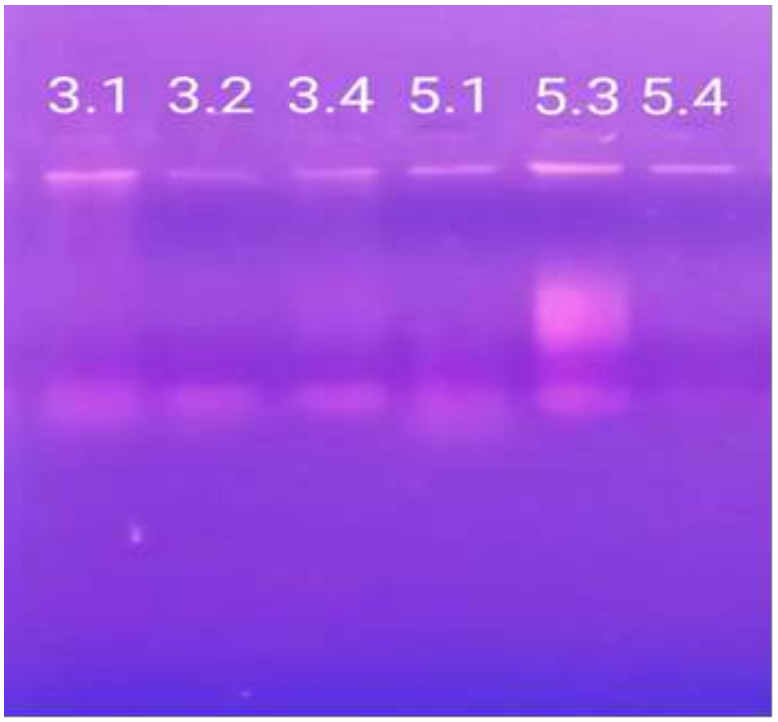

FIGURE 3.4: Bands of bacterial isolates in Gel Eectrophoresis

3.4.3 DNA Amplification by 16s rRNA PCR

DNA quantification through 16S rRNA PCR was performed in a thermocycler. The DNA samples, totaling six, were initially centrifuged for 5 seconds. Simultaneously, six new Eppendorf tubes were prepared, correctly labeled. Subsequently, 5 μl of DNA was combined with a Master Mix of 90 μl, Forward Primer 15 μl, Reverse Primer 15 μl, and PCR 30 μl. The PCR cycle was run for 2 hours and 18 minutes. Following the PCR, the DNA was visualized

3.4.4 Sequence Analysis and BLAST

The Basic Local Alignment Search Tool (BLAST), offered by NCBI, is a tool used for aligning a sequence with a reference sequence and calculating the similarity index based on matches, mismatches, and gaps.

3.4.5 Submission to the National Center for Biotechnology Information (NCBI)

Following the elimination of Sequences of low quality, the sequences were deposited in the National Center for Biotechnology Information (NCBI). NCBI, operated by the National Library of Medicine, serves as an extensive database, providing access to a diverse array of biological information and serving as a vital resource for researchers, scientists, and the broader public.

3.4.6 Scanning Electron Microscopy (SEM)

In order to obtain high-resolution, three-dimensional pictures of the surface of solid specimens, scientists and industry use scanning electron microscopy (SEM), a potent imaging technology. Due to its capability to offer intricate and detailed images, SEM plays a crucial role in diverse scientific and industrial applications, significantly enhancing our comprehension of the microscopic realm. SEM is a powerful tool for visualizing bacterial biofilms, providing detailed information about

their morphology, structure, and composition, which is crucial for understanding biofilm formation, dynamics, and interactions with the environment.

3.4.7 UV Spectrometry

UV spectrometry, also referred to as ultraviolet-visible (UV-Vis) spectrometry, is a widely applied analytical method employed to detect the absorption of ultraviolet and visible light by chemical substances. It finds extensive usage across various disciplines such as chemistry, biochemistry, environmental science, pharmaceuticals, and materials science. UV spectrometry offers a versatile tool for detecting bacterial biofilms on plastic surfaces by directly measuring their optical properties or monitoring changes in the surrounding environment caused by biofilm formation.

Chapter 4

Results

4.1 Culturing and Screening of Samples

Among all samples collected from dumping sites in Islamabad, only samples collected from I8 dumping site (5 samples) were chosen for experimentation and samples collected from Westridge were discarded. Only 2 samples 3 and 5 (each replicated 4 times) cultured in the nutrient broth showed the microbial growth while rest of the samples did not show any growth of microbial growth. Therefore, isolates from sample 3 and 5 were further used in this study while rest were discarded. The resulting tubes yielded a range of diminishing concentrations for each bacterial sample, suitable for diverse analyses. Isolation of bacterial colonies on the nutrient agar revealed clear growth of bacterial strains (Figure 4.1, 4.2. 4.3).

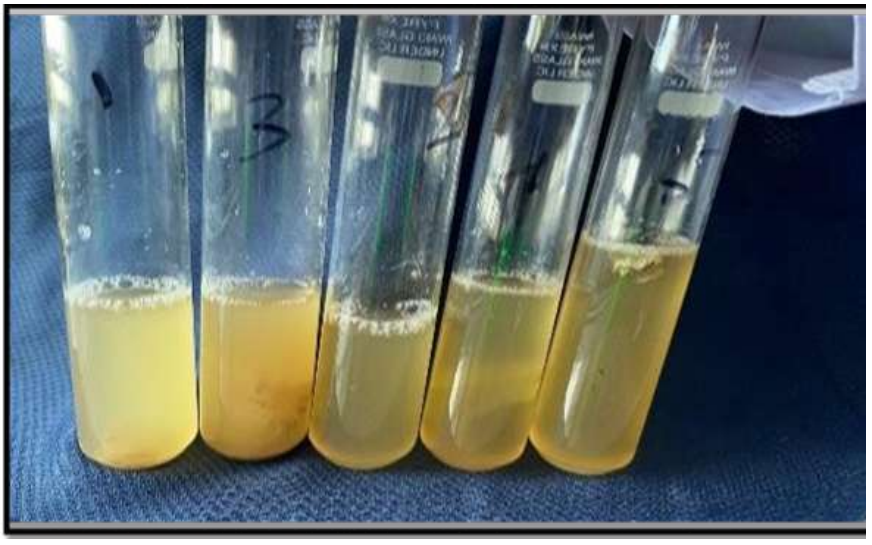

FIGURE 4.1: Bacterial Growth in Nutrient Broth

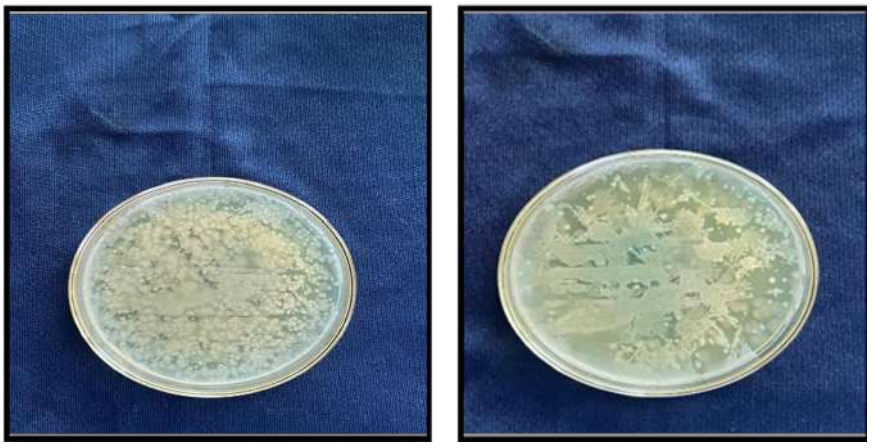

FIGURE 4.2: Bacterial Colonies (sample 3 and sample 5)

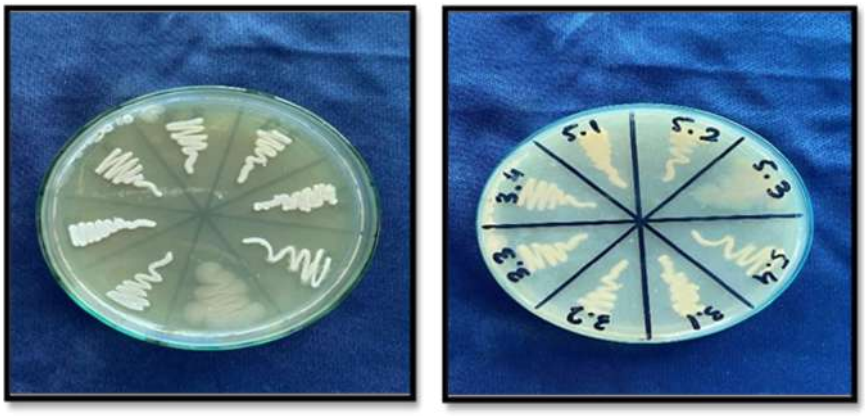

FIGURE 4.3: Bacterial strains on Nutrient Agar

4.1.1 Gram Staining Method

The examination of the slides was conducted using a compound microscope. S3.1 and S3.2 demonstrated a positive Gram-positive result, while the remaining samples (S3.4, S5.1, S5.3, and S5.4) exhibited a Gram-negative outcome. Gram-positive bacteria retained the crystal violet-iodine complex, leading to a purple or blue appearance when observed under a microscope. Their cell walls, characterized by a thick peptidoglycan layer, prevent the removal of the initial stain during the decolorization step, distinguishing them from Gram-negative bacteria. Microscopic analysis revealed that the majority of the bacteria exhibited a round shape, falling into the categories of cocci and coccobacillus (Figure 4.4, Table 4.1).

