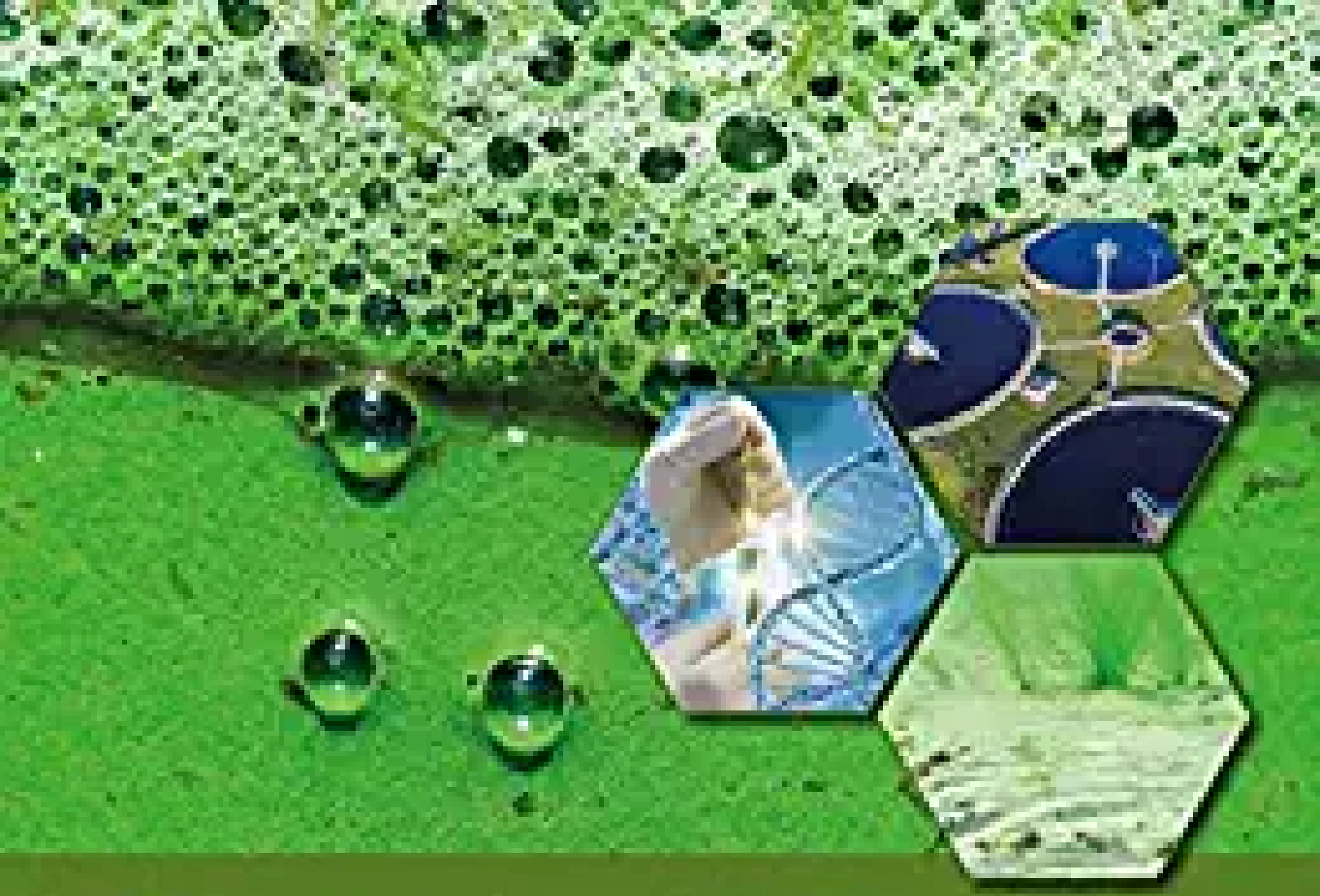

Algal Biotechnology

Integrated Algal Engineering for Bioenergy, Bioremediation, and Biomedical Applications

Edited by
Ashfaq Ahmad, Fawzi Banat, Hanifa Taher

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ALGAL BIOTECHNOLOGY

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CHAPTER

1

Algal engineering for bioremediation, bioenergy production, and biomedical applications

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OUTLINE

1 Introduction

Algae are photosynthetic aquatic plants that grow in ponds, streams, oceans, and even wastewater. Algae have a high tolerance for high temperatures, salinities, pH, and different

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light intensities and can grow alone or with other organisms because of their symbiotic relationship [1,2]. They are generally categorized as Rhodophyta (red algae), Phaeophyta (brown algae), and Chlorophyta (green algae). They can be grouped by sizes, e.g., macroalgae (seaweeds) are multicellular, large, and can be seen with the naked eye. In contrast, microalgae are unicellular, smaller in size, and can only be seen microscopically. Like conventional food crops, algae require water, sunlight, carbon dioxide (CO_2), and nutrients to grow. However, they have a higher growth rate than other plants and provide ecological benefits [3,4]. Microalgae can be prokaryotic such as cyanobacteria (Chloroxybacteria), or eukaryotic such as green algae (Chlorophyta). Fig. 1.1 shows the green marine microalgae *Nannochloropsis oculata* and the freshwater/terrestrial algae species *Eustigmatos splendida* and *Eustigmatos magnus* [5].

Algae can potentially be used to produce biofuel, bioproducts, medicines, and cosmetics as they are a rich source of carbon compounds [6]. Bioproducts produced by algae are polysaccharides, lipids, pigments, proteins, vitamins, bioactive compounds, and antioxidants that can be used for various purposes. Algae have extensive applications in industrial wastewater treatment and CO_2 sequestration [7]. Algae feedstock is deemed renewable and sustainable for biofuels, which has encouraged setting up biorefineries. Integrated algal engineering approaches for improving their growth rate and genetic modification can enhance their future applications for producing renewable bioproducts. Rapid climate change is being caused due to the burning of fossil fuels, the release of anthropogenic CO_2, and the increasing population worldwide. Microalgae and cyanobacteria can be promising biological tools to tackle these persistent problems [8,9]. Algal biotechnology aims to produce sustainable biofuels with zero CO_2 emissions. An algal strain can be modified through genetic engineering to enhance biofuel production by targeting either a single gene or multiple genes [10]. Fig. 1.1 presents

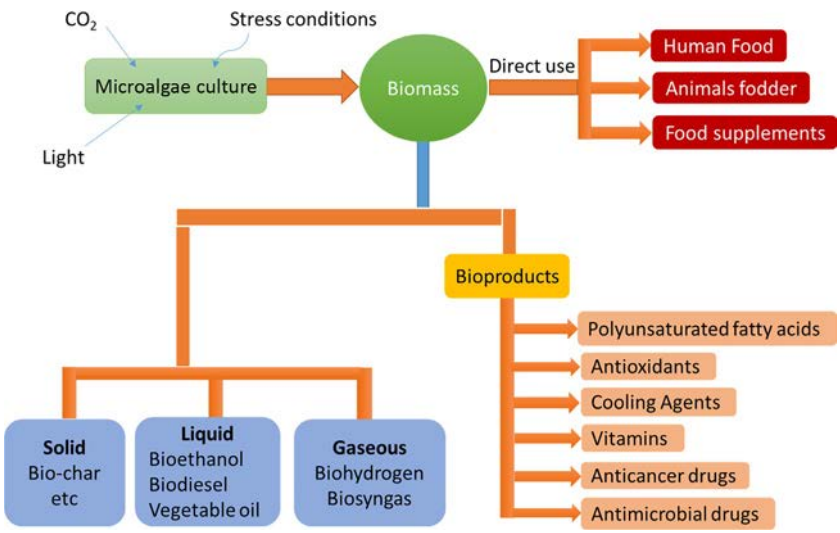

FIG. 1.1 Microalgae convert CO_2 into carbohydrates, lipids, and other valued bioproducts by using sunlight. *From M.I. Khan, J.H. Shin, J.D. Kim, The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products, Microb. Cell Fact. 17 (1) (2018) 36.*

the conversion of CO_2 into carbohydrates, lipids, and other valued bioproducts by using sunlight.

Commercial algae cultivation for generating biofuels and bioproducts has significantly increased recently [4]. An enormous amount of algae is being produced and sold for different purposes, such as the production of food and nutrient supplements. Algal extracts and by-products can be used in the pharmaceutical and cosmetic industries [5–7]. Algae feedstocks are proficient and desirable for biofuel production. They do not require vast lands for cultivation and can quickly grow in industrial wastewater. Algae do not contest human and animal food chains and mitigate atmospheric CO_2 [11–13]. Microalgae do not have lignocellulosic materials in the cell wall. This facilitates the pretreatment method and reduces production costs. Algae can grow in industrial wastewater and require less energy for their cultivation than the energy they can produce [14–16]. Production of second-generation biofuels from terrestrial plants is an immensely debated issue because biofuels' production from such food crops is expensive and competes with food and feed requirements. Moreover, crop foods need arable land and an enormous quantity of water, making biofuel production unsustainable. Therefore, liquid fuels from algae are an incomputable alternative [17,18]. Biofuel generation from microalgae is still in the developing phase, and a significant improvement is essential for its commercial application and attracting investors and consumers.

2 Industrial wastewater treatment

Several physical, chemical, and biological treatment techniques have been used for industrial wastewater treatment. Conventional methods for wastewater treatment involve intensive aeration for the oxidation of organic carbon and removal of other contaminants using microorganisms. An enormous amount of energy is required for the aeration of wastewater treatment plants, accounting for 50% or more of the total energy costs [19–21]. Numerous studies have suggested that algae can use various types of wastewater such as industrial, domestic, municipal, or agricultural wastewater. Combining sewage with the flue gas (atmospheric CO_2) enhances microalgae biomass productivity [22,23]. Organic carbon oxidation directly emits CO_2 into the atmosphere, whereas the energy used for the aeration of treatment plants can indirectly emit CO_2 [24,25].

Additionally, substantial quantities of potent greenhouse gases, such as nitrous oxide (N_2O), are also discharged in the latter case. One of the main constraints for traditional wastewater treatment is the recovery of N and P after the treatment [24,25]. Therefore, the algal wastewater treatment approach can be economical and ecologically friendly, mainly for removing and recovering N and P. Algae can produce oxygen (O_2) through photosynthesis and assimilate CO_2 during the photosynthetic process. They have a symbiotic relationship with bacteria. During the oxidation of organic carbon, the bacteria utilize the O_2 produced by photosynthetic algae, and the algae simultaneously assimilate the CO_2 generated by bacterial respiration. Therefore, the integration of algae in wastewater treatment can decrease aeration requirements and CO_2 emissions. Algae absorb N and P and photosynthetically fix carbon during their growth. This reduces the bacterial requirements for N and P removal and the associated aeration demands and N_2O emissions [21]. Another study has reported that algae

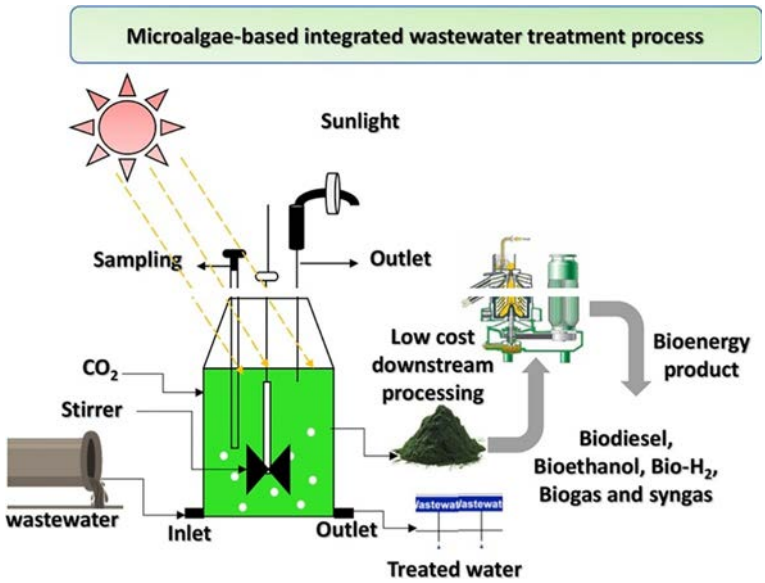

FIG. 1.2 An integrated approach of microalgae cultivation in different wastewaters for bioproducts application. *From R.K. Goswami, et al., Microalgae-based biorefineries for sustainable resource recovery from wastewater, J. Water Process. Eng. (2020) 101747.*

could be cultured via an integrated cultivation system using wastewater from the food industry and CO_2 from the atmosphere. Further, biomass can be used to produce bioenergy and bioactive compounds [22].

Moreover, algae biomass produced in the wastewater treatment process could be recycled for diverse applications, as shown in Fig. 1.2. Algal biomass contains lipids, carbohydrates, and proteins with high nutritious and calorific value. After its harvesting, it can be used as animal feed [26], slow-release fertilizer [27], or biofuel [28,29], thus turning waste into valuable resources. Biogas, such as biomethane and biohydrogen, can be produced through anaerobic digestion from wastewater. The biomass can also be utilized as supplementary feed for aquaculture and animals and as fertilizer for crops.

2.1 Removal of total nitrogen (TN) and total phosphorus (TP)

Fig. 1.3 explains the standard wastewater treatment process, including primary, secondary, and final processing stages. Raw wastewater usually comprises organic N that can quickly degrade into ammonium. Organic N and inorganic N, including urea, and such wastewater could be used to grow filamentous algae. Putatively, it is favorable to cultivate freely suspended cells of filamentous algae in raw wastewater. However, the existence of a suspended substance in wastewater makes it too turbid and blocks light penetration, inhibiting algae's photosynthetic activity. The solid wastes present in the wastewater can be typically removed through the primary process of sedimentation or dissolved air flotation to get a relatively perfect effluent that has soluble organic carbon and ammonium [21].

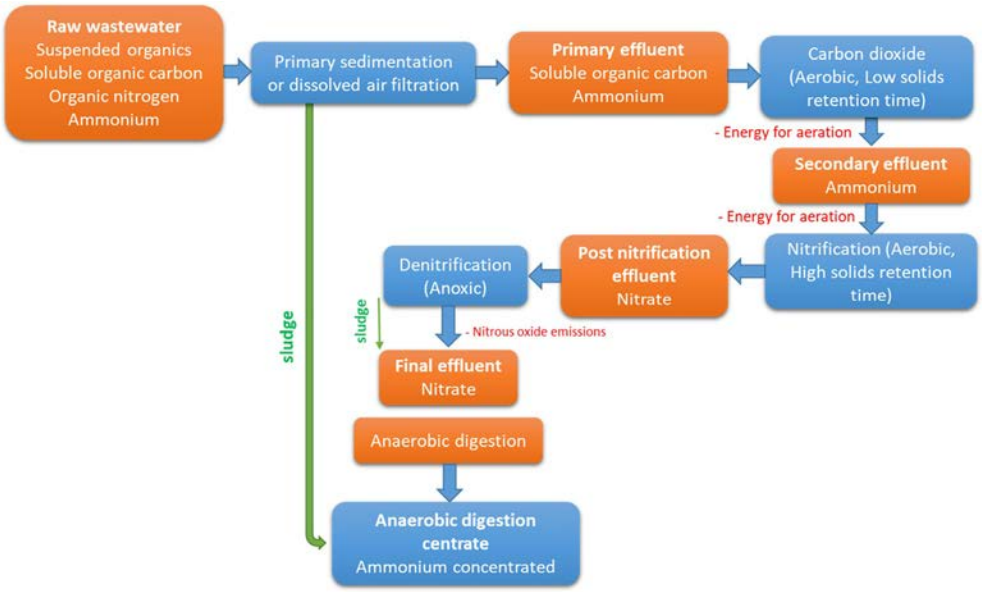

FIG. 1.3 Wastewater treatment process *(orange boxes, gray in print version)* and the different wastewater streams *(blue boxes, dark gray in print version)* in which algae could be cultivated. *From J. Liu, et al., Wastewater treatment using filamentous algae—a review, Bioresour. Technol. 298 (2020) 122556.*

Wastewater contains organic, and inorganic P. Algae utilize orthophosphate, polyphosphate, pyrophosphate, and metaphosphate for their growth [30]. N and P are important nutrients required for algal growth and are assimilated instantaneously. Table 1.1 presents the wastewater contamination removal rate for different species of microalgae. An N to P ratio (N:P ratio) is usually defined to identify whether they are the limiting nutrients for algal growth in certain wastewater. Microalgae *Pantanalinema* has been reported for N and P removal from wastewater under the dark-light condition. Around 86% of P was removed within a dark-light cycle of 6 h. Cellular and polyphosphate mechanisms of microalgal-bacterial granules were responsible for accumulating P. Approximately 70% of soluble P removal was reported due to polyphosphate development in *Pantanalinema* algal cells [31]. The algal-bacterial symbiosis (ABS) system has been reported to improve the removal of nutrients using a sequencing batch biofilm reactor (SBBR). The total N's elimination efficiencies increased from 38.5% to 65.8% and P from 31.9% to 89.3% using the algae-assisted SBBR. Moreover, chlorophyll-*a* (3.59 mg/g) production increased at a stable stage and was 4.07-fold higher than that of freely suspended cells. An analysis of the mechanisms proposed that the high removal of N and P is mostly due to enhancing both algal biomass and total biomass in the biofilm [32]. *Chlorella vulgaris* and *Neochloris oleoabundans* have been reported to remove the Chemical Oxygen Demand (COD), inorganic N, and total dissolved P at 36°C from primary and secondary effluents and centrate (CEN). The efficiency of COD's removal achieved with *C. vulgaris* was 51% from primary effluent, 55% from secondary effluent, and 80% from CEN. In contrast, the efficiency of COD's removal achieved with *N. oleoabundans* was 63% from primary effluent, 47% from secondary effluent, and 72% from CEN. Simultaneously, ammonia removal efficiencies (70%–84%) were obtained with both

TABLE 1.1 Microalgae species and their removal rate of contamination from different wastewater.

Wastewater	Microalgae	N (%)	P (%)	A (%)	COD (%)	Ref.
Industrial	*Spirulina platensis*	–	93	99	94	[40]
	Scenedesmus sp.	90	89	–	87	[41]
	Chlorella sp.	30–51	45	69–90	8–44	[42]
Municipal	*Coelastrum microporum*	35–88	42–89	–	60–80	[43]
	Chlorella sp.	89	81	94	91	[44]
	S. obliquus	–	55–83	97–100	–	[45]
	Blue-green algae	77–98	55–73	100	98	[46]
Agriculture	*Chlorella* sp.	76–83	63–75	100	28–38	[47]
	C. vulgaris	–	88	54	–	[48]
	Isochrysis galbana	58	–	–	77	[49]
	Pavlova lutheri	60	–	–	80	[49]

From N.S.M. Aron, K.S. Khoo, K.W. Chew, A. Veeramuthu, J.S. Chang, P.L. Show, Microalgae cultivation in wastewater and potential processing strategies using solvent and membrane separation technologies, J. Water Process Eng. 39 (2021) *101701.*

species in different wastewaters. High P concentrations that were removed from primary effluent were >84%. These were moderate in CEN (>22%) and less in secondary effluent (<15%). These studies confirmed that algae could grow in wastewaters at a hot temperature of 36°C and remove contaminants such as organic carbon, N, and P [33]. Algae-based membrane bioreactor (A-MBR) has been reported to cultivate algae with high cell density to remove P. The concentration of algae cells was increased from 385 to 4840 mg/L, and the average solids yield production rate of 32.5 g^{-3}/day was attained. Total P removal of 66% was achieved from wastewater in A-MBR. This study suggested that algae-induced phosphate precipitation is the key to removing P. The high-cell density of algal cultivation can produce P-rich biomass with brilliant harvesting properties [34]. The discharge of excessive P causes extensive eutrophication and water pollution that threatens both ecological and human health. However, P is an important component for all living microorganisms, but it is nonrenewable. Further, its natural reserves are depleting rapidly. Algae can sustainably reuse P from wastewater for their growth. Ultramembrane-treated landfill leachate can be utilized as a nutrient medium for culturing indigenous algal species with immediate elimination of P and N. Maximum N removal of 69% and P removal of 100% were achieved from 100 mg/L P-PO_4^{-3}-supplemented medium. Algae can be grown and used to sustain P and N from landfill leachate [35]. A study has recommended natural algae granulation in open sequencing batch reactors (SBRs) for treating synthetic wastewater to overcome the high separation cost of algae. High removal of P content (33 mg-P/g-TSS) with higher P bioavailability (92%) was achieved with algae granules as compared to seed algae (20 mg-P/g-TSS). The algae granules have a rich perspective for P rescue and reuse [36]. The anaerobic-aerobic-anoxic sequencing batch reactor (AOA-SBR) system has been suggested for instantaneous carbon, N, and P removal. High removal proficiencies of COD (97%), TN (96%), and TP (94%) were achieved with the AOA-SBR system in 6-h cycles [21]. Algae

immobilization has great potential to eliminate nutrients from wastewater. However, its commercial application is challenged by the high cost and the maintenance of many viable and active microalgal cells. Agar-immobilized microalgal cells efficiently removed NH_4^+-N (96%) and PO_4^3-P (99%) in both batch and continuous modes. The immobilized algal cells were still active and could eliminate 94% of NH_4^+-N and 66% of PO_4^3-P after being recycled for 8 cycles. Further, their nutrient removal efficiency was still more than 60% even after their preservation for 120 days under normal conditions. This study suggested that algae immobilization is simple, less costly, and practicable in preserving the microalgae at room temperature for a long time. Applying algae to remove nutrients in wastewater treatment is more suitable and advantageous [37]. Another study reported removing more than 94% ammonium-nitrogen and phosphate-phosphorus with the use of *Chlorella sorokiniana* cultivated in 10-L flat-panel bioreactors. This study indicated that controlled pH and high hydraulic retention time (HRT) could maximize the algae yield and improve the uptake of nutrients without magnesium (Mg) enrichment through continuous cultivation [38]. Outdoor cultivation of *C. vulgaris* in a thin-film flat plate photobioreactor (PBR) using digested piggery wastewater as the culture medium has also been reported. High levels of TN (72.48%), TP (86.93%), and COD (85.94%) removal were achieved with *C. vulgaris*. This study suggested that algal cells can adapt quickly to wastewater in outdoor conditions [39].

2.2 Heavy metal (HM) removal by algae

HM contaminants are increasing in water bodies due to urbanization, industrialization, and natural earth processes. HMs accumulate in the human body by consuming polluted water and food. The conventional methods reported for their removal are electrolysis, ion exchange, precipitation, chemical extraction, hydrolysis, polymer microencapsulation, and leaching. Several algal species are said to be useful biosorbent materials for removing HMs [50–55]. However, these approaches are not economical, especially on a large scale, due to continuous monitoring and control required and because of their lower HM-removal efficiency. Filamentous algae can adsorb HMs from wastewater. Algae can remove HMs by biosorption and bioaccumulation, as well as through physical and chemical mechanisms. HM removal by dried algal cells is called "biosorption," and the use of accumulating abilities of alive algal cells is called "bioaccumulation." A majority of the studies on this subject have focused on the biosorption of HMs from wastewater by utilizing the dry mass of filamentous algae as bio-adsorbent material. HM removal by inactive and dead biomass depends on the metal ions and the biomass's high affinities. Fig. 1.4 illustrates the complex mechanisms of metal ion binding. More precisely, the properties of algal cell wall constituents, such as alginate and fucoidan, are specifically responsible for metal ions' sequestration. The main functional groups existing in the brown and green algae, such as carboxyl, hydroxyl, sulfate, phosphate, and amine groups, play an essential part in metal binding [50,56].

The alginates in the cell wall and the intercellular substance of brown algae have a greater uptake for divalent cations such as Pb^{2+}, Cu^{2+}, Cd^{2+}, and Zn^{2+} [50]. In this case, the use of dried biomass of *Oedogonium*, *Spirogyra*, and *Cladophora* for the biosorption of lead (Pb), cadmium (Cd), nickel (Ni), and mercury (Hg) was reported [51–54]. Filamentous algae biomass performs the dual function of removing N from wastewater and subsequently being useful as

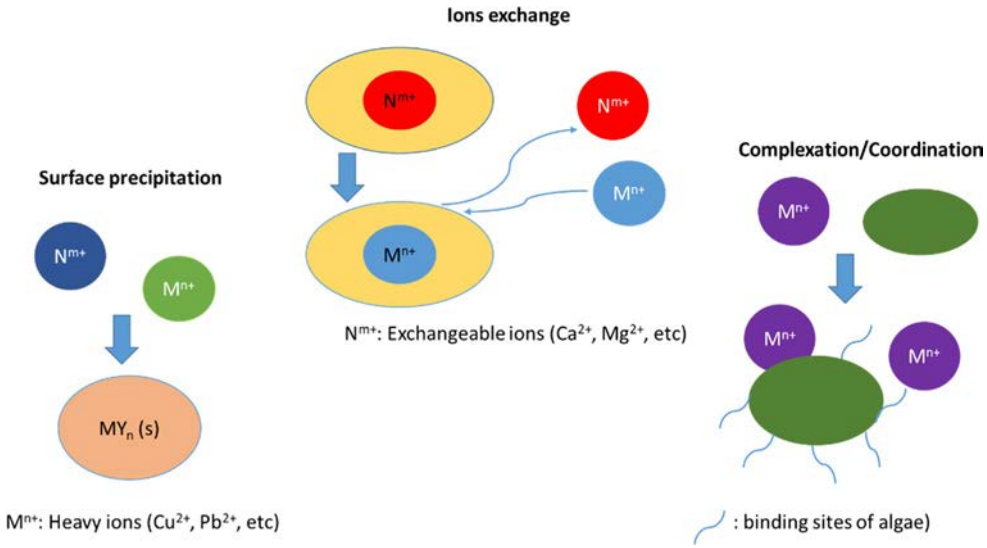

FIG. 1.4 The complex mechanisms of metal ion binding. *From J. He, J.P. Chen, A comprehensive review on biosorption of heavy metals by algal biomass: materials, performances, chemistry, and modeling simulation tools, Bioresour. Technol. 160 (2014) 67–78.*

a biosorbent for removing HMs from other contaminated water. Algae can offer an unconventional, sustainable, and environment-friendly approach for the bioremediation of HMs [57,58]. Numerous studies have reported the use of diverse filamentous algae species, including *Oedogonium*, *Rhizoclonium*, and *Hydrodictyon*, for maximum removal of HMs. The cultivation of these algal species in ash dam water confirmed more than 60 mg/g of HMs' accumulation from wastewater [59]. It was observed that the cultivation of *Cladophora* in synthetic wastewater removed more than 80% of Cd in batch and semibatch systems [60].

The alga *Distigma proteus*, isolated from industrial wastewater, has been reported to have a high tolerance against HMs such as Cd^{2+}, chromium (Cr^{6+}), Pb^{2+}, and copper (Cu^{2+}). The growth rate of the algae was found to slow down by day 8 against the metal stress of Cu^{2+} (90%), Cd^{2+}(84%), Cr^{6+} (71%), and Pb^{2+} (63%). The highest Cd^{2+} removal rate of 90% was achieved from the medium after 8 days with the use of the *Distigma* algae. The metal removal capability of *Distigma* can be explored for metal detoxification and environmental clean-up [61]. The use of freely suspended and immobilized cells of *Anabaena doliolum* and *C. vulgaris* has been reported for Cu and iron (Fe) removal. The immobilized algal cells showed a high removal rate for both Cu and Fe. This means that immobilization can protect the algal cells from toxic metals compared to the freely suspended cells. The immobilization technology can also ease the harvesting process and can potentially be used during repeated cycles [62]. Immobilized *Microcystis* has been used in the packed column for Cu^{2+} removal at different flow rates and metal ion concentrations. The highest reduction of Cu^{2+} (54%) was reached at a flow rate (0.75 mL/min) with an initial metal ion concentration of 30 μg/mL and biomass dosage of 0.016 g. The elimination of Cu^{2+} was found to be influenced by inlet metal ion concentration and biomass density. Increasing the biomass dosage from 0.016 to 0.128 g increased the removal percentage, and the Cu^{2+} adsorbed per unit dry weight

dropped [63]. The use of brown seaweeds, such as *Hizikia fusiformis*, *Laminaria japonica*, and *Undaria pinnatifida*, for the biosorption of heavy metal ions (Pb^{2+}, Cd^{2+}, Mn^{2+}, Cu^{2+}, and $Cr_2O_7^{2-}$) has been reported [64]. Dried biomass of brown marine algae *Ecklonia radiata* has been observed to uptake Cd from an aqueous solution. The maximum removal of Cd (1634 mg/g) dry biomass at an optimum pH of 4 and 50°C temperature has been reportedly achieved. Adsorption temperatures and pH levels can play an essential role in Cd uptake [65]. The biomass of the brown marine algae *Sargassum fluitans* has been reported to maximize Cr, Cu, and Al removal [66].

A native cyanobacterial species *Nostoc muscorum* has been reportedly used for Cu [67], Zn [67], Pb [67], and Cd [67] biosorption from an aqueous solution. The highest biosorption of Pb (96.3%) [67] and Zn (71.3%) [67] were obtained at 60 h of incubation with the algae. The biosorption of metals was attributed to passive biosorption and accumulation by the actively growing *N. muscorum* biomass. This study demonstrated that cyanobacteria could be used to remove metals from a multicomponent system [68]. Another study reported the use of dried biomass of the most common filamentous algae for the biosorption of HMs in the batch process. The removal of Pb^{2+} by 97% and 89% were reported with *Pithophora oedogonia* and *Spirogyra neglecta*, respectively, in 30 min from an initial concentration of 5 mg/L metal ions. The removal efficiency of Pb^{2+} decreased as the initial concentration of metal ions increased. Reduction of Pb^{2+} 70% was obtained with *S. neglecta* at an initial concentration of 75 mg/L of Pb^{2+}, while more than 75% of Pb^{2+} and Cu^{2+} reduction were achieved with *S. neglecta* and *P. oedogonia* from mixed-metal ions solutions. Some algal species, such as *Hydrodictyon reticulatum*, *Cladophora callicoma*, and *Aulosira fertilissima*, could not efficiently remove metal ions from mixed solutions [69].

Conversely, high concentrations of HMs can cause toxic effects on algal cells and hinder their growth and cellular metabolism. The absorption of HMs through algae could be determined by the latter's growth phase and other environmental settings. Numerous studies have reported that the use of dead algae shows more significant advantages than the use of living cells. Dead algae biomass comprises cellulose, glycoprotein, and pectins that act as biosorbents and adsorbents of HMs via the extracellular process [70].

3 Algae for CO_2 sequestration

CO_2 is continuously increasing in the atmosphere due to various anthropogenic activities. The burning of fossil fuels has contributed around 87% of CO_2; deforestation contributes 9% of it, whereas 4% is generated by industrial, manufacturing, and human activities [71]. In general, the three sectors that contribute to CO_2 emissions are transport, industrial production, and fuel combustion for some other activities [72,73]. Recent statistics have revealed that transportation and industrial production account for about 80% of fuel combustion, while the electricity sector accounts for the remaining 20% [74]. The rapid increase in atmospheric CO_2 levels is a key contributory factor for global warming, which is one of the leading challenges threatening environmental sustainability [75,76]. Several conventional technologies have been developed to deal with this challenge that involves the sequestration of CO_2, particularly along with energy retrieval, which has become an urgent need. The processes

involving the mitigation of CO_2 by chemical absorption, membrane separation, and physical adsorption and the cryogenic processes are all expensive and cause secondary pollution [75,77]. In the natural environment, CO_2 fixation can be conceivably done via photosynthetic land plants and microorganisms.

Algae and cyanobacteria have been reported for CO_2 fixation due to their fast growth rate and greater fixation efficiency than land plants [78]. Hence, the sequestration of CO_2 via photosynthetic algae and cyanobacteria is an ideal approach. The microalgae *Spirulina platensis* has been reported as a favorite strain for the fixation of CO_2 due to its fast growth rate, high resistance to high CO_2 concentration and temperature, nutrient deficiency, and pH flocculation. Additionally, it can produce precious bioactive compounds to improve immunity [79] and prevent aging [80]. Biofixation of CO_2 through algae is considered favorable as it can fix CO_2 and instantaneously yield value-added chemicals too [81].

Algae-based CO_2 sequestration offers an encouraging prospect to decrease CO_2 and convert carbon into bioproducts. Further, this process has fewer safety requirements than that of the storage of CO_2. Algae's high photosynthetic ability makes their CO_2 utilization 10–50 times greater than any other terrestrial plants using sunlight [82]. The bubbling-type photosynthetic algae microbial fuel cell (B-PAMFC) has been reported for wastewater treatment, and it facilitates CO_2 sequestration along with instantaneous power production. The highest fixation rate of CO_2 was achieved with *C. vulgaris* at 2.8 g/L urea in the B-PAMFC. The absorption efficiency of CO_2 and lipid productivity (105.9 mg/L/day) was enhanced due to urea's application. The highest net energy of 1.824 kWh m^{-3} was produced. This study demonstrated that B-PAMFC with urea as the N source offered a beneficial method for instantaneous CO_2 mitigation and bioenergy production [83]. Microalgae *Phormidium valderianum* BDU 20041 reported high tolerance at 15% CO_2, thus proving to be a suitable contestant for carbon capture. An increased amount of lipid at an elevated CO_2 level was achieved with actual flue gas conditions than that achieved in ambient air [84].

Mixed algal cultures in batch mode with an external supply of CO_2 from wheat straw fermentation have been reported. High sequestration of CO_2 (287 mg/L/day) has been observed with mixed cultures of algae. Removal of 87% ammonium, 78% phosphate, 68% COD, and 65% nitrate was also achieved. About 12.29% of lipids were produced with the help of enriched CO_2 and wastewater for the supply of nutrients. The total amount of chlorophyll and protein content achieved was 14.3 and 12.3 μg/L and 0.13 and 0.15 mg/L, respectively. This study indicated that algae consortia could be potentially used for CO_2 mitigation, wastewater treatment, and bioenergy production [85]. Freshwater algae *C. vulgaris* was cultivated in PBRs with low doses of sugars to enhance CO_2 mitigation under the light-emitting diode's illumination. Glucose addition at low concentration improved the photoautotrophic growth and biomass generation and CO_2 capture by 10%. A techno-economic analysis (TEA) suggested that LED-based PBRs are a feasible approach for transforming CO_2 into value-added algal biomass [86]. Another study reported the use of *Chlorococcum humicola*, *C. vulgaris*, and *Scenedesmus quadricauda* for total CO_2 fixation and chlorophyll, protein, and carbohydrate production. Sodium bicarbonate at a concentration of 0.2%–1% was used in the bold basal medium for the uptake of carbon, and it acted as a substitute source of atmospheric CO_2. A high amount of lipid content and CO_2 fixation rate of 0.4% were achieved, whereas the maximum amount of chlorophyll content was obtained at 0.6% of sodium bicarbonate concentration. This study showed that

S. quadricauda could be suitable for CO_2 mitigation and the high production of polyunsaturated fatty acid [87].

Microalgae cultivation has been reported in an outdoor raceway pond to produce biomass and for CO_2 mitigation from the flue gas under diverse conditions. Algal growth is affected by numerous physiochemical parameters, predominantly temperature, solar irradiance, CO_2, and cross-contamination. Lower algal biomass productivity in batch cultivation was achieved without the addition of external carbon supplementation. In contrast, feeding of flue gas at a concentration of 10% CO_2 v/v improved the dissolved medium carbon concentration, which enhanced the rate of CO_2 fixation. The semicontinuous method has been adopted to strengthen the performance of the system further. The highest biomass density of 0.42 g/L and a 3.5-fold improvement in areal productivity of 11.488 g/m^2 were achieved [88]. The effect of N:P ratios on the growth and CO_2 fixation of *Chlorella* sp. was evaluated in the bubble column PBR. The maximum biomass yield (3568 mg/L) was achieved at the N:P ratio of 15:1. The highest algae cell density of 105×10^6 cells/mL and sequestration of CO_2 at 28% was obtained after 92 h. This study demonstrated that organic and inorganic carbon could influence algal cultivation [89]. The marine algae *Nannochloropsis salina* has been reportedly used for CO_2 fixation and high lipid production. The optimum growth rate of *N. salina* was reported at the concentration of 6% CO_2, while some cells were found to grow well under the CO_2 concentration of 20%. Increasing the level of CO_2 caused acidification in the medium, which reduced the pigment and inhibited the cells' growth [90]. Conversely, CO_2 fixation and the production of specific lipids increased with the removal of O_2 from the inlet gas. Increasing the concentration of CO_2 from 25% to 100% caused inhibited cell growth entirely. These findings suggest that, in the future, a more efficient approach to algal biotechnology can be developed and applied for both CO_2 mitigation and biofuel production [90]. *Chlorella minutissima* cultivation in an indoor PBR for CO_2 absorption from the anaerobic digestion system has been reported. The intake of CO_2 by *C. minutissima* was found to be in the range of 75%–85% at a light intensity of 1296 μmol m^2/s and gas flows at 0.33 vvm [91].

The regeneration of different solvents used in the absorption of CO_2 is a significant challenge. A hybrid system of ammonia and microalgae for capturing CO_2 has been reported. The fixation of carbon over 85% was obtained with *Chlorella* sp. L166, L38, and UTEX1602 suggested that algae could be used in a chemical-biological hybrid system to capture CO_2. This study demonstrated that the new absorption-algae hybrid process could replace the conventional method for CO_2 absorption [92]. However, it was also found that slow diffusion of CO_2 in water for a short resident time could limit microalgae growth. Thus, polyethylene glycol (PEG) 200 enhanced the transfer of CO_2 from the gaseous phase to the liquid phase. The sequestration of CO_2 increased the growth of *Nannochloropsis oceanica* with the CO_2 bubbling of 15% vol. The maximum specific growth rate of *N. oceanica* (1.41/day) was achieved with 1 mmol of PEG 200 in the culture medium. The algae biomass increased by about 79% with increased TIC because of more CO_2 dissolution in the culture medium. Thus, as a CO_2 absorbent, PEG 200 can efficiently capture CO_2 from flue gas to grow algae [93]. A substantial amount of natural gases are generated in the process of oil extraction. The composition of natural gases generated during the process varied, but the most dominant ones were methane (CH_4) (80%–95%) and CO_2 [92].

Conversely, the release of both these gases is considered to be the primary cause of global warming and climate change. Therefore, it is necessary to convert natural gas into other

valuable products by the process of mitigation. A microbial consortium of algae and bacteria has been suggested for quick metabolization of the high levels of CO_2 and CH_4 and their transformation into value-added bioproducts. The consortium (algae and bacteria) isolated from mangroves can survive in 70% of CH_4 and 30% of CO_2 [94]. A novel airlift photosynthetic microbial fuel cell (AL-PMFC) with *C. vulgaris* has been reported for the biofixation of CO_2 and bioenergy. The maximum CO_2 fixation rate of 835.7 mg/L/day was achieved with AL-PMFC. The highest CO_2 fixation rate of 1292.8 mg/L/day, lipid productivity of 234.3 mg/L/day, and power density of 5.94 Wm^3 have been attained at the optimized *C. vulgaris* inoculum size, level of CO_2, and aeration rate. Thus, AL-PMFC can provide an attractive approach for CO_2 fixation and bioenergy production [95]. Cultivation of *N. oculata* in semibatch PBRs for the bioconversion of CO_2, wastewater treatment, and biomass production has been reported. The maximum growth rate of *N. oculata* with the productivity of 0.088 g/L/day was obtained at 18% of CO_2 and the optimal pH range of 5.5–6.5 [96].

4 Bioenergy from algae

With a growing population worldwide, bioenergy demands are increasing globally. Fossil fuels are reducing worldwide and are close to their exhaustion point due to high and unsustainable consumption and due to their nonrenewable nature. Therefore, biofuels are gaining much attention globally as an alternative to fossil fuels. Biofuels, including biodiesel and bioethanol, are now being produced commercially in several developed countries. Alternative biofuels can be produced from numerous renewable substrates such as food crops, crop or fruit wastes, woody parts of plants, garbage, and algae [97,98]. The key benefit of producing and using biofuels is that they are renewable and sustainable and can considerably reduce environmental pollution and global warming. A leading source of global warming is the emission of greenhouse gases, such as CO_2, generated due to the burning of fossil fuels. Approximately 29 gigatons of CO_2 are generated annually, and a total amount of 35.3 billion tons of it has been produced until now due only to the burning of fossil fuels [99]. Algal biofuels could be an alternative to fossil fuels as they have 10%–45% of O_2 and fewer sulfur emissions [100].

In contrast, petroleum-based fuels emit a high level of sulfur and do not have O_2. Biofuel does not create environmental pollution. Moreover, it is a readily available, sustainable, and reliable fuel produced from bioresources. Biofuel from algae is environment-friendly and nontoxic, and it is considered to be a strong product for fixing CO_2 worldwide. It is said that 1.83 kg of CO_2 can be fixed per kg of algae. Moreover, several algal species consume flue gases, such as SO_x and NO_x, together with CO_2 as nutrients [101]. CO_2 constitutes 50% of the dry weight of algae biomass. The assortment of algal biomass is vital for regulating biofuel production costs and optimizing the energy structure. The variety of algal biomass used in the generation of biofuels is directly associated with the emission of greenhouse gas and further with environmental and economic sustainability [102]. Algae are currently the most favored raw material for bioenergy, and they can cater to the growing demands for biofuels, food, feed, and valued compounds [98,99]. Several countries in Asia, Europe, and America are starting to produce bioenergy from algae commercially [103].

4.1 Biodiesel production

Most of the microalgal species are promising with regard to biodiesel production due to their high lipid content (50%–70%). The biomass of several algal species, including microalga *Botryococcus braunii*, can produce around 80% of oil, making biodiesel production appropriate [104–106]. Algae can yield up to 58,700 L/ha of oil, from which 121,104 L/ha biodiesel can be produced [101,107,108]. Alkaliphilic green alga *C. vulgaris* has been reported to be appropriate for biodiesel production. The highest productivity of biomass at 28–31 mg/L/day along with lipid content of 38% were achieved at a low light intensity (60–90 μmol/m^2/s). The main fractions of the fatty acids C16:0, C18:1, and C18:2 were observed after lipid transesterification. Biodiesel production from the same strain of algae can also be energy efficient [109]. Economical algal biodiesel production is directly associated with high operational, maintenance, harvesting, and conversion costs. Wastewater has been used as a cost-effective medium for culturing algae and biodiesel production by using magnetic nanocatalyst SO_4^{2-}/Fe_3O_4-Al_2O_3 [110]. The effect of storage temperature and time on the increase of the lipid composition of *Scenedesmus* sp. has been studied. This study found that the free fatty acid content in wet algal biomass increased from a trace to 62% when stored at 4°C. Preesterification and transesterification were used for a two-step catalytic conversion of algal oil having high free fatty acid content into biodiesel. A high conversion rate of triacylglycerols was obtained at a methanol-to-oil molar ratio of 12:1 during catalysis with 2% potassium hydroxide at 65°C for 30 min. According to Chinese National Standards, a biodiesel analysis confirmed the standard level after purification by bleaching earth [111]. Dairy farm wastewaters have been reported as potential resources for algae cultivation for biodiesel production. A consortium of native strains removes more than 98% of nutrients from the treated wastewater. Biomass production of 153.54 t ha/year and lipid content of 16.89% were achieved from the cultivated consortium in treated wastewater. Algal lipids of 72.70% obtained from the consortium can be converted into biodiesel [112]. A freshwater microalgae *C. zofingiensis* was cultivated in pilot-scale PBRs by using artificial wastewater as a medium for their growth and lipid and biodiesel production and treatment. The maximum removal rate of TN (92%) and TP (100%) was attained for a mixotrophic culture with acetic acid as a pH regulator. A high productivity of biomass (66.94 mg/L/day) and a specific growth rate of 0.260 mg/day were achieved at controlled pH. Higher productivity of lipid content (37.48 mg/L/day) was achieved at the optimal condition. A biodiesel yield of 19.44%, presenting a massive 16–18 carbon composition of FAME, was obtained. This study suggested that pH regulation with acetic acid is the most useful method for the growth of *Chlorella zofingiensis* in wastewater during winter for biodiesel production and the removal of nutrients [113]. Another study proposed a single-step subcritical methanol extraction (SCM) process for biodiesel production from *C. pyrenoidosa*. The highest yield of crude biodiesel (7.1 wt%) was achieved at 160°C in a 3-min reaction time along with an optimal (7 wt%) methanol ratio to algae. This study suggested that the SCM process does not require any pretreatment step or a catalyst making it economical and practical for large-scale biodiesel production from algae [114]. A study experimented with mono- or cocultivation of *C. vulgaris* and *Scenedesmus dimorphus* in media containing different sources of N for growth, lipid content, biodiesel production, and nutrient elimination. This study confirmed that algae cultivation in the media containing various sources of N indicated not only a high removal

efficiency but also increased biomass, lipid productivities, and biodiesel production. A trend of high lipid content was observed in the mixed culture (as against their mono-culturing) of *C. vulgaris* and *S. dimorphus* in the media that had the same N source. The main fatty acids of C16:0, C16:1, C18:0, C18:2, and C18:3, accounting for 79.6%–90.6% of the total, were achieved. Further, biodiesel production in the range of 8.5–11.2 g biodiesel/100 g dry weight was achieved, which demonstrates that the N source can affect the nutrient removal efficiency and biodiesel production of both mono- and mix-cultured microalgae [115].

Biodiesel production from algal oil via transesterification using various acids, base catalysts, and supercritical fluids has been reported. These catalysts are toxic and pose many challenges related to environmental contamination. A lipase-based enzyme extracted from a novel fungal strain *Cladosporium tenuissimum* with a molecular weight of ~46 kDa and specific activity of 37.2 U/mg has been reported for biodiesel production. A purified lipase as a biological catalyst was successfully used for transesterification of *Nitzschia punctata* oil into biodiesel. The highest conversion efficiency of 87.2% was achieved with lipase as biocatalyst, and it was 83.02% in the case of a conventional acid catalyst. Lipase has the potential for extensive scale applications to increase the transesterification process' conversion efficiency [116]. A quick algal growth rate, biofixation of CO_2, and no competition with food and high lipid content make them an appropriate feedstock for biodiesel production. Conversely, the high cost associated with dehydration, extraction, and biodiesel conversion can limit their application at the industrial level. Direct biodiesel production from pretreated wet algae *C. vulgaris* through esterification and transesterification has been reported. A high Fatty Acid Methyl Ester (FAME) yield of 80% was achieved with a small volume of methanol and catalyst (either HCL or NaOH) assisted by RF heating at 55°C for 20 min [117]. The ability of microalgal strain growth in various wastewaters to remove nutrients and accumulate lipid is demonstrated in Table 1.2.

TABLE 1.2 Removal of nutrient and lipid accumulation potential of different microalgae from wastewater.

Wastewater	Algae	COD (%)	N (%)	P (%)	Biomass productivity	Lipids content (%)	Comments	Ref.
Swine wastewater	*Tribonema* sp.	56.6	89.9	72.7	–	42.4	Wastewater was pretreated for the removal of color to increase light availability.	[118]
	Synechocystis sp.	68.6	75.8	71.4	–	26.3		[95]
	Tribonema sp.	52.2	100	68–74	2.04 g/L	55.4	The effluent was decolorized with a weak electric field and titanium dioxide (TiO_2) and pulse intense pulsed light (T-IPL).	[119]
	Tribonema sp.	55.6	89.9	72.7	–	42.4		[119]

TABLE 1.2 Removal of nutrient and lipid accumulation potential of different microalgae from wastewater—cont'd

Wastewater	Algae	COD (%)	N (%)	P (%)	Biomass productivity	Lipids content (%)	Comments	Ref.
Municipal wastewater	*Scenedesmus obliquus*	85.43	80.30	95.72	–	46.92	The lipid content was increased to 24% with N^+ ion implantation for mutagenesis.	[120]
	Scenedesmus sp.	90	90	79–88	4.65 g/L	–	Combined municipal wastewater and cattle manure digested effluent was used as a nutrients medium.	[121]
	Scenedesmus sp. HXY2	96	96.6	94.5	–	15.56	Algae were able to grow at high concentration of organic carbon and ammonia	[122]
	Chlorella minutissima	89.4	92	90	0.995 g/L/day	14	A stainless steel photo cavity reactor was used to stop the light scattering.	[123]
Palm mill effluent	*Chlorella sorokiniana* CY-1	93.7	98.6	96	5.74 g/L	14.43	A triangular photobioreactor was used to make algae harvesting easy by sedimentation.	[124]
	Chlorella vulgaris	53.7	55.6	77.3	2.04 g/L	16	Cocultured with *Pseudomonas* sp. to decolorize the effluent.	[124]
Textile wastewater	*Chlorella* sp. Wu G23	75	75	–	58 mg/L/day	16.6	High content of FAME was produced by culturing algae in the pH range of 9–11 under aeration.	[125]
	Anabaena ambigua	50	52.95	63.05	11.61 mg/L/day	–	Mostly studied algae have the potential to grow in 100% of textile wastewater	[126]
Pharmaceutical wastewater	*Tetraselmis indica* BDU 123	66.30	67	70	46.85 mg/L/day	16.40	Anaerobic treatment of wastewater was performed with MFC before aerobic treatment with algal	[127]

From S.K. Bhatia, S. Mehariya, R.K. Bhatia, M. Kumar, A. Pugazhendhi, M.K. Awasthi, A.E. Atabani, G. Kumar, W. Kim, S.O. Seo, Y.H. Yang, Wastewater based microalgal biorefinery for bioenergy production: progress and challenges, Sci. Total Environ. 751 (2021) *141599.*

4.2 Bioethanol production

Bioethanol is a significant and clean biofuel that can be utilized for transport. There are several benefits of bioethanol over fossil fuels. Bioethanol has a high octane number that can prevent cylinders from knocking in the engines, and it produces fewer greenhouse gases due to its high O_2 content. Bioethanol can be utilized directly in the present automotive industry without any alterations and blended with oil [128–130]. Biofuel production increased from 4.8 to 16 billion gallons from 2000 to 2007 [131]. Presently, the United States and Brazil account for about 75%–80% of the world's bioethanol production, and these countries are considered to be world leaders on this front [129,131]. The United States produces bioethanol commercially from corn grain in 187 plants [132]. In 2013, Brazil produced 37 billion liters of bioethanol with sugarcane as the primary feedstock. The European Union (EU) uses wheat and sugar beet as the ideal feedstock and has been producing 2 billion gallons of bioethanol annually [128,129]. The United States' Renewable Fuel Standard (RFS) is expected to achieve 36 billion gallons of biofuel from algae by 2022 [133]. Biofuels produced from renewable and sustainable feedstock will progressively become the future energy sources in place of liquid fossil fuels. Bioethanol production from corn and sugarcane sugars is now shifting to algal carbohydrates as potential feedstock [134,135]. Bioethanol production has recently improved from 1 to 39 billion gallons and is estimated to reach 100 billion gallons quickly [136]. Algae contain many diverse carbohydrates, such as glycogen, starch, agar, and cellulose, which can efficiently be converted into fermentable sugars for bioethanol production [137].

The production of bioethanol from algae is a unique struggle to develop sustainable biofuels. There are many challenges related to pilot-scale production for its commercial utilization as a clean biofuel. An assortment of suitable algal biomass, pretreatment methods, and an effective fermentation process need to be developed for bioethanol production for industrial applications. Bioethanol's fermentative yield significantly depends on the performance of potential microorganisms. The fermentation process needs to be carried out in sterile conditions to avoid contamination and increase the final production of bioethanol [138]. Algae's potential to be a suitable feedstock requires constant efforts to overcome the existing limitations regarding algal cultivation and the carbohydrate-rich biomass production from them in addition to their harvesting and pretreatment for the highest yield production. An economical algal culturing system can be established by developing and optimizing specific process parameters. Algal cell biomass and carbohydrate productivity must be improved for economically feasible bioethanol production [139]. Several carbohydrates-rich algae, such as *Chlamydomonas reinhardtii* and *C. vulgaris*, have been considered as potential options for a TEA of bioethanol production [140]. A TEA of commercial bioethanol production from algae has suggested the plant's suitability concerning total investment, cost, and net revenue [141].

Another study reported algae's economic feasibility for high bioethanol production via the fermentation process by considering numerous parameters. A critical factor, in this case, is to maximize the algal biomass production and reduce the operational and maintenance costs [142]. It is estimated that achieving algal biofuels' economic feasibility needs more than 10 years of research for developing a stable position [101]. Though algal biofuels are not yet economically practical, several companies in the United States, Europe, and other regions produce them on a commercial scale [101,143]. As per a TEA, biodiesel production from algae that costs less than $5/gallon is comparable to producing gasoline, while bioethanol

production at the cost of $2.95/gallon is financially feasible [144]. Several studies have proposed the production of biofuels from algae priced at $1/L to be practical and economical when compared to producing other fuels [11]. Many companies, such as Algenol, Sapphire Energy, and Seambiotic, are working on bioethanol's commercial-scale production with an output of 1 billion gallons/year that would cost 85 cents/L [145].

4.3 Biogas production

Algae biomass can be potentially used as raw material for biogas production. However, due to their cell walls, the interest in using algal biomass as an alternative biodegradable organic matter for biogas production has declined [146]. Cellulose or hemicellulose compounds in algal cell walls have high resistance to degradation under anaerobic digestion. Several algal strains produce toxic compounds that can harm anaerobic bacteria during digestion. An improper C:N ratio of biomass can also disturb the fermentation processes [147,148]. Nickel nanoparticles (Ni-NPs) were introduced to increase biogas production from the green algae *Enteromorpha* through anaerobic digestion. Some critical parameters, such as temperature in the range of 25–45°C, initial pH (5–9), and Ni-NPs in the concentration of 0.5–2 mg/L, were optimized in the batch mode. A high concentration of biogas yield of 346 mL was achieved at 1 mg/L of Ni-NPs compared to other concentrations and controls. This study confirmed that an initial concentration of Ni-NPs at 1 mg/L, a temperature of 35°C, and an initial pH of 7 had a better effect on the anaerobic digestion of green algae [149]. Several pretreatment methods for algal biomass degradation that require high energy and affect biogas production's overall efficiency have been reported [150]. Microwave [150] pretreatment of the biomass of algae *Enteromorpha* combined with metal nanoparticles (NPs) has been used for the algae's degradation. The highest total biogas production (54 mL/g-TS) was attained where Co-NPs plus MW pretreatment was used. In contrast, Ni-NPs plus MW pretreatment produced a maximum biohydrogen of 60% (v/v). Further, the energy analysis conducted during the study confirmed that the combined processes of MW pretreatment with metal NPs consumed less energy when compared to the MW pretreatment alone [151]. Anaerobic digestion of pure algae biomass, such as *Chlorophyta*, *Cyanoprokaryota*, and *Bacillariophyceae phylum*, has been used for biogas production under controlled conditions. An average biogas production yield of 396 dm^3/kg at the reaction rate of $r = 54.3$ cm^3/day was achieved from *Chlorophyta*. The CH_4 content in the biogas accounted for 59.7%. In contrast, a biogas yield of 382.5 dm^3/kg, biogas rate of $r = 97$ cm^3/day, and 63.1% of CH_4 were achieved from *Cyanoprokaryota*. On the other hand, a low concentration of biogas production of 357 dm^3/kg, a mean rate of biogas at $r = 51$ cm^3/day, and 58% of CH_4 were achieved when *Bacillariophyceae* was used as the feedstock. This study concluded that low biogas production with *Bacillariophyceae* algae was due to the latter's complex cell wall composition [152].

Adding algal biomass to a substrate mixture increases the C:N ratio for anaerobic digestion [153]. Yen and Brune [154] observed substantial biomethane production due to cellulose wastes' codigestion with algal biomass. Compared to the fermentation process solely using algae, the CH_4 production increased from 0.57 to 1.17 dm^3/m^3 day after adjusting the ratio of organic wastes to algal biomass (1:1). Vergara-Fernández [155] examined the anaerobic digestion of algal biomass *Macrocystis pyrifera* and *Durvillaea antarctica* with other substrates. The highest biogas production yield of 180.4 dm^3/kg d.m.d. was achieved when all the substrates

were used. In comparison, the biogas production was low (158.3 dm^3/kg d.m.d.) when the algae mixture was used separately. A total of 60%–70% of CH_4 was obtained. Yuan et al. [156] have demonstrated that CH_4 production from anaerobic digestion of blue-green algae (189.9 dm^3 CH_4/kg) was achieved.

Moreover, Zeng et al. [157] have analyzed biomethane production from algal biomass with a dominant *Macrocystis* sp. during the process of its codigestion with bovine liquid manure. A high yield of biomethane production (153.7 dm^3 CH_4/kg) was reported. Vergara-Fernández [155] reported a high concentration of biogas (16 mg/g d.m.) from *M. pyrifera* and *D. antarctica* (17 mg/g d.m.) with NH_3 due to the existence of a high level of N in the marine algae. Codigestion of the *Taihu* algae having a high carbon content substrate can balance the nutrients and ensure a smooth anaerobic digestion process. The highest biogas yield (389 mL/g-TS) was achieved at C:N ratio of 15:1 of algae and kitchen wastes. The codigestion of the *Taihu* algae and kitchen wastes increased the biogas production at a possible C:N ratio of 15:1 [158]. The wet biomass of *C. vulgaris* and hydrochar (HC) was reported to have increased the biogas production through the co-Hydrothermal gasification (co-HTG) process. The effects of the mixing ratio and HC quality on the biogas yield and composition were also reported. The total co-HTG gas yield increased from 19 to 47 mol/kg at a temperature of 65°C and a pressure of 300 bars using 5 wt% HC blending concentration, and it also reduced the volatile matter (24.6 wt%). The high yields of hydrogen (19.49 mol/kg) and CH_4 (2.98 mol/kg) and the carbon conversion ratio of 82.31% indicated a significant hydrothermal-upgrading potential in the case of wet and waste biomass feedstocks [159]. The green algae *Ulva rigida* has been reported as a good source for the production of biomethane. The highest output of biogas (408 mL) was achieved at the optimal inoculum by mixing decomposed algae with anaerobic sludge and water. A biogas yield of 375 mL containing 40% CH_4 was achieved in an SBR. Further, a high biogas yield of 114 mL g-VS added with 75% biomethane was achieved with the codigestion of algae with sugar wastewater in an anaerobic upflow bioreactor. In this study, the codigestion allowed the recovery of natural biomethane and provided a promising alternative to conventional anaerobic microbial fermentation using the *Tunisian* algae [160]. Anaerobic co-digestion of the freshwater microalgae *Chlorella* sp. with oil palm empty fruit bunches (OPEFB) has been used for palm oil mill effluent (POME) treatment and biomethane production. The maximum specific biogas production rate of 0.128–0.129 m^3/kg COD/day was reported [161]. Another study reported the maximum specific biogas production rate of 1.13–1.14 m^3 kg/COD/day with the cocultivation of the marine algae *N. oculata* and POME. Without the addition of *N. oculata* and OPEFB cocultivation, a 1.3-fold lower yield of biomethane was reported, while the specific biogas production rate remained constant at 1.13–1.16 m^3 kg/COD/day [162,163].

5 Biomedical applications

5.1 Antioxidant activity

Antioxidants are essential substances for the protection of the human body from the harmful properties of free radicals. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) [164] attack biomolecules such as DNA, proteins, and membrane lipids. Disturbances in these biomolecules can lead to numerous critical diseases such as cancer, coronary artery

disease, obesity, diabetes, ischemic stroke, and Alzheimer's disease [165]. Free radicals can cause lipid peroxidation and biological membranes, thus decreasing food materials' shelf life and nutritional value. Antioxidants help in preventing the oxidative destruction of free radicals and their effect on cells and tissues. The human body has a self-enzymatic antioxidant system that can stop this oxidative development and defend itself from the free radicals' harmful properties.

Conversely, when the body's natural antioxidants do not defend it from the effects of free radicals, it results in oxidative stress, which can cause several hazardous and lethal diseases. In such circumstances, the intake of antioxidants is highly essential. Several natural antioxidant compounds, such as flavonoids, carotenoids, vitamins, and tocopherols, have been reported. Numerous synthetic antioxidants that prevent the oxidation and peroxidation processes have not reported any side effects [166,167]. This has led to a greater focus on investigating the natural antioxidants for nutraceuticals and in pharmaceutical industries. Scientists are working on extracting antioxidants from natural resources. Some medicinal plants that have shown massive prospects in terms of their natural and biologically active production of antioxidants are also being duly researched. Algae have been reported to be the richest and cost-effective feedstock for extracting natural compounds having potent antioxidant properties [168]. The antioxidant properties that have been identified for these compounds include BTS, DPPH-free radicals scavenging assay, ferric reducing potential, and metal chelating assay.

The structural features responsible for these antioxidant qualities include a phytyl chain, a porphyrin ring, and conjugated double bonds [169]. Microalgae species produce chlorophyll, and their metabolites have been reported to have antioxidant activities [170,171]. Microalga *U. pinnatifida*'s pigment fucoxanthin and its derivatives, such as auroxanthin, have intense radical scavenging action [172]. The effect of fucoxanthin on rat liver and plasma suggests that it has high antioxidant properties compared to the β-Carotene. Fucoxanthin contains two hydroxyl groups in a ring structure, which reflects its active moiety against free radical scavenging [173,174]. Phycobiliproteins containing antioxidants, such as C-phycocyanin and R-phycoerythrin, derived from algae, also have intense antioxidant activity and are commercially used in the food and cosmetic industries as natural dyes [175,176]. Each of these great natural products having high antioxidant activity has raised algae's economic and dietary-use potential as far as the food, pharmaceutical, and nutraceutical industries are concerned.

5.2 Anticancer, antiangiogenic, and cytotoxic activities

Angiogenesis is the biological course in which new blood vessels are generated from existing blood vessels. This process occurs faster during processes such as uterus development, embryogenesis, and healing of wounds. Angiogenesis can break the contact between the cells and abolish the endothelium and extracellular matrix. Proliferation and relocation of endothelial cells and formation of capillary tubes occur during this process [177]. However, this typical process might become uncontrolled in some instances of cancer, atherosclerosis, arthritis, diabetic retinopathy, and ischemic stroke [178]. Pathologic angiogenesis stimulates tumors and supports their propagation [179]. Hence, angiogenesis is one of the leading causes of tumor growth and cancer proliferation. Some activators and inhibitors regulate the

angiogenesis processes. The main antigenic factors and proteins are VEGF, PDGF, angiopoietin-1, angiopoietin-2, platelet-derived growth factor, interleukin-8, bFGF, and angiotensin-II [180,181].

Several studies have recommended the use of natural products produced from microalgae that prevent the angiogenesis process to treat cancers and tumors. Fucoxanthin provided by several algal species can inhibit human blood cell production and human umbilical vein endothelial cells (HUVECs) tube formation. Fucoxanthin and fucoxanthinol have been reported to inhibit the angiogenesis process in the aortic ring of rats by destroying microvessels' growth [182]. Many algal species can produce siphonaxanthin, which also has antiangiogenic properties [183]. Another study reported fucoxanthin's therapeutic effects for diabetes as the former induced the synthesis of arachidonic acid and DHA content in mouse livers [184]. Furthermore, fucoxanthin has also been reported to protect DNA from photooxidation [185]. Aerucyclamide isolated from the *Microcystis aeruginosa* algae has been used as a pharmaceutical product for an antiplasmodial agent [186]. Blue-green algae have gained much attention in this regard and are now being considered as potential sources of bioactive materials for cancer treatment. Several studies have reported the bioactive compounds that can be extracted from these algae, and these compounds can be beneficial in terms of their anticancer properties. Anticancer agents derived from many algae can induce apoptosis in tumorous cells by abolishing the chromatin network that leads to cell death [187,188]. Cyanobacteria can yield different metabolites, including peptides or alkaloids, through the ribosomal or nonribosomal pathway [67]. Peptides, comprising but not limited to cyanobacteria, tend to be toxic substances having intense cytotoxic activity. The cytotoxicity of these compounds is essential for inducing apoptosis that leads to cell death [189].

Apoptosis induces compounds that can be produced from several species of cyanobacteria. Their definite morphology recognizes apoptotic cells that usually contain abundant cytoplasm and compacted organelles with chromatin materials' variations. Compounds from *Synechocystis* sp. and *Synechococcus* sp. have been shown to drive the HL-60 cells into apoptotic conditions. After being treated with algae-derived compounds, these cells expressed apoptotic markers such as fragmentation of the nucleus, shrinkage of cells, and discharge of apoptotic substances [188]. Correspondingly, *Lyngbya* sp. produces glycoside biselyngbyaside that drives the mature osteoclasts into apoptotic conditions [190]. Extracts of *Anabaena* sp. have also induced apoptosis in a leukemia myeloid cell line [191]. Several *Nostoc* species reported cryptophytes' production and demonstrated a positive effect on human colorectal cancer cells [192]. An extract from *Oscillatoria boryana* has been actively used against human breast cancer cells [193]. Bioproducts from *Microcystis* sp. can be potentially used in the fields of toxicology and pharmacology. Several biotechnological and toxicological applications of bioactive compounds from these species, including their anticancer activity, have been reported [194].

5.3 Antiobesity activity

Overaccumulation of adipose tissues in the body can cause obesity [192,193]. Several metabolic disorders can cause certain complications and diseases including cancer, cardiovascular disease, diabetes mellitus, and age-related ailments [195]. Adipogenesis causes abnormal

growth of the adipose tissue. The aforementioned complications/diseases can be controlled by protecting our body's cells against adipogenesis [196]. Several antihyperlipidemic and fat-reducing agents from natural sources, including from medicinal plants, have been reported. An antiobesity compound extracted from algae is now considered to be a potential source of these agents that have health benefits. Many researchers have also reported obesity progression due to ROS and NOS. Hence, obesity caused due to free radicals- can also be controlled by using antioxidants. Fucoxanthin and fucoxanthinol from algae can inhibit the differentiation of 3T3-L1 cells to adipocytes and stop adipocyte differentiation by downregulating peroxisome proliferator-activated receptor-c [197].

Neoxanthin and fucoxanthin have been reported for the inhibition of fat accumulation [198]. Adiposity in obese mice that were administered fucoxanthin was suppressed by activating the mitochondrial protein uncoupling protein1 [199]. Further, fucoxanthin was also reported in a clinical trial to help with considerable fat reduction in obese individuals [200]. Algal species, such as *Cylindrotheca closterium* and *Phaeodactylum tricornutum*, have been reported to be useful for fucoxanthin production. As such, they have shown their potential to be a good source of anticancer, antioxidant, antiobesity, antidiabetic, and antiinflammatory agents [201].

5.4 Antimicrobial activities

Bacteria and fungi can cause severe infections in plants, animals, and humans. Microbes can decrease crop yields and can cause significant food spoilage. In the last few years, various antibiotics have been developed to increase the resistance against microbes. The search for suitable antimicrobial agents is no less relevant. Not only have synthetic antibiotics reported unsatisfactory results vis-à-vis disease control but they are also known to have side effects. Further, they have also been reported to be expensive even as they carry the risk of generating severe resistance to pathogenic strains. Hence, scientists have been looking for natural and novel antibiotics derived from plants and microorganisms that would have relatively fewer or no side effects.

Natural bioactive products derived from microalgae are useful in both crude and purified forms. Antibacterial products from algae have been expressly used to inhibit gram-positive and gram-negative bacterial growth [202]. Several algal species can also yield compounds that have antifungal properties [203]. *Prorocentrum lima* and *Gambierdiscus toxicus* can produce okadaic acid and ciguatoxin, which can be used as active antifungal agents. Lipid metabolites produced by *Chaetoceros lauderi* can effectively prevent the growth of some bacterial strains. *M. aeruginosa* is a rich source of some toxic metabolites that have strong cytotoxic and antimicrobial properties. The crude extract of *M. aeruginosa* displays high antifungal and antibacterial activity. The bioactive compounds from *Dunaliella salina* can be actively used against numerous bacterial and fungal strains such as *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, and *Aspergillus niger* [204]. A compound produced from *D. salina* has considerably inhibited the growth of *Klebsiella pneumoniae*. *Dunaliella primolecta* substances too have exhibited antibacterial activity against *S. aureus* and other microbial strains [205]. Table 1.3 displays bioactive compounds from algal and their biomedical applications.

TABLE 1.3 Bioactive compounds from algal and their biomedical applications.

Compounds	Algae species	Biomedical applications	Ref.
Fucoxanthins	Brown algae *Myagropsis myagroides*	antidiabetic Antiangiogenic, Antiinflammatory	[206]
Fucosterols	*Ulva* sp., *P. siliquosa*, *U. pinnatifida*, *H. elongata*, *Porphyra* sp., *S. vulgare*, C. crispus, *Cystoseira* sp.	Anticancer, inhibition of cholesterol absorption, antiulcerative, antifungicidal, antidiabetic	[207]
Terpenes	*Sargassum* sp., *Bifurcaria* sp., *Dictyota* sp.	Antiviral antioxidant	[206]
Terpenoids Alkaloids, Phenolic compounds	*P. fragile*, *C. vulgaris Anabaena flos-aquae*, *N. humifusum*, *N. muscorum*, *S. platensis*, *A. oryzae*, *Oscillatoria* sp.	Anticancer, antioxidant	[208]
β-Carotene	*Haematococcus* sp., *D. salina*	Anticancer, antiinflammatory, antioxidant	[209]
Stigmasterol, Brassicasterol	*P. lutheri*, *I. galbana*, *Skeletonema*, *Chaetoceros*	Hypocholesterolemic activity	[210]

6 Conclusion and future outlook

This chapter has highlighted the broad applications for algae in diverse fields such as bioenergy and bioremediation and the pharmaceutical industry as well. This chapter also covers the challenges related to the increased and growing applications of algae and their extracts along with the barriers and solutions to make their use feasible for commercialization.

Microalgae are like tiny factories that produce renewable, sustainable, and economical biofuels, bioactive compounds, and food ingredients. Algae are valuable in the sequestration of CO_2 from the atmosphere and for industrial wastewater treatment. The cosmeceutical industry is now a profitable and continuously growing segment of the world economy. Consumer interest in natural products instead of synthetic products has been on the rise. Algae are also useful in producing bioactive compounds with antioxidant, antiinflammation, and antiallergic properties and those compounds that act as tyrosinase and hyaluronidase inhibitors.

Given the immense potential of algae/microalgae as a sustainable source of bioenergy, further efforts are needed to reduce the costs of environment-friendly algae-production processes so that these processes can be effectively commercialized to produce biofuel that requires large quantities of algae feedstock. Developing an in-depth understanding of microalgae physiology and biochemistry can open new research avenues for exploring the potential ways in which algae can be cultivated on a large scale for commercial applications.

Several researchers have opined that assessing nanotechnology's applicability to a microalgae biorefinery's various processes and its economic performance is essential to prevent the biorefinery's adverse environmental impact. Integrated nanotechnology with microalgae biorefinery techniques may provide a promising solution for enhancing microalgae-based products' economic productivity. Therefore, this combination needs to be explored, developed, and consistently applied in practical settings to achieve a new generation of biofuels and high-value microalgae products.

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CHAPTER

2

Microalgae biotechnology for bioremediation applications

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OUTLINE

1 Introduction

The increase in the global population and rapid development have caused a more significant environmental problem worldwide. Major ecological pollution, such as air pollution, land and water pollution, has contributed to the imbalance of the ecosystem and global warming. Several toxicities of various chemicals and pollutants, such as heavy metals, nuclear waste, chemical

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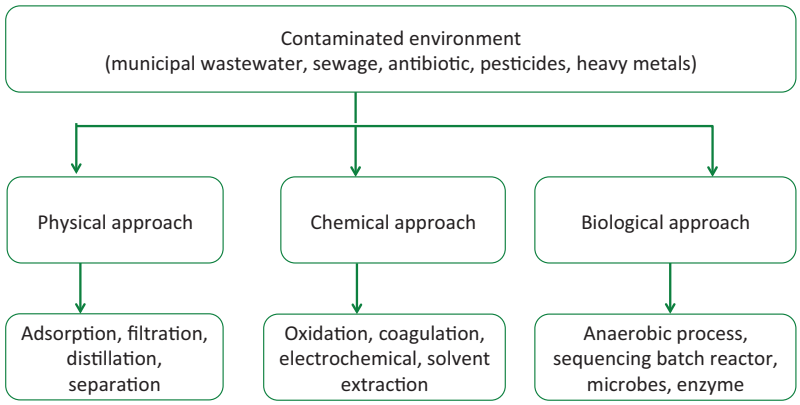

FIG. 2.1 Common remediation strategies to reduce contamination from the environment.

fertilizer, pesticides, hydrocarbons, and pharmaceutical by-products, can contribute to pollution. This main problem has caused considerable damage to the ecosystem. Therefore, it has become a serious challenge for the scientific community, requiring a comprehensive state of knowledge and technology to overcome this issue.

Several physicochemical and biological treatments have been introduced to remove or remediate contaminants and pollutants from the contaminated environment (Fig. 2.1). Remediation using a physicochemical approach such as membrane filtration, ion exchange, electrochemical treatment, osmosis, precipitation, and evaporation are among the technologies that have been employed. Nonetheless, the majority of these technologies are neither economically feasible nor environmentally friendly [1–3]. In addition, most of the technologies mentioned are expensive and energy-intensive.

Biological remediation, or bioremediation, is considered one of the most promising bioremediation technologies because it is cheap, green, environmentally friendly, and sustainable. Biological remediation, or bioremediation, is defined as a process that utilizes microorganisms to detoxify or reduce the presence of either organic or inorganic contaminant compounds in the environment. Different types of microorganisms such as bacteria, fungi, and microalgae are currently used in this process [4–6]. Generally, bioremediation involves complex mechanisms that require specific enzymes to remediate specific contaminants. Bioremediation can occur via two main mechanisms: biosorption and bioaccumulation (Fig. 2.2). The active process or bioaccumulation of chemicals, involves living organisms that are capable of taking up the compound across their cell walls. Some of the compounds need to be metabolized by microbial enzymes before accumulation into the microbe cell. On the other hand, the passive process of biosorption involves physicochemical mechanisms performed by either living or dead microbial cells.

Although there are many studies on the bioremediation of contaminated environments by several types of microorganisms, microalgal-based bioremediation technology has gained great attention as a promising technology due to its advantages. Bioremediation using microalgae not only remove pollution but the biomass produced from the process can be further converted into other value-added products. Therefore, this approach is considered the most promising approach for sustainable remediation, and it can also create a better circular economy. This chapter presents the role played by microalgae in the bioremediation of

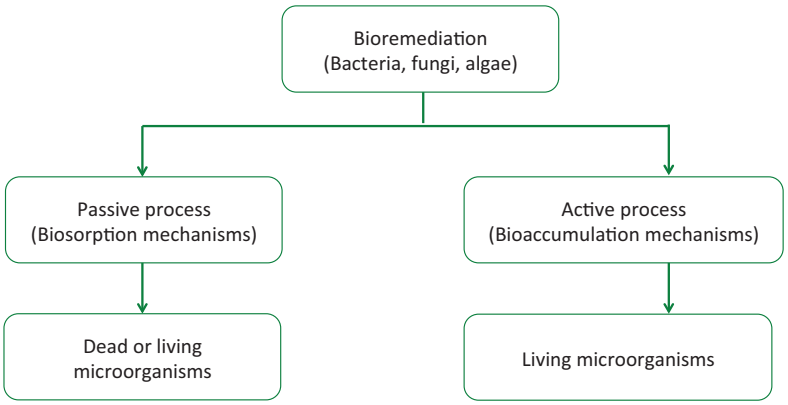

FIG. 2.2 Bioremediation mechanisms process by different microorganisms.

different contaminants. In addition, the chapter also discusses integrated bioremediation technology, carbon fixation, and microalgal biorefinery, which is considered a promising low-cost technology for a sustainable environment.

2 Microalgae

Microalgae is one of the microorganisms that has been proven to have the potential to be used in the bioremediation process. Microalgae are microorganisms that can be abundantly available in the environment, such as soil surfaces and water bodies, including fresh and marine water. These microorganisms can be classified into several groups, such as Bacillariophyceae, Chlorophyceae, Eustigmatophyceae, Prymnesiophyceae, Chrysophyceae, Cyanophyceae, and Euglenophyceae (Table 2.1) [7].

3 Bioremediation using microalgae

The cultivation of microalgae requires a huge volume of water and nutrients to support their growth. To date, the utilization of contaminated water as a medium for mass-scale microalgae cultivation has gained great attention as an alternative approach to reduce the cost of water

TABLE 2.1 Main class and microalgae species.

Group	List of strain
Bacillariophyceae	*Cyclotella*, *Rutilaria*, *Isthmia*, *Corethron*, *Navicula*, *Pinnularia*
Chlorophyceae	*Chlamydomonas*, *Volvox*, *Prasinocladus*, *Cladophora*, *Ulva*, *Pediastrum*, *Spirogyra*
Eustigmatophyceae	*Nannochloropsis* sp., *Eustigmatos*, *Pseudostaurastrum*
Prymnesiophyceae	*Pavlova lutheri* and *Isochrysis* sp.
Chrysophyceae	*Ochromonas* sp. and *Chrysosaccus*
Euglenophyceae	*Euglena spirogyra*

supply. Generally, the contaminated water or effluent generated by industries contains a high amount of carbon and nitrogen, which is believed to accelerate microalgae growth. On the other hand, the current approach to integrated carbon emission release of flue gas supplied and effluent into the cultivation system has proven to be a promising approach to act as a double-action for biomass and pollution remediation approaches.

4 Industrial wastewater

Wastewater generated by commercial industries is one of the most common by-products, and major pollution is discharged into the water stream. Most of the wastewater generated from commercial manufacture or production consists heavily of a wide range of pollutants, including minerals, volatile organic compounds, oil and grease, heavy metals, and pesticides, depending on the nature of the industries. In addition, the effluent or wastewater from industry or sewage is nutrient-rich waste (nitrogen and phosphorus) and the accumulation of this pollutant or contaminant will cause a major problem for the environment and affect both fresh water and sea-life [8,9]. Thus, advanced wastewater treatment is required to overcome this problem. Bioremediation using microalgal has been reported to have great potential to be applied as an alternative approach to treat the effluent generated by industries (Table 2.2). A wide number of microalgae have been used to treat wastewater, such as *Chlorella* sp. [17], *Scenedesmus* sp., *Nannochloropsis* sp. [11], *Chlamydomonas* sp. [18], and *Dunaliella salina* [19,20].

Most studies indicate that using wastewater as a cultivation medium for microalgae cultivation could accelerate microalgal biomass production and remove chemicals, including nitrate and phosphate [21,22]. The presence of nutrients in the wastewater provides nutrients for microalgae to grow and accumulate their biochemical components in their cells. The study indicated that the cultivation of microalgae using wastewater could reduce COD levels by up to 90%. According to Ibrahim et al. [23], COD value in the agriculture drain was reduced by 51.1% when the cultivation was conducted using *Chlorella* sp. Another study indicated that approximately 90% of COD in dairy farm wastewater was reduced when the treatment was conducted using microalgae [24]. The study also found that higher maximum nutrient removal was observed for the cultivation using diluted wastewater. A similar trend has also

TABLE 2.2 Bioremediation of different wastewater sources by different microalgae species.

Wastewater	Type of microalgae	COD reduction	Total nitrogen	References
Palm oil mill effluent	*Chlorella sorokiniana* C212	45	–	[10]
Palm oil mill effluent	*Nannochloropsis* sp.	93%	78%	[11]
Dairy wastewater	*Chlorella* sp.	75	95	[12]
Textile wastewater	*Chlorella* sp.	74	–	[13]
Municipal wastewater	*Scenedesmus obliquus*	–	97	[14]
Petrochemical wastewater	*Chlorella* sp.	38	51	[15]
Agriculture wastewater	*Cladophora* sp.	–	87	[16]

been reported for the bioremediation of raw blackwater, and winery wastewater by *Chlorella vulgaris* exhibited significant COD removal of up to 92% [25].

Bioremediation of wastewater using microalgae can be performed using free or immobilized cells. It has been reported that bioremediation using immobilized microalgal cells poses many advantages over free microalgal cells. For instance, this technique requires less space and ease during the harvesting process. A study on the dye decolorization of immobilized *Desmodesmus* sp. indicated that this microalgal is able to reduce methylene blue and malachite green up to 98% within 6 days of cultivation [26]. In addition, a study by Kassim et al. [27] on the decolorization of textile wastewater and nitrogen removal showed that 80% and 71% of the nitrogen was removed when the treatment was carried out at pH 12, lux intensity of 1000 lx using 150 microalgal beads. The potential of utilizing immobilized microalgae has also been reported in the bioremediation of wastewater using immobilized *Tetraselmis* sp. and *Chlorella* sp. Wu-G23 [28,29].

5 Antibiotic and hormone

It is estimated that there will be a significant increase in antibiotic consumption of up to 65% from 21.1 to 34.8 billion defined daily doses (DDD) in 2030 [30]. Generally, antibiotics are commonly used to prevent and treat infections caused by microorganism and are used in various industries, including pharmaceuticals and agriculture. Antibiotics such as penicillin, cephalosporin, quinolones, sulfonamides, and macrolides are among the common antibiotics that have been widely used globally [31]. Antibiotics such as penicillin, cephalosporin, quinolones, sulfonamides, and macrolides are among the most commonly used antibiotics worldwide. However, only a small fraction of the antibiotic is absorbed, and approximately 90% of the antibiotic is secreted either in urine or in feces [32]. Continuous overuse and release of the antibiotic fraction into the surrounding environment will eventually cause serious health problems, particularly the development of high-resistant antibiotic microorganisms.

To date, several techniques, such as chemical, physical, and biological treatments, have been introduced to remediate antibiotics from the contaminated environment [33,34]. Among these techniques, the biological treatment approach plays an important role in removing antibiotics from the antibiotic contaminated environment because this technique is cheaper and environmentally friendly. Currently, microalgal-based technology has gained great attention for reducing antibiotic fraction in the environment [35–37]. Antibiotic removal mechanisms are complex reactions and can be removed by microalgae via several main approaches, such as (1) adsorption, (2) accumulation, (3) biodegradation, (4) photodegradation, and (5) hydrolysis [14,38]. The bioadsorption process occurs when the antibiotics are absorbed into the microalgae cells or attached to the extracellular polymeric substance (EPS). On the other hand, bioaccumulation is an active metabolic mechanism in which antibiotics are transported into the microalgal cell membrane and bind to the intracellular protein. In contrast, biodegradation of antibiotics involves the breakdown of the antibiotic structure and transforming it into a small intermediate with the presence of an enzyme before transport into the microalgal cell. A previous study also reported that the biodegradation of antibiotics could occur extracellularly with the help of EPS released by the microalgal cell.

Many researchers have reported antibiotic removal by various microalgal strains. A study by Guo et al. [35] compared the removal of cephalosporin 7-ACA by three different

microalgae, *Chlorella* sp. Cha-01, *Chlamydomonas* sp. Tai-03, and *Myxhonasters* sp. YL-02, and found that the removal capability of microalgae is species dependent. The study found that the microalgal exhibited high removal and was tolerant to a high concentration of cephalosporin. The difference could be attributed to several factors, such as cell size, cell characteristic properties, and the specific surface area of the microalgae. Similarly, an investigation into antibiotic removals such as amoxicillin and cefradine by two microalgal strains, *Chlorella pyrenoidosa* and *Microcystis aeruginosa*, was conducted [39]. The study reported that removal of these antibiotics is species-dependent and that higher cefradine was observed when the treatment was conducted using *C. pyrenoidosa*, while higher amoxicillin was obtained for treatment using *M. aeruginosa*.

Excellent removal of amoxicillin was also observed when the bioremediation process was conducted using *Chlorella* sp. in batch and continuous systems [36]. Another study by Mojiri et al. [40] on removing different pharmaceutical products, including carbamazepine, sulfamethazine, and tramadol by *Chaetoceros muelleri*, indicated that approximately 64%–69% of the chemicals had been removed by this microalgal when the treatment was carried out using an integrated biochar and cultivation system. Other antibiotics and hormone removal by different types of microalgae such as *Chlamydomonas mexicana*, *Nannochloropsis oculata*, *Phaeocystis globosa*, *D. salina*, *Scenedesmus obliquus*, and *Platymonas subcordiformis* have also been reported [41,42].