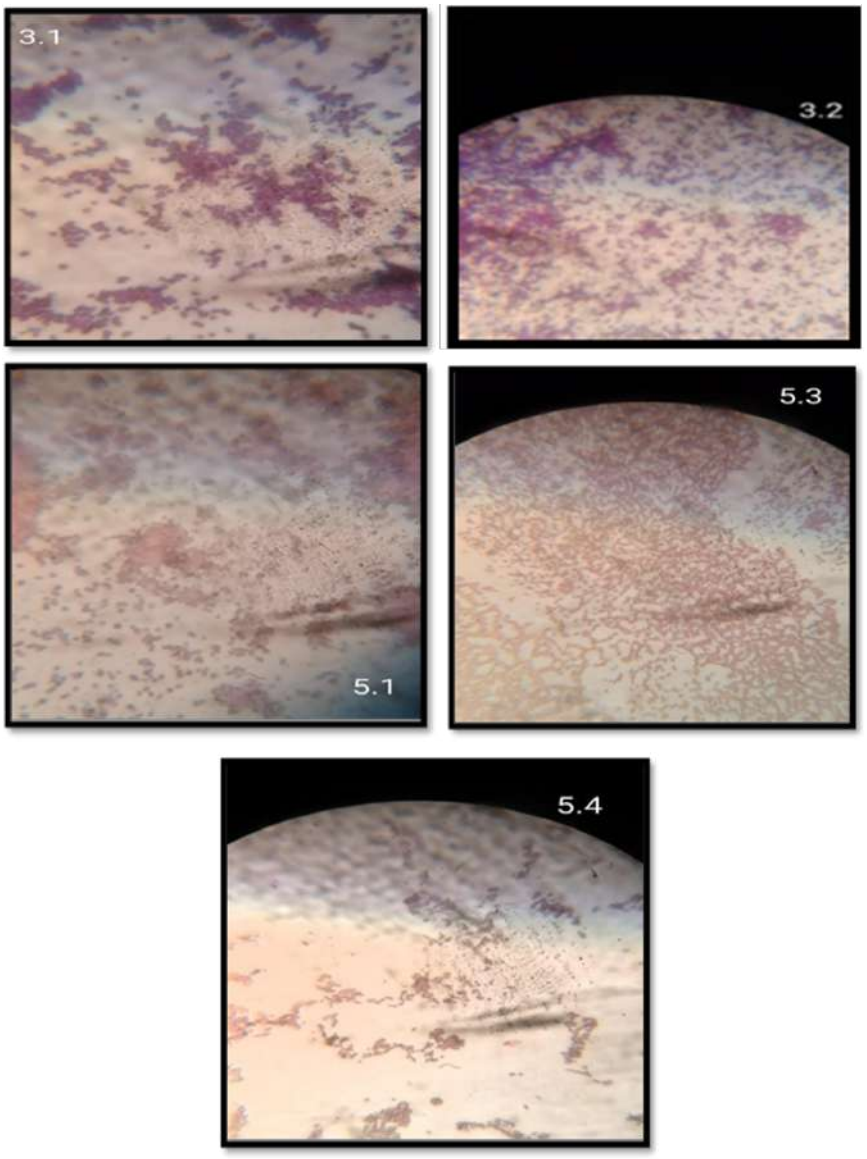

FIGURE 4.4: Gram Staining of bacterial strains isolate. Sample 3.1 and 3.2 showed positive result while sample 3.4, 5.1, 5.3, and 5.4 exhibited Gram-negative results

TABLE 4.1: Morphological Characterization of Gram staining bacteria

Sample IDs	**Gram Staining**	**Motility Test**	**Shape**
3.1	+ve	Non motile	Round, cocci
3.2	+ve	Non motile	Round, cocci
3.4	-ve	Non motile	Cocci
5.1	-ve	Non motile	Coccobacillus
5.3	-ve	Non motile	Coccobacillus
5.4	-ve	Non motile	Coccobacillus

4.2 Biochemical Tests

4.2.1 Catalase Test

The catalase test is conducted to distinguish bacteria that produce the enzyme catalase, which assists in the detoxification of hydrogen peroxide by decomposing it into oxygen and water. The presence of bubbles indicates a positive catalase result. Catalases play a role in protecting bacteria from oxidative stress by facilitating the decomposition of H_2O_2.

They are involved in various cellular processes such as metabolite production and cell development. All six isolated bacterial strains labeled 3.1, 3.2, 3.4, 5.1, 5.3, and 5.4 exhibited positive results in the catalase test, as evidenced by the formation of bubbles within 5-10 seconds after the addition of catalase enzyme (Figure 4.5).

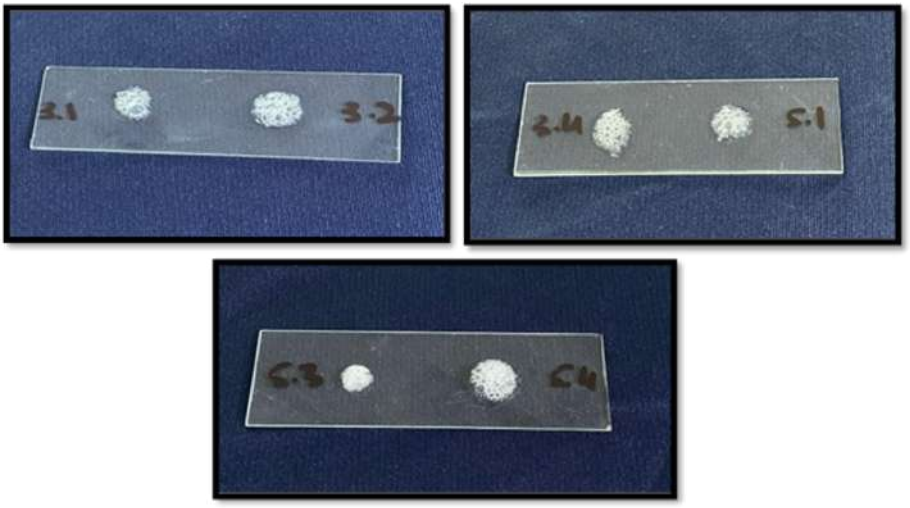

FIGURE 4.5: Catalase Test Result for isolated strains of bacteria

4.2.2 Oxidase Test

The oxidase test is used to determine whether cytochrome c oxidase is present. This enzyme is essential to some microbes' electron transport chain. This diagnostic method is essential for distinguishing and categorizing bacteria based on their oxidase activity, differentiating between oxidase-positive and oxidase-negative strains. A strong deep purple hue within 5–10 seconds indicates a positive

reaction; coloration within 10–60 seconds indicates a delayed positive reaction; and coloration not present or appearing later than 60 seconds indicates a negative reaction. All isolates displayed oxidase-positive results except for S3.1 (Figure 4.6).

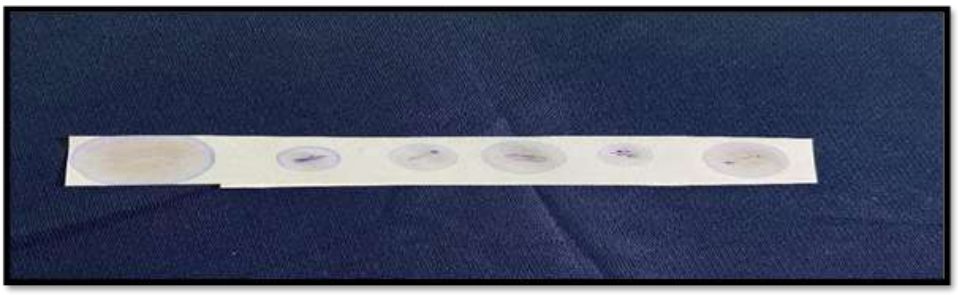

FIGURE 4.6: Oxidase Test Result

4.2.3 Motility Test

The motility test is employed to ascertain whether an organism possesses motility or is non-motile. Generally, bacilli are motile organisms, although a few cocci may also exhibit motility. A positive result in the motility test is identified by the presence of a red turbid region extending outward from the inoculation line, whereas a negative test is marked by red growth solely along the line of inoculation.