6 Heavy metal

Another main chemical pollutant that contributes to this serious environmental issue is heavy metal pollution. It is known that some heavy metals can be beneficial to humans and plants, such as Cu, Ni, Mn, Co, and Zn [43]. But, few other heavy metals have been identified to cause toxicity to human beings. These chemicals can be classified into three main groups: precious metals (Au, Pd, Pt, and Ru), radionuclides (U, Ra, Am, and Th), or toxic metals (Zn, Ni, Ag, Cu, Cr, As, Sn, Co, and Pb) [44]. Continuous and prolonged exposure of animals to these heavy metals will cause serious and deleterious health issues, especially for their body systems, such as the kidneys, liver, and brain.

Many heavy metal treatment technologies, including physical and mechanical approaches, have been introduced to remove heavy metals from water bodies. However, another alternative method is using a cost-effective and eco-friendly biological technique, which has also been introduced to treat the contaminated environment [45]. Bioremediation of metal contaminated by microalgae is considered a flexible and sustainable technique as the biomass produced during the process can be further converted into various products. On the other hand, the wide distribution of robust microalgae in the environment makes these organisms easily adapt to environmental conditions.

Generally, the capability of bioremediation of metal-contaminated environments by microalgae is species dependent. The metal interaction with the microalgal depends on the surrounding environment, enabling the algae to develop their extracellular or intracellular defense mechanisms. The microalgal cell contains a polyanionic cell wall structure that can bind heavy metals through the principle of adsorption (passive) onto the cell surface and absorption (active) into the cell with metal-binding peptides or contained in the vacuole.

TABLE 2.3 Heavy metal removal by different microalgae species.

Microalgae	Heavy metals	Removal (%)	References
Scenedesmus acutus	Cd, Zn, Cr	66, 85, 48	[46]
Chlorella vulgaris	Cd, Zn, Cr	57, 78, 34	[46]
C. vulgaris	Nickel	64	[47]
Nannochloropsis oculata	Lead (Pb)	55	[48]
Chlamydomonas reinhardtii	Copper	55	[49]
	Lead	45	[49]
Chlamydomonas reinhardtii	Pb	23.4	[50]

Table 2.3 shows the different types of microalgae that were previously reported to have the capability to remediate the metal-contaminated environment.

Apart from the microalgal strain, the efficiency of bioremediation of metal-contaminated environments can be affected by other factors, including pH, temperature, initial metal concentration, and contact time. The effects of pH and temperature are among the important factors influencing the efficiency of metal contaminated remediation. The pH value is important because it affects the solubility and toxicity of the metals in the water. Furthermore, changes in surrounding pH could affect the carboxyl group in the microalgal cell wall, influencing heavy metal biosorption performance by microalgae. A study by Kaparapu and Krishna Prasad [51] investigated cadmium biosorption using different pH ranges from 2 to 5, and indicated that high cadmium removal of 48% was achieved when the treatment was performed at pH 5. Low cadmium removal by *N. oculata* was observed when the treatment was performed at a higher pH value. A similar observation was reported on the influence of pH on heavy removal by *Oocysis* sp. and *Chlorococcum* sp. which indicated that higher removal of Cd^{2+} and Cu^{2+} was achieved when the investigation was conducted under an acidic pH value range [52]. Furthermore, a study by Han et al. [53] showed that higher biosorption of Cr (III) was obtained for the cultivation in the pH range of 2–4.5. Cultivation beyond the optimal pH range exhibited a significant reduction in Cr (III) removal. High heavy metal removal observed at low pH values can be attributed to several factors, such as changes in the microalgal cell wall charge surface and the heavy metal ion solubility in the surrounding liquid. Negative charges on the cell wall surface at acidic pH enhance electrostatic interaction between the cell surface and heavy metal cationic species, resulting in a higher metal biosorption process. On the other hand, most studies also suggest that the influence of heavy metal removal efficiency is species-dependent.

Temperature is one of the parameters that play a crucial role in heavy metal removal by microalgae. Changes in temperature beyond the optimal value could affect microalgae physiology and enzymatic activity involved in biosorption by the microalgae. In addition, the temperature also affects the metal ion solubility, where a higher temperature provides better surroundings for greater solubility of ions metals, resulting in lower biosorption of metal ions [54]. Several investigations into the influence of temperature on heavy metal removal by different microalgal strains have been reported. For instance, Lieswito et al. [55] indicated that a

maximum Cu^{2+} of 96.2% was achieved when the cultivation was conducted using a mixed microalgae culture of *Chlorella sorokiniana*, *Monoraphidium* sp., and *S. obliquus* at 35°C. On the other hand, cultivation of the microalgae beyond 35°C was found to reduce the Cu^{2+} removal. Another study on heavy metals such as Pb, Co, Cr, Cd, and Cu removal by *Chlorella kessleri* indicated that temperature value exhibited a significant effect on heavy metal removal and suggested that treatment at a lower temperature is favorable at a lower temperature [56]. According to this analysis, it is indicated that a lower temperature is more suitable to be applied for the heavy metal bioremediation process.

Contact time is one of the parameters that influences heavy metal removal from the contaminated environment. The removal of heavy metals by microalgae can occur in two stages. Firstly, the metal ion is passively absorbed into the microalgal cell and occurs within a few minutes. Later, passive biosorption occurs slowly until it reaches equilibrium. Continuous exposure for a longer contact time could increase the biosorption and lead to a relatively higher removal capacity. Several studies have been conducted on the influence of contact time on the removal of heavy metals by microalgae [57–59]. For example, a study by Kumar et al. [60] indicated that the biosorption of Hg (II) by *C. vulgaris* UTEX 2714 increased at the early stage of the experiment and started to be constant after 2 hours of treatment. A similar trend was also reported for heavy metal ions such as Cu^{2+}, Ni^{2+}, and Cd^{2+} removal from drinking water by immobilized *C. sorokiniana*, which found that the higher removal rate was observed within 2 hours of treatment and the biosorption rate started to relatively become constant afterward [61]. This phenomenon can be explained by the fact that continuous exposure of microalgae to heavy metal ions will affect pore diffusion. In addition, the optimum contact time for heavy metal removal can be varied according to the variation of functional cluster groups, cell surface properties, and pore volume.

7 Pesticide

The rapid increase in the human population and the development of the agriculture industry have led to the overuse of pesticides to control crop production. Pesticides, including herbicides, fungicides, and insecticides, are among the most common pesticides currently used in the modern agriculture industry (Fig. 2.3). However, overuse of these pesticides could contribute to environmental pollution, especially when sprayed. The chemicals are eventually absorbed into the soil, air, or water body and run off into the water stream. The accumulation of these chemicals could cause serious health issues, such as a link to respiratory problems and cancer, including non-Hodgkin's lymphoma, brain, breast, and testes cancer. Thus, further treatment is required to remediate the pesticide-contaminated environment and overcome this issue.

Bioremediation of pesticide-contaminated environments using microalgae has been reported to be an alternative approach to solving this problem. The utilization of microalgae to remediate pesticide-contaminated samples is believed to be the most environmentally friendly [62]. In addition, there are about a thousand microalgal species that have a special capability to remove pesticides from the environment. The efficiency of pesticide bioremediation was found to be higher than that using natural light [63]. Several studies have been reported on the capability of microalgae to biosorb pesticides, including aldrin, carbaryl, DDT, atrazine, and lindane [64–66].

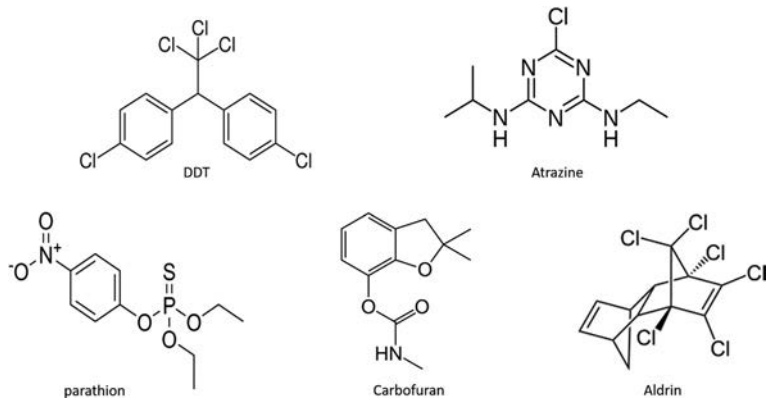

FIG. 2.3 Example of pesticides commonly used in the agriculture industry.

Previous study on microalgae *Nostoc muscorum* showed significant organophosphorus pesticides of up to 91% [64]. Another study indicated that microalgae *Nannochloropsis* oculate could remove up to 73% of lindane pesticides from the liquid medium [67]. In their study, Hu et al. [68] reported that the initial concentration significantly influences pesticide removal, such as atrazine by *Chlorella* sp. The maximum atrazine of 83% was obtained within 8 days of treatment. A study on the bioremediation of pesticides such as phenylamine using *Scenedesmus* sp. microalgae showed that the maximum pesticide removal was achieved using a low concentration of pesticide [69]. The study indicated that cultivation of *Scenedesmus* sp., under this condition, increased microalgal pigment such as chlorophyll, which could benefit further potential use. In addition, the study also indicated that the cultivation of *S. obliquus* using pesticide-contaminated wastewater could improve wastewater quality with a significant reduction in COD and pesticide up to 92% and 97%, respectively [70]. Microalgal bioremediation of pesticides can be done in both short and long-term treatments. According to Hussein et al. [71], in their study on the remediation of different types of pesticides such as *molinate*, *simazine*, *isoproturon*, *propanil*, *atrazine*, *pyriproxin*, and *dimethonate* using dead and living *Chlorella* sp. indicated that long-term treatment using dead microalgal achieves higher removal capacity up to 90% compared to those using living cells.

8 Integrated CO_2 biosequestration bioremediation and biorefinery

Currently, integrated microalgal-based bioremediation and biofuel production has gained great interest as an alternative to sustainable microalgae bioprocessing technology. The introduction of this technology is considered a promising approach to remediating the contaminated environment and producing a wide range of products. Microalgal biomass generated from the bioremediation process can be further converted into various value-added products, including biofertilizer, biofuel, and fine chemicals [72–74]. In addition, other valuable compounds accumulated in the microalgal cell, such as lipids, proteins, vitamins, pigments,

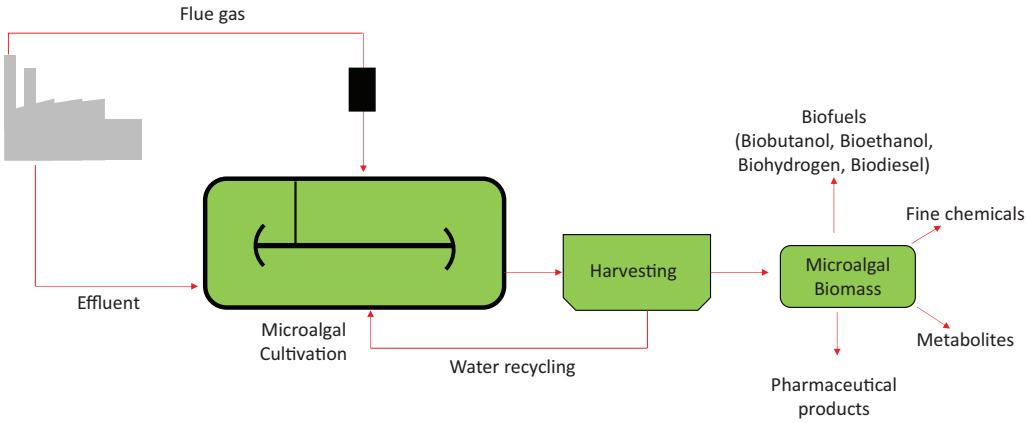

FIG. 2.4 Integrated CO_2 biosequestration and bioremediation for microalgal-based biorefinery.

and carbohydrates, are further used in various industries. Fig. 2.4 shows the typical flow process for integrated carbon emission and effluent treatment by microalgae.

In their study, Kassim and Meng [75] indicated that microalgal *Chlorella* sp. and *Tetraselmis suecica* biomass generated from cultivation for CO_2 biofixation can be further converted into various fine chemicals such as butanol, acetic acid, and butyric acid. The study also indicated that the microalgal lipid extracted from the biomass could be converted into biodiesel via the transesterification process. The previous study also suggested that cultivating microalgae using wastewater from industry could enhance the accumulation of biochemical compounds such as lipids and hydrocarbons in their cells [76]. A study by Cheah et al. [77] proved that the cultivation of microalgae *C. sorokiniana* CY-1 using palm oil mill effluent (POME) as a low-cost medium could improve lipid production from the microalgal cell. On the other hand, the study also found that the fatty acid distribution with a high percentage of C16 and C18 revealed their potential to be converted into biodiesel. Another study into aquaculture waste remediation for chemical production from *C. vulgaris* found that the higher microalgal pigments such as chlorophyll *a*, *b*, and carotenoid significantly increased for microalgae coculture using wastewater [78]. According to this review, it is clearly indicated that integrated bioremediation for effective pollutant removal and value-added chemical production will contribute to operational cost reduction and be important for the achievement of carbon-neutral conditions.

9 Conclusions and future prospective

The potential of microalgae to remove pollutants and other emerging chemicals, including antibiotics, hormones, heavy metals, and pesticides, has been shown to be an interesting approach. Apart from reducing the pollutants in the contaminated environment, the biomass generated from the process can also be converted into other valuable products. This method is considered important for achieving a cost-effective carbon-neutral technology. However, using microalgae for bioremediation applications on a large-scale continues to pose numerous challenges due to the fact that this process is species and chemical dependant. Additional

isolation to obtain a robust strain able to withstand a wide range of environmental fluctuations and a wide range of contaminants is required to assure the sustainability of microalgal bioremediation. Strain improvement can be accomplished by either conventional or genetic engineering approach. Engineering considerations, particularly those pertaining to the cultivation system and operational control for microalgal-based bioremediation technology, are also required. Further low-cost bioreactor development and acceptable technology with possible commercialization are also required. The successful development of low-cost and easy-to-handle bioreactor systems could encourage more industries to invest in this technology.

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CHAPTER

3

Bioremediation of wastewater using algae for potential renewable bioenergy cogeneration

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OUTLINE

1 Introduction

Domestic and industrial wastewater must be treated to remove nutrients, phosphorus, and hazardous chemicals before being discharged into receiving rivers and streams. Algae-based treatment is a promising alternative to the traditional activated sludge system for pollutant removal in wastewater. Algae can remove nutrients from wastewater through adsorption, accumulation, and immobilization in both freshwater and seawater [1–3]. Algal growth is a frequent occurrence in wastewater treatment plants (WWTPs). Algal growth in wastewater treatment plants (secondary sedimentation tanks) can result in color and odor problems, disinfectant degradation, and tank wall corrosion, all of which have a negative impact on the operation and management of wastewater treatment plants [4].

Algae are simple plants with no roots, stems, or leaves, instead of relying on a leaf-like thallus [5]. Algae, fungi, some liverworts, lichens, and the Myxogastria (plasmodial or acellular slime molds) all have leaf-like thallus, which are twig-shaped or green shoots made up of undifferentiated vegetative tissue [6]. The size of algae can range from microscopic (microalgae like green, brown, and red algae, as well as cyanobacteria or blue-green algae) to macroalgae (huge seaweeds like giant kelp) [7]. On the planet, more than 50,000 different species of microalgae have been identified [5].

2 Algae classification

Algae can be classified into photo-autotroph (photosynthetic and working autonomous/stand-alone) or heterotroph organisms. Autotrophic algae are able to synthesize food directly from simple inorganic substances (i.e., carbon dioxide) and water using energy from sunlight. Heterotrophic algae obtain nutrients from complex organic substances. Algae cells are divided into eukaryotes and prokaryotes. Algae are classified as eukaryotic (Eu = true) cells. Eukaryotic

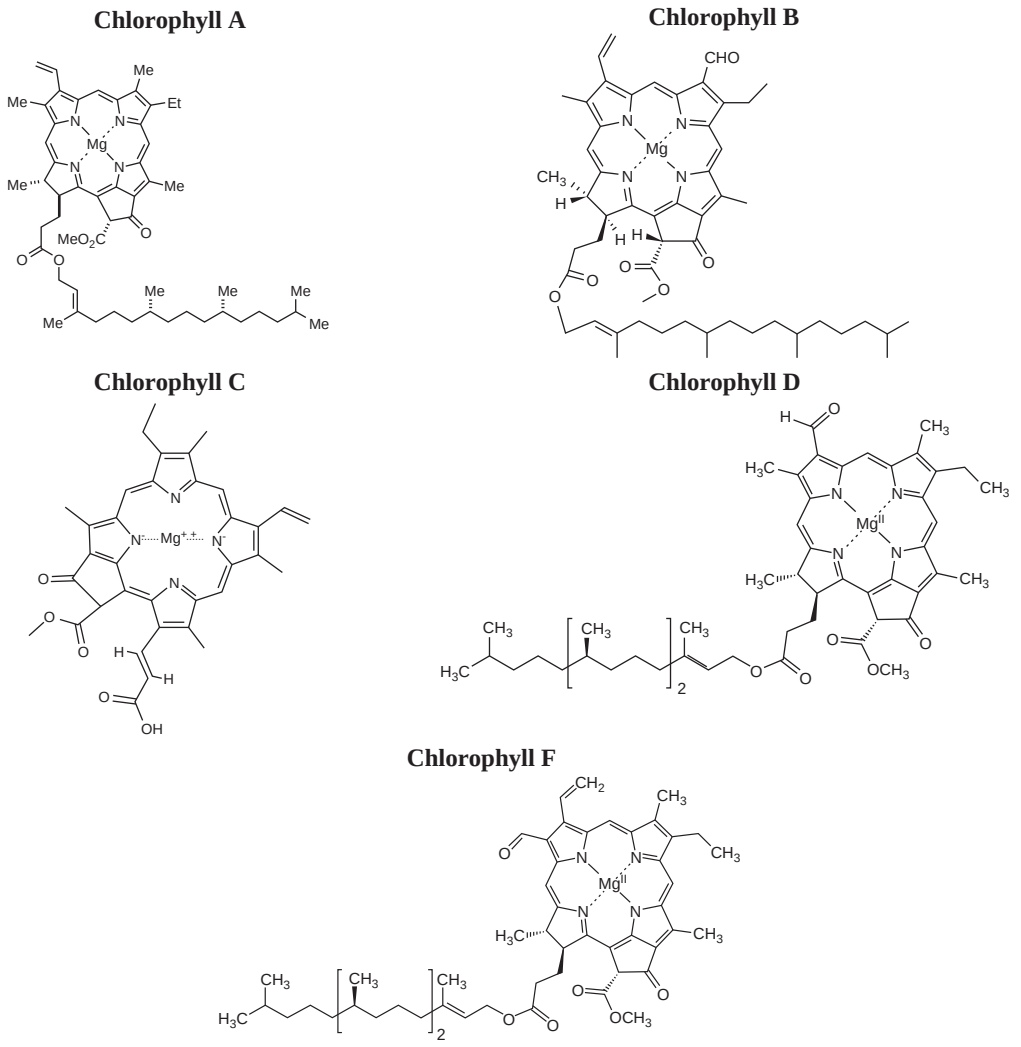

FIG. 3.1 Types of chlorophyll.

cells (e.g., animals, plants, fungi, seaweeds, and protists) have a plasma membrane, cytoplasm, and ribosomes similar to prokaryotic cells (e.g., bacteria and archaebacteria). Eukaryotes are 10–100 times larger than prokaryotic cells, have a true nucleus (DNA), and membrane-encased organelles [8]. Chloroplast or algae plastids are double membrane-bound organelles that contain chlorophyll pigments to trap light for photosynthesis. Chlorophyll (e.g., chlorophyll A, B, C, D, and F) is a liposoluble (i.e., fat-soluble, or soluble in lipids or organic solvents) pigment

that gives plants and algae their green color (Fig. 3.1). Different algae have different types or combinations of chlorophyll.

2.1 Chemical composition of algae

Algae is composed of polysaccharides and carbon, which account for 50% of its total weight. A polysaccharide is a large molecule composed of a number of monosaccharides. It converts inorganic nutrients (i.e., N and P) that exist in water bodies (wastewater, marine, and freshwater) into less hazardous compounds through natural biochemical processes and photosynthesis, producing oxygen as a by-product (Fig. 3.2). Algae is a source of cellulose with the highest grade of crystallinity (80%) as compared to bacteria (65%–79%), cotton (56%–65%), and ramie (44%–47%) [1].

Algae elemental composition contains all elements listed in the periodic table at different concentrations, such as major (oxygen (O), carbon (C), silicon (Si), hydrogen (H), potassium (K), sodium (Na), N, chloride (Cl), and sulfur (S) >1.0%), minor (calcium (Ca), magnesium (Mg), P, boron (B), aluminum (Al), iron (Fe), and strontium (Sr), 0.1%–1.0%), and trace elements (<0.1%) at various concentrations. Algae in the marine environment have more nutrients than those in freshwater [5].

Based on X-ray powder diffraction (XRD) analysis on marine microalgae at various thermal treatment temperatures (500, 700, 900, 1100, and 1300°C) by [5] and [9], the algae consisted of amorphous (24%–100%) and crystalline (0%–76%) content. In the crystalline phase, there were silicates (SiO^-_4), oxides (O^-_2) and hydroxides (OH^-), sulfates (SO_4^{2-}), carbonates (CO_3^{2-}), and chlorides (Cl^-). The SiO^-_4 consisted of quartz or silicon dioxide (SiO_2) ranged from 1% to 2% for 500–900°C. SiO^-_4 was not detected at the higher temperature of 1100–1300°C. The O^-_2 and OH^- consisted of lime (calcium oxide, CaO) (1%–2% for 700–1100°C) and portlandite ($Ca(OH)_2$) (9%–17% for 500–900°C). The SO_4^{2-} consisted of anhydrite (calcium sulfate, $CaSO_4$) ranged from 11% to 31% for 500–1100°C. The CO_3^{2-} consisted of calcite (calcium carbonate, $CaCO_3$) ranged from 5% (at 700°C) to 19% (at 500°C), while Cl^- consisted of halites (sodium chloride, NaCl) and sylvite (potassium chloride, KCl) ranged from 17% to 26% and 2% to 4% at 500–900°C, respectively.

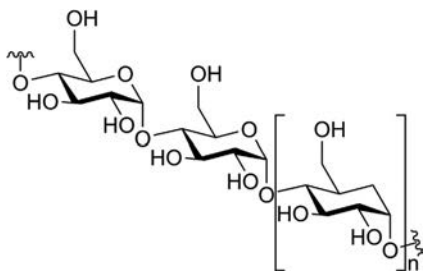

FIG. 3.2 Chemical structure of polysaccharides.

3 Bioremediation of wastewater using algae

Algal cells are naturally defensive when it comes to wastewater bioremediation. To cope with environmental stress, they will alter their appearance through metabolic activities. Surface area, volume, surface-to-volume ratio, growth rate, fluorescence intensity, Raman intensity, and maximum electron transport rate are all morphological characteristics that vary over time but remain constant [10]. Algal technology integration will be a complementary approach to existing wastewater treatment plants that use conventional or advanced oxidation processes to treat wastewater. This chapter discusses the application of aquatic organisms in live-cell forms for wastewater bioremediation. Literature on the designs of bioreactors and scientific investigation into the bioremediation of wastewaters using are presented in Table 3.1. The designs of bioreactors include Revolving Algal Biofilm (RAB) reactors [11], Algal-Sludge Bacterial-Membrane Bio-Reactor (ASB-MBR) [12], algae and cathodic photo-electro-catalysis [13], and application of encapsulated algae in Membrane Bio-Reactor (MBR) [14]. The scientific investigation of algae-based wastewater treatment involves specifying carbon dioxide (CO_2) leakage that disrupts the algae-bacteria symbiosis (ABS) system [15], incorporating calcium phosphate precipitation into the flocculation-sedimentation of algae [16], and biosorption of toxic metals using dried algae [17].

At the Metropolitan Water Reclamation District of Greater Chicago, Zhao et al. [11] investigated the use of filamentous cyanobacteria and green algae (i.e., *Scenedesmus*, *Pediastrum*, and *Chlorella*) in revolving algal biofilm (RAB) reactors using supernatant from dewatering activated sludge from sludge sedimentation (MWRD). At a hydraulic retention time of 7 days, total Kjeldahl Nitrogen (TKN) removal was 87% and phosphorus (TP) removal was 80% at rates of 0.5 mg/L/day and 0.8 mg/L/day, respectively. The TP removal rate of the 1.8-m RAB reactor was found to have increased dramatically to 3.4 mg/L/day (HRT: 1.3 days). For a 1.8 m RAB reactor, the highest productivity was achieved with 7.0 g/m^2/day of biomass generation (HRT: 1.3 days). However, the experimental parameters must be optimized, and the reactors must be operated in a greenhouse.

Sun et al. [12] developed the Algal-Sludge Bacterial-Membrane bioreactor (ASB-MBR) system with algae harvested from a municipal wastewater treatment facility's secondary clarifier wall in Harbin, China. The ASB-MBR resulted in a 25% increase in microorganism growth (algae and sludge bacteria) and a 25% improvement in sludge activity. Chemical oxygen demand (COD) removal efficiencies were 4.6%, ammonium nitrogen (NH_4^+-N) removal efficiencies were 6.7%, total nitrogen (TN) removal efficiencies were 10.1%, and phosphate ion (PO_4^3) removal efficiencies were 8.2%. The rate of increase in transmembrane pressure (TMP) was 50% lower in ASB-MBR than in Control-MBR (C-MBR), indicating that the algal system effectively prevented membrane fouling.

Jiaqi et al. [13] treated synthetic nitrogenous mariculture effluent (COD/N ratio: 0.5–1.0) by bioelectrochemical treatment. Algae and cathodic photo-electro-catalysis were used together. At UV254, the treatment removed NH_4^+-N with a removal efficiency of 94.05%, organic nitrogen with a removal efficiency of 77.35%, and organics with a removal efficiency of 76.66%. The NH_4^+-N concentration in the effluent was less than 2 mg/L. At UV254, the synergy of bacteria, algae, and cathodic treatment promote high pollutant removal efficiencies based

TABLE 3.1 Bioremediation of wastewater through algae from literatures.

Type of Algae Application/ Algae Composition (Ref)		RAB reactors [11]	ASB-MBR system [12]	Algae and cathodic photo-electro-catalysis [13]	Encapsulated algae in MBR [14]
		Filamentous cyanobacteria and green algae (Scenedesmus, Pediastrum, and chlorella)	The algae originated from the secondary clarifier wall	Bioelectrochemical treatment of nitrogenous mariculture wastewater	Macro-encapsulated algae (*Chlorella pyrenoidosa*, FACHB-28) in glass fiber spheres
Location		Metropolitan Water Reclamation District in Greater Chicago (MWRD)	Municipal wastewater treatment plant of Harbin located in Heilongjiang province, China	Seawater from Huanghai in Dalian province, China	Hangzhou, China
Description on Bioremediation	Type of Wastewater	Supernatant from dewatering activated sludge at sludge sedimentation stage	Synthetic wastewater	Synthetic mariculture wastewater	High-ammonia nitrogen wastewater
	Design of Bioreactor	- Greenhouse (atmosphere: 10–30°C, Influent: 10–20°C). - Vertical conveyor belt (1.8 m × 43.5 m × 43.5 m, and 0.9 m × 23.4 m × 23.4 m, speed: 4 cm s^{-1}) - Bottom region was submerged in 1000 L wastewater (2.43 m × 1.83 m × 0.22 m). - Upper region was exposed to the natural sunlight for photosynthesis.	*C-MBR (control) and ASB-MBR (with algae)* - Transparent glass bioreactor (200 mm × 150 mm × 270 mm) - 6 L of wastewater - Light treatment: 12 h light-12 h dark cycle - Hollow fiber membrane (polyvinylidene fluoride, surface area: 0.1 m^2, mean pore size: 0.2 μm) - Bottom aeration (0.15 m^3/h, DO: 2–4 mg/L) - Operating flux: 9 L/(m^2 h). - HRT: 8 h, 8 min suction, 2 min pause - SRT: 19 days (C-MBR), 15 days (ASB-MBR) - MLSS: 3300 ± 110 mg/L - Inoculation ratio: 1:10 (algae/ sludge) - MLSS: 300 ± 20 mg/L (algae) and 3000 ± 90 mg/L (sludge) - Temperature: 22 ± 3°C - Acclimation period: 50 days	*Tubular Reactor* - External circuit (titanium wire, Ø 0.8 mm, 500 Ω) - Data acquisition system (R7100, Anhui) - Carbon rode connecting cathode and anode - Upper cathode chamber - Bottom anode chamber - Bottom aeration - Middle part has layer made of sand:manganese (1:1 v/v) (with filter cloth above and below): separation, filtration and proton transfer layer - Marine sediment: granular activated carbon (with bacteria): sand: cathode chamber ➔ (4.0:5.0:1.6:4.7, v/v)	*MBR* - High ammonia nitrogen wastewater: 10 L - Mode of operation: - Operating flux: 8.2 L/(m^2 h), - HRT: 2.72 h, 10 min suction, 2 min pause - SRT: 30 days - Aeration rate: 5 L/min - Microfiltration flat modules: self-made Nylon-6 (N6) and cellulose acetate (CA). - TMP is used to study membrane fouling by microorganisms. - The encapsulated microcapsules were washed to remove the adhesive cells of surface.

			Reactor Maintenance: Membrane Permeability The biocake layer accumulated on the membrane will be cleaned with pure water once the transmembrane pressure (TMP) reached 30 kPa. The 5% (v/w) chlorine solution was applied on the membrane to retrieve the membrane permeability.		
	Environmental Parameters	Total Kjeldahl Nitrogen (TKN) Total Phosphorus (TP) Ortho-Phosphorus Phosphorus Ammonium (Lachat autoanalyzer) Total heavy metals (inductively coupled plasma atomic emission spectroscopy, ICP-AES)	Chemical oxygen demand (COD) Ammonia nitrogen (NH_4^+-N) Total nitrogen (TN) Phosphate (PO_4^{3-}-P) Zeta potential (ζ)	Nitrogen (NH_4^+-N) Organic pollutants at UV_{254} (UV-5500, METASH, Shanghai) Microbes in biofilm (16S rDNA Amplicon sequencing kit)	COD NH_3-N Light intensity (Digital lux meter, AS831)
Findings		*Removal Efficiency* TKN: 87% TP: 80% - 0.5 mg/L/day (HRT: 7 day) - 0.8 mg/L/day (HRT: 4.7 day) TP (1.8-m RAB reactor) - 3.4 mg/L/day (HRT: 1.3 day) - 7.0 g/m^2/day of biomass production	*Removal Efficiency of ASB-MBR* 25% faster growth of algae and bacteria. Enhanced the nutrient breakdown activity in sludge. COD: 4.6% NH_4^+-N: 6.7% TN: 10.1% PO_4^{3-}: 8.2%	NH_4^+-N: 94.05% Inorganic nitrogen: 77.35% Organics (at UV_{254}): 76.66% NH_4^+-N: <2 mg/L The synergy of bacteria, algae and cathode, promoted pollutant removal and made the system sustainable and efficient in treating mariculture wastewater.	Initial ammonia nitrogen: 50–100 mg/L *Removal efficiency* COD: 62.23% NH_3-N: 97.38%
Limitations		Optimization parameters are yet to be studied. Reactors need to be in greenhouse operated.	The algae system controls the membrane fouling in ASB-MBR. Optimization parameters are yet to be studied.	Wastewater has fluctuated concentration of pollutants. The maximum tolerant limit for the bacteria and natural grown algae on pollutant parameter is not mentioned.	The long-term stability of encapsulated microcapsules (30 days) in complicated environments provides an energy-saving solution to MBR's existing poor microbial activity and substantial biofouling concerns.

Continued

TABLE 3.1 Bioremediation of wastewater through algae from literatures—cont'd

Type of Algae/Algae Composition (Ref)		CO_2 stripping in the aeration process is the main cause of damage to the ABS [15]	Flocculation-sedimentation of algae in wastewater effluent by calcium phosphate precipitation [16]	Biosorption of toxic metals from industrial wastewater [17]
		Microalgae: *Scenedesmus obliquus*	*Chlorella* sp. (259,000 cells/mL) and cyanobacteria (*Merismopedia* sp. and *Planktothrix* sp.)	*Spirulina platensis* (SP) and *Chlorella vulgaris* (CV)
Location		Taiping wastewater treatment plant in Harbin, China	Leanyer/Sanderson municipal wastewater treatment plant, northern suburbs of Darwin, Northern Territory, Australia	The wastewater treatment plant in Doha industrial city, Qatar
Description on Bioremediation	Wastewater Type	Sludge from the secondary sedimentation tank	Waste stabilization pond (WSP) effluent	Effluents of secondary WWTP
	Design of Bioreactor	Four sequence bioreactors (SBRs, glass bottles of 0.5 L) contained 0.4 L working volume. Aeration intensity (AI): 0, 20, 50, and 100 mL/min Group 1 (1 reactor, control): AI, 0 mg/L Group 2 (3 reactors): AI, 20 mL/min, 50 mL/min, and 100 mL/min. The SBRs system: - Mixing: magnetic stirrer (50 rpm) - Reaction: reactor and a gas collection bag - Gas supply: a flow meter (nos: 3), an oxygen cylinder, a nitrogen bottle, and a gas supply pipe Flow meter: - 1 controls nitrogen gas - 2 controls oxygen gas - 3 controls total gas volume (20 mL/min, 50 mL/min, and 100 mL/min)	Flocculation (Phipps and Bird PB-900 jar tester) based on a standardized jar test technique (1 L of wastewater sample): - 5 min fast mixing while adding chemicals (100 rpm) - pH was raised to four distinct levels using three different ways (pH 7.0–8.0–9.0–10.0). - 30 min of moderate mixing (15 rpm) during flocculation to allow for floc development - 30 min for settling - Turbidity analysis and sample extraction (2 cm from bottom) to measure separation efficiency Preparing calcium phosphate stock solutions entails the following steps: - Calcium chloride ($CaCl_2$) and sodium hydrogen phosphate (Na_2HPO_4) in MilliQ water (Ca:P 1.33:1, 1.5:1, and 1.67:1). - To dissolve all produced precipitates, these stock solutions were acidified with HCl (37%) until all precipitates were dissolved. [1]. Photosynthesis increases pH;	Toxic metals (TMs): copper sulfate pentahydrate, nickel nitrate hexahydrate, and aluminum sulfate were spiked in wastewater Equilibrium tests: - Mixing of TMs (0.25, 0.5, 1, 1.5, 4, and 5 g/L) with dry biosorbents (SP or CV) in 20 mL of wastewater containing (150–200 rpm) - Time: 5, 15, 20, 40, 60, 80, 100, 120 min - pH: 4–7 - Algae was filtered - TM concentration in wastewater was measured via flame atomic absorption spectrophotometer (model AA-700). - Zeta potential (ζ) was determined via NanoBrook Zeta Potential Analyzer (NanoBrook 90Plus Zeta, United States) Zeta potential (ζ): - Ratio of 1 mL (algae + wastewater): 10 mL (Milli-Q water, v/v) - 1 mL of the mixture was added to the analyzer (25°C)

			[2]. 0.1 M sodium hydroxide is added to increase pH. [3]. 1.8 M slaked lime addition (100 g commercially available lime (CaO, 88% w/w) was "slaked" with 1 L MilliQ water)	- pH adjustment (if require): Additional of 1% HNO_3 or 1 N NaOH
	Environmental Parameters for	pH Dissolved oxygen (DO) NH_4^+-N Nitrate nitrogen (NO_3^-N) Nitrite nitrogen (NO_2^--N) Phosphate (PO_4^{3-}-P) TN Dissolved inorganic carbon (DIC) Total organic carbon (TOC) CO_2 (GC system, 7890A, Germany). Sludge flocs (Malvern particle size analyzer, 2000 UK) Extracellular polymeric substance (EPS) (EEM–PARAFAC analysis, F7000, Hitachi, Japan)	Separation efficiency (%) Phosphate concentration (mg/L)	Concentration of TMs (mg/L) The removal efficiency of TMs (%)
Findings		Due to CO_2 stripping, aeration intensity of 100 mL/min will degrade algal growth, resulting in a considerable amount (15.62 mg/L) of wasted nitrogen dioxide (NO_2) and nitrate (NO_3^-). In the ABS, a decreased aeration rate (20 mL/min) resulted in a more stable state and greater pollutants removal effectiveness.	Algal harvesting from urban wastewater using calcium phosphate precipitation was effective and allowed for phosphate recovery; however, this could not be accomplished in this work by relying simply on algal photosynthesis to raise pH. Flocculation caused by lime addition resulted in good algal harvesting and very high-quality effluent, while the cost of alkali addition was comparable to that of traditional coagulants. WSP effluent solids have a charge density that shows they are considerably distinct from most laboratory cultures of algae and cyanobacteria.	Removal efficiencies (Algae concentration: 4.8 g/L): - 95.0% for Al, pH 5.5 - 87.0% for Ni, pH 6.0 - 63.0% for Cu, pH 7.0
Limitations		Algae needs CO_2 for growth, not excessive aeration like common practice.	High calcium and phosphate doses were required, which were far greater than those reported in most laboratory trials to date.	Acidic treatment of dried algae is required.

on NH_4^+-N, organic nitrogen, and organics. It demonstrated that the system is capable of treating mariculture effluent in a long-term and cost-effective manner.

In Hangzhou, China, Qin et al. [14] used micro-encapsulated algae in a membrane bioreactor (MBR) to treat high-ammonia nitrogen effluent. *Chlorella pyrenoidosa* (FACHB-28) macro-encapsulated algae in glass fiber spheres having a capacity of 0.05 ± 0.016 g cells/g microcapsule. At initial ammonia nitrogen levels of 50–100 mg/L, the treatment achieved removal efficiencies of 62.23% for COD and 97.38% for ammonia nitrogen (NH_3-N). Encapsulated microcapsules' long-term stability (30 days) in complex environments provides an energy-saving solution to MBR's existing poor microbial activity and significant biofouling concerns.

Carbon dioxide (CO_2) stripping in the aeration process is the principal cause of damage to the algae-bacteria symbiosis (ABS) system, according to Zhang et al. [15]. *Scenedesmus obliquus* was the microalgae used in the experiment. The sludge was collected from the Taiping wastewater treatment plant's secondary sedimentation tank (WWTP, Harbin, China). Due to CO_2 stripping, aeration intensity of 100 mL/min will degrade algal growth, resulting in a considerable amount (15.62 mg/L) of wasted nitrogen dioxide (NO_2) and nitrate (NO_3^-). In the ABS, a decreased aeration rate (20 mL/min) resulted in a more stable state and greater pollutants removal effectiveness. Algae, in general, require CO_2, not extensive aeration, as is normal practice.

The cultivation of microalgae in municipal and agricultural wastewater provides an opportunity to treat the water while also converting nutrients into valuable biomass. Algae harvesting from such wastewater is difficult due to that fact that conventional coagulants (iron and aluminum salts) contaminate algae and lock up phosphorus, making it difficult for beneficial reuse. Algae and phosphate can be harvested from municipal wastewater using flocculation by calcium phosphate precipitation and reused. Algae harvested 93% of soluble phosphate from a waste stabilization pond effluent by initiating flocculation with 129 mg/L soluble phosphate and adding 364 mg/L quicklime to achieve a pH of 10.0 at a calculated cost of $0.05 USD per kL for alkali addition, which is comparable to the chemical addition costs of conventional coagulation. Phosphate could be recovered from urban wastewater by growing algae with calcium phosphate precipitation. Algal photosynthesis alone would not be sufficient to raise pH. Algal harvesting and effluent quality improve with lime addition coagulation, and the added cost of alkali is comparable to that of traditional coagulants. The charge densities of most WSP effluent solids show that they are very different from most lab-grown algae and cyanobacteria cultures [16].

Another study investigated the effects of an acidic treatment on the removal of toxic metals (TMrem) from wastewater by algae strains (Spirulina platensis (SP) and Chlorella vulgar (CV)) at various adsorbent dosages (0.2–2.5 g), pH values (4–8), and contact times (5–100 min). The acidic treatment (Ac-T) altered the functional groups on the algae's surface, promoting the formation of more electronegative groups and increasing the removal of Al, Ni, and Cu. At optimal pH values of 5.5, 6.0, and 7.0 and an adsorbent concentration of = 2.5 0.1 g/L, treated SP removed up to 95%, 87%, and 63% of Al, Ni, and Cu, respectively. TMrem values of 87%, 79.1%, and 80% were obtained with treated CV. The optimal operating conditions for maximum removal of TMrem at ($C_{algae} = 4.8\ g_{MNPs}.L^{-1}$, Ct = 88, and pH = 6) were determined using the response surface methodology. (RSM). The endothermic, spontaneous adsorption of TMs on algae is controlled by Langmuir and second-order kinetics. Zeta potential measurements indicated that electrostatic interaction is responsible for the toxic

metal (TM) adsorption mechanism between algal strains. As such, bio-sorption is an economically viable and environmentally friendly method of removing TM from wastewater [17].

AlMomani and Örmeci [18] investigated the use of microalgae to remediate wastewater in hot climates. Microalgae *C. vulgaris* and *Neochloris oleoabundans* were tested for their ability to eliminate soluble chemical oxygen demand (CODs), inorganic nitrogen, and total dissolved phosphorus in primary effluent (PE), secondary effluent (SE), and centrate (CEN) at 36°C. Significant differences in C.V. and N.O. growth patterns and rates were found in various wastewater samples. In CEN, C.V. grew at the fastest rate, followed by PE and SE. In PE, N.O. species grew at the fastest rate, followed by SE and CEN. In PE, SE, and CEN, the COD removals for C.V., 55%, and 80%, and for N.O., 63%, 47%, and 72%, respectively. The ammonia removal efficiency of C.V. and N.O. in various wastewaters was quite similar (70–84%). The removal of phosphorus by C.V. and N.O. was considerable in PE (>84%), moderate in CEN (>22%), and constrained in SE (15%). At 36°C, C.V. and N.O. growth rates were compared to those at 20°C, as reductions in CODs, ammonia, nitrate, and total dissolved phosphorus. The kinetics of algal strains accurately matched the growth profile and evolution of organic matter and nutrients, which were validated using experimental data, allowing process optimization and scale-up. According to the findings, microalgae can be successfully cultivated in a wide range of wastewaters at 36°C and can achieve natural wastewater treatment in hot climate zones to eliminate organic carbon, nitrogen, and phosphorus [18].

4 Bioenergy cogeneration using algal biomass

A study of sustainable harvesting of algal biomass for food supplements, biodiesel generation, and other purposes led to the introduction of algae into engineered conventional wastewater treatment systems (treatment performance and kinetics).

4.1 Biogas

Anaerobic digestion produces 55%, 70%, and up to 95% methane gas during organic degradation (e.g., agricultural waste, microalgae biomass). The production of methane is dependent on the organic composition of the waste as well as the method of digestion used [19]. Microalgae can be cultivated on a large scale in an open raceway pond or a closed-loop system of photobioreactors using brackish water, wastewater, or seawater water [20]. The closed-loop system of the photo-bioreactor provides high capacity in biomass production due to high photosynthesis efficiency, stability with low reliability (low contamination risk), low water evaporation, net energy ratio of more than 1 (energy output/input), and low area requirement [20].

4.2 Biodiesel

Biomass-derived renewable diesel contributes to nontoxic, biodegradable energy sources that release fewer greenhouse gases (GHG) than conventional diesel. First-generation

biodiesel derived from soybeans, sunflowers, palm oil, and corn; second-generation biodiesel derived from jatropha, animal fats, and waste cooking oil; and third-generation biodiesel derived from algae. The first-generation source (from crops) is unable to meet demand because it accounts for 70%–85% of total biodiesel production costs. The use of second and third-generation sources is increasing due to lower production costs [21,22]. The advantages of algae as biodiesel come from its high growth rate (15 and 25 tons/ha/year) when compared to first-generation sources, which have biomass production rates of 0.4 metric ton/ha/year for soybeans, 3.62 metric tons/ha/year for palm oil, and 4.14 metric tons/ha/year for jatropha [23]. Algae-based biodiesel has a high energy content (35,800 kJ/kg, or 80% of the energy in petroleum) [24], is noncompetitive in food markets (as compared to first-generation sources), and may grow by utilizing wastewater nutrients [25].

Dunaliella salina, *Ettlia oleoabundans*, and *Botryococcus braunii* are examples of green microalgae that can produce high amounts of lipids, which can be utilized for biodesel production. Given that microalgae cells contain 30% lipid, they will be able to generate a considerable amount of lipid, approximately 4.5 to 7.5 metric tonnes/ha/year [26]. Large-scale algae production, on the other hand, necessitates a lot of energy, water, and nutrients (e.g., NO_3^- and PO □ $^{3-}$). Fertilizer plants are known for producing large levels of these nutrients [27].

4.3 Bioelectricity

Fossil fuels contribute to 86% of the world's energy production [28]. Bioelectricity is one of the renewable sources that can be produced from waste biomass [29]. In tissue cell engineering, bioelectricity can be used by exploiting signaling to predict morphogenetic information of fetus growth, reprogram cancer development, and be beneficial for regenerative medicine of damaged organs [30], acquisition of electrocardiogram signals [31], and is important for photosynthetic rate evaluation [32]. The bioelectricity from microbial fuel cells (MFCs), sediment-type algae microbial fuel cells (SMFCs), and photosynthetic algal microbial fuels (PAMFCs) are among renewable fuel resources. These technologies are sustainable in the long run because they have integrated technologies with wastewater treatment plants that not only treat the wastewater but also produce hydrogen and consume the CO_2 by-product, reducing the carbon footprint. Not only that, they have a low cost for installation and maintenance without changing the existing infrastructure, as well as high process yields and energy conversion efficiencies [28]. In this case, the PAMFCs are highly recommended. Algae produce O_2 and simultaneously consume CO_2, N, and P during the daytime, and, hence, aeration is not necessarily needed like in MFCs and SMFCs. Excessive aeration will only slow down algae growth and efficiency. Nevertheless, bioelectricity based on photosynthetic activity can be produced in the nighttime due to the presence of other electron acceptors (nitrate and sulfate) in the cathode compartment.

5 Algal practicality and limitations

Algae has been explored in terms of its practicality, the advantages it gives in terms of carbon footprint reduction, and its limitations. Algae have different life cycles based on the

species they are. Algae will have decomposed and turned into biomass, resulting in anthropogenic eutrophication of the environment. When the concentration of N and P in a water body is high, eutrophication occurs. It is anthropogenic because it originates from human activities (directly or indirectly), detrimental to the biological organism and the environment. The decomposition process will also consume the dissolved oxygen (DO) in the water body, creating a large anoxic zone that will kill the aquatic living organisms below the surface of the water body. Moreover, some algae produce toxins that are neurotoxic, and detrimental to human health and aquatic life.

Plouviez et al. [33] reported that high-rate algal pond systems (HRAPs) such as waste stabilization ponds (WSPs) (900 L capacity) used for wastewater treatment (location: Palmerston North wastewater treatment plant, New Zealand) emit nitrous oxide (N_2O) at different levels, ranging from 21 to 138 g N_2O per capita per year at hydraulic retention time (HRT) of 7.5–10 days. The microalgae *C. vulgaris* is well-known for its ability to produce N_2O via nitrate reduction [33].

However, Kohlheb et al. [34] reported that this technology was found to be more energy-efficient (22% of its electricity consumption) than activated sludge-based sequential batch reactors (SBRs), economically (HRAP: 0.18 €/m^3, SBRs: 0.26 €/m^3) and environmentally friendly. Although the SBR provides a higher removal rate of nutrients as compared to HRAP, the estimated eutrophication potential of HRAP was lower than the SBR, equivalent to 146.27×10^{-3} kg CO_2 equiv./m^3 and 126.14×10^{-6} kg PO_4 equiv./m^3. The eutrophication potential for SBR was 458.27×10^{-3} kg CO_2 equiv./m^3 and 158.01×10^{-6} kg PO_4 equiv./m^3. The CO_2 supply for HRAP technology boosts microalgae productivity by calculating potential biomass generation based on the carbon-to-nitrogen ratios of microalgae and wastewater. However, under ideal conditions, the actual value of absorption into biomass was lower than the projected value, accounting for just 57% of the inflow of nitrogen. The concentration of nitrogen was lowered via nitrification and volatilization. The process performed well with higher biomass productivities due to the short hydraulic retention durations. In a dissolved air flotation device, the biomass produced was efficiently collected [35].

In the scenarios of manure management systems (MMS, encompassing materials, transport, energy, and emissions) for intensive pig farming systems, the nonconventional MMS scenario, which combines an integrated anaerobic mono-digester and an algae-based wastewater treatment (AWWT) process, is highly recommended. Several components of pig diets are replaced by algal biomass. Using life cycle assessment (LCA) methods, the total environmental impact was reduced by 35.5%–40%, and the nutrient recovery of nitrogen ratio was enhanced to 81%. Another study investigated the use of an integrated anaerobic codigester in conjunction with the AWWT method. The algal biomass is partially used in pig feed, with the remaining half flowing into the anaerobic codigester to improve biogas production. Algal biomass increased by 49%, while heat and electricity cogeneration increased by 26.6% and 7%, respectively [36]. Algae development in wastewater treatment plants is a common occurrence due to the presence of nitrogen and phosphorus in wastewater. Algae growth on secondary sedimentation tanks in wastewater treatment plants (WWTPs) can affect effluent color and odor, disinfectant usage during disinfection, and tank wall corrosion. Chen et al. [4] developed antialgae coatings by mixing quaternary ammonium compound (QAC) with aqueous enamel paints for stable operation and maintenance of WWTPs.

6 Conclusions and future outlook

Algae can be used to treat wastewater with high N and P content, such as wastewater from livestock (swine, bovine, and poultry) and biomass-rich agricultural processes, as well as effluent from urban and domestic areas. Algae is pH sensitive. Different species have different pH tolerance. The algae application needs to be conducted in a closed bioreactor system for a controlled environment because some algae species, such as *C. vulgaris* produce N_2O upon decomposition of algae. Therefore, the maintenance of the bioreactor needs to recycle the system before decomposition happens. The algae can be used as food for livestock as well as for biodiesel production due to their high lipid content. Due to the presence of extra electrolytes (nitrate and sulfate) that cause pollutants in wastewater, to decompose algae can be used in both day and night systems. Algal systems, namely PAMFCs, can produce bioelectricity based on photosynthetic activity. It not only treats wastewater in plants, but it also generates hydrogen, which is used to generate electricity. In general, algal application is a highly justified green technology that has the potential to significantly reduce carbon footprints and is long-term sustainable.

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CHAPTER

4

Microalgae for bioremediation of pesticides: Overview, challenges, and future trends

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1 Introduction

The increase in food production, fueled by the world's growing population, has made agricultural production highly dependent on pesticides. Pesticides are chemicals that are used to protect crops from pests and to achieve the required quality and efficiency in plant production [1,2]. However, excessive pesticide use can negatively impact the environment, resulting in soil, air, and water contamination. The accumulation of these compounds in soil, plants, microorganisms, and surface waters can also have harmful effects on human health [3,4]. Therefore, in recent years, efficient alternatives for remediation using microorganisms have been developed to provide a sustainable solution to mitigate the environmental impacts of pesticides [3,4]. Bioremediation is a technology based on biological materials (e.g., bacteria, fungi, microalgae, and cyanobacteria). This technology is used to remove, degrade, accumulate, or transform contaminants, such as pesticides and pharmaceuticals. However, although it presents numerous advantages, bioremediation is complex and influenced by several variables [5]. Microalgae stand out among the species used for bioremediation [6,7]. Microalgae are photosynthetic microorganisms found in marine or freshwater environments [8]. These organisms can purify different types of wastewater by using the wastes as substrates for their growth. Furthermore, during growth, these microorganisms can fix CO_2, and help to mitigate atmospheric carbon dioxide [9]. Microalgae such as *Chlorella* spp., *Scenedesmus* spp., and *Chlamydomonas* spp. have already been used to remove a range of organic pollutants from wastewater [1,7,9]. Microalgae can remove pesticides through bioadsorption, biodegradation, and bioaccumulation [6,10]. The rate of pesticide removal depends on the organism used, pesticide structure, and contaminated area [2]. Studies have shown the removal efficiency of pesticides by microalgae is greater than 90% [7,11]. However, the inhibition of microalgal growth by degradation products is a challenge to this technology [1]. The use of microalgae biomass resulting from bioremediation processes should be highlighted because of its various uses. Biomass can be valorized for the production of biofuels, such as biogas, using the biorefinery approach [3]. Therefore, this chapter provides an overview of the bioremediation of pesticides by microalgae. It focuses on different mechanisms and techniques of remediation by these organisms and suggests alternatives to increase pesticide removal and biomass valorization. Moreover, challenges and future perspectives are highlighted.

2 Contamination by pesticides

Pesticides are chemicals that, among other things, include herbicides, insecticides, and fungicides [6,12] and are used to prevent crop diseases, weeds, and insect pests [13]. These components have played an essential role in agricultural production for many years [6,13]. Glyphosate and atrazine are two of the most widely used herbicides in the world [1,14]. Each pesticide has specific chemical properties and mechanisms of action. Glyphosate can be used as a foliar postemergent herbicide, while atrazine absorption can be incorporated by roots in a preemergent manner [14]. Previous studies have reported persistent pesticides detected in aquatic environments [1,14]. Furthermore, pest resistance to chemicals caused by long-term pesticide use is another challenge to overcome [13]. Mahler et al. [14] investigated the

occurrence of the pesticides atrazine and glyphosate in agricultural and urban streams. The presence of herbicides in urban streams could be caused by drainage systems, such as direct water from storms to a nearby stream. Additionally, according to Nie et al. [6], pesticide application by "spraying" can facilitate environmental contamination.

The management of residual pesticides is challenging, and it is difficult for conventional processes to remove certain pollutants from water [2,15,16]. Pesticide residues in water and soil can have hazardous effects on living organisms and the environment [2]. Moreover, their presence in raw agricultural products or processed foods directly affects food safety. The amount of pesticide residues on food will be determined by various pesticide properties, such as solubility and vapor pressure [13]. High levels of pesticide exposure have been reported to potentially contribute to multiple diseases, such as cancer and endocrine disorders [1,14,17]. Chai et al. [9] reported that water scarcity appropriate for human consumption in some countries could be attributed to failures in wastewater treatment and effluent discharge. Wastewaters containing pesticide residues can originate from pesticide manufacturing and formulation (e.g., from cleaning activities) and agricultural industries. These wastes can pollute water bodies and groundwater with toxic compounds [17]. Thus, strategies to remove pesticides from water in a sustainable manner to decrease the health risks associated with pesticide contamination have recently been the focus of research.

3 Environmental fate of pesticides

The environmental fate of pesticides begins when they are sprayed into the environment. From this moment on, these compounds can be absorbed by plants or remain available in the soil matrix. In the environment, pesticides are subjected to different physicochemical and biological processes. Adsorption, desorption, biodegradation, photodegradation, and volatilization are examples of these processes [18,19] (Fig. 4.1). Some pesticides have volatile or semivolatile characteristics, and, as a result, they can be transported into the atmosphere, increasing their mobility [20]. Additionally, other processes, such as wind erosion and volatilization of these compounds present in the soil and plants, may occur. These parameters are influenced by the soil sorption characteristics, the lipophilic properties of the plants, and vapor pressure. Wind can also promote the mobility of pesticides even before they encounter the soil or plants [21]. Many factors influence the volatilization of pesticides in the soil, including the degree of adsorption, water content, soil properties, and environmental temperature [22].

Sorption and desorption processes are dependent on soil characteristics, such as pH, organic matter, moisture, clay, and silt composition, and are considered some of the most important parameters in the destination of pesticides. The slope of the soil, permeability, and depth of the water table also affect pesticide movement. These processes determine the distribution of pesticides in the solid, gaseous, and aqueous phases, controlling the availability of other transport and degradation processes [19,23]. Other chemicals have high water solubility, allowing them to be dispersed by rain, resulting in pesticide leaching into water resources and potentially affecting marine and other nontarget species [23,24]. Transportation and storage of pesticides can also contribute to possible contamination routes, as can the improper disposal of wastewater from the packaging washing step or during pesticide production [6,25].

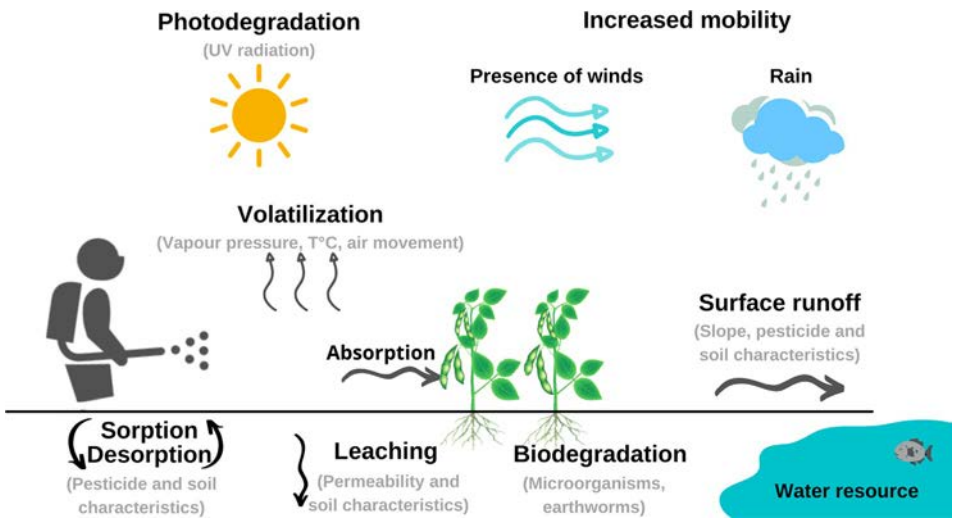

FIG. 4.1 Environmental fate of pesticides after application in agriculture.

4 Bioremediation of pesticides by microalgae

Microalgae are microscopic photosynthetic organisms found in freshwater and seawater [8] and utilize energy and carbon sources to grow [26]. These organisms can be applied to the production of food, animal feed, and various biofuels because of their flexibility in producing a range of compounds, such as proteins, fatty acids, vitamins, and pigments [8]. Among the various advantages of using microalgae are their ability to use solar energy more than terrestrial plants and their efficiency in capturing CO_2 from the atmosphere [4]. Furthermore, microalgae have been applied to wastewater biotreatment and studied regarding the assimilation of pesticides as an energy source for their growth [6,9]. Microalgae-mediated bioremediation is also known as phytoremediation, phycoremediation, and cyanoremediation. This technique not only accumulates or adsorbs pesticides but also transforms them into pollutants that are less toxic to the environment, depending on the microalgal species, metabolic pathways used, and characteristics of the pesticides [27,28]. Bioremediation mechanisms can occur through active or passive processes in which physicochemical interactions happen in the cell wall. The mechanisms involved in microalgal bioremediation include bioadsorption, bioaccumulation, and biodegradation processes, in addition to external degradation factors, such as photodegradation and volatilization [6,16,29]. Several studies have demonstrated the use of microalgae for the bioremediation of pesticides (Table 4.1). In most studies, the inhibition of microalgal growth at high concentrations of pollutants has been identified, as well as inducing oxidative responses and changes in cell contents and morphology [33].

4.1 Bioadsorption

Bioadsorption is a passive, nonmetabolic process in which interactions occur between the contaminant (positively charged) and the cell wall of the microalgae (negatively charged) in

TABLE 4.1 Mechanisms involved in the bioremediation of pesticides from microalgae.

Microalga	Pesticide	Concentrations of pesticide	Removal efficiency	Exposure time	Mechanisms involved in bioremediation	References
Chlorella vulgaris	Diazinon	0.5 mg L^{-1}	100%	12 days	Biodegradation + bioaccumulation	[7]
		5 mg L^{-1}	87.67%			
		20 mg L^{-1}	93.31%			
		40 mg L^{-1}	61.99%			
		100 mg L^{-1}	45.22%			
Chlamydomonas reinhardtii	Trichlorfon	2 mg L^{-1}	100%	10 days	Biodegradation	[11]
		10 mg L^{-1}	100%			
		40 mg L^{-1}	100%			
		60 mg L^{-1}	100%			
		100 mg L^{-1}	100%			
		200 mg L^{-1}	96.2%			
Oscillatoria limnetica	Glyphosate	5 mg L^{-1}	97.5%	7 days	Biodegradation	[30]
		10 mg L^{-1}	57.9%	14 days		
		15 mg L^{-1}	37.18%	14 days		
		20 mg L^{-1}	99.9%	35 days		
Synechococcus elongatus	Atrazine	0.025 μM	80%	12 h	Bioaccumulation	[31]
		0.1 μM	75%			
		0.75 μM	70%			
	Terbutryn	0.025 μM	80%	12 h	Bioaccumulation	
		0.1 μM	85%			
		0.75 μM	82%			
Chlorella vulgaris	Atrazine	0.025 μM	84%	12 h	Bioaccumulation	[31]
		0.1 μM	90%			
		0.75 μM	83%			
	Terbutryn	0.025 μM	85%	12 h	Bioaccumulation	
		0.1 μM	93%			
		0.75 μM	93%			
Chlorella saccharophila	Pyridaphenthion	10 mg L^{-1}	<4 mg L^{-1}	6 days	Bioconcentration	[32]
Consortium (*Chlorella* sp. and *Scenedesmus* sp.)	Chlorpyrifos	1 mg L^{-1}	97%	7 days	Sorption + biodegradation	[3]
	Cypermethrin		74%			
	Oxadiazon		88%			
Chlorella sp.	Atrazine	40 μg L^{-1}	87%	8 days	Bioaccumulation	[1]
		80 μg L^{-1}	84.9%			

their active or inactive state. A chemical affinity between the contaminant and the microorganism is necessary for this process to occur and for the control of external conditions such as pH and temperature. The chemical processes that affect bioadsorption also include complexation reactions, ion exchange, precipitation, and electrostatic interactions [6,16]. In microalgae, the bioadsorption processes of organic compounds occur mainly because of functional groups (e.g., carboxyl, amine, and phosphoryl), intercellular spaces, sulfated polysaccharides, and carbohydrates present in the cell wall [34,35]. Bioadsorption can also occur in extracellular polymeric substances (EPS) bound to microalgal cells or excreted in the extracellular medium [29]. These substances, formed mainly of proteins, lipids, and polysaccharides, are responsible for the aggregation of cells and the formation of film. This film protects cells from external stresses, such as high concentrations of contaminants and variations in temperature and pH, in addition to facilitating adsorption [36].

Adsorption processes generally have more significant advantages than bioaccumulation, such as lower cost and less time. According to Sutherland and Ralph [16], optimization of microalgal growth will positively influence the remediation of emerging contaminants. According to these authors, the more microalgae cells, the more bioadsorption is expected to occur. Furthermore, inactive cells do not require control of biological conditions for the growth of microorganisms and cellular metabolism, thus presenting greater versatility [37]. The disadvantages are related to the nonselectivity of these processes, which is a problem because of the diversity of contaminants present in wastewater. Thus, the presence of more than one contaminant can interfere with the adsorption processes and saturate the binding sites with nontarget contaminants, reducing the efficiency of removing the intended contaminants for bioremediation [16].

4.2 Bioaccumulation

Bioaccumulation occurs when pollutants are absorbed or transported through the microalgal cell wall. In this active process, pesticides bind to proteins and other intracellular compounds. This mechanism can be divided into (a) passive diffusion, which does not require energy from the cell because the contaminant, which has a low molecular weight and is nonpolar, diffuses through the cell membrane (hydrophobic). (b) Passive-facilitated diffusion occurs with the help of transporter proteins, facilitating the diffusion of contaminants through the cell membrane. The last mechanism is (c) active uptake, which requires energy from cells [16]. Bioaccumulation is the process posterior to bioadsorption and can occur simultaneously with biodegradation [38]. This process can be advantageous, allowing greater efficiency in removing the contaminant because of more binding sites (surface and inside of the cell) [37]. During bioaccumulation, high concentrations of pesticides can induce stress because of the generation of reactive oxygen species (ROS), which are responsible for numerous toxic effects on microalgal metabolism [6]. Among them are oxidative damage, cell dysfunction, changes in the content of chlorophyll and carotenoids, influence on antioxidant enzymes, such as superoxide dismutase and catalase, and even the death of microorganisms [7]. Therefore, the study of the appropriate concentrations of contaminants to avoid cell death and the selection of suitable microalgal strains is extremely important.

4.3 Biodegradation

Biodegradation is the most important process in the bioremediation of contaminants by microalgae because it is this mechanism that results in the metabolic transformation of complex contaminants into simple and less toxic molecules. Moreover, the biodegradation of contaminants eliminates additional problems associated with the treatment of contaminated biomass. Biodegradation can be divided into metabolic degradation and cometabolism [6,16]. Biodegradation occurs in three stages, resulting in more soluble and less toxic compounds. In the first phase, the oxidative enzyme cytochrome P_{450} plays an important role in metabolism and is related to the oxidation, reduction, and hydrolysis of pesticides. Other enzymes, such as polyphenol oxidases and peroxidases, can also act in these processes. In the second step, pesticides or metabolites are conjugated with sugar, glutathione, or amino acids. The compounds produced in this step are converted in phase three into less or nontoxic substances (in relation to the original compounds), which are useful for the growth of microalgae cells [18,39].

During biodegradation, other compounds with equal or greater toxicity can be formed. Some microorganisms can generate amino-methyl phosphonic acid from the most common decomposition pathway by metabolizing glyphosate [40–42]. Furthermore, some physicochemical treatments, such as the sono-Fenton processes, UV and H_2O_2 treatments, ultrasonic irradiation, and TiO_2-induced photocatalysis, may result in more toxic compounds from the oxidation of the pesticide diazinon [7]. However, Kurade et al. [7] reported that using the microalgae *Chlorella vulgaris* in the biodegradation of this contaminant results in less toxic compounds, such as 2-isopropyl-6-methyl-4-pyrimidinol.

4.4 Photodegradation and volatilization

Photodegradation and volatilization processes can occur in a contaminated water treatment system during the bioremediation carried out by microalgae. Both processes can be improved based on the conditions used for microalgal cultivation. Photodegradation occurs by photolysis and can be performed by the absorption of light or by photooxidative degradation (which occurs from interactions with hydroxyl radicals) [3,16]. Volatilization processes occur because of the loss of volatile organic compounds, which can be facilitated by microalgal cultivation conditions, such as adequate aeration and high temperatures [16,29]. However, volatilization processes also depend on contaminant characteristics [23].

5 Main factors involved in the bioremediation of pesticides by microalgae

Bioremediation of pesticides is related to microalgae survival conditions, contact time between microorganisms and pesticides, and environmental and operational factors. When it comes to the removal of organic pollutants, factors such as cell size, the microalgal strain, biological activity, and microalgal morphology can have a substantial influence [26]. The mechanisms of pesticide removal by microalgae can depend on their surface-active groups, cell wall properties (bioadsorption), and enzymes (biodegradation and bioaccumulation) [6]. During bioremediation, chlorophyll and carotenoids can be used as biomarkers to detect

the exposure of microalgae cells to toxic compounds from pesticides [7]. The selection of microalgal species is one of the first steps in the bioremediation process and, associated with cultivation techniques, can influence EPS production, which can aid or hinder the processes of bioadsorption of contaminants in the extracellular medium [36]. Other factors that influence the speed of bioremediation are related to the chemical structure of pesticides, functional groups, molecular weight, water solubility, concentration, and toxicity of the pesticide [27]. Understanding these factors can improve the operational conditions of the processes intended for biomass productivity, consequently improving bioremediation efficiency [10].

Generally, higher pesticide removal by microalgae occurs with higher exposure times [7]. Hultberg et al. [43] observed that the exposure of *Chlorella vulgaris* in the remediation of 10 pesticides for 4 days proved to be more effective than in the short-term (1 day). The authors observed a reduction of approximately half of the pesticides (e.g., carbofuran, terbuthylazine, carfentrazone ethyl, and difenoconazole). Hu et al. [1] applied *Chlorella* sp. to the removal of the herbicide atrazine (a highly used pesticide worldwide) and evaluated the bioaccumulation and toxicity of degradation products during this process. Within 8 days of exposure to atrazine, the removal efficiency was >83% (Table 4.1). In another study, the microalga *Chlorella vulgaris* exhibited higher removal (94%) of the pesticide diazinon than other strains (*Chlamydomonas mexicana*, *Scenedesmus obliquus*, and *Chlamydomonas pitschmannii*). The authors reported the biodegradation of diazinon by *Chlorella vulgaris* with removal efficiencies of 100%, 87.67%, and 93.31% at insecticide concentrations of 0.5, 5, and 20 mg L^{-1}, respectively (Table 4.1). The authors also observed the formation of a less toxic compound after bioremediation [7].

Bioremediation mechanisms depend on hydraulic retention time. Bioaccumulation, for example, can be increased by saturating the biodegradation capacity. Moreover, the bioaccumulation process generally does not occur at the beginning of remediation. Additionally, extended periods of exposure may result in microalgae adapting to contaminants, making them more resistant to the stress caused by high concentrations of pollutants [38]. Some species experience growth inhibition when exposed to high concentrations of pesticides, whereas others have a natural tolerance. For example, *Arthrospira fusiformis* and *Spirulina platensis*, exhibit resistance when exposed to glyphosate concentrations of 0.01–10 mM. However, these two strains cannot use phosphonate as the only source of phosphorus, which in turn does not allow the glyphosate molecule to break down. Unlike these strains, other cyanobacteria, such as *Anabaena* sp., *Leptolyngbya boryana*, *Microcystis aeruginosa*, and *Nostoc punctiforme*, were able to metabolize glyphosate [44]. The tolerance of some cyanobacteria can be explained by the resistant form of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, in addition to the ability of the strains to metabolize glyphosate, using it as a source of phosphorus [42,45].

Thus, to suppress the toxic effects of the contaminants, microalgae can be preacclimated using subtoxic concentrations of the contaminants before bioremediation. Acclimatization reduces the toxic effects of contaminants by improving cellular function and metabolic processes [10]. The improvement of the strains can also occur through the adaptive evolution of microalgae to specific contaminants. Studies have shown promising results using these methods [46,47]. Among the environmental and operational mechanisms involved in bioremediation, temperature stands out in bioadsorption, mainly because it is a thermodynamic process [10]. Temperature is also essential for the cultivation of microalgae, which can

increase the growth rate and the process of adsorption of contaminants [16]. Microalgae can adapt to a wide range of temperatures in general, but the most commonly used prefer temperatures between 25°C and 30°C [48].

In addition to temperature, pH is an intrinsic factor in microalgal culture and is related to the ionization or dissociation of contaminants in bioadsorption processes [29]. Additionally, the degradation of fungicides, such as iprodione, can be accelerated under abiotic conditions, such as in an alkaline medium to which microalgae are adapted [49]. Li et al. [46] demonstrated changes in pH through respiration and photosynthesis of *Chlorella pyrenoidosa* and the influence of these parameters on the degradation of 2,4-dichlorophenol, a compound used to produce pesticides. The authors reported that pH influenced the formation of chlorophenol compounds in water. Thus, for its degradation using *Chlorella pyrenoidosa*, a pH close to neutrality was considered ideal. Luminosity is another environmental factor of substantial importance to microalgal cultures (growth rate and production of biocomposites) and photodegradation processes. According to Remucal [50], light intensities relevant to photodegradation processes are wavelengths greater than 290 nm. Light intensity and photoperiod also have a considerable influence on microalgal growth. Higher light intensities affect the photosynthesis rate of microalgae, increasing the productivity of biomass and production of compounds, such as carbohydrates and lipids, which are used in the production of bioethanol and biodiesel, respectively [51,52].

6 Techniques used to increase pesticide removal from water

6.1 Consortia between microorganisms

The use of consortia between bacteria-microalgae/cyanobacteria is well known because, in practical applications of wastewater treatment and water resources, these microorganisms share the same space [6]. These systems create an equilibrium environment in which bacteria use the oxygen produced by microalgae from photosynthetic processes as an electron acceptor to degrade organic matter and organic carbon. By using oxygen for respiration, bacteria produce CO_2, which is accessible to microalgae, in addition to other compounds, such as siderophores, phytohormones, and vitamin B [53–55]. These consortia create cooperative interactions and competition, demonstrating greater resistance to the presence of invasive species and reducing costs for aeration [16].

However, the cultivation conditions of these microorganisms can be different, mainly concerning environmental factors, such as pH, temperature, and light intensity. Other factors are related to the availability of nutrients and the growth phases and conditions of these microorganisms. Furthermore, the production of toxic metabolites by microalgae (exotoxins) and bacteria (phycotoxins) can act as growth inhibitors [56]. Regarding the use of microalga-microalga consortia, the main advantages involve higher pollutant removal rates, higher productivity of biomass, and the production of compounds, such as lipids. This alternative is considered a promising strategy for the treatment of polluted water and biofuel production [57–59]. In these consortia, the cooperative interactions between microalgae increase, making the systems more resistant to external environmental conditions and invasion by other species,

and increasing the capacity for the absorption of nutrients and contaminants [60,61]. Avila et al. [3] evaluated the removal of hydrophobic pesticide compounds using a microalgae consortium (Table 4.1). The authors observed that total degradation of pesticides resulted in the removal of chlorpyrifos (35%), cypermethrin (14%), and oxadiazon (55%), with *O,O*-diethyl thiophosphate identified as the chlorpyrifos transformation product. The sorption of chlorpyrifos, cypermethrin, and oxadiazon to the solid phase of microalgal biomass was 62%, 60%, and 33%, respectively.

Consortia of fungi-microalgae were also used to remove organic contaminants, such as pesticides. In these systems, the degradation of pollutants with high molecular weights can be facilitated by the action of extracellular enzymes produced by fungi. Additionally, these systems can assist in the sustainable harvesting of microalgae and promote the biorefinery system [62]. Hultberg and Bodin [63] used *Chlorella vulgaris* with the fungus *Aspergillus niger* for the bioremediation of several pesticides. The authors reported that the formation of biopellets by these microorganisms make the treatment of 16 of the 38 pesticides evaluated more efficient. Among the pesticides, carfentrazone ethyl, phenmedipham, difenoconazole, and trinexapac ethyl were below the detectable limit. According to the study, this system (biopellets) could facilitate biomass harvest, directly impacting costs.

6.2 Immobilized microalgae cultivation

Suspended cultivation techniques are more common than immobilized cultures, mainly because of the cost of the process. However, issues related to the harvesting of microalgae could result in difficulties in applying this cultivation method. Thus, microalgal immobilization is an alternative for optimizing processes, protecting cells from photoinhibition, and offering greater resistance to stressful environments caused by salinity or toxicity [29,60]. The immobilization process consists of maintaining the cells alive and metabolically active within a natural or synthetic hydrophilic matrix, limiting the mobility of the cells, and allowing the filtration of water. In some cases, dead biomass can act as an adsorbent through a passive process [64]. Several natural matrices (e.g., alginate, luffa sponge, carrageenan, collagen, cellulose, agar, and agarose) and synthetic matrices (e.g., polyurethane, acrylamide, and polyvinyl) were considered for the immobilization of microalgae [65,66]. Polymeric matrices of calcium alginate are preferred for microalgal immobilization. Polymeric materials have characteristics, such as low cost and transparency, that are considered essential for microalgal cultivation [67,68]. However, although alginate matrices are easy to handle, several factors, such as insufficient alginate concentration, Ca^{2+}, and cell saturation, can affect the development of microalgal spheres. These factors can lead to the rupture of capsules and inefficiency in the harvesting process [68].

The efficiency of microalgae immobilized for bioremediation processes can also be influenced by high cell densities (e.g., 5 g L^{-1}), the number of matrices added to the medium, and factors related to microalgae survival conditions, such as light intensity and photoperiod. There are numerous challenges for immobilizing microalgae on a large scale, such as the stability of the capsules, use of suitable matrices, commercialization, and preservation of cells [69]. However, this process has great potential for wastewater treatment.

7 Challenges and future trends

The remediation of wastewater with pesticides using microalgae has demonstrated advances in recent years. Algae-based systems can be highlighted as an alternative to improve the environment. Sutherland and Ralph [16] described microalgae cultivation associated with the removal of emerging contaminants as a cost-effective option. However, other researchers reported that more investigation is needed regarding the quality of wastewater resulting from microalgae treatment. Furthermore, the need for life cycle analysis and an evaluation of bioremediation beyond laboratory conditions is also pointed out [10]. According to Sutherland and Ralph [16], multicontaminants displayed for microalgae remediation could be challenging for one species to metabolize; thus, consortia application could emerge as a much more viable solution. Achieving higher productivity could also help overcome economic issues [26].

Despite demonstrating the high removal content of pesticides, some microalgae were negatively affected by the pesticides or their degradation products. The inhibitory effects of these compounds were observed in a study conducted by Hu et al. [1]. In this study, degraded atrazine inhibited *Chlorella* sp. growth more than pure atrazine; however, inhibition occurred in both treatments. The authors attributed the inhibition caused by pure atrazine or its degradation products to the reduction in light absorption and utilization, resulting in lower photosynthetic efficiency. Kurade et al. [7] observed a reduction in *Chlorella vulgaris* growth and in pesticide removal efficiency with higher concentrations of diazinon (40 mg L^{-1} and 100 mg L^{-1}) (Table 4.1). According to the authors, high concentrations of the pollutant can cause cell membrane rupture and exposure of intracellular compounds to toxic compounds. Furthermore, Sutherland and Ralph [16] reported that the bioaccumulation of emerging contaminants by microalgae cells might lead to ROS production, and consequently, can cause cell death.

Regarding the mechanisms operated by microalgae during the remediation of pesticides, biodegradation can be considered the most promising because it does not result in biomass with pollutants. Instead, pesticides are transformed into less toxic compounds [10]. In this context, Sutherland and Ralph [16] suggested optimizing the conditions (e.g., enzyme properties and production) in certain microalgae species to stimulate higher degradation levels of the contaminants. In a recent review, Nie et al. [6], considered some interesting approaches to increasing pesticide removal by microalgae and were supported by other authors [10]. According to Nie et al. [6], novel approaches with a possible future could be screening and domesticating certain microalgae strains, cocultivation of microalgae and bacteria, and microalgae immobilization. According to the authors, the first refers mainly to the possibility of obtaining strains that grow in extreme conditions, such as wastewater, and can degrade toxic compounds. The second approach is also interesting; the authors exemplify the possibility of microalgae providing O_2 for bacterial growth, whereas bacteria offer CO_2 for microalgal use as a substrate. The third approach refers to the immobilization of microalgae to improve remediation steps by protecting the cells and allowing their reuse. Recently, Hu et al. [69] reported that *Chlorella saccharophila* agar-immobilized cells maintained high efficiency in wastewater treatment after recycling several times. The literature also suggests genetic modification of microalgae as another option for future researchers to address, aiming to improve pesticide removal [6]. Xiong et al. [35] indicated similar approaches for improving the removal of pharmaceutical contaminants by microalgae.

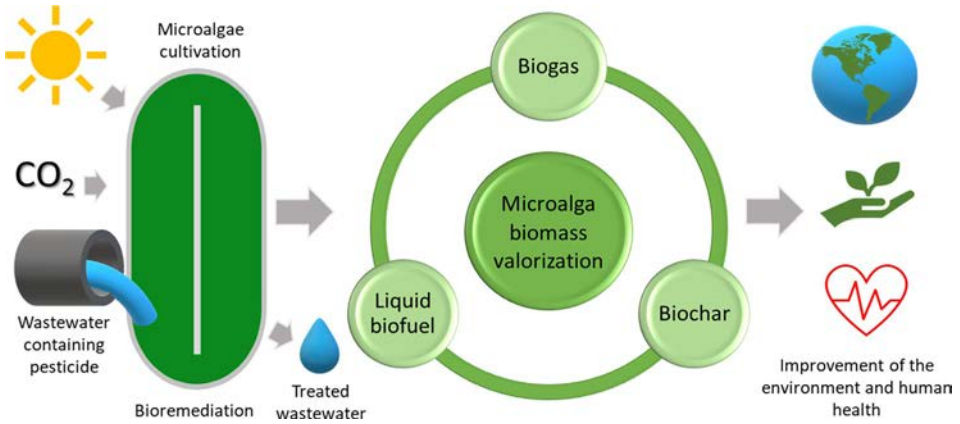

FIG. 4.2 Removal of pesticides by microalgae and possible alternatives for biomass valorization.

To improve the economic feasibility of the processes involving microalgae for bioremediation and wastewater treatment, the valorization of the resulting biomass is important and can be performed using the biorefinery approach [3,9] (Fig. 4.2). However, according to Nie et al. [6], toxic substances in biomass should be addressed to avoid negative effects during biomass application. In a recent study conducted by Avila et al. [3], after bioremediation of pesticide active compounds, the resulting biomass containing pesticides was used for biomethane production. The authors observed that biogas production was not inhibited by pesticides in the biomass, confirming the possibility of integrating these processes, resulting in biomass valorization.

8 Conclusions and future outlook

Microalgae have been exploited in recent years as important organisms that metabolize some pesticides, using them as energy sources while performing bioremediation of the environment. The removal of certain pesticides from soil and water generally results in the transformation of toxic compounds into less toxic products. This chapter summarizes the mechanisms involved in this process (biodegradation, bioadsorption, and bioaccumulation) and highlights the main factors that affect bioremediation. In general, the activity of the microalgae mechanism during bioremediation depends on surface-active groups, cell wall properties, and enzymes. Based on the present outcomes, *Chlamydomonas* spp. and *Chlorella* spp. are considered promising strains. Furthermore, more research should be conducted on new microalgae strains isolation, different consortia with microalgae, and immobilized microalgae to improve the feasibility of bioremediation by these microorganisms. Actions that promote process scale-up and assessment of the quality of effluents are also important when it comes to bioremediation by microalgae. This chapter also demonstrated that microalgae biomass originating from bioremediation could be considered a key to transforming this into a cost-viable process because the biomass can be used for bioproducts production. Thus, studies focusing on biomass valorization are welcome.

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CHAPTER

5

Algae harvesting: Application of natural coagulants

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OUTLINE

1 Introduction

Microalgae have been cultivated to produce diverse bioproducts for the food, agricultural, cosmetic, and pharmaceutical markets [1]. Their extracts include high-value compounds such

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as pigments, lipids (as omega-3 polyunsaturated fat acid), oils, natural dyes, sugars, and antioxidants [2]. The biomass can also be used as fertilizer, increasing the availability of nutrients (such as nitrogen, phosphorus, potassium, and other trace elements) on the soil, protecting against pathogens and pests, and stimulating plant growth and development [3]. Algae can also be utilized for bioenergy purposes, such as the production of biodiesel, bioethanol, and biogas [4].

Apart from these end-market products, microalgae can be grown to remove pollutants and recover highly valuable products found in wastewater (organic and inorganic compounds) [5,6]. Even though it is considered a secondary pollutant due to the inadequate discharge of domestic wastewater, microalgae have been shown to be a promising solution for the treatment of effluents. Microalgae can effectively assimilate nitrogen, phosphorus, CO_2, and other macro- and micronutrients from nutrient-rich wastewater to fuel their growth, which can be used to produce bioenergy, biofertilizers, bioplastics, dyes, and chemicals [7–9].

Microalgae growth can be inhibited due to the high-level presence of organic matter and oxygen dissolved in the wastewater, impairing the efficiency of the treatment [10,11]. For this reason, several studies have used a pre-treatment before the cultivation of microalgae, with an emphasis on anaerobic digestion (AD) [5,11–17]. At wastewater treatment plants (WWTP), microalgae are applied after the AD as a tertiary process to treat domestic wastewater (segregate black water). AD can recover the organic matter [18] but conserves the nitrogen and phosphorus in the wastewater [19] that can be incorporated by the microalgae during the post-treatment [20,21]. Macro and micronutrients present in the wastewater are recovered [22] as a response to the scarcity and depletion of resources and to encourage economic, social, and environmental sustainability [23]. *Chlorella* sp. and *Scenedesmaceae* are examples of species used at WWTP [24]. They have a fast growth rate, high economic value, and guarantee the quality of the final effluent [20], but its separation from the effluent continues to be a challenge as this step can represent up to 60% of the total process cost [25] (usually 20%–30%) [26].