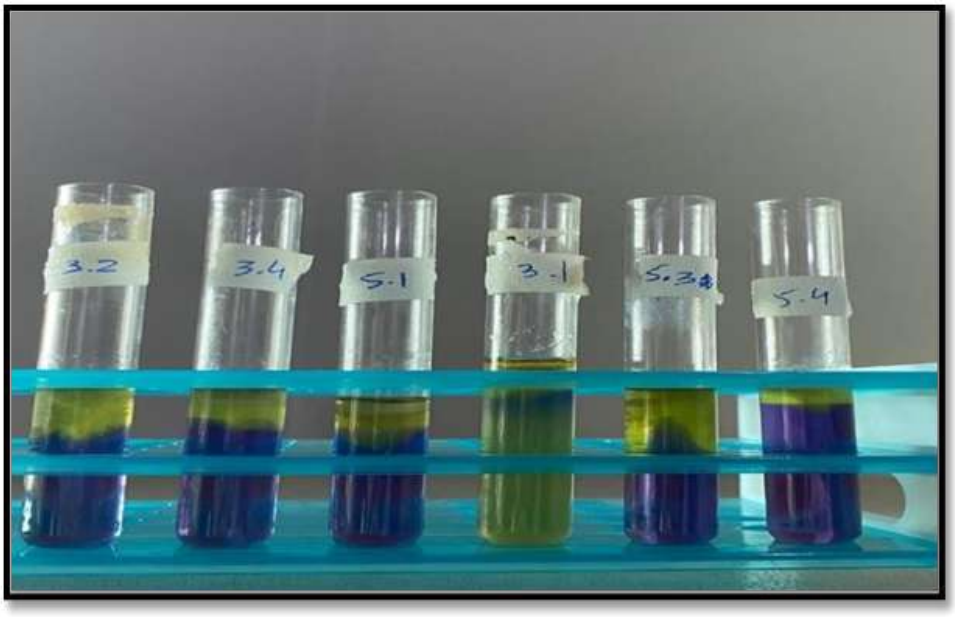

FIGURE 4.7: Motility Test Result

The ornithine decarboxylation test within the MIO test is vital for differentiating bacteria based on their metabolic traits. This biochemical assessment reveals the specific ability of certain bacterial species to decarboxylate ornithine. All samples except S3.1 shows positive result for ornithine reagent as they changed color from yellow to purple (Figure 4.7).

4.2.4 Voges-Proskauer (VP) Test

The Voges-Proskauer test is conducted to identify the presence of acetylmethyl carbinol resulting from glucose fermentation. In this test, naphthol and potassium hydroxide are added to VP broth (also known as glucose phosphate broth) that has been inoculated with bacteria. Positive results are indicated by strains displaying a brownish-red to pink color, while negative results are characterized by the absence of a red-pink color change. The results for the isolated bacterial strains 3.1, 3.2, 3.4, 5.1, 5.3, and 5.4 showed no change in color, indicating that all strains tested negative for the VP test (Figure 4.8).

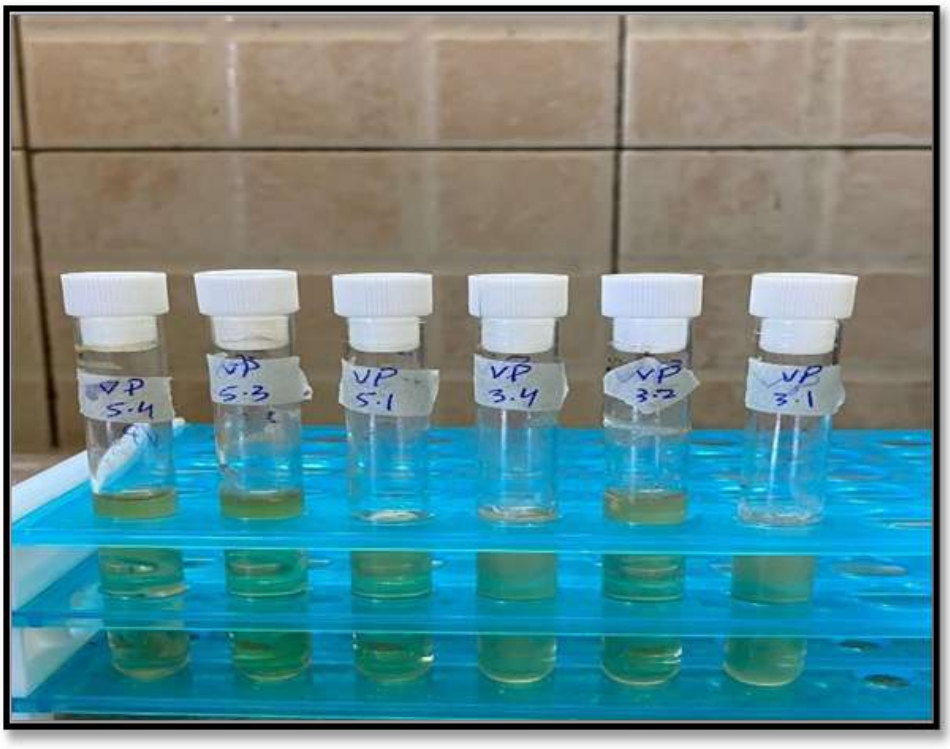

FIGURE 4.8: Voges-Proskauer Test Result for isolated bacterial strains

4.2.5 Methyl Red (MR) Test

The methyl red test is conducted to identify bacteria capable of undergoing the glucose fermentation pathway, leading to the production of various gases and acids, including acetic acid, formic acid, and lactic acid. A positive outcome is characterized by a color change to red in the test tube, while a red-orange hue indicates weak positivity, and a yellow color signifies negative results. As illustrated in Figure 4.9, isolated strains 3.2 and 3.4 displayed positive results, evident from the color change to red.

Conversely, strains 3.1, 5.1, 5.3, and 5.4 demonstrated negative results. If the bacteria produce stable acidic end products such as mixed acids (e.g., lactic acid, acetic acid), the pH of the medium drops, and methyl red turns red at a pH below 4.4 and if the bacteria produce neutral end products like acetoin, the medium remains near its initial pH and methyl red remains yellow. This indicates that the bacteria do not perform mixed acid fermentation.

FIGURE 4.9: Methyl Red (MR) Test results

4.2.6 Urease Test

The purpose of the urease test is to determine whether microbes can manufacture the enzyme urease, which catalyses the conversion of urea into ammonium and carbon dioxide, among others. One way to observe this is to see if the tubes are starting to become pink. A positive result is indicated by a change in the medium's color to pink or magenta, denoting urease production and an alkaline pH. In samples 3.4 and 5.3, a light pink color change was observed. Conversely, a negative result is determined when there is no color alteration or the retention of the initial yellow color, as seen in samples 3.1, 3.2, 5.1, and 5.4, suggesting the absence of urease enzyme.

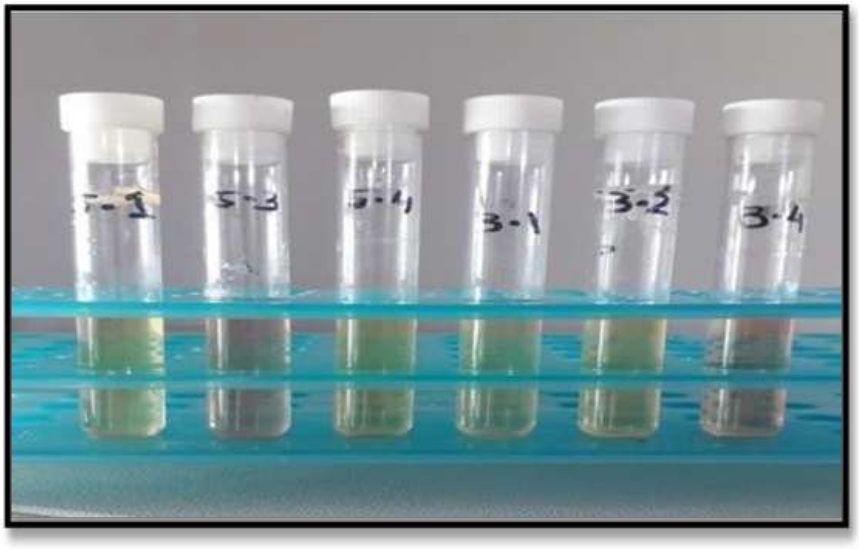

FIGURE 4.10: Urease Test Result

4.2.7 Casein Hydrolysis Test

The purpose of the the casein hydrolysis test is to evaluate a microorganism's capacity to enzymatically degrade casein, a protein present in milk. The clear zone surrounding the bacterial growth, which indicates proteolysis, was seen in cultures using milk agar plates. When microbes are seen breaking down casein enzymatically, the casein hydrolysis assay yields a good result. This is indicated by the formation of a transparent zone around bacterial growth on the medium, signifying the production of proteolytic enzymes that break down the casein protein into smaller, soluble compounds. In Figure 4.11, only S3.1 and S3.2 exhibited positive results by forming clear zones.

FIGURE 4.11: Casein Hydrolysis Test Result

4.2.8 Gelatin Hydrolysis Test

The gelatinase enzyme works by breaking down gelatin into simpler components. The test aims to confirm whether the microorganism under examination possesses the ability to hydrolyze gelatin. A positive result, indicated by an empty space surrounding the colonies becomes visible upon the introduction of mercuric chloride. ($HgCl_2$), was observed on agar plates. Conversely, a negative result, signifying the absence of gelatin hydrolysis, was represented by the absence of clear zones around the colonies after the addition of mercuric chloride (Figure 4.12). All six isolates tested negative for gelatinase activity.

FIGURE 4.12: Result of Gelatin Hydrolysis Test

4.2.9 MacConkey Agar Plate

The primary objective of MacConkey agar is to isolate and differentiate various bacterial types, particularly fermenting gram-negative bacteria. This medium is both selective and differential, fostering the growth of gram-negative species while providing distinctions among them. Bacterial pathogens like *E. coli*, *Enterococcus*, *Acetobacter*, and *Pseudomonas* can flourish on MacConkey media. The fermentation of lactose by these bacteria results in the production of organic acids, particularly lactic acid, causing a decrease in the pH of the agar.

MacConkey agar incorporates a pH indicator which turns pink under acidic conditions. Consequently, gram-negative bacteria fermenting lactose form pink colonies, while non-lactose fermenters produce white colonies. In Figure 4.13, all samples displayed lactose fermentation, with strains exhibiting as red or pink colonies encircled by an area where bile has precipitated due to acidity.

FIGURE 4.13: Growth on MacConkey Agar Plate

4.2.10 Eosin-Methylene Blue (EMB) Agar Test

EMB agar serves as a medium specifically designed to favor the growth of gram-negative bacteria and is particularly useful for the isolating and distinguishing different gram-negative bacilli, including coliforms and fecal coliforms. It enables the identification of lactose-fermenting bacteria through the formation of colored colonies, whereas non-lactose fermenters present as colonies without color .The

key components of EMB agar include enzymatic digest of gelatin, lactose sugar, dipotassium phosphate, eosin Y indicator, agar, and methylene blue. The dyes, particularly methylene blue, possess reversible oxidation-reduction potential and are toxic to bacteria. Gram-negative bacteria engaging in lactose fermentation generate acid, leading to a decrease in pH that facilitates the uptake of dyes by the colonies, resulting in a dark purple appearance. All six isolates demonstrated positive outcomes on EMB agar (Figure 4.14).

FIGURE 4.14: Growth on Eosin-Methylene Blue (EMB) Agar Test

TABLE 4.2: Results of biochemical characterization

Sample ID	VP	Methyl Red	Simon Citrate Agar	MacConkey Agar	EMB	Catalase Test	Urease Test	Gelatinase Test	Oxidase Test	Casein Hydrolysis Test
3.1	-ive	Yellow	+ive	Pure Fre-mentre	-ive	+ive	-ive	-ive	-ive	+ive
3.2	-ive	Red	+ive	Pure Fre-mentre	-ive	+ive	-ive	-ive	+ive	+ive
3.4	-ive	Red	+ive	Pure Fre-mentre	-ive	+ive	+ive	-ive	+ive	-ive
5.1	-ive	Yellow	+ive	Pure Fre-mentre	-ive	+ive	-ive	-ive	+ive	-ive
5.3	-ive	Yellow	+ive	Pure Fre-mentre	-ive	+ive	+ive	-ive	+ive	-ive
5.4	-ive	Yellow	+ive	Pure Fre-mentre	-ive	+ive	-ive	-ive	+ive	-ive

4.3 Molecular Characterization using 16S rRNA

From the obtained samples, five bacterial strains were identified through the analysis of their 16S rRNA gene sequences. The identified strains include *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, exhibiting a sequence similarity ranging from 92% to 100%. The sequencing results for the isolated strains

are presented in the provided table (Table 4.3). These strains have successfully submitted in NCBI with accession numbers *Acinetobacter baumannii* OR827196, OR826264, OR826261, *Pseudomonas aeruginosa* OR826267 and *Uncultured bacterium* OR826194. An "uncultured bacterium" refers to a bacterium that has been detected or identified through sequencing techniques, particularly the 16S rRNA gene sequencing, but has not been successfully cultured or grown in laboratory conditions.

TABLE 4.3: Molecular Characterization using 16S rRNA

Sr No.	**Sample ID**	**Scientific Name**	**Accession No.**	**Query Cover**	**% Identity**
1	3.1	*Acinetobacter baumannii*	OR827196	100%	100%
2	3.2	*Psedomonas aeroginosa*	OR826267	99%	99.76%
3	3.4	*Acinetobacter baumannii*	OR826264	99%	99.8%
4	5.1	*Acinetobacter baumannii*	OR826261	92%	92.0%
5	5.3	*Uncultered bacterium*	OR826194	99%	99.07%

4.4 Biofilm Formation Potential

The visual representation depicts the biofilm-forming capacities of five bacterial strains engaged in plastic degradation. On the x-axis, each bacterial strain is plotted, while the y-axis denotes the values for biofilm-forming potential. The unit of absorbance for biofilm forming potential plotted in y-axis is measured in Lambda (λ). The graph, which may take the form of a scatter plot or a bar graph, showcases the variability in biofilm-forming capabilities across the strains. Interpretation involves examining the position of each data point or bar.

A higher placement on the y-axis indicates a more substantial biofilm-forming potential for the respective bacterial strain. The bars exhibit patterns, trends, or distinctions in biofilm formation, offering valuable insights into the relative biofilm-forming capabilities of the bacterial strains. Notably, Sample 5.3 demonstrates the highest potential for biofilm formation, while Sample 3.1 exhibits the lowest.

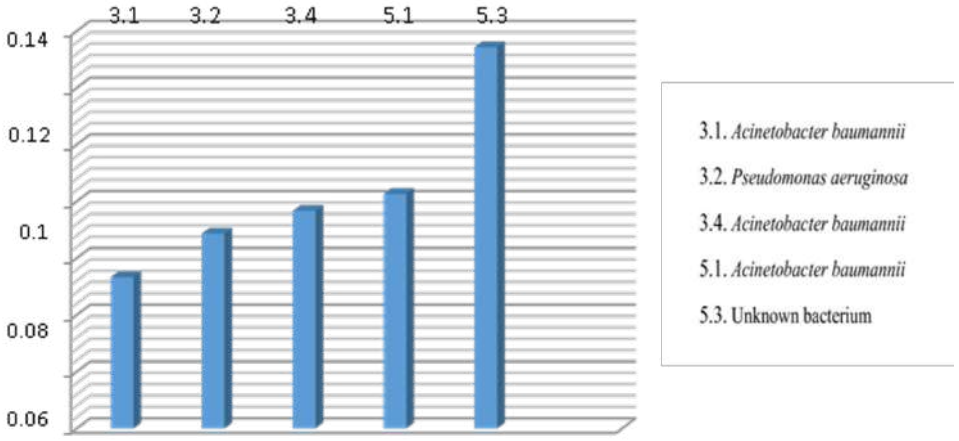

FIGURE 4.15: Biofilm formation potential graph

4.4.1 Biofilm formation as Consortium

All the five strains of bacteria in combination showed maximum potential for biofilm formation and are involved in plastic degradation. The correlation between higher values and increased biofilm potential allows for pattern identification and the selection of combinations with superior biofilm-forming characteristics.

Beyond numerical aspects, the interpretation encapsulates microbial community dynamics and emphasizes the importance of considering emergent properties in biofilm ecology. The combinations represent different groups of strains being considered together for potential biofilm formation. Practical implications include informing strategies for biofilm management and influencing strain selection on the basis of plastic degradation.

The highest biofilm formation potential was revealed by $1+2+3+4+5$(*Acinetobacter baumannii*+ *Pseudomonas aeruginosa*+ *Acinetobacter baumannii*+ *Acinetobacter baumannii*+uncultured bacterium) followed by 2+3+4 (*Acinetobacter baumannii*+ *Acinetobacter baumannii*+ *Acinetobacter baumannii*). The lowest value was recorded 3+5(*Acinetobacter baumannii*+ Uncultured bacterium) as shown in fig 4.16. The unit of absorbance for biofilm forming potential plotted in y-axis is measured in Lambda (λ).

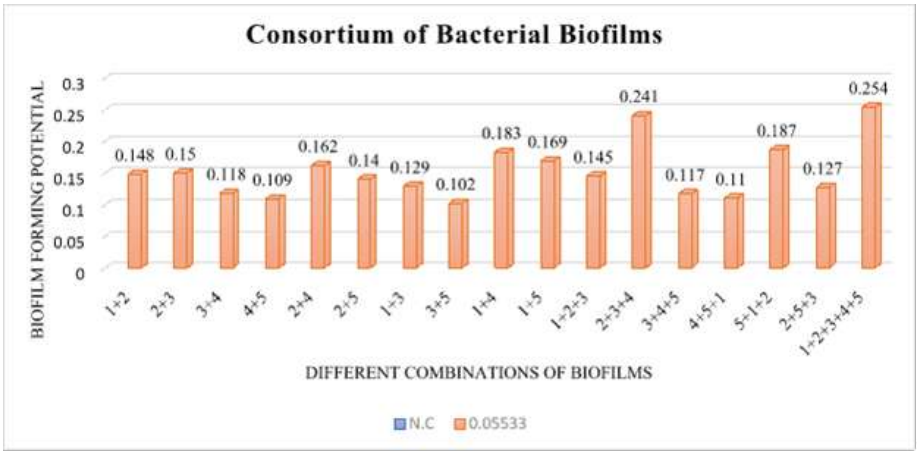

FIGURE 4.16: Biofilm forming potential of Different bacterial strains in combinations. 1+2+3+4+5(*Acinetobacter baumannii*+ *Pseudomonas aeruginosa*+ *Acinetobacter baumannii*+ *Acinetobacter baumannii*+uncultured bacterium) with highest potential, 3+5 (*Acinetobacter baumannii*+ Uncultured bacterium) showing lowest potential

4.5 Scanning Electron Microscopy

Prior to the bio films being mechanically detached, the plastics were also examined using scanning electron microscopy. The results are shown in figs. 4.24- 4.30 for sample 3 and 4.21–4.26 for sample 5, where each image displays the plastic attached to the bio film at 1, 2, 10, 20, 50, and 500 scale, respectively. Prokaryotic cells of varying morphologies adhered to all plastics, together with filamentous structures and detritus signifying a colonization of bio films. Numerous cavities were seen on the molded surfaces of the same samples when they were examined at 500.