The high cost of algal biomass production associated with the stages of cultivation, harvesting, and processing, is the main bottleneck to commercial-scale implementation. Therefore, cultivation and commercial applications of biomass strategies are fundamental for the consolidation of technological routes and economically and technically viable production [27,28].

Harvesting methods have been studied for more than 50 years [29]. They can be chemical, physical, or biological [30] and they should guarantee that both biomass and effluent are recovered for further use [31]. The separation process should strive for low cost, ease of operation, low energy consumption, and large-scale applicability [32]. For example, in the case of biodiesel production, the microalgae must be concentrated as much as possible to simplify the following steps, such as the cell disruption process aimed at amplifying the recovery of lipids [33].

There are several methods available, such as coagulation/flocculation (bio, electro, or auto), sedimentation, flotation, centrifugation, filtration, and combinations of those [34] followed by dewatering (using a rotary press or filter press) [35]) and drying by freeze-drying (on lab scale), spray drying, convective drying, or solar drying [36]. The selection of the dewatering and drying processes suitable for the microalgae will depend on their suspension properties (as concentration, pH, cell size, shape, density, and others), the final target products (downstream process), and costs associated [36].

The most conventionally applied technology for microalgae biomass separation and harvesting on a large scale is coagulation, flocculation, and gravitational sedimentation steps [33]. Coagulation and flocculation are more attractive than centrifugation, for example, but there are challenges associate to the use of efficient techniques and the problems of contamination of biomass, especially when used for the production of food, drugs, and cosmetics [37]. The choice of the coagulant is a determinant for both the separation performance and biomass harvesting. A coagulant is usually chosen according to the solution (pH, cell concentration, ionic strength) and the microalgae species (surface charge of the particle), although price and availability are common constraints [38]. The coagulant can affect subsequent processes and its characteristics could make unfeasible further biomass applications.

Chemical coagulants are more widely utilized and consist of aluminum or iron salts (aluminum sulphate, ferric chloride, and others) [39]. These coagulants are cost-effective and provide high coagulation performance (improving the catalyst process for thermochemical conversion). However, they are inorganic, potentially toxic, non-biodegradable, and their presence could generate contamination in the medium and on the final algae biomass, damaging the cell structure, which reduces their utility [40] and increasing the biomass ash content, with a consequent reduction of their heating value [39].

Organic coagulants, or natural-based coagulants (NBC), are presented as an ecological alternative with low toxicity and the capacity to be incorporated into the microalgae biomass bioproduct [25]. They are biodegradable, non-toxic, do not add metals to the biomass, generate less sludge, and guarantee the safety of the treated effluent [41]. Commonly used natural coagulants are based on tannin, chitosan, *Moringa oleifera* Lamarck seeds (MO), and others.

The coagulant characteristics and properties can impact directly on the final use of the biomass sludge (microalgae and coagulant) after sedimentation, as it can change the composition, pH, and other biomass features. Therefore, this chapter aims to address the application and characteristics of NBC, including its properties, coagulation mechanisms, and impact on the biomass characteristics.

2 Coagulation/flocculation mechanisms

A prerequisite for successful algae harvesting is the aggregation and removal of microalgae in solution via coagulation and flocculation processes. Coagulation makes the suspended algae cells' nature unstable, at the same time as flocculation increases particle growth through the formation of aggregates (also known as flocs). Poor harvesting results from inefficient coagulation and flocculation processes, potentially jeopardizing the entire process [42].

The coagulation and flocculation processes are affected by the coagulation characteristics (such as type, dosage, quality, lifespan, makeup solution, and dilution), medium characteristics (such as quality, concentration, temperature, and pH), and physical conditions (as flotation time and mixing intensity) [43].

The use of coagulation and flocculation for harvesting algae has been studied for a long time [44]. Algae have been harvested mainly with chemical coagulants, such as aluminum sulphate, with decades of specific tests on the coagulation process [45]. On the other hand,

polymers have been widely applied in physical-chemical processes for water treatment since the 1950s [46]. Flocculation and filtration aids are described as the main uses of polymers in the water industry [47], although their application as primary coagulants has been reported [48]. Recently, the harnessing of using naturally occurring or environmentally friendly materials, known as "green" coagulants, in the water industry has gained attention [49]. The intrinsic renewable properties, low toxicity, and ease of biodegradation of natural coagulants are especially attractive features of natural-based polymers for algae harvesting purposes.

Microalgal cells are hydrophilic, small in size (3–25 mm), and remain in suspension due to their low mass and negative charge balance [50,51]. The negative surface cell charge, as in other biological materials, is due to the cell wall composition (of polysaccharides, proteins, and lipids); and depends on the species, cultivation medium, pH, and other environmental conditions [52]. Polymeric flocculation is a natural technique for harvesting several kinds of microalgae [50]. The coagulants' function is to destabilize the cell's particles by reducing their repulsive forces and allowing the attractive forces to act in order to form aggregates [52] and their performance depends on medium pH, algae concentration, algae characteristics, and other parameters [51].

Charge neutralization and electrostatic bridging are important mechanisms for algae destabilization and flocculation via chemical aids [50], and adsorption is a crucial requirement for both mechanisms [48]. The electrostatic patch is a specific technique for charge neutralization that has been developed. For all mechanisms, charge density (CD) and molecular weight [53] of ionic polymers are determinants. These mechanisms are presented in Fig. 5.1.

Polymer adsorption on the surface occurs at many attachment points and is often considered irreversible. At equilibrium, distinct types of configurations are assumed for the polymer chain: trains attached to the surface; tails, projected into the solution and loops between trains can be formed between the polymer and the algae surface. The extent of each one depends upon polymer-solvent interactions, such as electrostatic, hydrogen bonding, and ion binding [48].

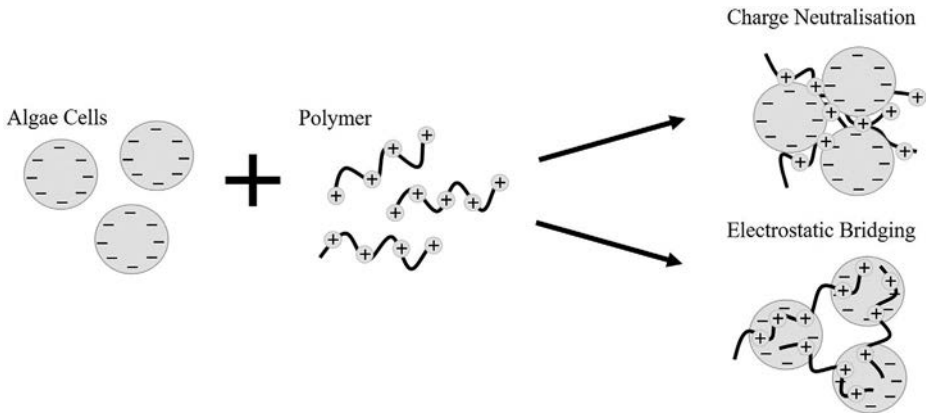

FIG. 5.1 Main coagulants mechanisms for algae harvesting [54,55].

Polymer coagulants can be produced by the polymerization method in the form of liquid, beads, powder, emulsion, and dispersion, with a linear, branched, or cross-linked structure chain [43]. Natural polymers have intrinsic cationic properties with CD generally pH-dependent [48], and they are more suitable for inducing neutralization of negative charges of algae [50], with the reduction of the electrical repulsion between particles [48]. Solubility, dosage, and algal solution characteristics are equally important in charge neutralization, and charge reversal can also occur. Aggregates produced from charge neutralization are small, and binding is not as strong as bridging, but re-flocculation is more likely to occur once flocs are broken [48]. Polyelectrolytes with high CD are more likely to succeed in this mechanism.

The loops and tails projected into solutions for a long-chain polymer during adsorption can favor the attachment to other particle sites during flocculation. This phenomenon is known as polymer bridging and is very important in practice [48]. Much stronger and bigger aggregates are formed during the bridging mechanism than those formed through charge neutralization, with higher settling velocities and shear resistance.

3 Organic coagulants

The stage of selection of the flocculating agent with the aim of commercial exploitation of algae biomass plays a crucial role. In addition, both the toxicity of the flocculating agent and that of the culture medium employed are decisive for its commercial application. Although the benefits, such as the reduction of costs and humidity linked to the biomass collection stage, are well known, there are also negative impacts, and the final quality of the biomass must be attested for its commercial targeting [54].

Chemical coagulants and synthetic polymers, such as aluminum sulphate ($Al_2(SO_4)_3$), ferric chloride ($FeCl_3$), aluminum polychloride (PAC), and others, are commonly used in treatment systems, as previously described. Therefore, the applications of these inorganic compounds can contaminate the biomass with metallic salts and produce toxic wastewater, in addition to presenting unacceptable conditions for reusing generated by-products [30,56,57].

It is possible to observe that the selection of the coagulant to be applied is of great relevance since the ideal is to apply a low-cost, effective in low doses, non-toxic, and extracted from renewable resource products [56–58]. Several scientific studies have focused on using natural-based coagulants (NBC), as they are biodegradable, efficient, and economically viable products that do not harm the environment [47,57,59–61]. However, these studies are still scarce in the definition of parameters for the separation process [62] and characteristics of by-products for further application [63].

Natural polymers (such as tannin and chitosan) are the most common NBC studied, but developing countries have been focusing their studies on the different species of plants present in their territory that can be applied as NBC [58]. Such species can be applied in a simple and traditional way, or after some change in their chemical structure. An advantage of using local plant species is the availability throughout the year and the price of the coagulant [64,65].

Even with the advantages of NBC applications, there are some barriers to their commercialization on a large scale, mainly due to the extraction method standardization, including the time consumed and controllability during their production [58]. Chemical, physical, and thermal characteristics impact the NBC's performance and the way they act predominantly through electrostatic bridging and charge neutralization [56,66–68]. Therefore, studies have been conducted to improve extraction methods, coagulant characteristics, and application performance [58].

The following sections discuss natural polymers (tannin and chitosan), cationic starch (CS), directly plant-based coagulants (such as *Moringa oleifera* seeds), and microbial flocculants (MBF).

3.1 Tannin

Among the natural coagulants, those derived from tannins have stood out [69,70]. Tannins are natural polymers derived from secondary plant metabolites such as bark, fruits, leaves, and seeds. They are usually extracted from the bark from of *Acácia*, *Castanea,* and *Schinopsis* trees [71]. The compounds present in the barks are modified by physical-chemical processes, due to the introduction of an amine group in its chemical structure, making it possible to guarantee its coagulant properties [72]. The coagulant prepared from vegetable tannins is characterized by being cationic, low molecular weight, dark in color, and high viscosity [73].

Studies indicate that the vegetable tannins extracted from *Acácia Mearnsii* can guarantee a good coagulation/flocculation of microalgae, even for marine species. AFlok-BP1 which demonstrated effective results (more than 90% efficiency) to harvest *Nannochloropsis* sp. [67], and Tanfloc SG which was used to to harvest *Monoraphidium contortum* (with a 99% efficiency) [74].

Brazil stands out to produce tannin-based coagulants due to the highest concentration of tanning trees in the world. Therefore, the country has been increasingly exploring the use of tannin as an NBC for the treatment of water and wastewater [66]. In Brazil, the company TANAC produces tannin-based coagulants (Tanfloc), essentially of vegetable origin, with low molecular weight and acting at pH 4.5–8 that could be used to separate microalgae grown in different culture media, presenting a satisfactory biomass recovery with good sedimentation speeds [73].

3.2 Chitosan

Chitosan is a non-toxic natural biopolymer produced from chitin, a component found in the exoskeleton of crustaceous animals (such as shrimp, crabs, and lobster shells) by the alkaline deacetylation process [75]. It has been considered the second most abundant biopolymer globally, after chitosan, as there is a large amount of chitin produced by the seafood industry without destination [30,76]. Due to its biological and physicochemical properties, Chitosan can be applied in the cosmetics, pharmaceutical, biotechnology, and nutrition industries [76].

Chitosan has a high cationic charge, with long polymeric chains, in which it provides a satisfactory removal of algal cells due to the presence of the amine functional group, making it possible to adsorb microalgae surfaces to strongly destabilize them. This coagulant has been

used in water treatment [77,78], industrial wastewater treatment [79], and has had positive results in harvesting microalgae even at small concentrations (99% harvesting of *Chlorella* sp. applied at 10 ppm) [56]. More research on algae harvesting from domestic WWTP is required, but chitosan application has already presented effective results for harvesting several microalgae species, demanding low dosage rates for several industrial tertiary wastewater treatments [30,76,79].

3.3 Cationic starch

Cationic Starch (CS) is obtained from natural starch sources (corn, maize, wheat, potatoes, rice, oat, and cassava) [80–82] by a simple and economic synthesis where glucose hydroxyl groups of the starch are substituted by quaternary ammonium groups through etherification done using glycidyltrimethylammonium chloride (GTAC) or another cationic compounds such as 3-methacryloyl amino propyl trimethyl ammonium chloride (MAPTAC) [37,83]. The Hydra Greenfloc 120 and Cargill C*Bond HR 35.849 are two commercial CS examples, with the first typically used in wastewater treatment and the second in the paper manufacturing industry [80].

Using CS presented good efficiency in the microalgae flocculation. Some examples are presented as follows. *Chlorella protothecoides* were harvested with commercial CS (Greenfloc 120) [84], while cationic cassava starch was successful in harvesting *Chlorella* sp. [53], and CS produced from wheat, potato, and corn presented high efficiency (with no significant difference between them) on the harvesting of *Chlorella pyrenoidosa* and *Botryococcusbraunii* [85]. Cationic maize starch obtained similar results to $Al_2(SO_4)_3$ on the harvesting of microalgae from a WWTP; in this case, it had the advantages of being applied in lower concentrations than the chemical coagulant and being able to inhibit the growth of *E. coli* [81].

Cationic starches do not significantly alter the pH value and are less toxic than chitosan [80]. Even though the synthesis is not expensive, and it adds a chemical modification compared to other-directed used coagulants, which increases the costs. However, the primary influence on the final price of the coagulant is the starch price [53,85]. Additionally, it is widely available and did not damage the algae's properties. Therefore, the harvested biomass could be used used as a bioproduct to further applications [53,81]. One disadvantage of cationic starches is that their use competes with more priority uses, as starches are consumed as food or used by the paper and adhesives industries [53,80].

3.4 Plant-based coagulants

Organic polymers can be produced by a huge variety of plants and their components (from the seeds to the fruit). They are natural and water-soluble coagulants and can be cationic, anionic, poly-ionic, or non-ionic [68]. Examples of plant species that have coagulating properties are the family of *Acanthaceae*, *Anacardiaceae*, *Annonaceae*, *Araceae*, *Cactaceae*, *Capparidaceae*, *Malvaceae*, *Moringaceae*, *Papilionideae*, and *Tliaeae* [86].

Hibiscus esculentus fruit (ripe and dried), known as "Okra" can be used as an anionic coagulant. The polysaccharides that make up the fruit of mucilage are water-soluble and have the ability to coagulate algae cells. Okra can also be used as a gum produced from the liquid fraction from the seeds grinding [87]. Okra has an efficiency similar to other natural coagulants,

commonly applied for the treatment of domestic and industrial effluents and raw water for public supply. However, just like cationic starches, its use competes with more priority uses, such as its consumption in food, and may limit its use as a natural coagulant [88,89].

Moringa Oleifera (MO) is another plant-based coagulant that has been standing out for microalgae harvesting. MO is one of the 14 tropical plant species belonging to the *Moringaceae* family. The species *Moringa Oleifera Lamarck* and *Moringa Stenopetola* present the best coagulant properties compared to the other species [90]. Being a widespread species with good adaptation conditions in different regions of Brazil and other countries of the world, such as India, Egypt, Philippines, Thailand, Malaysia, Pakistan, Singapore, Jamaica, and Nigeria, it is considered an easily accessible raw material [91].

The cultivation of MO does not require any kind of pre-treatment before germination [92]. It is easily grown and adapted to low rainfall and well-drained soils [93,94]. Its trees can reach between 5 and 12 m in height, the fruit reaches 30 cm in length, and the seeds without the husk are white and soft when freshly harvested [95].

In addition to being a renewable resource, their seeds have low toxicity [96], a protein content that is comparable to cationic polyelectrolytes, and an isoelectric pH of 9–10, which when added to water ensures effective turbidity removal and contributes to biomass recovery [97–99]. The seeds achieve 23% to 40% proteins [40,100], divided into globulin, albumin, and prolamin as the main constituents of positive charges that cause the destabilization of negatively charged particles [99]. These seeds' coagulant properties promote the removal of suspended particles, bacteria, metals, and algal cells in the wastewater, and therefore, guarantee a sludge with satisfactory characteristics for reuse [72,93,96,101,102]. The seeds can be applied as a whole powder, defatted powder, in an aqueous solution, or saline solution [103]. In order to achieve the optimum coagulation properties, the seeds must have a white color, good texture, and consistency, without the presence of moisture, fungi, or bacteria [103]. The seeds must be freshly harvested, because their biodegradability, and therefore, their coagulant properties may decrease over time [104,105].

The coagulation mechanism by which MO seeds can function has been thoroughly studied by the scientific community. The seeds prepared in the form of integral powder, defatted powder, and aqueous solution act as the mechanism of neutralization of charges [101,102,106,107]. A distinct case applies to seeds prepared in saline solution, in which the bridging mechanism operates [101].

When used for the coagulation of microalgae, MO (at a 30 mg/L concentration) achieved a removal efficiency of 93.6%, higher than chitosan and poly-aluminum chloride (PAC) [108]. MO was also successful in harvesting *Chlorella sorokiniana* from digested wastewater [109]. The preparation and application of MO seeds as a natural coagulant are inexpensive and straightforward, and MO does not negatively impact the environment or microalgae biomass, which is an ideal characteristic for its further application [96,98,104,110].

3.5 Microbial flocculants

Microbial Flocculants (MBF) have been applied in industrial and domestic wastewater treatment, fermentation, and algae harvesting [111]. They can be divided into extracellular metabolic, intracellular extractive, and bacterial flocculants [112]. MBF is polymer substrates produced from bacteria, fungi, or algae. Some species reported in the literature are *Bacillus*

sp., *Chlorella* sp., *Corynebacterium* sp., *Paenibacillus* sp., *Rhodococcus* sp., *Scenedesmus* sp., *Trichoderma* sp., and others [113]. MBF is an effective flocculant from a low-cost security source [112].

This method requires a large amount of energy due to the long mixing time [51]. It usually also needs the addition of an organic substrate to induce the growth of the microbial coagulant in situ. They can be acetate, glucose, or glycerine, which could be obtained as by-products from anaerobic digestion or biodiesel production, which would reduce the costs [111]. An example of the use of this harvesting method was demonstrated with *Pleurochrysiscarterae* with 90% efficiency by the addition of organic substrate in a low concentration (0.1 g/l) and a mixing time of 24 h [111]. Algae self-immobilization can also happen by an aerobic granulation process that generates high settleability algae granules with high phosphorus content [114].

Research on the application of MBF is still on a laboratory scaled-up and needs to be scale-up to become feasible and more economical than the application of other coagulants. In addition, the flocculation mechanisms need to be studied in-depth, as they can also include chemical reactions [112,113].

4 Harvested biomass

The harvested biomass can generate diverse bioproducts. Therefore, making full use of biomass and implementing biorefineries to stimulate the circular economy and expand the sustainability of processes and products is required. A promising alternative is the recovery of high-added-value biomolecules for their use by the pharmaceutical, nutraceutical, and cosmetics industries and the use of residual biomass in energy conversion processes and/or animal feed production (fish, shrimp, poultry, etc.) [115–118]. Previous studies on the characterization of algae biomass are fundamental to the selection of target species for biotechnological and energy purposes and, therefore, commercial exploitation.

Fig. 5.2 illustrates a biorefinery proposal using algal biomass harvested with NBC. It demonstrates the recovery of target biomolecules, the use of residual biomass in energy conversion processes (ethanol production, biogas, biodiesel, synthesis gas, etc.), and the possibility of integration with waste biorefineries.

The production of bioactive compounds usually requires the use of monocultures and controlled cultivation systems [119–121]. Currently, the generation of algal biomass from effluents or other contaminated sources limits the purpose of marketable products. Among other commercial uses, leftover ash from the energy conversion process may be used in civil construction and asphalt materials to produce energy products.

There is generally no preferred method for collecting biomass, as factors such as energy consumption and necessary capital must be considered [33]. For products with low added value, such as biodiesel, the most promising option for collecting microalgae would start with flocculation (pre-concentration stage) combined with later sedimentation stages, ending with centrifugation or filtration. For high value-added products, continuous centrifugation (the technique with the highest operating cost) is used in industrial plants because it is fast and has greater efficiency (even though it is an energy-intensive and, consequently, high-cost

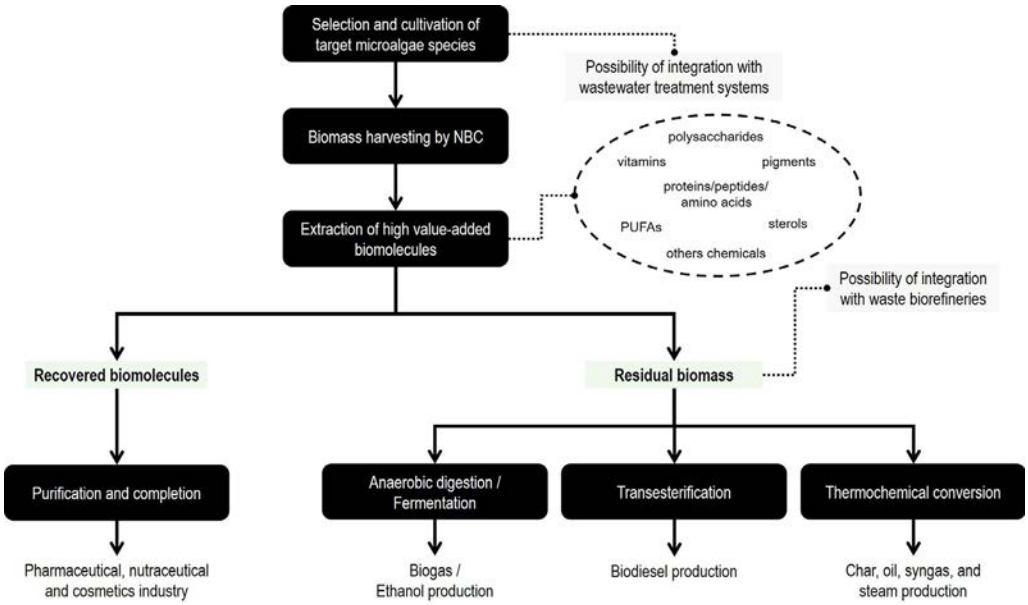

FIG. 5.2 Application routes for algal biomass harvested with natural coagulants (NBC).

methodology), in addition, it guarantees the quality of nutraceutical and/or cosmeceutical products [122–124]. However, the exposure of cells to high magnitudes of gravitational force and shear stress can damage the cell structure, making it impossible to store it for long periods [33].

4.1 Biomolecules

Tables 5.1 and 5.2 show that potential high value-added biomolecules obtained from microalgae include, but are not limited to, astaxanthin, β-carotene, zeaxanthin, lutein, lycopene, phycobiliproteins (phycocyanin, allophycocyanin, phycoerythrin, and phycoerythrocyanine), and polyunsaturated fatty acids (omega-3, eicosapentaenoic acid—EPA, and docosahexaenoic acid—DHA) [27,125–127].

The costs of producing algae biomass and high-value added biomolecules are linked to the characteristics of the selected target species (photosynthetic efficiency, growth rate, acclimatization potential in adverse conditions, etc.), to the cultivation systems (closed or open), to the cultivation conditions (pH, temperature, carbon source, etc.), and among other factors. Therefore, the selection of biomass cultivation, harvesting, and processing techniques that are most appropriate and aligned with the specificities of the desired target product, directly influences the economic and energy feasibility of the production process. In addition, the reuse of the culture medium after collection is an action that can reduce production costs and increase the sustainability of the production chain [128,129].

TABLE 5.1 Biomolecules are obtained from the biomass of microalgae and their main uses for food, medicines, and cosmetics.

Use	Biomolecules	Main commercial uses
Food	Folic acid, astaxanthin, β-carotene, carbohydrate, inositol, proteins, pro-vitamin A, vitamin E, B vitamins (B1, B2, B3, B7, B6, and B12)	• Production of food colors, teas, soups, pasta, bread, cookies, sweets, and ice cream; • Elaboration of human health dietary supplements such as pills, tablets, capsules, and fortified nutritional mixtures; • Feeding of fish, shrimp, mollusks, and crustacean larvae; • Vegan food.
Medicine	γ-Linolenic acid, polyunsaturated fatty acids (DHA and EPA), essential amino acids (isoleucine, leucine, and valine), astaxanthin, α-carotene, β-carotene, cryptoxanthin, phycocyanin, glucan, glutathione, lutein, pro-vitamin A, vitamin B1, vitamin B12, vitamin K, zeaxanthin	• Hypocholesterolemic and hepatoprotective agents during malnutrition; • Prevention of schizophrenia, dermatitis, multiple sclerosis, diabetes, rheumatoid arthritis, and viral infections; • Anti-tumor, antioxidant, anti-hypertensive, and anti-inflammatory action; • Reduction in cholesterol and blood sugar levels; • Production of analgesic drugs and to improve the immune system.
Cosmetic	Phenols (gallic, caffeic, salicylic, p-coumaric, and ferulic acid), glycerol, pigments (α-carotene, β-carotene, and phycocyanin)	• Preparation of facial and body regenerating creams and moisturizers; • Production of hair lotions, sun protection, and stimulating collagen synthesis.

Adapted from W.G. Morais Junior, M. Gorgich, P.S. Corrêa, A.A. Martins, T.M. Mata, N.S. Caetano, Microalgae for biotechnological applications: cultivation, harvesting and biomass processing. Aquaculture 528 (2020) 735562.

NBC has been proven to have no adverse effects on algal characteristics when used as a bioproduct [60,61]. However, the market still prefers centrifugation because biomolecules are high-valued products whose extraction might be hampered by the added coagulant mass [122]. More specific research in this field is required.

4.2 Fertilizers

The algae biomass or residues from its processing also have the potential for end-use as fertilizers [17]. The biomass's potential as an organic fertilizer is assessed based on the composition of primary and secondary macronutrients and trace elements. Microalgae-based fertilizer production is satisfactory when it is possible to hydrolyze proteins, amino acids, and peptides while preserving the phyto-stimulants contained in the biomass itself and complying with the commercialization regulations in terms of nutritional content [130]. Microalgae fertilizers are able to increase the availability of nitrogen, phosphorus, potassium, and trace elements in the soil, therefore closing nutrient cycles, stimulating soil enrichment and plant development [3,17].

TABLE 5.2 Biomolecules obtained from microalgae and examples of potential species.

Components	Species
Protein-rich biomass	*Aphanizomezon flos-aquae*; *Chlorella vulgaris*; *Spirulina pacifica*; *Spirulina platensis*; *Schizochytrium limacinum*
Carotenoids	
• Astaxanthin	*Chlorella zofingiensis*; *Chlorococcum* sp.; *Haematococcus pluvialis*, *Haematococcus* sp.
• β-Carotene	*Dunaliella bardawil*; *Dunaliella salina*; *Haematococcus* sp.; *Scenedesmus almeriensis*
• Phycocyanin	*Gracilaria gracilis*; *Porphyridium* sp.; *Spirulina platensis*
• Fucoxanthin	*Chaetoceros* sp.; *Odontella aurita*; *Sargassum siliquastrum*
• Lycopene	*Chlorella marina*
• Lutein	*Chlorella protothecoides*; *Chlorella zofingiensis*; *Chlorella sorokiniana*; *Chlorococcum citriforme*; *Dunaliella salina*, *Muriellopsis* sp.; *Neospongiococcus gelatinosum*; *Scenedesmus almeriensis*
• Zeaxanthin	*Chlorella saccharophila*; *Nannochloropsis oculata*; *Porphyra* sp.; *Scenedesmus almeriensis*; *Synechocystis* sp.
Polyunsaturated fatty acids	
• Docosahexanoic acid—DHA	*Chlorella ellipsoidea*; *Chlorella pyrenoidosa*; *Crypthecodinium cohnii*; *Crypthecodinium*; *Isochrysis galbana*; *Rhizosolenia setigera*; *Schizochytrium* sp.; *Schizochytrium*; *Spirulina platensis*; *Thalassiosira stellaris*
• Eicosapentaenoic acid—EPA	*Amphora* sp.; *Monodus subterraneus*; *Nannochloropsis oculata*; *Nannochloropsis* sp.; *Nitzschia* sp.; *Phaeodactylum tricornutum*; *Porphyridium cruentum*
Phycobiliproteins	
• Phycocyanin, allophicocyanin, phycoerythrin, and phycoerythrocyanine	*Amphanizomenon floa-aquae*; *Arthrospira platensis*; *Spirulina platensis*; *Spirulina* sp.

Adapted from A. Aslam, T. Fazal, Q.U. Zaman, A. Shan, F. Rehman, J. Iqbal, N. Rashid, M.S. Ur Rehman (2020) Chapter 13—Biorefinery of microalgae for nonfuel products, in: A. Yousuf (Ed.), Microalgae Cultivation for Biofuels Production. Academic Press. 197–209; G.L. Bhalamurugan, O. Valerie, L. Mark (2018) Valuable bioproducts obtained from microalgal biomass and their commercial applications: a review. Environ. Eng. Res. 23(3), 229–241; E. Jacob-Lopes, M.M. Maroneze, M.C. Deprá, R.B. Sartori, R.R. Dias, L.Q. Zepka (2019) Bioactive food compounds from microalgae: an innovative framework on industrial biorefineries. Curr. Opin. Food Sci. 25, 1–7.

Microalgae fertilizers produced by chemical hydrolysis and acidic conditions result in a product of inferior quality. Enzymatic methods and dehydration cause cells to rupture, and consequently they are able to extract microalgae compounds for the preparation of various specific fertilizer products [25]. Microalgae obtained via anaerobic digestion with MO exhibited nutritional properties equivalent to other fertilizers on the market [109,131].

Therefore, although the examined species have a chemical composition (macro and micronutrients) comparable to that of other forms of organic fertilizers, further studies need to be performed in addition to the composition to demonstrate the potential for this application [17,109]. To guarantee the safe use of this material in the soil, the type of cell membrane of the cultured strain, the presence of metabolites or carapace, and the chemical speciation of metals, in addition to the toxicity of the flocculating agent, must be evaluated.

4.3 Energy

A possible alternative use for the harvested biomass is as a fuel source to energy production. The implementation of ventures focused on the production of energy products must take into account economic barriers as well as the competition with fossil fuels. The centrifugation process consumes about 8 kWh/m^3 energy to obtain biomass [124]. The centrifugation of large volumes of cultivation to collect biomass on a commercial scale implies high implementation, operation, and maintenance costs. Thus, it is considered economically viable only for the production of high-value products [124]. Therefore, it is necessary to evaluate the possibility of pre-concentration stages of biological material to reduce the volume to be centrifuged for the use of algae biomass for energy purposes in order to promote its economic viability.

Both the reduction of production costs and the energy demand of the process should be aimed at in order to guarantee a positive energy balance of the final fuel. It is noteworthy that the production costs vary mainly depending on the cultivation system (open lagoons or photobioreactors), the collection technique, the drying step (when necessary), and processing. One of the alternatives to reduce costs is the adoption of an integrated system with a biorefinery, where co-products with high added value can be produced concurrently with energy products [132–134].

Regarding energy feasibility, life cycle assessment (LCA) studies have different technological bottlenecks. While the drying step is crucial for a positive energy balance [35], the evaluation of the collection method for the use of co-products is also essential in the final energy balance of biodiesel production [135].

Several biomasses have been reported as promising for the production of biofuels in order to reduce the consumption of fossil fuels and greenhouse gas emissions, mainly CO_2. In the case of algae biomass, several studies highlight its potential for the production of biofuels because some species have higher productivity than terrestrial crops such as soy and canola [121,136].

Solvents and biofuels (such as ethanol and biodiesel) have been produced from microalgae, and the harvesting method directly impacts the bioproduct yields. It was reported that using potato and corn cationic starch produces a higher yield of acetone, butanol, ethanol, and biodiesel than using an aluminum coagulant to harvest *Scenedesmus obliquus* which demonstrates the benefits of NBC to downstream processing [37].

Contaminated algae biomass, due to its production in wastewater or contaminated waters, can be directly burned for the production of steam in industrial plants to meet thermal demand or for the production of energy in steam plants. In the case of biomasses that contain an inorganic flocculating agent, this conversion process will be impacted by an increase in the ash content produced during combustion, resulting in higher maintenance costs. Another

critical issue is the moisture content of the biomass. The lower the humidity, the greater the calorific value and the better the performance of the process. Data on moisture content, ash content, and elemental ash composition, among other factors, are of paramount importance for the design of the process [134].

The physical-chemical and thermophysical characterization of biomass is required to assess its energy potential and use in energy conversion technologies. In this sense, the following analyses stand out: immediate analysis; elementary analysis (carbon, hydrogen, oxygen, nitrogen, sulphur, chlorine); bulk density, real and apparent; differential thermal analysis and differential scanning calorimetry; calorific power; elementary ash analysis, etc [137].

The immediate analysis establishes the moisture and ash content (% by mass) and, by difference, the fuel fraction (% by mass), which is subdivided into volatiles and fixed carbon (Fig. 5.3). The volatile matter will lead to flaming combustion and fixed carbon to incandescent combustion. Wet biomass burns only after the evaporation of most of the moisture contained in its composition. Therefore, adequate procedures must be followed to guarantee a quick and complete drying of the biomass. Based on this information, it is possible to establish, for example, the amount of moisture to be evaporated before combustion and the size and type of equipment required for ash removal, depending on the energy conversion technique to be employed [137,138].

For this purpose, inorganic flocculants should be avoided, as their presence in the biomass will result in a higher ash content after the combustion process, which will reduce its calorific value. The use of NBC to remove toxic microalgae from blooms and its application for energy purposes presented promising results [140]. The calorific value and humidity values were within a desirable range for their use in energy conversion systems. However, both the

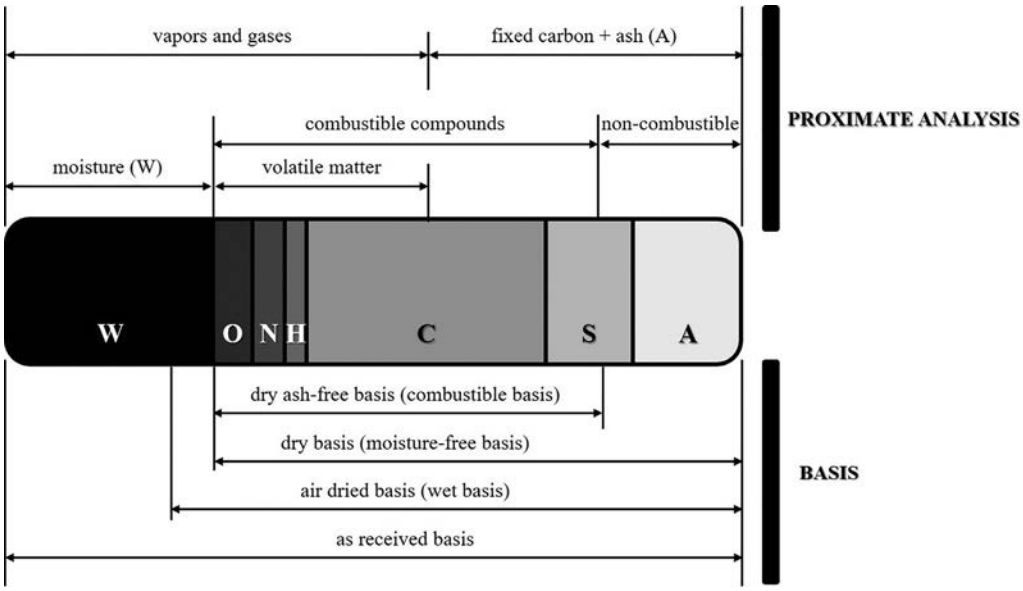

FIG. 5.3 Relationship between elemental (CHNSO) and proximate analysis of fuels [137–139].

ash and the pollutants produced by the combustion process must be analyzed to confirm that the toxins included in the biomass have been thermally degraded. Otherwise, the use of biomass from toxic strains should not be used for commercial purposes, principally for energy purposes, where exposure to highly toxic substances will be widely disseminated. Therefore, it is extremely important to detect toxins in biomass, in the culture medium, or in the residues from microalgal processing [141].

5 Conclusions and future perspective

The chapter has covered microalgae harvesting using natural-based coagulants (NBC), as well as their sources, applications, and mechanisms. Moreover, the possible applications of the harvested biomass have been explored. NBC is a category of non-toxic and biodegradable polymers, and many of them have been applied to replace inorganic coagulants (such as aluminum sulphate). Tannin and Chitosan natural polymers are the most common NBC, but different plant-based coagulants (such as *Moringa oleifera* seeds), cationic starches, and microbial flocculants are being studied, especially by developing countries, due to their low cost, availability, and easy application. Their predominant coagulation mechanisms are charge neutralization and electrostatic bridging, and adsorption plays a crucial role in both cases. NBC derived from renewable resources, is effective at low dosages, economical, and unlike inorganic coagulants, does not contaminate biomass for future application. Literature is still scarce in the parameters' definitions for the harvesting process, and studies have been developed to improve extraction methods and application performance. Microalgae biomass harvested with NBC showed good characteristics that could be applied to the production of high-value biomolecules, fertilizers, or even energy (as biofuel or directly burned). These applications depend on efficient coagulation/flocculation and separation processes and the properties of the obtained biomass. However, further tests that analyze the characteristics of the by-products case-by-case are required to confirm these applications.

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CHAPTER

6

Microalgae cultivation in wastewater from agro-industries: An approach integrated for bioremediation and biomass production

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1 Introduction

Agriculture and industrial food processing are required to supply the growing global demand for food. This increase has generated an enormous amount of liquid waste that is called effluents or wastewater. These effluents generated by various human activities contain a high chemical demand for oxygen and nutrients, in addition to a high load of polluting compounds [1].

According to each country's regulations, the techniques used to clean wastewater prior to disposal involve an additional cost, which is often considerable. As previously mentioned, a massive amount of effluent is produced, which may pose serious environmental issues. Therefore, wastewater treatment efficiency must be improved expeditiously.

Bioremediation is a wastewater treatment that combines chemical and/or microbial flocculation processes to reduce the organic load containing total solids and nutrients. This process has stimulated, and also the biotechnological microalgae use has attracted the spotlight of applied research to minimize the release of carbon into the atmosphere. At the present, a noticeable trend in the worldwide market is the demand for carbon-neutral industries and companies.

The "renewable energy" is a consensus in the major world countries and economies that marks the final step to reach the Paris Agreement and the Sustainable Development Goals (SDGs) by the United Nations (UN). For this reason, investment in clean sources and low-carbon energy should be at the top of your list of public policy priorities [2]. Even though different biomass sources are available for sustainable bioenergy production through renewable processing, the amount varies according to the country's climate and socioeconomic conditions, leading to a gradual increase in this substitution [3].

Two waste remediation strategies may be developed via a combination of ecoindustrial parks and industrial symbiosis. The eco-industrial park has been highlighted by several experts. It can be defined as a grouping of a systematic set of companies that share resources and/or products, seeking to increase profitability with a significant reduction in environmental impact [4]. This concept of agriculture-related eco-parks was applied in a study that addressed several case studies focusing on the ecological integration of food production with waste and wastewater treatment systems, aiming at the recovery of the natural conditions of the environment [5]. To consolidate these industrial ecoparks, several factors must be considered, including scientific and technical studies, societal participation, public and business political actions, and government policies that are relevant in decision making and the process's success [4].

Regarding the second concept of waste remediation, the use of waste and by-products to replace the raw material [6]. These authors gave an example of Portugal's industrial symbiosis and suggested that using waste and by-products as raw material substitutes is expected to grow, aiming to use wastewater bioremediation and the carbon dioxide (CO_2) depleted by microalgae production for fertilizer production. For instance, this symbiosis between different industries, such as food, livestock, aiming at bioremediation using microalgae, has a positive balance, but it needs to increase collaborative research projects in the productive and industrial sectors to achieve the goals of clean production. Microalgae systems for biomass cultivation are ideal for the generation new energy technologies and their transition to future low-carbon production arrangements in a circular economy-based industry [7].

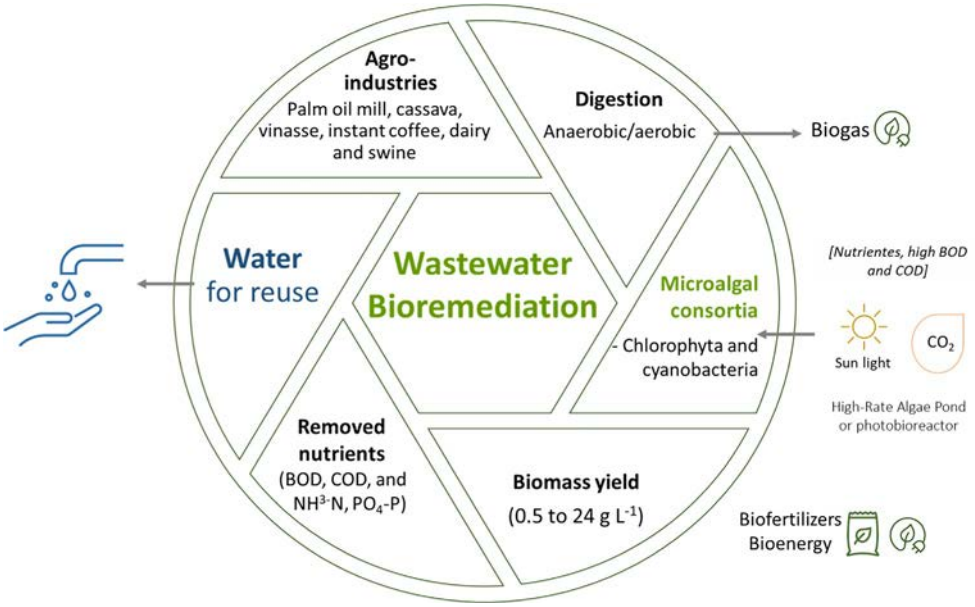

FIG. 6.1 Flow diagram proposed for microalgae wastewater bioremediation.

Brazil is a country in which there is potential for both eco-industrial parks and industrial symbiosis, integrating existing ethanol production biorefineries with the cultivation of microalgae, aiming at bioremediation and lowering the release of CO_2 in addition to the production of biofertilizers [6–8]. Furthermore, there are favorable climatic conditions for the cultivation of microalgae in open ponds [8–10].

In this chapter focuses on the bioremediation of wastewater generated from the food processing industries of palm oil, instant coffee, cassava, sugarcane, dairy, and, agricultural activity such as pig farming. The bioremediation of wastewater through the production of microalgae biomass on an industrial scale can result in profitable agriculture and healthy environmental management, with carbon emissions close to zero and water reuse (Fig. 6.1).

2 Wastewater and nutrient's recovery

Eutrophication occurs if wastewater is discharged into waterbodies without being treated to remove nutrients and organic carbon. It promotes the growth of undesirable organisms such as aquatic macrophytes and toxin-producing cyanobacteria [11].

When treating wastewater, it is critical to understand not only the amount of each nutrient but also their relationship. For example, the ratio of N:P:K for critical for the growth of microalgae growth [10,11]. The reuse of wastewater directly in agriculture without prior treatment has limitations [12], mainly because it has a high load of organic matter and nutrient imbalance, in addition to contaminants with heavy metals from wastewater discarded from some industries [11,13].

For the purposes of this discussion, waste is classified according to its origin: waste from the plant-based products industry, waste from swine farming, and waste from the dairy product manufacturing process. In the plant-based industry, processing includes palm mill oil (POME), instant or lyophilized coffee, cassava (flour and starch), and vinasse from the sugar cane biorefinery. In general, for both types of effluents, anaerobic fermentation is widely used as the first step in initial remediation due to a large amount of biogas produced, which can be used as a source of bioenergy, lowering the operating cost by adding value to the process. Fig. 6.2A–F shows the range of the main components into wastewater from agro-industries of POME, Instant coffee, Cassava flour/starch (manipueira), and sugar cane (vinasse).

At this time, the demand for the water resource has increased and the first proposed goal to expand the treatment of wastewater is to reach close to zero with the waste of this natural resource. The second proposed goal is to reduce the costs of remediation by removing elements chemicals that are in excess, by generating co-products that can be used in other sectors.

2.1 Palm oil mill effluent

Oil palm (*Elaeis guineensis*) is an oleaginous plant with high productivity and adaptability in humid tropical regions [61,62]. The world production in 2019 of oil palm fruit was approximately 410 million tons [63]. Brazil has stood out in the production of palm oil, concentrating most of its plantations in the Amazon forest, which can be an energy alternative to replace fossil fuels due to its high productivity, in addition to the social structure of production, generating environmental benefits [14]. Furthermore, the biomass can be used for renewable and bio-based products, which offer new hope as a generation of waste biomass that is linked to local production [64].

The production of crude palm oil needs a huge amount of water, leading to the generation of large volumes of wastewater [65]. Palm oil mill effluent (POME) is the wastewater produced from the production of palm oil industrial processing [15] that has been produced in a large amount by the palm oil industry [16]. POME has been treated in general using two sequential biochemical processes consisting of anaerobic acid fermentation followed by aerobic digestion [14,17,66]. The treatment of POME is carried out in low-cost pond systems and, in addition, there is the possibility of using the biogas generated during anaerobic digestion in the first step of the treatment. However, even after aerobic digestion, the effluents contain a high concentration of nutrient organic carbon that is not suitable for discharge into rivers.

Aiming at the production of pigments such as astaxanthin by microalgae, POME was suggested as an alternative source of nutrients due to the nitrogen and phosphorus contents and low values of heavy metals [17]. However, the physical–chemical composition of wastewater from aerobic digested POME showed that C:N:P ratio needs adjustments for microalgae cultivation [18].

Based on data from the literature, the physicochemical composition of wastewater from the palm oil extraction industry is given in Fig. 6.2A.

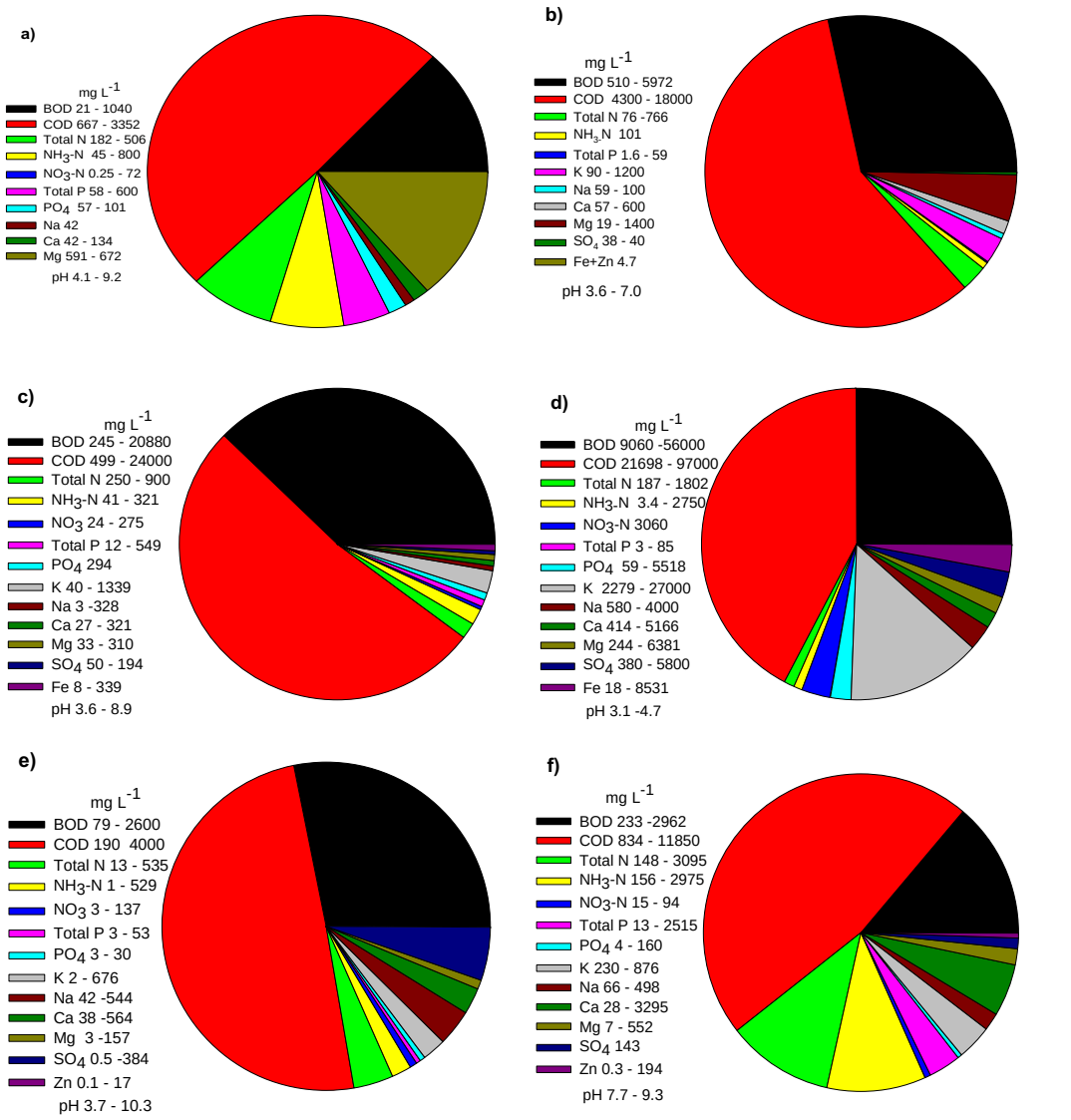

FIG. 6.2 Physicochemical composition of wastewater from the agro-industries: (A) palm oil extraction (POME), (B) instant coffee (C) cassava flour/starch/etanol, (D) sugar cane (vinasse), (E) dairy products and (F) swine farming. Note: BOD, Biochemical Oxygen Demand; COD, Chemical Oxygen. *Sources: Graphs constructed with mean values extracted from several studies in the literature [14–60].*

2.2 Instant coffee industry wastewater

The coffee industry uses large amounts of water, approximately 40 to 45 liters per kilogram of coffee is processed [67,68], and it generates large amounts of wastewater, which is exacerbated by the fact that this product is the second-most marketed globally [68]. The residue from the production of lyophilized or instant coffee decomposes slowly and has high water content. In Brazil, the instant coffee residue has been used as a soil additive or fuel, but there is a serious problem due to the high C/N ratio and its toxicity [69]. In Brazil in 2018, the effluents generated by the instant coffee industry were approximately 624,911 m^3 [19].

The instant coffee industry generates huge volumes of brown-colored wastewater containing compounds such as caffeine, fat, and peptic substances, as well as many different macromolecules such as lignin, tannins, humic acids, and polyphenols [20,67], which are difficult to degrade using conventional biological treatment processes. This occurs due to the presence of organic material that demands great quantities of oxygen to degrade. Hence, instant coffee industry wastewater represents a pollution risk if discharged into the environment.

A combination of chemical coagulation, flocculation, and oxidation processes that has been shown to be efficient for the removal of organic material, including recalcitrant organic compounds, reducing COD [70], but, in this remediation process, there is a need to use chemical compounds. For coffee wastewater treatment, anaerobic digestion has been studied as an ecofriendly technology using up-flow anaerobic sludge blanket reactors [20] and pH adjustment [22]. Publications with physical–chemical characteristics of instant coffee industry wastewater are scarce, as shown in Fig. 6.2B.

2.3 Cassava processing wastewater

Cassava (*Manihot esculenta*) is the world's most significant staple root crop, with an estimated 303 million tons of unprocessed roots produced in 2019 [63].

This crop, also called manioc or tapioca plays a key role in food security and income generation. Among the largest producers of cassava are the countries of Nigeria, Brazil, Thailand, and Indonesia. Most of the production of cassava roots is for the industry of flour and other derivatives, such as starch. According to [64] cassava is the primary staple food for the majority of indigenous Amazon people in the north of Brazil because its roots provide carbohydrates after traditional processing.

The cassava industrialization process generates significant amounts of wastewater, which an average of 11,000 L is produced per ton of cassava processed [64]. In Brazil, in 2018, the effluents generated by the cassava processing were estimated at 4 million m^3 $year^{-1}$ [19]. This cassava processing effluent, also known as *manipueira*, has a high chemical oxygen demand due to the high organic loads, but also contains significant nutritional content that is suitable for microalgae cultivation and integrated bioremediation. The anaerobic treatment of wastewater from the cassava starch industry has been shown to be efficient for producing biogas and for removing organic material [71].

Fig. 6.2C shows the composition of wastewater, which takes into consideration data from several studies. The physical–chemical composition of cassava processing wastewater has a range of variations in the content of nutrients and other compounds depending on the season

of the raw material and the industrial processing type used. Overall, wastewater from the food processing industry has a high content of nutrients and organic carbon, which is suitable for heterotrophic microalgae growth.

Bioremediation of cassava processing wastewater as a substrate to grow microalgae under heterotrophic conditions is described as acting as an effective CO_2 capturing and bioremediation agent for biodiesel and bioethanol production. From potential to reality, the integration between the cassava industry and the microalgae production chain has grown due to an increase information on this topic, as described in the following studies: using *C. pyrenoidosa* [24], *Phormidium* sp. [25], *Scenedesmus* sp. [28], *C. sorokiniana* [19], *Spirulina platensis* [27] and, using open pond pilot-scale treatment with *Acutodesmus obliquus* [26]. All these studies have shown significant removal of COD and chemical elements such as nitrate, phosphate, sulfate, chloride, calcium, potassium, magnesium, sodium, phosphorus, ammonia, and organic carbon, while simultaneously producing microalgal biomass.

2.4 Sugar cane wastewater: Vinasse

The world's sugarcane production in 2019 was approximately 1 billion tons in a harvested area of 26 million hectares [63]. According to Albarelli et al. [72] the expansion of sugarcane crops and industrial processing are linked to different environmental impacts, such as land competition with food crops, the displacement of farming to protect forest environments, and a high water footprint.

For every 1 L of sugarcane alcohol produced, approximately 13 L of vinasse is discarded [73], which is already widely used as liquid fertilizer by irrigation in Brazil. According to physical–chemical composition data from several studies, vinasse has a low pH ranging from 3 to 5, and a high chemical and biochemical oxygen demand (Fig. 6.2D). Vinasse is a rich medium containing N, P, and K for microalgal mixotrophic growth, e.g., *C. vulgaris* [39,74], but a study by Garcia et al. [31] has shown that sugarcane vinasse contains high concentrations of toxic compounds such as metals and low pH.

A biorefinery approach combining microalgae cultivation with vinasse, also called stillage, a byproduct of ethanol distillation, appears to be a sustainable option for the production of bioenergy and biofertilizers [10,39]. The integration of the sugarcane crop and industrial processing using a microalgae open pond system with vinasse as nutrients to grow represented approximately 20% of the CO_2 captured from the sugarcane biorefinery [72].

2.5 Dairy industry wastewater

Dairy farming and industrialization processes are an important activity in several countries, such as Brazil, where in 2018, the quantity of effluents generated by dairy processing was estimated at 104 million m^3 ano^{-1} [19]. Like most of the agro-industries wastewater, biological treatment of dairy wastewater may not be straightforward due to high variations in flow and chemical characteristics.

Wastewater pH is a key factor in any biological treatment process because most microorganisms have defined acidity or alkalinity ranges for optimal growth [75]. By combining enhanced biological remediation with algal biomass production as a treatment option for dairy

wastewater, it is important to evaluate phosphorus and nitrogen removal. Currently, the use of chemical compounds for cleaning has undergone a significant change due to restrictions on phosphorus content discharge regulations that were introduced in many countries to avoid impact on wastewater characteristics.

Fig. 6.2E summarizes dairy industry wastewater physicochemical composition based on data from different countries.

Aiming to establish the applicability of the integration between the microalgae and dairy production industries through the recycling of nutrients from dairy effluents. Labbé et al. [48] studied a consortium of *Chlorella* and *Scenedesmus* in systems indoors and outdoors in a pilot plant. The author concluded that due to the variation, there is a need to establish a remediation efficiency indicator based on nutrient reduction in the wastewater to be released.

In order to assess the viability of unsterilized dairy-derived liquid digestate as a raw material for simultaneous biofuel feedstock production and contaminant removal, *Chlorella vulgaris* was used to grow, showing higher biomass production. In addition, microalgal growth reduced the bacterial community in the wastewater [55]. The authors calculated that for every ton of *C. vulgaris* biomass produced, about 102 tons of dairy-derived liquid digestate can be treated with simultaneous N and P removal in this integration between microalgae production and the dairy sector.

2.6 Swine farming wastewater

Regarding animals, there is an increase in demand for meat since this is the main protein source for the vast majority of the world population. Worldwide, pork or pork meat stands out as a relatively inexpensive source of animal protein, and swine production has become a key in the livestock industry. In 2019, there were approximately 850 million pig heads [63]. The high demand for protein has increased livestock, causing environmental impacts [56]. Consequently, there are huge demands on agricultural land for the application of swine digestate. In many countries, authorization for the application of digestate produced from the digestion of animal byproducts and wastes is very difficult to obtain [76]. In this swine wastewater, the main chemical elements are carbon, phosphorus, and nitrogen, and to clean the water, some studies have suggested the use of diverse species of microalgae (Fig. 6.2F).

Microalgae are autotrophic and heterotrophic microorganisms having a high photosynthetic efficiency, a fast growth rate, a wide adaptability and tolerance to extreme environments, and excellent development in intensive cultivation, which make them effective in capturing CO_2. In addition to water and other factors, to express this potential, microalgae need essential nutrients to reach higher rates of biomass yield. Comparing the growth of *Chlorella pyrenoidosa* strains in swine farming wastewater diluted with that in Bristol's medium, it was shown that there is a high potential for bioremediation with lipid production [42]. By combining the swine wastewater-based microalgae with a biodigester technology in a circular economy approach, there are huge economic and environmental benefits through microalgae growth, converting the wastewater into the raw material of added-value products [59].

Wastewater treatment by high-rate algal ponds can be energy positive if the biomass is co-digested with primary sludge to generate electricity or heat [77]. From this system, there are other valuable byproducts such as biofertilizers, lower bioremediation costs. Among the

most common microalgae used, *Chlorella*, and *Scenedesmus* stand out due to their capacity to grow in these conditions (Table 6.1). In nonaxenic conditions, piggery wastewater contains contaminants. For the swine digestate treatment process, Mezzari et al. [78] suggested using *Scenedesmus* spp. cultivation as an alternative to removing *Salmonella enterica* serovar Typhimurium.

Some studies have suggested first adapting the microalgae to wastewater conditions. According to Wang et al. [50], by using *Neochloris aquatica* to remove nutrients from swine wastewater, the highest COD removal and NH_3-N removal were achieved using an N/P ratio of 1.5/1, showing that it is important to have a nutrient balance for microalgae to grow. Swine digested by the anaerobic process contains a substantial amount of N, P, and micronutrients for microalgae growth. For piggery wastewater, a study by Ran et al. [60] proposed an improved bioconversion system using *C. vulgaris* at a mixotrophic growth rate that increases biomass production to an average of 2.56 g L^{-1}.

Regarding sustainable wastewater infrastructure facilities, the mixotrophic algal wastewater treatment system was ranked as the most preferred one, followed by the membrane bioreactor [101]. For swine wastewater bioremediation, studies with different species of microalgae, such as using *Tribonema* sp., *Synechocystis* sp. [57], and *Chlorella sorokiniana* [56] have shown a high capacity to remove excess nutrients and organic load with high biomass production.

3 Potential microalgae to grow in effluents

Microalgae are microorganisms, either in mono- or multicellular form that comprise eukaryotic protists and prokaryotic cyanobacteria. The eukaryotic group includes both diatoms and green algae. The other group, so-called prokaryotic microalgae, comprises the cyanobacteria [102]. Wastewater bioremediation with microalgae (phytoremediation) is a novel and sustainable technology that combines biomass production, pollutant removal or biotransformation, and CO_2 fixation, as well as the production of bioenergy and biofertilizers.

This group of photosynthetic microorganisms are primary producers due to their ability to fix carbon dioxide (CO_2) using light as the only source of energy, and excel in the production of biomass rich in essential oils and other compounds such as pigments. Furthermore, several species of microalgae are able to carry out chemoheterotrophic or mixotrophic metabolism, allowing their use in the bioremediation of wastewater with a high organic load [103].

Seeking to lower the production costs of microalgae cultivation and bioremediation, several species of microalgae have shown the ability to grow in wastewater as an alternative culture medium, but one goal is that they are capable of achieving high cell growth [42]. So, the use of microalgal cultivation in wastewater has been proposed over the last decade. Several algal species, including *Chlorella pyrenoidosa*, *C. protothecoides*, *C. vulgaris*, *Scenedesmus* sp., *Tetradesmus obliquus*, *Chlamydomonas* sp. and *Nanochloropsis* sp. have been successfully cultivated in wastewater [96,104–109].

Studies with species of *Chlorella* and *Scenedesmus* growing under mixotrophic conditions in wastewater from a wide range of agricultural industries, such as from dairy products [110], tofu [111], food anaerobically digested [112], corn steep liquor, vinasse, and cheese whey [73]

TABLE 6.1 Biomass production by microalgae under different wastewaters

Microalgae species	Wastewater type	Biomass ($g\ L^{-1}$)	Reference
Desmodesmus sp.	Vinasse	4.0	De Mattos and Bastos [33]
Chlorella vulgaris	Vinasse	6.9	Jasmin Nivetha et al. [79]
Chlamydomonas polypyrenoideum	Dairy industry wastewater	2.2	Umamaheswari and Shanthakumar [80]
C. vulgaris	Tertiary wastewater by Forward osmosis membrane photobioreactor	5.0	Yu et al. [81]
C. vulgaris	Synthetically-made municipal wastewater	2.2	Mujtaba et al. [82]
C. vulgaris	Biologically treated swine wastewater	0.5	Abou-Shanab et al. [43]
C. vulgaris	Textile wastewater	0.7	Bhatt et al. [83]
Scenedesmus obliquus	Brewery wastewater	0.9	Mata et al. [84]
C. protothecoides	Whey permeate from dairy industry	9.1	Espinosa-Gonzalez et al. [85]
Nannochloropsis salina	Walne medium	1.9	Marudhupandi et al. [86]
C. pyrenoidosa	Anaerobic digestate of sludge	2.6	Tan et al. [87]
Chlorella sp.	Glucose recovered from enzymatic hydrolysis of food waste	6.9	Wang et al. [88]
C. vulgaris	Stillage	9.8	Mitra et al. [89]
	Soy whey	6.3	
	Modified basal medium	8.0	
S. obliquus	Municipal wastewater	0.4	Ansari et al. [90]
C. protothecoides	Sugarcane bagasse hydrolysate	24.0	Mu et al. [91]
C. sorokiniana	Wine waste	11.0	León-Vaz et al. [92]
C. vulgaris	Swine wastewater	4.2	Wang et al. [93]
S. obliquus		2.3	
C. reinhardtii	Influent, effluent, and centrate wastewaters	2.0	Kong et al. [94]
Neochloris aquatic	Undiluted swine wastewater	3.7	Wang et al. [50]
Chlamydomonas sp.	Industrial wastewater	1.3	Wu et al. [95]
C. polypyrenoideum	Dairy industry wastewater	1.6	Kothari et al. [96]
C. vulgaris (UTEX-265)	Brewery wastewater	2.3	Farooq et al. [97]
S. obliquus	Cheese whey permeate	4.9	Girard et al. [98]
Tribonema sp.	Swine wastewater	2.0	Huo et al. [58]

TABLE 6.1 Biomass production by microalgae under different wastewaters—cont'd

Microalgae species	Wastewater type	Biomass ($g\ L^{-1}$)	Reference
Dunaliella sp.	Poultry wastewater	0.7	Han et al. [99]
C. vulgaris	Municipal wastewater	5.1	Lee et al. [100]
C. pyrenoidosa	Cassava fermentation	3.73	Yang et al. [24]
Nannochloropsis sp.	Palm oil mill effluent (POME)	1.27	Emparan et al. [66]

and using wine waste [92] have highlighted the higher microalgae productivities of biomass, lipids, starch, and proteins than under photoautotrophic conditions.

Among green microalgae, the *Scenedesmus* genus has been highlighted as a producer of several secondary metabolites, such as carotenoids (e.g., α-carotene, β-carotene, and lutein). Another advantage is that these species are able to increase biomass production, and consequently, the productivity of pigments while performing bioremediation of different types of wastewater [113–115]. *Scenedesmus obliquus* capacity to thrive in a broad range of nutrient concentrations qualifies it as a species able to resist the physiological stressors associated with the majority of wastewater [116,117].

Chlamydomonas species are widely distributed worldwide in soil and freshwater. *Chlamydomonas reinhardtii* is an especially well-studied biological model organism, partly due to its ease of cultivation and the ability to manipulate its genetics. A practical advantage of *C. reinhardtii* (and other *Chlamydomonas* sp.) is its rapid propagation under mixotrophic conditions using acetate. The growth rate in the mixotrophic mode generally exceeds the sum of the heterotrophic and autotrophic rates [118,119]. In addition, a *Chlamydomonas* sp. was found growing in palm oil mill effluent, further indicating the utility of this genus [120]. Continuous anaerobic digestion of untreated low N biomass of the model alga *C. reinhardtii* was defined by a stable and efficient process with low levels of inhibitory substances (i.e., ammonia and volatile fatty acids); it resulted in extraordinary methane productivity [121].

Nannochloropsis is considered promising algae for industrial applications because of its ability to accumulate high levels of polyunsaturated fatty acids [122]. The species has primarily been studied in the marine environment, but they can also be found in fresh and brackish water [123]. *Nannochloropsis* cultivation using desalination concentrate (DC) as a source of water and nutrients is of special interest in the valorization and recycling of brine waste loaded with valuable compounds [124]. Marine *Nannochloropsis gaditana* performed well when exposed to a high DC concentration of 75% (~8.0 mS/cm), at which lipid productivity (12.10 mg/L/day) was higher than the lipid productivity of *Chlorella vulgaris* (7.37 mg/L/day) and *Spirulina platensis* (6.97 mg/L/day). These examples of the influence of DC on algal growth suggest that DC is a good medium for marine algae strains such as *Nanochloropsis gaditana*, but that the ionic concentration of DC is too high for the cultivation offreshwater *Chlorella vulgaris* and *Spirulina platensis* [125,126].

4 Microalgae biomass from wastewater

As mentioned earlier, several species of microalgae grow under nutrient-deficient stress and in a wide range of climatic conditions, without any detrimental effects on their productivity [127,128]. As microalgae require nitrogen and phosphorus for protein synthesis and heavy metals as micronutrients for growth, they present a great capacity for the uptake of inorganic nutrients [112,129]. Microalgae-based biotechnologies for bioremediation appear to be an attractive environmental solution due to their effective fixation of inorganic compounds, including carbon dioxide, heavy metals, and the ability to allow water reuse [130–133].

Algal biomass is rich in lipids, proteins, carbohydrates, pigments, enzymes, and other compounds that can be applied in different areas. From this point of view, intensive microalgae cultivation takes advantage of the microalgae's ability to efficiently consume nutrients, such as nitrogen and phosphate, during its growth. Studies have shown that once the wastewater treatment is complete, the biomass produced by microalgae can be recovered and used for other purposes, such as energy generation after anaerobic digestion, biofertilizers for plant growth, or soil improvers [134].

Given that microalgae thrive on both organic and inorganic media, cultivating these microorganisms has both environmental and economic advantages. Chew et al. [135] reported that inorganic medium can be replaced with a 25% food waste mixture, and C. vulgaris produced more biomass and had higher lipid and protein levels in its cells.

Several procedures have been used in phycoremediation, most notably the selection of microalgae strains and culture methods. By analyzing the growth and bioremediation of eleven microalgae species in three distinct wastewaters, the study demonstrated the critical nature of using microalgae isolated from the waste [136].

Studies focusing on the production of microalgae biomass when growing in swine wastewater, as well as the effects of conditions inherent to this alternative culture medium during the growing process, have shown the high potential of this source of organic carbon and nutrients [50]. One of the problems with using this wastewater for microalgae growth has been the imbalance of nutrients and often the presence of heavy metals, antibiotics, hormones, and bacteria that can adversely affect the initial rate of microalgae growth and biomass productivity [137,138]. As for wastewater from industrial food processing, *C. vulgaris* and *S. obliquus* are examples of species most cultivated in swine wastewater due to their high tolerance to environmental stresses, and a high potential for accumulation of biomass and lipids [93,139]. Furthermore, as they are efficient in CO_2 capture and wastewater bioremediation, green microalgae have a high potential as a raw material for renewable energy generation [140].

In order to minimize microalgae harvesting costs and increase bioremediation capability with nutrient recovery, the application of microalgae in the form of granules has been described as highly efficient for phosphorus removal, and the recovery and reuse of this P-rich microalgal biomass produced for biofertilizers [141].

Table 6.1 shows the biomass obtained by microalgae in different wastewater conditions.

5 Application of microalgal biomass in the agricultural activities

The primary problem for food and energy contemporary production (sustainable agriculture) is to ensure that natural resource sources are efficient, low-cost, and nonpolluting. In recent years, bio-inputs have stood out in this role for nutrition and biocontrol. Thus, microalgae can be adequate in two categories, microbial inoculants, and biofertilizers. The first is microalgae species having a defined and characterized association with a plant, contributing to plant development and production via, for example, hormonal control and biologic nitrogen fixation. Second, the biomass of microalgae contributes to soil nutrients as well as plant fertilization and nourishment (e.g., organic matter and nutrients).

Inoculants using cyanobacteria, which is a procaryote microorganism able to fix biological nitrogen in the absence of combined nitrogen [142] have been used. A classic example is *Anabaena* sp. in symbiosis with rice, and many other cyanobacteria can fix atmospheric nitrogen [143], Cyanobacteria, such as *Nostoc*, may also promote maize growth, yield, N-soil levels, and mineralization [144]. *Anabaena cylindrica* is also advantageous in bacterial co-inoculation with *Azospirillum brasilense* in maize production [145].

This second strategy, using biofertilizers, already poses a problem for large-scale applications utilizing microalgal biomass for plant fertility and nutrition. Microalgae biomass production protocols have been described using large-scale open and closed systems. According to Brennan and Owende [146] mixing and circulation are required to stabilize algae growth and productivity. Raceway pond systems run a continuous production cycle using the nutrients necessary for the microalgae to grow, which are diluted in water and introduced in front of the paddlewheel and circulated through the loop to the harvest extraction point; in addition, the paddlewheel is in continuous operation to prevent sedimentation. Closed systems (bioreactors) are also viable, especially as the culture conditions are more adjusted and and the space requirement smaller for a corresponding biomass production amount.

The biotechnological challenge is to verify if the microalgae biomass can provide nutrients to crops. Macronutrients such as N, P, and K are the most expansive and have the greatest need for agricultural production; by microalgae biomass, considerable quantities of these nutrients are required for plants. Initially, increasing organic matter values in tropical agricultural soils is already a significant advantage of microalgae-biofertilization biotechnology. Furthermore, a study by Castro et al. [147] showed that using microalgae biofertilizer in millet crops has a similar response to N-mineral fertilizer. Therefore, the use of microalgae biomass as a biofertilizer is comparable to the use of conventional fertilizer. Additionally, there is an enhancement in terms of nutrition for the culture, particularly N and P; this results in an advantage for millet based animal feed.

In horticulture, the application of microalgae-biofertilizers also has consistent advantages. Using microalgal bacterial flocs and *Nannochloropsis* sp. a study by Coppens et al. [148] indicated the feasibility of microalgae-based fertilizers. In another study, microalgae extracts increased the germination rate of several crops and, also acted as biopesticide [109].

6 Challenges in reducing wastewater treatment costs

Industrial activity can generate a huge quantity of effluents or wastewater, which are released into the environment. Almost all over the world, legislation on wastewater treatment is strict, and industries must treat contaminated wastewater before returning it to the environment to avoid the contamination of streams and rivers.

The most commonly used methods of water decontamination from industrial effluents are (i) adsorption, (ii) evaporation, (iii) chemical precipitation and filtration, (iv) reverse osmosis, (v) oxidation and biological reduction, (vi) oxidation and chemical reduction, (vii) electrochemical treatment, and (viii) ion exchange. In general, the most ordinary methods are ion exchange and adsorption with activated carbon, although these procedures are efficient, they are relatively expensive, especially due to due to the high cost [149]. Although widely used, chemical oxidation seems to be an expensive system to be used for wastewater remediation [67]. Increasing the framework of materials that can be used for the decontamination of water from industrial effluents is an important and necessary measure. They can reduce the cost of this process, would make it much easier to apply. Therefore, the cultivation of microalgae in wastewater from industrial processes, in addition to a sustainable alternative of bioremediation and treatment of effluents, is an opportunity to generate income because in these processes, microalgae biomass will be produced.

Bioremediation from microalgae culture is an alternative for wastewater treatment that uses the algae's capacity to remove contaminants, making its utilization an ecofriendly revenue potential with a low-cost approach [150]. Unlike the conventional methods of wastewater treatment, which are excessively capital-cost and not sustainable. Microalgae culture propose a low-cost alternative that fulfills two purposes: (i) phyco-remediation, and (ii) valuable biomass production [116]. Microalgae usage is sustainable, low-cost wastewater treatment [151] and eco-friendly technology, [152] not only for industrial wastewater treatment but can also for the treatment of urban (municipal) and agricultural wastewater [153].

For the water treatment processes, microalgae have been shown to be an efficient method with some advantages, such as developing innovative bioproducts [154]. Likewise, microalgae have the ability to assimilate nitrogen, phosphorus, heavy metals, toxic compounds, fix CO_2 [151,153], and detoxify organic and inorganic pollutants from wastewater [155] which can also benefit the environment.

Microalgae produce a variety of compounds with biochemical properties that have uses in a variety of fields, including food and health, and have a high added value to biomass production [153]. Some of these products are also useful for the pharmaceutical and agricultural sectors, and one main product is the feedstock for the production of bioenergy or biofuels derived from microalgae [156]. With microalgae cultivation, many co-products can be produced like foods, biofuels, and bioactives with zero pollution and the potential to clean the environment through CO_2 sequestration [151].

Growing microalgae for simultaneous bioremediation of wastewater contaminated with heavy metals and biomass production appears to be a biological system with a positive balance for the circular economy, as it performs CO_2 fixation and oxygen production via photosynthesis, in addition to the possibility of reusing water [157] and reducing greenhouse gas emissions [158,159].

This circular process involves wastewater treatment, microalgae growth, and microalgae biomass production. During this process, the wastewater receives an appropriate treatment that can be reused or returned to the environment, and in addition, microalgae biomass is produced. Combining the process of wastewater treatment with the production of co-products is a way to make the use of microalgae more sustainable, feasible, and cost-efficient. In this so-called circular economy, effluent treatment involves the growth of microalgae for biomass production. While the effluent may be reused or returned to the environment after being treated for COD and nutrients, it also produces microalgae biomass that can be processed or sold in nature. In short, combining wastewater treatment with the production of co-products is a way to make the use of microalgae more sustainable, viable, and cost-effective [157]. Also, microalgal biomass can be used for energy production [158] by consuming the gases from anaerobic digestion and fermentation [160], which converts algal biomass via a hydrothermal process [161].

Regarding costs, the integration of urban wastewater treatment and the cultivation of microalgae for biofuel in large open ponds was suggested to lower the operating costs of both processes [162]. In addition, microalgae's culture and its potential biomass can reduce the system's energy consumption [163] mentioned before, because of the co-generation of energy and biofuels.

The major cost of microalgae wastewater treatment is harvesting [116]. The microalgae biomass production has to be harvested with some frequency to maintain good levels of wastewater treatment, and this process could be more costly if not done adequately. However, harvesting cost are one of the most expensive costs in the microalgae treatment system, after the cost of installation, mainly when using photobioreactors for large-scale microalgae growth [146,151]. Nonetheless, the efficiency of the wastewater treatment system and its superior performance when compared to traditional methods make microalgae a low cost approach [164]. Considering the world's environmental issues, microalgae biomass production during wastewater treatment could be one potential solution and provide an opportunity in energy sustainability, high-added-value products, recycling, and pollution control [165]. Furthermore, the cultivation of microalgae, which are able to adapt to different conditions, can lead to carbon dioxide fixation [166]. With that stated, the wastewater microalgae treatment system is a low-cost and cost-effective solution for effluent treatment, providing environmental advantages via wastewater treatment and zero-carbon-emission, as well as economic benefits such as biomass, co-products, and energy production.

7 Conclusions and future outlook

Agricultural waste has been shown in the literature to be an excellent source of microalgae biomass and for bioremediation, with these photoautotrophic microorganisms functioning as an effective method for organic pollutant removal from water. Agro-industry effluent is a source of water that may be reused, preventing the depletion of drinking water. Furthermore, the biomass produced is a nutrient-dense bio-fertilizer that may be used in agriculture, making a circular economy possible. Despite multiple studies on microalgae growth using different types of effluent from the agriculture sector, large-scale deployment in industrial facilities is uncommon.

In the current context, the main challenge for the establishment and exploitation of microalgae is still to determine practical large-scale cultivation systems and methods of production of metabolites with high added value from microalgal culture through biomass and/or the medium itself. Residual crops are capable of economically competing with other metabolites of traditional interest. Therefore, significant improvements are needed in our knowledge of the existing potential in biodiversity, aiming at the selection and improvement of high-yielding algal strains, as well as the establishment of optimized protocols for the growth, harvesting, and processing of biomass and other useful products. In this way, metabolic engineering pathways for several already well-established microalgal species can be better adapted for specific purposes. However, more research into microalgal metabolic engineering would be needed to overcome some of the challenges of microalgal wastewater bioremediation. The production of microalgae by providing effluent and/or flue gas, instead of freshwater and/or CO_2 (uneconomic input), would conventionally alleviate the likely impacts of industrial emissions and waste disposal in natural, aquatic, terrestrial, and air ecosystems. Integrating commercial cultivation with phytoremediation would preserve the environment and, at the same time, save water treatment plant management costs. In this sense, efforts should be made to structure algal biomass production systems in a biorefinery context, taking advantage of all available components such as lipids, fatty acids, sugars, carotenoids, proteins, and enzymes for the production and development of processes effectively involving the different areas of the industrial sector. The assumption is that, in the medium or long term, commercial cultivation of microalgae will become a reality and will become the raw material of choice for biomass production in a variety of strategic sectors.

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CHAPTER

7

Microalgae-based systems applied to the dual purpose of waste bioremediation and bioenergy production

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OUTLINE

1 Introduction

The overexploitation of natural resources has led to the depletion of fossil fuel reserves as an energy source. The extensive use of fossil fuels, such as oil, natural gases, and coal, has

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been estimated to be exhausted in the next 50 years [1]. It is also reported that around 60% of greenhouse gas emissions originate from the burning of fossil fuels in the energy and industrial sectors, and although the rebounds indicate that this share will decrease, these categories will still be responsible for the high demand for CO_2 emissions in the coming years [2]. At the same time, the vast extent of industrialization and the significant increase in population also ended up resulting in the release of an enormous amount of wastewater, which represented a serious threat to the environment and its entire ecosystem [3]. Driven by the green agenda and the commitment of current governments to reduce problems caused by the environment, effluents for productive purposes have been studied worldwide. Integrated management of wastewater treatment plants with reuse practices, especially in the energy area, can help prevent pollution of water bodies by nutrients while providing productive activities and environmental sustainability [4].

Wastewater is rich in nutrients, mainly nitrogen, phosphorus, and carbon [5]. Benefiting from the abundance of these nutrients, the cultivation of microalgae in wastewater represents an attractive opportunity for the valorization of effluents through the use of the produced biomass, crucial for approaching a circular bioeconomy [6]. The cultivation of microalgae in wastewater has gained enormous popularity due to its efficiency in the production of biomass with reduced costs and less environmental impacts since nutrients and freshwater are not required [7]. Consequently, microalgal biomass is considered a potent source of bioenergy and the generation of high-value bioproducts due to its ability to accumulate higher oil and carbohydrate content, a high photosynthetic rate, no competition for agricultural land, and be easy to manage [8,9].

Presently, commercially available biofuels are primarily produced from crops of edible foods (such as sugarcane, soybeans, rapeseed, wheat, and others) [6]. However, there are several concerns about the competitive economic behavior of crop-based biofuels versus fossil fuels and their implications for food security, because the high demand for these crops may generally lead to higher prices and, as a result, food shortages [10,11]. In this sense, biofuels derived from microalgae have been privileged and proposed as an alternative approach without any negative or adverse effects on most production systems [3].

2 Sources of waste

The generation of waste has become a significant challenge worldwide due to the increasing diversity of its properties, increases in production processes, the lack of implementation of waste policies, and rapid urbanization [12]. Waste management problems are associated with increased production and insufficient material disposal due to the difficulties in locating suitable places for deposit or disposal [13]. Industrial activities and population growth are generating several solid, liquid, and gaseous residues. In fact, waste management has become a significant challenge for most countries worldwide, as waste generation in developed countries is much greater when compared to developing countries [14]. The increase in these residues is due to the wide variety of sources related to the most diverse sectors and segments, which have increased in recent years.

The generation of waste has become a significant challenge worldwide due to the increasing diversity of its properties, increases in production processes, the lack of implementation of waste policies, and rapid urbanization [12]. Waste management problems are associated with increased production and insufficient material disposal due to the difficulties in locating suitable places for deposit or disposal [13]. Industrial activities and population growth are generating several solid, liquid, and gaseous residues. In fact, waste management has become a significant challenge for most countries worldwide, as waste generation in developed countries is much greater when compared to developing countries [14]. The increase in these residues is due to the wide variety of sources related to the most diverse sectors and segments, which have increased in recent years.

Agricultural wastewater is mainly generated by animal production. Notably, approximately half of the nitrogen in animal waste is in the form of ammonium and the other half is in the form of organic nitrogen. The excess of nutrients accumulates in the soil, increasing the loss of nutrients through runoff, resulting in eutrophication in the receiving waters. In addition to manure, agricultural runoff may also contain herbicides, fungicides, insecticides, and nitrate and phosphate components from agricultural operations [15]. Agricultural waste is a great source of pollution, pathogens, odors, and greenhouse gases produced by manure, silage, oil processing, veterinary drugs, pesticides, and fertilizers [16].

Municipal wastewater sources are generated from domestic water use, manholes, sanitary sewage, and others [17]. Cleaning products, such as detergents, are estimated to generate one of the main residual loads in municipal treatment plants. Waste food, in turn, is also a source of solid waste worldwide, accounting for around 30%–60% [18]. Typically, wastewater and flue gas pollution are addressed by physical, chemical, and other methods. On the other hand, although these methods are fast and effective, they are expensive and ecologically destructive. Thus, biological methods have become a greener and more sustainable alternative to different types of waste [19]. The following section presents the main technologies for waste treatment.

3 Technologies for waste treatment

It is not new that the waste generated by human activities has been accumulating in the environment, causing terrestrial subsystems' pollution. The consequences of poor waste management highlight the need for appropriate tailings management. In light of this, several treatment methods based on the characteristics of the waste have been developed. The treatment process includes physical, chemical, and biological methods, and each method has its own advantages and disadvantages [20]. Details of the conventional waste treatment process and available conventional and emerging methods are discussed below.

3.1 Conventional technologies

Effluents are waste originating mainly from domestic and industrial activities and, often contain dangerous elements that, without proper treatment, end up being released into the environment, causing damage to human health. Liquid effluents, in particular, need to

undergo appropriate treatment to be released into bodies of water without significant environmental impacts. The conventional treatment of liquid effluents, also called wastewater, consists of a combination of physical, chemical, and biological methods used in the different levels of treatment. Namely, the treatment levels are (i) preliminary, (ii) primary, (iii) secondary, and (iv) tertiary [21]. These levels are used in sequence to increase the treatment degree, and each level aims at the removal of specific compounds and is based on the compound to be removed from the chosen treatment method (Fig. 7.1).