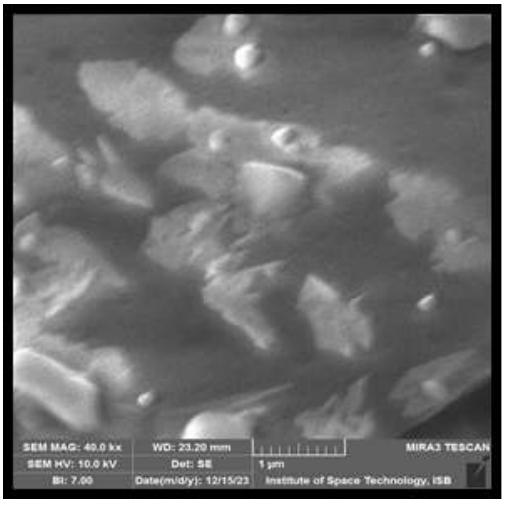

FIGURE 4.17: SEM of sample 3 at 1 μm

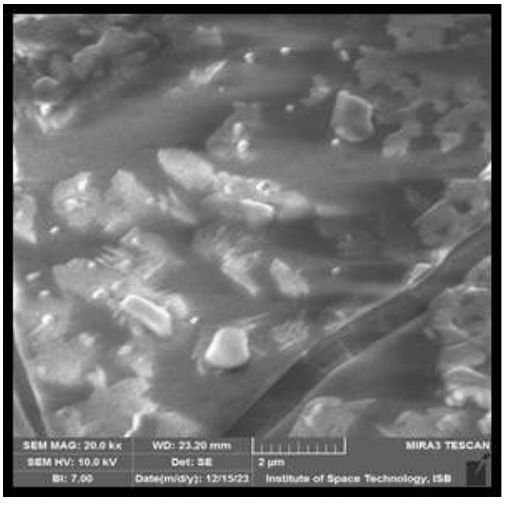

FIGURE 4.18: SEM of sample 3 at 2 μm

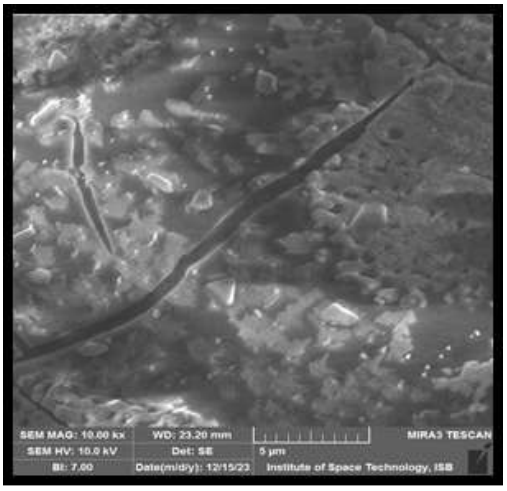

FIGURE 4.19: SEM of sample 3 at 5 μm

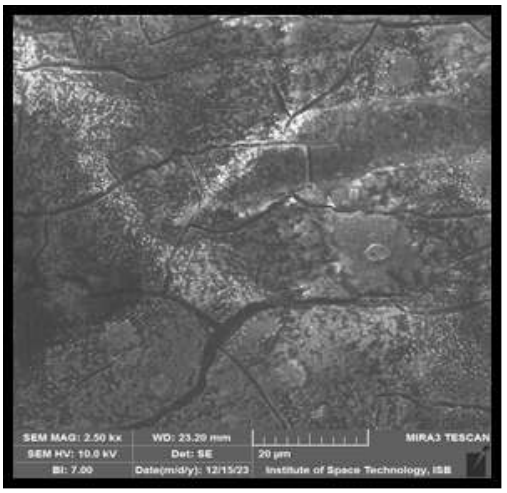

FIGURE 4.20: SEM of sample 3 at 20 μm

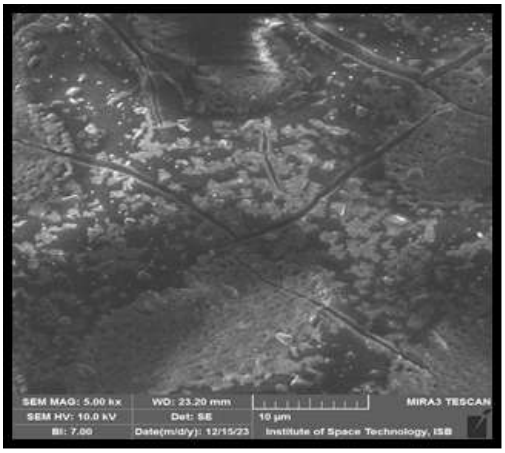

FIGURE 4.21: SEM of sample 3 at 10 μm

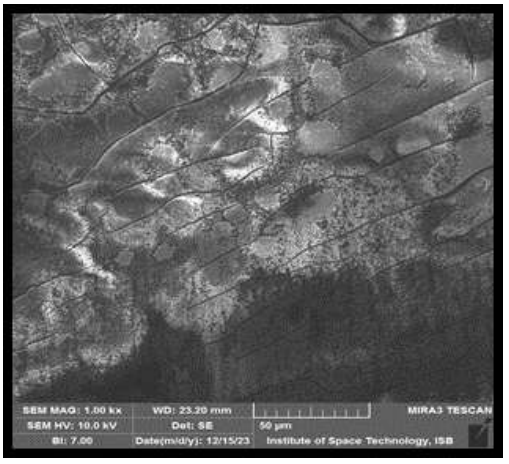

FIGURE 4.22: SEM of sample 3 at 50 μm

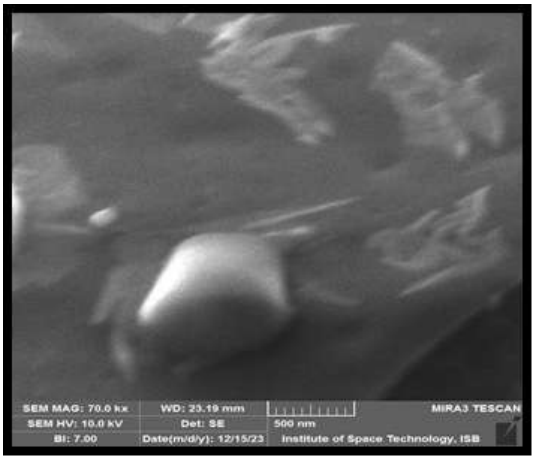

FIGURE 4.23: SEM of sample 3 at 500 μm

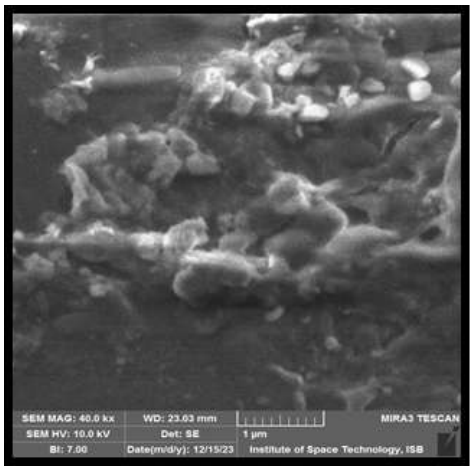

FIGURE 4.24: SEM of sample 5 at 1 μm

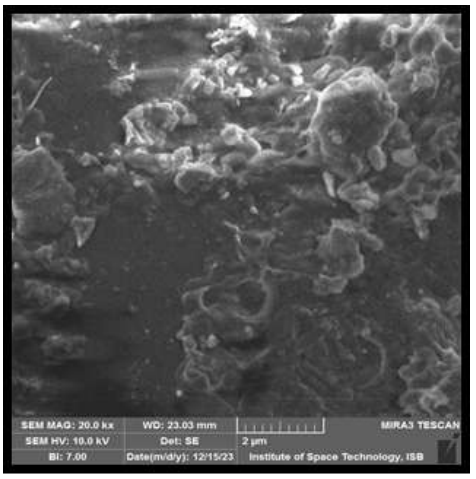

FIGURE 4.25: SEM of sample 5 at 2 μm

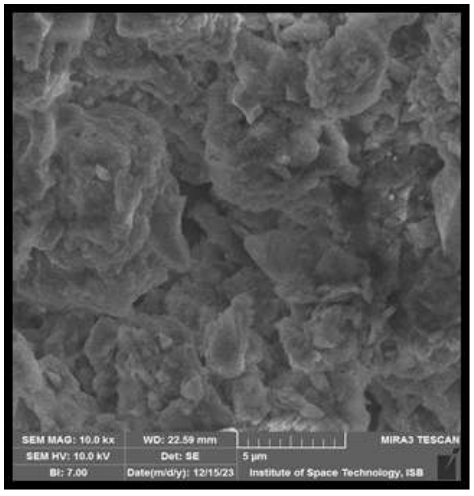

FIGURE 4.26: SEM of sample 5 at 5 μm

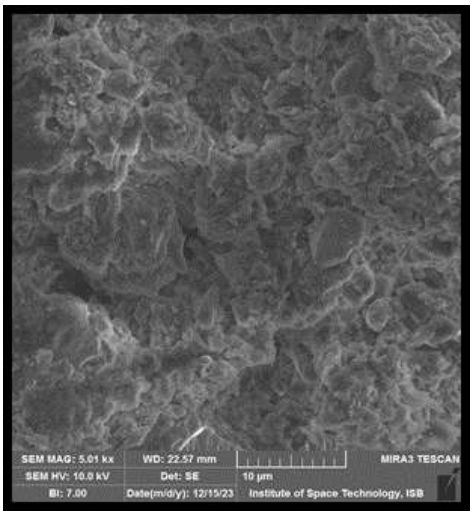

FIGURE 4.27: SEM of sample 5 at 10 μm

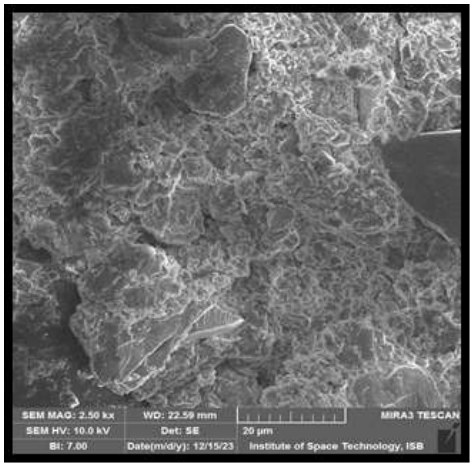

FIGURE 4.28: SEM of sample 5 at 20 μm

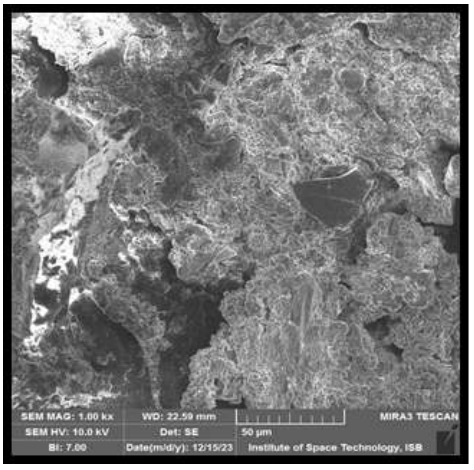

FIGURE 4.29: SEM of sample 5 at 50 μm

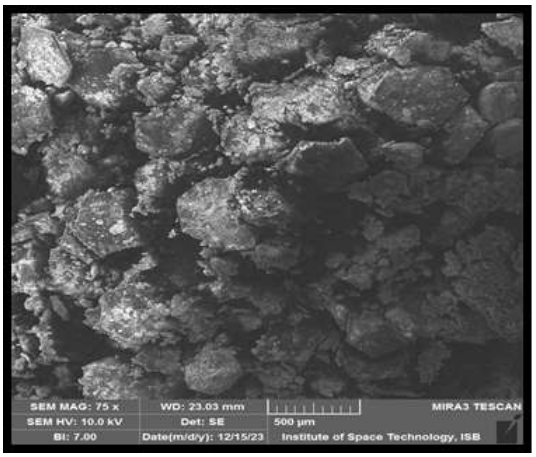

FIGURE 4.30: SEM of sample 5 at 500 μm

4.6 UV Spectrophotometry

UV spectrometry was employed to detect and quantify biomolecules, such as proteins, nucleic acids, and polysaccharides present in biofilms. The absorbance of these biomolecules were examined at specific UV wavelengths (490 nm and 510 nm), providing information about the composition and density of biofilms on plastic surfaces. Results are reported in fig 4.16 in which the graph shows the plastic degradation potential by bacterial biofilms at two different wavelengths (490 nm and 510 nm). All plastics showed Changes in the spectra which indicated that all the plastic samples have potential to degrade plastic over time. On comparing both wavelengths, Samples at wavelength 490nm showed more degradation potential than the wavelength 510 nm. The unit of absorbance for biofilm degradation potential plotted in y-axis is measured in Lambda (λ).

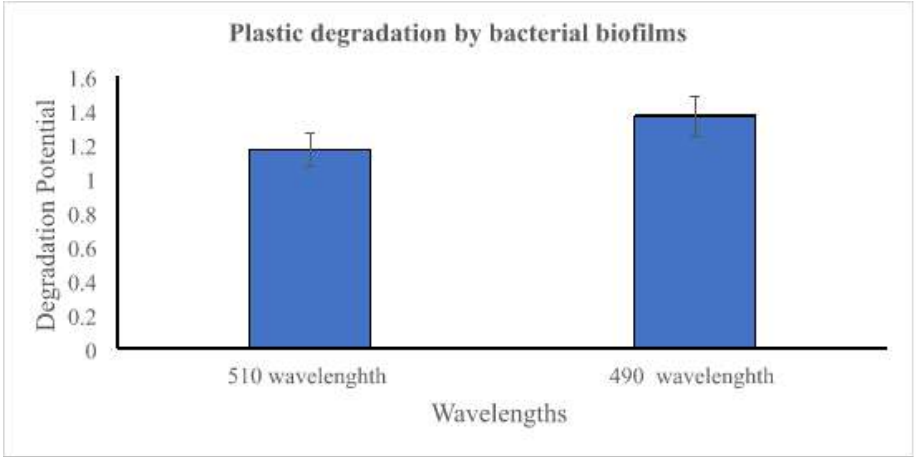

FIGURE 4.31: Graph of plastic degrading bacteria by bacterial biofilms at wavelength 510 nm and 490 nm

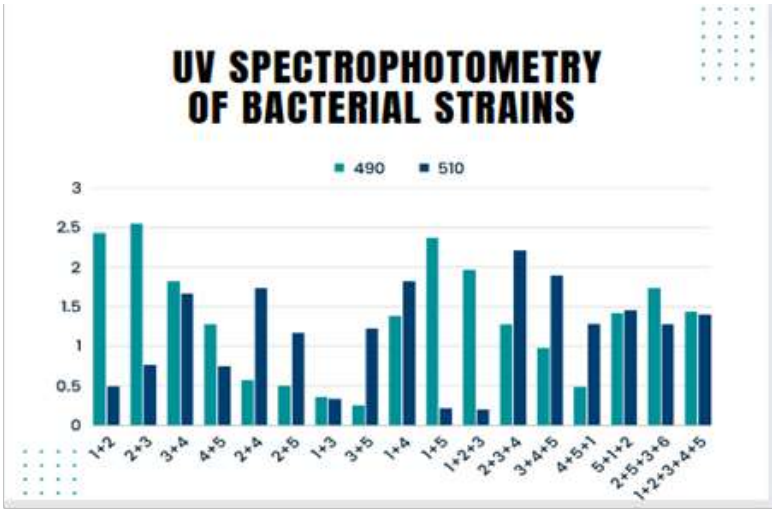

FIGURE 4.32: UV Spectrophotometry of bacterial strain consortiums at wavelength 490nm and 510nm

When plastic degrades, it undergo changes that affect its ability to interact with light. These changes may result in increased absorption of light at specific wavelengths, leading to higher absorbance values. Conversely, if degradation process lead to the formation of transparent or less absorbing products, transmittance values may increase. In general, an increase in absorbance and decrease in transmittance can indicate changes in the chemical composition or structure of the plastic material. These changes might be associated with degradation processes such as oxidation, chain scission or the formation of degradation products.

In figure 4.32, UV spectrophotometry of consortium species at wavelength 490nm showed that Combination 2+3 (*Pseudomonas aeruginosa*+ *Acinetobacter baumannii*), 1+5 (*Acinetobacter baumannii*+ Uncultured bacterium) and 2+5+3+6 (*Pseudomonas aeruginosa*+ uncultured bacterium+ *Acinetobacter baumannii*+ unknown strain) showed lowest transmittance at 0.2nm with absorbance (2.555, 2.372, 1.734) respectively. While at wavelength 510nm, consortium 3+6 (Unknown strain + *Acinetobacter baumannii*), 4+5+1 (*Pseudomonas aeruginosa*+ Uncultured bacterium+ *Acinetobacter baumannii*) and 2+3+4 (*Pseudomonas aeruginosa*+ *Acinetobacter baumannii*+ *Acinetobacter baumannii*) showed low transmittance values 0.2nm, 0.5nm and 0.6nm respectivelty with absorbance of 1.118, 1.287 and 2.215 respectively (Table 3 and 4).

Chapter 5

Discussion

Plastic packaging has been a prevalent part of society for an extended period, serving diverse functions like bags, bottles, food packaging, and toys. However, a concern arises when these plastics end up in the environment post-use. Polymer degradation, involving chemical, physical, or biological reactions Lead to the breaking of bonds and Consecutive changes, can be classified into various types, including photochemical degradation, Thermodegradation, Ozone-mediated degradation, Mechanical degradation, Catalyst-assisted degradation, and Microbial degradation [133]. Biodegradable polymers, also termed bioplastics, possess a chemical structure conducive to direct enzymatic degradation or microbial processes. These polymers can mineralize into CO_2, CH_4, H_2O, inorganic compounds, or Organic matter by the enzymatic action of particular microorganisms like bacteria and fungi in Suitable environmental settings, biochemical reactions take place [134].

The intricate process of breaking down the long-chain polymer into CO_2 and water renders it vulnerable and susceptible to additional oxidation facilitated by enzymes released by microorganisms. The comprehensive biodegradation process is categorized into four stages:

(a) Biodeterioration involves the formation of carbonyl groups by oxidative enzymes either released by microorganisms or induced by external factors. Further

oxidation leads to the production of carboxylic acids. (b) Biofragmentation entails the breakdown or cleavage of polymer carbon chains, liberating intermediate products through enzymes produced by microorganisms. (c) Bioassimilation occurs when bacteria absorb and metabolize small hydrocarbon fragments generated during biofragmentation. (d) Mineralization involves integrating hydrolysis products into the cell wall, intracellularly converting these products into microbial biomass, and subsequently releasing carbon dioxide and water from the cell [135]. Throughout this process, the polymer undergoes structural modifications, yielding oxidized oligomers, which microorganisms then assimilate as small cleavage fragments.

Microbial initiation of polyethylene degradation involves adherence to its surface, followed by the production of extracellular enzymes. Under aerobic conditions, the final degradation products include CO_2, water, and microbial biomass. In contrast, under anaerobic or methanogenic conditions, the end products consist of CO_2, water, methane, and microbial biomass [136].