As can be seen in Fig. 7.1, the type of treatment is based on the compound to be removed at each level for which the method is chosen. Usually, the preliminary treatment employs physical methods; in primary and tertiary treatments, physical, chemical, and/or physicochemical methods, and in secondary treatment, in turn, biological, chemical, and/or physicochemical methods. Here, it is worth mentioning that the level of treatment and the treatment method to be applied depend on the peculiarities of the wastewater, which include the mechanisms by which the compounds are removed. Besides that, it depends on the intended destination of the treated wastewater, that is, whether it will be returned to hydric bodies or used for potable or not potable reuse [22].

Fortunately, advances in wastewater treatment technology have allowed their reuse. The use of advanced methods of treatment, such as microfiltration and advanced oxidation, allows the potable reuse of wastewater. However, the application of such methods is limited by the high cost, and is applied mainly in developed countries. In fact, most uses of treated wastewater are nonpotable. This is because it is not necessary to apply complex and expensive treatment methods, making it a more attractive and economically viable option [23,24]. Of the conventional physical methods of wastewater treatment, the most commonly used include screens, sedimentation, flotation, decantation, and filtration. The screens are typically

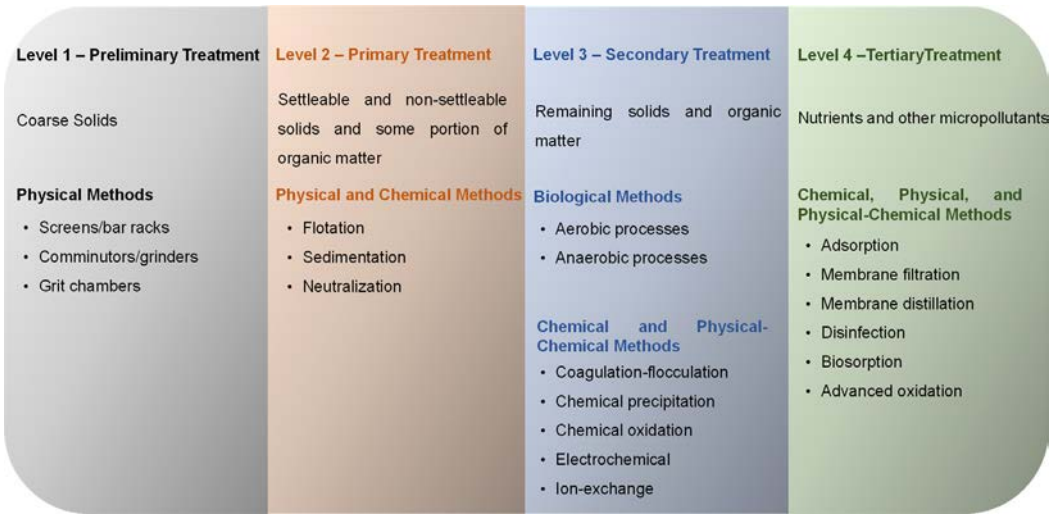

FIG. 7.1 Characteristics of the compounds and treatment methods involved in the different levels of wastewater treatment. *Based on A. Ullah, S. Hussain, A. Wasim, M. Jahanzaib, Development of a decision support system for the selection of wastewater treatment technologies, Sci. Total Environ. 731 (2020) 139158.*

used in the preliminary treatment to remove coarse solids when present, and the rest in the primary treatment to separate heterogeneous mixtures that can be of the liquid-liquid and/or liquid-solid type. Regarding filtration, it is important to highlight the pressure-driven membrane processes, which are microfiltration, ultrafiltration, nanofiltration, and reverse osmosis. These processes are generally applied for tertiary treatment, and the applicability of each one is based on the size of the particles and molecules. By way of curiosity, ultrafiltration is a process that can potentially remove colloids, bacteria, and viruses [25].

Of the conventional chemical and physicochemical methods of wastewater treatment, one can mention coagulation-flocculation, adsorption, chemical oxidation, chemical precipitation, ion exchange, and electro-chemicals. On the other hand, conventional biological methods include aerobic and anaerobic processes that take advantage of the actions of bacteria and other microorganisms to clean the water. All of these methods are applied at the secondary or tertiary treatment level, where the objective is to remove basically remaining all solids, organic and inorganic nutrients, and pathogens. As mentioned earlier, it is based on the characteristics of the wastewater and its destination or intended use that we select the most appropriate methods to be applied in the treatment process. This is also done while considering technical and economic aspects, as well as legal, social, and environmental aspects [26].

3.2 Emerging technologies

Concerns with the scarcity of hydric resources and with environmental pollution are pressing the development and implementation of new wastewater treatment technologies. The membrane aerated biofilm reactor (MABR) is one of the innovative wastewater treatment solutions. The MABR is an emerging technology that is now commercially available. This technology produces high-quality treated tertiary effluent that can be reused for nonpotable purposes, such as irrigation. The MABR, without a doubt, provides a long-term biological treatment solution capable of revolutionizing current technology [27,28].

The application of nanomaterials in the different treatment methods to remove organic and inorganic compounds has also received considerable attention. The nanomaterials have remarkably effective properties that can contribute to the development of high-efficiency technologies. Silver nanoparticles and titanium dioxide as photocatalysts, nanomaterials based on carbon and metal oxides as adsorbents, and nanocomposite membranes have all been investigated as promising alternatives for pollutant removal. There is a prospect that nanomaterials can meet the new demand for enhanced wastewater treatment technologies. However, although this is true, there are unresolved bottlenecks that are under consideration for full-scale application to be possible [29]. In conformity with the prospects of revolutionizing wastewater treatment, is the recent progress in advanced oxidation processes (AOPs). Currently, AOPs are established at full-scale, such as the O_3, O_3/H_2O_2, UV/H_2O_2, and Fenton processes. However, there is an even greater variety being considered for implementation, and those are on a lab-and pilot-scale. Among the electrochemical and physical AOPs being tested on a lab-scale are SnO_2-doped electrodes, PbO_2-doped electrodes, TiO_2-doped electrodes, microwaves, and electron beams [30].

In parallel, in line with the new trends in wastewater treatment, is also the emerging process of bioremediation. In particular, biosorption has been widely investigated for the

removal of pollutants in aqueous media. The method is environmentally benign and is based on the passive absorption of pollutants through biological materials such as fungi, bacteria, and microalgae. The practical limitations to be overcome for the biosorption method to become technologically feasible were discussed in detail by Vijayaraghavan and Balasubramanian [31].

Finally, microalgae have stood out not only for their biosorption capacity but as a technology capable of offering multiple benefits. These microorganisms can remove excess nutrients from wastewater in tertiary treatment and, in parallel, produce biomass that can be used for several purposes. Besides that, combining wastewater treatment and microalgae cultivation is a strategy that can benefit conventional microalgae production processes [32]. The use of wastewater can reduce the costs associated with the culture medium and improve the economic viability of low-value products such as biofuels [33]. In addition, it is worth mentioning here that these microorganisms are excellent biological carbon fixers [34,35]. Considering all this, microalgae are likely to become a technology capable of meeting the new demand for enhanced environmental solutions [36].

4 Bioremediation potential of microalgae

Many species of microalgae are able to grow effectively in wastewater conditions through their ability to recover and recycle the nutrients contained in them while producing biomass [37]. As an example, it is possible to produce about 1 and 10 kg/m^3 of dry biomass-based waste sewage and manure, respectively. This means that microalgae have been successfully used as a waste-based nutrient cycling technology [38].In a simplified scheme of the process, microalgal cells perform photosynthesis using sunlight as a booster for oxygen production (O_2) and consum inorganic compounds, finally producing biomass [38]. These microorganisms have simple cultivation requirements, high rates of cell growth and productivity, develop quickly and efficiently, inhabit environments of extreme conditions, and do not require arable land or freshwater. Thus, its distinct structural, physiological, and morphological characteristics expand the possibilities of its use in different processes and of obtaining high-value products [39].

In general, the use of microalgae can be combined with other waste treatment processes or as an additional step in the process to increase efficiency. The bioremediation process promoted by microalgae is more environmentally responsible and sustainable since it does not generate additional pollutants and offers a successive opportunity for the efficient recycling of nutrients. For example, algae biomass rich in N and P recovered can be used as a biofertilizer or as animal feed [40]. Furthermore, another significant advantage of these systems is the potential cost savings since they use a cheap and available culture medium for the control and production of the microorganism itself.

Consequently, to maximize the assimilation of inorganic compounds and benefit the treatment of wastewater, the productivity of microalgae must be optimized [41]. In this case, the design and operation of the type of microalgae cultivation must be adequate. Currently, there is a substantial amount of research in the literature demonstrating the various reactor designs and operational conditions for the cultivation of microalgae, such as open and closed systems. However, for waste recovery, open systems are the most frequently studied [42]. The reason is that this type of reactor has a much simpler design for its implementation. Currently, different

TABLE 7.1 Cultivation of microalgae in different sources of waste.

Water sources	Microalgae	Biomass (g/L)	Removal (%)			References
			COD	Nitrogen	Phosphorus	
Industrial wastewater						
Tannery wastewater	*Tetraselmis* sp.	1.40	56.7	71	97	[44]
Soybean wastewater	*Chlorella* sp.	0.78	65	80	100	[45]
Textile industrial	*Chlorella pyrenoidosa*	–	63	82	87	[46]
Dairy wastewater	*Chlorococcum* sp.	0.80	82–93	–	–	[47]
Paper and pulp/dairy industrial	*Symbiodiniumminutum*	1.40	67–92	96–99	91–99	[48]
Brewery wastewater	*Chlorella vulgaris*	2.26	–	90	18	[49]
Agricultural wastewater						
Palm oil mill effluent	*Chlorella sorokiniana*	0.10	80	80	72	[50]
Piggery effluent	*Acutodesmus obliquus*	6.00	50	60	70	[51]
Anaerobic digested piggery effluent	Algal consortium	2.50	–	97	–	[52]
Municipal wastewater						
Municipal wastewater	*Chlorella sorokiniana*	1.00	–	60	100	[53]
Municipal wastewater/biogas slurry	*Chlorella zofingiensis*	2.50	–	93	90	[54]
Treated municipal wastewater	*Oedogonium intermedium*	9–15	–	36	65	[55]
Pretreated urban wastewater	*Chlorella* sp.	1.34	–	42	6	[56]
Municipal wastewater	*Auxenochlorella protothecoides*	1.20	90	60	81.5	[57]

COD, chemical oxygen demand.
Based on S.B. Ummalyma, D. Sahoo, A. Pandey, Resource recovery through bioremediation of wastewaters and waste carbon by microalgae: a circular bioeconomy approach, Environ. Sci. Pollut. Res. 1–20 (2021).

models are being investigated, such as circular ponds, shallow lagoons, and ponds, mixed ponds, and inclined systems, but raceway ponds are traditionally used for commercial purposes [43]. Although most research on the cultivation of microalgae comes from laboratory scales, a wide range of studies has examined the growth of microalgae and the potential for removing polluting compounds from a variety of residual sources, such as industrial, agricultural, and municipal effluents (Table 7.1). In parallel, these studies also evaluated the

production of biomass and the potential to reuse all of this raw material for the generation of high-value products, such as biofuels. Microalgae, under certain conditions, undoubtedly provide a fundamental path to meeting the urgent global need for sustainable, clean, and safe energy sources, while also reducing anthropogenic carbon emissions [58,59].

5 Bioenergy technologies and applications

It is estimated that 80% of human needs are met through the generation of fossil fuels. Consequently, immersed in this constant energy dependence, goals associated with the search for sustainable bioenergetic technologies have boosted the acceleration of the energy transition and, therefore, the generation of clean energy has become a global obsession in recent decades [60]. Thus, numerous technologies for the generation of bioenergy have been proposed. However, several studies report that, regardless of the applied technology, by far, the most latent substitute is biofuel, although several substitutes are being explored to supplant the current high pollutant, diesel. Therefore, finding the ideal raw material for the production of biofuels has led to the intensified promotion of research in the area of bioenergy [61]. From this perspective, the metabolic versatility of microalgae allows its biotechnological applicability under the most different technologies. This is because the ability of microalgae to use nutrients from waste, whether they come from wastewater or even exhaust gases, as an energy source for the production of biomass makes this bioproduct form with a high potential for exploration. Thus, the biomass resulting from bioremediation processes is undoubtedly seen as a target object to be investigated [62].

However, it is important to note that the characteristics of the elemental composition of microalgal biomass play an important role in the quality and yield of the biofuels formed. Depending on the cultivation conditions and effluents used for bioremediation, these biomasses will differ in terms of chemical composition, which will have different properties in terms of thermal stability and morphology of the macromolecular structure and, therefore, will undergo different reactions at variable and different temperatures, giving rise to a wide range of products [63]. Therefore, the technology for conversion to bioenergy will directly influence the selection of the technology to be used.

Given this scenario, technologies for converting microalgae biomass into a means of generating biofuels have been examined and reported in the academic literature. Among the main bioenergy technologies, two main routes are considered: thermochemical and biochemical conversions. Then, each of these conversion pathways, as well as the processes for obtaining biofuels, will be detailed and discussed in a context based on the biomass derived from microalgae bioremediation.

5.1 Thermochemical conversion

Thermochemical conversion is understood as a route where chemical processes at high temperatures are applied to biomass, to obtain biofuels with high-quality and densified energy content. It is common to say that thermochemical conversion is based on the energy released through the combustion/decomposition of carbon. The most promising thermochemical routes

for the conversion of biomass are roasting, carbonization, pyrolysis, combustion, and gasification (Fig. 7.2) [64]. Besides, the application gains from these processes are numerous, which can be cited as advantages regarding environmental footprints, efficient recovery of nutrients, short reaction times, and the ability to deal with a variety of residues and mixtures.

However, regardless of the chosen conversion route, the parameters of temperature, heating rate, and residence time are considered the key parameters of the process. Given this, they can often serve as a basis for differentiating between the technologies to be used. In addition, another determining factor in the choice of technology is based on the fraction of the elemental composition contained in the raw material in question. This is due to the fact that, depending on the carbonaceous concentration of the fuel, the combustion temperatures, as well as the technology to be used, might vary. Based on the above, Fig. 7.3 shows the intervals for these differentiation parameters of the technologies for conversion to bioenergy.

Additionally, it is important to note that among these conversion techniques, there is currently an applicational tendency for combustion as the main dominant technology in the context of bioenergy supply (~80% of world energy). This is because, other techniques such as gasification and pyrolysis still face obstacles to being consolidated, highlighting the high operating costs, in addition to the low efficiency of energy conversion. Therefore, the following

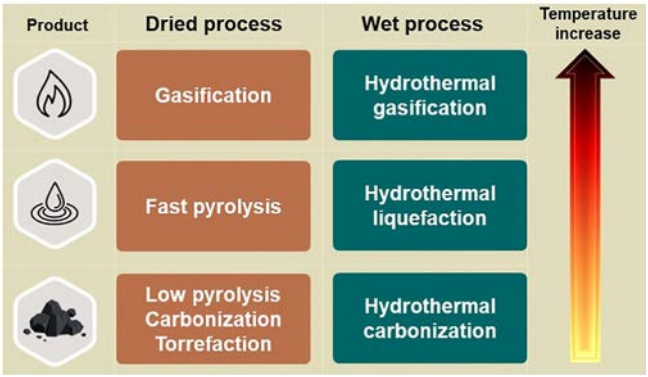

FIG. 7.2 Dried and wet processes of the thermochemical routes and their respective generated products.

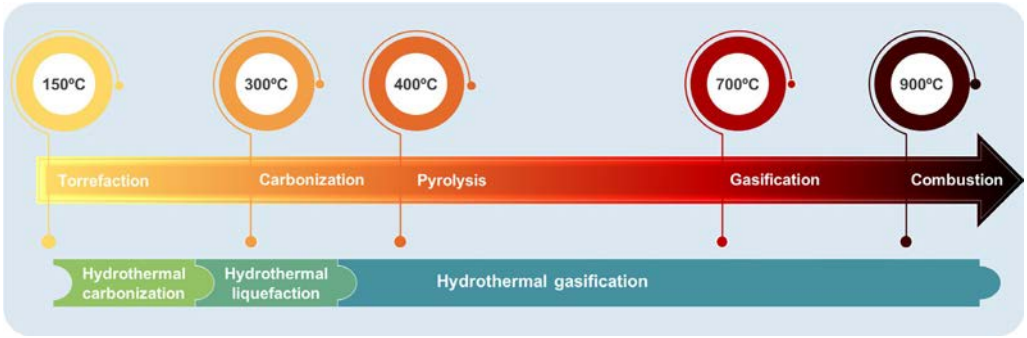

FIG. 7.3 Temperature ranges for thermochemical bioenergy conversion techniques.

will discuss the basic principles of each of the thermochemical conversion techniques and their interactions in the context of the application of microalgal biomass.

5.1.1 Torrefaction and carbonization

Torrefaction and carbonization are thermochemical technologies that often cause confusion in much of the literature. Both are practiced in environments without the presence of oxygen, as well as pyrolysis and liquefaction. However, the target bioproduct of these technologies consists of the generation of solid fuels such as charcoal and biochar.

However, a slight difference between them is the justification for applying the method and the objective of the target to be achieved. In this way, torrefaction can be defined as the process that aims to maximize the total energy and the biomass yields without severe decomposition of their structure. On the contrary, carbonization tends to maximize the fixed carbon and, therefore, minimize the hydrocarbon content of the treated biomass. It can be assumed that carbonization removes most volatiles from biomass, while roasting retains most of it, removing only the chemical water and low energy dense volatile compounds. In addition, it is widely recommended that carbonization reactions should be avoided during the roasting process. On the other hand, both torrefaction and carbonization are considered pretreatment processes for raw materials at mild temperatures, so catalysts are usually not essential for these processes [65].

Currently, conventional torrefaction is one of the most common technologies in the generation of solid biofuels from microalgae. Some studies suggest that the operational peculiarities should be standardized at temperatures between 200°C and 300°C, with a retention time of around 10–60 min, in the presence of an inert gas or carrier gas, generally nitrogen [65]. As a result, torrefaction produces biochar, that is, solid fuel with properties similar to coal fuel. Among the advantages are high reliability and stability with easy control. However, longer residence time and operating costs are the main challenges when competing with other methods [66]. In contrast, the carbonization process consists of the reaction of biomass, under the elevation of the temperature between 400°C and 1000°C. Charcoal, a bioproduct formed from heating, is generally used as a fuel raw material in gasification, and as a reducing agent in metallurgical processes.

5.1.2 Pyrolysis

Similar to previous technologies (roasting and carbonization), pyrolysis is operated in conditions without oxygen. However, the process requires higher temperatures, and as a result, the fuel generated is composed of its liquid form, commonly known as bio-oil. In addition, depending on the process conditions, pyrolysis also carbonizes biomass in biofuels such as biochar (solid) and gas, simultaneously. Thus, because of the versatility of biofuels generated, this process has increasingly stood out among the thermochemical processes. Based on the heating rates, different classifications for the pyrolysis process can be assigned, namely slow pyrolysis, fast pyrolysis, catalytic pyrolysis, and microwave pyrolysis. Slow pyrolysis is characterized by a slow heating rate (~10 K/min), with a long residence time for vapors (>1 min), under moderate temperatures, around 300–400°C. On the other hand, rapid pyrolysis occurs at higher temperatures (500–600°C) and an approximate heating rate of 104 K/min, with a short residence time for the vapors (2–5 s). However, catalytic pyrolysis usually occurs at lower temperatures (~300°C), under the action of a catalytic agent (usually a metal), whereas

microwave pyrolysis takes place over a temperature range of 300–800°C, overtime of residence between 10 and 120 min [67].

However, in general, solid or biocoal products are formed at lower pyrolytic temperatures, whereas liquid or bio-oil conversion requires a temperature range of 400–550°C and a short-term residence permit. In contrast, a higher range of pyrolytic temperature is more likely and feasible for the production of biogas [68]. Additionally, the breakdown of carbohydrates and proteins occurs at a temperature below 400°C, but the breakdown of lipids occurs at a temperature above 550°C during the pyrolysis process [67]. Thus, given the potential for the chemical composition of microalgae, the main species that contribute to the conversion of algae to biofuel are the species of *Chlorella* and *Scenedesmus*.

5.1.3 Hydrothermal processes

The hydrothermal conversion consists of a conversion process whose biomass passes through various thermochemical routes in compressed hot water under different conditions to form solid, liquid, and gaseous fuels. Depending on the variation of the temperature ranges and the applied reaction pressures, these processes assume subclassifications, being divided into hydrothermal carbonization, hydrothermal liquefaction, and hydrothermal gasification (Table 7.2) [69].

Thus, in terms of the aspect of the application for biomass from microalgae, the hydrothermal conversion technology under gasification tends to be the most promising. This is because the possibility of using biomass without drying could dramatically reduce downstream processing costs [70]. As a result, microalgal biomass could be converted directly into fuel gas under conditions beyond the critical point of water. On the other hand, disadvantages regarding the deposition of solids and corrosion in the reactor resulting from the operational conditions of the process prevent the consolidation of these technologies [68].

5.1.4 Gasification

Gasification is one of the main technologies for converting biomass into flue gases. In addition, recent research has heavily investigated its performance as a low-carbon technology. This is because the carbonaceous material contained in the biomass is converted into synthesis gas (usually carbon monoxide and biohydrogen), under elevated temperatures, that is, above 700°C, which is characterized by being an endothermic process. Also, according to the final application of the final bioproduct, oxidizing agents may differ, with air, oxygen, or steam being commonly used. Subsequently, the synthesis gas can be used for burning or synthesized to produce other liquid biofuels [71].

TABLE 7.2 Differentiation parameters between subclassifications of hydrothermal processes.

Hydrothermal processes	Temperature range (°C)	Reaction pressure (MPa)	Residence time (min)	Biofuel
Hydrothermal carbonization	200	2–10	60	Solid fuel
Hydrothermal liquefaction	300–350	5–20	5–60	Bio-oil
Hydrothermal gasification[a]	>374	>22.1	15–45	Flue gas

[a] *Microalgal biomass can be used with a moisture content of ~80%.*

When the process uses microalgae biomass, some preprocessing steps are necessary, since gasification only occurs under ambient conditions and dry raw materials. Thus, treatments for the dehydration of biomass are unavoidable requirements for the gasification process to enhance the efficiency of the process [72]. However, it is important to note that the main bottleneck of this technology is the formation of soot. Soot is formed from the burning process, and therefore its characteristics influence the yield of the process and also vary according to the chemical composition of the biomass, which makes the research even more complex [73].

5.1.5 Direct combustion

To date, direct combustion has been the most frequently employed globally due to efforts in research, development, and advances in bioenergy conversion technology [74]. Basically, direct combustion occurs through the burning of crude biomass, under excess air (oxygen) for the final production of heat and electricity. However, it is essential to consider the release of polluting compounds, such as nitrous oxide and carbon dioxide, under real conditions into the atmosphere. In more detail, the combustion route results in the release of the volatile compounds present in the biomass, and therefore, when heated, they ignite when mixed with oxygen. The combustion of gaseous volatiles is fast compared to the combustion of solid coal, and a high proportion of volatiles decreases the residence time of the fuel in the furnace. The remaining coal will predominantly retain its original shape and, during burning, will be reduced to ashes [75].

Similar to gasification, the direct combustion of microalgae biomass also requires predrying treatments. In addition, another factor to be circumvented is the resistance to the storage of the generated heat, since the generated energy must be used immediately, considering that the available options are not yet viable options [76].

5.2 Biochemical conversion

As seen in the sections above, there are several technologies for converting biomass into bioenergy. However, biochemical conversions stand out as providing a favorable direction in the face of environmental aspects, due to their ability to emit less pollutants into the atmosphere, beyond reducing energy requirements for operation. Technically, biochemical conversions are known to use biologically and biochemically based processes to generate biofuels. In a subclassification, we can list anaerobic digestion, fermentation/distillation, and photolysis. In addition, other technologies, such as chemical transesterification, direct production of electricity by specific microorganisms, and technologies beyond the generation of energy through the production of volatile organic compounds, are also part of this hierarchy.

Additionally, although they have environmentally friendly characteristics, the bottlenecks inherent to the biochemical conversion processes include issues associated with the biomass stocks available for the required energy demand, recalcitrance of some specific compounds contained in the commonly used biomasses, in addition to the specific characteristics of the enzymes and microorganisms used [77].

In this way, the following will briefly describe and elucidate questions regarding each of these subcategories of the biochemical conversion processes.

5.2.1 Biophotolysis

Biohydrogen gas has been considered the biofuel with the greatest potential, in the medium and long term, for the replacement of fossil fuels. Its process of obtaining safer, in environmental terms, can be attributed to the process of biophotolysis. In short, during this process, water is converted directly into biohydrogen gas, by a biological system in the presence of light so that solar energy is converted into chemical energy.

Biophotolysis can occur in two ways|: considered direct biophotolysis and indirect biophotolysis. Direct biophotolysis occurs in the presence of sunlight, which is captured by photosystems I and II to perform oxygen photosynthesis. In this process, biohydrogen is directly produced from the breakdown of the water molecule, with the concomitant release of oxygen. In contrast, in the indirect biophotolysis process, the generation of biohydrogen occurs from the breakdown of the carbohydrate molecule, usually, glucose, previously synthesized by the biological system in the presence of water and carbon dioxide absorbed from the atmosphere. Thus, the breakdown of carbohydrates generates hydrogen and carbon dioxide gases [78].

In the case of green microalgae, conversion can occur either directly or indirectly. On the other hand, cyanobacteria have a predilection for the indirect pathway. However, both can be used concurrently for the bioremediation of industrial waste and energy production. Nevertheless, one of the biggest bottlenecks to be circumvented in the microalgal biohydrogen production processes is the incompatibility between oxygenic photosynthesis and anaerobic hydrogen gas production due to the high sensitivity of the enzyme hydrogenase to oxygen. However, some studies have suggested that hydrogen photoproduction may be favored by the deprivation of sulfur and phosphorus in cultivation.

5.2.2 Fermentation

It is well known that the fermentation process, that is, the conversion of biomass into a given bioproduct, occurs in the presence of a microorganism or active enzyme, and is an essential part of the biochemical conversion. Thus, fermentation for the production of biofuels uses aerobic fermentation (presence of oxygen) or anaerobic fermentation (absence of oxygen) to originate specific compounds of hydrocarbon precursors, such as ethanol and butanol, among others [79].

The main biofuel obtained from the fermentation process is bioalcohols, more precisely bioethanol. Generally, the sugars contained in the biomass are extracted and serve as nutrients for the yeast to perform the molecular breakdown, and therefore convert them into methanol. Subsequently, it is necessary to apply a distillation process in order to remove water and other impurities in the product (10%–15% ethanol). Finally, the concentrated bioethanol is removed and condensed into its liquid form.

Microalgae with higher levels of carbohydrates are the most suitable raw materials for the production of bioethanol. In addition, studies report that the yields of bioethanol produced from microalgae are two to five times higher than ethanol produced from sugar cane and corn, respectively [80]. On the other hand, fermentation routes can also give rise to biofuels such as biohydrogen and biomethane. For biogeneration to occur, strict anaerobes or facultative anaerobes must be present, which carry out the fermentation of carbohydrates, giving rise to dark fermentative biohydrogen and other parallel products such as volatile fatty acids

and alcohol [81]. However, although this route has potential for production, to date, researchers have not yet been able to expand studies on a laboratory scale and consolidate this technology at industrial levels.

5.2.3 Anaerobic digestion

Anaerobic digestion is a biological process used to decompose organic matter in oxygen-free conditions and produce renewable energy—biogas—mainly methane and carbon dioxide [82]. In recent years, it has been approached as a cutting-edge technology to minimize the recovery of microalgae resources [83]. This is because the generation of biogas is commonly applied to organic wastes with a high moisture content (~90%). Therefore, it is applicable for converting biomass from wet microalgae, which is an environmentally viable way to create a renewable energy source for domestic and industrial consumption. The biochemical conversion route of anaerobic digestion takes place through four stages, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis, where the carbonaceous, nitrogenous, and lipidic content of microalgal biomass is used as a fundamental raw material for biogeneration. Thus, the higher the content fractions of these compounds, the better the process yield. In addition, studies report that the higher the lipid content of biomass, the better the yield when compared to carbohydrates and proteins [84].

5.2.4 Transesterification

The production of biodiesel from the lipid content of microalgae biomass is a technology aimed mainly at the generation of an economically viable biofuel [85]. The conversion process from transesterification chemically takes place, wherein the presence of methanol and triglycerides, under the action of a catalyst, results in methyl esters of fatty acids and glycerol. The reaction parameters are ensured by the conditions of temperature, residence time, alcohol fraction, and catalyst. However, although transesterification is a simple process, the prestage of extraction of the lipid fraction is a bottleneck in the use of microalgal biomass. This is due to the high costs and low yield of extraction techniques, which keep this technology from becoming a true industrial consolidation. Faced with this, and willing to circumvent this bottleneck, researchers developed a one-step transesterification method. Thus, this method results in higher yields (>98%) than the conventional method.

5.2.5 Microbial fuel cells

Microbial fuel cells can be understood as photosynthetic microorganisms that are capable of converting solar energy into electricity through metabolic reactions. Thus, the use of microalgal cells has been the target of research for this purpose [86]. The process of biogeneration of electricity consists of two chambers known as an anode and a cathodic chamber, separated by a proton exchange membrane. The anode side contains electrochemical active microorganisms, while the cathode is abiotic. The microorganisms will act as biocatalysts that motivate the degradation of organic materials for the production of electrons that travel to the cathodic side of the electrical circuit. In this way, electrons pass through the external circuit, reaching the cathode, and the hydrogen ions move to the cathode and react with oxygen to form water in the internal circuit [87].

Concomitantly, the generation of electricity from wastewater using a microbial fuel cell based on microalgae has attracted a lot of attention from researchers today [88]. In fact, this ability of microalgal cells makes it possible to circumvent the problems of energy demand, in addition to enhancing environmental mitigation. This is because, through photosynthesis, these microorganisms also capture and convert carbon dioxide into energy, making them versatile in the most diverse biotechnological fields.

5.2.6 *Volatile organic compounds*

The most recent biochemical conversion route to generate bioenergy consists of the secondary metabolism of microalgae, resulting in the generation of volatile organic compounds (VOCs). In short, these compounds are released into the environment during the cultivation of microalgae. Some studies suggest that this compost belongs to the most diverse chemical classes. However, the most common are alcohols, aldehydes, esters, and hydrocarbons [89]. In turn, these compounds have a substantially and considerable energy content. Studies developed by Severo et al. [90] suggest that the volumetric energy potential of VOCs in a photobioreactor integrated with a biofuel system is approximately 15,247.78 kJ/m^3/day. On the other hand, Jacob-Lopes et al. [91] evaluated the potential for the production of volatiles in heterotrophic microalgae systems, and the potential for energy generation of the volatiles produced was estimated at 10,580.50 kJ/m^3/day. In terms of comparison, the energy potentials of gasoline, liquefied petroleum gas, diesel oil, and natural gas are close to 47,300, 46,100, 44,800, and 39,360 kJ/kg, respectively.

However, although they are bioenergetic compounds with high application potential, to date, the main bottlenecks involving this technology are the lack of recovery technologies for biomolecules in exhaustion, in addition to the low work volumes of microalgal cultivation, and consequently, small VOC productivities.

6 Future perspectives and conclusion

The integration of wastewater treatment using microalgae cultures can be an attractive approach for both the treatment of effluents and the production of energy simultaneously. This synergy between wastewater treatment and the cultivation of microalgae effectively reduces processing costs and therefore can benefit both sectors. Microalgae have been used to produce biomass and a multitude of bioproducts with excellent results. However, commercial initiatives for this purpose will depend on the composition and volume of the effluent the selected microalgae species, and the conditions used. Depending on the cultivation conditions and the effluents used for bioremediation, these biomasses will be different in terms of chemical compositions and properties that will directly influence the final result. In addition, the initiatives will also depend on the specific biofuel of interest to each region or that is necessary for local consumption. Therefore, each situation must be analyzed individually.

Finally, the integration of bio-based processes with established industrial processes is an appropriate and innovative method to meet the requirements of green technology,

prioritizing preventive and remedial actions with a reduction in the use of energy and inputs. However, these approaches are still in their infancy, with the biggest challenge being the understanding of material flows and their value in biological systems and bioprocesses, for the expansion of these circuits based on the aspirations of the modern bioeconomy.

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CHAPTER

8

Direct utilization of lipid and starch from wet microalgae (*Chlorella vulgaris*)

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1 Introduction

Crude oil reserves are expected to be depleted by 2050, affecting the production of fossil oil-based fuels and materials. As a result, alternative energy and material resources have become critical [1]. There are more than one million species of algae growing in the water (maritime, brackish, and freshwater) and on land [2]. Among them, most species are microalgae. The advantages of growth rate, fixation of CO_2, high lipid and/or carbohydrate content of some species, and no or less competition with food make them excellent candidates for sustainable resources [3]. Mata et al. [4] reported that the lipid content of most common lipids containing microalgae is 20%–50% (weight of dry mass), with a maximum of 75% found in *Botryococcus braunii*. There are 12 lipid-containing microalgae genera mostly reported: *Chlorella, Crypthecodinium, Cylindrotheca, Dunaliella, Isochrysis, Nannochloris, Nannochloropsis, Neochloris, Nitzschia, Phaeodactylum, Porphyridium, Schizochytrium,* and *Tetraselmis.* Among these 12 genera, *Chlorella,* a genus of single-celled green algae belonging to the family of *Chlorellaceae,* is intensively studied as a resource for fuel and biomaterial production.

There are at least 13 species in the genus of *Chlorella: Chlorella autotrophica, Chlorella colonials, Chlorella lewinii, Chlorella minutissima, Chlorella pituita, Chlorella pulchelloides, Chlorella pyrenoidosa, Chlorella rotunda, Chlorella singularis, Chlorella sorokiniana, Chlorella variabilis, Chlorella volutis*, and *Chlorella vulgaris* [5]. Despite the fact that it was discovered and reported in 1890 by a Dutch microbiologist and botanist, Dr. Martinus Willem Beijerinck, with a color illustration, *Chlorella vulgaris* is thought to have survived on Earth for more than 2.5 billion years [6].

There are numerous studies that discuss how to use microalgae lipids as feedstock to produce biofuel (mostly biodiesel) or how to use microalgae carbohydrates to produce reducing sugars. However, few studies have been conducted to investigate the comprehensive utilization of both lipid and carbohydrate in microalgae. This article will discuss the use of lipids and carbohydrates in microalgae, as well as their comprehensive utilization, with a focus on the *Chlorella* genus.

2 Utilization of lipid

The genus of *Chlorella* is the most studied microalgae for its use in the production of fatty acid methyl ester (FAME, biodiesel) because it is rich in lipid. In the early 2000s, due to the relative easiness of transesterification and esterification, studies on FAME production started with dried microalgae as feedstock. However, more and more studies shifted to wet microalgae in the 2010s because of the high energy consumption required to dry wet microalgae.

2.1 Dried microalgae as a feedstock

There are two methods for using dried microalgae as feedstock to produce biodiesel: the fractional step method and the one-step method.

Fractional step method: The typical steps of utilizing microalgae to produce biodiesel in the early 2000s were harvesting, drying, extraction of lipid with a solvent (normally hexane), and transesterification and esterification of lipid in the presence of a catalyst (normally NaOH) [7]. In a few studies, a fermentation method was adopted to replace chemical transesterification. Li et al. vacuum dried *Chlorella protothecoides* at 40°C and then extracted lipids with *n*-hexane as a Soxhlet solvent [8]. Extracted lipids were then bio-catalyzed at 38°C for 12 h by fixed lipase from *Candida* sp. 99–125 to produce biodiesel. The conversion rate was as high as 98.15% from oil to monoalkyl esters of fatty acids, though it was much slower than with chemical methods.

Onestep method: In order to reduce operating steps and simplify the production of biodiesel from microalgae, and subsequently to reduce the time and cost, a one-step method or in situ/direct transesterification (extraction of lipid and transesterification/esterification to FAME take place simultaneously) was explored by researchers. Velasquez-Orta et al. explored a one-step method to transfer lipid from *Chlorella vulgaris* to biodiesel using NaOH as a catalyst [9]. The molar ratio of the methanol to the lipid to the NaOH was 600:1:0.15. Carvalho Júnior et al. also employed a one-step method to produce biodiesel from spray dried *Chlorella vulgaris* with a ratio of methanol to dried microalgae of 204 mL:2 g (v:w) [10]. High FAME yields were achieved by these studies. However, large amounts of methanol, catalyst, and drying (especially spray drying) were required, which are costly.

2.2 Wet microalgae as feedstock

Theoretically, the elimination of water facilitates transesterification. However, the drying process is an energy-consuming operation [11]. About 2260 kJ of energy is required to thermally remove 1 kg of water at 100°C under 1 atm. In order to save energy for drying, the production of biodiesel directly from wet algae has been gaining a spotlight [12–15]. There are two methods for utilizing wet microalgae as feedstocks: the fractional step method and the one-step method.

Fractional step method: This method saves a large amount of energy during the drying operation of wet algae, which is good. However, extraction of lipid from wet microalgae raises new challenges that are not encountered by dried biomass. Normally, nonpolar organic solvents, including hexane and chloroform, and solvent mixtures such as chloroform and methanol (1:1 v/v) are used to extract lipids from biomass. However, the polar water phase reduces the extraction yield of lipids due to the low immiscibility of water and nonpolar organic solvents [16]. Another major difficulty for microalgae, especially for the wet ones, is that they have a tough cell wall. In order to reduce this negative impact, pretreatments using physical and enzymatic methods are normally employed to disrupt the cell wall before lipid extraction. The physical methods primarily include: (1) high temperature and pressure: typically, an autoclave is operated at a temperature of 125°C under a pressure of 1.5 MPa for 5 min or longer, (2) bead-beat: a bead beater is operated at a high-speed above 2500 rpm for 5 min or longer, (3) microwave heating: a microwave oven is usually used to heat the wet microalgae to ~100°C and hold it for 5 min, (4) sonication: a sonication machine is normally operated at ultrasonic frequencies for several minutes, and (5) osmotic shock: a

shaker/vortex is used to shake microalgae in a 10% NaCl solution for a couple of minutes and then settles for 48 h [17]. Lysozyme and cellulase are used in enzymatic methods to disrupt the cell wall [18].

One step method: In order to further reduce the production steps, consequently reducing the consumption of energy and time, concurrent extraction and reaction, also known as direct transesterification or in situ transesterification, is becoming more and more popular. Special containers were adopted in these studies due to the high temperature and pressure required by operation steps [19–21]. Macías-Sánchez et al. studied the direct biodiesel production from wet microalgae (75% moisture content) in a 5 L pressure proof reactor with stirring throughout the whole process. Their optimized reaction conditions were at a temperature of 100°C, for a time of 105 min and under a pressure of 2.5 atm [13]. Pan et al. used deep eutectic solvent technology with a pressure-proof reactor to improve the extraction yield of lipid from wet microalgae (65%–67% moisture content) [22]. Im et al. used a 14 mL pressure proof Teflon-seal tube in their study to conduct the reactions [23]. But these harsh situations and the requirement for special devices seriously limit the industrialization of both batch and continuous processes.

3 Utilization of carbohydrate

Microalgae not only possess lipids but also carbohydrates. Therefore, microalgae are also known as the third generation of starch resources, which has a trend to substitute the first and second generations of starch resources (cornstarch and lignocellulosic feedstock) to produce reducing sugars [24,25]. In microalgae, carbohydrates exist mainly in the form of starch in the cytoplasm or in the form of cellulose in the cell wall [1,25].

3.1 Saccharification and fermentation

There are two approaches to using starch in microalgae for bioproduction: separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) [25].

Separate hydrolysis and fermentation (*SHF*): In this category, hydrolysis (also known as pretreatment) is conducted to reduce starch into reducing sugars, followed by fermentation with bacteria or yeast [24]. Hydrolysis of starch is normally conducted via three main pathways: chemical, enzymatic, and physical hydrolysis, or a combination of two of the three methods.

Chemical hydrolysis, using acid or alkali, usually possesses the features of fastness, simplicity, and cheapness. However, it will degrade the starch too much and produce unwanted inhibitors for the microbial fermentation stage if a large amount of chemicals are used, and the reaction temperature is too high and the time is too long [26].

Zhou et al. [27] mixed a small amount of wet *Chlorella* sp. TIB-A01 with HCl at a concentration of 2% to 6% in a stainless steel cylindrical reactor with a total volume of 15 mL. Then, the mixture was incubated at 120°C for 60 min, or 180°C for 10 min followed by microbial fermentation using *Saccharomyces cerevisiae* Y01 at 30°C to produce ethanol. Kumar et al. [28] prepared a 30 mL polytetrafluoroethylene (PTFE)-lined container by mixing 0.2 g of

dried *Chlorella vulgaris* powder with 5 mL of 1–5 M concentration HCl. Then the mixture was incubated in a household microwave oven and operated at 1200 W for 5–30 min at temperatures of 80°C, 100°C, or 120°C, respectively.

Besides HCl, H_2SO_4 is also frequently used in chemical hydrolysis. The procedure is similar to that used in the determination of the carbohydrate content, except for lowering the concentration of acid remarkably (from 4% to 1%). Ho et al. [25] hydrolyzed freeze-dried *Chlorella vulgaris* FSP-E with 1% H_2SO_4 at 121°C for 20 min, followed by a 24 h fermentation with Zymomonas mobilis ATCC 29191 at 30°C.

Harun et al. first reported using alkali to hydrolyze microalgae [29]. Five grams of dried microalgae powder of *Chlorococcum infusionum* (another carbohydrate rich (32.52% w/w) species) was mixed with 0.75% (w/*v*) of NaOH, and incubated at 120°C for 30 min. The usage of alkali instead of acid is believed to favor lower temperature and pressure during hydrolysis of biomass [30], and minimize the degree of inhibition during fermentation thereafter, consequently cutting production costs [31].

Enzymatic hydrolysis is regarded as more environmentally friendly than chemical hydrolysis. Moreover, higher glucose production can be achieved from raw materials without producing inhibitors, but it is not as fast as chemical ones [25]. However, in order to improve sugar yield, disruption of the cell wall is normally needed before enzymatic hydrolysis due to the tuff structure of the microalgal cell wall. Disruption of the cell wall normally includes physical, chemical, and enzymatic methods, and sometimes a combination of two of the three methods. Ho et al. [25] pretreated dried *Chlorella vulgaris* FSP-E powder with an acetate buffer solution (pH = 6) by sonication for 10 min, followed by incubation using an autoclave at 121°C for 20 min. After pretreatment, enzymatic hydrolysis was carried out at 45°C with an enzyme mixture of endoglucanase, β-glucosidase, and amylases. A maximum glucose yield of 0.461 g/g biomass (90.4% of total glucose) was obtained after 72 h.

Simultaneous saccharification and fermentation (*SSF*): In addition to SHF, Ho et al. [25] attempted an SSF operation as a comparison. They mixed sonication-pretreated *Chlorella vulgaris* with an acetate buffer solution at a pH value of 6.0 at a biomass concentration of 20 g/L. Then, the mixture was autoclaved and the solution was inoculated with *Z. mobilis* at an inoculum size of 10% (or optical density, OD 600 = 2.0). Meanwhile, a filter-sterilized enzyme solution (mixture of endoglucanase, 0.61 U/mL; b-glucosidase, 0.30 U/mL; amylase, 0.75 U/mL) was added into the mixture. The SSF was then conducted at 30°C in a desktop fermenter for 36 h. An ethanol titer of 4.17 g/L (87.1% theoretical yield) was obtained.

4 Comprehensive utilization of lipid and starch

Based on the aforementioned studies, it could be concluded that either the production of biodiesel from lipids or the saccharification of starch in microalgae is technically feasible when conducted separately. However, there are few reports discussing the technical feasibility of comprehensive utilization of lipid and starch, i.e., using both components of lipid and starch in microalgae, not to mention economic advantages. The ideal of comprehensive utilization of lipid and starch fits an integrated biorefinery concept of microalgae raised by Wijffels et al. in an outlook on microalgal biofuels [32,33]. In order to reduce costs and energy

consumption, all production procedures should be simplified and all components of microalgae, such as lipids, carbohydrates, proteins, minerals, and even water, should be utilized. In essence, the concept is actually the "comprehensive utilization" that people usually refer to, although it is defined as a fancy term for biorefinery by Wijffels et al. [32].

Lee et al. [34] conducted a study on using the residuals of *Chlorella* sp. KR-1 to produce bioethanol. Several grams of freeze-dried microalgae were mixed with methanol and dimethyl carbonate (3:7, v/v) to extract lipid at 60°C for 12 h. After lipid extraction, the residual was subjected to either enzymatic or diluted acidic hydrolysis. In enzymatic hydrolysis, 5% (w/v) of the microalgae residual was mixed with an enzyme cocktail containing polygalacturonase (Pectinex Ultra SP-L with 9500 PGU/mL), amyloglucosidase (AMG 300 L with 300 AGU/mL), cellulase (Celluclast 1.5 L with 700 EGU/mL), and Viscozyme L (with 100 FBG/Viscozyme L was added at an amount of 0.08–2.4 mL/g of the dry biomass residual. Viscozyme L was added in an amount from 0.08 to 2.4 mL/g of the dry biomass residual. The mixture was then incubated at different temperatures (35–55°C) and pH values (3.5–6.5). In diluted acidic hydrolysis, 5% (w/*v*) of the microalgae residual was mixed with either HCl or H_2SO_4 at various concentrations (0.1, 0.3, 0.5, 0.7, 1 N) and autoclaved at 121°C for 15 min. The sugar content after each hydrolysis was determined using the dinitro salicylic acid (DNS) method. A fermentation using *Saccharomyces cerevisiae* KCTC 7931 (ATCC 20626) was carried out after hydrolysis to complete the separate hydrolysis and fermentation (SHF) processes. For comparison purposes, SSF was also carried out by mixing 5% of the residual with an enzyme cocktail, *Saccharomyces cerevisiae*, and sodium acetate buffer. The ethanol yield via SSF was 82.3% versus 79.3% through SHF. Lee et al. [34] concluded that compared with the SHF process, the SSF method was more suitable for residuals of *Chlorella* sp. KR-1 after lipid extraction due to simpler operation, less operation time, and a higher ethanol production yield. It is believed that during the lipid extraction process, the cell walls of the microalgae, *Chlorella* sp. KR-1, have already been disrupted, which increases the accessibility of the enzyme cocktail to starch in microalgae cells. Therefore, lipid extraction from microalgae rich in both lipid and starch followed by either a hydrolysis or an SSF saves as a cell wall disruption step, and consequently, lowers production costs.

Based on the integrated biorefinery concept of microalgae, the integrated production of biodiesel and bioethanol from *Scenedesmus* sp. (rich in both lipid and starch) was carried out following either (1) an order of direct transesterification first followed by starch hydrolysis, or (2) an order of starch hydrolysis first followed by direct transesterification [16]. In direct transesterification, 1 g of ultrasound pretreated freeze-dried biomass was mixed with dimethyl carbonate (DMC), lipase immobilized on Celite material with an enzyme activity of 182 U/g and distilled water. In starch hydrolysis, 1 g of ultrasound pretreated freeze-dried microalgae was mixed with 0.3 N H_2SO_4 and autoclaved at 120°C for 30 min. In bioethanol fermentation, a mixture of 20 mL of hydrolysate from starch hydrolysis, 5 g/L yeast extract, 10 g/L peptone, and 47.5 mL of distilled water in a 125 mL conical flask was autoclaved. Then, 2.5 mL of *Saccharomyces cerevisiae* was added into the mixture, and the mixture was incubated at 30°C for 72 h in an incubating shaker at 180 rpm. For the first combined operation, direct transesterification was carried out first. The residual after transesterification was washed, ready for starch hydrolysis, and subsequent bioethanol fermentation. For the second operation order, starch hydrolysis was carried out first followed by the separation of supernatant and solid biomass using centrifugation. The supernatant from starch hydrolysis was used for bioethanol fermentation, and the solid residual was washed and dried for direct

transesterification. The results indicated that the combined method with the order of direct transesterification first, followed by ethanol fermentation yielded 92% and 93% of methyl ester and ethanol, respectively, which were higher than those with the other order (starch hydrolysis first followed by direct transesterification). Authors attributed this observation to a reduction of some phenolic compounds that occurred in direct transesterification, which acted as inhibitors in the fermentation process.

Ma et al. explored the feasibility of comprehensive recovery of lipid and carbohydrates from wet microalgae *Chlorella vulgaris* both technically and economically [11] which is the first report on the comprehensive usage of lipid and starch from wet microalgae. The key innovations of the study are: (1) it is the first article reporting comprehensive utilization of lipids and carbohydrates directly from wet microalgae so far; (2) all steps adopted in the study (including cell destruction, in situ transesterification of lipid for biodiesel, and enzymatic saccharification of starch for reducing sugars) were carried out at a temperature below 100°C and pressure of 1 atm, and no specific apparatus was required; (3) the combination of radio frequency (RF) heating assisted pretreatment and enzyme CTec2 had a synergistic effect on cell wall destruction; and (4) adsorption kinetics of the optimized enzyme combination on untreated microalgae (negative control), RF heated microalgae (positive control), and microalgae residual were measured and the adsorption isotherm was calculated with the Freundlich equation. In addition, mass balance, energy balance, and brief techno-economics were analyzed.

4.1 Experimental design of biodiesel production followed by enzymatic saccharification of starch

Based on a literature review, past research [35–37] and preliminary experiments, a flow chart of comprehensive usage of lipid and starch in microalgae was developed (Fig. 8.1). Wet microalgae were disrupted in a phosphoric acid solution by RF heating (details in Section 4.2). Then, the lipid part was converted into biodiesel through esterification and transesterification assisted by RF (details in Section 4.3), and the starch part was hydrolyzed into fermentable sugars (details in Section 4.4).

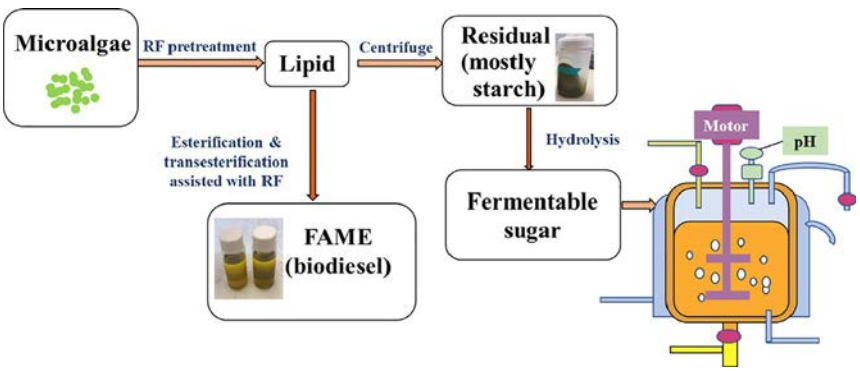

FIG. 8.1 Flow chart of direct utilization of lipid and starch from wet microalgae.

4.2 Pretreatment of wet microalgae

4.2.1 Disruption of cell wall using RF heating

The presence of large amounts of water and tough cell walls are the two major barriers that hamper the use of wet microalgae. In order to reduce the negative impact due to tough cell walls, pretreatments using physical, chemical, and enzymatic methods are normally employed to disrupt the cell wall. A certain amount of wet microalgae (80.3 ± 0.4 wt%) was mixed with 2.5% (weight of dry microalgae) phosphoric acid (85%) in a big enough glass beaker for the synergistic wall disruption purpose. Instead of H_2SO_4 or HCl, H_3PO_4 was used because inorganic phosphate is a major component of nucleic acids, nucleotides, phospholipids, lipopolysaccharides (LPS), and teichoic acids in microorganisms. Moreover, H_3PO_4 is usually used as one of the nutrient sources in fermentation thereafter [38–40]. The wet microalgae with phosphoric acid were then heated using an RF heater with a maximum output power of 6 kW (SO6B; Strayfield, Berkshire, England) operated at a 27.12 MHz frequency. A magnetic stirring system was employed to stir the wet mixture to enhance heat transfer and uniformity. Fiber-optic sensors (Neoptix, Inc., Quebec City, Quebec, Canada) were used to measure the temperature and control the pretreatment. Once the temperature reached 90° C, the RF heater was switched on and off to maintain the temperature of the mixture at around 90°C for 20 min [11]. The container was covered either with a piece of glass or a piece of Parafilm to maintain the moisture content.

A scanning electron microscope (SEM) was used to illustrate the changes in *Chlorella vulgaris* cells before and after the disruption of the cell wall, assisted by RF heating. According to SEM images shown in Fig. 8.2A, the cells were clear and intact before the disruption operation, but the surfaces of the cell walls became very fuzzy with many small dents on them after the disruption (Fig. 8.2B), indicating damage. Furthermore, plenty of extracellular substances were observed, which should leak from the inside of the cells, confirming the breakage of the cell wall caused by the synergistic disruption effect of phosphoric acid and RF heating. The breakage of the microalgae cell wall resulted in an easy leakage of lipids inside microalgae cells during the biodiesel production phase, thus increasing the biodiesel conversion rate.

4.2.2 Disruption of cell wall using enzyme CTec2

After disruption of the cell wall using phosphoric acid and RF heating, a separation of the liquid portion and solid portion of the microalgae mixture was conducted by a high-speed centrifuge (Thermo Scientific Sorvall Legend RT+, Waltham, MA, United States) at 9900 rpm for 10 min. The liquid phase was saved for further production of biodiesel, and the solid phase was dried by air flow in a fume hood for 2 days before saccharification. In the solid phase, the enzyme cocktail (CTec2) was added to hydrolyze cellulose and hemicellulose (if any) to synergize the disruption of the cell wall further and enhance the starch release from the cell. According to the SEM images of the microalgae samples, it is obvious that the cells with the addition of CTec2 were destructed more severely and hydrolyzed more thoroughly compared to those without CTec2 [41].

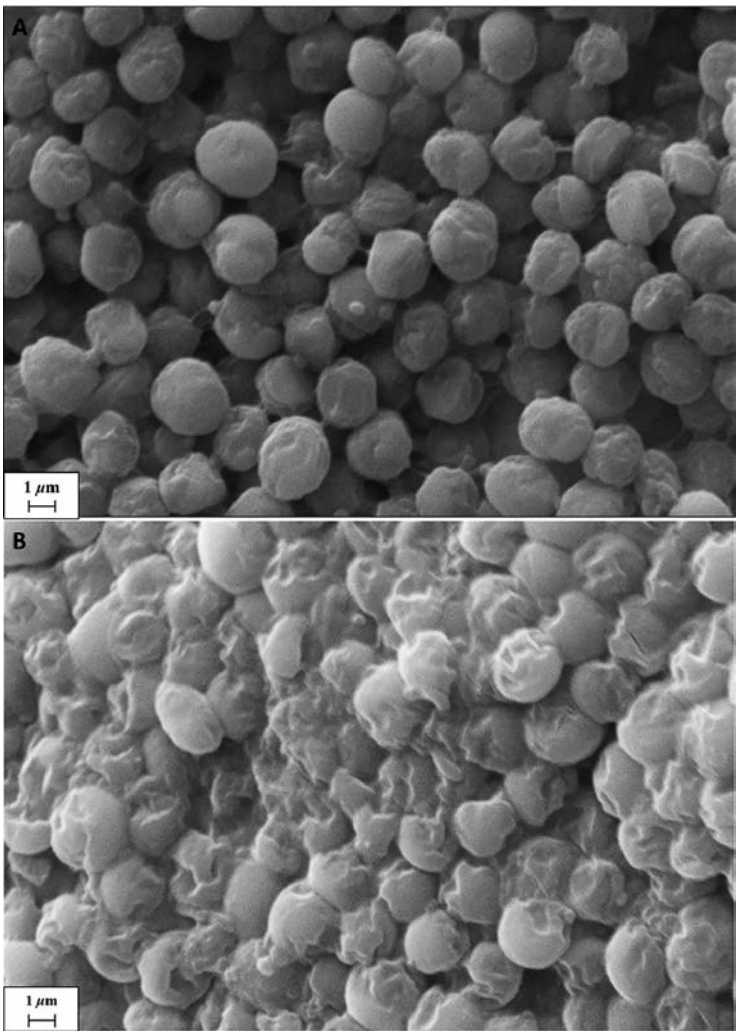

FIG. 8.2 (A) SEM image of raw microalgae and (B) after pretreatment [11].

4.3 Direct biodiesel production from wet microalgae

The comprehensive usage of microalgae consists of two parts (Fig. 8.1): the conversion of lipids to biodiesel and the saccharification of starch to fermentable sugars. In the biodiesel production part, when there are both free fatty acids (FFAs) and triacylglycerols (TAGs), plus a high concentration of water at the same time, the conversion process becomes very complicated. According to chemical principles, biodiesel is produced from FFA through esterification and TAG through transesterification. In esterification, if an alkali (such as NaOH) is used as a catalyst, saponification will be significant when notable FFA and/or water are present.

Therefore, it is more appropriate to use acid as a catalyst for esterification when wet algae are involved in the system. On the contrary, if TGA is used as a raw material to produce biodiesel through transesterification, it's better to use a base (such as NaOH) as a catalyst because the reaction is considerably quicker than using acid as a catalyst. Therefore, to obtain desirable reaction efficiency, a two-step conversion of biodiesel from lipids of microalgae was designed [11].

In the first step, a mixture of pretreated wet algae (5 g), methanol (2 mL), catalyst (8 mL of HCl-MeOH solution (36% HCl: MeOH = 5: 95 in volume) and hexane (10 mL) were placed in a 125 mL Erlenmeyer flask. The mixture was mixed using a magnetic stirring system for 5 min, and then heated by an RF heater to 50°C and maintained the temperature for 20 min. During the stirring and RF heating, a condenser was used to prevent MeOH evaporation. After the RF heater was switched off, the stirring continued for 5 more min to ensure the reaction was adequate. Then, 20 mL of hexane was added, and the flask was sealed with a piece of Parafilm and shaken in a shaker operated at 200 rpm and room temperature for 1 h to extract FAMEs, TAGs, and remaining FFAs. After the extraction, 10 μL of methyl tridecanoate was added to the mixture as an internal standard, along with 30 mL of deionized water. The mixture was centrifugated at 9900 rpm for 10 min. The liquid phase was taken out for the second step of biodiesel production, and the solid phase was used later as a carbohydrate source for saccharification. In the second step, the hexane in the liquid phase was recovered using a rotary evaporator operated at 45 °C. After this, 10 mL of NaOH-MeOH solution (NaOH: MeOH = 0.5 g: 100 mL) was added to the flask, and the mixture was heated by an RF heater to 55°C and maintained the temperature for 20 min. Finally, 10 mL of hexane was used to extract the FAMEs produced in this step for analysis.

The production yields of FAME were 58.8 ± 1.2% and 79.5 ± 3.0% after the first and second stages, respectively. Although the hypothesis of the two-step conversion of biodiesel from wet microalgae was directly worked out, the relatively low FAME yields (79.5 ± 3.0%) strongly suggested a study on optimization of the production conditions should be conducted to achieve a higher FAME yield.

4.4 Fermentable sugar production from residual of wet microalgae

The solid phase (residual after biodiesel production) obtained from the centrifugation (Section 4.3) was dried by air flow in a fume hood for 2 days before saccharification to remove the organic solvent. Before enzymatic hydrolysis, the pH value of the solid phase was adjusted to 5.0 using NaOH. The hydrolysis was carried out by mixing about 10 g of residual with concentrated sodium citrate buffer. The final total volume of the mixture was 50 mL, the pH value was controlled at 5.0, and the final sodium citrate concentration was controlled at 50 mM. Several sets of enzyme combinations with different dosages of each enzyme were tested on different samples to optimize the enzyme combination [34,42,43]. After optimization, the most efficient enzyme combination of α-amylase (1500 FAU/g glucan), amyloglucosidase (100 U/g glucan) and CTec2 (40 FPU/g glucan) was selected. The enzymatic hydrolysis of residuals was conducted using an incubator shaker operated at an agitation speed of 200 rpm, and a temperature of 50°C for 3 days. The mixture was sampled at certain intervals to determine the reducing sugar concentration with an HPLC.

The carbohydrates of the *Chlorella vulgaris* used in the study consisted of 31.5 ± 0.4% glucose and 10.2 ± 0.2% galactose (calculated on dry biomass). After the FAME production, glucose was the major reducing sugar in the solid residual, with a concentration of 23.0 ± 0.7% (calculated on dry biomass). A glucose yield of 54.5 ± 1.2% after 72 h with the enzyme combination listed above was achieved.

4.5 Mass balance, energy balance and brief techno-economics

The biomass balance analyses on lipid and carbohydrates were calculated by dividing the total weight of biodiesel (g) by the total neutral lipid (g), and the reducing sugars by the total dry weight of microalgae.

The mass flow chart of raw microalgae to biodiesel and to reducing sugars in this study (Fig. 8.3) demonstrates that out of every 1 kg of wet microalgae, 0.0538 kg of lipid can be extracted and converted into biodiesel, and 0.021 kg of reducing sugar can be produced. The lipid was extracted directly from the raw wet microalgae using a one-step (also known as direct transesterification or in situ transesterification) method, the same as what most other studies reported. However, the reducing sugars were obtained from microalgae residuals (which were normally rendered as low-value plant fertilizers or even as landfills) after the lipid was removed. The additional monetary value obtained by using the carbohydrates in the residual in this study took the advantage of comprehensive usage.

The energy consumption of RF heating in the pretreatment/disruption was calculated using the following Eqs. (8.1), (8.2) [44]:

$$Q = m \cdot c_p \cdot \Delta T \tag{8.1}$$

$$c_p = 4.187\,X_w + 2.093\,X_f + 1.256\,X_s \tag{8.2}$$

where Q is theoretical sensible heat [45] needed in the process, m is mass (kg) of wet microalgae, ΔT is temperature difference of the wet algae (°C, the temperature difference

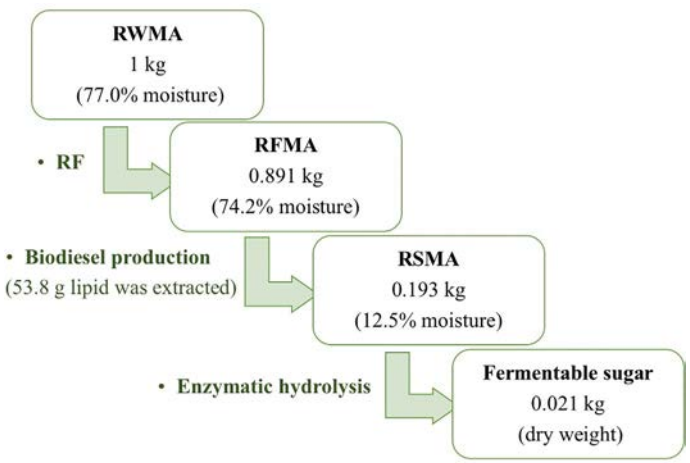

FIG. 8.3 The mass flow chart of raw microalgae to biodiesel, and to reducing sugars. The value was calculated on 1 kg of raw wet microalgae [41].

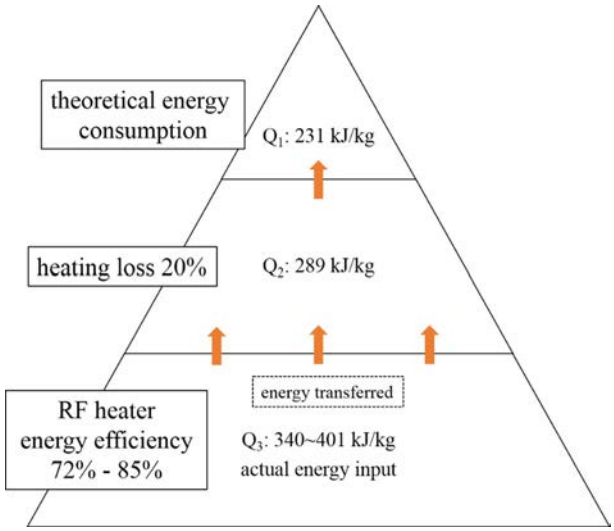

FIG. 8.4 The energy consumption of the radio frequency heating [41].

between 25°C and 90°C during RF heating), c_p is specific heat (kJ/[kg °C]) for wet microalgae, X is mass fraction, of which the subscripts w is water, f is fat, and s is nonfat solids. A heat loss was assumed at 20% and a range of heating efficiency of RF heater was set at 72%–85%. Based on results from the energy pyramid (Fig. 8.4), the input energy needed to heat 1 kg of raw wet microalgae from room temperature to 90°C and hold it at 90°C for 20 min ranged from 340 to 401 kJ/kg. If spray dried or freeze-dried microalgae were used to replace the wet algae, besides the sensible heat required to raise the microalgae temperature from 25 to 100°C, it requires about 2260 kJ more latent heat to remove 1 kg of water at 100°C under 1 atm by evaporation. Based on the chemical composition of the algae samples used in this study, it was calculated that about 1740 kJ of energy was required to dry 1 kg of wet microalgae. Apparently, biodiesel production directly from wet microalgae reduces energy consumption and costs dramatically.

The electrical power ranging from 0.094 to 0.111 kWh was needed for RF heating during the pretreatment of raw wet microalgae when the heat amount was converted into electricity. The average retail price of electricity in Alabama, United States was 0.119 USD/kWh in the year of 2019 (Consumer Electronics Control, 2020). Thus, moneywise, the total electrical cost of RF heating was from 1.11 ¢to 1.32 ¢/kg wet microalgae. The tough structure and complex chemical compositions of cell walls have major negative impacts on the use of lipid and starch by microalgae [46]. Pretreatment should disrupt the cell wall of microalgae and enhance the leakage of lipid and starch into the cell, which leads to increased production efficiency and reduced downstream processing costs. However, no matter whether they are physical, chemical, or enzymatic approaches for algal biomass processing, the pretreatment always contributes a significant fraction to the overall cost [47–49]. Fortunately, the above data shows the disruption of the cell wall using RF heat is technically efficient and economically inexpensive.

In this study, biodiesel production required disruption of the cell wall in the pretreatment no matter whether the solid residual (starch) would be used to produce reducing sugars.

Therefore, no additional cost in terms of pretreatment was required for the saccharification, which greatly reduced the production cost of reduced sugar. With the additional value of the reducing sugars, it diluted the unit cost of biodiesel and reduced sugars versus producing them separately. The current practice is to dispose of the microalgae residual as waste by a paid service after the lipid is used. Therefore, the raw material cost for reducing sugar here should actually be a negative value (disposal cost), but it was treated as a zero value in the calculations. Compared with other studies on reducing sugar production, only three kinds of enzymes in a relatively small amount were used thanks to efficient cell wall breakage by RF heating. The reduced operation costs in the fermentation stage lower the total cost too. Nevertheless, compared with other methods using integrated methods, Ma et al.'s method can still achieve comparable or even better sugar yields.

5 Conclusions and future perspectives

The results show that it is both technically and economically feasible to comprehensively utilize lipid and starch in wet microalgae to produce biofuel and biomaterials. The lipids were used for biodiesel production, and the carbohydrates were saccharified into fermentable sugar, which could be further utilized as the raw material to produce either biofuel or biochemical products. Although this comprehensive usage method was developed based on *Chlorella vulgaris*, it can be applied to any other species of microalgae that possesses lipid and carbohydrate content. The optimal utilization of microalgal components includes at least three major components, including lipid, carbohydrate, and protein. Only the combination of lipids and carbohydrate has been described so far. In the future, it would be fascinating to explore the combination of lipid and protein, carbohydrate and protein, or even the three-component combination of lipid, carbohydrate, and protein.

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CHAPTER

9

Algae: An emerging feedstock for biofuels production

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OUTLINE

1 Introduction

The concept of using green algae as a feedstock for biofuel production is gaining attention due to spiraling prices of petroleum, rapidly diminishing natural oil reserves, and more notably, the emerging lethal problems associated with global warming caused by the burning

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of fossil fuels. According to the Organization of Petroleum Exporting Countries (OPEC) World Oil Outlook (WOO) report of 2020, it has been predicted that oil is expected to remain the dominant fuel and its global demand will increase from nearly 100 million barrels per day (mb/d) in 2019 to around 109 mb/d in 2045, while the present petroleum reserves of OPEC members are diminishing very rapidly [1]. In the future, fossil fuels resources will no longer be available in the same amount and at the same price. Given the critical dependence and limited availability of fossil fuels, world economists have consistently urged the search for such renewable energy resources that are sustainable, eco-friendly, emit no or little carbon dioxide, and meet a significant portion of global energy demand at prices comparable to current petroleum prices [2]. These are the key factors that triggered the initiation of first-generation biofuel production from food crops such as sugarcane, maize, soybeans, and vegetable oils. However, the limited availability of biomass resources, technological constraints, poor energy balance, higher costs, and the food-energy dispute in the future steered the focus toward the second generation of biofuels. The second-generation biofuels are obtained from nonfood biomass resources such as grass straws, wood, jatropha, switchgrass, and organic wastes with a higher net energy yield per acre than the first-generation ethanol from maize and sugarcane. However, the complex thermochemical and biochemical processes used to generate biofuel from such resources result in reduced yields and increased production costs. Therefore, to solve these critical issues, researchers have explored the use of photosynthetic thallophytes as the ultimate source of third-generation biofuels. This not only concludes the food versus fuel debate but also eliminates the dependency on terrestrial biomass for biofuel production.

Algal feedstock has proved to be a prominent and sustainable source of renewable fuel. The application of algal biomass for the production of biofuel started in the early 1970s. Green algae are considered to be a promising oleaginous flora, containing 20%–70% lipids, and have extraordinary potential for cultivation as energy crops [3]. Their growth and oil-producing efficiency are much higher than usual oilseeds and other conventional crops. For instance, microalgae containing 30% oil could produce 58,700 L/ha and those with 70% yield 136,900 L/ha of oil per year, much greater than the oil obtained from rapeseed or soybeans grown in the same area [4]. The annual macroalgae biomass production has tremendously increased over the past 50 years worldwide, reaching 32.67 Mt [Fresh Weight (FW)] in 2016. Most of the macroalgal biomass (97%) comes from China and Indonesian aquaculture, whereas the remaining 3% is obtained from wild harvesting, which is led by Chile, China, and Norway [5]. Available data on global microalgae biomass and oil production is almost nonexistent, and information is scattered and difficult to access. The worldwide biomass production of microalgae in 2004 was 7000 t/year [6,7]. However, it is pertinent to mention that the quantity of biofuels produced currently from algae is far less than that of conventional fuels.

A variety of fuel types can be produced from algae, such as biodiesel, kerosene (aircraft fuel), bioethanol, biobutanol, methane, and biogas. Apart from biofuel production, algae have many other advantages, such as mitigation of environmental CO_2, release of O_2 for other living organisms, bioremediation of heavy metals and organic wastes in water sources, storage of energy in the form of carbohydrates and lipids, and production of other valuable products such as pigments, nutraceuticals, bioactive pharmaceutical compounds, rare earth metals, agar, fertilizers, and lubricants, etc. In addition, they can be cultivated on nonarable land under difficult agro-climatic conditions, unlike terrestrial plants. Algae can be cultured year-round under optimum conditions of light and temperature. Some strains grow fast, doubling

their biomass in less than 2 days. These unique properties make algae a promising feedstock for biofuel and other industries.

Keeping in view the high oil content and other useful bioproducts, different countries like the USA, China, India, and the UK, Singapore, Denmark, and Belgium have initiated algae utilization as a biofuel source on an industrial level [8]. Other countries, such as Argentina and Brazil, are also accelerating biofuel generation from algae [9]. According to the summary report of the Center for Climate and Energy Solutions, algae products could reach a potential market size of $320 billion by the year 2030 [10].

2 Types of algal biomass for biofuel production

The term "algae" refers to a diverse group of photosynthetic thallophytes that includes members of both prokaryotic (e.g., blue-green algae or cyanobacteria) and eukaryotic (e.g., *chlorella*, diatoms, etc.) organisms. They grow in aquatic environments and carry out photosynthesis to generate biomass. Unlike terrestrial plants whose vegetation is influenced by precipitation and temperature, algal growth is mostly dependent on light and nutrients. When there is an abundant supply of nutrients, as in some polluted waters, algal cells multiply rapidly and produce an abundant biomass. Two different types of algae can be produced to make biofuels: microalgae and macroalgae.

2.1 Microalgae

Microalgae are a large and diverse group of photosynthetic eukaryotes with a simple cellular structure, ranging from unicellular to multicellular. More than 25,000 microalgal species have been isolated and identified [11]. About 80% of all algal species are comprised of microalgae. They are ubiquitous in distribution and found everywhere in soil, rivers, lakes, springs, and oceans, subject to the availability of sunlight and dissolved nutrients in water [5]. The commercially important microalgae are categorized as Cyanophyceae (blue-green algae), Chlorophyceae (green algae), Rhodophyceae (red algae), Phaeophyceae (brown algae), and Bacillariophyceae (diatoms) [12]. These species can grow freely in a suspended medium and do not need a substance to attach to; therefore, they are commonly known as phytoplankton.

Microalgae represent one of the promising sources of biofuel feedstock due to several unique properties, such as:

- Microalgae contain a potential amount of lipids (20%–70%) per unit weight of dry biomass [13].
- They are fast-growing and can multiply in as little as 8–24 h, depending upon the species and the availability of nutrients and sunlight [4].
- They grow everywhere in fresh, saline, brackish, and seawater and utilize nutrients from various sources, including agricultural, municipal, and industrial wastewaters.
- They are highly efficient photosynthetic organisms, converting sun power, water, and CO_2 into organic matter. They can tolerate stressful conditions and have evolved multiple

pathways for nutrients and other growth factors utilization to facilitate growth and lipid accumulation.

- They can be grown in open ponds, tubular photobioreactors, or vertical reactors depending upon the available space to maximize annual biomass productivity per unit area.
- Microalgae can be grown on noncultivable land by utilizing fresh as well as wastewater resources. Any type of wasteland can be used for algae farming and wastewater resources such as municipal wastewater, industrial wastewater, and cattle waste effluents can be fed to algal ponds for self-purification and to reduce process expenditure.
- They are the most effective biological systems for CO_2 sequestration, thus protecting the environment from the major greenhouse gases emitted from burning fossil fuels and other sources.
- Microalgae can produce other valuable products such as pigments, protein, vitamins, polysaccharides, animal feed, manure, biopolymers, etc.

Microalgae exhibit three types of metabolic pathways to meet their nutritional requirements: autotrophic, heterotrophic, and mixotrophic types of mode of nutrition. Autotrophic microalgae acquire their needed organic matter from CO_2 and H_2O in the presence of sunlight (Fig. 9.1). Heterotrophic microalgae utilize organic compounds such as glucose and glycerol, for their growth, whereas mixotrophic species simultaneously show both autotrophic and heterotrophic behavior depending upon the growth conditions. For example, if monosaccharides are available in the culture medium, mixotrophic algae start feeding on these simple sugars and shift from autotrophic mode toward heterotrophic nutrition to save energy.

The key features that are focused on by researchers to explore the most adequate microalgal species for biofuel production include high lipid content, rapid growth rate, and enhanced biomass production. Nutrient stress such as N, P, and K deficiency may trigger

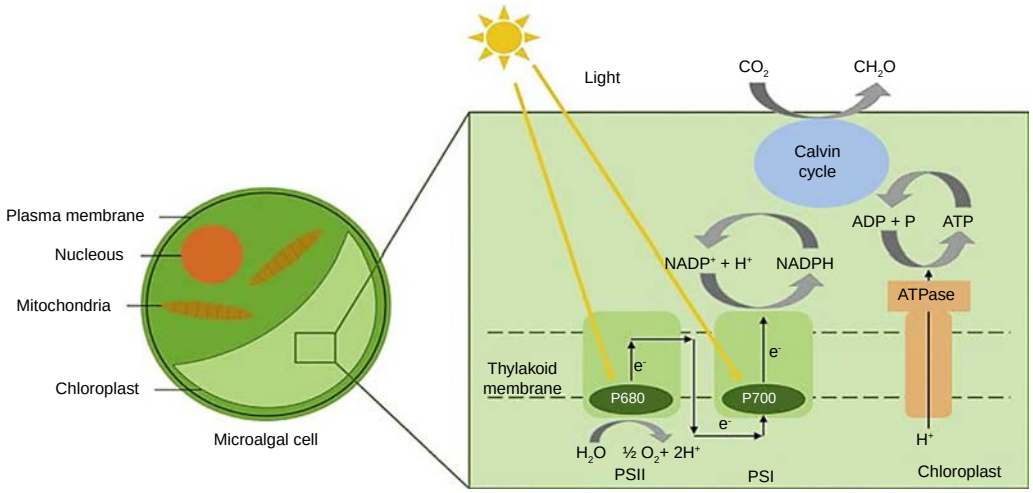

FIG. 9.1 Schematic presentation of the photosynthetic process taking place in the microalgal cell. *Adapted from M.A. Vale, A. Ferreira, J.C.M. Pires, A.L. Goncalves, CO_2 capture using microalgae, in: M.R. Rahimpour, M. Farsi, M.A. Makarem (Eds.), Advances in Carbon Capture, Woodhead Publishing, UK, 2020, pp. 381–405.*

lipid synthesis in microalgae. The microalgal species *Botryococcus braunii*, *Scenedesmus obliquus*, *Chlamydomonas reinhardtii, Chlorella emersonii*, *Chlorella vulgaris*, *Dunaliella tertiolecta*, *Nannochloropsis oculate,* and *Schizochytrium* sp. have been reported as high oil yielding species in various studies [13–16]. However, powerful downstream processing technology is needed for biofuel production from such a promising microalgal feedstock.

2.2 Macroalgae

Macroalgae, also known as seaweed, are large multicellular organisms that exhibit many forms and sizes. Their size may vary from a few millimeters to more than 70 m (as in the case of some kelp species) in length. Of the 72,500 known species of algae, 14,500 belong to macroalgae, which make up 20% of the total algal flora. They are mostly harvested from wild stock and several are cultivated in aquaculture systems. Based on their pigmentation, they are usually categorized into three main classes: Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae). The annual global biomass production of macroalgae has significantly increased over the last 50 years and reached 32.67 Mt in 2016, the majority (97%) of which is obtained from aquaculture. The increase in annual biomass production of macroalgae is largely attributed mainly to expansion and innovation in the aquaculture system. According to an estimate, an approximately 20 Mt increase in global aquaculture production of macroalgae has been recorded over the last 20 years. China was the main aquaculture producer. However, Indonesia showed the largest increase in macroalgal production during this period. Interestingly, the top major macroalgal-producer countries through aquaculture belonged to Asia during the years 2014–16, whereas Chile produced the maximum macroalgal biomass (0.364 Mt) through wild harvesting during this period [5]. Currently, the USA, Germany, France, Canada, and the Netherlands are interested in establishing large-scale cultivation of macroalgae.

Despite the fact that macroalgae have long been used for numerous purposes, such as food production and medicine, they have not been given due attention as an energy source. There are several advantages to using macroalgae as a fuel stock, such as:

- Macroalgae show higher sunlight conversion efficiency (approximately 3%–8% against 0.5% for terrestrial plants), producing higher biomass production per unit area. Their biomass productivity is 10 times higher than microalgae and terrestrial plants. The annual productivity varies from 1 to 15 kg/m^2 of dry weight [17].
- Planktonic species may have a maximum chlorophyll content of 3 g/m^2 and an algal biomass of 10 $kg/m^{2,}$ thereby accelerating the photosynthetic rate.
- They usually rely on natural sources of nutrients for their growth and can withstand fluctuations in temperature, salinity, light, pH, depth, and ocean current. Thereby, several macroalgal species appear to be suited for mass cultivation under the natural environment.
- They use marginal land unsuited for agricultural use (e.g., seashore and desert land), thereby not competing with cultivable land for food production.
- The direct uptake of HCO_3 instead of CO_2 for their growth makes them more efficient photosynthetic flora. Thus, they quickly mitigate CO_2 and restore balance to the environment [18].

- Wide range cultivation in seawater and natural ponds offers an efficient way for large-scale production of macroalgae as a fuel feedstock.
- The biomass can be harvested batch-wise round the year; so, its production is not limited to a specific season.
- They can be cultivated without the use of fertilizers and pesticides, thereby minimizing the cost of production and causing no environmental pollution.
- Seaweed cultivation on a commercial scale has been practiced for a long time, revealing successful outcomes for about 200 species globally. The intensively cultivated species include *Undaria pinnatifida*, *Laminaria japonica*, *Porphyra* sp., *Kappaphycus* sp., *Gracilaria* sp., *Enteromorpha* sp., *Eucheuma* sp., and *Ulva*. However, the use of these species for biofuel production is still under investigation [19].

Macroalgae usually yield less than 5% oil on a dry weight basis, so they are not considered a viable feedstock for biodiesel production. On the other hand, they have a significantly higher carbohydrate content (>50% dry weight), making them suitable for bioethanol production via fermentation [20]. In certain species of red algae, the presence of agar (a galactose polymer) strongly influences the carbohydrate content, making it difficult to release simple sugars. However, some recent research has attempted to develop saccharification methods that could be used to easily release glucose from cellulose and galactose from agar to increase bioethanol production by fermentation. Likewise, some species of brown algae such as *Saccharina japonica* and red algae such as *Gelidium amansii*, are promising sources of biohydrogen through anaerobic fermentation [21].

Macroalgae are generally larger in size and so more easily harvested. In certain cases, maceration is needed for easy collection and pumping. The presence of hydrocolloids such as alginates is an added complication that becomes highly viscous in solution, making macroalgal biomass collection more laborious and energy-consuming. Another drawback with macroalgae is the presence of high ash and chloride content, which may promote chloride stress corrosion.

3 Algal cultivation and biomass production

The major factors that influence algal growth are availability and intensity of light, temperature, nutrient concentration, and availability of CO_2. In addition, other factors, such as availability of O_2, pH of the medium, water quality, salinity, cell density, fluid dynamics and hydrodynamic stress, depth, gas exchange, toxic chemicals, dilution rate, competition by other algae, and the presence of pathogens such as fungi, bacteria, and viruses, etc., also affect algal growth. Generally, algae show three types of cultivation modes, which are autotrophic, heterotrophic, and mixotrophic. In the autotrophic mode, light and inorganic carbon are the primary sources of energy for algal growth, whereas, in the heterotrophic mode, algae obtain their energy from the oxidation of organic carbon sources. Mixotrophic algae show a mix of both autotrophic and heterotrophic modes, i.e., the carbon sources can be in both organic and inorganic forms.

Light of varying intensity and wavelength is required for the growth and efficient production of biomass of autotrophic and mixotrophic species. For example, Metsoviti et al. [22]

attained a 25%–100% increase in biomass of *C. vulgaris* and a 25.6% and 24.7% increase in its lipid content in June and September when the solar radiance was at its maximum. Likewise, Lv et al. [23] demonstrated that light intensity of 60 mmol/m^2/s significantly enhanced the biomass content and lipid concentration of *C. vulgaris*. The temperature must generally remain within the range of 20–30°C for optimum growth of all three types of algae, whereas the essential nutrient elements for algal growth include C, N, P, Fe, and, in some cases, silicon (Si) [13].

Microalgae can grow in a variety of habitats, such as ponds, lakes, rivers, oceans, wastewater, and even deserts. However, mass cultivation of microalgae is usually carried out either in open or closed systems. The open pond system can be grouped into natural systems (e.g., lakes), artificial ponds, and containers. Traditional or open-field cultivation of many autotrophic species is carried out in open ponds constructed of concrete, clay, brick, or plastic sheets. In general, shallower ponds (0.15–0.45 m depth) are preferred so that the microalgae are exposed to sufficient sunlight. Different types of ponds are commonly used, such as circular ponds, tanks, and raceway ponds. Raceway ponds are the most popular among the algae farming community [13]. A raceway pond is typically made of a closed-loop circulation channel about 0.3 m deep. The algal cells and nutrients are mixed with the help of a paddlewheel. The flow of water is guided around by baffles placed in the flow channel (Fig. 9.2). The frontal space of the paddlewheel is the feeding point for microalgae culture into the pond. The culture is uniformly distributed around the pond in the direction of the flow of water, and on completion of the circulation loop, the broth is harvested behind the wheel. Raceway ponds are less expensive to operate, but they have several shortcomings as compared to closed bioreactors. For example, they have low biomass productivity, low CO_2 use efficiency, substantial water loss through evaporation, and difficulty maintaining temperature and can be easily polluted by other algae and bacteria [24,25].