The degradation process may occur aerobically, utilizing oxygen as an electron acceptor, or anaerobically in its absence. Aerobic microorganisms decompose organic chemicals into smaller compounds through the presence of oxygen, whereas anaerobic biodegradation takes place in the absence of oxygen. Throughout degradation, polymers become brittle, forming small fragments known as microplastics—particles with a diameter less than 5 mm, resulting from the breakdown of larger plastics. These microplastics have been detected in various environments, including food, drinking water, soil, and the air [137].

Synthetic plastics, non-biodegradable and derived from petrochemicals, possess high molecular weight due to the continuous repetition of monomer units. In contrast, Biodegradable plastics are made from sustainable sources and naturally undergo decomposition. Certain bacteria, such as *Pseudomonas fluorescens* and *P. aeruginosa*, showcase significant polyethylene degradation capabilities in aquatic environments at pH 7 and temperatures between 30–37 ^{o}C. Acidic conditions adversely affect their performance compared to alkaline conditions [138].

The isolates of a baumannii's uses LDPE as its only carbon source in order to thrive. Without the need for any additional ingredients or treatments, the bacteria may quickly break down LDPE [139].

Plastic packaging has been a prevalent part of society, when these plastics end up in environment post-use. Polymer degradation involving chemical, physical or biological reactions result in bond breakages. These polymers can mineralize into CO_2, CH_4, H_2O, inorganic compounds, or biomass by the enzymatic activity. Biodegradable plastics are made from sustainable sources and naturally undergo decomposition. Certain bacteria, such as *Pseudomonas* species, *Vibrio*, *Bacillus*, *Flavobacterium* species, and *A. baumannii* showcase significant polyethylene degradation capabilities in isolated and in consortium form [140].

5.1 Advantages of Bacterial Biofilms

1. Bacteria within biofilms often exhibit increased resistance to antibiotics and disinfectants compared to planktonic (free-floating) bacteria. This resistance arises due to various factors such as the physical barrier provided by the extracellular polymeric substances (EPS) matrix and the presence of dormant or slow-growing cells within the biofilm.

2. Biofilms can protect bacteria from various environmental stresses, including desiccation, UV radiation, and fluctuations in temperature and pH. The EPS matrix provides a physical barrier that shields the bacteria from adverse conditions.

3. Bacterial biofilms enable microbes to colonize and survive in diverse and harsh environments, including medical devices, industrial equipment, and natural habitats such as soil and water.

4. Within biofilms, bacteria can engage in cooperative behaviors, such as metabolic cooperation and quorum sensing-mediated communication. These interactions can lead to increased nutrient availability, enhanced metabolic efficiency, and improved resistance to environmental stresses.

5. Biofilms play a crucial role in bioremediation processes by facilitating the degradation of pollutants and contaminants in soil, water, and air. Bacteria within biofilms possess diverse metabolic capabilities that can be harnessed for the removal of organic and inorganic pollutants from industrial effluents and wastewater [141].

5.2 Disadvantages of Bacterial Biofilms

1. Bacterial biofilms are implicated in numerous chronic infections, including those associated with medical devices (e.g., catheters, implants), respiratory tract infections (e.g., cystic fibrosis), and oral diseases (e.g., dental plaque). Biofilm-associated infections are often recalcitrant to treatment and can lead to persistent inflammation and tissue damage.

2. Biofilms can cause biofouling of surfaces in various industrial and marine applications, leading to reduced efficiency and performance of equipment such as pipes, membranes, and ship hulls. Biofouling can result in increased energy consumption, maintenance costs, and operational downtime.

3. Eradicating bacterial biofilms can be challenging due to their inherent resistance to antimicrobial agents and the presence of persister cells, which are metabolically dormant cells that are tolerant to antibiotics.

4. Bacterial biofilms formed on food contact surfaces and in water distribution systems can serve as sources of contamination, contributing to foodborne illness outbreaks and waterborne diseases. The presence of biofilms in food processing facilities and water treatment plants necessitates stringent hygiene practices and disinfection measures to prevent microbial contamination.

5. Bacterial biofilms serve as reservoirs of antibiotic resistance genes and can facilitate the horizontal transfer of resistance determinants between bacterial species. This phenomenon contributes to the spread of antibiotic resistance in clinical settings and environmental reservoirs [142].

Chapter 6

Conclusion and Future Perspectives

The amount of plastic garbage that has accumulated globally is concerning, raising concerns all throughout the world. Plastic solid waste still finds up in landfills, despite concerted attempts to alleviate the issue through separated collection and recycling. This environmental problem is made worse by the persistent existence of traditional plastics in the natural world, the widespread use of single-use plastics, and insufficient waste management techniques. Pollution from plastic poses a major threat to aquatic as well as terrestrial ecosystems, affecting wildlife populations. The polymerization of hydrolysable plastics in moderate conditions, enabled by microbial enzymes that catalyze plastic biodegradation, is a potentially effective solution to this issue. During this process, bacteria settle on the plastic's surface and create a bio film. Extracellular enzymes secreted by these microbes break the down the polymer, producing shorter chains and oligo-, di-, and monomer that can be taken up by other microorganisms. In this work, the ability of microbial strains isolated from the plastisphere of terrestrial substrates to break down different kinds of plastics was evaluated.

The first objective of the study was the isolation of bacterial strains from the samples collected from the landfills. For this purpose, plastic pieces when cultured on nutrient broth and nutrient agar showed microbial growth only in samples 3 and

5 while the other samples did not exhibit any growth. Gram staining of samples 3.1 and 3.2 demonstrated a positive Gram-positive result, whereas samples 3.4, 5.1, 5.3, and 5.4 displayed a Gram-negative result. Biochemical characterization indicates that to achieve the second objective 16s RNA sequencing was performed. 16s RNA sequencing of 5 strains revealed that 3.1 (100%), 3.4 (99%) and 5.1 (92%) have sequence similarity with *Acinetobacter baumannii* (OR827196) (OR826264) and (OR826261) respectively whereas sample 3.2 (99%) has sequence similarity with *Pseudomonas aeruginosa* (OR826267) and sample 5.3 was uncultured bacteria with sequence similarity 99% (OR826194). Third objective was meant to evaluate the potential of recovered strains for biofilm formation. All samples showed significant potential as an individual biofilm formation with sample 5.3 highest and 3.1 comparatively with lowest potential and as a consortium, 3+5 (*A. baumannii + uncultured bacterium*) showed lowest biofilm formation capacity while 1+2+3+4+5 (*A. baumannii+ P. aeruginosa + A. baumannii+ A. baumannii+ uncultured bacterium*) showed highest biofilm formation capacity. Results of UV spectrophotometry of consortium species at wavelength 490nm showed that Combination 2+3 (*Pseudomonas aeruginosa+ Acinetobacter baumannii*), 1+5 (*Acinetobacter baumannii+ Uncultured bacterium*) and 2+5+3+6 (*Pseudomonas aeruginosa+ uncultured bacterium+ Acinetobacter baumannii+* unknown strain) showed lowest transmittance at 0.2nm with absorbance (2.555, 2.372, 1.734) respectively. While at wavelength 510nm, consortium 3+6 (Unknown strain + *Acinetobacter baumannii*), 4+5+1 (*Pseudomonas aeruginosa+ Uncultured bacterium+ Acinetobacter baumannii*) and 2+3+4 (*Pseudomonas aeruginosa+ Acinetobacter baumannii+ Acinetobacter baumannii*) showed low transmittance values 0.2nm, 0.5nm and 0.6nm respectivelty with absorbance of 1.118, 1.287 and 2.215 respectively.

The study identified five bacterial strains that demonstrated the ability to form biofilms and degrade various types of plastics. This discovery may enhance the rates at which microbial strains degrade plastic and may have consequences for the breakdown of plastic in settings with limited carbon and nutritional supplies. The discovered microbial strains have a great deal of potential to further the creation of effective and long-lasting processes for recycling plastic waste. The study was

only focused to bacterial population involved in plastic degradation, other plastic degrading potential microbes including fungi, and algae should be investigated.

1. Isolated strain should be evaluated for the specificity of type of plastics that they degrade.

2. Whole genome studies should be carried out to identify the strain with 92% similarity and to identify the novel enzymes in isolated strains with the potential of biofilm formation responsible for the degradation of plastics.

3. Genetic modification and synthetic biology techniques could be employed to optimize bacterial enzymes involved in plastic degradation.

4. 4. By utilizing isolated strains bacterial consortia can be constructed, which involve multiple bacterial species working together. Such consortia may have synergistic effects on plastic degradation and could be more effective than individual strains.

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Appendix A

Following is the table of absorption of UV spectrometry of bacterial combinations done on different wavelengths 510nm and 490nm and their mean values.

Table 1: Absorption of UV spectrometry of bacterial combinations done on different wavelengths

510 wavelength Absorbance	**490 wavelength Absorbance**
1.267	1.327
1.253	1.788
0.964	1.573
1.23	1.339
1.143	2.365
0.96	1.827
0.497	2.437
0.768	2.555
1.667	1.821
1.941	2.71
0.753	1.281
1.815	1.763
1.192	1.203
0.126	0.424
0.83	1.82
1.169	0.502
1.735	0.577

510 wavelength Absorbance	**490 wavelength Absorbance**	
1.226	0.252	
0.216	2.372	
1.118	0.393	
0.338	0.363	
1.971	0.215	
1.287	0.493	
1.821	1.387	
0.205	1.197	
2.215	1.28	
1.897	0.981	
1.638	1.364	
0.512	1.486	
0.572	1.363	
1.284	1.734	
1.462	1.422	
1.403	1.443	
1.165909091	1.365363636	Mean
0.561219229	0.680862635	SD
0.097695728	0.118522972	SE

Following is table of t-test showing a significant difference between the means of two wavelengths 490 nm and 510 nm.