Cultivation in closed systems can be carried out in photobioreactors (PBRs) of various configurations. Closed photobioreactors have increased efficiency and higher biomass production as compared to open pond systems [26]. They can be built either outdoors in sunlight or indoors with an artificial light system. Closed photobioreactors have several advantages

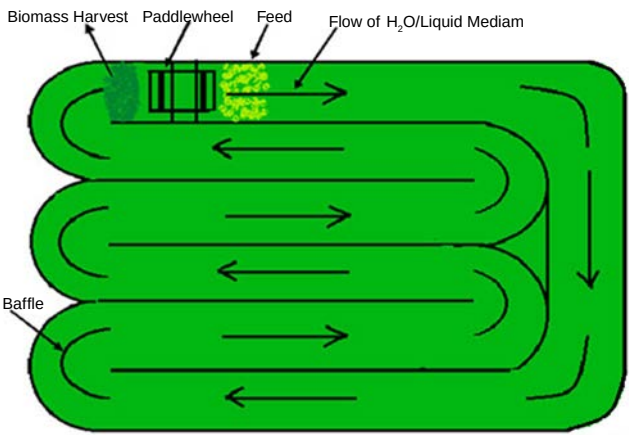

FIG. 9.2 Schematic diagram of a raceway pond.

over open pond systems: they minimize external contamination, prevent CO_2 loss, and water evaporation, permit high cell concentration, and offer better control over cultural conditions such as pH, light, temperature, CO_2 and nutrient concentration, etc. However, closed PBRs require significant start-up investment and can only be used to cultivate an axenic culture of specific microalgal species with compatible physiologies [27], which is why such technology was not adopted until recent decades [26]. Several types of closed PBRs have been designed and developed for the cultivation of microalgae, including tubular or vertical columns, horizontal photobioreactors, and flat plate photobioreactors. The detailed description and their advantages and weaknesses are further discussed by Carvalho et al. [28], Spolaore et al. [6], Chisti [13], Gouveia, [29], Sun et al. [30], Yen et al. [31], Chen et al. [25], and Veeramuthu and Ngamcharussrivichai [16].

For macroalgal biomass production, submerged cultivation is preferred. Rocky substrates are their natural habitats, where they form stable, multilayered perennial vegetation, capturing all available photons. The best way to cultivate macroalgae (seaweed) is in the sea or a similar body of water body. Their cultivation requires much less capital cost and is easy to maintain, but due to heavy biomass production, the process is much more laborious. For offshore culture, the bottom of artificially constructed ponds should be made of sandy clay to reduce the organic load. The culture must be submerged in seawater maintained in the pond at a depth of 0.3–0.5 m with a specific gravity of around 1.01. For optimal growth, a slightly alkaline pH (7.0–8.0) and temperatures between 15°C and 30°C are preferred. Pools, crab ponds, bays, and straights are chosen as algal cultivation sites. The existing culture naturally releases the spores into their habitats, and when the conditions become favorable, germination takes place. The most common method of seaweed cultivation is the longline technique, which uses two parallel lengths of rope held together and afloat by large plastic floats, which are attached at intervals of approximately 10 m in the sea. Threads of seaweed are spread on the upper rope, which gradually establishes themselves as the growth progresses. The plantlets then grow, ultimately covering the whole area between the two ropes. This method has been successfully employed for the cultivation of *Alaria esculenta* in Ireland and produced a yield of 15 kg per meter of rope over a period of 5 months [32]. Commercial farming of seaweed has a long history, especially in Asia. With 4.2 million tons of production, the kelp *Laminaria japonica* is the most prominent macroalgae cultivated mainly in China [33]. About 10 species of seaweed are used, including *Monostroma* and *Enteromorpha* (green algae), *Undaria pinnatifida* and *Laminaria japonica* (brown algae), and *Porphyra* sp., *Kappaphycus* sp., *Eucheuma* sp., and *Gracilaria* sp. (red algae).

4 Biomass harvesting and dewatering

Algae biomass harvesting refers to the separation of algae from water for subsequent biofuel production. Because of low density (generally less than 1 g/L in open ponds) and carrying a negative charge, the algal cells are normally found in a suspension state, which makes the harvesting process expensive and energy-consuming. The harvesting cost may contribute to 20%–30% of the total production cost [34], which is one of the major hurdles in large-scale algal biomass production. Because of the minute cell size (1–20 μm) and suspension in water, algae biomass harvesting is challenging. Therefore, researchers have explored several types

of harvesting methods in order to achieve maximum biomass during harvesting. Algal biomass harvesting may be achieved in one or more steps and involves several physical, chemical, and biological processes to accomplish solid-liquid separation. However, the overall harvesting process can be divided into two distinct phases: 1) bulk harvesting, to collect algal biomass from suspension, and 2) thickening, to concentrate the algal slurry after harvest [35,36].

The existing methods for microalgal biomass harvesting can be broadly classified into physical, chemical, and biological methods. The physical methods are further divided into four categories: (1) Sedimentation, which uses gravitational forces to separate solids (cell biomass) from liquids. (2) Filtration uses a medium that is permeable to allow the liquid to pass through while retaining the algae biomass. The process is usually accomplished by applying pressure generated by a vacuum or gravity across the filter. (3) Centrifugation involves centrifugal force to separate solids from liquids while spinning the suspension of algae cells. This method is currently considered too costly and energy-intensive for the primary harvesting of microalgae. However, it is useful in secondary harvesting to concentrate an initial slurry (10–20 g/L) into an algal paste (100–200 g/L). (4) Floatation is a physicochemical gravitational separation process in which gas bubbles pass through a liquid-solid suspension, causing the microalgae to float to the surface by adhering to the gaseous bubbles [37]. In this method, the size of the algal cell is of prime importance, as smaller cells would easily be lifted to the top of the medium by the bubbles. This method is usually recommended for suspensions having particle sizes of less than 500 μm [38].

Currently, flocculation is the most promising method for large-scale microalgal biomass harvesting. In this process, suspended cells are accumulated together into large aggregates, utilizing the chemical aspects of microalgae cells. The negative surface charge on the algal cells can be countered by the addition of chemicals known as flocculants. The increased particle size, thus formed, facilitates settling or agglutination with floatation bubbles, leading to the quick separation of algal biomass. The commonly used flocculants include inorganic cationic salts such as aluminum sulfate [$Al_2(SO_4)^3$], ferric chloride ($FeCl_3$), and ferric sulfate [$Fe_2(SO_4)^3$] or organic polymers such as chitosan. The effectiveness of these multivalent salts depends on the strength of the ionic charges they carry. Apart from chemical flocculation, the self-flocculation of cells of certain species also takes place in response to environmental stimuli, carbon limitation, nitrogen stress, and alterations in pH and level of dissolved oxygen [37].

The biological method of microalgal biomass harvest, also known as bio-flocculation, utilizes other microorganisms such as bacteria and fungi to adhere to the surface of algae, causing the weight to increase and resulting in the settling of the cells to the bottom of the vessel. The use of microorganisms for the flocculation and sedimentation of algal cells has been reported by Schenk et al. [39], Lee et al. [40], Kim et al. [41], Salim et al. [42], Zheng et al. [43], Ummalyma et al. [44], Nazari et al. [45], etc.

Macroalgae, on the other hand, are harvested with comparatively simple but laborious methods. Macroalgae are either attached to a solid substrate or float in a fluid suspension. The stagnant biomass can be directly harvested from the medium, which can be easily done with nets. The nets are raised from the bottom of the pond and rolled over a rotary cutter mounted on the harvesting boat. In this way, the mass is collected and shifted to a land-based facility for dewatering and drying. The freshly harvested biomass of both micro- and

macroalgae usually contains 70%–80% water, and the remaining 20%–30% is comprised of solid materials. This huge amount of water can cause spoilage of the biomass, especially in a hot environment. Therefore, immediate dewatering is recommended after harvest to prevent the spoilage of biomass. The harvested biomass is commonly dried under the sun, which works well in low-humidity climates. Nevertheless, complete drying of algae biomass cannot be achieved with sun drying due to high moisture content. In addition, sun drying is time-consuming, during which the biomass may be contaminated by bacteria and fungi, leading to bad smelling. In addition to sun drying, drum drying, spray drying, and freeze-drying, etc. are some other methods used for drying algae biomass [46]. These methods are more efficient but require energy input, in contrast to natural sun drying. Removal of 1 kg of water from freshly harvested algal biomass needs over 800 kcal of energy. Therefore, dewatering is one of the crucial steps that imposes a significant economic burden on the entire process of algal biofuel production.

5 Lipid extraction and biofuel production

Algae is a potential source of lipids and the oil content of some microalgae can exceed 70% by weight of dry biomass (Table 9.1). Lipid productivity represents the mass of lipids produced per unit volume of microalgae culture per day and depends upon the algal growth rate and the oil content of the biomass. Microalgal species with higher lipid productivities are chosen as the preferred feedstock for biofuel production. The lipid profile of microalgae varies with cultivation conditions, temperature, aeration, and N and P starvation, etc. However, the main lipids found in microalgae include triglycerides, free fatty acids, phospholipids, lipoprotein, glycolipids, sterols, pigments, and hydrocarbons [48].

Selection of appropriate species, mode of cultivation, biomass harvest, drying, lipid extraction, fractionation, and conversion are the key determinants for successful biofuel production from algae. The overall process from algal biomass production to harvesting and drying (upstream) to biofuel preparation (downstream) is presented in Fig. 9.3.

5.1 Lipid extraction and purification from algal biomass

Due to the tough exterior of algal cells, the cells first need to be physically disrupted to make it possible for an organic solvent to significantly penetrate and extract the lipids from algal biomass. Cell disruption is usually carried out by mechanical pressing, bead milling, osmotic shocks, ultrasound, microwaves, and enzymatic hydrolysis, etc. The bead milling process involves the application of fine beads to rupture the algal cells. A thick suspension of algae cells is rapidly stirred in the presence of glass or ceramic beads, exposing the cells to the crushing action of the beads when they become colloid with them. The cells are sheared apart, releasing the internal contents. A biomass concentration of 100–200 g/L gives the best economical results for the bead-beating process [55]. Lee et al. [56] achieved 28.1% oil extraction with the bead-milling process from *Botrycoccus braunii*, *Chlorella vulgaris*, and *Scenedesmus* sp. The expeller pressing squeezes the cells under high pressure and can extract nearly 75% of the oils from algal cells in a single-step process. Similarly, osmotic shock treatment is the best

TABLE 9.1 Lipid productivity (g/L/day) and oil content (%) of some prominent microalgae species presented in descending order of oil content.

Microalgal species	Lipid productivity (g/L/day)	Oil content (%)	Reference
Botryococcus braunii	0.020	75	[11]
Scenedesmus quadricauda	0.140	73	[47]
Dunaliella tertiolecta	0.120	71	[11]
Auxenochlorella protothecoides	–	70	[48]
Parachlorella kessleri	0.590	66	[49]
Neochloris oleoabundans	–	65	[11]
Chlorella emersonii	0.036	63	[11]
Chlorella protothecoides	2.000	57	[11]
Phaeodactylum tricornutum	1.900	57	[11]
Desmodesmus sp. S81	0.020	48	[50]
Chlorella vulgaris	0.042	48	[51]
Nannochloropsis oceanica	0.057	46	[52]
Parachlorella kessleri	0.500	25	[53]
Chlamydomonas reinhardtii	0.081	21	[54]

way to disrupt the algal cells, especially those lacking cell wall synthesis. In this method, a sudden change in osmotic pressure is created due to which cells are ruptured, releasing intracellular fluid, including oil. Ultrasonic-mediated extraction is another way to recover oils from microalgae cells through cavitation generated by ultrasonic waves. During this process, high-intensity bubbles are created around microalgae cells in the slurry under reduced pressure. The pressure is gradually increased, which enlarges the bubbles' sizes and eventually burst them. The shock wave, thus produced, disrupts the cell walls and releases the oil into the solvent. Similarly, microwaves shatter the cells using the shock of high-frequency waves. Likewise, enzymatic hydrolysis uses enzymes to hydrolyze the cell wall, using water as a solvent. Autoclaving [56], use of supercritical CO_2 [57], vacuum-shelf drying [58], pulse electric field technology [59], and electroporation technique [60] are other techniques used to extract lipids from algae biomass. After mechanical disruption of cells, the algal biomass is exposed to solvents.

5.1.1 Solvent extraction

Due to their nonpolar nature, most of the lipids are insoluble in water but soluble in organic solvents such as petroleum ether, hexane, chloroform, benzene, and acetone, etc. Therefore, the solubility of lipids is of critical importance, and must be given due importance during their extraction. The choice of extraction solvent depends on the type of lipid present in the sample, i.e., nonpolar lipids such as triglycerides or polar lipids such as phospholipids and glycolipids. Usually, hexane or petroleum ether is used in the case of the Soxhlet and

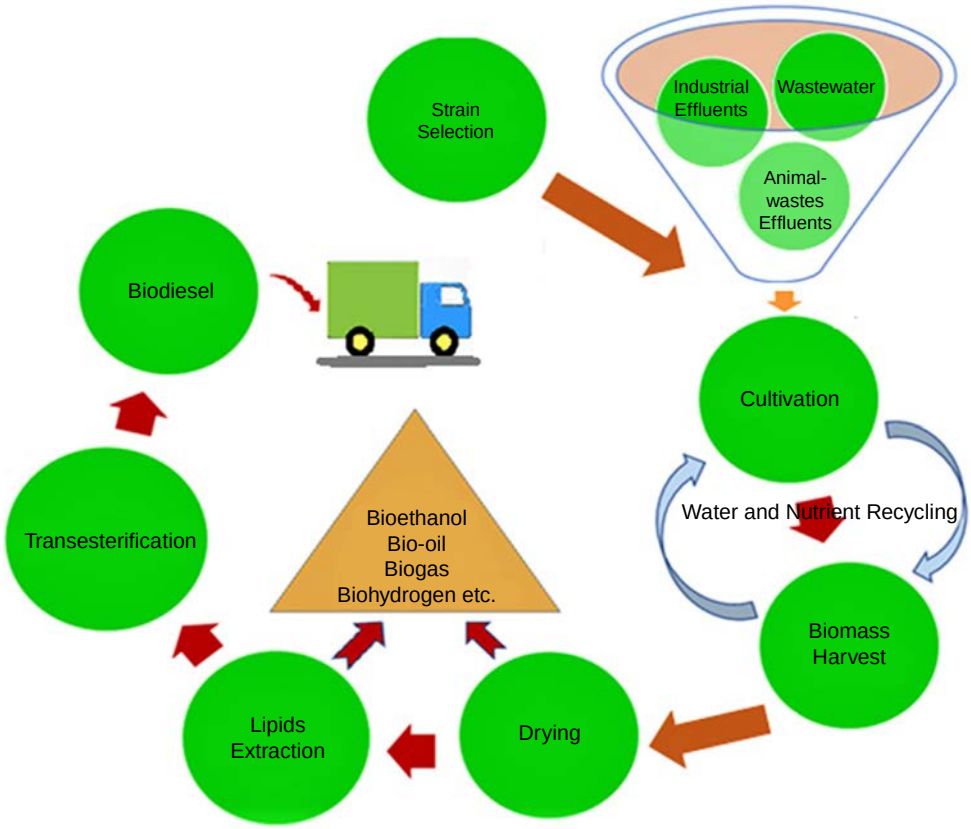

FIG. 9.3 Process flow diagram of microalgal biofuel production.

Goldfish methods, whereas chloroform-methanol-water or chloroform-methanol mixture is used in the Bligh and Dyer method or Folch method [59]. Apart from extraction with nonpolar solvents, Samori et al. [61] have recently devised a novel extraction method that uses interchangeable polarity solvents for lipid extraction. This method can extract 70%–80% of the lipids from diluted algal cultures.

5.1.2 Soxhlet extraction

This method is frequently used for the extraction of nonpolar lipids using petroleum ether (bp 40–60°C) or hexane as solvents. This is a semicontinuous process during which the sample is soaked in solvent for 5–10 min in the extraction chamber of Soxhlet's apparatus. The solvent is recovered by siphoning it back into the boiling flask. Nonpolar lipids are also extracted with the Goldfish extraction procedure, where the solvent continuously flows over the sample instead of semicontinuous application as in the Soxhlet method.

5.1.3 Hydrothermal liquefaction

Hydrothermal liquefaction uses hot, compressed water to convert algal biomass into liquid biocrude [62]. During this process, the algae cell suspension is heated at a high temperature (200–300°C) under 15–20 MPa pressure to bring the water content of the broth to a subcritical condition and to prevent latent heat vaporization. The complex molecules of algal biomass are broken down under these conditions and then repolymerized into oily compounds. This approach is especially suitable for the extraction of lipids from high-moisture content algal biomass.

5.1.4 Wet lipid extraction

This extraction procedure is similar to solvent extraction, but in this method, wet algal biomass is extracted with a suitable solvent or a combination of solvents. This procedure has the advantage that it eliminates the drying step, saves energy, and reduces process expenditure. However, the lack of a wide range of applicability of solvents to wet biomass and the interference of moisture content during extraction are the major limitations of this procedure.

5.1.5 Acid hydrolysis

Acid hydrolysis is a proven, simple, and effective method for extracting lipids from algal biomass. A variety of acids, such as HCl, H_2SO_4, and HNO_3, can be used for hydrolysis. This method is best suited for the extraction of bound lipids as it mainly targets lipid-protein and lipid-starch interactions, thereby releasing bound lipids [48,63].

5.1.6 Ionic liquids

Extraction of lipids from moist biomass of algae with ionic liquids is a relatively new approach that employs green solvents that are capable of withstanding temperatures of up to 140°C without decomposition. Chemically speaking, ionic liquids are salts that are composed of inorganic anions and organic cations. The use of ionic liquids for lipid extraction from algal biomass has been discussed in detail by Pojo et al. [48], Chen et al. [64], Olkiewicz et al. [65], and Orr et al. [66].

5.2 Technologies for algal biomass conversion

Important biofuels obtained from algae include biodiesel, bioethanol, biogas, and biohydrogen. Chemical conversion or transesterification of algal oil yields biodiesel, whereas the direct combustion of algal biomass can produce electricity. Similarly, biochemical conversion technology can be used to generate methanol and ethanol from algal biomass through anaerobic digestion and fermentation, respectively. Likewise, thermochemical conversion of algal biomass leads to the production of bio-oil and charcoal (pyrolysis), syngas or fuel gas (gasification), and bio-oil (liquification process) [7,67]. The various processes involved in the conversion of algal biomass into various biofuels are shown in Fig. 9.4.

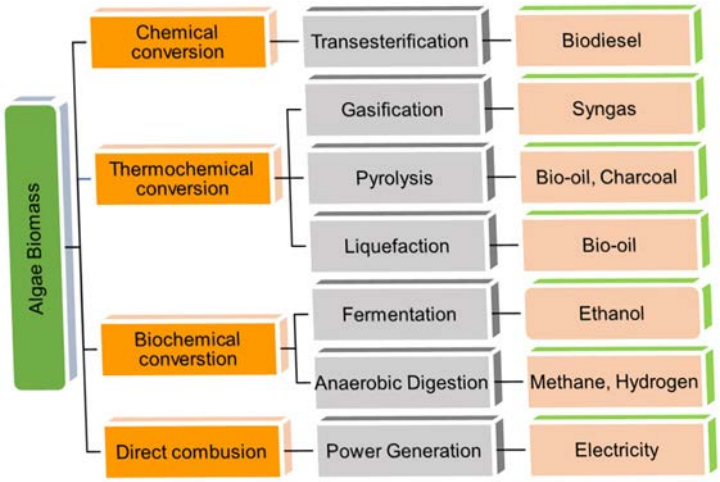

FIG. 9.4 Production of various biofuels from algae biomass.

5.3 Transesterification of algae oil to biodiesel

The crude oil obtained from algae usually has a higher viscosity than diesel oil, which makes it unfit for direct use in engines. Therefore, chemical conversion, known as transesterification, is needed to reduce the viscosity and increase the fluidity of algal oil. Esterification is any reaction between a fatty acid (or organic acid) and alcohol that results in the production of an ester, whereas transesterification is a further reaction between an ester and alcohol to replace the alkoxy group, producing fatty acid-alcohol esters. This is a stepwise process, during which triglycerides are first broken down into diglycerides, then into monoglycerides, and finally into glycerol. During transesterification, small and straight-chain ester molecules, which are similar to diesel fuel, are obtained from the large and branched triglyceride molecules [68].

Transesterification of algae oil to biodiesel is accomplished by the reaction of triglycerides with alcohol (typically methanol) in the presence of a catalyst (usually alkali or acid) to produce fatty acid methyl esters (FAME) and glycerol. The overall reaction can be represented as (Fig. 9.5).

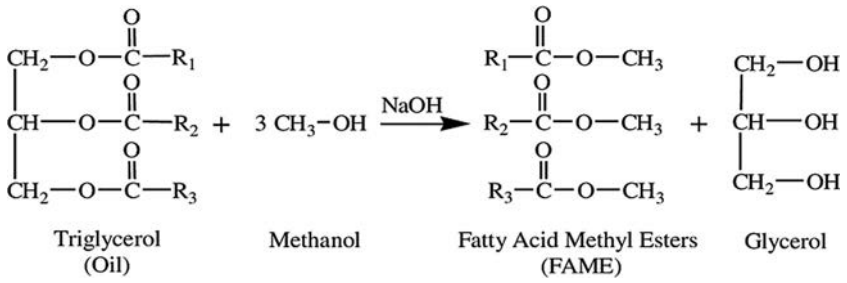

FIG. 9.5 Transesterification reaction showing the conversion of triglycerides to fatty acid methyl esters (FAME).

The commonly used alcohols in transesterification are methanol, ethanol, propanol, butanol, and amyl alcohol. However, due to its low cost and physical and chemical advantages, methanol is widely used. The nature of the catalyst also significantly influences the rate of reaction and efficiency of the conversion process. Acid, base, or enzyme-based transesterification reactions are mostly used in practice for the conversion of algae oil into biodiesel. Acid-based (H_2SO_4/HCl) transesterification is carried out at low to moderate temperature and pressure, with a high alcohol-to-oil ratio and a high catalyst concentration, resulting in the slow production of FAME [69]. Enzyme-based transesterification is accomplished with the help of the enzyme lipase [70]. However, the complex instrumental setup needed, and the costliness of enzymes make the process limited. The most viable and efficient transesterification of algae oil is done by using a base catalyst (hydroxides or carbonates). At normal atmospheric pressure and temperatures of 60–70°C in the presence of excess methanol, base-catalyzed transesterification is 4000 times faster than acid-based transesterification [59,71,72].

6 Techno-economic analysis of algal biofuel production

Capital investment, operating costs, and earnings or sales are the key components that form the basis for the economic analysis of algae biofuel production. Thus, the sum of capital and operating costs minus the revenues generated from all products obtained from algae determines the cost of producing algae biofuels [73]. In addition to biofuel, the sale of other by-products of the process, e.g., residual biomass, glycerol, and other value-added products such as protein and carbohydrates, could generate substantial revenue and reduce the final cost of biofuel production [74]. Nonetheless, the techno-economic and life cycle analysis shows that microalgae-derived fuels are not cost-competitive in comparison with petrochemical fuels [16]. Despite the potential of microalgae, some challenges need to be overcome to expand the production and commercialization of algae biofuels. For example, cultural conditions should be further optimized for enhanced growth rate, biomass yield, and ability to compete for nutrients versus lipid content. Likewise, after biomass harvest and dewatering, nutrient supply and water recycling are also essential for the sustainable production of microalgae biofuel [75].

A preliminary economic analysis conducted by Chisti [13] showed that the estimated cost of production of algal biomass is $2.95/kg and $3.80/kg for closed PBRs and open raceways, respectively. Similarly, Norsker et al. [76] calculated the production costs of algal biomass raised in open ponds, horizontal tubular PBRs, and flat-panel PBRs to be 4.95, 4.15, and 5.96 € per kg, respectively. Regarding oil production, the cost incurred per liter of oil obtained from biomass cultured in closed PBRs has been estimated to be ~ $2.80/L, assuming that the algal biomass contains 30% oil. The estimated cost could further be reduced to $0.72/L for algal biomass that contains 70% oil [13]. The following formula can be used to estimate the cost of algae oil where it can be a competitive substitute for petrodiesel [13]:

$$C_{\text{algal oil}} = 25.9 \times 10^{-3}\, C_{\text{petroleum}}$$

where: $C_{algal\ oil}$ is the price of microalgal oil in dollars per liter and $C_{petroleum}$ is the price of crude petroleum in dollars per barrel. The above equation is based on the fact that the energy value of algae oil is approximately 80% of the calorific value of crude petroleum. For example, in order to be competitive with petrodiesel in the market, the price of algae oil should not surpass \$0.47/L if the petroleum price is \$68/barrel (as of the existing petroleum price in May 2021).

The cost analysis by the U.S. National Renewable Energy Laboratory indicated that the cost of algae biodiesel was in the range of \$0.53–0.85/L (2012 USD values), whereas Nagarajan et al. [77] estimated a final cost of algal biodiesel in the range of \$0.42–0.97/L [77]. In a recent study, Branco-Vieira et al. [74] estimated the cost of biodiesel production from algae oil to be 0.33€/L and that of biomass production at 2.01€/kg in a 15.247 ha facility. Considering a scaled-up size of 100 ha, the total investment cost has been reported as varying from \$48/m^2 for open raceways to \$66/m^2 for tubular PBRs [76]. Likewise, the economic approximation of biofuel production from microalgae has also been published in other literature by Medipally et al. [7], Douskova et al. [78], Stephens et al. [79], Singh and Gu [80], Williams and Laurins [81], Acien et al. [82], and Heo et al. [83]. All these studies indicate that biofuel production from algae is currently more expensive than fossil fuel and needs further innovations in cultivation and downward processing to save energy and minimize the cost. A possible way to minimize the cost of production is to integrate the microalgal cultivation system with wastewater treatment to avoid the use of fertilizers, reduce utilities and labor charges, improve CO_2 use efficiency, design more productive PBR and raceway systems, and control depreciation, especially the cost of harvesting equipment such as centrifuges. In addition, the valorization of high-value bioproducts such as protein, carbohydrates, rare earth metals, and pharmaceutical ingredients, etc. from residual algae biomass can compensate for the production cost, decreasing the final cost of biofuel production.

7 Prospects and challenges

A significant number of research studies explain and validate the technical feasibility of algae biofuel production. The economic viability of algae biofuels on a large scale is yet to be ascertained for a long time or in the near future. Nevertheless, as a strategic opportunity to meet future energy demands, algae technology must be consistently developed into a sustainable and eco-friendly renewable source of high-energy biofuel. Even though the lipids and biomass productivity of algae are superior to terrestrial oleaginous crops, the cultivation, harvesting, drying, and biofuel conversion processes are quite complicated and consume a substantial amount of energy input. The positive opportunity of using algae biomass to generate alternative and sustainable fuel is mostly overclaimed. Therefore, further innovations and improvements are needed to address the feasibility of utilizing this renewable feedstock for commercial use. For instance, system automation for cultivation, biomass harvest, and product extraction are opportunities that could be further explored for improvement to address the process of industrialization. The integration of algae farming with improved technologies can cut down on product and production costs. The overall process economics could be positively influenced by the integration of algae biofuels with wastewater treatment and

simultaneous coproduction of valuable byproducts such as carbohydrates, protein, lipids, pharmaceutical and cosmetic ingredients, etc. These byproducts could serve as raw materials for other industries to produce alcoholic and ester fuels, medicines, nutraceuticals, and cosmetics. Likewise, the utilization of entire biomass to make the concept of zero waste schemes will bring privileged improvements favoring commercialization. The existing technological limitations could further be reduced by mapping the availability of compatible resources, allocating the corresponding resources to algal farms, increasing accessibility, and incorporating technology into the overall process. Moreover, consistent support from technology developers, policymakers, and politicians, as well as public acceptability are the key motives for continuing to drive algae technology for solving future energy crises.

8 Conclusions and future outlook

Algae represents an emerging source of biofuels and could potentially replace fossil fuels in the future. Green algae offers several advantages as a fuel supply. Algae, for example, contains a significant amount of lipids, utilizes wasteland and wastewater resources, produces O_2, and produces a variety of valuable products such as pigments, pharmaceutical compounds, fertilizers, agar, and lubricants. Considering the importance of algae, the global production of algal biomass has increased significantly in the last 50 years. However, due to the higher costs associated with the production technology, the low economic viability of algal biofuels restricts their production on a commercial scale. Several obstacles still need to be overcome to bring algae biofuel technology on par with the petroleum industry. Cultivation system design, strain selection, space, availability of water, efficient light and CO_2 utilization, algal growth and nutrient uptake, biomass harvesting and drying, oil extraction, and biofuel conversion technologies are some of the key areas that demand considerable attention. There is a need to explore new algal strains and genetically improve the existing strains, for both higher oil content and overall productivity, as well as resistance to pathogens and environmental stresses. Integration of the algal fuel production process with other technologies, especially wastewater reclamation, is a promising strategy to be considered for economic viability and cost optimization. Likewise, the cost of biofuel could further be reduced by coextraction of valuable byproducts in concurrence with biofuel. At present, the large volume of active research in conjunction with the significant interest in algae biofuel production by the public and private sectors attest that this technology will ultimately gain economic viability and will be able to replace some proportion of fossil fuels in the near future. However, the commercialization of algal fuel production is still in the developmental stage, and more research should be focused on the economic feasibility of full-scale production. The commercialization of algae production could redefine the future of global energy generation and the mitigation of greenhouse gas emissions through algae carbon capture.

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CHAPTER

10

Microalgal biofuels: A sustainable pathway for renewable energy

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1 Introduction

Global energy demand is expected to increase by 44% from 2006 to 2030 [1,2]. The prerequisite for clean energy, which serves as a substitute for fossil fuels, is a basic necessity for increasing populations and technological advancement [3]. Fossil fuel combustion accounts for over 80% of the world's energy. Among these, only the transportation sector accounts for up to 58% of the total utilization of fossil fuels [4–6]. The extensive use of fossil fuels could lead to a fast rate of depletion of these resources and high greenhouse gas emissions associated with environmental degradation and climate change [2]. The utilization of fossil fuels in the transportation sector accounts for 15% of greenhouse gas (GHG) emissions worldwide [7]. Therefore, the world is exploring for an alternative renewable source of energy that can meet the world's increasing energy demands while also reducing greenhouse gas emissions. These renewable energy resources are a part of energy security, socioeconomic development, and environmental sustainability [2].

A variety of alternative renewable energy sources, for instance, wind energy, solar energy, geothermal energy, hydropower, and biomass, have been explored and gained remarkable attention for energy security and reduction of GHG emissions in a sustainable way [8]. Biomass is a major carbon-neutral resource that is abundant on the planet and can be easily converted into a variety of solid, liquid, and gaseous energy forms. It can reduced emissions of GHG and is an environmentally friendly source of energy [2]. Biofuels are types of energy resources derived from biomass. In 2006, biofuels accounted for around 13% of total global energy demand. Furthermore, biofuels account for roughly 90% of total transportation energy consumption [2]. Biofuels are considered a cheap alternative to fossil fuels because they provide different forms of energy, abundance, and minimization of factors associated with environmental degradation and climate change. There are four types of bioenergy biomass: first-generation biofuels (1G), second-generation biofuels (2G), third-generation biofuels (3G), and fourth-generation biofuels (4G) [2,6,9–12].

Algal biomass, including microalgae and macroalgae, is being investigated as a possible source of 3G biofuels and has generated considerable attention worldwide due to its potential to address the challenges associated with 1G and 2G biofuel feedstock [2]. Algal biofuels have many advantages, such as higher yields of oil, less land requirements, no competition with edible feedstock, low recalcitrance, the potential for growth in every type of environment, from saline to freshwater, and reduced GHG emissions due to carbon sequestration [2,13,14]. Technological developments have made microalgae cultivation, processing, and biofuel production more efficient, resulting in bioethanol, biodiesel, syngas, bio-oil, and biohydrogen. However, the large-scale production of microalgal biofuels is still not feasible and needs additional improvements in biofuel production processes [15]. This chapter highlights biofuels produced from microalgae and the promising attributes of microalgae as a remarkable feedstock for third-generation biofuels. Furthermore, it discusses the cultivation, harvesting, and conversion technologies along with the valuable biofuels produced from microalgal biomass. In addition, it also shed light on the advantages of biofuels, their sustainability, challenges and future outlook, and conclusion.

2 Biofuels

Historically, man has had an intimate association with and dependence on energy resources for several millennia. Energy plays an important role in the establishment, wellbeing, and development of human societies. Throughout historical times, man used wood as a primary energy source. Then, the man switched to coal and later oil-based fuels. The steady rise in energy demand and consumption leads to the depletion of fossil fuels and an increase in greenhouse gas emissions. So, there is a rise in the security risk for the global world, in terms of social, economic, and the environment. At present, there is an urgent need to move in an alternative way for the sake of energy security and the protection of the environment. Recently, renewable energy is the only source of available energy that meets the ever-pressing requirement for sustainable development [16]. The term "biofuel" is assigned to a liquid fuel that is derived directly or indirectly from biomass feedstock and can be used as an alternative to petroleum-based fuels [6,17,18]. "Biomass" refers to biological material obtained from living organisms or recently lived organisms. According to the United Nations Framework Convention on Climate Change, biomass is defined as nonfossilized and biodegradable living matter obtained from animals, plants, and microorganisms. It also includes products, coproducts, residual and waste materials from agriculture and forestry, as well as industrial and municipal organic waste fractions. It further includes liquids and gases emitted from the decomposition or degradation of nonfossilized and biodegradable organic matter. So, the fuels obtained from biomass through conversion processes are termed biofuels [6,10].

Biofuels are hydrocarbon fuels that are manufactured from organic matter in a short stretch of time. As fossil fuels are limited in availability, biofuels are an efficient substitute for fossil fuels, as well as a way of achieving energy security in an environmentally friendly manner. Biofuels are essential resources for energy-rich fuels since they are greenhouse gas neutral or carbon neutral [10]. Recently, the production of biofuels has increased along with the increase in demand for transportation fuel. Various biofuels are used as a substitute for petroleum-based fuels. Alcohol fuels are considered as an alternative to gasoline, while biodiesel is a potential alternative to the petroleum-diesel used in compression ignition engines [17]. Bioethanol is a clean biofuel and can be used directly in the automotive industry [19]. Globally, the production of bioethanol increased from 4.8 to 16.0 billion gallons in the period from 2000 to 2007. Currently, Brazil and the United States are the main countries for bioethanol production and contribute approximately 75%–80% of the total bioethanol production in the world. The United States mainly produces bioethanol by processing corn grain [20,21]. While Brazil produces bioethanol by utilizing sugarcane as a potential feedstock [19]. Concerns about increased biofuel production include loss of agricultural and forest areas, biodiversity loss, and higher demand for water supplies [17].

2.1 The generations of biofuels

Biofuels are divided into four generations, which are referred to as first-, second-, third-, and fourth-generation biofuels [9,10]. These are classified based on the source of biomass, the

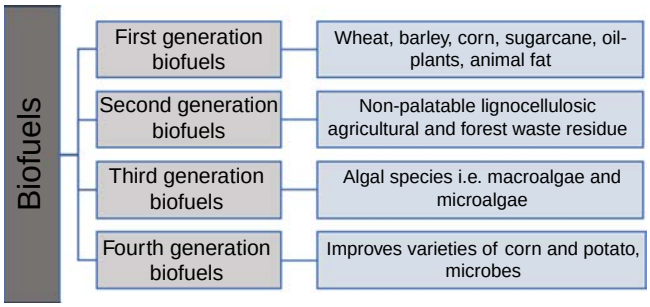

FIG. 10.1 Classification of biofuels and their biomass sources.

potential constraints of each generation as an energy source, and technological advancements. Fig. 10.1 shows the classification of biofuels along with their potent feedstock.

The first-generation biofuels, which are also termed conventional biofuels, mainly include bioethanol, biodiesel, and bioethanol. They are obtained by utilizing conventional food crops, oil crops, and animal fats [22]. The conversion processes are either by fermentation of sugars and starches of conventional crops such as corn, sugarcane, sugar beet, and wheat, or chemical conversion of oil crops such as coconut, sunflower, soya bean, palm oil, rapeseed, and animal fats [23,24]. The fermentation of starch and sugar produces ethanol, which is used as an additive because it has one-third of the energy content of gasoline. Ethanol is a green fuel and produces less greenhouse gas emissions than gasoline. Biodiesel is obtained by the transesterification process of plant oils and animal fats and is used as a substitute for petroleum-based diesel in engines. However, first-generation biofuels have some limitations. The escalating price of food crops is the main concern because of the food shortages around the world. Furthermore, there exists a competition between edible food and biofuel feedstock because both need the same amount of land and water. The pressure on water resources increases as production and processing require large quantities of water. Furthermore, increased agricultural and land harvesting resulted in bare land, forest clearing for agriculture, habitat destruction, loss of indigenous biodiversity, and polluted air [4,6].

The second-generation biofuels, also termed advanced biofuels [10], are mainly derived from nonpalatable biomass and lignocellulosic feedstock. Forest and agricultural residues, manure, and municipal solid waste are the most important biomasses for second-generation biofuels [6]. The second-generation biofuels, such as bioethanol, can be obtained by either biochemical or thermochemical conversion of sugar present in plant fibers. Syngas is produced by the thermochemical conversion of straw and forest waste residue. Second-generation biofuels outperform first-generation biofuels because they can be cultivated on infertile land and do not compete with food supply [25,26]. Nonetheless, certain limitations are associated with second-generation biofuels since they have the same growth requirements as food crops. However, their processing is more difficult than the biomass of first-generation biofuels because they need pretreatment to release trapped sugars. They have complex structures, therefore the conversion is inadequate [6,24].

Third-generation biofuels are the most feasible and cost-effective, mainly derived from algae species. Algae, both macroalgae and microalgae have the ability to produce biofuels [6]. Gaurav et al. [5] reported that algae have the capacity to produce crude oil, which, after

further processing, and can be converted into diesel and gasoline [5]. Microalgae require sunlight, water, carbon dioxide, and nutrients for optimum growth and to increase their growth rate. They need less land and energy as compared to first and second-generation biofuels. Microalgae have no competition with edible crops for their growth. The conversion processes involve the thermochemical, biochemical, chemical, and direct combustion of biomass. Several biofuels are produced, such as biodiesel, bioethanol, syngas, hydrocarbons, and bio-oil. Third-generation biofuels have the advantage of reducing greenhouse gases as microalgae have the potential for carbon sequestration from the atmosphere. Hence, algal-derived biofuels are cost-effective, less time-consuming, and environmentally friendly green renewable biofuels.

It is unusual for biofuels to be classified as fourth generation. In this type, genetically modified crop types such as corn and poplar, as well as microalgae species, are used to produce biofuel. They capture CO_2 from the atmosphere and trap it in their trunks, branches, and leaves. The biological conversion process, such as fermentation, is involved in the production of biomethane, biohydrogen, and synthetic biofuel [9,10]. Table 10.1 summarizes the four generations of biofuels based on the potential source of biomass, conversion methods, and biofuels produced [9,10,16,27,28].

2.2 General perspective of microalgae

Generally, algae are considered a diverse group of organisms, either prokaryotic or eukaryotic, ranging from small unicellular species such as *Chlorella* to multicellular giant species such as *Kelp* (a brown algal species, which can grow to a length of up to 50 m) [14]. Algae is classified into two distinct forms: macroalgae and microalgae. Macroalgae are large,

TABLE 10.1 The summary of classification of biofuels.

Categories	Potential source of biomass	Example of biomass	Conversion technologies/ methods	Biofuels produced
First-generation biofuels	Conventional food and nonfood crops, oil crops, animal fat	Corn, sugarcane, sunflower, soya beans, sugar beet, rapeseed, potato	Fermentation, transesterification, saccharification	Bioethanol, biobutanol, biodiesel, vegetable oil
Second-generation biofuels	Nonfood biomass, lignocellulosic feedstock	Corn stalks, palm oil, switchgrass	Gasification, catalytic cracking	Biogas, Biohydrogen, Fischer-Tropsch diesel, biomethanol
Third-generation biofuels	Algal strains	Microalgae	Alcoholic and anaerobic fermentation, gasification, liquefaction, transesterification, thermochemical conversion, direct combustion	Biodiesel, bioethanol, biogas, biosyngas, Biohydrogen, hydrocarbons
Fourth-generation biofuels	Modified nonfood crops, microbes	Genetically engineered varieties of corn and poplar, microalgae strains	Bioconversion methods, like fermentation	Biohydrogen, biomethane, synthetic biofuels

multicellular, and can be seen with the naked eye. Seaweeds are mainly included in macroalgae and are measured in inches. While microalgae are unicellular and small-sized organisms that can be measured in micrometers [29].

Microalgae are considered the oldest living organisms. For instance, thallophytes are plant-like structures without roots, stems, and leaves and contain chlorophyll as the primary photosynthetic pigment [30]. Microalgae have the ability to survive in almost all types of environmental conditions, so they are present in almost all types of ecosystems, such as freshwater, marine, riverine, brackish, soda lakes, saline, thermophilic, and hypersaline environments [31,32]. More than 50,000 species of microalgae are present, among which only about 30,000 microalgae species are used for research purposes [2,33–35]. They have a similar photosynthetic mechanism to that of plants and convert solar energy, into chemical energy which is then stored in their cellular structures [30]. Microalgae, for nutrition, can either be autotrophic or heterotrophic organisms [35]. Autotrophic microalgae can either be photoautotrophic, i.e., using sunlight as an energy source, or chemoautotrophic, i.e., using inorganic substances as an energy source. Heterotrophic microalgae use organic compounds to obtain energy. In addition, microalgae also have the ability to fix CO_2 from the atmosphere, acting as an excellent sink of carbon [30].

2.3 Microalgae as a source of biofuels

Microalgae are defined as photosynthetic unicellular organisms, having the ability to store CO_2 as a carbon sink and transform it into energy-intensive substances like fatty acids, starch, or cellulose [36]. Among other feedstock for biofuel production, microalgae are regarded as an efficient source for biofuels [5]. Different forms of biofuels such as biodiesel, bioethanol, biohydrogen, or biomethane can be produced by microalgal species [35,37–39]. Microalgae have a wide range of benefits that make them an efficient for biofuel producer, such as:

- Microalgae production is substantially higher than that of conventional plants or oil seed crops because microalgae can grow throughout year and are less influenced by environmental factors [35,40].
- They can act as carbon sink and taking CO_2 from the atmosphere and converting it into energy-rich substances such as triacylglycerides or fatty acids and storing it in their cellular structure which is then transesterified into biodiesel [35]. To produce 100 tons of microalgal biomass, about 180 tons of CO_2 are captured from the environment [37,41].
- The cultivation of microalgae is easy as compared to other feedstock and needs basic nutrients such as water, light, CO_2, nitrogen, and phosphorus [35].
- The growth rate of microalgae is fast, and they can double their biomass in a single day. They have almost 10–20 times higher productivity rates in comparison to traditional biofuel crops like palm oil, sugarcane, or corn [42].
- Microalgae can grow in diverse environmental conditions. Its cultivation does not necessitate fertile land, but it can grow anywhere there is an abundance of water and sunlight [35].
- Microalgae take up CO_2 from the atmosphere and have a high CO_2 sequestering potential, thereby, reducing greenhouse gas emissions [40].

- Microalgae have a remarkable potential for phytoremediation, and they absorb nitrogen and phosphorus from the wastewater by minimizing the nutrient load, thus limiting the process of eutrophication [40,43].
- They mainly grow in aqueous cultures and use sunlight as an energy source, so both are easily available for large-scale production of microalgae [40].
- Microalgae do not need chemicals like herbicides or pesticides for their growth and survival [44].
- The lipid content is higher than in vascular plants. Some species of microalgae have greater than 50% lipid content, making them an efficient source of biofuels [40,45].
- Besides biofuels, microalgae is a potent source of value-added products, such as fertilizers, animal feed, therapeutics, nutraceuticals, protein supplements, vitamins, and aquaculture [30,40].
- Many useful by-products, such as biochar, methane, or hydrocarbons, are also formed during the conversion of algal biomass into biofuels. These useful by-products serve many purposes for electricity generation [45].

2.4 Composition of microalgae

Currently, almost 30,000 microalgal species have been used for biofuel production [34]. Generally, microalgae contain 20%–50% oil concentrations, but in some algal strains, this content reaches up to 80% [2]. Table 10.2 depicts potential microalgal species and their biochemical composition expressed as a percentage of dry mass [2,6,29,46]. Microalgae contain three main ingredients in their biomass, which are: lipids, carbohydrates, and proteins. The carbohydrates and lipids present in microalgal strains are converted into useful biofuels such as biodiesel, methane, syngas, bioethanol, and solid biochar [2]. In contrast, the biomass of red algae and certain species of green algae constitute lignin as a component of their cell walls [47]. Based on elemental analysis, microalgae contain carbon, nitrogen, oxygen, hydrogen, and sulfur in their biomass. The molecular formula of microalgae is presented as $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ [37,46].

2.4.1 *Carbohydrates*

Carbohydrates are mainly present in the algal cell wall in the form of polysaccharides and cellulose. The algal cell wall is divided into two layers: the outer layer and the inner layer [48]. Among these, carbohydrates, produced as a result of photosynthesis, such as cellulose and hemicellulose, are present in the inner layer of the cell wall [49]. However, the carbohydrate content varies in different algal strains. For instance, *Chlorella vulgaris* contains 51% carbohydrates, *Porphyridium cruentum* contains 40%–57%, and *Spirogyra* sp. contains 33%–64% carbohydrates in its cell wall [2].

2.4.2 *Lipids*

Microalgae species contain about 20%–60% lipid content in their biomass. Microalgae contain both polar and nonpolar lipids, but most of the species contain lipids that are nonpolar in nature [2]. Lipids are either storage lipids or membrane lipids. Storage lipids are mainly fatty acids or triacylglycerides, and constitute up to 50% of the cell dry weight. Membrane lipids

TABLE 10.2 Potential microalgal species and biochemical composition expressed in % dry weight [2,6,29,46].

Microalgal species	Lipids	Protein	Carbohydrates
Anabaena cylindrica	4–7	43–56	25–30
Botryococcus braunii	33	–	–
Chaetoceros calcitrans	15–40	–	–
Chaetoceros muellerii	34	–	–
Chlamydomonas rheinhardii	21	48	17
Chlorella protothecoides	15–58	–	–
Chlorella pyrenoidosa	2	57	26
Chlorella vulgaris	14–22 5–58	51–58 –	12–17 –
Chlorella sorokiniana	19–22	–	–
Chlorella emersonii	25–63	–	–
Dunaliella bioculata	8	49	4
Dunaliella salina	57	32	6
Dunaliella primolecta	23	–	–
Euglena gracilis	14–20	–	–
Ellipsoidion sp.	14–20	39–61	14–18
Haematococcus pluvialis	25	–	–
Isochrysis galbana	7–40	–	–
Nannochloris sp.	20–56	–	–
Neochloris oleoabundans	29–65	–	–
Phaeodactylum tricornutum	18–57	–	–
Porphyridium cruentum	9–14	28–39	40–57
Prymnesium parvum	22–39	28–45	25–33
Scenedesmus obliquus	12–14 11–55	50–56 –	10–17 –
Scenedesmus dimorphus	16–40	8–18	21–52
Scenedesmus quadricauda	47	–	1–9
Spirogyra sp.	11—21	6–20	33–64
Spirulina maxima	6–7	60–71	13–16
Spirulina platensis	4–9 4–17	46–63 –	8–14 –
Synechococcus sp.	11	63	15
Schizochytrium sp.	–	–	50–77
Tetraselmis maculate	3	52	15

are polyunsaturated fatty acids and constitute up to 40% of the cell's dry weight. Lipids are then extracted from wet algal biomass through a process of transesterification [41].

2.4.3 Proteins

The protein content present in microalgae is polypeptides-rich and complex in nature. Algal cell walls contain 20% to 50% of the protein in their dry weight [41]. For instance, *Chlorella vulgaris* constitutes 61.24% of the protein content of its dry biomass. Thus, microalgal protein content mainly depends on the growth conditions and types of culture medium on which microalgal species are cultivated [2].

2.5 Basic requirements for microalgae production

The large-scale cultivation of microalgal biomass leads to the generation of biofuels along with value-added by-products [19]. Many species of microalgae have been cultivated by various methods under different conditions. Mostly, sunlight is a source of energy for microalgae, which is then converted into chemical energy via photosynthesis. About 20%–50% of the end products of photosynthesis are utilized in algal growth and become a part of algal biomass either as cellular components or as storage products [37]. Among these, nutrients like nitrogen and phosphorus are necessary for algal growth and account for 10%–20% of the algal biomass. Wastewater also provides a medium with necessary nutrients for microalgal growth [19]. Different strains of microalgae have different requirements for growth. However, the basic requirements are similar for most microalgal strains, such as light, nutrients, a carbon source either organic or inorganic, optimum temperature, mixing, and an aqueous medium or water [50]. The following are some important parameters for microalgal culture that greatly influence the overall productivity of biomass.

2.5.1 Light

Light is the basic source for photosynthesis, and light intensity and duration directly affect the biochemical composition of algal biomass [51]. The growth of microalgal biomass increases with the increase in intensity and duration of light until the saturation point is reached. At saturation point, the intensity and duration of light do not affect the growth of microalgae. Khoeyi et al. [52] in their experiments proved the change in growth patterns by changing light intensity and found a decrease in growth by decreasing light intensity [52]. The intensity of light is measured in units of photon flux density (μE or μmol m^{-2} s^{-1}) or photon absorption rates (μE or μmol kg^{-1} $cell^{-1}$ s^{-1}) [41,53,54]. For most algal species, the optimum light intensity ranging from 200 to 400 μM photons/m^2/s [55]. Kitaya et al. [56] reported that light intensity of about 100 μmol/m^2/s is optimum for the production of microalgal biomass [19,56]. The optimal intensity of light is necessary for CO_2 assimilation and transformation into sugars [57]. Energy-rich biomolecules such as ATP and NADPH are synthesized as a result of the light reaction of photosynthesis. The energy of these molecules is utilized to drive the dark reaction of photosynthesis, and carbohydrates are produced as a final product. The carbohydrates are then utilized by microalgae to increase their biomass and are incorporated into the cellular structures of microalgae [58].

2.5.2 Temperature

Temperature is another important factor that is required for maximum biomass production. Any temperature variation directly alters the growth rate of microalgal biomass [41]. The optimum temperature for most of the microalgal species is 20–30°C [59]. However, some microalgal species, for instance, *Anacystis nidulans* and *Chaetoceros*, have the ability to withstand temperatures up to 40°C [60]. The growth of microalgal biomass at nonoptimal temperatures leads to a loss of productivity, especially in outdoor cultivation systems [19,61]. Regular monitoring of temperature is necessary for open pond cultivation systems because a minor change in temperature results in a loss of biomass [62]. A decrease in temperature causes a reduction in carbon dioxide assimilation, while an increase in temperature inactivates the photosynthetic proteins [63]. Kitaya et al. [56] mentioned that the optimum temperature for various microalgal strains is between 27°C and 31°C [56].

2.5.3 Water

Water provides an aqueous medium for the growth of microalgal biomass. Water is also required for the photosynthetic mechanism where water molecules are split and energy emitted from this reaction is converted into the production of energy-rich molecules such as ATP and NADPH. Water also balances the temperature of the cultivation system through evaporation. Wastewater is considered a potential source for microalgal biomass growth because it contains the necessary ingredients or nutrients for maximum growth.

2.5.4 Carbon

Microalgae have the ability to assimilate inorganic carbon as CO_2 for photosynthesis. This CO_2 is transformed into sugars and stored in the body in the form of carbohydrates and lipids, which is a potential source of biofuel. To assimilate CO_2 for photosynthesis, microalgae must be grown in an aqueous suspension [64]. CO_2 dissolves in water in several forms depending on the pH of water. The chemical Eq. (10.1) is [41,65]:

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{-2} \tag{10.1}$$

Microalgae mainly grow in an aqueous medium having a neutral pH, so the carbon must be dissolved in the form of CO_2 and carbonate ions [41].

2.5.5 Nutrients

Microalgae need a variety of macronutrients in varying concentrations for optimum growth. The basic macronutrients are nitrogen and phosphorus [19]. Nitrogen is the most important element for microalgal structural protein synthesis after carbon. Phosphorus is essential for cellular metabolism and causes pigment aggregation in microalgal biomass [66]. The main sources of nitrogen and phosphorus are nitrates and phosphates present in water. Urea is also a cost-effective source of nitrogen [19,41]. The decline in nitrogen and phosphorus concentrations in chlorella species resulted in a reduction in growth [67]. Further, some marine species also need silicon as a macronutrient for their increased growth [19]. Among macronutrients, microalgae also require trace amounts of micronutrients such as K, Fe, Mg, Zn, Mo, and B. These micronutrients may be required only in small concentrations, but their

impact on microalgae growth and enzymatic activity is much higher [68]. Iron is an essential micronutrient and is involved in photosynthesis for the transportation of electrons [69].

2.5.6 Mixing

Proper mixing and aeration in microalgal cultures are essential because they allows uniform distribution of the required nutrients, air, and CO_2. It also enables the effective penetration of light deep inside the culture medium and prevents the accumulation and setting of biomass [19,70]. Mixing is essential as it keeps conditions optimum for growth and increases productivity. But, increased turbulence causes damage to biomass [71].

2.5.7 pH and salinity

The change in pH can alter the growth of algal biomass because most microalgal species are pH sensitive and only grow in the optimum range of pH. The optimum pH for most species is 6–8.76. *C. vulgaris*, for example, can tolerate pH and is most productive at pH 9–10 [72]. The increase in pH can also enhance salinity, which is harmful to algal biomass [19,73].

2.5.8 Estimated production cost

The cost of production of biofuels from microalgal can be estimated based on some factors, such as the total production of algal biomass from the culture medium, the total oil content, the scale of cultivation of algal species, and the total cost of extracting oil from algal biomass. Furthermore, it adds the cost of harvesting, dewatering, maintenance and operational costs, infrastructure costs, and rental costs of the production area or land [74]. Currently, the production of algal oil is still a more expansive approach than other petroleum diesel fuels. Algal fuel has a high production cost [41]. The cost of cultivation and harvesting is greater due to the fact that algal fuels are far from market use [74,75]. It is reported that the production and harvesting of algal biomass account for 50%–60% of the total production cost [76]. Further treatment processes such as extraction of oil, preesterification, cleaning, and distribution could account for 15%–25%, 10%–15%, 2%–3%, and 2%–3% of the total production cost, respectively [74].

Chisti [37] in his study, estimated the cost of algal oil by an equation. This equation is used when the algal oil price and the price of petroleum diesel oil are the same and both are used as alternative competitive fuels. Eq. (10.2) is as follows [37]:

$$C_{\text{algal oil}} = 25.9 \times 10^{-3}\, C_{\text{petroleum}} \tag{10.2}$$

here, $C_{\text{algal oil}}$ is the microalgal oil price measured in \$/gallon and $C_{\text{petroleum}}$ is the crude oil price measured in \$/barrel [37].

3 Technologies for microalgae cultivation

Microalgae are photosynthetic organisms, which means they require light and carbon dioxide as a source of energy and carbon. This mode of culture is usually referred to as photoautotrophic. However, some species of algae have the ability to grow in darkness as they use organic carbon (like glucose or acetate) as a source of energy and carbon. This mode of culture is referred to as heterotrophic [30]. Among light and carbon dioxide, several

nutrients, such as nitrogen, zinc, phosphorus, potassium, and calcium, are required to produce biomass through the process of photosynthesis. In photoautotrophic cultivation, light energy is converted into chemical energy through a chain of photosynthetic reactions. Similarly, in heterotrophic cultivation, organic carbon materials are used to get energy for growth. In mixotrophic cultivation, the organisms live either autotrophically or heterotrophically, and it depends on the available light intensity and nutrient concentration [6].

Among these, various other factors are also considered important in the selection of an algal cultivation system. These factors include the land availability and cost, volume of water, nutrients, energy, labor, and climate of the area. The characteristics of the cultivation system, such as its hydrodynamics characteristics, the efficiency of utilizing light energy, temperature maintenance, and ability to balance mono-algal cultures are also considered important [77]. Therefore, there are three main methods for cultivating microalgae on a pilot and industrial scale. (1) Photobioreactors are closed systems in which the algal culture has little or no direct exposure to the atmosphere. (2) Photobioreactors are closed systems in which the algal culture has little or no direct exposure to the atmosphere [6,30,41,78]. (3) Hybrid cultures, in which algal cultures are grown either autotrophically or heterotrophically depending on the needs and conditions [6,40]. Further, among these cultivation methods, open systems are commonly used because they are economically feasible and effective for the large-scale production of microalgae [6].

3.1 Open microalgae cultivation system

Open ponds for microalgae cultivation are mostly used for algal growth because of their ability to utilize light and carbon dioxide from the atmosphere. The open-air ponds are composed of closed-loop recirculation pathways, with an average depth of 10–50 cm. The ponds are shallow to enhance the penetration of solar light for photosynthesis. Furthermore, it is provided with paddle wheels to prevent settling and effective mixing. As the ponds are open, temperature regulation is done by evaporation of water. The required algal nutrients are also provided in these ponds [6,79]. There are different arrangements of cultivation systems for enhanced biomass and phytoremediation of wastewater. The most commonly used systems for microalgae cultivation for research and industrial purposes are as follows:

i. The raceway pond system
ii. The circular pond tank system
iii. The shallow big pond system
iv. The closed pond system [40]

In an open pond system, the location of the pond is important as it requires sufficient sunlight and carbon dioxide availability from the atmosphere [40]. Open pond systems are very effective because they are cost-effective as they require low construction, operational, and energy requirements. However, these systems also have some drawbacks as they require large land areas for production. There are greater chances of contamination by animals, bacteria, fungi, etc. [37,80]. Other operational parameters, such as controlled temperature and evaporation of water, are also affected [42].

3.2 Closed microalgae cultivation system

A closed cultivation system is also known as a photobioreactor (PBR) [6,40]. In this system, microalgae are cultivated in a recycling reactor. It has well-controlled conditions for growth and increases the availability of light. The enhanced mixing allows the light to be within the optimum range for cell growth and improves gaseous exchange. Photobioreactors have solved many problems related to open cultivation systems [4,40]. Based on their shape and construction, photobioreactors have a wide range of variations. Dragone et al. [80] provide details of different forms of PBRs, which are as follows:

i. Tubular PBRs
ii. Flat PBRs
iii. Bubble column PBRs [80]

A tubular PBR consists of an array of parallel arranged transparent tubes that help to capture sunlight for photosynthetic reactions. The variations in algal growth are due to changes in day length, optimum temperature, the oxygen concentration in the medium, and temperature during the night [81]. Jorquera et al. [79] reported that PBRs have greater volumetric output in comparison to open-air systems because they efficiently capture solar energy and use less land area. The controlled growth of microalgae in bioreactors prevents contamination. However, PBRs are expensive in terms of capital and operational costs as they need a lot of energy [80]. Zhou et al. [82] designed a multilayer PBR that not only minimizes the occupied space but also mitigates sunlight penetration issues [82].

3.3 Hybrid microalgae cultivation system

Hybrid microalgae cultivation is a combination of both open pond and PBR systems and has the advantages of both systems [6]. The hybrid system minimizes the limitations of the open pond system and also overcomes the high capital and operational costs of the closed system [83]. It is used to produce a high yield of algae. In this system, microalgae are first grown under closed photobioreactors under controlled conditions and then transferred to an open system to increase productivity [84].

4 Harvesting methods for microalgae

Microalgae harvesting means the separation of algae from the growth medium after cultivation. The harvesting of microalgae depends on various factors, such as the type of algae cultivated, density, size, and nature of the end product obtained from algae [50,85,86]. Microalgae cells are present in a suspension of microscopic cells in the growth medium. Algal biomass is estimated at up to 0.4 g/L in open pond systems and 3 g/L in closed systems [4]. The algae concentrations obtained are relatively low, ranging from 1 to 5 g/L, and the diameter of algal cell biomass ranges from 2 to 20 μm [30]. This shows that about a liter of water from the culture medium is required to be removed to get algal dry biomass [87]. Therefore, the process of harvesting accounts for 30% of the total cost of biofuel production [88].

Algal biomass is present as a suspension of insoluble cells in the aqueous culture medium. The presence of lipids in algal cells also makes them immiscible in water. Principally, the algal oil detachment from the aqueous medium is a voluntary process and needs little energy. But, in practice, the detachment of algae from the aqueous medium and then the segregation of lipids from algal cell biomass is a time-consuming and energy-intensive procedure. The requirements of intensive energy could be met by making improvements to algal strains by engineering methods [87]. Table 10.3 explains microalgae harvesting methods along with their features, pros, and cons. Harvesting methods of algae depend on whether macroalgae or microalgae are cultivated. About 400 years ago, harvesting of macroalgae was done [93] through low-energy input mechanical processes and it is continuing with innovations [87]. For microalgae, the growing cycle is 1–10 days, and it produces a higher quantity of biomass [94]. The harvesting of microalgae involves two major steps:

1. Detachment and concentrating of algal cell biomass from the aqueous suspension medium by sedimentation, flotation, flocculation, micro strainers, and electrophoresis.
2. Dehydrating the microalgae slurry by filtration, or centrifugation [95].

In flocculation, the separation is done based on density, and the flocculating agents cause microalgae to assemble. The aggregates of microalgae either settle at the bottom or float on the surface of the aqueous medium. Several organic or synthetic flocculating agents are used, which trigger aggregation by changing the pH or chemical activators [87]. Photobioreactors require 73 kg of water to cultivate 1 kg of microalgae biomass and require high energy consumption [94]. The flocculation process helps to reduce this high energy requirement. Different types of flocculation processes are used, such as auto flocculation, chemical flocculation, inorganic flocculation, electrolytic method, etc. [96]. But, this method has the disadvantage that the detachment of microalgae from chemicals is difficult, which alters the quality. Another disadvantage is that the chemical flocculation process is costly for large-scale algae production [97]. In the flotation technique, the microalgae cells are carried to the surface of the aqueous medium by air bubbles from where they are collected [94]. For filtration, micro strainers and vibrating screens are used. Vibrating screens show a 95% harvesting rate [97]. Filtration alone is not very effective because microalgae cells can clog the filter paper and make the process insufficient [87]. So filtration and centrifugation can be used as a combined treatment, and it is gaining focus [98,99]. Centrifugation is an effective method for harvesting, but it has high capital and processing costs [87]. Under optimum conditions, about 95% of algal biomass recoveries could be achieved by the centrifugation technique [100]. Other harvesting methods include electrophoresis methods such as electrolytic coagulation and electrolytic flocculation, but their reliability rates vary [101–103].

The microalgae species are harvested at 5%–15% of the aqueous suspension, and then they are further subjected to a dehydration process [95]. Drying is considered important to enhance the shelf-life of algal cell biomass and prevent spoilage after harvesting [35,104]. The drying process accounts for 84.9% of the total energy spent on the complete production process [85,105] and requires 11.22 MJ/kg of energy to dry algal cell biomass [94,106]. It is necessary because moisture present can cause hindrance to further processing like lipid extraction and trans-esterification. Some effective dehydrating techniques are spray-drying, freeze-drying or lyophilization, sun drying, vacuum drying, convective drying, rotary drying, cross-flow drying, flashing drying, fluid bed drying, and toroidal drying [3,85,94,95].

TABLE 10.3 Summarizes some silent features of different harvesting methods [6,29,87,89–92].

Harvesting methods	Concentration of suspended solids (%)	Operational cost (per gallon of water)	Biomass harvesting efficiency	Types of algal species	Advantages	Disadvantages
Filtration/ screening	Medium to high	Medium to high	20%–90%	Algae which have a large cell size (>5 μm)	Cost-effective, suitable for the production of larger species of algae	Time taken, dependent on types of algal species, clogging of the filter, fouling, limited capacity for harvesting algal species
Flocculation	Low to medium	Low to medium	50%–90%	Algae having low density	High capacity to harvest biomass, easy to scale-up	Flocculating agents are costly, toxic outputs in high concentration will impact the quality and recycling ability of water and the final product
Sedimentation	Low	Low to medium	10%–90%	Algae having high density	Cheap, less energy requirements, easy to install and process	Time consuming, end product concentration is low, not suitable for small-sized species
Centrifugation	High	Very high	>90%	All species of algae	Easy to install and process, quick and efficient, good recovery	High cost and energy requirements, less reliable, algal biomass specific
Flotation	Low to medium	Low to medium	30%–90%	Algae having low density	Cost-effective, process efficient than sedimentation	Less reliable, added agents might be expensive, toxic end products, requires greater energy for air bubble formation
Bioflocculation	Low to medium	Low	About 90%	–	Effective, verity of flocculants present	Toxic end products, cause environmental concerns and recycling issues
Microfiltration/ ultrafiltration	Low	Very high	>90%	Small-sized species of algae	Used as a preliminary treatment prior to centrifugation, very effective and 98% dewatering ability	High cost of installment and operation, fouling of the membrane

Sun drying is not very effective because algal biomass contains a lot of water. However, Prakash et al. [107] devised a solar drying device and successfully removed moisture up to 90% from Spirulina and Scenedesmus species [107]. But, it is not effective in countries with short-day periods. Further, when the algal cells cease to dry, the nutrient content in algal species dissipates [97].

5 Conversion technologies of biomass into biofuels

The rise in energy demand has led to the development of new technologies to produce bioenergy. These technologies involve the conversion of biomass into fuel, heat, and power [108]. Microalgae contain mainly carbohydrates, proteins, and lipids, and these components are converted into biodiesel, syngas, and bioethanol through four main conversion processes, which are thermochemical conversion, biochemical conversion, chemical conversion, and direct combustion conversion [2]. Fig. 10.2 highlights microalgal conversion technologies into biofuels [2,6,35,80].

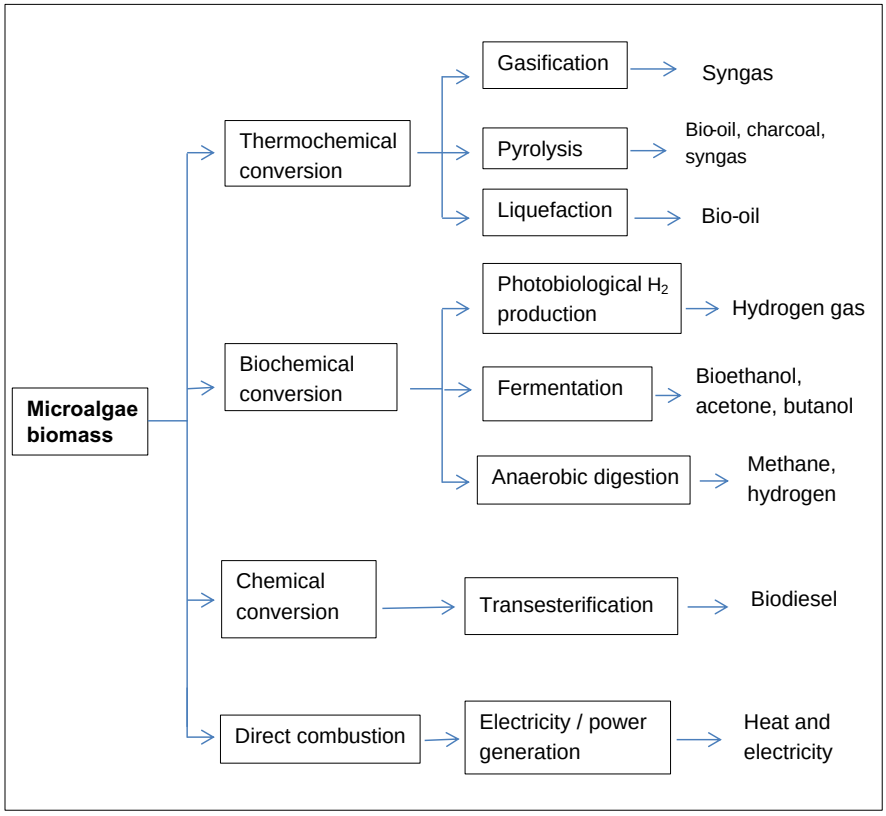

FIG. 10.2 Microalgal biomass conversion technologies into biofuels.

5.1 Thermochemical conversion

Thermochemical conversion involves the high-temperature chemical altering of organic matter from microalgae into solid, liquid, and gaseous biofuels. The algal biomass is converted into biochar (solid), a highly oxygenated bio-oil (liquid), and synthetic gas (gaseous) biofuels through the breakage of bonds at high temperatures. The three main processes for the thermochemical conversion process are gasification, pyrolysis, and hydrothermal liquefaction [108,109]. So, for this reason, it is gaining attention in industries [110]. It has many advantages, such as being less time-consuming, less consumption of water, and it converts plastic waste into energy that is not digested by microorganisms [111].

5.1.1 Gasification

Gasification is a process in which biomass is converted into biofuels in an oxygen-deficient environment. The heating of biomass is done at extreme temperatures (about 500–1400°C) and 33 bars of atmospheric pressure [108]. The partial oxidation of oxygen, air, or steam yields a combustible mixture of gases [6]. In the gasification process, the carbonaceous components of algal biomass are transformed into hydrogen, methane, and carbon monoxide (also known as syngas) in the presence of gasification agents and a catalyst [6,108,111].

It is shown that gasification is suitable for the production of hydrogen gas [110]. It is observed that the gasification technique is able to recover high energy and high heat capacity in comparison with pyrolysis and combustion. Further, hydrogen and carbon monoxide conversion by pyrolysis and combustion is less efficient because of secondary reactions, complex processes, and reliance on operational conditions [112]. The gasification process is suitable for the conversion of different types of biomass, varying from waste products of agriculture, kitchens, industries, and farms [108]. The gasification technique is accompanied by two main types, which are: conventional gasification technique and supercritical gasification technique (SCWG) [6]. The difference between these two types is shown in Table 10.4.

5.1.2 Pyrolysis

Pyrolysis is the thermal decomposition of microalgal biomass in the absence of oxygen at temperatures ranging from 400 to 600°C with an atmospheric pressure of 0.1 MPa for 30–60 min. The end products are solid (char), liquid (oil), and gaseous (gas) [2]. The major difference between pyrolysis and gasification is the end-product formed. Pyrolysis produces liquid oil, mainly termed pyrolysis oil (py-oil) or bio-oil, which is used as an alternative fuel oil for static heating and power generation applications. Whereas, the end product of gasification is fuel gas that is combusted for heat production. Further, liquid oil can easily be handled and transported as compared to fuel gas [119].

Bio-oil is present in a dark brown colored liquid that has greater viscosity. It has a low calorific value and comprises acids, aldehydes, alcohols, phenols, and oligomers that are derived from lignin [108,120]. The bio-oils produced from the pyrolysis of algal species are more stable in comparison to those produced from lignocellulosic biomass [121]. The microalgae bio-oil has a higher heating value ranging from 31 to 42 MJ/kg with a specific viscosity of 0.060 Pa s. It mainly comprises hydrocarbons and nitrogenous compounds derived from lipids and proteins, respectively [122,123]. The conversion rate is affected by temperature, holding time, catalyst, pressure, and pyrolysis type [124].

TABLE 10.4 Difference b/w conventional and supercritical gasification technique.

Sr. no.	Conventional gasification	Supercritical gasification (SCWG)	References
1	High temperature ranges from 800 to 1000°C in a fluidized bed or fixed bed	Less operational temperature of 375 to 550°C at 22.1 to 36 MPa	[2,6,112]
2	Comprises of four processes: drying, pyrolysis, combustion, and reduction	SCWG does not need a drying process	[2,6,113]
3	It requires drying the algal biomass before conversion into biofuels	It directly converts algal biomass into a gaseous mixture beyond the critical point of water	[2,6,113]
4	For drying process, takes a lot of heat energy and has high heat requirements	It consumes less heat	[2,6,111,114]
5	The yield and composition of gas formed depends on the feedstock composition	The yield of gas, carbon, and energy could be enhanced by higher operating temperature, longer time, high load ratio, and using a suitable catalyst	[6,113]
6	It takes time from the conversion process	It is less time consuming and residence time varies from seconds to minutes	[6,113]
7	The by-products produced are tar, ash, and solids	This process limits the production of tar and char as by-products	[111,115,116]
8	Less effective	More effective by using NaOH or KOH or a suitable catalyst	[117,118]
9	Less cost-effective as compared to SCWG	More cost-effective	[6,114]

Pyrolysis is divided into the following main types, which are: slow or conventional pyrolysis, fast pyrolysis, flash pyrolysis, and microwave pyrolysis. These types differ based on operational conditions. Fig. 10.3 shows the types of pyrolysis, their operational conditions, and the products obtained from pyrolysis. Slow or conventional pyrolysis is achieved at low temperatures ranging from 400 to 600°C [113]. It mainly yields biochar and enhances the production of gaseous products such as methane and carbon dioxide [121]. It has a low heating rate and a long residence time for vapors [108]. Fast pyrolysis is achieved at a controlled temperature of approximately 500°C. It has high heating rates (>200°C s1) and short residential time (2 s) [108,125]. It produces crude bio-oil [125]. The fast pyrolysis of *C. protothecoides* and *Microcystis aeruginosa* yields 18% and 24% liquid products, respectively, at operating conditions of 500°C temperature, the heating rate of 600°C s1, and residence time of 2–3 s [6]. In flash pyrolysis, operating conditions are greater than in fast pyrolysis. The heating time is very short and the heating rate is greater [108]. Microwave pyrolysis has an advantage over other types of pyrolysis.

The temperature is up to 800°C. The heating time is short and uniform. The process is targeted and controlled and depends on the strength of microwaves [6]. Li et al. [126] investigated that the increase in microwave power strength from 750 to 2250 W results in greater production of gas by 39.45% and a reduction in the solid residue of up to 22.055 [126].

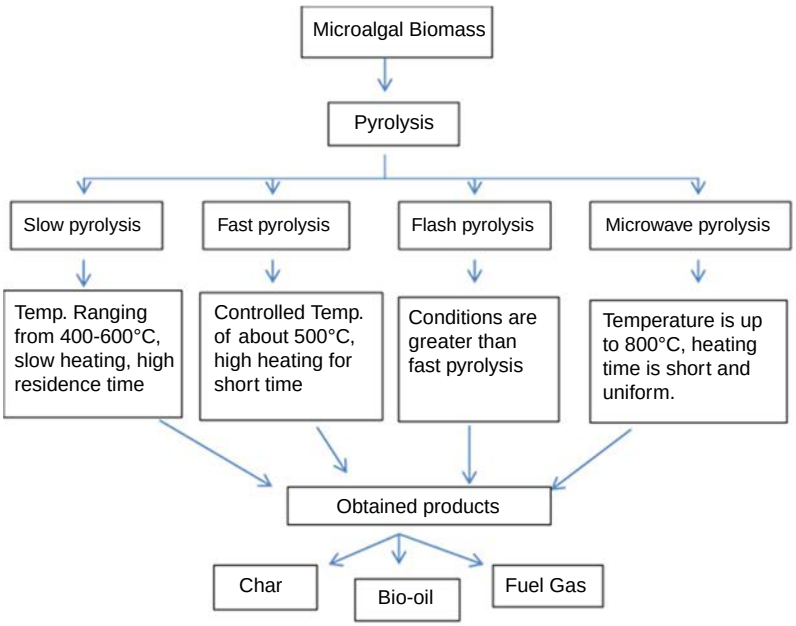

FIG. 10.3 Types of pyrolysis along with operational conditions and produces products.

5.1.3 Liquefaction

Liquefaction is a process in which algal biomass is converted into bio-oil or crude oil at a low temperature ranging from 300 to 350°C, high pressure of 5 to 20 MPa for a time required of 5 to 60 min in the presence or absence of a catalyst [109,111,113]. The end products of liquefaction are bio-oil and methane, which is produced as a by-product [6]. Hydrothermal liquefaction (HTL), also known as hydrous pyrolysis, is a type of liquefaction that is based on subcritical water (SCW) at 250 to 374°C temperature and 40 to 220 bar pressure for biomass conversion into bio-oil. HTL is a decomposition and repolymerization reaction [6,108]. The high pressure helps to keep the water in liquid form, whilst the mixing of biomass at high pressure and temperature helps to reduce the dielectric constant and density of the slurry, which keeps the hydrocarbons dissolved in water [127].

HTL is a cost-effective process because it feeds biomass that comprises high moisture, so it does not require drying or dewatering of biomass [108]. The microalgae biomass is suitable for the liquefaction process because it contains 80%–90% moisture content and the reactor is fed in a slurry form [128]. Microalgae are a more efficient feedstock for HTL than lignocellulose because the thermal breakdown of lignin results in the solid residue, which leads to low final yields and a long time of residence [129]. Studies have shown that the HTL of microalgae species *Spirulina* sp. only yields 54% bio-oil, which is lower than pyrolysis and gasification [121,130]. Matsui et al. [130] reported that the yield could be increased by up to 66.9% in the presence of Fe $(CO)_5$—S as a catalyst [130].

5.2 Biochemical conversion

Biochemical conversion of microalgae requires the use of microorganisms such as bacteria, yeast, etc., and enzymes to transform algal biomass into liquid biofuels [6]. The algal biomass is converted into biogas, alcohol, biofuels, and other chemicals [131]. Biochemical processes are environmentally friendly, require less temperature, and the rate of reaction is slow [132]. Biochemical conversion can be done in three ways: anaerobic digestion, fermentation, and photobiological H2 production [2,6,108,132]. Among these, anaerobic digestion and fermentation need pretreatment for the conversion process.

5.2.1 Anaerobic digestion

Anaerobic digestion of algal biomass is a process in which organic algal biomass is transformed into biomethane and carbon dioxide with a trace amount of hydrogen sulfide. It is also produced by the use of microorganisms and enzymes. High moisture content biomass is suitable for anaerobic digestion [4,6,108]. Microalgae have high polysaccharides in the absence of lignin and 80%–90% moisture content [4]. Many factors affect the process of anaerobic digestion, such as the design of the anaerobic digester (batch or continuous), types of microorganisms based on temperature (mesophilic (30–38°C) or thermophilic (49–57°C)), hydraulic retention time, carbon/nitrogen ratio, protein content, and the pH of the medium [133]. Anaerobic digestion is divided into three steps: hydrolysis, acetogenesis, and methanogenesis [6,108,134]. Hydrolysis involves the breakdown of complex biomolecules into simple biomolecules. Acetogenesis, or fermentation, utilizes these simple biomolecules into alcohols, fatty acids, acetic acid, which is volatile in nature, and a mixture of H_2 and CO_2 gas. Methanogenesis uses this mixture of gases and produces biogas consisting of CH_4 (60%–70%) and CO_2 (30%–40%). Methanogenesis can be enhanced by the presence of main nutrients (carbon, phosphorus, and nitrogen) and trace elements (iron, zinc, and cobalt) in the algae biomass [134].

The hydrolysis process is the rate-determining step because the algal cell wall is difficult to hydrolyze. So, the loading rates and retention times are highly influenced by the selection of microalgae species. In the methanogenesis step, the increase in pH helps to increase the methane ratio. In fermentation, the formation of ammonia causes an increase in pH, which results in the dissolution of carbon dioxide, which leads to an increase in methane concentration in biogas. Further, an increase in temperature also favors microbial activity and the production of methane. For instance, in *Spirulina maxima*, the rise in temperature from 15 to 52°C results in enhanced methane productivity and a reduction in volatile solids by 35% [135]. El Asri et al. [136] investigated *Gracilaria bursa-pastoris*, *Caulerpa prolifera*, and *Colpomenia sinuosa* species of macroalgae and found that *G. bursa-pastoris* and *C. sinuosa* have the potential to produce 86.35 and 74.68 mL/g VS of biogas, respectively [136].

5.2.2 Fermentation

In the process of fermentation, bioethanol is produced by the alcoholic fermentation of carbohydrates such as simple sugars, starches, or cellulose. Microalgae species such as *Chlorella*, *Scenedesmus*, *Chlamydomonas*, *Spirulina*, and *Dunaliella*, have a high quantity of starch, glycogen, and cellulose, so they are suitable for bioethanol production by fermentation [6,108]. This polysaccharides is complex and difficult to metabolize, so hydrolysis is done

to convert complex molecules into simple sugars [137]. Hydrolysis is performed by either acid/alkali or by enzymes. Acid hydrolysis treatment is cheap, fast, and suitable, but it could change the sugar composition. In contrast, the enzymatic hydrolysis process is expensive and slower, but it does not affect the sugars [108].

Fermentation and anaerobic digestion are similar to each other because they both require the pretreatment of algal cell walls for further processing [121]. Kim et al. [138], for example, reported that using pectinase enzyme from *Aspergillus aculeatus* may help to achieve a 79% saccharification yield in *C. vulgaris* hydrolysis at 50°C for 3 days [138]. The process of fermentation is achieved by four main steps. Initially, glycolysis involves the formation of two pyruvate molecules and water. Hydrogen ions are released as a by-product and reduction of coenzymes (adenosine diphosphate (ADP) and nicotinamide adenine dinucleotide (NAD_+) converts them into ATP and NADH during glucose breakdown. In the second step, the pyruvate is converted into acetaldehyde, carbon dioxide, and H_+. The reaction is catalyzed by the enzyme pyruvate decarboxylase. In the third step, ethanol anion is formed by acetaldehyde with NADH as a coenzyme. In the last step, the protonation of the ethanol anion is achieved by the hydrogen ion, and ethanol is formed [6,121]. It is noted that the higher concentrations of yeast facilitate the microalgae to produce an improved quality of ethanol. Based on this fact, Harun et al [139]. reported that the *Chlorococum* sp. of microalgae produces the highest concentrations of 3.58 g/L of ethanol when a 14.25 g/L concentration of yeast was used. Further, he concluded that the dried or pure microalgae biomass has a lower concentration of ethanol than lipid-extracted microalgae species [139].

5.2.3 *Photobiological hydrogen production*

Some species of microalgae have the ability to naturally produce H2 gas by oxygenic photosynthesis in the presence of sunlight [2,108,134]. This oxygenic photosynthesis is achieved by the utilization of nitrogenase or hydrogenase enzymes [139]. Firstly, microalgae split water molecules into H^+ and O_2. Then, under anaerobic conditions, H+ is reduced into H_2 with the help of the hydrogenase enzyme. The O_2 emitted from this photosynthetic process causes an interruption in the release of hydrogen gas by inhibiting the hydrogenase enzyme [108,134]. There are two different approaches to extracting hydrogen gas. The first method involves the simultaneous generation of O_2 and H_2 by the utilization of emitted electrons by the hydrogenase enzyme in the presence of sunlight. This method has higher productivity than hydrogen gas [140]. In the second method, the two-phase system first grows microalgae in a normal environment and then promotes the continuous production of H_2 under anaerobic and sulfur-deprived environmental conditions [140].

In the two-phase method, the generation of H_2 decreases after 60 h [141]. This short production duration could be resolved by the addition of sulfur, which improves the microalgae cells and their PSII system [142]. The addition of sulfur could increase H_2 production 3–4 times by the application of sulfur at 5 intervals for a time period of 1 month [143].

5.3 Chemical conversion

The chemical conversion of microalga biomass is achieved by a process in which lipids or triglycerides are chemically transformed into biofuels known as transesterification.

5.3.1 Transesterification

Transesterification is also termed "chemical changeover of microalgae biomass" and involves the transformation of microalgae biomass into biodiesel [6]. It is achieved by utilizing the triglycerides of fatty acids and converting them into biodiesel [144]. This process has higher productivity of up to 98%, which is further increased by the separation of biodiesel from methanol and then transforming glycerol into glycerol carbonate [145]. In the transesterification process, the triglycerides combine with monoalcohol in the presence of an appropriate catalyst, which may be acid, alkali, or enzyme, to form fatty acid methyl ester (FAME) and glycerol [137,146,147]. The process is very efficient, but the cost of the enzyme is very high [6]. Glycerol, which is a by-product of the transesterification process, is used in the pharmaceutical and cosmetic industries [146]. Fig. 10.4 describes the chemical equation of the transesterification process. The transesterification process using an alkaline catalyst is the most feasible for biodiesel production [148]. Milano et al. [137] used two species of microalgae, which are *Spirogyra* and *Oedigonium*, and gained up to 90% conversion of FAME in the presence of an alkaline catalyst [137]. The process of transesterification is achieved by two methods, which are ex-situ transesterification and in situ or direct transesterification.

The ex-situ transesterification process needs pretreatment for extracting lipids, so it is time-consuming and it takes up to 80% of the total preparation cost [149]. Jazzar et al. [149] reported that in situ transesterification is the best option to overcome the pretreatment lipid extraction issue and it is economically feasible [149]. In situ transesterification of *Chlorella* sp. in the presence of 20 wt% sulfuric acid resulted in 96%–98% production of FAME in a time period of 4 h [6].

In another study, nearly 96%–99% of FAME yields were obtained using a nanocatalyst of calcium methoxide Ca $(OCH_3)_2$ with the addition of 3% wt of catalyst loading and a 30:1 M ratio of methanol-to-lipid. The reaction was conducted at a hydrothermal temperature of 80°C and the time required was 3 h [150]. Ehimen et al. [151] showed that the continuous mixing during the in situ transesterification process increases the yield of biodiesel from microalgae [151].

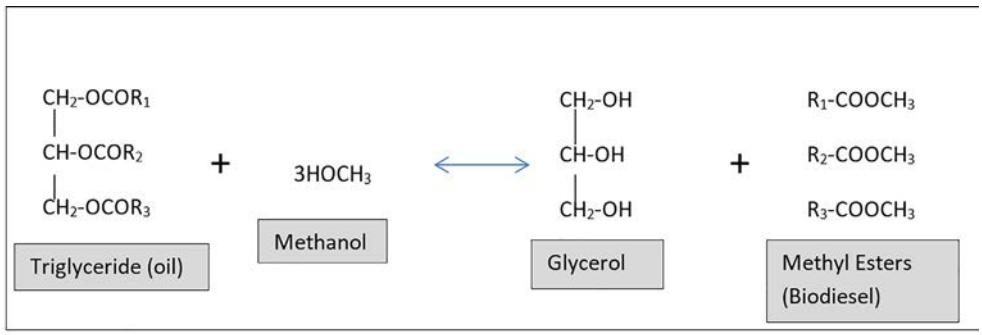

FIG. 10.4 The process of transesterification for biodiesel production.

5.4 Direct combustion

In the process of direct combustion, the microalgae biomass is burned in a furnace, boiler, or steam turbine at a high temperature of 1000°C with excessive air supply to completely convert the chemical energy of the microalgae biomass into heat, energy, or electricity [6,108,146]. Only those species of microalgae that have less than 50% moisture content of their dry weight [2,109]. However, this process also has a limit in the pretreatment of the microalgae biomass, for instance, drying, which consumes a lot of energy and makes this process ineffective [6]. Instead of direct burning, cofiring of microalgae biomass and coal has been proven to be an effective treatment for electricity generation. In the cofiring process, microalgae are first cultivated and then fed, along with coal, to the power plant for combustion [137]. The addition of coal to cultivated algal biomass can help to minimize the release of greenhouse gases [152].

6 Potential bioenergy products of microalgae

Microalgae have a unique potential for producing a wide range of biofuels along with the production of value-added by-products and phytoremediation of wastewater. Many species of microalgae have been selected for producing biofuels. The selection depends on various factors, such as the type of microalgae strain, the nature of biofuel produced, potential yield, downstream processing, and environmental conditions, along with the adaptability of algal strains to yield the required biofuel. Algal biofuels are unique and depend on the component of algal biomass from which biofuels are obtained by a conversion process [40,153]. Fig. 10.5 illustrates the variety of biofuels produced from microalgal biomass and also the by-products.

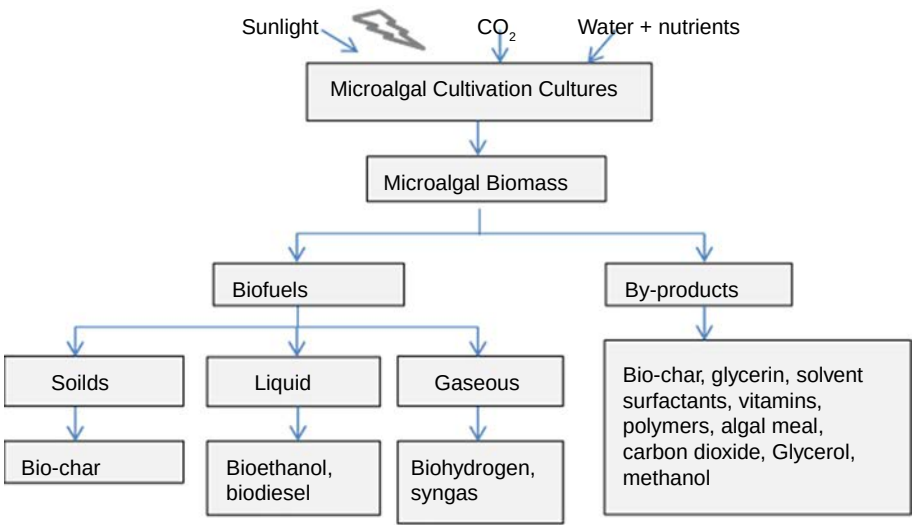

FIG. 10.5 Significant products and by-products obtained from microalgal biomass.

6.1 Biodiesel

Biodiesel is regarded as a substitute for petroleum diesel and is derived from renewable biomass and unused lipids [6,137]. The composition of lipids in the algal biomass determines the quality and potential yield of biodiesel, and also its potency to be used as an alternative fuel to petroleum-based diesel [137]. Furthermore, biodiesel derived from algal biomass, like petroleum diesel, is sulfur-free and has lower particulate matter emissions and greenhouse gas emissions [55]. However, the oxidative stability of algal biodiesel is low, which causes poor performance in cold weather conditions [6]. In their study, Milano et al. [137] proposed producing biodiesel from microalgae strains with high oleic acid concentrations in their fatty acids. Biodiesel is produced from microalgae species such as *Chlorella* sp., *Nannochloropsis oculata*, *Botryococcus* sp., *Scenedesmus* sp., *Picochlorum* sp., and *Saccharomyces cerevisiae*, which have higher oleic acid, and thus help to improve the oxidative stability of biodiesel [137]. Biodiesel is mainly derived from microalgae by a transesterification process. In this process, lipids, specifically triacylglycerides (TAG), are transesterified in the presence of alkali or acid and yield biodiesel and glycerol [154]. The selection of microalgal strains is a crucial factor for biodiesel production [6]. The quality of the yielded biodiesel is determined by the cetane number (CN), viscosity, calorific value, and melting point, as all these factors greatly impact the performance of biodiesel in engines [155]. Chung et al. [155] suggest many strategies for improving the quality of biodiesel, some of which include the bioengineering of strains of microalgal [155].

6.2 Bioethanol

The microalgae cell wall is a rich source of lipids and carbohydrates such as cellulose, mannans, sulfated glycans, xylans, and starch. These complex components are decomposed into simple sugars either chemically or via enzymatic activity, which is then transformed into bioethanol under anaerobic conditions [156]. Pure ethanol is a substitute for gasoline but has a higher octane number and heat of vaporization than gasoline, which makes it a better fuel than gasoline. It has the same energy content as 66% of gasoline by volume [46]. Microalgae are considered as an efficient feedstock for bioethanol production in comparison to conventional crops like corn, soya beans, or sugarcane [157,158]. The potential yield of bioethanol produced from microalgae is almost double the yield of sugarcane and five times the yield of corn [159]. Several species of algae, like *Chlorococcum* sp., *Spirogyra* sp., *Gelidiumamansii*, *Sargassum* sp., *Gracilaria* sp., *Laminaria* sp., and *Prymnesium parvum* have been considered important for bioethanol production [34,35]. Bioethanol is produced from microalgae by several processes, such as selection and culturing of algal biomass, pretreatment, liquefaction, saccharification, anaerobic fermentation, and then, distillation for purification of bioethanol [159]. Further, the production of bioethanol from microalgae could be improved by improving strategies. Fig. 10.6 depicts the bioethanol production process steps.

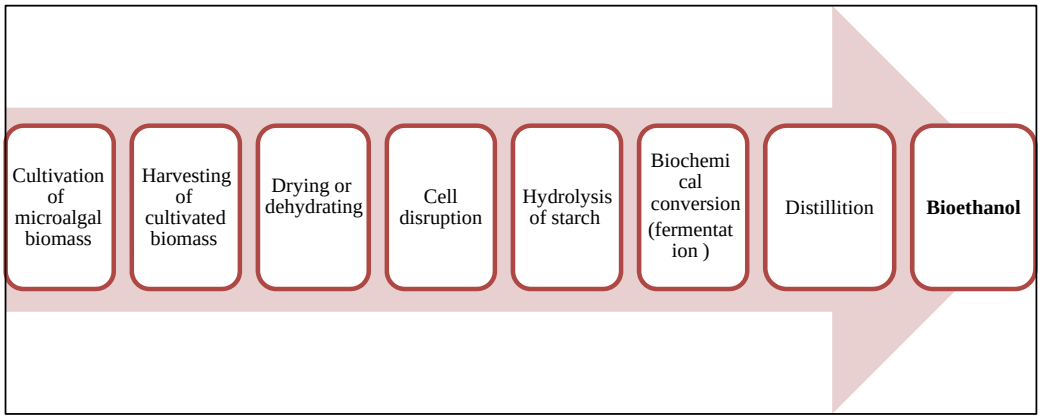

FIG. 10.6 The production process of bioethanol from microalgal biomass.

6.3 Biogas

The end product of anaerobic digestion is biogas or biomethane, which mainly constitutes 55%–75% of methane and 25%–45% of carbon dioxide [40]. It is also produced as a by-product of anaerobic fermentation [6]. Microalgae are considered a remarkable source for biogas generation as they constitute higher concentrations of polysaccharides and lipids, zero lignin, and lower cellulose concentrations. Furthermore, microalgae are easy to harvest with lower land usage, simple to transform into biogas, and have high growth rates in comparison with lignocellulosic biomass. In addition, anaerobic digestion produces solid residue that is used as a soil additive [10,160,161]. Seaweed has excellent potential for the production of biogas. Many algal species, such as *Scenedesmus*, *Euglena*, *Spirulina*, and *Ulva*, have been used for biogas production [162,163]. Biogas is produced in a three-step process which includes hydrolysis, acetogenesis, and methanogenesis [4,35,161]. However, certain limitations make biogas generation inappropriate. The risk of eutrophication causes hindrance to biogas production. Furthermore, the production of toxic substances, unwanted C: N ratio, and the formation of ammonia [40,160]. The low C: N ratio leads to the formation of ammonia, which affects anaerobic microorganisms such as bacteria. Also, sodium ions inhibit microbial activity. So, it is suggested to use salt-tolerating microorganisms for the production of biogas through anaerobic digestion of microalgal biomass [4,35].

6.4 Biohydrogen

Microalgae have the ability to produce hydrogen in the presence of light and water, under anaerobic conditions. Hydrogen is considered an efficient energy fuel as it is free from greenhouse gas emissions and also produces water [164]. The energy content of 2.2 lb of hydrogen is the same as that of 6.2 lb of gasoline [165]. Among all the fuels, the gravimetric energy density and conversion efficiency of hydrogen are high, which is 142 MJ/kg and 94% respectively [166]. Certain species of microalgae have the hydrogenase enzyme, which reduces protons by

splitting water to produce hydrogen. This process takes place in the dark, thereby rendering it anaerobic [166]. However, this method has the limitation of a short life of reaction due to the production of oxygen, which immediately deactivates the hydrogenase enzyme. Moreover, better strategies are needed to improve the oxygen tolerance of the hydrogenase enzyme, which needs further technological advancement [6,166].

6.5 Biosyngas

Syngas is considered a mixture of hydrogen, methane, and carbon monoxide. It is produced under high temperatures, usually 800°C to 1000°C, by the partial oxidation of algal biomass by a process known as gasification [6,108,111]. In this process, the algal biomass reacts with water (steam) and oxygen and decomposes into a mixture of gases known as syngas [35]. Syngas has low calorific value and is produced from microalgal strains that have less than 20% moisture content [167]. It is used to generate electricity in turbines and boilers [14].

6.6 Bio-oil

The pyrolysis of algal biomass under anaerobic conditions at high temperatures yields bio-oil. The composition of bio-oils depends on the nature of the feedstock and operational conditions [168,169]. Different factors, such as biomass composition, temperature, pressure, vapor residence time, water, and ash content, alter the productivity of bio-oil [170]. Demirbas [171] showed that bio-oil produced from microalgae is more stable than that of wood [171]. Bio-oil can be used for power generation.

6.7 Hydrocarbons

Microalgae have the potential to yield hydrocarbons which are then further transformed into diesel, gasoline, and kerosene. The microalgae strain *Botryococcus braunii*, a freshwater species, has excellent oil potential [172]. It can tolerate various salt concentrations. Further, hydrocarbon extraction from this species is easy because it stores oil outside the cell [173,174].

7 Advantages of algal biofuels

Microalgae are regarded as diverse, single-celled photosynthetic organism that enables us to deal with the shortage of liquid fuel for transportation by providing a variety of solutions through a large number of avenues. Microalgae species have the potential to grow in a wide range of aqueous environments. Algal's ability to efficiently assimilate CO_2 from the environment makes it responsible for carbon fixation of up to 40% worldwide. Marine microalgae play a significant role in CO_2 assimilation [175]. The growth rate of microalgae is very fast and it can double its biomass in a couple of hours, and many species exhibit double productivity in a day [37,176]. All algal species can produce energy-rich oils and some species can accumulate oils in high concentrations in their total dry biomass [177,178]. For instance,

Botryococcus spp. has the ability to store long hydrocarbon chains up to 50% of dry mass [179]. Algae provides an alternative platform for renewable energy production. The biofuels derived from microalgae are a potent substitutes for first and second-generation biofuels. Microalgae growth and production processes do not harm agricultural productivity. Microalgae consume solar energy for the production of biomass such as carbohydrates, proteins, and lipids. Microalgal fuels are mainly green fuels, as algae behave as carbon sinks, utilizing atmospheric CO_2 and producing oxygen through the process of photosynthesis. Furthermore, microalgae utilize nitrogen and phosphorus as macronutrients for their effective growth in water bodies, thus limiting pollutants and toxic chemical concentrations in these bodies [178,180].

The extensive use of fossil fuels leads to the depletion of these energy sources, so there is a need for an alternative source of energy that would fulfill the increasing energy demand. Microalgae biofuels are renewable and can be produced in abundance [180]. Algal biofuels ease the burden on traditional fuel crops because they need fewer requirements for land and nutrients for their bulk growth [177]. The biofuels produced from plants have not been proven to be effective because of conflicts with food chain supply. So, microalgae biofuels appear to be a viable substitute [181]. The establishment of an algal biorefinery is a good initiative. In a biorefinery, the cultivation, harvesting, extraction of oil, and further purification processes are performed very efficiently. Among biofuels, many value-added coproducts are formed during algal processing [46]. The coproducts have high market demand and price. In addition, microalgae strains have the ability to be bioengineered, in which many beneficial improvements to algal strains are made and useful products are obtained [182,183]. All these advancements make algal biofuels an efficient source of bioenergy and allow them to economically compete with petroleum-based biofuels.

8 Environment and sustainable perspective

Sustainability is regarded as a key element in natural resource management. The main aspects of sustainability are operational efficiency, reduction in environmental impacts, and social-economic perspectives, and all these aspects are interrelated and interdependent. The continued utilization of fossil fuel energy resources leads to the depletion of these resources, which is unsustainable. It causes greenhouse gas emissions and a shortage of energy resources for future use. Due to this, the global world is facing an issue regarding unsustainability. Therefore, vigorous efforts are made to resolve these issues and the development of renewable and environmentally friendly solid, gaseous, and liquid biofuels as alternative energy resources. In this regard, first-generation biofuels were derived from terrestrial crops like maize, sugarcane, sugar beet, and corn. But, these biofuels greatly impact the environment through the destruction of food crops, shortages of water supplies, and deforestation of the world's forest coverage. The second generation of biofuels is based on the same aim and is derived from lignocellulosic crops and forest residues, as well as nonfood crops solid waste. But, these biofuels also contribute to the same concerns and also cause land-use changes. Therefore, to overcome the issues of first and second-generation biofuels, third-generation biofuels, obtained by using microalgae as an energy source, have proved to be a

viable resource of energy devoid of the pitfalls associated with terrestrial crops and lignocellulosic and nonfood feedstock [4].

Algal biofuels may contribute to improved energy security, minimize greenhouse gases emissions, and enhance environmental quality [87]. Biofuels act as a substitute for petroleum-based fuels and thus diminish the dependence on fossil fuels. However, biofuels have certain limitations. A large area of land with the necessary nutrients (sunlight, water, temperature, N, and P) is required for the production of algae species for biofuel production. It is noted that biofuels reduce greenhouse gases emissions, but if a large area of land is cleared for biofuel production, then it is not an effective approach. Biofuels, which are produced without increasing arable land or deforestation of tropical rainforest would be considered sustainable. However, algae may provide this opportunity with advanced technological improvements [184].

9 Challenges of algal biofuels and future outlook

The use of algal biomass for the production of biofuels has various advantages over first and second-generation biofuels. Several advancements have been made to improve the production process of biofuels from algae. However, the cultivation of biomass is still not completely improved for large-scale production and needs some efficient techno-economic improvements [2]. Currently, the high cost associated with algae cultivation, as compared to other biofuel feedstock, is one of the most prominent challenges for the production of algal biofuels. The contamination of algal cultures in open raceway pond systems causes a loss of biomass productivity [185]. Further, the techniques of harvesting and dewatering the algal biomass require high costs and energy. Algal cultures grow in aqueous suspension, so settling and separation are also challenge in biofuel production [29]. The process of harvesting accounts for 20%–30% of the total production cost [111]. Moreover, the methods of harvesting are not feasible and effective in terms of cost and productivity and are also poorly developed. So, there is a need to develop methods that have low cost and high efficiency.

Algae have the ability to grow in saline and nutrient-rich wastewater, so, algal biomass cultivation facilities should be near the waste source, such as an industrial wastewater treatment plant or municipal wastewater drain. The algae utilize the nutrients present in the wastewater and decrease the primary or secondary treatment requirements [85]. Different algal strains have different compositions of biomass, mainly carbohydrates, proteins, and lipid content. The production of various types of biofuels demands a different amount of biomass. For instance, the production of biodiesel from microalgae requires a high content of lipids. The content of algal biomass depends on the cultivation conditions. For example, the microalgal biomass contains high lipid and carbohydrate content and is cultivated in a low nitrogen environment. The low productivity of biomass and lipid content is a barrier to the viable yield of algal biodiesel. Therefore, an in-depth study is needed to select algae strains that have a high growth rate and a high yield of biofuels. In addition, detailed research for the optimization of growth parameters to increase productivity is a basic necessity so that it can be applied on a large scale to obtain higher productivity in a short time. The stress management strategy should also be concerned with enhancing the growth of algal biomass [2,29].

The cell wall of microalgae is rigid in structure and requires pretreatment for the production of bioethanol. Similarly, the segregation of lipids is necessary for biodiesel production. These pretreatment processes have high costs, high energy requirements, and are time-consuming. Therefore, extensive research is needed for cost-effective pretreatment technologies that not only increase productivity but also minimize the consumption of energy and time [2]. The biomass conversion technologies have their own specifications and convert a specific composition to produce various forms of biofuel. The selection of an appropriate process for obtaining biofuel is necessary so that it produces biofuel efficiently and cost-effectively. The cost of production of a unit of algal biofuel must be less than that of petroleum-based fuels for their commercial applications [29]. The production of by-products is also dependent on the conversion process. However, currently, there is no efficient conversion process that efficiently transforms algal biomass into biofuels in an environmentally friendly manner. Therefore, new strategies for techno-economic analysis, treatment designs, and production efficiency should be required [2]. Dutta et al. [193] suggested the need to obtain higher-value by-products so, the process of biofuel production becomes more economically viable.

10 Conclusions

This chapter highlights a brief review of the biofuels produced from microalgae. The three generations of biofuels, with a detailed analysis of microalgae as a potential source of biofuels, are enlisted. The study identified various aspects of microalgae, biomass composition, growth requirements, and potential cost for the production of microalgal cultures. A sequential review of the entire production process has been conducted, including cultivation, harvesting, dewatering, pretreatment, and biomass conversion technologies. Algal cultivation system along with specifications, advantages, and disadvantage have been discussed. A variety of algal harvesting technologies have been reevaluated on account of their potential benefits and pitfalls for assistance in the selection of technology. Algal biomass conversion technologies have been outlined, with a detailed analysis of operating conditions and end products produced. This chapter also discusses the potential biofuels produced from microalgal biomass and their applications as an energy source. Furthermore, the advantages of algal biofuels have been examined. Moreover, the techno-economic challenges for producing algal biofuel and the future outlook are discussed. In the end, a few recommendations have been made based on the bottleneck of the current situation.

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CHAPTER

11

Thermal treatment kinetics of microalgae for energy production

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OUTLINE

1 Introduction

In recent years, fossil fuel (e.g., coal, oil, and gas) use has been drastically increased due to the rise in population and industrial development [1–4]. With the increasing use of fossil fuels

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in the world, their reserves are decreasing, which not only leads to the depletion of energy but also has a great impact on the climate [5–8]. Therefore, it has become essential to look for renewable and environmentally friendly alternative energy options. Because fossil energy is nonrenewable, people have begun to pay attention to biomass energy, because this energy is renewable energy and is in line with sustainable development [1]. Microalgal biomass is considered an important material (called the third generation) that can be used to produce alternative energy. It surpasses first-generation raw materials such as grain crops and second generation raw materials such as lignocellulosic biomass in many aspects, such as renewability, energy level, and growth cycle [6,9].

There are many ways in thermochemical conversion technology, including gasification and pyrolysis, to convert microalgae biomass into various biofuels, for instance, biodiesel, bioethanol, and syngas [10–13]. Pyrolysis refers to the thermal transformation of biomass in an inert atmosphere to produce high-value target products, such as biochar, biooil, and biogas. In the process of biofuel production, pyrolysis technology is commonly used because of its high conversion, adaptability, and short process. Another common method is gasification, in which biomass is converted into syngas by heat under the action of gasification agents such as steam, carbon dioxide, air, or oxygen.

At present, with the continuous development of research, catalytic gasification, catalytic pyrolysis, and co-pyrolysis are used to promote the thermochemical conversion efficiency of microalgae and the productivity of bio-hydrogen and bio-oil [14,15]. To further understand the fundamentals of microalgae thermochemical conversion and the effect of process parameters, kinetic parameters are required. In thermal conversions with catalysts, kinetic parameters are also necessary measures to quantify the impact of catalysts and the conversion efficiency. Generally, thermogravimetric analysis (TGA) is used to analyze the decomposition behavior of biomass samples [16], and then the "kinetic triplets" are obtained, including the reaction model of three kinetic parameters, preexponential factor, and activation energy [17], so as to study the basic principle of biomass thermal decomposition reaction. The main methods to evaluate activation energy are model-fitting, model-free (isoconversional), DAEM, etc.

Although the gasification and pyrolysis kinetic parameters of microalgae will be calculated according to the DAEM method, model-free (isoconversional) method and model-fitting method, the review of its mechanism explanation is very rare. This chapter mainly introduces the thermochemical conversion kinetics of microalgae and their mechanism in relation to pyrolysis and gasification of microalgae. Dynamic methods such as DAEM method, model-free method, and model-fitting method are systematically analyzed and studied in order to ensure effective utilization of microalgae for higher productivity.

2 Chemical composition of microalgae

Microalgae biomass has many unique advantages, including prominent solar energy conversion rate, growth capacity on noncultivated land, and productivity, so it has significant advantages as a source of next generation biofuels [18]. Microalgae biomass can be used as raw materials for the production of biofuels such as syngas, biohydrogen, biogas, bioethanol, and biodiesel [19]. The great potential of microalgae for fuel production is closely related to its main components. In microalgae, three main biological compounds account for more than

half of the mass, including (i) protein (25%–70%), (ii) lipid (0%–45%), and (iii) carbohydrates (8%–65%) [20]. Table 11.1 lists the elements and chemical compositions of some common microalgae. Microalgae with high oxygen content (30%–50%) would produce oxygen-containing compounds (such as esters, aldehydes, ketones, acids, furans, etc.) in the bio-oil [28]. Excessive oxygen content in microalgae can also lead to more tar products during gasification, which can cause problems such as plugging the filter, forming coke, and corroding the equipment [29]. An interaction between proteins and carbohydrates during pyrolysis, called the Maillard reaction, can produce aroma compounds that play an important role in the food industry. The composition of lipids includes peptide glycolipids, peptide lipids, glycolipids, sphingolipids, phospholipids, and neutral lipids [30]. High quality bio-gas and bio-oil can be obtained by the pyrolysis of low-fat microalgae [31]. Volatile matter is the main contributor to energy products. Fixed carbon could be regarded as the source of activated carbon, which has been widely used in pollutant removal, energy storage, chemical purification, etc. Biomass ash, which is composed of various metal oxides and silica, may have catalytic effect by reducing the reaction activation energy [32]. Microalgae cell walls are composed of cellulose, mannan, xylan, and sulfated polysaccharides. These polysaccharides with other minor components can be decomposed into monosaccharides by chemical or enzymatic action, and then converted into ethanol [33]. In addition, microalgae biomass includes a large number of nutrients such as phosphorus and nitrogen. Microalgae waste can not only recover nutrients through anaerobic digestion but also produce biomethane [19].

3 Thermo-chemical conversion

3.1 Pyrolysis

The pyrolysis process is the thermal degradation of biomass under anoxic conditions, so as to obtain coke, bio-oil, pyrolysis gas, and other products [25]. Pollution is easier to control in the pyrolysis process than in the direct combustion process. In addition, the combustion of biomass gas and biomass oil exhibited higher energy efficiency than the traditional direct combustion of biomass. Thermogravimetric analysis (TGA) is widely used in the pyrolysis kinetics of microalgae. Its main principle is to detect the real time mass change of samples in a high-temperature environment and an inert atmosphere and calculate the pyrolysis kinetic parameters in this way [26].

3.1.1 Copyrolysis

The principle of copyrolysis is to pyrolyze no less than two kinds of raw materials, so that they can achieve a certain synergistic effect.

Although microalgae pyrolysis has many advantages in producing biofuel over conventional energy sources (e.g., fossil fuels), it requires a huge investment that surpasses the energy production cost of fossil fuels. Besides, the product quantity of single algae pyrolysis is generally low. Hence, copyrolysis of two different materials could compensate for the drawbacks of each other, which could reduce the cost of biofuel production and improve product quality and quantity [34]. Many studies have demonstrated that the copyrolysis of solid biomass and waste plastics has a certain synergistic effect, that leads to the improvement of product quality. With

TABLE 11.1 Basic properties of various microalgae.

	Elemental analysis (wt%)				Chemical composition analysis (wt%)			Proximate analysis (wt%)				
Feedstock	**C**	**H**	**O**	**N**	**Carbohydrate**	**Protein**	**Lipid**	**Volatile matter**	**Ash**	**Moisture**	**Fixed carbon**	**Reference**
Chlorella vulgaris	49.4	7.2	34.6	8.7	20.2	50.5	14.6	77.6	6.2	2.7	13.5	[21]
Nannochloropsis oculata	47.1	6.9	37.7	8.2	10.8	45.8	20.6	71.9	13.2	1.6	13.3	[21]
Spirulina platensis	42.6	7.2	33.6	9.9	–	–	–	76.5	6.8	6.1	10.5	[6]
Haematococcus pluvialis	55.2	8.2	26.7	1.7	–	–	–	85.3	1.2	5.5	8.0	[22]
Seawater *Spirulina*	45.7	6.7	36.9	9.7	–	–	–	78.4	4.4	2.3	14.9	[12]
Spirulina	49.8	6.6	31.9	11.0	23.4	57.8	2.9	73.5	6.6	6.7	13.2	[23]
Chlorella sp.	35.0	9.2	2.5	3	–	–	–	32.4	12.8	2	–	[24]
Dunaliella salina	47.4 ± 0.5	6.7 ± 0.3	40.1	3.5 ± 0.1	–	–	–	73.9 ± 1.6	7.7 ± 0.2	5.4 ± 0.5	12.8	[25]
Isochrysis galbana	47.6	7.2	38.3	5.9	–	–	–	86.1	8.3	–	–	[26]
Nannochloropsis gaditana	58.6	9.0	22.9	8.8	–	–	–	84.0	10.5	–	–	[26]
Nannochloropsis limnetica	58.1	8.6	23.7	9.0	–	–	–	81.5	9.1	–	–	[26]
Phaeodactylum tricornutum	54.5	8.9	25.5	9.1	–	–	–	62.1	25.5	–	–	[26]
Nannochloropsis sp.	38.3	5.6	35.1	6.1	9.0	62.0	18.0	66.6	14.9	–	18.5	[27]

the help of low oxygen carbon ratio (O/C) and a high hydrogen carbon ratio (H/C) in plastic waste, it can offset the inherent low (H/C) and high (O/C) of solid biomass in copyrolysis stage, which can effectively improve the uniformity and overall quality level of products [35]. In addition, the apparent activation energy of copyrolysis reaction may increase continuously, and the overall level is relatively high. Copyrolysis might produce more pyrolytic carbon. Moreover, the enhancement effect of hydrocarbons obtained in the copyrolysis stage will also be affected to some extent [36].

3.1.2 *Catalytic pyrolysis*

Catalyst play a very important role in the sustainable chemical industry of clean bio-oil production [11]. In other words, the catalyst is added during the cracking stage of microalgae, that is, the catalytic cracking process. Adding a catalyst can greatly reduce the activation energy and energy consumption of the reaction, achieve a constant reaction rate, and significantly improve the quality and products of biofuels.

If microalgae contain more protein compared with the general lignocellulose biomass, it will generates more nitrogen in the production of bio-oil. At the same time, if there are high nitrogen compounds, it will affect the quality of bio-oil and greatly reduce its HHVs [11]. Therefore, the removal of the nitrogen portion in bio-oil is important before it is exploited as a transport fuel to avoid nitrogen oxide emissions [37]. The liquid fuel produced from microalgae pyrolysis cannot be burnt directly due to some disadvantages, such as low calorific value, poor stability, and high viscosity [38]. Therefore, by adding catalysts to the pyrolysis stage, the overall quality of liquid fuel is improved, which has attracted more and more attention.

3.2 Gasification

Biomass gasification is actually a thermochemical process. In the presence of a gasifying agent such as water vapor, oxygen or air, carbon containing materials are thermochemically reacted accordingly, including pyrolysis, oxidation, reduction, and reforming, so as to obtain syngas [12]. Among various analytical techniques, mass spectrometer thermogravimetric analysis is the most effective and economical technique, which can perceive the decomposition behavior of materials, including the real time released gas distribution [39]. Gasification was generally implemented in fluidized reactors [40–42], in which temperature, residence time, and gas composition played dominant roles in the gasification products. The gasification kinetics provided important guidance for designing the structure of the reactor and optimizing the parameters of operation.

4 Basic formulas and models of kinetics

4.1 Basic kinetic formulas

The pyrolysis and gasification kinetics of microalgae are essential in understanding the thermal degradation, transformation, and product formation, as well as in-process rate prediction, determination of operating parameters, and determination of experimental design

parameters [43]. The thermal behavior of microalgae is related to the thermal stimulation process, which is caused by the temperature change. In fact, the main variables included in the process rate are parameterized as follows: the thermal treatment time, t; the extent of conversion, α; and the temperature, T:

$$\frac{d\alpha}{dt} = k(T)f(\alpha) \tag{11.1}$$

where $k(T)$ represents the rate coefficient, $f(\alpha)$ is the reaction model. The specific expression of $k(T)$ generally follows the Arrhenius equation:

$$k(T) = A\exp\left(\frac{-E}{RT}\right) \tag{11.2}$$

In the above formula, R is the gas constant, E is the activation energy, and A is called the preexponential factor.

The conversion can be easily determined as the fraction change of any physical properties related to the reaction process. When the thermal process is monitored as mass change through thermogravimetric analysis, α represents the ratio of current reacted mass to total reactive mass [44]:

$$\alpha = \frac{m_0 - m}{m_0 - m_f} = \frac{\Delta m}{\Delta m_{tot}} \tag{11.3}$$

In the above formula, m is the mass at time t, and m_f and m_0 are the initial and final masses.

The temperature program can be either nonisothermal or isothermal. In general, the nonisothermal program is to carry out linear heating at a constant heating rate [17]:

$$\beta = \frac{dT}{dt} = \text{const} \tag{11.4}$$

where β represents the heating rate.

Based on the correlation of Eq. (11.4), Eq. (11.5) is the first-order derivative of conversion with respect to temperature:

$$\frac{d\alpha}{dT} = \frac{d\alpha}{dt}\frac{dt}{dT} \tag{11.5}$$

where dT/dt represents the heating rate β, $d\alpha/dt$ represents the isothermal reaction rate, $d\alpha/dT$ represents the nonisothermal reaction rate. Combining Eqs. (11.1), (11.2), (11.4), and (11.5), the following differential formula of nonisothermal rate law can be obtained:

$$\beta\frac{d\alpha}{dT} = A\exp\left(\frac{-E}{RT}\right)f(\alpha) \tag{11.6}$$

Combining Eqs. (11.1) and (11.2) leads to:

$$g(\alpha) = \int_0^\alpha \frac{d\alpha}{f(\alpha)} = A\int_0^t \exp\left(\frac{-E}{RT}\right)dt \tag{11.7}$$

In the above formula, $g(\alpha)$ is defined as the integral form of the reaction model.

TABLE 11.2 A variety of typical kinetic models in heterogeneous dynamics [46].

Model code	Model name	$f(\alpha)$	$g(\alpha)$
A2	Avrami-Erofeev	$2(1-\alpha)[-\ln(1-\alpha)]^{1/2}$	$[-\ln(1-\alpha)]^{1/2}$
A3	Avrami-Erofeev	$3(1-\alpha)[-\ln(1-\alpha)]^{2/3}$	$[-\ln(1-\alpha)]^{1/3}$
A4	Avrami-Erofeev	$4(1-\alpha)[-\ln(1-\alpha)]^{3/4}$	$[-\ln(1-\alpha)]^{1/4}$
An	Avrami-Erofeev	$n(1-\alpha)[-\ln(1-\alpha)]^{n-1/n}$	$[-\ln(1-\alpha)]^{1/n}$
D1	1-D diffusion	$1/(2\alpha)$	α^2
D2	2-D diffusion	$-[1/\ln(1-\alpha)]$	$(1-\alpha)\ln(1-\alpha)+\alpha$
D3	3-D diffusion	$[3(1-\alpha)^{2/3}]/[2(1-(1-\alpha)^{1/3})]$	$1-(2/3)\alpha-(1-\alpha)^{2/3}$
F0	Zero order	1	A
F1	First order	$(1-\alpha)$	$-\ln(1-\alpha)$
F2	Second order	$(1-\alpha)^2$	$[1/(1-\alpha)]-1$
F3	Third order	$(1-\alpha)^3$	$(1/2)[(1-\alpha)^{-2}-1]$
Fn	n-th order	$(1-\alpha)^n$	$[1/(n-1)]\,[(1-\alpha)^{-n+1}-1]$
P2/3	Power law	$2/3\alpha^{-1/2}$	$\alpha^{3/2}$
P2	Power law	$2\alpha^{1/2}$	$\alpha^{1/2}$
P3	Power law	$3\alpha^{2/3}$	$\alpha^{1/3}$
P4	Power law	$4\alpha^{3/4}$	$\alpha^{1/4}$
R2	Contracting area	$2(1-\alpha)^{1/2}$	$1-(1-\alpha)^{1/2}$
R3	Contracting volume	$3(1-\alpha)^{2/3}$	$1-(1-\alpha)^{1/3}$

In fact, with a constant heating rate, the integral relative to temperature can be taken to replace the integral relative to time, as follows:

$$g(\alpha)=\frac{A}{\beta}\int_0^T \exp\left(\frac{-E}{RT}\right)\mathrm{d}T \tag{11.8}$$

The model can effectively describe specific reaction types for solid-state reactions, so as to obtain the corresponding mathematical equations. On the basis of certain mechanistic assumptions, solid reaction kinetics have been mathematically derived [45]. Several typical reaction models are listed in Table 11.2.

5 Isoconversional method

5.1 Integral method

In Eq. (11.8), the temperature integral is the integral on the right, and the expression symbol is $\Lambda(T)$. Even though $\Lambda(T)$ does not have an analytical solution, its approximate solution could

be achieved. By defining a variable $u = E/RT$, from Eq. (11.8) the integral could be rewritten as:

$$g(\alpha) = \frac{A}{\beta}\int_0^T \exp\left(\frac{-E}{RT}\right)\mathrm{d}T = \frac{AE}{\beta R}\int_\infty^u \frac{-e^{-u}}{u^2}\mathrm{d}u = \frac{AE}{\beta R}p(u) \tag{11.9}$$

From the integration by parts:

$$p(u) = \int_\infty^u \frac{-e^{-u}}{u^2}\mathrm{d}u = \frac{e^{-u}}{u^2}\left(1 - \frac{2!}{u} + \frac{3!}{u^2} - \frac{4!}{u^3} + \cdots\right) \tag{11.10}$$

many approximations of temperature integral are derived from Eq. (11.10) which are summarized in Table 11.3.

5.1.1 Flynn-Wall-Ozawa method

The Flynn-Wall-Ozawa method uses Doyle's temperature integral approximation by taking the first two items in the right-hand brackets of Eq. (11.10) and takes the logarithm on both sides:

$$\ln P(u) = -u + \ln(u-2) - 3\ln u \tag{11.11}$$

TABLE 11.3 List of $p(u)$ expressions and temperature integral approximations [47].

Number	Name	$p(u)$	$\int_0^T \exp\left(\frac{-E}{RT}\right)dT$
1	Frank-Kameneskii	$e^{-u}\left(\frac{1}{u^2}\right)$	$\frac{RT^2}{E}e^{-E/RT}$
2	Coats-Redfern	$e^{-u}\left(\frac{1}{u^2}\right)\left(1-\frac{2}{u}\right)$	$\frac{RT^2}{E}\left(1-\frac{2RT}{E}\right)e^{-E/RT}$
3	Doyle	$0.00484e^{-1.0516u}$	$\frac{E}{R}0.00484e^{-1.0516E/RT}$
4	Gorbachev	$e^{-u}\left(\frac{1}{u}\right)\left[\frac{1}{u+2}\right]$	$\frac{RT^2}{E+2RT}e^{-E/RT}$
5	Lee-Beck	$\frac{e^{-u}\left(\frac{1}{u^2}\right)\left(1-\frac{2}{u}\right)}{\left(1-\frac{4}{u^2}\right)}$	$\frac{RT^2}{E}\left[\frac{1-2\left(\frac{RT}{E}\right)}{1-4\left(\frac{RT}{E}\right)^2}\right]e^{-E/RT}$
6	Agrawal	$e^{-u}\left(\frac{1}{u^2}\right)\left[\frac{1-\frac{2}{u}}{1-\frac{5}{u^2}}\right]$	$\frac{RT^2}{E}\left[\frac{1-2\left(\frac{RT}{E}\right)}{1-5\left(\frac{RT}{E}\right)^2}\right]e^{-E/RT}$
7	Li Chung-Hsiung	$e^{-u}\left(\frac{1}{u^2}\right)\left[\frac{1-\frac{2}{u}}{1-\frac{6}{u^2}}\right]$	$\frac{RT^2}{E}\left[\frac{1-2\left(\frac{RT}{E}\right)}{1-6\left(\frac{RT}{E}\right)^2}\right]e^{-E/RT}$

From $20 \le u \le 60$, $-1 \le \frac{u-40}{20} \le 1$ and $v = \frac{u-40}{20}$:

$$u = 20v + 40 \tag{11.12}$$

Substitute the Eq. (11.12) into Eq. (11.11) and approximate the logarithmic expansion by first order:

$$\ln P(u) = -u - 3\ln 40 + \ln 38 + \ln\left(1 + \frac{10}{19}v\right) - 3\ln\left(1 + \frac{1}{2}v\right) \approx -5.3308 - 1.0516u \tag{11.13}$$

$$\ln P(u) \approx -5.3308 - 1.0516\frac{E}{RT} \tag{11.14}$$

Combining Eqs. (11.14) and (11.9) gives:

$$\ln\beta = \ln\left(\frac{AE}{Rg(a)}\right) - 5.3308 - 1.0516\frac{E}{RT} \tag{11.15}$$

Eq. (11.15) is the final FWO expression.

Gong et al. [22] calculated the average activation energy of *Haematococcus pluvialis* by the FWO method, and the activation energy was found to be 201.57 kJ/mol. Rasam et al. [1] performed copyrolysis of scrap tires, sucrose and *Spirulina*. The average activation energy of ternary mixtures was calculated by FWO analysis method, and the activation energy was 171.90 kJ/mol.

5.1.2 Kissinger-Akahira-Sunose method

There are some differences between KAS and FWO equations, the KAS method uses the Coats-Redfern approximation, $p(u)$ was expressed as follows [26]:

$$p(u) = \int_{\infty}^{u} \frac{-e^{-u}}{u^2}\mathrm{d}u \approx \frac{\exp(-u)}{u}\sum_{n=0}^{\infty}\frac{(-1)^n 2^n}{u^{n+1}} \tag{11.16}$$

Based on the variable $p(u)$ and $u = E/RT$, the right integral of Eq. (11.8) can be expressed as:

$$\int_0^T \exp\left(\frac{-E}{RT}\right)\mathrm{d}T = \frac{E}{R}p(u) \approx T\exp\left(\frac{-E}{RT}\right)\left[\frac{RT}{E} - \frac{2R^2T^2}{E^2} + \ldots\right] \tag{11.17}$$

In the pyrolysis stage, RT/E is generally a small number, and the higher-order of RT/E is even smaller, therefore, only the first item of the summation is sufficient to approximate the integral properly, and the Coats-Redfern approximation is obtained:

$$\int_0^T \exp\left(\frac{-E}{RT}\right)\mathrm{d}T \approx \frac{RT^2}{E}\exp\left(\frac{-E}{RT}\right) \tag{11.18}$$

Combining Eqs. (11.18) and (11.8) leads to:

$$g(\alpha) = \frac{ART^2}{\beta E}\exp\left(\frac{-E}{RT}\right) \tag{11.19}$$

Rearranging Eq. (11.19) gives:

$$\frac{\beta}{T^2}=\frac{AR}{Eg(\alpha)}\exp\left(\frac{-E}{RT}\right) \tag{11.20}$$

Take the logarithm of Eq. (11.20) to obtain KAS equation:

$$\ln\left(\frac{\beta}{T^2}\right)=\ln\left(\frac{AR}{Eg(\alpha)}\right)-\frac{E}{RT} \tag{11.21}$$

In the above formula, E can be estimated by a slope of the linear plot $\ln(\beta/T^2)$ against $-1/RT$.

Chen et al. [48] conducted a study on the copyrolysis treatment of kitchen waste and *Chlorella vulgaris*. Higher average activation energy and lower mass loss are obtained. When the ratio of the *C. vulgaris* and kitchen waste was 1:1, the average activation energy of 228.21 kJ/mol (KAS) was the highest. Azizi et al. [49] also used various biomasses such as *C. vulgaris*, wood, and propylene for copyrolysis, and the thermal decomposition process was inhibited due to material interaction. However, the copyrolysis of the three materials can show a certain synergistic effect at temperatures of 300°C and 400°C. Then the average activation energy was calculated by the KAS method, and the average value was 131.228 kJ/mol. Tang et al. [36] mixed rural solid waste and *C. vulgaris* for copyrolysis, and added three different catalysts (MgO, CaO, and HZSM-5) for catalytic pyrolysis. To determine the activation energy, the FWO and KAS methods were used. The activation energy showed a decreasing trend at a mass ratio of 1:1 for MgO and HZSM-5, while increasing the amounts of CaO improved the quality of bio-oil.

5.2 Differential method

5.2.1 *Kissinger method*

If the reaction model follows order law $f(\alpha)=(1-\alpha)^n$, Eq. (11.1) is expressed as follows:

$$\frac{\mathrm{d}\alpha}{\mathrm{d}t}=Ae^{-E/RT}(1-\alpha)^n \tag{11.22}$$

Differentiating Eq. (11.22) with respect to time yields:

$$\begin{aligned}\frac{\mathrm{d}}{\mathrm{d}t}\left[\frac{\mathrm{d}\alpha}{\mathrm{d}t}\right]&=\left[A(1-\alpha)^n\frac{\mathrm{d}e^{-E/RT}}{\mathrm{d}t}+Ae^{-E/RT}\frac{\mathrm{d}(1-\alpha)^n}{\mathrm{d}t}\right]\\&=\frac{\mathrm{d}\alpha}{\mathrm{d}t}\left[\frac{E\frac{\mathrm{d}T}{\mathrm{d}t}}{RT^2}-An(1-\alpha)^{n-1}e^{-E/RT}\right]\end{aligned} \tag{11.23}$$

When the reaction rate $\mathrm{d}\alpha/\mathrm{d}t$ reached its peak, the corresponding temperature T_p could be obtained from $\frac{\mathrm{d}}{\mathrm{d}t}\left[\frac{\mathrm{d}\alpha}{\mathrm{d}t}\right]=0$:

$$\frac{E\frac{\mathrm{d}T}{\mathrm{d}t}}{RT_\mathrm{p}{}^2}=An\left(1-\alpha_\mathrm{p}\right)^{n-1}e^{-E/RT_\mathrm{p}} \tag{11.24}$$

Kissinger assumes that the decomposition follows the first order model, thus $n(1-\alpha_p)^{n-1}$ value is 1, so Eq. (11.24) becomes:

$$\frac{E\beta}{RT_p{}^2} = Ae^{-E/RT_p} \tag{11.25}$$

Take the logarithm of Eq. (11.25) leads to Kissinger equation:

$$\ln\left(\frac{\beta}{T_p{}^2}\right) = \ln\frac{AR}{E} - \frac{E}{R}\frac{1}{T_p} \tag{11.26}$$

where E is estimated as a slope of the linear plot $\ln(\beta/T_p^2)$ against $1/T_p$.

5.2.2 *Friedman method*

Combining Eqs. (11.1) and (11.2), we can obtain:

$$\frac{d\alpha}{dt} = A\exp\left(\frac{-E}{RT}\right)f(\alpha) \tag{11.27}$$

From the logarithms on both sides of Eq. (11.27), Friedman's equation can be calculated:

$$\ln\left(\frac{d\alpha}{dt}\right)_{\alpha,i} = \ln[f(\alpha)A_\alpha] - \frac{E_\alpha}{RT_{\alpha,i}} \tag{11.28}$$

For each, α, the value of E_α depends on the slope of the curve of $\ln(d\alpha/dt)_{\alpha,i}$ against $1/T_{\alpha,i}$. Each temperature program is index i, $T_{\alpha,i}$ is under the temperature program i, and the value of the temperature degree of conversion is α. In the case of a linear nonisothermal procedure, i determines the heating rate [17].

Yu et al. [21] used the Friedman method to calculate the average activation energies of two microalgae, *C. vulgaris* and *Nannochloropsis oculata*. Based on this method, the activation energy values are 120.52 kJ/mol and 107.67 kJ/mol. Zhao et al. [39] calculated the average activation energies of *C. vulgaris* and *Spirulina* by Friedman method and obtained the activation energies of 199.03 and 216.99 kJ/mol, respectively.

Compared with the differential method, the integral method is more common because it has a higher tolerance to experimental noise and it is easier to calculate activation energies. The reaction rate from the first-order derivative of TG data by the differential method is sensitively affected by noise, thereby reducing the accuracy of the results. However, the integral method also has an inevitable fallacy in its derivation. The error mainly occurs in the variable separation process from Eq. (11.27) into Eq. (11.7). In the mathematical process of separating variables, all variables of t should be arranged on the right, and all variables about α are arranged on the left, so as to facilitate the calculation of subsequent integrals. In Eq. (11.27), E and A on the right are still function about α, so it is not completely separated. Therefore, only when A and E are considered irrelevant to α, Eq. (11.27) can be implemented in the integral method.

5.3 Compensation effect and master plots

The estimation of activation energy E has been described above. Regarding the reaction mechanism function $f(\alpha)$ and preexponential factor A, the master plots method or compensation effect method are usually used for corresponding calculation.

5.3.1 Compensation effect

The dependence of preexponential factor A_α on α could not be determined from Eq. (11.28) because A_α and $f(\alpha)$ are lumped together as $\ln[A_\alpha f(\alpha)]$. However, isolation of A_α on α turns out to be possible by employing compensation effect [39]. From Eq. (11.28):

$$\ln\left(\frac{1}{f_j(\alpha)}\frac{\mathrm{d}\alpha}{\mathrm{d}t}\right) = \ln A_{\alpha,j} - \frac{E_j(\alpha)}{RT} \tag{11.29}$$

When selecting any model $f_j(\alpha)$ from Table 11.2, a pair of $\ln A_{\alpha,j}$ and E_j will be generated. The subscript j represents the selected reaction model in Table 11.2. E_j and $\ln A_{\alpha,j}$ will eventually fall on a compensation line, and the real E and $\ln A_\alpha$ will also arrive on this line. The slope a and intercept b of the compensation line can be obtained by linear fitting of these $\ln A_j$-E_j pairs:

$$\ln A_j = aE_j + b \tag{11.30}$$

Using compensation effect to calculate preexponential factor is generally divided into the following four steps: (1) Using isoconversional method to obtain E. (2) Single-heating-rate method is used to determine the data of $\ln A_j$ and E_j. (3) Fitting $\ln A_j$ and E_j. (4) The real A can be obtained by substituting E into Eq. (11.30).

The mechanism function $f(\alpha)$ can be obtained by substituting E_α, A_α, T_α, and $\mathrm{d}\alpha/\mathrm{d}T$ measured at experimental heating rate β into Eq. (11.6):

$$f(\alpha) = \beta\left(\frac{\mathrm{d}\alpha}{\mathrm{d}T}\right)_\alpha \left[A_\alpha \exp\left(\frac{-E_\alpha}{RT_\alpha}\right)\right]^{-1} \tag{11.31}$$

Zhao et al. [39] determined the real A_α of two microalgae *C. vulgaris* and *Spirulina* by compensation effect, and obtained a series of $\ln A_{\alpha,j}$ and E_j by substituting different $f_j(\alpha)$ models in Eq. (11.29), as shown in Fig. 11.1. Substituting the activation energy E_α into Eq. (11.30), the logarithmic form of A_α can be obtained from the compensation line (Fig. 11.1), as shown in Fig. 11.2.

5.3.2 Master plots

Another classical approach to estimate the reaction model is using the master plots method. In the master plots method, the best-fitting model can be selected for the single-step process with the expression of invariant $g(\alpha)$ [50]. The applicability of this method depends on the variability of E_α. The E_a value is constant relative to α. Taking the reference at point $\alpha = 0.5$, the following can be obtained based on Eq. (11.9):

$$g(0.5) = \left(\frac{AE}{\beta R}\right)p(u_{0.5}) \tag{11.32}$$

In the above formula, $u_{0.5} = E/RT_{(0.5)}$. Divide Eq. (11.9) and Eq. (11.32) to obtain:

$$\frac{g(\alpha)}{g(0.5)} = \frac{p(u)}{p(u_{0.5})} \tag{11.33}$$

Theoretical plots of $g(\alpha)/g(0.5)$ could be plotted according to $g(\alpha)$ functions (Table 11.2). Using Doyle approximation $p(u) = 0.00484\mathrm{e}^{-1.0516u}$, Eq. (11.33) shows that for a given α, when selecting a reasonable kinetic model, the theoretical value of $g(\alpha)/g(0.5)$ is consistent with the

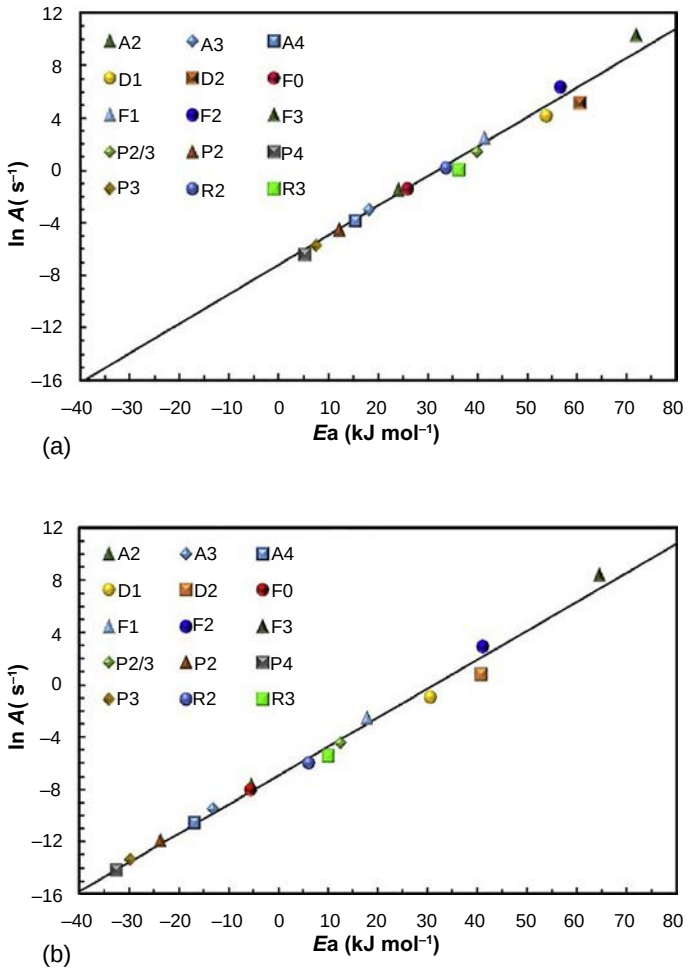

FIG. 11.1 Arrhenius parameter compensation line: CV (A) and SP (B). *From M. Zhao, A. Raheem, Z.M. Memon, A.K. Vuppaladadiyam, G. Ji, Iso-conversional kinetics of low-lipid micro-algae gasification by air, J. Clean. Prod. 207 (2019) 618–629.*

experimental value of $p(u)/p(u_{0.5})$ [51]. Therefore, the model of which $g(\alpha)/g(0.5)$ shows the closest fit to $p(u)/p(u_{0.5})$ should be the most likely reaction model. The integral master plots method can be used to determine the kinetic model of microalgae decomposition reaction.

Zou et al. [52] used the master plots method in fitting the pyrolysis model of *Dunaliella tertiolecta*. Fig. 11.3 shows the fitting analysis results. The master plots method was used to analyze the reaction mechanism, and it was found that *Fn* model was the most reasonable reaction mechanism.

6 Model-fitting method

The isoconversional method is commonly used to estimate the kinetic parameters without any hypothesis of a reaction model. However, when the classification of the reaction model has been known based on references, using the model-fitting method is also an effective approach to study the reaction kinetics.

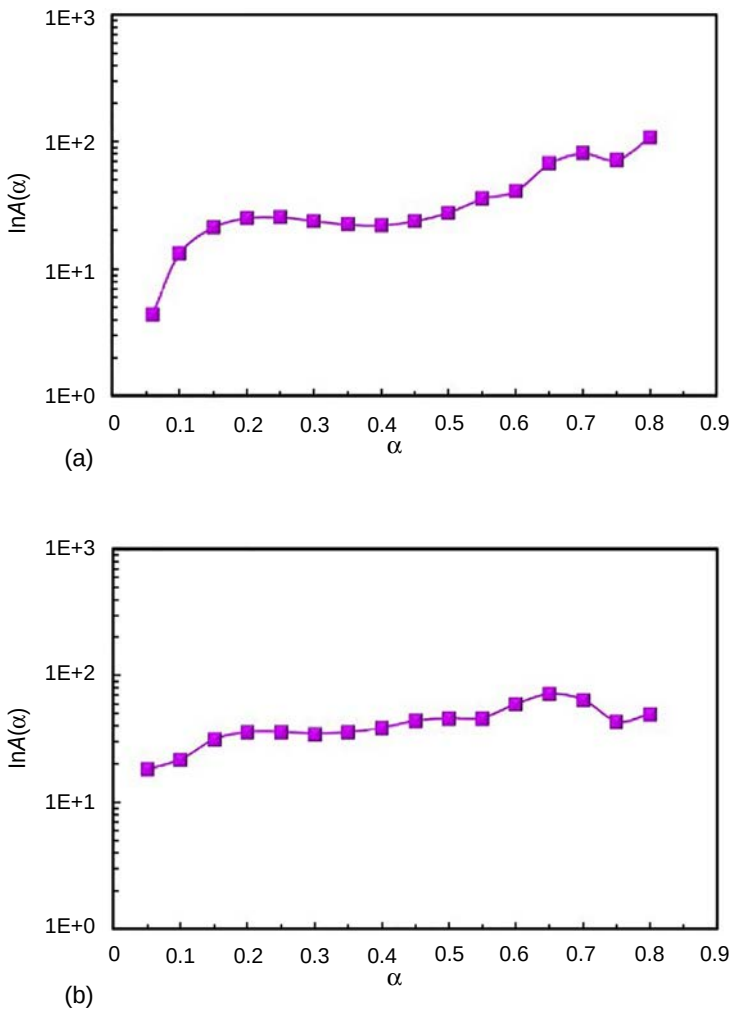

FIG. 11.2 Calculated logarithm of preexponential factors CV (A) and SP (B). *From M. Zhao, A. Raheem, Z.M. Memon, A.K. Vuppaladadiyam, G. Ji, Iso-conversional kinetics of low-lipid micro-algae gasification by air, J. Clean. Prod. 207 (2019) 618–629.*

6.1 Single reaction model

The single reaction model assumes that all components in microalgae have consistent thermal reactivity. In other words, the pyrolysis process of algae can be described by using a single reaction of biomass [21]:

$$\textbf{Algae} \xrightarrow{k} \text{Char} + \text{Volatile} \tag{11.34}$$

The most common $f(\alpha)$ are summarized in Table 11.2. Among them, reaction-order model $f(\alpha)$ is relatively common, and the specific form is:

$$f(\alpha) = (1-\alpha)^n \tag{11.35}$$

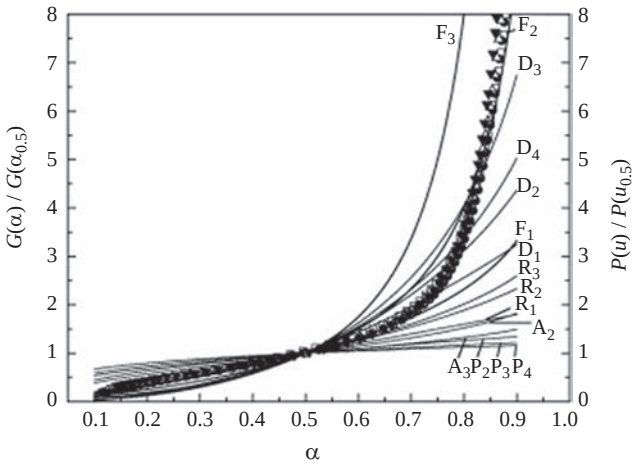

FIG. 11.3 According to the experimental data obtained at different heating rates, the experimental main diagrams of $p(u)/p(u_{0.5})$ against a, the theoretical main diagrams of $g(\alpha)/g(0.5)$ against α for various reaction models (Table 11.2). *From S. Zou, Y. Wu, M. Yang, C. Li, J. Tong, Pyrolysis characteristics and kinetics of the marine microalgae* Dunaliella tertiolecta *using thermogravimetric analyzer, Bioresour. Technol. 101 (1) (2010) 359–365.*

In the above equation, n represents the reaction order.
Eq. (11.27), combined with the reaction-order model leads to:

$$\frac{d\alpha}{dt} = A\exp\left(\frac{-E}{RT}\right)(1-\alpha)^n \tag{11.36}$$

Using the average activation energy obtained by the differential and integral isoconversional methods, the reaction order is estimated by optimizing Eq. (11.36). By adjusting the value of the reaction order, the best fitting reaction order can be found when the predicted value is the closest to the experimental value. Although the model is very convenient to use, the fitting quality is usually poor.

6.2 Independent parallel reaction models

Eq. (11.37) is the basic expression of the independent parallel reaction (IPR) model. This model is mostly used to describe the pyrolysis of microalgae by kinetic fitting model. It originated in analyzing the pyrolysis process of lignin, cellulose, and hemicellulose in lignocellulosic biomass [26]. Different microalgae fractions will lead to different reaction quantities in the model. At the same time, the pyrolysis factors of lipids, proteins, carbohydrates, and other microalgae secondary components should be analyzed. Algae pyrolysis is described by parallel reactions of all pseudo-components of biomass:

$$\textbf{Algae}\begin{cases} \text{Comp1} \xrightarrow{k1} \text{Char1} + \text{Volatile1} \\ \text{Comp2} \xrightarrow{k2} \text{Char2} + \text{Volatile2} \\ \vdots \\ \text{Comp}N \xrightarrow{kN} \text{Char}N + \text{Volatile}N \end{cases} \tag{11.37}$$

Reaction-order model is generally used in independent parallel reactions. This model is a common mechanism of the solid reaction. Substituting Eq. (11.35) into Eq. (11.6) gets [53]:

$$\frac{d\alpha}{dT}=\frac{A}{\beta}\exp\left(\frac{-E}{RT}\right)(1-\alpha)^{n} \tag{11.38}$$

The components in microalgae are expressed as follows:

$$\frac{d\alpha}{dT}=\sum_{I}c_I\frac{d\alpha_I}{dT},\ I=1,2,\ldots,N \tag{11.39}$$

$$\frac{d\alpha_I}{dT}=\frac{A_I}{\beta}\exp\left(\frac{-E_{\alpha_I}}{RT}\right)(1-\alpha_I)^{n_I} \tag{11.40}$$

In the above equation, N represents the total number of components; c_I represents the mass fraction of each composition; $d\alpha/dT$ represents the total conversion (K^{-1});

In the effectiveness analysis of ($E_{\alpha I}$, A_I, and c_I), the experimental curves and prediction curves are compared and analyzed. Using the nonlinear least square method, the objective function (OBJ) and fitting quality (Fit) equations are obtained as follows:

$$OBJ=\sum_{I=1}^{N}\left[\left(\frac{d\alpha}{dT}\right)_{\exp,I}-\left(\frac{d\alpha}{dT}\right)_{\mathrm{cal},I}\right]^2 \tag{11.41}$$

$$Fit(\%)=\left(1-\frac{\sqrt{\frac{OBJ}{N}}}{\left[\left(\frac{d\alpha}{dT}\right)_{\exp}\right]_{\max}}\right)\cdot 100\% \tag{11.42}$$

In the above equation, $\left(\frac{d\alpha}{dT}\right)_{\mathrm{cal},I}$ and $\left(\frac{d\alpha}{dT}\right)_{\exp,I}$ are calculated and experimental conversion rates, respectively.

Yu et al. [21] predicted the activation energies of two microalgae, *C. vulgaris* and *N. oculata* using the four-pseudo-component model, and the activation energies were 106.15 and 97.15 kJ/mol, respectively. Andrade et al. [37] calculated the kinetic parameters of the microalgae *Chlamydomonas reinhardtii* using the IPR model. The activation energies of lipids and pigments, hemicellulose, cellulose, proteins and completely decomposed lipids were 93–99 kJ/mol, 116–119 kJ/mol, 116–117 kJ/mol, 123–125 kJ/mol, and 130–133 kJ/mol, respectively. Chen et al. [53] used an IPR model to study the pyrolysis kinetics of *Chlamydomonas* sp. JSC4, *Nannochloropsis oceanica* CY2 and *C. vulgaris* ESP-31. The activation energies of lipids, proteins and carbohydrates were 40.21–59.23 kJ/mol, 142.61–188.35 kJ/mol, and 52.28–53.30 kJ/mol, respectively.

For the estimation of kinetic parameters, due to the diversity of biomass components and the limited role of the isoconversional method, the independent parallel reaction method is encouraged to be used for kinetic analysis [54]. The application of independent parallel reaction has also achieved a good fitting effect [21,37,53–55]. Therefore, the independent parallel reaction method, and the isoconversional method can be complementary to each other.

7 DAEM

Distributed activation energy model (DAEM), mainly used to describe the decomposition process of liquid fuel and solid fuel, is a commonly used multireaction model. It is assumed that the decomposition process is based on a large number of parallel and independent first-order reactions, and the different activation energies can characterize the different bond strengths of the materials [56].

7.1 *n*th DAEM

The basic assumption of the application of DAEM in biomass pyrolysis and gasification is that the biomass could be classified into a number of groups based on the difference in the activation energy: $s = 1, \ldots, K$. V_s is the release mass fraction of group sth. The decomposition of sth group is described by the pseudo-nth-order rate equation. Based on Arrhenius's law, the corresponding decomposition rate formula can be obtained as [57]:

$$\frac{\mathrm{d}(V_s/V_s^*)}{\mathrm{d}t} = k_s\left(\frac{V_s^* - V_s}{V_s^*}\right)^n = A_s \exp\left(\frac{-E_s}{RT}\right)\left(\frac{V_s^* - V_s}{V_s^*}\right)^n \tag{11.43}$$

In the above equation, n represents the reaction order; V_s^* represents the total released mass fraction for the sth group.

Convert Eq. (11.43) into integral form as:

$$\frac{V_s}{V_s^*} = 1 - \left[1 - (1-n)\int_0^t A_s \exp\left(\frac{-E_s}{RT}\right)\mathrm{d}t\right]^{1/(1-n)} \quad n \neq 1 \tag{11.44}$$

The proportion of the chemical groups of which the activation energies are between E and $E + \Delta E$ is $\int_E^{E+\Delta E} f(E)\mathrm{d}E$. At a given time t, the mass fraction of potential volatile substances is:

$$\mathrm{d}V^* = V^* f(E)\mathrm{d}E \tag{11.45}$$

Then, V_s are replaced by $\mathrm{d}V$, and V_s^* are replaced by $\mathrm{d}V^*$. Here is to turn all discrete expression into continuous differential forms. Then Eq. (11.44) becomes:

$$\mathrm{d}V = V^*\left\{1 - \left[1 - (1-n)\int_0^t A \exp\left(\frac{-E}{RT}\right)\mathrm{d}t\right]^{1/(1-n)}\right\} f(E)\mathrm{d}E \quad n \neq 1 \tag{11.46}$$

After rearrangement, Eq. (11.47) was achieved:

$$\alpha = \frac{V}{V^*} = 1 - \int_0^\infty \left[1 - (1-n)\int_0^t A \exp\left(\frac{-E}{RT}\right)\mathrm{d}t\right]^{1/(1-n)} f(E)\mathrm{d}E \quad n \neq 1 \tag{11.47}$$

In the above equation, α represents the degree of conversion.

With a linear heating rate β Eq. (11.47) can be expressed as Eq. (11.48):

$$\alpha = 1 - \int_0^\infty \left[1 - (1-n)\int_{T_0}^T \frac{A}{\beta} \exp\left(\frac{-E}{RT}\right)\mathrm{d}T\right]^{1/(1-n)} f(E)\mathrm{d}E \quad n \neq 1 \tag{11.48}$$

Using the least square method, the kinetic parameters of DAEM can be estimated [58]:

$$O.F = \min \sum_{i=1}^{N_d} (x_c - x_e)^2 \tag{11.49}$$

where x_c and x_e represent x in calculation and x in the experiment, and N_d represents the total number of experimental data points used for fitting.

Kirtania and Bhattacharya [59] used nth DAEM to describe the slow weight loss kinetics of *Chlorococcum humicola* at the end of pyrolysis, and the activation energy distribution was shown in Fig. 11.4 which demonstrated that the activation energy range covered 140–240 kJ/mol, and the distribution peak located at 190 kJ/mol.

7.2 Miura-Maki DAEM

When $n = 1$ in Eq. (11.43), Eq. (11.47) becomes [56,60–62]:

$$1 - \alpha = \int_0^{\infty} \exp\left(-A \int_0^t e^{-E/RT} dt\right) f(E) \mathrm{d}E \tag{11.50}$$

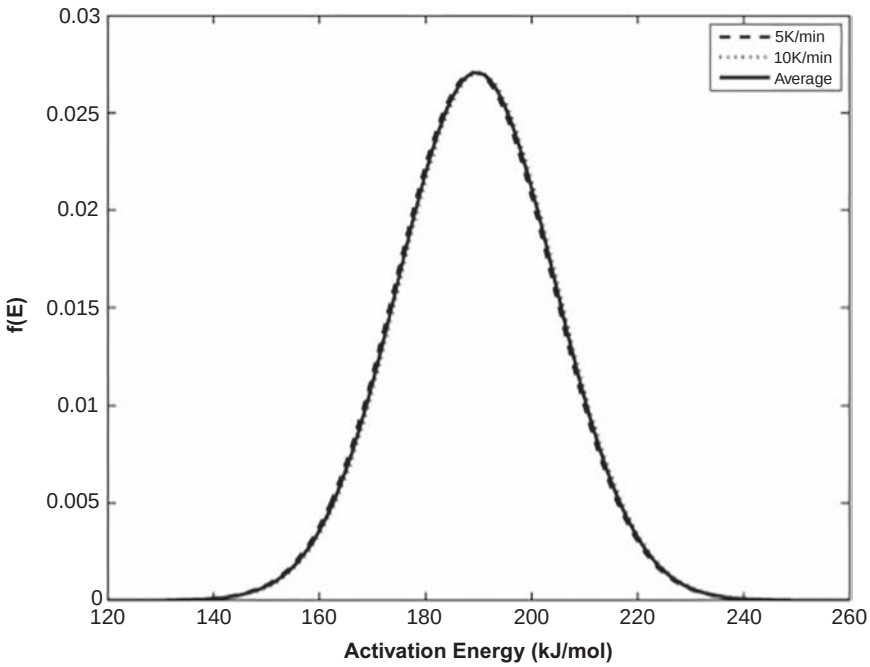

FIG. 11.4 Activation energy distribution of *C. humicola* at various heating rates during pyrolysis. *From K. Kirtania, S. Bhattacharya, Application of the distributed activation energy model to the kinetic study of pyrolysis of the freshwater algae* Chlorococcum humicola, *Bioresour. Technol. 107 (2012) 476–481.*

When temperature increases at a constant rate β Eq. (11.50) is rearranged as:

$$1-\alpha=\int_0^{\infty}\exp\left(-\frac{A}{\beta}\int_0^{T}e^{-E/RT}dT\right)f(E)\mathrm{d}E \tag{11.51}$$

A function Φ is defined to name the exponential term on the right side of Eq. (11.51):

$$\Phi=\exp\left(-\frac{A}{\beta}\int_0^{T}e^{-E/RT}\mathrm{d}T\right) \tag{11.52}$$

In fact, in order to reduce the complexity of the model, a variety of simplification methods are proposed [43].

The following equation is treated by using the approximate values of Redfern and Coats [61]:

$$\Phi=\exp\left(-\frac{A}{\beta}\int_0^{T}e^{-E/RT}\mathrm{d}T\right)\approx\exp\left(-\frac{ART^2}{\beta E}e^{-E/RT}\right) \tag{11.53}$$

The Φ function expression can be approximated as a step function at $E=E_s$. Considering the normalization criterion of activation energy probability density function $f(E)$, the following expression of pyrolysis conversion rate α is obtained:

$$\alpha=1-\int_{E_s}^{\infty}f(E)\mathrm{d}E=\int_0^{E_s}f(E)\mathrm{d}E \tag{11.54}$$

The value change of step function Φ should be established, that is, Φ value could be switched from 0 to 1 from $E<E_s$ to $E>E_s$. The critical value E_s is the function of T, which means that the temperature T is reflected in the integral limit E_s. At this time, the Arrhenius equation of simplified DAEM can be obtained in this way:

$$\ln\left(\frac{\beta}{T^2}\right)=\ln\left(\frac{AR}{E_s}\right)+0.6075-\frac{E_s}{R}\frac{1}{T} \tag{11.55}$$

Ji et al. [5] analyzed the gasification stage of *Spirulina* and *C. vulgaris* based on primary DAEM. Fig. 11.5 shows the distribution of activation energy for these two types of microalgae.

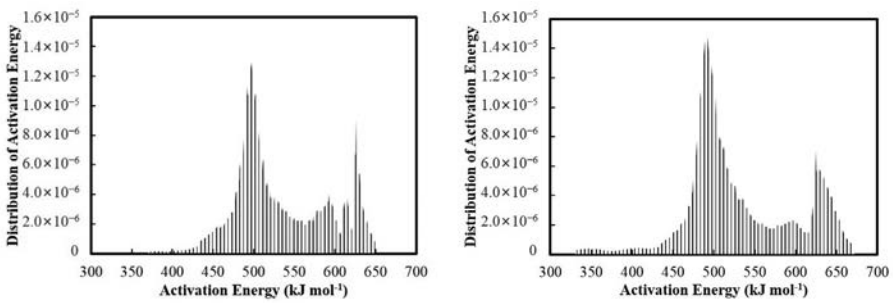

FIG. 11.5 Activation energy distribution of gasification *Chlorella* (A) and *Spirulina* (B) [5].

All of them have a large number of components when the activation energy is 500 kJ/mol. There are many inactive components in *Spirulina* and it requires a higher temperature than 500°C to convert thses components. While *C. vulgaris* gasified completely before 500°C.

7.3 Avrami-Erofeev DAEM

The traditional DAEM model assumes that the algae decomposition follows a reaction-order model. However, not all algal biomass is decomposed by this reaction order mechanism. It is possible that some algal biomass follows other solid reaction models during decomposition. It was reported by Raheem et al. that *C. vulgaris* follows nucleation and nuclei growth when it is gasified by air [63]. The Avrami-Erofeev model can replace the *n*th-order model to describe the nucleation and growth stages [64]:

$$\frac{\mathrm{d}(V_s/V_s^*)}{\mathrm{d}t}=A_s\exp\left(\frac{-E_s}{RT}\right)m\left(\frac{V_s^*-V_s}{V_s^*}\right)\left[-\ln\left(\frac{V_s^*-V_s}{V_s^*}\right)\right]^{m-1/m} \tag{11.56}$$

The volatile release $\mathrm{d}V$ can be obtained by integrating Eq. (11.56), as shown in Eq. (11.57):

$$\mathrm{d}V=V^*\left\{1-\exp\left\{-\left[\int_0^t A\exp\left(\frac{-E}{RT}\right)\mathrm{d}t\right]^m\right\}\right\}f(E)\mathrm{d}E \tag{11.57}$$

After integration, Eq. (11.58) is obtained:

$$\alpha=\frac{V}{V^*}=1-\int_0^\infty\exp\left\{-\left[\int_0^t A\exp\left(\frac{-E}{RT}\right)\mathrm{d}t\right]^m\right\}f(E)\mathrm{d}E \tag{11.58}$$

Under linear heating rate β, Eq. (11.59) can be obtained which is the final derived equation of DAEM based on Avrami-Erofeev model.

$$\alpha=1-\int_0^\infty\exp\left\{-\left[\int_{T_0}^T\frac{A}{\beta}\exp\left(\frac{-E}{RT}\right)\mathrm{d}T\right]^m\right\}f(E)\mathrm{d}E \tag{11.59}$$

It is proven that the DAEM well describes the complex chemical reactions. If a series of parallel reactions have different activation energies, the DAEM has a good ability to determine the kinetic parameters. Compared with other models, the DAEM model has a better fitting effect. DAEM also has some problems that affect its application. Firstly, the use of DAEM must assume an $f(\alpha)$ expression, but the actual process does not necessarily follow this $f(\alpha)$ model. Second, DAEM ignores the compensation effect between kinetic triplets; that is, a set of experimental results can be best fit by a variety of kinetic parameters.

8 Conclusion and future outlook

This chapter summarizes the fundamental methods of microalgae pyrolysis and gasification kinetics, focusing on several common methods such as the DAEM method, model-fitting method, and isoconversional method. Besides, the basic principle and formula derivation are

explained in detail. In addition to elaborating the calculation of activation energy E using the above three methods, two new calculation methods for the mechanism function $f(\alpha)$ and preexponential factor A such as master plots and the compensation effect method are introduced. In fact, in the pyrolysis kinetics of microalgae, the independent parallel reaction model has received increasing attention and has been widely used, demonstrating that it has significant advantages in characterizing the pyrolysis kinetics of various components in microalgae and has a similar fitting effect to DAEM. However, the model-fitting method is more complex than the isoconversional method, so it was recommended to combine it with various methods to further obtain more accurate kinetic parameters, so as to verify and interpret the experimental observations.

Besides understanding the reaction mechanism, another objective of studying algae conversion kinetics is to aid the design and operation of reactors. However, a main deficiency of current kinetic studies is that the heating rate of samples in lab research is far from the real heating rate in practical application. The heating rate in lab TGA test is limited to only 1–2 K/s, but the feedstocks in reactors could be heated at a rate of over 100 K/s. Therefore, it is of great interest to investigate the thermal kinetics of algal biomass at extremly high heating rates in future studies. Kinetic data obtained from more realistic heating rates will be much more reasonable and valuable for guiding the application of pyrolysis and gasification technology.

Acknowledgment

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CHAPTER

12

Microalgae: The challenges from harvest to the thermal gasification

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OUTLINE

1 Introduction

The incredible biodiversity of microalgae offers numerous applications, including technologies related to biomass conversion. In this respect, microalgae are important since they absorb solar energy and CO_2 from the atmosphere to grow [1]. Therefore, such biomasses may be a by-product of the wastewater treatment process because they can meet ideal conditions to grow naturally [2]. Microalgae can produce energy-rich substances such as lipids for biodiesel production, calorific gases such as H_2, CH_4, and CO by water photolysis, biological, or

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thermochemical gasification. Especially for thermochemical routes, these types of biomass can partially meet the need for liquid and gaseous fuels for integration with existing power generation infrastructure [3]. However, microalgae harvest for fuel production processes involves significant constraints and still challenges scientists and engineers [4].

The harmonious coexistence of aerobic bacteria and microalgae—which are indigenous to the type of wastewater—favors the increase in microalgae-bacteria biomass productivity. Hence, some scenarios have been suggested in the last few years [5]. Among these scenarios, two potentials arise to justify the ongoing studies in the field. One is growing microalgae using water and nutrients as culture media; the other, the start point to be discussed in this chapter, is recovering microalgae from the high-rate open ponds of wastewater treatment plants (WWTP). It is essential to note that WWTP's biomass comprises bacteria and different indigenous microalgae strains. From domestic wastewater produced in southeastern Brazil, diverse indigenous microalgae strains are reported in the literature, such as *Tetradesmus* sp., *Chlorella* sp., *Chlorococcum* sp., and *Scenedesmus* sp. [2,5,6]. Wastewater treatment facilities are naturally colonized by these species or microalgae belonging to the Bacillariophyta and Cyanobacteria groups [7].

Toward and challenging the feasibility of the thermal conversion process, harvesting and drying are necessary subprocesses to make biomass production a reliable feedstock for gasification. Remember that nearly 7% of the total energy produced in the world is consumed in WWTPs [1,8]. Furthermore, the most consumed energy is for electrical equipment (i.e., pumps and compressors) [9]. This energy consumption represents 5%–30% of the operating costs and is globally the most significant proportion of WWTP's costs [10,11].

Those plant operation costs justify why the microgeneration concept is often discussed in energy cost reduction for WWTP. This concept relates to the size and configuration of small to medium energy systems (such as gasification) [12,13], which use renewable energy sources such as microalgae biomass [14]). Also, it can operate independently of grid-supplied power [15]. Microgeneration offers higher environmental and energy efficiency by quickly converting all biomass fractions (lipid, protein, and carbohydrates) into useful products. Besides, it achieves low CO2 emissions and relatively higher H2 production—a calorific gas promised as an excellent future substitute for conventional fuels [16,17].

Nevertheless, the coagulants used for biomass harvesting can interfere in the thermal gasification process as they can affect the gasification reactions and other chemical and biological processes [4,18]. Soares et al. [4] summarized the typical chemical elements comprising the ash of postcombustion microalgae from WWTP, Table 12.1.

Forcibly, some catalytic effects may result in posterior processes given the abundant number of chemical elements, some from contaminants and others on purpose incorporated (such as coagulants) in algal biomass. The yield in biofuel production from flocculated microalgae using ferric sulfate and centrifuged microalgae differs [19], as differs the yield of bioproducts produced from microalgae harvested using aluminum sulfate and cationic starch [20]. Another known case is that aluminum chlorides inhibit transesterification reactions, negatively affecting biodiesel production [21]. In the same sense, aluminum sulfate and ferric chloride affect the yield of biogas production in anaerobic digestion [20]. Despite the mentioned drawbacks, the incorporated coagulants can also act positively on thermochemical conversion, acting as a catalyst, catalyst promoter, catalyst support, or as sorbents in the product cleanup [12,22–24]. This chapter discusses characterization based on the drawbacks and advantages of coagulants in gasification efficiency.

TABLE 12.1 Ash composition of post combustion microalgae.

	No coagulant	Polyquaternium polymer	Aliphatic amines polymer	Tannin-based polymer	Ferrous aluminum sulfate	Aluminum polychloride	Ferric chloride	Hydrated lime
Al	–	–	–	–	40.25	49.81	–	–
Ca	28.02	29.07	32.29	16.30	8.78	12.71	1.69	95.86
K	25.22	23.33	17.88	13.01	4.81	7.58	2.44	0.20
P	20.74	21.41	19.01	17.89	19.12	19.92	6.68	–
Cl	9.02	–	14.56	–	–	–	–	–
Fe	7.35	11.14	7.46	17.04	19.96	3.60	87.46	0.53
Si	5.54	9.21	4.72	29.08	3.58	4.52	–	2.26
Ti	1.26	1.84	0.97	2.08	0.39	0.32	–	–
S	0.93	2.22	1.52	3.08	2.45	1.01	1.58	0.52
Zn	0.21	0.24	0.23	0.59	0.31	0.17	–	0.02
Mn	0.46	1.06	0.74	0.38	0.13	0.18	0.12	0.09
Zr	0.72	0.02	0.01	0.02	–	–	–	–
Sr	0.19	0.29	0.31	0.08	0.08	0.08	0.02	0.38
Cr	0.13	–	–	0.08	0.09	0.05	–	–
Cu	0.09	0.08	0.09	0.21	0.03	0.02	0.01	0.02
Br	0.04	0.02	0.05	–	–	–	–	–
Rb	0.03	0.03	0.03	0.03	–	0.01	–	–
Ag	0.01	0.03	0.02	–	–	0.02	–	–
Ni	0.02	0.02	–	0.08	–	–	–	–
V	0.01	–	0.01	0.04	0.03	–	–	–
Mo	–	–	–	0.01	–	–	–	–
Sc	–	–	–	–	–	–	–	0.12
Co	–	–	–	–	–	–	–	0.01
Pd	–	–	–	–	–	0.01	–	–
Ba	–	–	0.10	–	–	–	–	–

From R.B. Soares, M.F. Martins, R. F. Gonçalves, Thermochemical conversion of wastewater microalgae: the effects of coagulants used in the harvest process, Algal Res. 47 (2020) 101864.

2 Microalgae thermochemical characteristics

2.1 Wastewater microalgae harvesting

To emphasize the impact of WWTP's biomass characteristics on the thermal gasification process, the microalgae characterized in this section (different microalgae species and bacteria under coagulant presence) were produced in high-rate algae ponds of 13.7 m^3 in volume, with 10 m long channels (two) per 2.4 m in width, resulting in 22.8 m^2 of superficial area. The pond was fed with the effluent obtained after wastewater treatment into an up-flow anaerobic sludge blanket (UASB) reactor (1.0 m in diameter × 4.8 m usable height, 3.8 m^3 net volume, and a 0.14 L/s average flow rate). Harvesting was carried out in a continuum coagulation-flocculation system using 50 mg/L of a tannin-based polymer. Further details can be seen in [4,18].

The biomass was dried at 60°C in an outdoor furnace to eliminate odors, sanitized at 105°C in an indoor furnace for at least 12 h, and then characterized. Complete moisture removal was done to avoid degradation of the biomass during the storage period until the gasification experiments were carried out. Fig. 12.1 shows the wastewater microalgae biomass before and after drying. The biomass naturally fragments during the drying process, resulting in solid pieces of firm consistency and a dark color, similar to coal. Most solid pieces (52.5%) were particles between 4.7 and 9.5 mm in size, Fig. 12.2; therefore, this size range was used in the gasification process without additional adjustments.