TABLE 2: T-test significant difference between the means of two wavelengths 490nm and 510nm.

t-Test: Two-Sample Assuming Unequal Variances		
	1.267	1.327
Mean	1.16275	1.366563
Variance	0.32478729	0.493433
Observations	32	32

t-Test: Two-Sample Assuming Unequal Variances	
Hypothesized Mean Difference	0
df	59
t Stat	-1.274590545
P(T<=t) one-tail	0.103725669
t Critical one-tail	1.671093032
P(T<=t) two-tail	0.207451338
t Critical two-tail	2.000995378

Following are the NCBI submitted strains with their Accession numbers

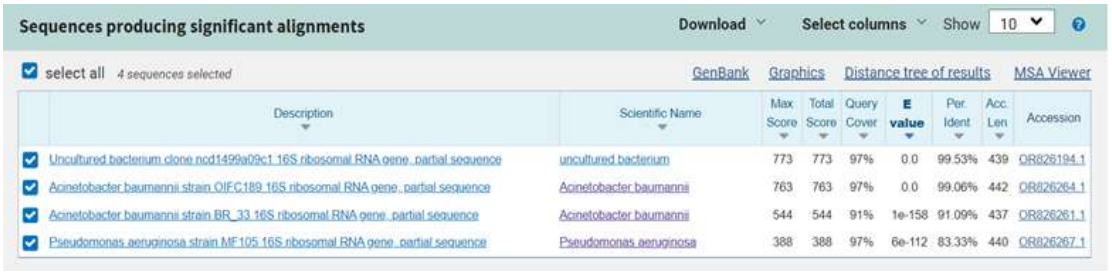

FIGURE 1: NCBI submitted strains with their Accession numbers

TABLE 3: Wavelength 490nm

No	**Sample**	**Name of combination strains**	**T%**	**Absorbance**
1	1	*Acinetobacter baumannii*	4.7	1.327
2	2	*Pseudomonas aeruginosa*	1.6	1.788
3	3	*Acinetobacter baumannii*	2.7	1.573
4	4	*Acinetobacter baumannii*	4.6	1.339
5	5	*Uncultured bacterium*	0.4	2.365
6	6	*Unknown strain*	0.3	1.827
7	1+2	*Acinetobacter baumannii + Pseudomonas aeruginosa*	1.5	2.437
8	2+3	*Pseudomonas aeruginosa+ Acinetobacter baumannii*	0.2	2.555
9	3+4	*Acinetobacter baumannii + Acinetobacter baumannii*	1.6	1.821
10	5+6	*Uncultured bacterium + unknown strain*	1.7	2.710

No	Sample	Name of combination strains	T%	Absorbance
11	4+5	*Acinetobacter baumannii+ Uncultured bacterium*	6.4	1.281
12	6+1	*Unknown strain + Acinetobacter baumannii*	8.4	1.763
13	6+4	*Unknown strain + Acinetobacter baumannii*	1.5	1.203
14	2+6	*Pseudomonas aeruginosa + unknown strain*	1.5	0.424
15	1+6	*Acinetobacter baumannii + unknown strain*	6.5	1.820
16	2+5	*Pseudomonas aeruginosa + uncultured bacterium*	6.0	0.502
17	2+4	*Pseudomonas aeruginosa+ Acinetobacter baumannii*	0.4	0.577
18	3+5	*Acinetobacter baumannii+ Uncultured bacterium*	4.4	0.252
19	1+5	*Acinetobacter baumannii+ Uncultured bacterium*	0.2	2.372
20	3+6	*Unknown strain + Acinetobacter baumannii*	1.6	0.393
21	1+3	*Acinetobacter baumannii + Acinetobacter baumannii*	1.2	0.363
22	2+4	*Pseudomonas aeruginosa+ Acinetobacter baumannii*	3.3	0.215
23	4+5+1	*Pseudomonas aeruginosa+ Uncultured bacterium+ Acinetobacter baumannii*	7.2	0.493
24	1+4	*Acinetobacter baumannii + Acinetobacter baumannii*	6.4	1.387
25	1+2+3	*Acinetobacter baumannii + Pseudomonas aeruginosa+ Acinetobacter baumannii*	8.5	1.197
26	2+3+4	*Pseudomonas aeruginosa+ Acinetobacter baumannii+ Acinetobacter baumannii*	4.3	1.280
27	3+4+5	*Acinetobacter baumannii+ Acinetobacter baumannii+ uncultured bacterium*	4.0	0.981
28	3+1+5	*Acinetobacter baumannii+ Acinetobacter baumannii + uncultured bacterium*	8.6	1.364

No	Sample	Name of combination strains	T%	Absorbance
29	1+3+4	*Acinetobacter baumannii + Acinetobacter baumannii+ Acinetobacter baumannii*	3.3	1.486
30	2+6+4	*Pseudomonas aeruginosa+ unknown bacterium + Acinetobacter baumannii*	4.2	1.363
31	2+5+3+6	*Pseudomonas aeruginosa+ uncultured bacterium+ Acinetobacter baumannii+ unknown strain*	0.2	1.734
32	5+1+2	*Uncultured bacterium+ Acinetobacter baumannii+ Pseudomonas aeruginosa*	8.0	1.422
33	1+2+3+4+5	*Acinetobacter baumannii+ Pseudomonas aeruginosa+ Acinetobacter baumannii+ Acinetobacter baumannii+uncultured bacterium*	3.6	1.443

Table 4: Wavelength 510nm

No	Sample	Name of combination strains	T%	Absorbance
1	1	*Acinetobacter baumannii*	5.4	1.267
2	2	*Pseudomonas aeruginosa*	5.5	1.253
3	3	*Acinetobacter baumannii*	10.9	0.964
4	4	*Acinetobacter baumannii*	4.8	1.230
5	5	*Uncultured bacterium*	2.7	1.143
6	6	*Unknown strain*	4.0	0.960
7	1+2	*Acinetobacter baumannii + Pseudomonas aeruginosa*	1.2	0.497
8	2+3	*Pseudomonas aeruginosa+ Acinetobacter baumannii*	5.9	0.768
9	3+4	*Acinetobacter baumannii + Acinetobacter baumannii*	7.0	1.667
10	5+6	*Uncultured bacterium + unknown strain*	7.5	1.941
11	4+5	*Acinetobacter baumannii+ Uncultured bacterium*	1.4	0.753
12	6+1	*Unknown strain + Acinetobacter baumannii*	6.7	1.815

No	Sample	Name of combination strains	T%	Absorbance
13	6+4	*Unknown strain + Acinetobacter baumannii*	1.7	1.192
14	2+6	*Pseudomonas aeruginosa + unknown strain*	4.8	0.126
15	1+6	*Acinetobacter baumannii + unknown strain*	1.9	0.830
16	2+5	*Pseudomonas aeruginosa + uncultured bacterium*	6.8	1.169
17	2+4	*Pseudomonas aeruginosa+ Acinetobacter baumannii*	1.8	1.735
18	3+5	*Acinetobacter baumannii+ Uncultured bacterium*	2.6	1.226
19	1+5	*Acinetobacter baumannii+ Uncultured bacterium*	3.1	0.216
20	3+6	*Unknown strain + Acinetobacter baumannii*	0.2	1.118
21	1+3	*Acinetobacter baumannii + Acinetobacter baumannii*	5.5	0.338
22	2+4	*Pseudomonas aeruginosa+ Acinetobacter baumannii*	6.2	1.971
23	4+5+1	*Pseudomonas aeruginosa+ Uncultured bacterium+ Acinetobacter baumannii*	0.5	1.287
24	1+4	*Acinetobacter baumannii + Acinetobacter baumannii*	2.2	1.821
25	1+2+3	*Acinetobacter baumannii + Pseudomonas aeruginosa+ Acinetobacter baumannii*	4.3	0.205
26	2+3+4	*Pseudomonas aeruginosa+ Acinetobacter baumannii+ Acinetobacter baumannii*	0.6	2.215
27	3+4+5	*Acinetobacter baumannii+ Acinetobacter baumannii+ uncultured bacterium*	5.1	1.897
28	3+1+5	*Acinetobacter baumannii+ Acinetobacter baumannii + uncultured bacterium*	7.2	1.638
29	1+3+4	*Acinetobacter baumannii + Acinetobacter baumannii+ Acinetobacter baumannii*	1.4	0.512
30	2+6+4	*Pseudomonas aeruginosa+ unknown bacterium + Acinetobacter baumannii*	9.6	0.572

No	**Sample**	**Name of combination strains**	**T%**	**Absorbance**
31	2+5+3+6	*Pseudomonas aeruginosa+ uncultured bacterium+ Acinetobacter baumannii+ unknown strain*	4.8	1.284
32	5+1+2	*Uncultured bacterium+ Acinetobacter baumannii+ Pseudomonas aeruginosa*	8.2	1.462
33	1+2+3+4+5	*Acinetobacter baumannii+ Pseudomonas aeruginosa+ Acinetobacter baumannii+ Acinetobacter baumannii+uncultured bacterium*	3.6	1.403