2.2 Wastewater microalgae characterization

The microalgae that produced main energetic characteristics were evaluated using the techniques, devices, and methods summarized in Table 12.2. The values obtained per technique are presented in Table 12.3. Also, Fig. 12.3 shows the thermal degradation behavior of microalgae recovered using a tannin-based polymer. The thermochemical behavior could be interpreted by taking advantage of the perspective brought by the microalgae's characterization.

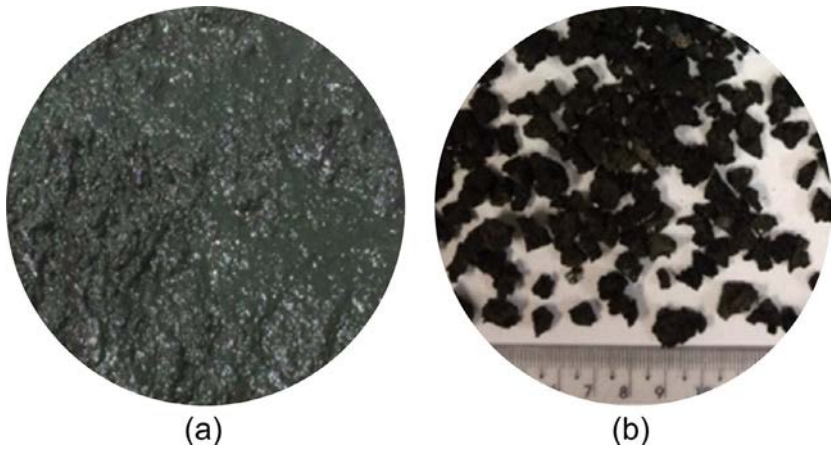

FIG. 12.1 Microalgae biomass from WWTP: aspect before (A) and after (B) drying.

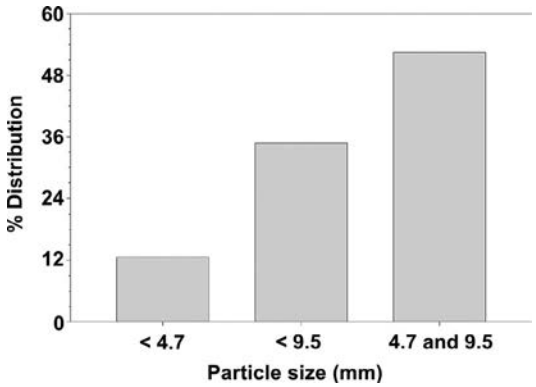

FIG. 12.2 Primary particle distribution selection for gasification: 4.7 mm < pd < 9.5 mm.

TABLE 12.2 Summary of characterization methods.

Technique	Manufacturer	Details
Higher heating value (HHV)	C2000, IKA-Werke	0.5 g of sample
X-ray fluorescence spectrometer	EDX720, SHIMADZU	Rhodium (Rh) X-ray tube and Si(Li) detector operating at 15–50 kV and 1000 mA
Proximate analysis	TGA701, LECO	ASTM D7582-15
Ultimate analysis	PE 2400 series II, PerkinElmer	ASTM D5373-*08*
Thermogravimetric analyses	Q600, TA INSTRUMENTS	Heating rate of 10°C/min to a temperature of 900°C, with a nitrogen flow of 50 mL/min, and a 25 mg sample

In terms of calorific value, the characterization revealed that the microalgae biomass from WWTP is equivalent to Pinus wood biomass, with an HHV of around 18 MJ/kg. The microalgae's calorific value can be improved by improving the harvesting efficiency. For instance, the high silica content indicates dust and sand particles incorporated during the harvesting stage, resulting in 10 wt% of ash, although the fixed carbon and volatile matter hover around 25 and 59.3 wt%, respectively. Other elements found in the chemical analysis were P, Fe, Ca, and K, among other traces.

The first evidence is that the degradation profile in Fig. 12.3 was comparable to those reported in the literature for *Micractinium*, *Scenedesmus*, and *Chlorella vulgaris* microalgae [25–27]. Two clear peaks in the DTG curve can be observed at a temperature below 500°C. The first is associated with moisture removal, accounting for a weight loss of approximately 14 wt%. The second peak is, in effect, two overlapped peaks, from 200°C to 500°C, attributed to the pyrolysis of lipids, carbohydrates, and proteins in the microalgae, resulting in a mass loss of around 61 wt%. The dehydration stage is also observed in the DTA curve, whose endothermic character is proved by the negative heat flux. The DTA curve from 200°C to 500°C

TABLE 12.3 Microalgae thermochemical and chemical characteristics.

	Value (wt%)
HHV (MJ/kg)	18.6 ± 0.7
Bulk density (kg/m^3)	1200 ± 62
Proximate analysis	
Moisture	5.4
Volatile matter	59.3
Fixed carbon	24.7
Ash	10.6
Ultimate analysis	
C	47.7 ± 2.5
H	5.9 ± 0.5
N	6.3 ± 0.3
O	29.5 ± 3.3
Ash analysis	
Si	29.08
P	17.89
Fe	17.04
Ca	16.30
K	13.01
Others	6.68

Data are shown as mean (n = 3) ± standard deviation.

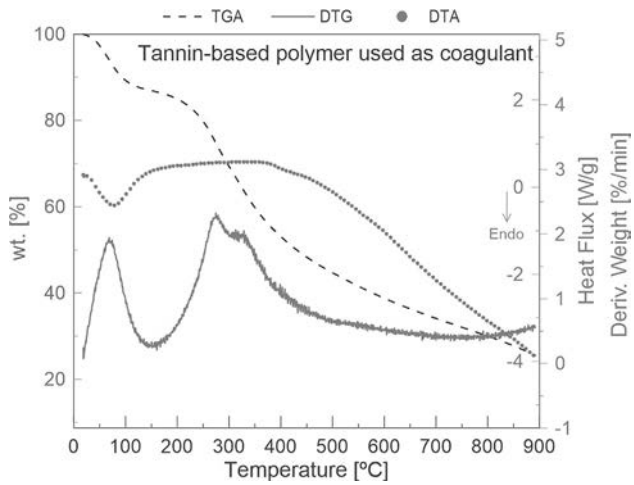

FIG. 12.3 Thermochemical behavior of microalgae biomass from WWTP.

exhibits the exothermic characteristics of microalgae biomass pyrolysis. As the sample loses mass, the heat is released accordingly and spreads along with the temperature range. According to Renan et al. [4] data, depending on the coagulant type used for the harvesting process, the pyrolysis's exothermicity can be more evident but never endothermic.

In Fig. 12.4, the comparison of three other coagulants with the tannin-based polymer coagulant was established. The results suggest the catalytic effect of coagulants increases or reduces the conversion rate by observing the mass percentage per minute. The use of the coagulant polyquaternium polymer increased the conversion rate by more than two times compared to the tannin-based coagulant rate. On the other hand, the aliphatic amine polymers reduced the reaction rate in the range of 200–400°C. However, they have stimulated a remarkable conversion rate in the range of 600–750°C.

3 Gasification of microalgae from WWTP

3.1 Gasification evaluation index

The gasifier used in this research was an All Power Labs Power Pellet GEK 20 kW downdraft biomass gasifier designed and manufactured in California/USA by All Power Labs Inc. [28]. The full description and images of the device can be accessed at the manufacturer's website [29]. Further details about the used protocol can be found in [30].

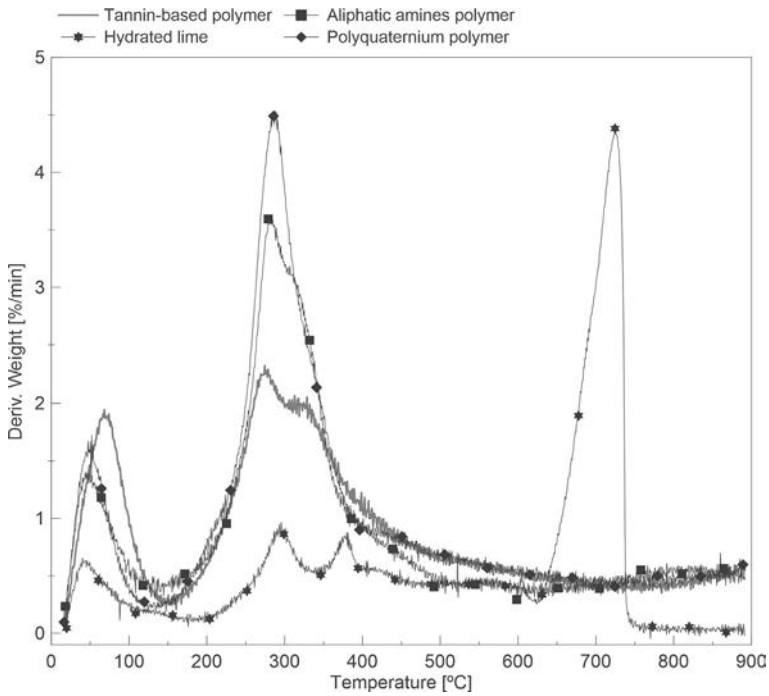

FIG. 12.4 DTG for microalgae recovered using different types of coagulants.

Batch gasification experiments were conducted. The runs were performed once with distinct equivalent ratios (ER = 0.14, 0.23, 0.28, and 0.47), Eq. (12.1), to define the performance curve for the process by plotting ER as a function of the syngas HHV. After defining the performance curve, the runs were repeated in the best ER region to confirm the results.

$$ER = (F_{air} * T_{run})/M_{air}, \tag{12.1}$$

where F_{air} = flow rate of air supply, T_{run} = runtime, and M_{air} = mass input of air stoichiometric.

The syngas volume produced was calculated by Eq. (12.2),

$$V_{syngas} * xN_2 = V_{air} * 0.79, \tag{12.2}$$

where V_{syngas} = syngas volume, xN_2 = nitrogen volume fraction in syngas, and V_{air} = air volume. Eq. (12.2) is based on the premise that the nitrogen in the air input was inert and had the same nitrogen yield as in the syngas [12]. Although this consideration may not be entirely accurate, the number of moles would be minimal and would represent less than 6% of the total N_2 fed in the process.

Finally, as suggested in [12,31], the performance indicator of the gasifier can be determined by the cold gas efficiency (CGE), Eq. (12.3):

$$CGE = \left(V_{syngas} * HHV_{syngas}\right)/\left(M_{bio.dry} * HHV_{bio.dry}\right), \tag{12.3}$$

where V_{syngas} = the syngas volume, HHV_{syngas} = the syngas HHV, $M_{bio.dry}$ = the mass of dry wastewater microalgae biomass, and $HHV_{bio.dry}$ = the dry wastewater microalgae biomass HHV.

3.2 Syngas characteristics

The temperature range for gasification was 873–949°C, typical for large-scale gasification projects described in the literature [32,33]. Temperatures below and above 900°C were observed for ER less than 0.23 and ER equal to or greater than 0.23. Still, it was not possible to observe a linear correlation between ER and temperature. It is noteworthy that the average bed temperature can be decreased when there is a higher amount of reagent fed at room temperature; this may justify the relatively low temperatures found at the highest ER. The experimental results at a higher temperature (949°C) produced the best results: high CO and H_2 content, consequently higher syngas HHV and CGE, see Fig. 12.5. Asadullah [34] mentioned that high-temperature gasification leads to a desired high CO and H_2 while reducing the tar content. The increase in H_2 production is due to the tar thermal cracking reaction and promoting endothermic reactions. Therefore, the results suggest that the hydrocarbon's endothermic reforming reactions were improved with the increasing temperature.

Also, Fig. 12.5 shows the CGE and syngas production rates at different ERs. While CGE presented a maximum point, syngas production increased linearly as the ER rose. The efficiency decreased for ERs above 0.24, despite the higher syngas volume due to high nitrogen content, and consequently lower HHV. The CGE varied from 31.9% to 87.0% due to changes in the syngas composition for different ER. Usually, the literature reports CGE ranging from 30% to 60% for downdraft gasifiers [31,35]. No value below 30% was shown here, yet

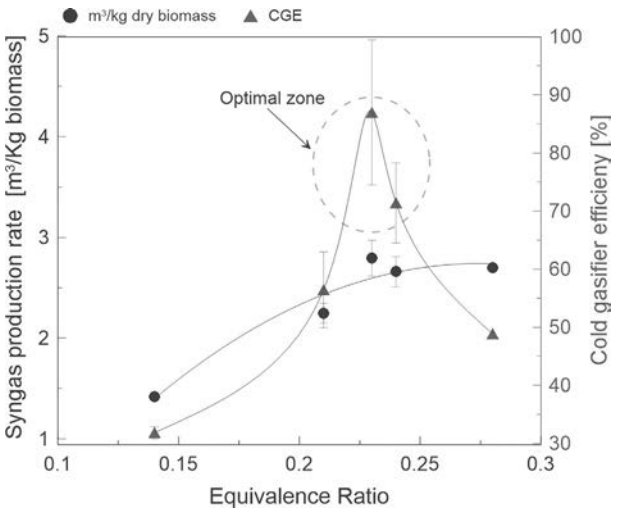

FIG. 12.5 Cold gasifier efficiency and syngas production rate versus the equivalence ratio. Error bars represent mean ($n = 3$) ± standard deviation.

efficiency of over 60% was achieved with ER of 0.23 and 0.24. Such results may indicate that the gasification of WWTP microalgae can deliver high efficiency once the ER is adjusted correctly. The higher syngas HHV and lower tar concentration in the syngas are of principal concern for achieving high efficiency. Therefore, ER optimization is still necessary [30,31].

3.3 Gasification challenges

The moisture content of the biomass was adjusted before each run, adding water to 30 wt%, the maximum value recommended in the gasifier's specifications. Gasifying biomass with the maximum moisture supported in the gasifier means less energy consumption in the drying process during feedstock conditioning. Particle size control is another challenge in gasifying microalgal biomass. As seen in Fig. 12.2, just 52% of the particles obtained, naturally, can be considered consistently sized feedstocks after the drying process. The remaining 48% can be pelletized to obtain a homogenized and uniform feedstock size. Although pelletizing can improve process controllability—a particular advantage for waste—it requires drying, grinding, and pressing the material, which means additional energy requirements other than a priori processes such as harvesting and drying.

Wastewater microalgae contain metals that could play a catalytic role in the gasification process, increasing or decreasing conversion, as seen in thermogravimetric studies, Fig. 12.4. The use of catalysts decreases the pyrolysis temperature and the formation of undesired compounds (such as tar) by catalyzing their breakdown or preventing their formation and increasing microalgae conversion to gaseous products [36,37]. Alkaline metals and alkaline earth metals, for example, play a catalytic role in the gasification process, increasing conversion [37,38]. On the other hand, silicon and phosphorus are inhibitory because they form inactive alkaline silicates and phosphates. As a result, the real influence of metals will depend on the balance between the elements present [24,38].

4 Conclusions and future outlook

This chapter explored the issues of harvesting and thermal gasification. The gasification of microalgae biomass collected by coagulation-flocculation processes and sewage recovery as a nutritional medium was discussed. The general advantages and disadvantages of the pictured scenario are highlighted as follows:

(a) ***From harvesting process***

- Coagulants could modify the properties of microalgae biomass and, thus, influence the energy recovery process, for example, by affecting the HHV due to high ash incorporated into the microalgae biomass;
- If energy recovery from microalgae is desired, coagulants must be well evaluated before being applied in the harvesting process.
- Coagulant also affects thermochemical behavior. The catalytic effect increases the reaction's heat, conferring an exothermic characteristic on the microalgae pyrolysis.

(b) ***From gasification process***

- The characteristics of the microalgae biomass for gasification, such as particle size, moisture, and ash content, do not create operational constraints;
- The need for processing microalgae biomass into briquettes was not necessary for 52 wt% of the microalgae recovered. During the drying process, the biomass naturally gains the ideal particle size for gasification;
- The gasification was carried out at the maximum moisture content recommended by the manufacturer, which was 30 wt%. Nonetheless, the gasification process went relatively smoothly;
- The fact that coagulants and contaminants result in considerable ash content may affect the gasification reactions. Downdraft gasifiers are not the best reactors for gasifying high-ash biomass;
- Since both high ash and moisture content in algae biomass result in a great amount of energy lost in the overall process, the harvest process must be well managed to ensure proper drying and avoid excess inorganic coagulants and the incorporation of sand and dirt during the production of microalgae;
- Finally, the H_2/CO ratio in the syngas was 0.61 at ER 0.23, which is very close to the 0.60 suggested for synthetic fuel production (Fischer-Tropsch gasoline and diesel).

In terms of future perspective, the WWTP microalgae gasification route seems to face the same challenges as other routes, that is, distrust related to the contradictory conclusions on microalgae biomass productivity, as well as its actual technological performance and cost effectiveness—due to the complexities of cultivation, harvesting, and drying. Nevertheless, the truth is that, in the scenario pictured in this chapter, the cost of cultivation and harvesting might have a smaller weighting factor on the total cost of accountability.

Another doubt that emerges is that, although biomass gasification is considered a good process and has the best cost/efficiency ratio for converting biomass into bioenergy, this technology is still facing resistance. There is a gap between implementation and the communicated success of this technology. Finally, the remaining core point is that it is still necessary to develop a sustainable drying process.

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CHAPTER

13

Harnessing the potential of microalgal species *Dunaliella*: A biofuel and biocommodities perspective

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OUTLINE

1 Introduction

Microalgae are considered a potential feedstock source for biofuels and bioactive compounds to address novel renewable sources for the sustainable supply of food and

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energy [1]. The novel approach involving integrated biorefinery deals with such issues and turns microalgae processes more profitable, focused on biofuel and other products.

The talotolerant microalga, *Dunaliella*, is the most common unicellular green alga found mostly in hypersaline environments. However, a few species may be found in freshwater or slightly brackish water habitats worldwide [2]. Michel Fliex Dunal first discovered it on the South Mediterranean coast of France in 838, and later, in 1905, Teodorescu gave the name of genus after the name of discoverer [3]. *Dunaliella* is a unicellular biflagellate green alga belonging to the phylum *Chlorophyta*, the order *Chlamydomonadales*, and the family *Dunaliellaceae* [4].

Dunaliella algae have a thin elastic plasma membrane instead of a rigid polysaccharide cell wall. Due to the lack of a polysaccharide membrane and various pigmented molecules, species of this genus have the characteristics feature of changing shape and color [5]. Thus, it becomes very intricate to deduce and discriminate between the species based solely upon only physiological and morphological characteristics. The recent interest in and development of novel molecular biology techniques and phylogeny analysis led to the discovery of the taxonomy and utilization of the diversity of *Dunaliella* species. Based on the latest data obtained from the algae base (http://www.algaebase.org), the genus *Dunaliella* is composed of around 29 species, including *Dunaliella tertiolecta*, *Dunaliella salina*, *Dunaliella bardawil*, and so on [6].

One of the characteristic features that made commercial exploitation of the *Dunaliella* genus likely is the ability to persist in extreme habitat conditions, specifically varied salt concentrations and high temperatures [7]. The adaptability of these microorganisms to various severe circumstances in global habitats makes them an ideal candidate for various applications. *D. salina* is the most famous and commonly used species among various *Dunaliella* species.

In addition to this, the higher tolerance of *D. salina* as compared to other planktonic algae for various contaminated sites generated by petroleum and other industries enhances its applicability [8]. Moreover, some species, such as *D. acidophila* can thrive in a very acidic environment (pH 0–1). Similarly, some strains of *D. salina* can tolerate high light intensities and *Dunaliella antarctica* can cultivate at subzero temperatures [9].

Also, the complex life cycle of *Dunaliella* involving sexual and vegetative reproduction enables this genus to accumulate various active metabolites such as a varied range of carotenoids, polyunsaturated fatty acids, chlorophyll, and glycerol [10]. Several species of the genus *Dunaliella*, such as *D. salina* and *D. bardawil* are known for their human and animal consumption as these species do not produce toxins and can accumulate large quantities of carotenoids (8%–14% of total dry weight) [11–13]. High amounts of pro-vitamin A carotenes are also reported in the *Dunaliella* genus [14]. On the other hand, *D. tertiolecta* has been reported for the presence of xanthophylls, mainly lutein [15,16].

During the past decade, a rapid expansion in the number of scientific publications expressing the capacity of *Dunaliella* for biofuel and biocommodities has been observed (Fig. 13.1, Pubmed Search).

This chapter aims to discuss various applications of *Dunaliella* strains along with studies on the selection of elite strains based upon the choice of applications. Moreover, an analysis based upon optimal culture conditions and optimized upstream and downstream processes for commercial application will also be presented. A consolidated flowchart of the process is represented in Fig. 13.2.

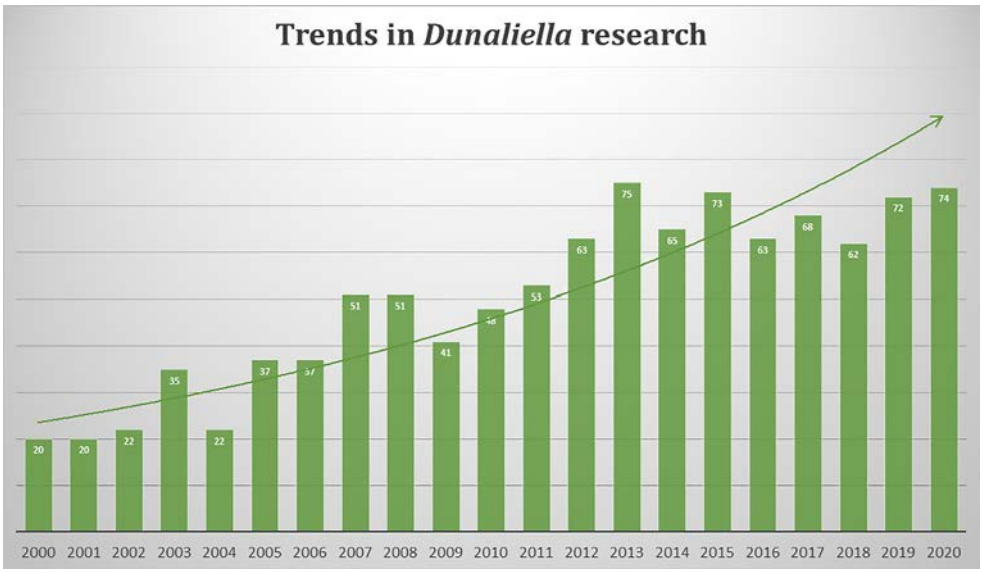

FIG. 13.1 PubMed publication trend analysis, demonstrating increased scientific interest in research related to *Dunaliella*. The data were retrieved from pubmed (https://www.ncbi.nlm.nih.gov/pubmed) on 4th May 2021 and cover the time period 2000–2020.

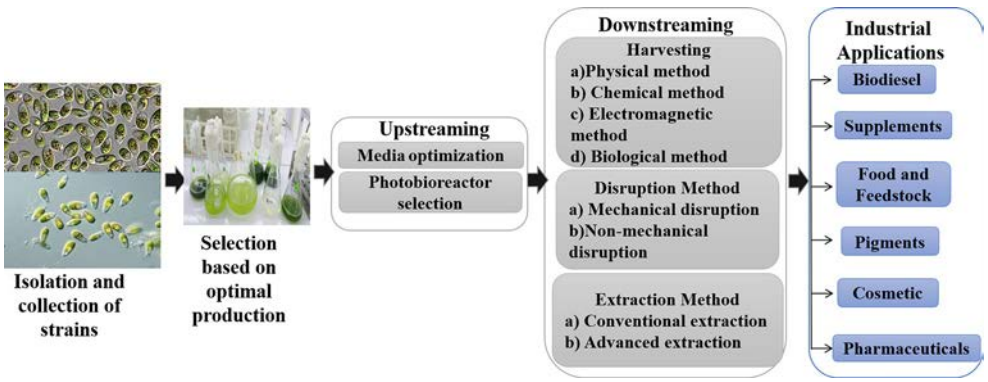

FIG. 13.2 A typical consolidated flowchart of the *Dunaliella* research and commercial process along with its applications.

2 Selection of elite strain and improvement

Most of the species of this genus are isolated from marine habitats like sea, and salt lakes, having a wide range of salinities and chemical compositions that construct the mechanism of halotolerance in this eukaryote genus. *Dunaliella* species can adapt to a wide range of salinities, pH, and salt stress due to various intracellular changes that occur when exposed to the aforementioned conditions [17,18]. Due to these changes, the production of favorable

metabolites cooccurs as a stress response in the cells of *Dunaliella*. Various strains of *Dunaliella* are well known for accumulating higher amounts of economically important chemicals like carotenoids, glycerols, lipids, fatty acids, and proteins [19].

Among microalgae, *D. salina* has attracted increasing research attention because of its inherent advantages [20]. *D. salina* is well-known for its high content of carotenoids and glycerol. Nevertheless, *D. salina* also has a high content of lipids, including polar lipids, and large-scale farming of *D. salina* is easy and inexpensive, which increases the applicability of this strain for nutraceutical/cosmeceutical applications.

2.1 Selection based on bioactives

Various species of *Dunaliella* are known to produce pigments such as β-carotene [21], violaxanthin [21], zeaxanthin [22], neoxanthin, and xanthophylls. Some of the species of *Dunaliella* accumulate lipids, polyunsaturated fatty acids, and lutein, which are considered important nutraceuticals and antioxidants that have benefits for humans and animals. *Dunaliella* is also a rich source of protein, essential polyunsaturated fatty acids, which are considered safe for human consumption as evidenced by GRAS (Generally Recognized as Safe) recognition [23].

Among all the isolated strains of the *Dunaliella* genus, the most explored one is *D. salina* due to its capability of producing carotenoids like β-carotene and its isomers [24,25], fatty acids, and proteins. *D. salina* is reported to accumulate approximately 14% of total dry weight β-carotene, making this strain ideal for mass production of this highly valued carotenoid [26]. This strain also accumulates essential pigments used in the pharmaceutical and health sectors, such as α-carotene, cryptoxanthin, and glutathione, which are known for their anticancer, antioxidant, and analgesic properties.

Considering the capability of tolerating high salinities ranging from 0.05 to 5.0 M, *D. salina* has become a significant species in high salinity environments, with depleting contamination with predators and competitors in aquaculture. *D. salina* not only produces a large amount of β-carotene, but it also accumulates a large amount of total lipids and fatty acids useful for biofuels, such as stearic acid, linoleic acid, and so on, which are then commercially produced [26,27].

Other strains like *D. bardawil*, *D. tertiolecta*, *D. primolecta*, and *D. viridis* are among those which are being explored by researchers nowadays, as they have shown promising results of producing bioactives similar to that of *D. salina*. It has been highlighted by Leon and coworkers that *D. bardawil* species can accumulate approximately 47 g/L culture phytoene [28].

Another strain of choice is *D. tertiolecta*, which produces important carotenoids and pigments. This strain accumulates various carotenoids when appropriate cultivation conditions are provided. Some carotenoids which are produced by this strain are trans- violaxanthin (0.45 mg/g), lutein (1.59 mg/g), β-carotene (0.62 mg/g), α-carotene (0.04 mg/g), and 9-cis-β-carotene (0.13 mg/g) [29].

Microalgae are a well-known source of available protein sources. Their protein content can vary from 20% to 60% of their dry weight. The protein content in *D. salina* is reported to accumulate in a range from 39% to 61% [30], whereas in *D. tertiolecta* it can be found in higher amounts (27%–62%) which are reported by researchers in different forms of edible products like cakes [31]. From all the secondary metabolite-producing strains of *Dunaliella* species,

D. tertiolecta is considered to produce the best protein content with 27.2% [32], 20% [33], and 49% [16] per its dry weight of total biomass accumulation. Many studies reveal that microalgae, along with protein accumulation, produce essential and nonessential amino acids. Amino acids are an important dietary product for humans and for animals as well. Strains of *Dunaliella* are also among the microalgae that produce essential amino acids such as *D. salina* (41.2%), *D. tertiolecta* (22.61), and *Dunaliella* sp. (41.3% of essential and 66.2% of nonessential amino acids) [34,35].

Microalgae are also known for their crude glycerol production, which has been accounted by several researchers. This accumulation acts as an osmotic pressure regulator between cells and their surroundings. *Dunaliella* species also produce significant amounts of crude glycerol when exposed to various salinities to regulate production. Many halotolerant species of *Dunaliella* have been accounted for the production of glycerol. One such study accounted for 17% glycerol in total dry weight of *Dunaliella* sp. on supplementation with 25% NaCl in the cultivation medium [36]. Many micronutrients play a vital role in glycerol accumulation as a response to several stress factors. In the synthesis of glycerol in *D. salina*, it is reported that regulation of Cu^{2+} ion concentration in the medium is responsible for the remarkable glycerol excretion compared to other nutrient starvation. One such study indicated by Lustigman et al., found a total of 150% increase in the glycerol content of the species when exposed to 50 ppm copper ions in the medium used for its growth [37].

Potential agents used as emulsion stabilizers and bioflocculants are polysaccharides. It helps in the removal of heavy metals from polluted water. *Dunaliella* sp. produces and excretes polysaccharides in higher amounts. The best species to produce polysaccharides is *D. salina* which produces 0.94 g/L of polysaccharides [5].

In addition to the bioactive compounds from *Dunaliella* sp., enzymes from *D. salina* (dihydroxy acetone reductase) have been extracted and sold commercially [25].

2.2 Selection based on biofuel

As the global economy runs on energy, the economic growth with the rising a population has led to an increase in demand for global energy. The oil content of many oleaginous microalgae has exceeded major oil-producing crops [38]. Biofuel production from microalgae depends on the rate of biomass production and its lipid content. The rate of lipid production depends on several factors, such as nutrient composition, light intensity, and cultivation in closed or open photobioreactors. Microalgae tend to capture excess solar energy through photosynthesis, which is stored mainly as lipids with a significant amount of triglycerols (TAGs) [38].

Microalgae lipid content ranges from 1% to 85% of dry weight [39,40]. Lipid accumulation in algae occurs under environmental stress and growth under nutrient starvation conditions. *Dunaliella* species are known to respond to nitrogen starvation by increasing lipid production [40,41].

Many studies have revealed that *Dunaliella* sp. produces lipids in a significant amount, which can be turned into biofuel. The oil content of many strains was found to be more than 40% of their dry weight [42]. For example, *D. tertiolecta* is reported to have accumulated a high amount of lipids, up to 71% per dry cell weight, which makes this species suitable for

feedstock for biofuel production [43]. *D. salina* reported to produce stearic acids (6.63%) and oleic acids (4.15%) out of 19% total fatty acids in high light along with carotenoids.

Another study found that when *D. tertiolecta* is exposed to high light intensity and a nitrate deficiency, it produces more essential saturated and monounsaturated fatty acids while producing less polyunsaturated fatty acids. It has been found that fatty acid compositions comprised of C16:0 (palmitic acid), C16:1 (palmitoleic acid), C18:0 (stearic acid), and C18:1 (oleic acid) were in the highest concentration as compared to C16:3 (hexadecatrienoic acid), C16:4 (hexadecatetraenoic acid), and C18:4 (stearidonic acid) [44].

Dunaliella sp. is known for their biofuel production as they are reported to yield high levels of lipids and oil as reported in *D. tertiolecta*, the oil yield produced was 37% [45]. Properties that determine whether such strain is suitable for biofuel production are cetane number (CN), saponification values, degree of unsaturation (DU), long-chain saturated factor (LCSF), and cold filter plugging point (CFPP) [45]. One such study reported the biodiesel property of *D. salina*, with CN (55.40), DU (91.83), LCSF (4.85), and CFPP (1.24) with total PUFA content of 34.47% [46].

3 Prerequisite: Optimal growth conditions

One of the crucial preconditions to achieve maximum productivity from microalgal cultivation is medium screening and optimization of growth conditions. The composition and growth for their cultivation considerably influence the biomass and value-added product yield (Table 13.1 and Fig. 13.3). Some of the parameters that may affect microalgae growth greatly are salinity, light, pH, temperature, and media composition.

3.1 Salinity and light

Effect of variation in salinity can be observed in different species as depicted in Table 13.1. Salt concentration is usually considered an important factor for growth. As for marine microalgae species such as *Dunaliella*, the salinity levels for the growth medium are adjusted to mimic those found in the sea. This has proven to be advantageous for the commercial production of such halotolerant strains due to the high salt levels in the closed system causes corrosion. Based on salinity ranges, the production of varied carotenoids and lipids has been categorized along with the stress which induces their production [62]. In *D. tertiolecta*, optimum salinity levels of the culture medium produced greater quantities of carotenoids, whereas, in *D. viridis*, carotenoids accumulate only in the higher range. It has also been shown in *D. salina* that due to salinity, the overall beta carotene content per dry weight gets affected [63]. Salinity not only affects pigment content but an increase of 2.5-fold in the carbohydrate content in *D. salina* has been reported [5]. When salinity is increased, the nitrogen and carbon content in the medium also increases that triggering microalgal cells to produce compounds like glycerols and amino acids to resist in a certain higher saline environment [64]. *D. salina* is reported to have produced at 270.03 μg/mL of glycerol when supplemented with 2.33 mol/L of salinity [52]. In another study, with a 10% increase in salinity in the medium, 9.19% of docosahexaenoic acid content was reported [65]. In one study, *Dunaliella* sp. was reported

TABLE 13.1 Examples of effect of different media composition and growth condition on value added products.

S. No.	Strain	Light intensity/ photo period	pH, temperature	Media composition and salinity	Metabolites[a]	References
1	*D. bardawil*	15,000 lx	7.5, 25°C	MH medium	β-Carotene—58.01 mg/L	[47]
2	*D. salina* CCAP19/12	70 μmol m/s^2	8.1, 25°C	F/2, 33.6 g/L artificial salt	Neutral lipids—5.88%, SFA—29.925, USFA—71.25%	[48]
3	*D. bardawil*	16:8 light/dark	7.5, 21°C	Johnson's medium	β-Carotene—9.2 μg/mL, 20% lipid productivity	[49]
4	*D. salina*	55 μmol m/s^2	7.5, 20°C	Modified Johnsons,2M salinity	Proteins—43.5 mg/L/day, total carotenoids—0.59%	[50]
5	*D. salina*	2500 lx, 12:12	8.5, 25°C	ASW	Total carotenoids—5.206 μg/mL	[51]
6	*D. salina*	16 h:8 h	7.76, NR	Salinity-2.33 mol/L	Glycerol—270.03 μg/mL	[52]
7	*D. salina* CCAP 19/30	50–1500 μmol m/s^2; 12 h:12 h	7.5, 20°C	MJ media	Carotenoids—0.81 pg/cell, glycerol—23,026 pg/cell, protein—26.23 pg/cell, starch—23.71 pg/cell	[53]
8	*D. salina*	245.6 μmol m/s^2	NR, 22°C	Modified Johnsons	β-Carotene—0.11 g/L	[54]
9	*D. salina*	NR	8.5, NR	10%NaCl conc.	Carotenoids—14.95 μg/mL, protein—186 μg/mL	[55]
10	*D. salina* CCAP 19/18	40–400 μmol m/s^2, cool white fluorescent light	8.1, 25°C	ASW, 1.5 M NaCl	β-Carotene, zeaxanthin	[22]
11	*D. bardawil* LB 2538	40–400 μmol m/s^2, cool white fluorescent light	8.1, 25°C	ASW, 1.5 M NaCl	β-Carotene, zeaxanthin	[22]
12	*D. salina*	52.8 μmol m/s^2, 14 h light	NR, 25°C	Modified hypersaline medium, saline soil extract	β-Carotene—56.25 ng/cell; 0.153 mg/mL	[56]
13	*D. bardawil*	52.8 μmol m/s^2, 14 h light	NR, 25°C	Modified hypersaline medium, saline soil extract	β-Carotene—52.91 ng/cell; 0.11 mg/mL	[56]
14	*D. salina*	150 μmol m/s^2, 12 h:12 h	8, NR	0.05–4.0 M salinity, MH medium	Amino acids	[57]

Continued

TABLE 13.1 Examples of effect of different media composition and growth condition on value added products—Cont'd

S. No.	Strain	Light intensity/ photo period	pH, temperature	Media composition and salinity	Metabolites[a]	References
15	*D. tertiolecta*	150 μmol m/s^2, 12 h:12 h	8, NR	0.05–4.0 M salinity, MH medium	Essential amino acids	[57]
16	*D. bardawil*	35 μmol m/s^2, 16:8	7.5, NR	AS100 medium $NaHCO_3$ (2 g/L)	Lutein—51.3%/DW, β-carotene—70%/DW	[58]
17	*D. tertiolecta*	8 W white light	7.8, 23°C	Erdschriebr's Medium, carbon dioxide flow 60 mL/min	SFA—27%, UFA—73%	[59]
18	*D. tertiolecta*	NR	NR	N 0.026 g/L, NH_4Cl	PUFA—73%	[59]
19	*D. tertiolecta*	NR	NR	Phototrophic	Lipid content—16.7%	[30]
20	*Dunaliella* sp.	NR	NR, 25°C	5 M NaCl	0.94 g/L polysaccharide	[5]
21	*Dunaliella* sp.	NR	NR	25% NaCl	17% (dry weight) glycerol	[36]
22	*D. tertiolecta*	50 μmol m/s^2	7.5, 34°C	ASW, NaCl (0.05–3 M)	Total carotenoids—3.75 mg/L	[60]
23	*D. salina*	50 μmol m/s^2	7.5, 34°C	ASW, NaCl (0.05–3 M)	Total carotenoids—3.57 mg/L	[60]
24	*D. tertiolecta*	20,000 lx	8.0, 27°C	ASW, NaCl (29.22 g/L), 3% CO_2	Oil yield—43.8%	[45]
25	*D. salina*	NR	NR	50 ppm Cu^{2+}	150% increase in glycerol production	[37]
26	*D. bioculata*	11 W/m^2/12 h:12 h	7.5, NR	ASW	60% glycerol conversion	[61]

[a] *Values shown in the tables are reported as per the referred articles in literature.*
ASW, *artificial sea water;* DW, *dry weight;* MH, *modified hypersaline;* MJ, *modified Johnsons;* N, *nitrogen;* NR, *not reported;* PUFA, *polyunsaturated fatty acid;* SFA, *saturated fatty acid;* UFA, *unsaturated fatty acid.*

to produce 53.5% stearic acid and 3.3% linoleic acid when supplemented with various salt levels with the presence of various carotenoids such as astaxanthin, luteoxanthin, violaxanthin, and zeaxanthin [66].

Microalgae can overproduce lipids and carotenoids under stress conditions such as high light, high salt, or nutrient limitations [67]. Lipid accumulation in *Dunaliella* sp. was increased by up to 70% under high salinity stress [67]. When salt concentration, was increased from 4% to 9%, it yielded β-carotene up to 30-fold in *D. salina*. The light requirements of different micro-algae differ widely, from high to moderate. *Dunaliella* species are known to be obligate autotrophs, which means light is the only energy source for their metabolism to function [68]. The cultivation of *Dunaliella* requires a sufficient amount of light, either growing in open ponds or in closed bioreactors. In open culture ponds, sunlight is the only cheap light source, whereas in closed systems, artificial light sources such as white fluorescent lamps, tube lights,

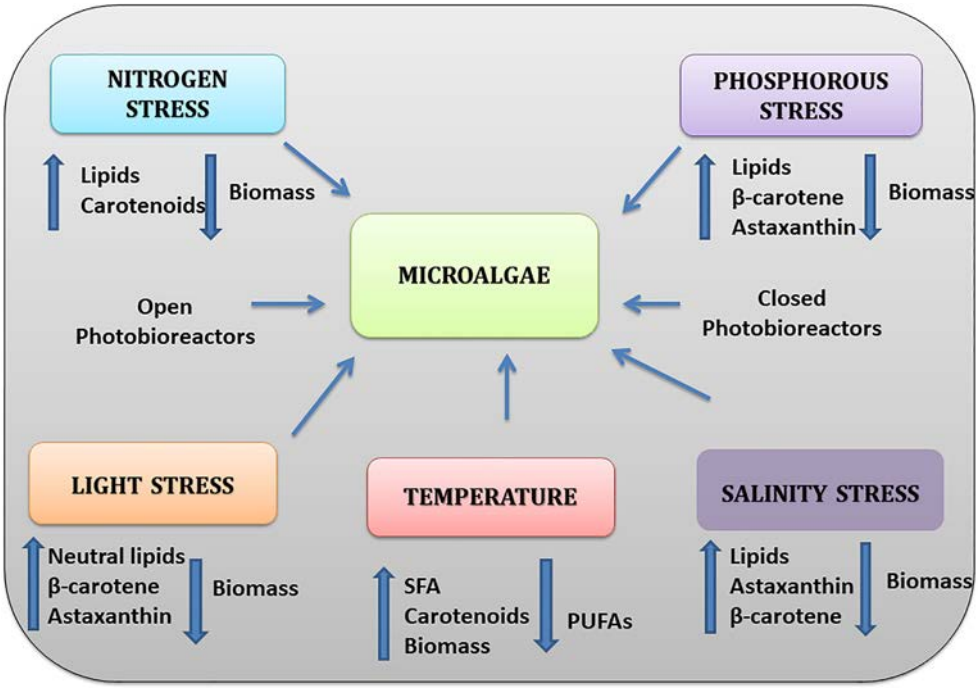

FIG. 13.3 Effect of various growth parameters on biomass and metabolites production.

or even sunlight can be a source. The growth of cultivation and metabolite accumulation respond differently to the quality, quantity, and intensity of light is provided. Carotenoid synthesis depends upon the light intensity but not on the wavelength given to microalgal cells. High light provided to microalgae culture can stimulate the production of neutral lipids, β-carotene, and astaxanthin, whereas it decreases the chances of accumulation of polar lipids, lutein, and biomass. *Dunaliella* sp. response to various light intensities and photoperiods (dark and light phase) has been accounted for in several studies (Table 13.1). *Dunaliella* sp. response to various light intensity and photoperiods (dark and light phase) has been accounted for in several studies (Table 13.1). Also, not only white fluorescent light contributes to the growth and production of valuable products, but a combination of different lights such as red and blue with an intensity of 85–170 $\mu E/m^2/s$ respectively, also contributes to carotenoid production as reported in *D. salina* [69].

3.2 Temperature

It is one of the most important factors that plays a role in the growth of microalgae. The specific growth rate of any microalgae species directly depends upon the gross rate of oxygen fixation, or its production (photosynthesis) and its respiration rate. Both these processes are temperature-dependent. With an increase in respiration rate, temperature also starts to increase. This factor influences the accumulation of biomass, proteins, lipids, and valuable metabolites in microalgae. *Dunaliella* can thrive in a varied ranges of temperature from below 0°C

to around 45°C. Temperature is important in terms of both biomass and fatty acid accumulation in the species. Usually, higher temperature favors increased cell growth and lower temperatures favor the production of fatty acids. Temperature also plays an effective role in changing the qualitative and qualitative carotenoid composition in *Dunaliella* species. *D. bardawil* accumulated 9 cis/all trans-β-carotene with 16,536 μg/L of total carotenoids at 26°C, whereas, at 15°C, the production of β-carotene (88.4%) was reported. Some Chilean strains of *D. salina* accumulated 3.5 times more α-carotene at 15°C than at 26°C [70].

3.3 pH

The adjustments in pH of the cultivation medium are made to prevent the cross contamination of other microorganisms (different microalgae species thriving in similar kind of environments) and keep the monoculture. The effective adsorption of the nutrients from the media is also controlled by pH, as it affects the availability of elements in the media and their effective utilization [71]. *Dunaliella* species grow well in wide ranges of pH, tolerance from 0 to 11 but the optimum pH for the growth of the cells is found to be 8. The pH range might increase from 8 with the utilization of media components and the production of metabolites over the course of time. The rising pH results in precipitation of utilized chemicals for the growth and metabolite production, which further hinders the overall cell growth. Hence it is necessary to avoid further pH increase from 11. Table 13.1 describes the production of different compounds at a varied range of pH in *Dunaliella* sp. One such study in which a pH range of 6.5–7.5 was subjected to *D. salina* cells along with red light, reported producing 5.12 mg/g of total carotenoids, 0.83 mg/g of astaxanthin, 1.02 mg/g of β-carotene, PUFA content (71.67%) with essential fatty acids such as α-linolenic acid (21.8 mg/g), γ-linolenic acid(2.53 mg/g), and linolenic acid(8.43 mg/g) [72].

3.4 Media composition

Nitrogen and phosphorous are the key nutrients that directly affect the overall growth and accumulation of metabolites from microalgae, as shown in Table 13.1. The combination of both these chemicals in a culture medium is responsible for the increased or decreased amount of biomass production of microalgae. When nitrogen supplementation is high, lipids and carotenoids accumulate in greater amounts while biomass decreases. Phosphorus deficiency in microalgae results in lower biomass production and higher lipid and pigment yields (β-carotene and astaxanthin) [62].

The best source of nitrogen for the cultivation of *D. salina* is nitrate. Higher amounts of $NaNO_3$ or KNO_3 favor the optimal growth of the algae. Limiting the nitrate concentration is one of the most common ways to reduce biomass yield and enhance carotenoid production. Nitrogen limitation in the culture medium often results in the production of important fatty acids in *Dunaliella* sp. One such study was reported in *D. salina* in which N-deprivation in the medium produced 39.6% of linolenic acid [59]. Manipulation of the amount of NH_4 and NO_3 in the cultivation medium for *D. salina* was reported to produce 64% of PUFA, out of which 55% is linolenic acid, which is an important fatty acid along with 38% of lutein content

[73] Modifications to the media composition on different days have also been reported to affect the production of metabolites, as demonstrated by one study in which nitrogen deprivation on the 7th day of cultivation resulted in 37 mg/L of biomass production, 33.8 % linolenic acid, and % eicosapentaenoic acid in *D. salina* [74].

A phosphorus source is added in the form of KH_2PO_4 or NaH_2PO_4, giving the best results in terms of growth and metabolite production. In one study, the effect of phosphorous concentration in *D. salina* culture media from 0.018 mM NaH_2PO_4 and 0.036 mM of NaH_2PO_4 content resulted in the production of 58.10% and 68.54% of unsaturated fatty acids, respectively [48]. Another study accounted β-carotene production (17.4 mg/L) in 145.2 μM of NaH_2PO_4 with the best biomass production (0.24 g/L) in 290 μM of NaH_2PO_4 content supplemented to *D. salina* KU XI [54]. The micronutrients essential for its growth and cultivation are zinc, magnesium, copper, manganese, iron, and sulfates form of these micronutrients. Micronutrients such as K^+, Ca^{2+}, Mg^{2+}, Cl^-, Na^+, chelated iron, and some trace elements are required for good growth and carotene synthesis in *Dunaliella* species [75]. *Dunaliella* also needs a high concentration of sulfates for maximal growth. Sulfates of various elements like magnesium, manganese, and iron are added, but this is generally not required in open pond cultivation due to the presence of these elements in natural water sources [76].

4 Downstream process

One of the key challenges for obtaining various metabolites from microalgae is selecting efficient and economically feasible processes and technologies. The selection of appropriate downstream processing techniques in *Dunaliella* sp. resulted in establishment of sustainable model for obtaining high-value products [77]. There are many steps involved in this process, including harvesting, cell disruption, and extraction [78].

The first stage of microalgae downstream processing is harvesting and biomass concentration [79]. The harvesting step requires special consideration in the case of *Dunaliella* sp. This is because of several reasons such as lack of rigid cell wall, low cell density, and small cell size. These features impose great challenges in applying routine harvesting procedures such as cellulose filtration and sand filtration that can be easily applied in other species [80]. Small cell size and low cell density in *Dunaliella* sp. clogs the various membranes and makes the process more tedious [81,82]. The only filtration method that exhibited some success in *Dunaliella* sp. is diatomaceous earth which can then be directly used for extraction purposes [83].

Similarly, the implementation of flocculating agents for harvesting has promised some practical outcomes: nevertheless, removal of these flocculating agents increases the cost of the process [84]. One of the most feasible and amicable harvesting methods that have been labeled as practical for obtaining *D. salina* cells from mature cultures is continuous-flow centrifugation [80]. The only proven large-scale industrial method used for harvesting *Dunaliella* for the purpose of β-carotene recovery is salinity-dependent hydrophobic binding [85].

After harvesting, the next step in the downstream cycle is drying the algal cell mass to extend the shelf-life of the biomass. The most common methods are drum drying, sun drying, freeze-drying, and spray drying. Although the selection of a drying technique is usually

based upon the nature of the end product needed. The sun-drying method is not a method of choice because of the rapid degradation of β-carotene pigment in presence of a higher temperature and light. Spray drying is the most appropriate drying method for industrial applications due to its low cost as compared to freeze-drying and optimum carotenoid yield [86].

The next step in the process is the extraction of desired compounds from the microalgal cell [87]. The selection of efficient extraction processes is another vital phase that demands careful consideration. Conventional solvent extraction using hexane or a similar solvent is the most commonly used method for the extraction of carotenoids from dried *Dunaliella* biomass. Extraction of carotenoids using edible oil is one of the several methods that have been reported for carotenoid extraction from *Dunaliella* biomass [88]. Recently, the separation of specific -carotene isomers from Dunaliella biomass was reported using novel extraction processes such as supercritical solvent extraction (SFE) and pressurized fluid extraction (PLE) [89,90]. In a recent study, supercritical extraction at 70°C and 500 bar with 10% ethanol as a cosolvent yielded more than 90% pigment recovery [90]. Similarly, selective extraction of β-carotene from *D. salina* can also be achieved with proper selection of cosolvent for supercritical extraction [91]. It has also been reported that pressure selection is a more crucial factor than the selection of temperature to achieve the best extraction yield. At 400 bar and 55°C the yield obtained was 115.43 μg/g dry algae [92].

5 Metabolites production and their applications

Microalgae contain a plethora of enormously significant bioactive molecules which have gained considerable attention from biotechnological and pharmaceutical groups owing to their potential applications. Various applications associated with microalgae are biomass production for food and feed industries, biorefinery applications, and bioactive metabolites for medical and pharmaceutical industries [93]. *Dunaliella* is a well-studied genus due to its ability to survive in harsh environments, exceptional adaptability, and physiological aspects, as well as its numerous biotechnological applications [94]. Some of the most common applications associated with *Dunaliella* sp. are discussed in the following section.

5.1 Feed application

Considering the high nutritional value of microalgae, it has been consumed worldwide for a very long time. These microalgae are available in many commercial forms for different applications, such as dry algae powders, pastes, and extracts. Algae pastes are the most widely employed formula and are used as a protein supplement in the aquaculture industry and animal nutrition to feed shrimp, larval fishes, molluscs, etc. On the other hand, powders and flakes have several applications and are mainly consumed in the food, nutraceutical, and feed industries [94].

Dunaliella is mainly composed of approximately 30%–60% protein, 35% carbohydrates including glycerol, lipids (about 10%), essential amino acids (up to 8%), primarily a huge amount of beta-carotene (up to 14% of dry weight), and 8% fat as hydrocarbons [95]. For

instance, difference in the compositional contents has been observed in different species of *Dunaliella*. *D. salina* contains 57% proteins and 32% carbohydrates while lipid content is only 6% and is found to be relatively very low. On the other hand, *D. bioculata* contains 49% proteins, 4% carbohydrates, and 8% lipids. Similarly, *D. tertiolecta* contains 29% proteins, 14% carbohydrates, and 11% lipids [96]. High protein and fatty acid content, high levels of β-carotene and other carotenoids, lack of an indigestible cell wall, and GRAS status are some of the distinguishing characteristics that make *Dunaliella* meal the first choice for aquaculture and poultry feed. The prolific nutritional suitability, rich protein, and carbohydrate content of *D. salina* inferred this species as a promising species for feed application. It is mainly used in cattle and aquatic food, along with its use in human food consumption.

This species is also used to provide natural pigmented growth environments for ornamental fish, trout, and shrimp [97]. The addition of these pigments as an animal feed supplement improves the yolk of eggs, flesh color, and improves the fertility and health of grain-fed cattle [98]. It also provides zero-plankton as animal feed needed for larval culture environments [99].

5.2 Cosmetic application

The *Dunaliella* algae group is considered the paramount reserve of assorted natural carotenoids, which include β-carotene, α-carotene, lutein, zeaxanthin, violaxanthin, neoxanthin, xanthophylls, and cryptoxanthin. Besides this, these microalgae also contain essential nutrients like minerals, vitamins, proteins, and essential fatty acids. Various cosmeceutical applications of these microbial products can be easily expounded in light of diverse pharmacological activities recognized with these biomolecules.

Several studies have emphasized the antioxidant and immune-boosting activities of the carotenoid mixture obtained from various *Dunaliella* species and proposed to be utilized in various skincare products having free radical damage protecting action and prevention from premature skin aging. Also, these carotenoids are known to reduce the effect of harmful radiation, soothe sunburns, and considerably diminish the number of photo-aging markers.

The growing need for natural colors in cosmetics and personal care products draws attention to the accelerated usage of β-carotene derived orange color pigmentation, which is accompanied by antioxidant properties. Given these market trends, *D. salina*-derived β-carotene is an excellent substitute for natural color in the cosmetic industry. In addition to this, *D. salina* also comprises a substantial quantity of sporopollenin organic, which behaves as an amino acid and thus can be efficiently utilized against ultraviolet rays [100]. Similarly, β-cryptoxanthin, a unique carotene found in *D. salina*, act as an anti-inflammatory agent. β-Cryptoxanthin also induces the synthesis of a glycosaminoglycan molecule named hyaluronic acid, which assists in skin hydration and thus improves skin texture. Recently, a formulation based upon *D. salina* lipid-based ingredients with potent cell proliferation activity was launched by Pentapharm in skincare products to increase energy metabolism and antiaging effect on the skin (Basel, Switzerland) [101].

Proteins, vitamins, fatty acids and phytonutrients like chlorophyll helps in the natural detoxification of the body. Also, these ingredients are frequently utilized as peeling and hydration agents in various cosmetic products [102].

5.3 Pharmaceutical applications

As discussed already in the abovementioned sections, *Dunaliella* sp. is *a* rich source of numerous carotenoids. Along with these carotenoids, *Dunaliella* sp. also contains several other bioactive metabolites such as retinal, aldehydes, ketones, epoxides, and apocarotenoids [8]. These carotenoids and other bioactive metabolites have several biological functions and can participate in several vital mechanisms, for instance, immune response, gene modulation, growth regulation, and metabolic enzyme modulators [103].

It has been reported in the literature that natural β-carotene which contains two different isomers, a mixture of "9-cis" and "all-trans" isomers is better in its pharmacological properties as compared to synthetic β-carotene which is mainly composed of all-trans isomers. Although there are numerous sources of natural β-carotene except for microalgae such as fruits, vegetables, and the fungus *Blakeslea* [104], its concentration in these sources is comparatively less compared to microalgae. It has also been observed that *Dunaliella* cell can store considerably great quantities of β-carotene than other sources [105]. Furthermore, 50% of the total isomers of β-carotene in *Dunaliella* species are 9-cis isomers, increasing its bioavailability and activity [68].

Furthermore, β-carotene is also reported to inhibit various types of tumors such as oral tumors [106], melanoma [107], breast cancer [107], and other related diseases. Similarly, carotenoids, specifically β-carotene play an important role in managing cholesterol levels and thus reducing the risk of cardiovascular disorders [108].

Besides, another therapeutic effect associated with β-carotene is free radical scavenging potential, thus preventing lifestyle disorders and immunomodulatory effects.

In this way, these metabolites warrant several health benefits, including diminishing the risk of neurodegenerative diseases, eye-sight enhancement, strengthening the immune system, hypolipidemic activities, antimicrobial, anticancer, and antioxidant properties. Although several species of *Dunaliella* are reported for various pharmacological activities, *D. tertiolecta* and *D. salina* have been extensively studied for various nutraceutical and pharmaceutical applications [109]. In one of the studies, different extracts of *D. salina* exhibited excellent antimicrobial activity against various micro-organisms, for instance, *Aspergillus niger*, *Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus* [110].

Apart from β-carotene, microalgae are also rich sources of xanthophylls, namely zeaxanthin and its stereoisomer lutein. These compounds have rich nutraceutical values and protect against macular degeneration and eye disorders [111]. Microalgae offer a commercial source for zeaxanthin with the advantages of fast and inexpensive growth and the possibility of simultaneous production of other valuable compounds.

D. salina is also a rich source of several enzymes. One such unique enzyme is dihydroxy acetone reductase, which has been explored commercially [112]. Besides these two species, crude extract from *D. primolecta* has been found to significantly inhibit the growth of *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, and *Enterobacter aerogenes* [113]. Phytoene, which is an intermediate compound in the biosynthesis of carotenoids, can be obtained for microalgae using a combination of *D. salina* with Norflurazon, a well-known bleaching herbicide. This compound is known to have very potent cancer-preventing and antioxidant activities in mammalian cells [114].

5.4 Biofuel

Recent literature has highlighted the use of microalgae for biofuel. Microalgae offer a suitable alternative to comprehend the requirements of sustainable and clean sources of energy. Among various microalgae, *Dunaliella* strains are believed to be valuable feedstock intended for biofuel production. Several species of *Dunaliella* have been investigated for evaluate their potential for biodiesel feedstock. The huge oil accumulation and swift growth rate of this group of microalgae render these organisms a noteworthy and valuable resource for biodiesel production [39]. Amidst several species, *D. tertiolecta* has a high CO_2 absorption rate and thus a higher growth rate. Similarly, it has been reported that *D. salina* produces an equivalent amount of hydrocarbons to *Botryococcus braunii*, which is a well-known species to produce liquid fuels. It has also been reported that optimization of salt concentration in *Dunaliella* can enhance the lipid content of the cell by up to 70%.

6 Challenges and integrated biorefinery approach

Dunaliella has received an enormous amount of attention as a potential cell factory for the accumulation of bioactive compounds like carotenoids and fatty acids. However, many challenges are being faced in the cultivation of *Dunaliella* for its commercial exploitation. It is not yet widely practiced in open systems because of its qualitative approach. Metabolite production is still sought in closed systems because these systems are more advantageous, despite their high cost. Many innovative and modified bioreactors have been developed by researchers based on the targeted metabolites, biomass production that can accommodate basic microalgal growth requirements such as light intensity, photoperiod, temperature, nutrients, mixing, etc. [114]. However, the need to develop a better strategy and innovative technologies in terms of *Dunaliella* cultivation and commercial exploitation is still lacking. The focus of current studies has shifted to making the whole process economically viable. Systems are being customized for reduced challenges. Many plants are under development in India, Chile, Mexico, Cuba, Iran, Taiwan, Kuwait, and Japan. The ability to induce, modify, and scale-up strategies for *Dunaliella* to produce a series of uncommon carotenoids of high nutritional and medicinal value, like phytoene, phytofluene, xanthophylls, and many more essential fatty acids, could open a new field in the area of *Dunaliella* biotechnology and its commercial exploitation. However, the economic viability of the process used to obtain these biocommodities from these microbial resources is a major challenge.

One of the strategies to exploit these resources for maximum utilization is the involvement of this microbial resource in integrated biorefineries. The algal biorefinery concept came up with a solution to obtain bioenergy and bioactive components from microalgal biomass as feedstock. One such example of the biorefinery model implemented in *Dunaliella tertiolecta* has been reported by Söyler and coworkers in 2017 to obtain lipids, fatty acids, phytosterol, and β-carotene followed by pyrolysis to obtain bio-oil and biochar from defatted biomass [115]. Such a consolidated approach might help in obtaining an exhaustive valorization of microalgae biomass. It also aids in obtaining cheaper and competitive resources for algal culturing, which eventually leads to the sustainable production of bioactive molecules and energy [116].

7 Conclusion and future scope

The production of metabolites, especially carotenoids, by *Dunaliella* species makes it a potential candidate for various commercial biotechnological applications such as animal feed, dietary supplements, pigments, cosmeceuticals, and pharmaceuticals. Considering its ability to produce multiple components, microalgal biomass is considered a promising raw material that can be utilized for various industries. However, maximum exploitation of this species can only be carried out with detailed research and in-depth investigation of various bioprocesses involved in microalgal cultivation, selection of elite strains, processing, and an economically viable downstream process that can bring about its effective utilization and commercialization aspects [47]. Furthermore, the implementation of novel genomic engineering tools can further enhance the application of this potential genus. In conclusion, looking toward the capacities of *Dunaliella* species and the extensive attention that they have received in the past decade, one can expect novel discoveries in this field yet to come.

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CHAPTER

14

Algae cultivation for biomedical applications: Current scenario and future direction

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1 Introduction

Algae encompass the most primitive inhabitants on earth. Comprising chlorophyll pigment, they are well-known for photosynthesis [1]. Algae are known to occupy various habitats, ranging from freshwater, saline environments, frost regions, rocks, and even in living organisms, namely plants and animals [2]. They are involved in fixing carbon dioxide as well as nitrogen oxides. Algae can be diversified into microalgae and macroalgae. Microalgae is widely used in the formulations of nanomedicines [3]. Algae holds potential significance in the research and development field. Due to the presence of disparate metabolites such as carotenoids, carbohydrates, lipids, proteins, and nucleic acids, they are greatly in demand for effective utilization in different industries [4]. Algae-derived components are considered high-value products because they serve different significant purposes in the pharmaceutical industry. A wide range of processes is carried out in industries to derive valuable products, as depicted in Fig. 14.1. These processes are comprised of upstream and downstream processing. Upstream operations cover multiple factors, namely the selection and isolation of algal species. Furthermore, it involves the purification and assortment of strains, cultivation, and harvesting of biomass. Downstream processing consists of disrupting selected algae to extract intracellular components of interest, transforming biochemical components, and recovering the products [5].

Additionally, optimizing cultivation conditions is also required to promote the development and build-up of metabolites by incorporating adequate nutrients, carbon dioxide, luminescence, temperature, and pH [6]. These physicochemical factors are crucial for promoting the synthesis and accumulation of significant metabolites. The costs of producing algal biomass are a critical factor in determining which upstream and downstream processes to use.

The magnificent glory of algae-based derivatives illustrates refined biocompatibility and availability attributes defining their biomedical utilization. The differences lie in the structural features, composition, and explicit cellular activities that favor algal exploitation for therapeutic interventions [7]. The physicochemical properties enhance the scope of the promising potential of bioactive products to function as a magical tool for novel strategies. From food to medicine, the biomedical utility of algal compounds dates back to the ages [8].

FIG. 14.1 Steps involved in bioprocessing for biopharmaceuticals.

The multifunctionalization of the algal species demarcates imminent antioxidant and immunomodulatory hallmarks, supporting biomedical evidence of application [9]. The identifiable algal treatment emphasizes the approachable measures employed in various aspects of medicine. Aside from its usage in food and fuel, it has been recognized for antiinflammatory, antiaging, and skin-treatment goals, creating a benchmark of overall health implications [10,11].

Algal applications have contributed a lot to the flourishing biotechnological sector, promoting their utility. An array of defensive properties depicted by the integrated algal system has gained scientific attention. The functional components of these photosynthetic organisms possess active metabolites that have biomedical significance [12]. Fig. 14.2 briefly illustrates the essential bioactive compounds of algae-like proteins, polysaccharides, carotenoids, vitamins, polyphenols, phycobilin, fatty acids, and astaxanthin, which account for the pivotal essence of nutritional assets. Subtle changes in lifestyle patterns have immediately caused a spike in mortality rates among individuals due to metabolic and chronic diseases [13,14]. The algae-derived bioactive compounds sustain efficient pharmacokinetic and exciting biological activity, integrating their formulation into clinical medicines [15]. The prompt algal bio-products have showcased nutraceutical as well as pharmaceutical benefits that have heightened their incorporation into the market. The emerging function of biomass constituents of algal species in treating diabetes, hypertension, cancer, chronic infections, and even in cosmetic practice, has highlighted their exceptional importance in medicine. A variety of algae have unique medicinal capabilities, affirming a rich source of beneficiaries for the pharmaceutical industries globally [16]. This chapter aims to shed light on the various strategies and technologies involved in algae strain cultivation, harvesting, and associated biomedical applications.

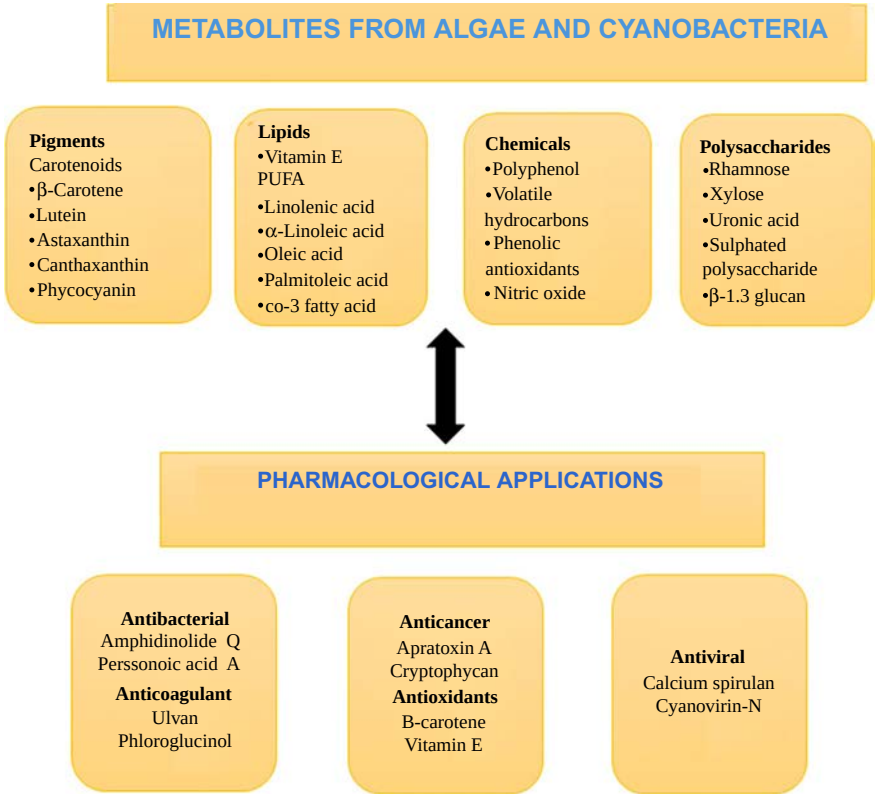

FIG. 14.2 Algal and cyanobacterial metabolites.

2 Considerations for choosing an algal strain

The selection of algal strains is one of the critical steps that define and progress the algae processing approach to success. With the efficient selection of algal strains, there are higher chances to promote growth and productivity of the algal biomass and obtain high-value-based products. This step influences the operational cost, which can be declined by effectively isolating and recovering the specific metabolites from the algae biomass [17]. This can be done by actively recognizing and selecting the algae strains and performing disparate screening activities. It can be influenced by selecting superior strains that are more adaptable and appropriate for the environment and climate than inferior strains [18]. Bioprospecting of algal strains belonging to the indigenous region requires deep knowledge of the cultural conditions required to enhance growth. Strains can be selected from geographic locations and culture collection centers for algae [19]. Table 14.1 gives detailed advantages and disadvantages of selecting the algae strain from the indigenous and cultural collection centers. Consideration of location is another crucial factor for selecting microalgae that influence the productivity of the algal biomass. This selection is based on the requirements of the algae strains

TABLE 14.1 Pros and cons of algal strains collected from indigenous regions and culture collection centers [20].

Algae strains source	Pros	Cons
1. Indigenous region	• Adaptable to local climate and environment • Accessible from the natural environment • The culture medium used for algae growth, such as BG-11, F/2, and BBM • Availability of a wide range of species • Not costly	• Do not adapt laboratory stimulated conditions. Few strains cannot be cultured • Locating strains from collection sites is a time-consuming, cost-involving process • Culturing of samples involves trials and errors • Requires appropriate characterization and understanding of growth rates and conditions
2. Culture collection centers	• Pure strains • No purification step involved • Adaptable to laboratory conditions • Can be grown in bioreactors • Mimicking of growth conditions can be done for the characterized species	• Involves purchasing cost • Involves inactivation or death to the exposure of extreme circumstances for growth • Availability of less number of strains • Difficult to grow in natural environmental conditions.

facilitating the growth, such as required nutrient supplementation, pH, temperature, and photoperiod.

Studies have reported that microalgae acquired from the marine environment exhibit significant properties for treating cancer [21]. Microalgae isolated from marine environments are more modifiable to metabolic manipulation. Thus, its bioactive compounds are beneficial for biomedical applications [22]. Therefore, choosing marine microalgae is considered a good option to incorporate its utilization into the biomedical domain.

3 Physicochemical conditions in algae growth

3.1 Culture medium

Culture medium plays a vital role in containing the required nutrients and vitamins that assist in developing algae in laboratories. The culture medium is made up of essential micronutrients and macronutrients present in various proportions and concentrations in different media used to grow algae. Culture mediums, namely Walne medium as well as Guillard's F/2 medium, are widely used for growing and producing algae [23].

3.2 Light

Light serves as a major component in influencing the development of microalgae. It serves the significant function of photosynthesis. The provision of adequate light can be provided through fluorescent lamps. The experiments show that algae grow well in red light as compared with other wavelengths of light. More algal cells and weight can be produced [24].

3.3 pH

The culture medium employed for the growth of algae requires an optimum pH ranging from 7 to 9. Optimization of pH is vital for facilitating better algae growth. However, extremely high and low pH can alter the functioning of vital enzymes, disrupting the healthy development and growth of algal cells. However, high pH is effective in degrading the biological contaminants in the culture medium [25].

3.4 Aeration/mixing

An efficient mixing/aeration facilitates the provision of nutrients and luminescence to algal cells in uniform concentrations [26]. Thus, this leads to the promotion of gas dispersion in the cultural medium. Adequate stirring must be done manually with hands or stirrers and mechanically using orbital shakers. Vigorous mixing influences the growth of some algal strains.

3.5 Temperature

Culture medium must be maintained in the temperature range of 20–24°C. Optimization of temperature should be maintained to control the metabolic and growth rate. Fluctuations in temperature can affect the growth rate. Temperature ranges lower than 16°C, which deteriorates the growth of algal cells, whereas temperatures above 30°C result in the death of the algal cells [27]. The elevated temperature can be lowered by providing cold water or maintaining the culture in air-conditioned rooms.

3.6 Salinity

Marine microalgae are considered the better choice for biomedical applications as they are more adaptable to the salty environment. Studies report that lipid production has been increased with the induction of salinity stress on algae. However, salinity stress can have an impact on the growth of freshwater algae [28]. It has been noted that marine microalgae attained the highest growth in the culture medium in which the dilution of seawater is done with water [29].

3.7 Carbon dioxide

The growth of microalgae can be augmented by adding higher proportions of carbon dioxide to the environment. Thus, it ensures the productivity of the algal biomass. Optimization of carbon dioxide must be maintained. Maximized growth rates can be obtained with a CO_2 concentration of 6% [30]. Carbon dioxide provision can be made by passing it over the medium in the suspensions of cultures.

3.8 Sterilization

Sterilization is vital in enhancing algal growth as it assists in preventing contamination of the culture medium. Glassware used in the preparation of culture media needs to be treated with the help of detergents or HCl. The autoclave can be used for sterilizing glassware [31].

4 Culturing of algae

Cultivation of microalgae can be carried out in two systems: open ponds and photobioreactors (PBRs).

4.1 Open ponds

Open ponds are chosen more often in industries due to the involvement of lower cost for maintaining them. It is simpler and easy to operate. Moreover, fewer energy requirements are associated with its operation, and, in turn, scaling up is easier for commercial purposes. Open systems can be of various types, comprising natural water in lakes and ponds and artificial water bodies, such as circular and raceway ponds. In open systems, natural sunlight is available to the algae; thus, they adequately carry out the photosynthesis process. The regulation is maintained by the natural water systems by maintaining the natural process of evaporation [32]. However, the ineffectiveness of maintaining these parameters leads to an impact on algae growth. This system requires efficient harvesting techniques, as from natural water bodies, less concentrated cells are obtained. Limitations are associated with an open system involving rainwater runoff, resulting in declining algae growth due to the alterations in pH and saline requirements. Additionally, the growth of algae can be impacted by water turbidity, cross-contamination, and cytotoxicity.

4.2 Circular ponds

Cultivation of algae on a large scale can be done in circular ponds, which are static with rotating agitators. It facilitates the mixing of nutrient constituents and inhibits the sedimentation of algae biomass. Due to the large size of ponds, more water resistance is created, impeding the algal growth due to the sheer forces of the rotating agitator. This system involves the composition of expensive mechanical parts and needs more energy for operation. Chlorella cultivation is done on a large scale in Japan and Taiwan by utilizing a circular pond system [33].

4.3 Raceway ponds

This system is composed of closed-loop channels. In this system, a paddlewheel is utilized for uniform circulation and mixing of nutrient components in equal proportions, reducing sedimentation of algal biomass. It can be exploited for cultivating algae in open systems. This is because of the fewer energy requirements. One paddle wheel can carry out an agitation process in a large tank. In Columbus, Ohio, this system is widely used to produce dried algal biomass [34].

4.4 Photobioreactors

PBRs are closed systems utilized in the pharmaceutical industry for commercial purposes. With the utilization of PBRs, algae growth can be maintained by adjusting the specific required conditions. PBRs can be operated easily in comparison with open ponds. Due to the fulfillment of nutrient composition and other physicochemical requirements, algal biomass productivity is enhanced in PBRs. It ensures the proper diffusion of air and fluids. These are potential candidates for commercial purposes. This can be attributed to the fact that PBRs are acquired in large volumes, which facilitates more algae cultivation. It does not pose contamination, thus reducing the fatal impact on algae and improving algae production compared with the open pond culture approach [35]. It has been evaluated that lipid production is higher in algae culturing in PBRs than in open ponds. It involves the use of artificial light sources, which means more energy demands.

5 Algae harvesting

Algae harvesting is a cost-involving process as it requires operational equipment and energy consumption. Various types of harvesting techniques can be employed, such as centrifugation, flocculation, and filtration.

5.1 Centrifugation

In centrifugation, algal separation is influenced by gravitational force, which leads to the accumulation of algal biomass at the bottom. It facilitates the separation process based on the characteristics of algal cells' size and density. It is a highly efficient method widely used to harvest algae. However, it is expensive, comprising higher energy demands and equipment costs [36]. This method is widely used in the biomedical and pharmaceutical industries as it involves the separation of high-value metabolites from algae. This technique can be employed for a wide range of algal species, although it can cause damage to algal cells.

5.2 Flocculation

Flocculation involves the clumping of algal cells due to sedimentation, which facilitates the separation from the medium and is attributed to the addition of chemicals called flocculants. Flocculants are employed in industries comprising aluminum sulfate and ferric sulfate. The efficiency of harvesting by flocculation is generally based on the hydrophobic properties of algal cells. Nowadays, bio-flocculants are widely utilized in industries due to their low costs and lack of contamination. Examples of bio-flocculants include microscopic living organisms such as bacteria, fungi, and yeasts. The flocculation technique is one of the effective techniques that can be employed for harvesting different algal species [37]. However, disadvantages are associated with flocculation that include toxicity of flocculants and huge energy requirements, thus increasing operational costs and reducing the efficiency of harvesting and recovering algal biomass.

5.3 Filtration

The filtration process comprises the passing of microalgal suspension through the filter medium. With the effect of the driving force, the algal slurry is retained in the porous medium as filtrate, and the left-out fluid flows down the filter. It facilitates the separation of algae biomass based on driving forces influenced by pressure, gravity, and vacuum. It is a promising strategy that can be employed in the pharmaceutical industry. It is a cost-effective process because of the minimum energy requirements and reagents that are not required, as well as the high recovery of shear-sensitive algal biomass [38]. However, filtration is associated with membrane clogging, so there is a need to replace the filters, which involves higher costs.

5.4 Floatation

This method of floatation is utilized for separating micro-algal cells. In this harvesting method, small gaseous bubbles adhere to the algal cells, which facilitates the floatation of the cells [39]. As an outcome, algal cells get separated. The separation of the constituents is based on cell size. Cells that are minute in size will float more in the medium because of the influence of attached bubbles than the higher-sized particles [40]. This approach is efficient for solutions having smaller-sized constituents that are below 500 μm [41]. The floatation method is used in industries due to its high harvesting efficiency and because it can be operated easily for commercial purposes [42].

6 Extraction and purification of high-value based metabolites

Postharvesting algal biomass is obtained. Drying methods are employed to prolong the shelf life of the algal product. Various drying methods can be used for desiccating the algal biomass, namely, spray drying and freeze-drying. The spray drying method is most often used for drying algal biomass to obtain high-value-based metabolites [43]. Freeze-drying, also called lyophilization, is one of the drying methods employed in industries for drying microalgae [44]. Thus, after freeze-drying the product, dried biomass is obtained, which enables the extraction of oil. Solvent-based extraction methods are considered a finer option for extracting intracellular components, such as lipids, from the dried biomass compared with the wet biomass [45]. Cell disruption methods such as expeller press, bead beating, ultrasonic and microwave waves have major use in extracting lipids from algae. Extracting oil by employing mechanical means is an effective approach. It does not contaminate or pose toxicity to algal cells. Moreover, it is a cost-effective approach as there is no involvement of chemicals and enzymes to extract oil [46]. However, intracellular metabolites cannot be extracted by mechanical means. The solvent extraction method is the most preferred method for extracting high-value-based metabolites from algae. Solvents such as hexane, ethanol, chloroform, and diethyl ether are primarily utilized in the pharmaceutical industry for extracting fatty acids [47].

Purification of the obtained bio-product is facilitated by using different types of chromatographic methods, such as ion-exchange chromatography. For extracting intracellular metabolites, supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) are more

often utilized to ensure the high recovery of products. In this SFE technique, supercritical carbon dioxide is used. Articles state that by applying high pressure, SFE is also effective in extracting carotenoids and pigments such as astaxanthin [48]. The PLE method is used for extracting secondary metabolites derived from microalgae [49]. Acid hydrolysis is done with the utilization of HCl to separate the lipids from carbohydrates and supplementary components. Further, after the purification of the bio-product, it is packaged and made ready to be delivered to the markets for pharmaceutical use. An integrated algal approach emphasizes conceptualizing and interlinking different aspects of the biomedical domain. This enhances the commercial utilization of algae, providing maximum benefits.

7 Biomedical applications of algal extracts: An integrated approach

7.1 Auspiciousness of polysaccharides

The substituted percentage of polysaccharides varies in algae, generally 4%–76% dry weight in seaweed and 8%–64% dry weight in microalgae, as per the species and time of harvest [7]. Fig. 14.3 gives a glimpse of cell-wall-derived sulfated polysaccharides as indispensable compounds that play a crucial role in promoting remedial properties. The polysaccharides are vital bioactive molecules that are prominently derived from sugar-based materials, extensively curtailing the effects of severe metabolic syndromes. These are extracted from a cascade of species of marine algae, namely carrageenan in the *Rhodophyceae* (red algae), laminarin, and fucoidans from *Phaeophyceae* (brown algae), and *Ulvans* in the *Chlorophyceae* (green algae). Each one of them demonstrated outstanding fitness traits required to protect human health. The optimal biological potential of different polysaccharides has ushered in a naive aspect of biotechnological solicitation in healthcare [50]. The demonstration of the multidimensional applicability of these algal extracts that contribute to the antiviral, antibacterial, antiinflammatory, anticancerous, and immunomodulatory features alleviates the ill-effects of diseases [51].

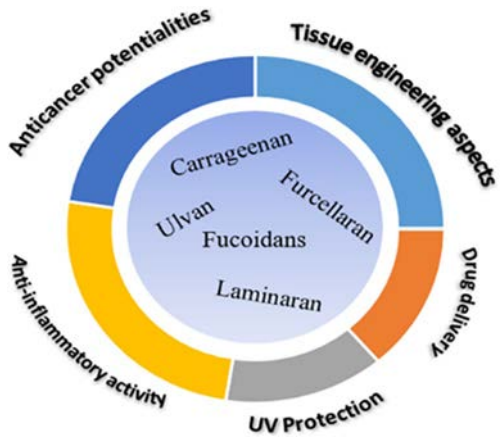

FIG. 14.3 Efficiency of algal derived polysaccharides.

7.2 Retaining antiviral and antibacterial assets

Adverse viral and bacterial diseases have emerged to date, leading to unfavorable health impacts. Infections like HIV and herpes require medicated drugs that can impressively inhibit viral replication and show effect against the virus. Microalgae species are widely used in the pharmaceutical industry due to the bioactivity of polysaccharides found in their cell walls [50]. The virucidal attributes of the algae, accompanied by the enzyme restricting venture, promote the development of the syncytium, in which the level of sulfation is proportionate to the anti-HIV purpose. An effective *virucidal negotiator*, *cyanovirin-N*, a holistic compound obtained from cyanobacteria, acts against HIV by obstructing the interactivity between viral glycoprotein gp120 and CD4 [52]. The qualitative composition of sulfated functionality favors exceptional biological specifications. According to Rizwan et al. [53], the sulfated polysaccharide calcium *spirulan* extracted from *Spirulina platensis* halts the ingression of viruses, showcasing viral resistance activity, aiding in the case of measles and herpes. The marine brown algae, *Undaria pinnatifida*, constitute significant polysaccharide fucoidans, which dominantly account for L-fucose and sulfate groups and a trace of galacturonic acid, xylose, galactose, and mannose. Fucoidan is known to foster bone amplification by inhibiting osteoblastic cell differentiation. *Gigartina skottsbergii*, red algae eliciting carrageenan, is efficient against enveloped and nonenveloped viruses [54].

The microalgae compound substantially retards the pathogenic ballooning of bacteria in the water that intercepts the emergence of the bacterial infection. A critical growth declination is observed in *Escherichia coli* and *Salmonella typhimurium* on the application of laminarin polysaccharide, which is selectively evoked by the brown algae Laminaria hyperborean as well as *Ascophyllum nodosum* [55]. Extracts of *Chlamydomonas pyrenoidosa* and *Chlorella vulgaris* have been found to have antibacterial properties against both Gram-negative and Gram-positive bacteria [53]. A few microalgal species demonstrate antimicrobial activity in separate ways. Rizzo et al. [56], stated that the polysaccharides extracted from *Ulva fasciata* green algae and *Dictyopteris polypodioides*, brown algae, have been examined to be active against *Aeromonas salmonicida* and *Vibrio alginolyticus*, which are potent producers of neurotoxin. Another crucial marine red alga, *Amphiroa rigida*, is popularly found on the southern seaside of Tamil Nadu, India. The polysaccharide mediated by *Amphiroa rigida* is well known for its outstanding antibacterial activity against *Salmonella typhimurium* [54]. The specificity of bacterial resistance can be accounted for by different attributes, including temperature, pH of the culture medium, and light for the production of divergent metabolites that uphold an adequate place in human well-being [53].

7.3 Drug delivery

The sulfated polysaccharides have surpassed the potential of drug formulation as they play an elemental role in drug delivery systems. The marine algal cell wall is composed of rich polysaccharides, namely carrageenan found in red algae, fucoidan in brown algae, and ulvan in green algae [57]. Out of the three, carrageenan has been a topic of interest for years as it provides a wide range of application implementation as an emulsifier, thickener, or stabilizer. The Fucoidan implications for the agricultural and therapeutic industries have a cheap commercial value. Ulvan's contribution is still unexplored as it has been significant in

agricultural and drug avocation [8]. The propensity of these polysaccharides, specifically carrageenan, enables them to fulfill the requirements of a particular drug delivery system by comprehensively supporting the adhesive properties so that they can be vitally used in formulating nanoparticles, beads, and film implants [57]. Polysaccharides have evoked a new era in the biomedical field by promoting regenerative medicine, tissue engineering, and drug delivery techniques. The key polyelectrolyte behavior provides tailored activities for devising objectives that trigger the structural capability [58].

Grounded on the nanoparticle cargo-carrying platform of the microalgae concept, the drug delivery for the chemotherapeutic aspect to the target tumor cell is defined. Microalgae linkage with the cargo was directed by magnetic action along with the property of biocompatibility in *Chlamydomonas reinhardtii* by peptide photocleavage under ultraviolet light irradiation [4]. It ensures accurate chemo drug transport to the metastatic region.

7.4 Sanative competency of algal extracts: Lipids and pigments

A wide range of saturated and unsaturated fatty acids are remarkable components found in the chloroplasts that are chiefly used as secondary metabolites (Table 14.2). The constituent essential lipids include MUFA (monounsaturated fatty acids) and PUFA (polyunsaturated fatty acids) algal fatty acids [15]. The established lipid content of the algae accounts for 0.12%–6.73% of dry weight [60]. They are acquired from glycolipids, phospholipids, and nonpolar glycerolipids [61].

7.5 Clinical relevance of algae fatty acids

The fatty acids supplements major health benefits to humans are in ensuring relief from various ailments and treating chronic illnesses, including cardiovascular diseases, neurological illness, renal disease, and hypertension [62]. The prime fatty acids biologically include omega-3 (ω-3) and omega-6 fatty acids (ω-6). The indispensable fatty acids that are not produced within the human body are obtained from outside sources [63]. They are α-linolenic

TABLE 14.2 Fatty acids/lipids: The bioactive compound [59].

Fatty acid	Source	Use
α-Linolenic acid	*Spirulina platensis*	Antiinflammatory and antioxidative property
ϒ-Linolenic	*Arthrospira* sp.	Used for newborns Nutritional supplements
Docosahexaenoic acid (DHA)	*Phacodactylum* sp. *Crypthecodinium* sp. *Schizochytriam* sp.	New-born formulae Nutritional supplements
Arachidonic acid	*Porphyridium* sp.	Nutraceuticals and cosmetics
ω-3 Fatty acids	*Sargassum fusiforme*	Anticoagulant properties Antiinflammatory properties

acid, linolenic, γ-linolenic, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid. They assist in the regulation of cellular activity [61].

ω-3 fatty acids can do wonders and have some mystic properties that ensure the proper development of the baby in pregnant women. It can reduce the risk of maternal depression in women during pregnancy by maintaining neuroinflammatory actions [64]. In their study, Chen et al. illustrated that the medicinal use of *Sargassum fusiforme* can be advised for treating cardiovascular diseases [59]. They also have anticoagulant and antiinflammatory properties [65]. The Food and Drug Administration (FDA), has certified the formulation of omega-3-based medicated drugs for the prevention of coronary heart diseases and is highly trending. Epanova and Lovaza are the stimulating compounds owned by the fish oil, which is composed of seaweed oil [66]. Key components of the drug are eicosapentaenoic acid and docosahexaenoic acid which are responsible for restricting fatty acid esterification. It suggest that these drugs are suggested to minimize triglyceride levels in patients with elevated hypertriglyceridemia [67].

7.6 Valuable virtue: Algal pigments

Commercial microalgal pigments have premier applications in the pharmaceutical industry [68]. Fig. 14.4 shows distinct pigments entail qualitative criteria showing divergent antioxidant, anticancerous as well as antiinflammatory activities [69–71]. Among them are the commonly known pigments, β-carotene, astaxanthin, phycobiliprotein, alkaloids, and terpenoids [72]. *Dunaliella salina* is the prime source of β-carotene; approximately 14% of its biomass contains this pigment [53]. It is a pragmatic source of vitamin A that contains antioxidative properties that can assist in oxidative stress protection. *Dunaliella salina* is a necessary microalga that consists of photosynthetic pigment, β-carotene and is an outstanding source of vitamin C [69–72]. Another holistic biomedical pigment, Astaxanthin, a red-orange colored pigment richly found in green microalgae *Haematococcus pluvialis* has recently gained recognition due to its vigorous bioactivities anticancer, immunomodulatory, and antioxidant

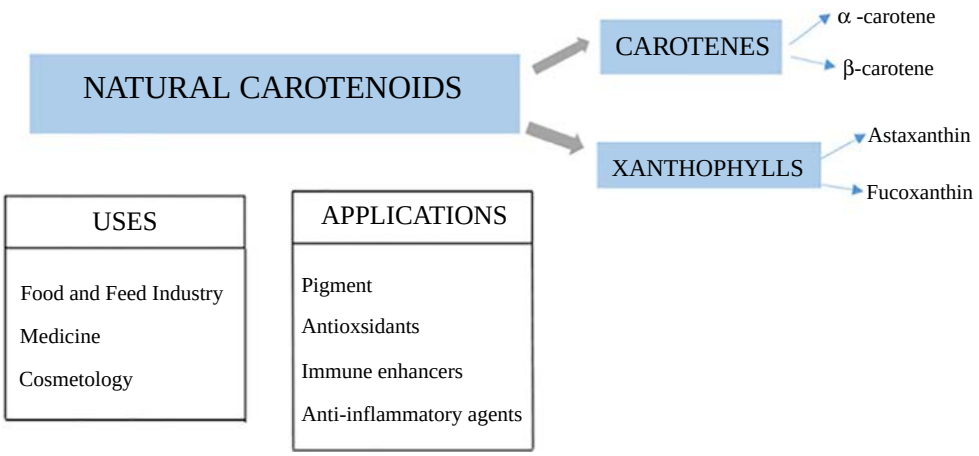

FIG. 14.4 Algae pigments and their applications.

[69,73,74]. The isolation of violaxanthin from *Dunaliella tertiolecta* inhibits the growth of the MCF-7 breast adenocarcinoma cell line by acting as reactive oxygen scavengers (scavengers of singlet electron species). This enhances their antioxidant activity, which enables them to inhibit cancer proliferation [20]. Among the numerous applications, astaxanthin compounds have demonstrated vigor for immune-stimulatory responses, acting as an immunomodulator in the body [10]. Phycobiliproteins are highly stable pigments, found in the chloroplasts of cyanobacteria and red algae, which are well-known for their antioxidative property. *Nostoc spongiaeforme* owes special pigments to allophycocyanin and phycocyanin whoes versatility possesses the potential to quench hydroxyl, superoxide, and alkoxy-radicals [37].

7.7 Unraveling the polyphenolic content

Polyphenols are the fundamental compounds that are categorized into two types, phenolic acids and flavonoids, and are profoundly involved in the healthcare industry [61]. These wondrous phenolic compounds exhibit antidiabetic, antiproliferative, and hemolytic properties with a degree of polymerization that eminently excels in the domain of biomedical application [75–77]. Divyashree et al. have observed that polyphenols, including glycoproteins and lipopeptides, have been screened for drug development and are efficient in inhibiting cancer cell proliferation [15]. Brown algae produce phlorotannins as a phenolic component, which is a biopolymer composed of 5%–30% of dry weight within the cell [61]. According to Korzeniowska and others, the division of the phlorotannins is done based on monomer blending [61]. The phlorotannins with the phenyl linkages are proposed as fuels. The phlorotannins constituting the ether linkages are termed phloroethols, whereas the phlorotannins with the dibenzodioxin chain with phenoxyl substitution are defined as Eckols [78]. Phlorotannins determine the prospects of pharmacology, promote health in numerous ways, and perform antitumor actions by inhibiting metastasis and stimulating the apoptosis of cancerous cells [79].

They have emphasized chelators and halted the activities of many enzymes. Phlorotannin is unique in itself because it is only found mainly in brown algae. It has an excellent structural framework with a functional ability that identifies it as a promising agent against bacterial and viral activities [8]. Phlorofucofuroekol is a phlorotannin derived from Ecklonia cava and other subspecies that acts as an antiinflammatory, possessing anticancer and antidiabetic features [61]. It triggers stimulation of adipocytes glucose uptake by protecting β-pancreatic cells against high glucose oxidative stress [78,79]. The metabolic syndromes are specifically related to other disorders that can be controlled by fostering nutraceuticals that may reduce antidiabetic complications. Other brown algae extracts, specifically Sargassum wightii, inhibit α-amylase and α-glucosidase activity [80]. The surging rates of neurodegenerative diseases among individuals make it elemental to have a drug providing neuroprotective action [81]. It can affect cognitive as well as neural abilities, leading to lifetime illness in a person. The phlorotannin inhibitory effect on the neurotransmitters produced during Alzheimer's and Parkinson's disease has outshined its clinical role [82]. Red algae, *Symphycladia latiuscula* have an array of multiple biological activities. It is a hub of bromophenols that precisely inhibits the cholinesterase enzyme, which causes Alzheimer's disease [83]. The pharmacological

attributes of phlorotannins are utilized in the therapeutics of sleep disorders. They are administered as a source of hypnotic drugs utilized for sedation and insomnia medication [84]. The functionality of the polyphenolic compounds is precious in itself, as only a few types of algae are present around the world from which these bioactive compounds can be derived. Due to their enormous potential in the therapeutic and cosmetic sectors, polyphenols, including phlorotannins, have emerged in a new era of biotechnology [85].

7.8 Budding potential of algae in cosmeceuticals

The changing lifestyle pattern has harmfully damaged the skin, causing terrorizing effects. The severity of these skin conditions can range from a lesion to cancer [61]. The propelling features of algal-derived derivatives have excelled in their utilization in skin treatment as well. Starting from scratch, the microalgae owe wound healing properties to their implementation in the formulation of skincare products [53]. Algal phytochemicals assist in treating these skin disorders. The umbrella of skin conditions detrimentally pacifies, inter alia, pigmentation, erythema, wrinkles, carcinoma, and more [86]. The cosmeceutical aspect of pharmacy has primarily encompassed the utility of different derivatives, phlorotannins, sulfate polysaccharides, −3 fatty acids, and carotenoids. The physiological hallmarks of these compounds make them eligible, comprising antiinflammatory, antiallergic, antiaging, antiwrinkle, and whitening attributes [87].

7.9 Revealing the antiaging secret

Everyone wants flawless skin, a shining face with no discoloration and no dryness problems. With increasing age, skin loses its elasticity due to exposure to heavy metals and lack of nutrition, which pessimistically affects the epidermis [88]. In recent years, analysis has been done on biopolymer compounds of microalgae, namely Fucoidan, which protects the skin from aging and has enhanced its future in biomedicine. The revitalization of the skin via an extract of *Chlorella vulgaris* stimulates collagen formation in the skin, leading to wrinkle reduction [86,87]. The oral administration of astaxanthin essence from *Hematococcus pluralism* has proven beneficial due to its antioxidant properties, which favor a balance between the oxidative and nonoxidative forces initiating wound healing [69]. The algal species that enhance the skin's features are *Colpomenia, Gracilaria,* and *Padina* [88]. Vitamin-E algal products provoke skin rejuvenation because of their antioxidative properties. An interesting microalgae component has fascinated scientists with its ultraviolet-protection facilitation. These are the mycosporine-like amino acids, abbreviated as MAAs, which are found in *Porphyra umbilicalis* [89].

7.10 Algal wonders in skin whitening and moisturizing

Arthospora and *Chlorella* are commonly used in the formulation of cosmetics [53]. Various algal polyphenols like phlorotannins obtained from brown algae, *Ecklonia cava,* or *Eisenia arborea* possess a certain antiallergic property capable of inhibiting hyaluronidase which causes itchy skin and is attributed to histaminic activity [61]. The harmful UV radiations

radiation is soaked up by the melanin pigment present in the skin, which act is a guarding barrier. The sun's irradiation aids in tyrosine synthesis, which catalyzes various reactions whose oxidation leads to the production of additional melanin, which must be restricted [88]. The phlorotannin content is involved in the maintenance of the skin pigmentation process. It inhibits the activity of the tyrosinase enzyme, which initiates the skin pigmentation process. The pigments from brown algae, *Laminaria japonica*, also reduce tyrosinase activity [87].

Skin bruising and drying ultimately deteriorate the skin's integrity, causing eczema. Skin quality can be retained by the utilization of moisturizers with certain acids like hyaluronic acid that help in keeping the skin hydrated [90]. Some nontoxic, economical algal polysaccharides like carrageenan and fucoidans are known to regulate the skin by maintaining water circulation in the skin (Table 14.3). Other microalgae species like *Chondrus crispus* provide a soothing effect on the skin and help with water absorption that ultimately keeps the skin moisturized in extremely hot environments [91].

8 Future direction and challenges

As discussed, algae are enriched with bioactive substances such as polysaccharides, carotenoids, sterols, phlorotannin, fatty acids, minerals, and vitamins. Moreover, it contains

TABLE 14.3 Biomedical dermal applications of pigments [88].

Application	Organism	Type of algae	Bio-active compound (pigment)
Antiwrinkling	• Sea lettuce (*Ulva lactuca*) • Sea palm (*Postelsiapal maeformis*) • *Ascophyllum nodosum* • *Spirulina* sp.	• Green algae • Brown algae • Brown algae • Blue-green algae/ cyanobacteria	• Chlorophyll-a, chlorophyll-b, β-carotene • Chlorophyll-c, fucoxanthin • Chlorophyll-c • Phycocyanin
Antiaging	• *Ascophyllum nodosum* • *Chlorella vulgaris*	• Brown algae • Green algae	• Chlorophyll-c • Chlorophyll-a, chlorophyll-b, β-Carotene
Antioxidant	• *Spirulina* sp. • *Isochrysis* sp.	• Blue-green algae/ cyanobacteria • Brown algae	• Phycocyanin • Canthaxanthin, fucoxanthin
Moisturizing and thickening agent	• *Chlorella vulgaris* • Sea lettuce	• Green algae • Green algae	• Chlorophyll-a, chlorophyll-b, β-Carotene • Chlorophyll-a, chlorophyll-b, β-carotene

immune-adjusting and disease-fighting properties; hence, it has been utilized in food regimens and nutritional therapies for a long time. The potential of algae as a medicinal and cosmeceutical component has recently received a lot of attention. Because their antiacne, antioxidant, antiaging, antiinflammatory, melanogenesis suppression, UV photoprotective, and antimelanoma capabilities, algae have a lot of promise in dermatological therapy as well [10,11]. Algae have clear benefits as a source of bioactive compounds due to their enormous diversity, rapid and simple cultivation when compared to their terrestrial counterparts, which also consist of biomolecules of biomedical importance [11,92]. Hence, it would not be amiss to state that algae-derived bioactive compounds seem to have a prominent future in the pharmaceutical and cosmeceutical markets.

However, there are certain drawbacks in order to take full advantage of algae bioactive substances. To begin with, extracting useful components from algae is typically challenging and time-consuming. Therefore, more efficient and cost-effective extraction techniques for bioactive compounds must be developed to commercialize them. Nonetheless, the regulation, profitability, and traceability of algae-derived products are critical [93]. Furthermore, modification of algae culture techniques is required to attain increased productivity and high bioactive content. Also, traditional approaches to developing high-performance algal strains for bioactive synthesis may be inefficient. As a result, substantial advancements in this field may require long-term study. However, for the algae-based bioactives in the biomedical business to mature, the market must test the true effects.

9 Conclusion and future outlook

As stated in the present chapter, microalgae owing to their high-value-based metabolites are utilized in various industries, ranging from food, pharmaceuticals, and biorefinery industries. However, there are some obstacles in the manufacturing process. The growth of algae in photobioreactors on a large scale necessitates a significant investment. It may be possible to build a sustainable approach to growing algae on a large scale while lowering manufacturing costs. Furthermore, optimizing the parameters of algae growth and the bioreactor for fermentation can be investigated. Microalgal species can be tested in order to obtain the appropriate algal biomass. Hence, there is a need to develop more efficient operations and maintenance of equipment units, that involve reduced costs and high recovery of bio-products.

Also, with the improvements in technologies and equipment used in the bioprocessing of algae, the utilization and applications of algae can become broader and wider in other domains of the industry. By adopting novel biotechnological strategies in culturing methods, the overall algae yield can be promoted. The cost involved in the production of algae and investments in equipment are among the major factors that limit its use. Harvesting methods that are highly effective and involve low energy requirements must be employed for the extraction of high-value-based metabolites from algae. The integrated algal system is potentially recognized as an efficient weapon against several diseases in a cost-friendly way. The

multidimensional approach to utilizing the valuable extracts of algae has systematically established a new paradigm shift in improving the biotechnology sector. Nonetheless, the heightened employment of algae species in today's era will modify the biotechnological aspect of medical essence.

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CHAPTER

15

Biochemical profiling, transcriptomic analysis, and biotechnological potential of native microalgae from the Peruvian Amazon

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1 Introduction

Microalgae are a ubiquitous and diversified group of photosynthetic microorganisms with a cosmopolitan distribution. About three decades ago, some researchers estimated that there are ~124,000 microalgae species [1,2], and these microorganisms are even more interesting because their endemism is high [2–4]. However, only a small fraction of microalgae cells have been isolated to date and are conserved in microalgae culture collections worldwide [5,6]. Only a few hundred of these "domesticated" microalgae biochemical profiles have been studied [7–13], and the genomic resources exist only for a more limited number of microalgae species, such as the model microalgae *Chlamydomonas reinhardtii* [14,15] and some commercially important species [16–20].

Therefore, it is important to increase efforts in bioprospection, isolation, biochemical, and molecular characterization of native microalgae in order to understand their genetic and metabolic capabilities to sustainably exploit them using biotechnological approaches. Motivated to overcome these needs, our research group has initiated primary research and established a collection of isolated native microalgae (approx. 100 strains) from the Peruvian Amazon. Some of these microalgae have been characterized at biochemical and molecular levels and determined their potential biotechnological use to produce biodiesel, nutrients, and several biochemical compounds with human health-promoting potential [9,21–26]. Consequently, the objective of this book chapter is to provide updated information based on published and unpublished results of our research team on the biochemical profile, transcriptome analysis, and biotechnological potential of native microalgae of the Peruvian Amazon.

2 Sampling, isolation, purification, and culture of native microalgae cells

Samples of superficial freshwater were collected from three river basins of the Peruvian Amazon (Amazon River [03°41′0.6″ S, 73°14′8.9″ W], Itaya River [03°43′1.4″ S, 73°14′17.8″ W] and Nanay River [03°42′0.2″ S, 73°15′32″ W]), then, to enrich the collected water samples with microalgal cells (stimulate cell division and growth), the water samples were proportionally mixed with Chu medium No. 10 [27] and cultured for one month in a culture area at $25 \pm 1°$ C, with a photoperiod regimen (12 h light: 12 h dark), light intensity of 80 μE m^{-2} s^{-1} provided by cool white fluorescent light, and constant homogenization at 110 rpm. Later, unicellular microalgae were isolated and purified, combining the cell washing, the serial dilution, and the streak plate techniques [6,28,29]. Repeated streaking on a solid medium was realized to ensure the purity and axenicity of the cultures. Further, axenic colonies emerging on agar dish were transferred into Chu liquid medium No. 10 and cultured, at the described conditions, in increasing volumes of culture medium according to their cell densities.

3 Morphological and molecular identification

Purified microalgae cells were identified using morphological characters according to Bicudo and Menezes [30]. Cells were recognized using a Carl Zeiss microscope (AxioLab. A1) and photographically recorded with an AxioCamERc 5 s digital camera.

For molecular identification, first, the genomic DNA of microalgae cells was purified using the cetyltrimethylammonium bromide method [31], and its quality and quantity were determined by standard optical density measurements [32] using a Nanodrop 2000 UV–Vis Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). In addition, genomic DNA intactness was evaluated by electrophoretic analysis [32]. Second, the rDNA second internal transcribed spacer (ITS-2) was amplified as reported by Kaur et al. [33] using the forward primer SQITS1 (5′-GAGCATGTCTGCCTCAGC-3′) and reverse primer SQITS2 (5′-GGTAGCCTTGCCTGAGC-3′) in a thermal cycler Realplex S^4 (Eppendorf, Hamburg, Germany). Amplicons were resolved by agarose gel electrophoresis and purified with ExoSAP-IT (Affymetrix, Santa Clara CA, USA). Finally, both DNA strands of amplicons were sequenced using SQITS1 and SQITS2 primers according to the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Further, the cleaned sequencing products were analyzed on a Genetic Analyzer 3130XL (Applied Biosystems, Foster City, CA, USA). The rDNA ITS-2 gene sequences were blasted against the GenBank [34–36], and the direct fold and homology models were obtained with the ITS-2 ribosomal RNA database [37–39].

The results on the taxonomic identification, commonly at the genus level, of the native microalgae and strains isolated from the Peruvian Amazon displayed typical morphological characteristics and were corroborated with molecular analysis based on the rDNA ITS-2 gene sequences (Fig. 15.1).

4 Biochemical profiling

4.1 Proximate composition analysis

Microalgal biomass was oven-dried at 70°C and its weight was measured gravimetrically utilizing an analytical balance, Kern ABJ 220-4NM (Kern & Sohn GmbH, Balingen, Germany). Total lipids were extracted and purified from 0.05 g of dried biomass according to Bligh and Dyer [40] and then determined gravimetrically with a semi-micro analytical balance (Sartorius, MSU225S-000-DU, Foster City, CA, USA). Total carbohydrates were determined from 0.005 g of dried biomass following the proposed method of DuBois et al. [41], and the protein quantity was assayed from 0.005 g of dried biomass as reported previously [42]. Finally, ash quantities were determined gravimetrically after incinerating 0.1 g of dried biomass based on the AOAC method [43]. All proximate composition analyses were achieved using three replicas, and results are displayed as mean plus–minus standard deviation.

The results of the proximate composition analysis (Fig. 15.2) of the eight microalgae species showed that carbohydrates ($23.81 \pm 8.58\%$) are the biomolecules most abundant compared with proteins ($20.23 \pm 5.68\%$), lipids ($13.34 \pm 6.09\%$), and ashes ($3.10 \pm 1.07\%$). Also, it is evident that these main biochemical compounds present marked differences in both species and strain levels. Similar observations have been reported by several research groups worldwide [10,44–46].

4.2 Fatty acid analysis

First, fatty acid methyl esters (FAMEs) were produced by acid transesterification as reported by Ichihara and Fukubayashi [47] and then resolved by a gas chromatograph, Varian CP-3800 GC (Agilent Technologies, Santa Clara, CA, USA) assembled with an automatic

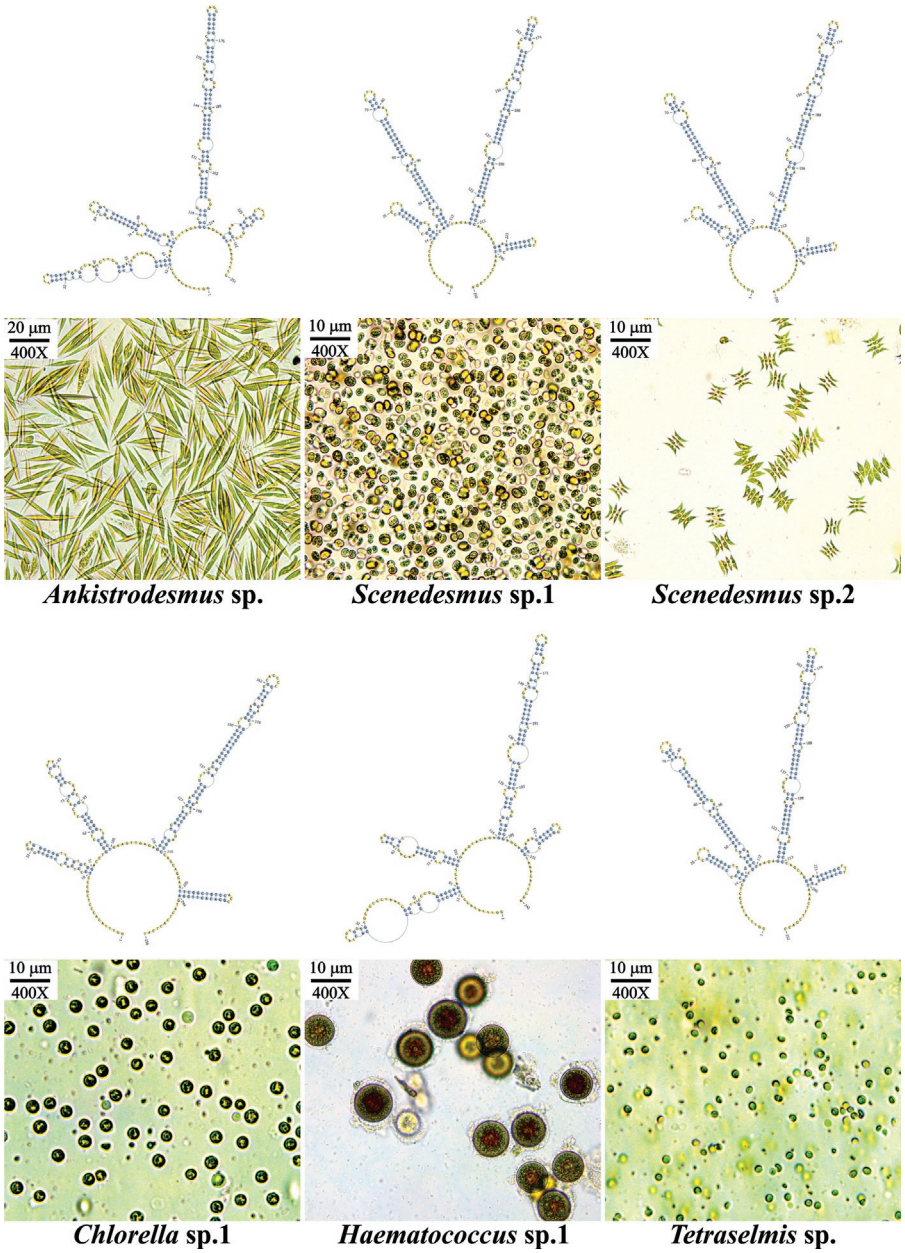

FIG. 15.1 Photomicrographs and typical ITS-2 secondary structures of six native microalgae strains of the group chlorophyte isolated from the Peruvian Amazon.

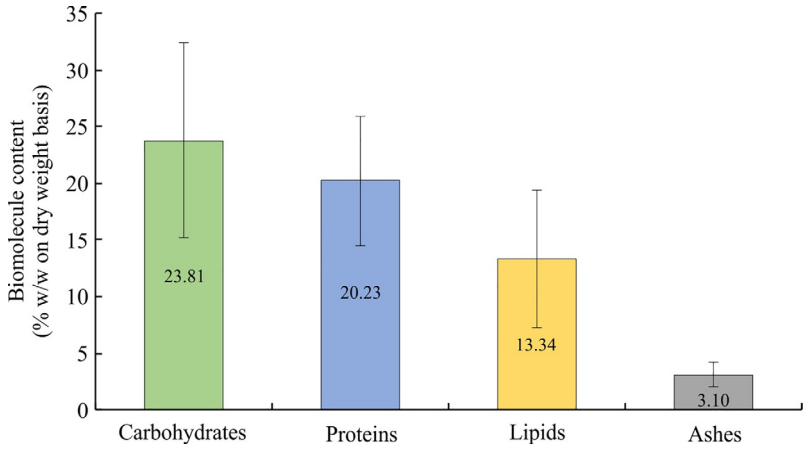

FIG. 15.2 Proximate composition (total carbohydrates, proteins, total lipids, and ashes content) of the dried biomass of eight native microalgae strains isolated from the Peruvian Amazon.

sampler/injector, a flame ionization sensor, and a Stabilwax capillary column of 30 m × 320 μm × 0.25 μm (Restek, Bellefonte, PA, USA). Fatty acids (FA) were identified by comparing their retention times with FAMEs standards (Nu-Check Prep, Elysian, MN, USA). Tricosanoic acid methyl ester (Sigma-Aldrich, Saint Louis, MO, USA) was incorporated into the samples as an internal standard. Chromatograms generated were examined with the software Galaxie Chromatography Data System Version 1.9.3.2 (Agilent Technologies, Santa Clara, CA, USA).

The microalgae strains are composed of saturated FA, monounsaturated FA, and polyunsaturated FA (PUFAs), although with distinct FA profiles [9]. Variations in the FA profiles are a frequent pattern in microalgae strains isolated from aquatic sources [48–52]. With regard to the content of FA, the three microalgae strains with the maximal FA content are *Scenedesmus* sp. 2 (approx. 200 mg g^{-1} of mbdw), *Chlorella* sp. 1 (approx. 85 mg g^{-1} of mbdw), and *Ankistrodesmus* sp. (approx. 81 mg g^{-1} of mbdw). Related to PUFAs, the microalgae of the chlorophyte group biosynthesize six types of PUFAs with variable quantities in interval values from 0.11 ± 0.06 to 12.81 ± 5.99 mg g^{-1} of mbdw (Fig. 15.3). With regard to very long-chain PUFAs, exclusively the microalgae of the *Haematococcus* genus produce eicosapentaenoic acid. However, none of the studied microalgae biosynthesize docosahexaenoic acid.

4.3 Amino acid analysis

To determine the amino acid profiles, 30 mg of dried biomass was acid-hydrolyzed (2 mL of 6 M HCl) at 112°C for 22 h [53]. Then, the free amino acids were chemically modified with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate according to Cohen and Michaud [54]. Finally, derivatized amino acids were identified utilizing a Hitachi Elite LaChrom HPLC System (Hitachi High Technologies, San Jose, CA, USA) assembled with an L-2130 HTA pump, L-2350 column oven, L-2200 autosampler, L-2485 fluorescence sensor, L-2455 DAD, and a Thermo Scientific Hypersil GOLD C18 Selectivity HPLC Column of 15 cm × 4.6 mm × 5 μm

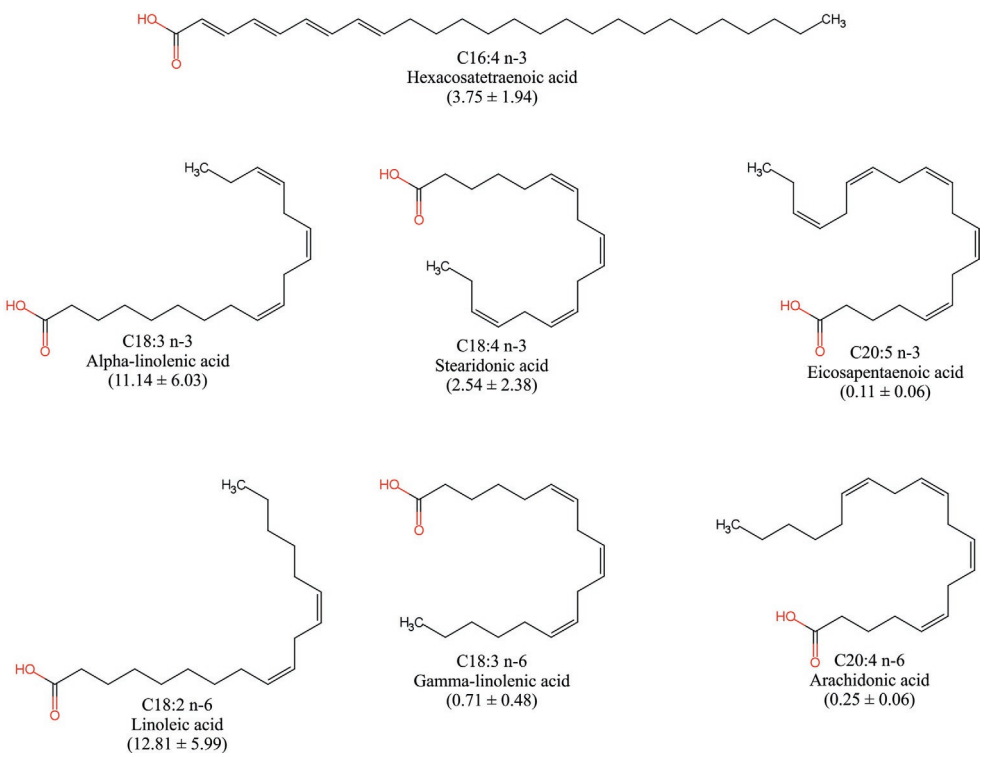

FIG. 15.3 Types and mean content of PUFAs of eight native microalgae strains isolated from the Peruvian Amazon. The values of PUFAs content within parenthesis are expressed as mg g^{-1} of mbdw.

(Thermo Fisher Scientific, Waltham, MA, USA). Chromatograms produced were analyzed with the EZChrom Elite software v 3.2.1 (Agilent Technologies, Santa Clara, CA, USA).

The amino acid analysis results show that native microalgae strains of both groups (cyanobacteria and chlorophyte) contain essential amino acids (EAA) and nonessential amino acids (NEAA). Also, the amino acid composition profiles are variable, and the quantities of single amino acids differ significantly. The cyanobacteria group had the highest amino acid content (from ~468 to ~487 mg g^{-1} of mbdw), while the chlorophyte group had the lowest ($\leq$433 mg g^{-1} of mbdw). In the EAA group, the most abundant amino acids were leucine and phenylalanine (plus tyrosine), whereas methionine (plus cysteine) and histidine were scarcer (Fig. 15.4). In the NEAA group, the major amino acids were Asx (aspartic acid + asparagine), serine, and alanine, and the minor was proline [9]. These findings are consistent with previous studies that found microalgae strains capable of biosynthesizing the 20 amino acids contained in proteins [10,55–57]. Also, this is in agreement with the transcriptomic analyses of the microalgae *Ankistrodesmus* sp. UCP001, which presents all the metabolic pathways for the biosynthesis of these amino acids [22].

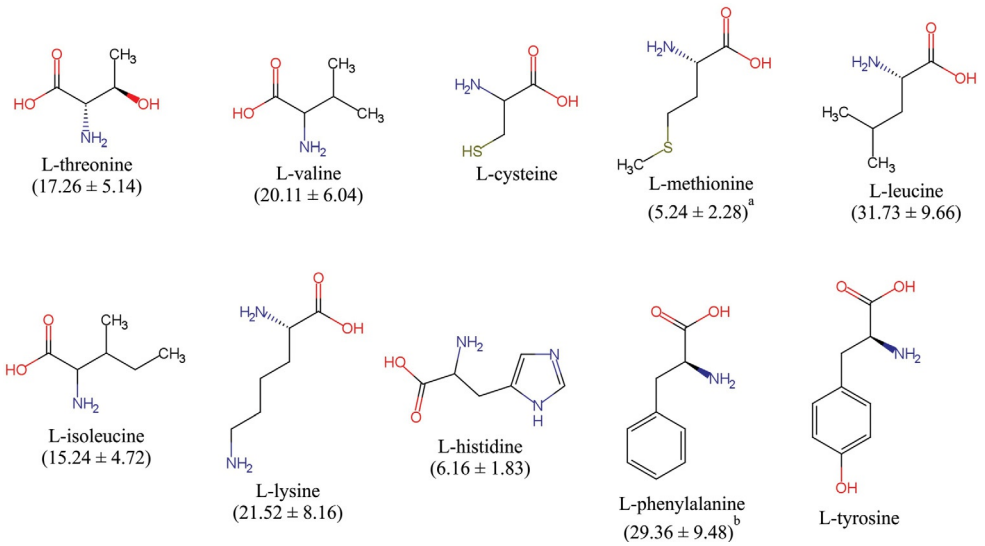

FIG. 15.4 Types and mean content of essential amino acids of eight native microalgae strains isolated from the Peruvian Amazon. The values of amino acid content within parenthesis are expressed as mg g^{-1} of mbdw.

4.4 Antioxidant activity and total phenols content analysis

Two hundred milligrams of dried biomass were finely ground in a mortar and pestle, adding 2 mL of hydromethanol solution (methanol 80% + water 20% [*v*/v]). Then, ground biomass was placed into a 15 mL tube containing 2 mL of hydromethanol solution, homogenized for 15 min at 25°C in a vortex (VM-10, Daihan Scientific, DKI Jakarta, Indonesia), then centrifuged (5000 × g, 15 min, 5°C) and the supernatant was separated. The hydromethanol extraction was repeated once more, the pooled supernatants were put into a graduated cylinder, and methanol was added to reach 5 mL. The antioxidant activity of the hydromethanolic extracts was assayed according to Sharma and Bhat [58]. This method is based on the capability of hydromethanolic extracts to scavenge the free radical generator DPPH (2,2-Diphenyl-1-picrylhydrazyl hydrate). Total phenol content was estimated by the Folin–Ciocalteu method [59], which is based on the redox reaction, under alkaline conditions, between the phenolic compounds and phosphomolybdic/phosphotungstic acid complexes. The reduced metal oxides generate a blue color with a maximum absorbance peak at 765 nm [60,61].

Results of antioxidant activity and total phenol content assays of eight native microalgae showed that the hydroalcoholic extracts have ability to neutralize free radicals, with considerable variation among microalgae of the chlorophyte group (Fig. 15.5). The major values of antioxidant activity were recorded in *Chlorella*, *Haematococcus*, and *Tetraselmis* genuses (from 131.39 ± 12.07 to 425.25 ± 36.07 μM of trolox equivalent g^{-1} of mbdw), but the minor values were registered in the *Scenedesmus* genus (< 100 μM of trolox equivalent g^{-1} of mbdw) [9]. The antioxidant activity of hydroalcoholic extracts obtained from marine and freshwater

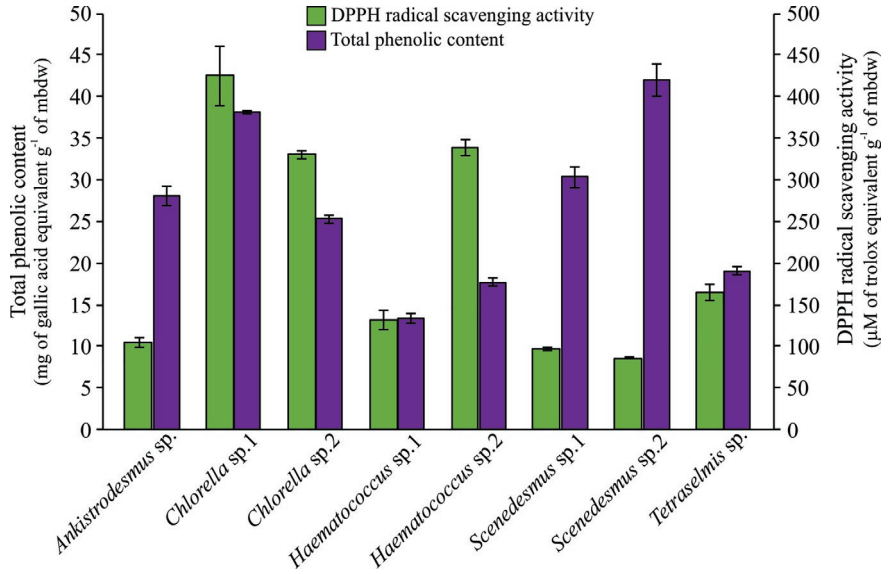

FIG. 15.5 DPPH scavenging activity and total phenolic content of eight native microalgae strains isolated from the Peruvian Amazon.

microalgae has been previously evidenced around the globe [62–65]. Additionally, the hydroalcoholic extracts are composed of phenols in variable quantities from 13.35 ± 0.53 (*Haematococcus* sp. 1) to 41.90 ± 1.95 (*Scenedesmus* sp.2) mg gallic acid equivalent g^{-1} of mbdw [9]. These bioactive molecules are common constituents in microalgae cells of marine and freshwater origin [63,66–69]. Together, this scientific evidence suggests that these microorganisms have biosynthetic metabolic pathways to produce phenolic compounds [70].

4.5 Chlorophylls a and b content analysis

Chlorophylls were extracted from 50 mg of the dried microalgae biomass with 5 mL of 100% acetone. Acetonic extracts were filtered with 4.5 μm PTFE syringe filters, and then 20 μL of the filtered organic solutions were injected and analyzed utilizing a Hitachi Elite LaChrom HPLC System (Hitachi High Technologies, San Jose, CA, USA) assembled with an L-2200 autosampler, L-2350 column oven, L-2130 HTA pump, L-2485 fluorescence sensor, L-2455 DAD, and a Thermo Scientific Acclaim C30 LC reversed-phase HPLC Column of 25 cm × 4.6 mm × 5 μm (Thermo Fisher Scientific, Waltham, MA, USA). Chromatograms generated were analyzed with the EZChrom Elite software v 3.2.1 (Agilent Technologies, Santa Clara, CA, USA).

The results of the chlorophyll pigment analysis showed fluctuations in the content of both chlorophyll a (from 31.45 to 430.15 μg $g^{-1}$of mbdw) and chlorophyll b (from 5.37 to 281.57 μg $g^{-1}$of mbdw). The average contents were 305.81 and 108.29 μg $g^{-1}$of mbdw for chlorophyll a and chlorophyll b, respectively. Also, both chlorophylls displayed a typical absorption spectrum and marked differences in their elution times under the HPLC analysis (Fig. 15.6).

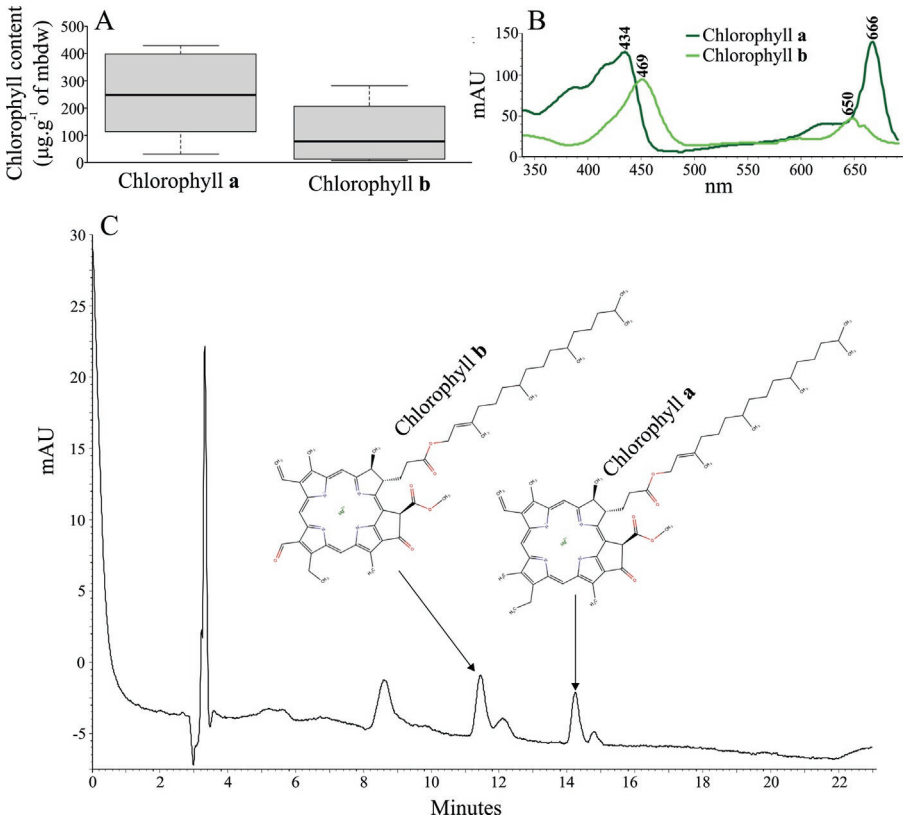

FIG. 15.6 Types and content of chlorophylls extracted from eight native microalgae strains isolated from the Peruvian Amazon. (A) Mean content, (B) absorption spectrum and (C) elution times under HPLC analysis of chlorophylls a and b.

5 De novo transcriptomic analysis

For this analysis, first, total RNA of *Ankistrodesmus* sp. UCP0001 was purified from fresh microalgae biomass [71], and its quality and integrity were assayed by electrophoretic [32] and spectrophotometric methods using a NanoDrop 2000 Spectrophotometer (ThermoFisher Scientific, MA, USA). Also, mRNA molecules were obtained from 20 μg of total RNA utilizing the Sera-Mag magnetic oligo (dT) beads (Illumina). Second, mRNA molecules were fragmented, then cDNA was generated according to the SuperScript double-stranded cDNA synthesis kit (Invitrogen, CA, USA). Further, synthesized cDNA was end-repaired, phosphorylated, and adenylated in 3′ termini with Klenow fragment (3′ → 5′ exo minus), and paired-end adapters were ligated to the ends of these 3′-adenylated cDNA fragments. Fifteen cycles of PCR amplification were carried out with Thermo Scientific Phusion High-Fidelity DNA Polymerase. Later, the cDNA library was constructed with a 200 bp insertion fragment, validated using an Agilent 2100 Bioanalyzer (Agilent Technologies, CA,

USA), and finally, the sequences were produced utilizing an Illumina HiSeq 2000 Sequencing Platform (Illumina Inc., San Diego, CA, USA).

Raw sequence reads were cleaned with Trimmomatic v0.36 [72]. Then, high-quality sequences were de novo assembled using Trinity with default settings [73]. The functions of the unigenes obtained were predicted using the annotation tool Blast2GO [74] to ascribe Gene Ontology terms, Enzyme Commission numbers, and functional domains to query sequences (cutoff $E_{value} \leq 10^{-6}$). Metabolic pathways were reconstructed by mapping the sequences with assigned Enzyme Commission numbers to the Kyoto Encyclopedia of Genes and Genomes metabolic pathway database [75]. Additionally, to enrich the metabolic pathway annotations and identify the BRITE functional hierarchies, assembled sequences were also submitted to the Kyoto Encyclopedia of Genes and Genomes Automatic Annotation Server [76].

The results of the de novo transcriptome analysis of *Ankistrodesmus* sp. UCP0001 showed that 38,414 unigenes (mean size = 508 bp, N50 = 1038 bp) were generated [22]. Based on the assembled and annotated transcriptome, several metabolic pathways have been reconstructed, such as those involved in fatty acids biosynthesis and elongation (e.g., polyunsaturated fatty acids), triacylglycerol biosynthesis, ascorbate and aldarate metabolism, nitrogen compound metabolism (e.g., amino acids, purine, pyrimidine, etc.), carotenoid biosynthesis, phenylpropanoid biosynthesis, porphyrins biosynthesis, and multiple additional pathways [22,25]. Of these metabolic pathways, details of the chlorophyll a and b biosynthetic pathways are shown in Fig. 15.7A. In this pathway, two 5-aminolevulinate molecules, the universal precursor for tetrapyrroles biosynthesis (e.g., chlorophylls, vitamin B12, heme, etc.) [77] condense to form porphobilinogen. Further, four porphobilinogen molecules are assembled to produce hydroxymethylbilane. Hydroxymethylbilane cyclizes to generate uroporphyrinogen III, which by consecutive decarboxylation and oxidative decarboxylation produces protoporphyrinogen IX and by oxidation, generate, protoporphyrin IX. Then, a magnesium chelatase (EC: 6.6.1.1) incorporates Mg to the macrocycle of protoporphyrin IX to produce Mg-protoporphyrin IX. The last compound is esterified at the propionate moiety to biosynthesize Mg-protoporphyrin IX 13-monomethyl ester. Then, one methyl propionate side chain (between the C and D rings) is cyclized to produce divinyl protochlorophyllide, and this compound is transformed to monovinyl protochlorophyllide by a reductase (EC 14.3.1.75). Subsequently, both divinyl and monovinyl protochlorophyllides are modified to chlorophyllide by the stereoselective reduction of the C17=C18 double bond. This biochemical reaction is a key step in chlorophyll biosynthesis and is catalyzed by two structurally unrelated enzymes in microalgae (Fig. 15.7B) and several photosynthetic organisms: the dark-operative protochlorophyllide oxidoreductase (DPOR; EC 1.3.7.7) and the light-dependent protochlorophyllide oxidoreductase (LPOR; EC 1.3.1.33). DPOR is a three-subunit enzyme complex (subunits B, L, and N) encoded in the chloroplast genome and powered by ATP hydrolysis [77–79]. In contrast, LPOR is a single polypeptide enzyme encoded in the nuclear genome, that is powered by light (photoenzyme) and requires NADPH for its catalytic activity [80,81]. Next, chlorophyll synthase (EC 2.5.1.62) catalyzes a covalent union of a polyisoprene phytol tail with a propionate moiety located in the D ring of chlorophyllide a, producing a mature chlorophyll a molecule [77,82]. To produce chlorophyll b, the enzyme chlorophyllide a oxygenase (EC 1.14.13.122) catalyzes two sequential hydroxylations at the 7-methyl group of chlorophyllide a producing the aldehyde hydrate, which spontaneously

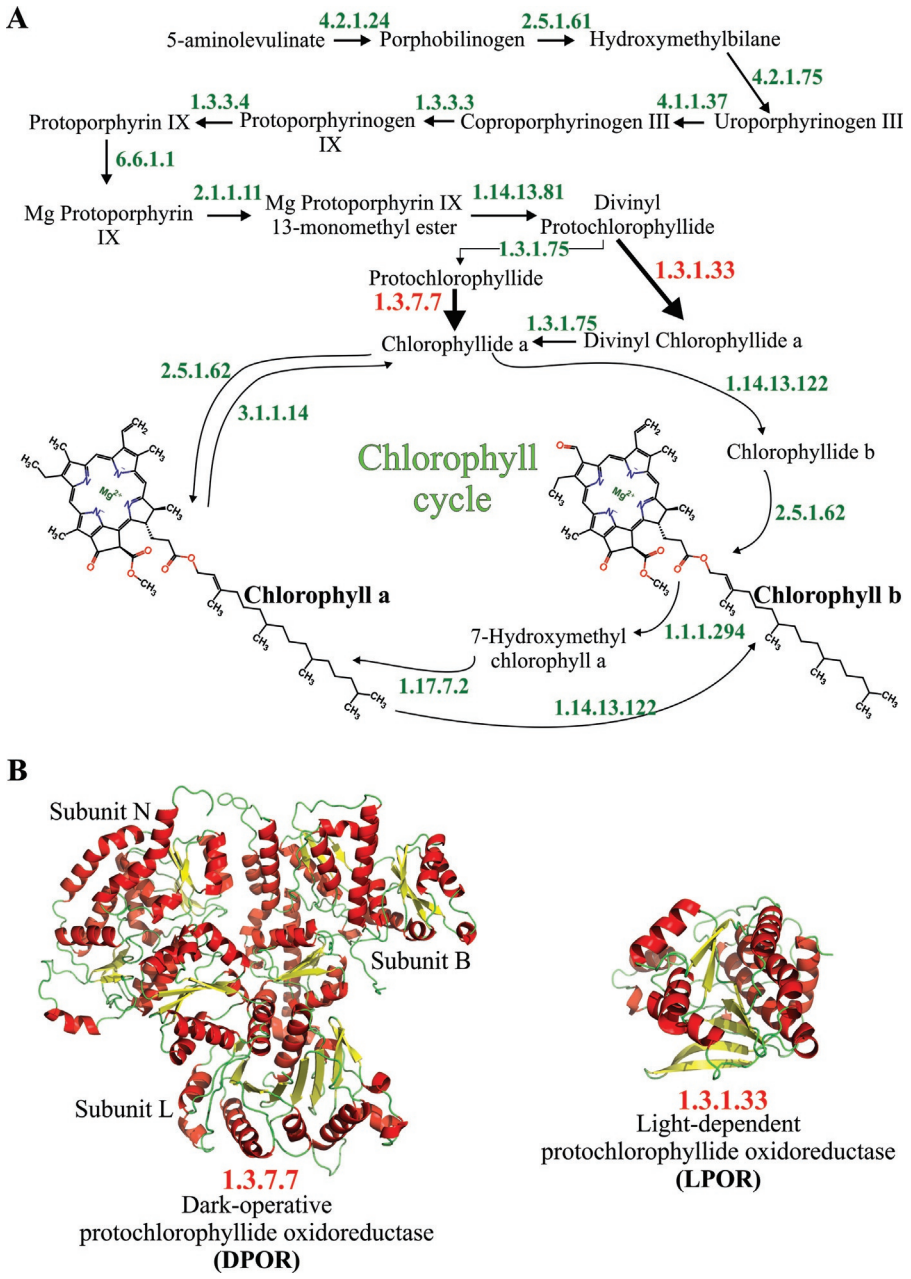

FIG. 15.7 Chlorophylls a/b biosynthesis, chlorophyll cycle pathways, and key enzymes predicted based on the assembly and annotation of the *Ankistrodesmus* sp. UCP001. (A) Metabolic pathways and (B) 3D predicted structures of two key enzymes involved in the production of chlorophyllide.

releases a water molecule and forms chlorophyllide b [83,84]. This compound is also, through their propionate moiety of the D ring, is covalently linked to a polyisoprene phytol tail to produce chlorophyll b. Finally, reduction of chlorophyll b to chlorophyll a by the consecutive action of two enzymes (EC 1.1.1294 and EC 1.17.7.2) and its hydrolysis to chlorophyllide a by chlorophyllase (EC 3.1.1.14) completes the chlorophyll cycle [85–87]. The chemical structures of the two main chlorophyll pigments differ only in one side chain of the tetrapyrrole: chlorophyll a has a methyl group and chlorophyll b a formyl group at the corresponding position in the B ring [86].

6 Biotechnological potential

The native microalgae strains isolated from the Peruvian Amazon to date have multiple potential biotechnological applications. First, because these strains are cultivable under laboratory conditions and have shown axenic characteristics. These potential biotechnological applications include:

(1) *Biofuels production*: Some microalgae strains, such as *Ankistrodesmus* sp. and *Scenedesmus* sp., could be a source of renewable feedstocks for biodiesel and bioethanol production because of their high content of triacylglycerol and carbohydrates, respectively. Biofuel production and exploitation could help us to adopt the sustainable development goals in our country as a feasible option to the petroleum extraction industry. Nonetheless, it will be indispensable to create innovative culture methods for an extensive scale for microalgal biomass production of selected native strains and/or de novo microalgae strains generated with novel approaches (i.e., CRISPR/Cas, Transposon system) to produce biofuels more efficiently.

(2) *Nutraceuticals and bioactive compounds production*: Several microalgae and cyanobacteria isolated showed the ability to biosynthesize useful nutraceuticals and bioactive compounds such as polyphenols, essential amino acids, essential fatty acids, and several other compounds. Several of these compounds could be efficiently exploited using biorefinery approaches.

(3) *Recombinant protein production*: Our research group recently sequenced and functionally annotated some organellar genomes of *Ankistrodesmus falcatus*, both mitochondrial [26] and chloroplast (unpublished results) genomes. Based on this information, we could design appropriate transformation and expression vectors targeting these organellar genomes to produce several recombinant proteins for biotechnological applications. To date, our research team has designed in silico a chloroplast expression vector based on endogenous functional elements (i.e., promoters, terminators, 5′UTR, 3′UTR regions). However, to verify its functionality, it will need to be experimentally tested in a laboratory.

7 Conclusions and future outlook

Native microalgae isolated from the Peruvian Amazon have promissory characteristics for biotechnological applications, such as (1) producing suitable renewable feedstocks for biofuel generation, (2) biosynthesizing various human essential nutrients (e.g., PUFAs, amino acids,

etc.) and human health-promoting compounds (e.g., antioxidants, phenols, pigments, etc.), and (3) generating recombinant protein for multiple applications (e.g., petroleum spills, mercury released by illegal mining, etc.). However, in order to achieve these sustainable and environmentally beneficial biotechnological applications, high-level basic scientific research employing multiomic approaches is needed. This scientific data will allow us to develop de novo microalgal strains that can be utilized entirely with biorefinery strategies in the near future.

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CHAPTER

16

Algae in medicine and human health

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OUTLINE

1 Introduction

Marine algae, also known as seaweed, is a photosynthetic eukaryotic organism that lives in the marine environment. It is useful in the bioremediation of wastewater in the wastewater engineering field due to its physicochemical properties [1–3], as well as providing an abundance of medicinal benefits to humans due to the presence of numerous bioactive compounds [4]. Protein and amino acids, lipid and fatty acids, carbohydrates, and pigments (carotenoids, chlorophylls, and phenolic compounds) are all common bioactive chemicals found in algae [5]. Microalgae have been found to have bioactive substances that can treat chronic diseases like HIV, cancer, pulmonary arterial hypertension, hepatitis, diabetes, systemic lupus erythematosus (SLE), asthma, Alzheimer's, and cerebrovascular diseases (e.g., stroke) [6–10]. The cardiovascular [11], reproductive [12], and digestive systems [13] are examples of major organ systems that have been successfully treated with biopharmaceutical active compounds from algae. It includes poly-amino-saccharides (e.g., chitin and chitosan) that have antilipidemic and membrane-stabilizing capabilities, as well as a considerable reduction in the low-density lipoproteins/high-density lipoprotein (LDL/HDL) ratio in the blood for cardio-protective activities [14,15]. It supports pro-biotic bacterial development and has a gastric protective impact on the digestive system, as well as enhancing cell renewal for reproductive failure. It stimulates wound healing and acts as an antibiotic (e.g., phlorotannin). It is utilized in cosmetics and cosmeceutical products due to its antioxidants and antiaging properties [16,17]. Furthermore, it is the only phyto-source of arachidonic acid, a chemical compound that is regularly found in animals [18]. Arachidonic acid is a polyunsaturated acid that protects the brain from oxidative stress and maintains hippocampal cell membrane fluidity. Sulfated polysaccharides, methanolic extracts, fucoxanthin, fucoidan, alpha-amylase, and acidic polysaccharides are among the bioactive substances discussed in this book chapter.

Polysaccharides are polycarbohydrates made up of numerous monosaccharides that are linked together by glycosidic linkages. Sulfated polysaccharides can be applied as topical dermatology and muscular tissue relief [19]. Sulfated polysaccharides, also known as sulfated galactans, are polysaccharides made up of branched or unbranched chains of repeating galactose units. These types of polysaccharides have large molecular weights [20]. Despite this, the anticoagulant activities of the sulfated polysaccharides with molecular weights ranging from 61.9 to 216.4 kDa were similar to those of the original sulfated polysaccharide. The anticoagulant activity of polysaccharide fragments is reduced when their molecular size is reduced [21]. According to Mourão [20], sulfated galactans with less than 5 kDa (units in

kilodaltons for molecular masses of proteins, nucleic acids, and polymers) in molecular weight showed antithrombotic capabilities but no procoagulant activity. Partial acid hydrolysis can be used to synthesize sulfated galactans of a particular molecular weight [20].

Aging, cardiovascular disease, diabetes, and cancer are all caused by oxidative stress. Synthetic antioxidants such as propyl gallate, butylated hydroxyanisole, tert-butylhydroquinone, and butylated hydroxytoluene have negative effects on human health and, hence, their applications are recommended. Natural antioxidants containing methanolic extracts (MEC) such as flavonoids, phenols (catechin, gallic, and *p*-coumaric acids), and tannins have the capability to reverse oxidative stress in human lipids and lipoproteins caused by harmful chemicals in the human body [22]. The healing mechanisms provided by natural antioxidants are free radical quenching, catalytic metal binding, and oxygen scavenging, while providing safe antiinflammatory treatments [23].

Brown seaweed produces abundant carotenoid and fucoxanthin. It produces approximately 10% of the carotenoids in nature. In photosynthesis, fucoxanthin is a component of the light-harvesting complex [24]. Fucoxanthin molecules have been shown to have anticancer, antioxidant, antihypertensive, antiinflammatory, radioprotective, and antiobesity effects [25]. Fucoidans are a group of sulfated polysaccharides with a complicated structure. Brown algal cell walls are a good place to look for them. Antioxidant, antiinflammatory, antiallergic, antitumor, antiobesity, anticoagulant, antiviral, antihepatopathy, antiuropathy, and antirenalpathy actions are among its bioactivities [26].

Alpha-amylase is a starch-degrading protein. Antioxidant activity in the human body is beneficial. By this enzyme, internal 1,4-glycosidic connections in polysaccharides are hydrolyzed into low-molecular-weight products, such as glucose, maltose, and maltotriose (trisaccharide) units. It has a random effect on starch, glycogen, and similar polysaccharides or oligosaccharides [27].

Several sulfated seaweed polysaccharides have synergistic effects of antiviral properties against enveloped viruses, including herpes simplex virus (HSV), human immunodeficiency virus (HIV), and dengue virus [28]. The biochemical activities of sulfated seaweed polysaccharides are based on their origins and chemical properties such as glycosidic chains, molecular weight, sulfate content, and chemical configuration [29]. Their presence in marine algae has been linked to salt stress, mechanical, ionic, and osmotic regulation, which aids algal survival in the sea [30]. Ascophyllan (As), alginate oligomer (Ao), S fucoidan (S-Fu), A-fucoidan (A-Fu), dextran sulfate (De-S), and dextran (De) are acidic polysaccharides found in brown algae, while porphyran is found in red algae. This book chapter discusses algal species, bio-active compounds that are beneficial as a cure for chronic diseases and organ systems, and the bio-process of seaweed polysaccharides and their efficiencies. The applications of algae or seaweed in medicine and human health was discussed in the following chapter and summarized in Table 16.1.

2 Bioprocess of seaweed polysaccharides

The antiaging properties of the green algae *Ulva lactuca* and *Enteromorpha prolifera* containing sulfated polysaccharide fragments with low molecular weight were investigated by Liu et al. [31]. Fujian Haixing Health Food Co., Ltd. collected and identified seaweeds in

China's Yellow Sea. Seaweeds were collected, washed, air-dried, and ground at 50 Hz to make powdered seaweeds. To produce supernatant, the powders were extracted using ultrasound-assisted extraction (1:40 g/mL, 60°C, 1 h). The supernatant was centrifuged (4500 rpm, 10 min) and 95% ethanol (supernatant:ethanol, 1:4) was added to precipitate the crude polysaccharides (24 h, 4°C). Enzymatic hydrolysis with alkaline protease (50°C, 2 h, enzyme loadings, 2.0×10^5 U/g polysaccharide) was used to eliminate the proteins from the isolated crude polysaccharides. The bicinchoninic acid (BCA) protein assay kit was used to determine the effectiveness of protein removal. To extract the oligosaccharides, the polysaccharide solution was dialysis (filter and purify: to remove proteins and contaminants) and degraded (0.05 M sulfuric acid (H_2SO_4) and hydrochloric acid (HCl), 1.5 h, 100°C). Using ultrafiltration, the oligosaccharides were segmented based on their molecular mass (1000 Da, 1000–3000 Da, and 3000 Da). According to Liu et al. [31], oligosaccharides from *U. lactuca* and *E. prolifera* can be used in functional foods and pharmaceuticals to prevent aging because they increase glutathione, superoxide dismutase, catalase, and telomerase levels and total antioxidant capacity while decreasing malondialdehyde and advanced glycation end products.

Alkhalaf [22] reported on the anticancer properties of reddish-brown seaweed obtained from the Red Sea, Kingdom of Saudi Arabia (KSA), and contained methanolic extracts (MEC) (e.g., flavonoids, phenols (catechin, gallic and *p*-coumaric acids) and tannins). The dried algal powder (10 g) was immersed in methanol (80%, 100 mL) and stirred using an orbital shaker (24 h). The antioxidant composition, cytotoxic (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte tetrazolium bromide or MTT test) and antiinflammatory effects of MEC against cancer cells were determined using the extracted extract (HepG2, MCF7, Caco-2, and A549). MEC (200 g/mL) was tested for cytotoxicity by growing cells in 96 well plates in Dulbecco's modified eagle's medium formulation (DMEM) culture media (24 h), followed by incubation with MTT (5 mg/mL) in a CO_2 incubator (4 h, 37°C). The cells were washed in phosphate-buffered saline (PBS) before being incubated in dimethyl sulfoxide (DMSO) (1 h). The absorbance of the produced purple color was determined (540 nm). The absorbance of cells treated with MEC versus control cells was used to calculate the percentages of cell viability. The 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays are the gold standards for assessing antioxidant efficiency. MEC Trolox, and BHT standards were compared in terms of free radical scavenging activity. The ABTS and DPPH assays revealed that the proportion of ABTS and DPPH free radicals inhibited by MEC extract of *Chondrus* sp. increased progressively with increasing extract concentrations. The inhibition efficiencies at 200 g of MEC were 120.29% (ABTS), 100.25% (Trolox), 84.17% (DPPH), and 91.51% (BHT). The highest total antioxidant capacity was observed at 400 mg of MEC, as was the highest total antioxidant capacity. The ability of MEC to suppress the denaturation of bovine serum albumin was used to determine its anti-inflammatory activity (BSA). Inhibition was likewise dosage dependent. At 200 g of MEC, the greatest antiinflammatory impact was seen. The inhibitory efficiencies were 80.78 g/g (nondenatured BSA/MEC) and 80.48 g (nondenatured BSA/total BSA (g)). MEC (100 mg) at various doses inhibited proliferation in HepG2 (81.9%), MCF7 (47.8%), Caco-2 (64.5%), and A549 (71.8%) cells. Meanwhile, the sorafenib treatment inhibited the growth of HepG2 (69.1%) and A549 cells (70.6%).

Wang et al. [12] reported on the medicinal benefits of *Sargassum glaucescens* (brown seaweed) (Pro-Algue Marine Ltd.) containing fucoxanthin, which is useful in the treatment of

testicular injury. Fucoxanthin was extracted from *Sargassum glaucescens* dry powder (500 g) by swirling the seaweed in 1.5 L of 70% acetone in ethanol (v/v, 48 h, in the dark) and filtering through filter paper. To obtain the extract, the filtrate solution was concentrated using a vacuum rotary evaporator (40°C). The concentration was then redissolved in 90% ethanol (100 L). The extract was partitioned into ethanol: water: hexane (9:1:10, v/v/v) and washed with 70% ethanol four times. The ethanol-water layer was collected, and the organic layer was separated in the separatory funnel with an equal volume of ether. Finally, a fucoxanthin-rich extract was obtained by lyophilizing the solution (FXE). FXE was fed to 80 male Syrian hamsters (control and CP-induced reproductive damage) for 5 days. The FXE treatment successfully reduced reactive oxygen species and malondialdehyde levels in cells (RAW 264.7), testis, and mitochondrial membrane potential. The medication increased testosterone levels, alpha-glucosidase activity, and sperm count while decreasing aberrant sperm. The morphology of the seminiferous tubules improved. FXE is an effective alternative treatment for testicular injury.

Wang et al. [13] reported on the antilipogenesis properties of *Sargassum* species algae (brown seaweed) harvested from the Penghu coastline area in Taiwan. Seaweeds were harvested, washed, oven-dried (60°C, 48 h), and crushed into *Sargassum* powder (SP). The SP (with 35% water content) was subsequently processed using a single-shaft extruder (115°C, feeding rate: 10 kg/h, screw speed: 360 rpm) to break down the cell wall under high temperature and pressure. The SP was powdered, sieved (20-mesh sieve), packaged in a light-proof aluminum foil bag, and stored at 4°C. After centrifuging and extracting the seaweed powder (1 g), it was extracted with 95% ethanol (liquid/solid ratio, 10 mL/g, 4 h). Hot water bath (100°C, 20 liquid/solid ratio, 1 h, orbital shaker), ultrasonic (10 mm horn microtip, 950 W, 21–25 kHz, immersed 1 cm into the solution), and microwave-assisted extractions were used. To get the crude extract, the supernatant was collected, dialyzed (14 kDa dialysis membrane, 24 h), and filtered (0.22 m filter). The polysaccharide from brown algae was precipitated up to 20% of the final ethanol concentration (95% pure ethanol). The mixture was spun in a centrifuge (9000*g*, 30 min). To obtain crude fucoidan, the supernatant was collected and treated up to 50% of the final ethanol content (95% pure ethanol). The supernatant was removed, and the particle was dried before being analyzed further. Methods for removing protein from crude *Sargassum fucoidan*: (1) Isoelectric point precipitation: protein precipitation to the isoelectric point (adding acid pH 6 to 3, 4 h), (2) Salting out: ammonium sulfate solution was added up to 80% of final concentration (4°C, 12 h), and (3) Trichloroacetic acid (TCA) denaturation: TCA solution was added up to 10% of final concentration (4°C, 12 h) (4 h). The sample(s) was/were centrifuged (6000 rpm, 3 min). To eliminate low molecular weight chemicals, the supernatant was dialyzed (14 kDa cut-off membranes). Uronic acid was removed by adding various quantities of calcium chloride (CaCl: 1%, 2%, 3%, and 4%), incubating (4°C, 4 h), and centrifuging (6000 rpm, 3 min). After that, the supernatant was dialyzed, filtered, and lyophilized. 300 mg fucoidan was dissolved in 10 mg/mL distilled water and applied to a DEAE Sephadex A-25 column equilibrated with distilled water. Following fucoidan loading, elution was carried out with sodium chloride (NaCl: 1, 2, 3, and 4 M, flow rate: 1 mL/min). The total carbohydrate content was evaluated using a colorimetric method based on phenol-sulfuric acid (490 nm). The purity of crude fucoidan's polysaccharide content increased from 33.3% to 64.5%, with a total sugar recovery of 50.3%. The purity of the final sulfated polysaccharide product is nearly 84.0% based on the sugar and sulfate

groups (the major components of fucoidan), and this purified fucoidan product was found to display biological activities linked to antilipogenesis with a 28.9% decrease in lipid synthesis in comparison to the free fatty acid-induced control.

Agatonovic-Kustrin and Morton [7] investigated the medicinal value of fresh algae samples obtained from Blue Lagoon beach, Teluk Kemang, Port Dickson, Malaysia, which included alpha-amylase chemical, which is beneficial for antioxidant activity in the human body. Fresh algae (Port Dickson): 8 samples, dried algae (local market in Kuala Terengganu): 2 samples, 0.2–0.5 kg wet weight were collected in insulated containers containing seawater, morphologically identified, washed with filtered seawater (3 times, 24 h), divided into 50–200 g portions, frozen (80°C), and lyophilized. The powder samples (5 g) were extracted and filtered using 50 mL of organic solvent (ethanol or ethyl acetate, 5 times, 15 min). The solution was concentrated to 10 mL and placed into volumetric flasks (25 mL) with an adjusted final volume. The product was stored at a cool (4°C) temperature. When compared to dried samples, extracts from fresh samples have stronger antioxidant and alpha-amylase inhibitory activity.

Ueno et al. [6] investigated the infection and replication activities of acidic polysaccharides isolated from alginate (brown algae: ascophyllan (As), alginate oligomer (Ao), S fucoidan (S-Fu), A-fucoidan (A-Fu), dextran sulfate (De-S), and dextran (De), red algae: porphyran (Po)) on the infection and replication activities. The acidic polysaccharides were obtained from a commercial supplier and produced from SA (e.g., *A. nodosum*, *F. vesiculosus*, and *P. yezoensis*). *A. nodosum* was used to create As and A-Fu. SA was used to create Ao. Sigma-Aldrich provided S-Fu (fucoidan from *F. vesiculosus*, F8190) and De-S (197-08362, MW 5000); and De (31387-25G, MW 15,000–25,000). Po was derived from tainted nori (*P. yezoensis*). Acidic polysaccharides such As, S-Fu and A-Fu effectively reduce the early stages of HIV-1 (R9 and JR-FL), HBV (dose-dependently), and HCV infections. There was no inhibition of HTLV-1 replication found. The Ao, on the other hand, had no discernible inhibitory effects. Overall, depending on the polysaccharides, acidic polysaccharides are capable of inhibiting the early stages of viral infections, but not in a species-specific manner (Table 16.1).

3 Conclusions and future outlook

Polysaccharides found in seaweed are complex. It comprises numerous monomers (e.g., galactose, rhamnose, xylose, fucose, uronic acid, etc.) that require specific fragmentation via bioprocesses such as extraction, purification, modification, and characterization. The physicochemical features (e.g., polysaccharide molecular weight distributions, monosaccharide composition, sulfate concentration, and location) and therapeutic effects of polysaccharides differ depending on fragmentation. Organic solvents are extensively employed in extraction. Furthermore, extraction techniques such as microwave-assisted, supercritical carbon dioxide, and ultrasonic-aided extractions, as well as membrane separation technologies, are being employed to produce polysaccharides from seaweed. Fragmentation is required because low-molecular-weight polysaccharides have been discovered to be more bioactive than high-molecular-weight polysaccharides. However, not all of the bioactive chemicals found in seaweed have therapeutic properties. Previous research has shown that the molecular size of the polysaccharide has the greatest influence on anticoagulant activity.

TABLE 16.1 Algae in medicine and human health from literatures.

Ref.	Liu et al. [31]	Alkhalaf [22]	Wang et al. [12]
Types of algae	Green algae: *Ulva lactuca* and *Enteromorpha prolifera*	Red algae: *Chondrus crispus*	*Sargassum glaucescens*
Location	Yellow Sea near Qingdao of China	Red Sea, KSA	Québec, Canada
Chemical compound	Sulfated polysaccharides fragmented at the low molecular weight	Flavonoid, phenolic (catechin, gallic, and *p*-coumaric acids), and tannin extracts in methanol	Fucoxanthin
Benefits for medicine and human health	Antiaging	Antioxidant, antiinflammatory, and cytotoxic properties in several human cancer cell lines	Treatment of testicular damage
Type of extraction	– The seaweeds were collected, cleaned, air-dried, ground, and extracted (ultrasound-assisted extraction, 1:40 g/mL, 60°C, 1 h) to produce supernatant – The supernatant was centrifuged (4500 rpm, 10 min) and 95% ethanol was added (1:4, supernatant:ethanol) (24 h, 4°C) – Enzymatic hydrolysis using alkaline protease (50°C, 2 h, enzyme loadings: 2.0×10^5 U/g polysaccharide) – The efficiency of protein removal: BCA protein assay kit – The polysaccharide solution was dialysis and degraded (0.05 M H_2SO_4 and HCl, 1.5 h, 100°C) to obtain the oligosaccharides. The oligosaccharides were segmented: $\leq$1000 Da, 1000–3000 Da, and $\geq$3000 Da using ultrafiltration treatment	– The algae were hand-picked and rinsed – The dried algal powder (10 g) was soaked and stirred in methanol (80%, 100 mL) (24 h) – The extracted MEC was tested for antioxidant content, cytotoxicity (MTT assay), and antiinflammatory effects against cancer cells	– Fucoxanthin was extracted from *Sargassum glaucescens* dry powder (500 g) by swirling the seaweeds in 1.5 L of 70% acetone in ethanol (v/v, 48 h, in the dark) and filtering through filter paper – The filtrate solution was concentrated using a vacuum rotary evaporator (40°C) – The concentration was then redissolved in 90% ethanol (100 L) – The extract was partitioned in ethanol:water:hexane (9:1:10, v/v/v) and washed with 70% ethanol four times – The ethanol-water layer was collected, and the organic layer was separated in the separatory funnel with an equal volume of ether – The fucoxanthin-rich extract was lyophilized
Molecular size	1% agarose gel electrophoresis using hepatin as standard	n.a.	n.a.

Continued

TABLE 16.1 Algae in medicine and human health from literatures—cont'd

Characterization of physicochemical properties	FTIR, GCMS	HPLC to determine phenolic components in the MEC	n.a.
Immunohistochemical analysis	n.a.	– MEC (200 g/mL) cytotoxicity was determined by culturing cells in 96 well plates in DMEM culture media for 24 h, followed by treatment with MTT (5 mg/mL) in a CO_2 incubator for 4 h at 37°C. Cells were washed with PBS before being incubated with DMSO (1 h) – The absorbance of the produced purple color was determined (540 nm), treated with MEC	The hamster testis seminiferous tubular was fixed with 10% neutral buffer formalin and embedded in paraffin. After that, the slices were stained with hematoxylin and eosin. A phase-contrast microscope was used to examine the seminiferous tubular morphology (Olympus CK-2, Japan)
Efficiency	These oligosaccharides increased glutathione, superoxide dismutase, catalase, and telomerase levels, as well as total antioxidant capacity, while decreasing malondialdehyde and advanced glycation end products	Inhibition efficiencies (200 μg of MEC): 120.29% (ABTS), 100.25% (Trolox), 84.17% (DPPH), and 91.51% (BHT) The antiinflammatory effect of MEC (200 μg): 80.78 μg/g (nondenatured BSA/MEC) 80.48 μg (nondenatured BSA/total BSA found with aspirin (g)) The growth inhibition by MEC (100 mg): 81.9% (HepG2), 47.8% (MCF7), 64.5% (Caco-2), and 71.8% (A549 cells)	The FXE successfully improved the seminiferous tubules morphology
Ref.	Wang et al. [13]	Agatonovic-Kustrin and Morton [7]	Ueno et al. [6]
Types of algae	Brown algae: *Sargassum siliquosum*	3 Chlorophyta, 4 phaeophyta, and 3 rhodophyta	*Ascophyllum nodosum*
Location	Penghu, Taiwan	Teluk Kemang, Port Dickson, Malaysia	
Chemical compound	Fucoidan	Alpha-amylase	Acidic polysaccharides
Benefits for medicine and human health	Antilipogenesis	Antioxidant activity	Antiviral: HIV-1, HBV, HCV, and HTLV-1

TABLE 16.1 Algae in medicine and human health from literatures—cont'd

Type of extraction	– Seaweeds were harvested, washed, oven-dried (60°C for 48 h), and pulverized into SP – A single-shaft extruder (115°C, feeding rate: 10 kg/h, screw speed: 360 rpm) was used to process the SP – The SP was powdered, sieved (20-mesh sieve), packaged in a light-proof aluminum foil bag, and stored at 4°C. Extraction was conducted (95% ethanol liquid/solid ratio, 10 mL/g, 4 h) – The supernatant was collected and dialyzed (14 kDa dialysis membrane, 24 h) before being filtered (0.22 m filter). The polysaccharide mixture was precipitated up to 20% of the final ethanol concentration and centrifuged (9000*g*, 30 min). The supernatant was collected and treated to 50% of the final pure ethanol concentration. The pellet was dried. Protein extraction methods from crude *Sargassum fucoidan* were tested. The samples were centrifuged (6000 rpm, 3 min) and dialyzed (14 kDa membranes). Uronic acid was removed by adding various quantities of calcium chloride (CaCl: 1%, 2%, 3%, and 4%), incubated (4°C, 4 h), and centrifuged (6000 rpm, 3 min). The supernatant was dialyzed, filtered, and lyophilized. 300 mg fucoidan was dissolved in distilled water (10 mg/mL) and applied to a DEAE Sephadex A-25 column that had been previously equilibrated with distilled water. – Elution was carried out with NaCl concentrations of 1, 2, 3, and 4 M (1 mL/min)	Fresh algae samples (Port Dickson): 8 dried algae samples (from a local market in Kuala Terengganu) 2 samples (0.2–0.5 kg wet weight) were collected fresh in seawater-insulated containers, washed with filtered seawater (3 times in 24 h), divided into 50–200 g pieces, frozen (80°C), lyophilized, and ground to a fine powder. The powder samples (5 g) were extracted and filtered using organic solvent (50 mL, either ethanol or ethyl acetate, 5 times, 15 min). Using a Büchi rotary evaporator ModelR-200, the solution was concentrated to 10 mL and placed into volumetric flasks (25 mL) with an adjusted final volume. The product was stored at 4°C	*A. nodosum* was used to produce ascophyllan and A-fucoidan (fucoidan). Because of the high viscosity of alginate aqueous solution, alginate oligomers were produced from sodium alginate (31132-75, 1000-cps; Nacalai Tesque Inc., Kyoto, Japan). Sigma-Aldrich supplied S-fucoidan (*F. vesiculosus* fucoidan, F8190) and dextran sulfate (197-08362, MW 5000). (St. Louis, MO). Wako Pure Chemical Industries, Ltd. supplied Dextran (31387-25G, MW 15,000–25,000). (Osaka, Japan). Porphyran was created from discolored nori (*P. yezoensis*)

Continued

TABLE 16.1 Algae in medicine and human health from literatures—cont'd

Molecular size	n.a.	n.a.	Western blotting
Characterization of physicochemical properties	The total carbohydrate content was determined based on phenol-sulfuric acid colorimetric method (490 nm)	High-performance thin-layer chromatography	n.a.
Immunohistochemical analysis	n.a.	n.a.	n.a.
Efficiency	The purity of crude fucoidan's polysaccharide content increased from 33.3% to 64.5%, with a total sugar recovery of 50.3%	When compared to dried samples, extracts from fresh samples have stronger antioxidant and alpha-amylase inhibitory activity	Acidic polysaccharides such as As, S-Fu, and A-Fu dramatically reduced the early stages of HIV-1 (R9 and JR-FL), HBV (dose-dependently), and HCV infections

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CHAPTER

17

Microalgae biotechnology: Emerging biomedical applications

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1 Introduction

Microalgae are single-celled organisms that live in salt or freshwater, and their main energy source is light. Microalgae are photosynthetic microorganisms that are able to rapidly generate biomass from solar energy, CO_2, and nutrients in bodies of water [1]. Microalgae rule the oceans of the planet despite their small size. They could be found in all water bodies, generating 50% of global oxygen, an important factor for marine as well as for freshwater ecosystems. Nonetheless, with over 200,000 scientifically known microalgal species, only a few are commercially explored. Microalgae often produce complex and unique compounds for their safety and development. Microalgae have proven beneficial to humans and the ecosystem as they use photosynthesis to reduce carbon dioxide and release oxygen, which benefits the whole world.

These algae consist of various unusual properties, that aid in solving problems and making our lives easier. Several microalgal species have been identified, including Dunaliella salina, Chlorella vulgaris, spirulina, and many others. Many of these algal and cyanobacteria genera are distinctly beneficial to humans and the environment. Several nutritional compounds that promote human health and commodities on the market associated with microalgae have been reported. This is due to the presence of sugars, vitamins A, B1, B2, C, E, and B3, valuable minerals, and polyunsaturated fatty acids. As a result, microalgae can be used as a nutritional supplement. Currently, microalgae-derived foods are marketed as healthy foods and are available in the industry as capsules, tablets, powders, and liquids [2].

Although there are many reports on microalgae's benefits, this review will concentrate on their effects on fitness and illnesses. Alzheimer's disorder may trigger depression, apathy, social isolation, mood fluctuations, distrust of others, irritability, aggression, changes in sleeping habits, wandering, or illusions that something has been stolen. Another prominent condition associated with Alzheimer's is cardiovascular disease (CVD), leading to coronary heart disease, stroke, peripheral artery disease, and aortic disease. These diseases can be contained with microalgae utilization, which does not involve invasive surgery or excessive pharmaceuticals.

Microalgae have many benefits, including the ability to aid in the treatment of many diseases. Many metabolites isolated from these microorganisms have shown biological activities and potential health benefits [3]. In addition, the nutritional value of microalgae is beneficial in enhancing the immune system by reacting against pathogens and eliminating them. Besides being used in the treatment of diseases, microalgae can also be consumed by anyone. Typically, microalgae can be turned into a diet product suitable for all, as they contain carotenoids that enhance and strengthen the immune system. Microalgae are high in antioxidants, which help the body remove toxic and heavy metal compounds, in addition to carotenoid (a provitamin A).

The amounts of minerals, vitamins, and other components within microalgae can be increased via genetic engineering. Microalgal genome sequence information has become highly valuable for the development of efficient genetic engineering tools that can be used to produce a large number of transgenic microalgae [4]. In biochemical engineering, biotic parameters such as light, temperature, pH, and other essential compounds could be optimized to provide the desired characteristics. Photosynthesis and carbohydrate metabolism may be improved via genetic modification, while microalgal cultivation can be introduced

at a certain stage. Many obstacles and problems must be overcome to accomplish those incredible goals, which is time-consuming for the researchers.

Although it is easy to grow microalgae naturally, their growth and nutrient upgrade require a more complex process. Hence, the utilization of modern genetic engineering techniques would aid in resolving the issue. Previously, scientists and researchers discovered a method and approach for increasing the amount of components or compounds found in microalgae. Therefore, microalgae could contain double nutrient compounds. With this in mind, microalgae could be beneficial in various sectors and industries.

1.1 Nutrition

Microalgal nutritional content is an extremely important characteristic, causing them to be referred to as a superfood. Their nutrient content depends on the microalgal species, growth conditions, and how the supplements are processed [5]. Previous studies have found that microalgae like *chlorella* are very nutritious and produce 50% more protein than most plants and animals [6]. As we eat proteins, our digestive enzymes in the stomach break them down into amino acids, and our amino acids are used to synthesize muscle tissue proteins, which are then absorbed by our intestinal walls.

Microalgae such as chlorella, *D. salina*, and *spirulina* also contain vitamins, minerals, and antioxidants that can be made into supplements and meet our nutritional needs. Moreover, microalgae act as a health supplement and benefit people with iron deficiency, anemia, Alzheimer's disease, and cardiac disease [7]. Since microalgae are high in nutrients such as fiber, vitamins, and minerals, they can also help defend against oxidative cell damage by acting as antioxidants in our bodies [8]. Chlorella, a microalga, is high in copper, copper, fiber, zinc, potassium, magnesium, folic acid, and vitamin B [9]. The lack of fiber consumption is associated with certain health conditions like heart failure and diabetes [6].

1.2 Immune system against disease

The immune system protects aganist infectious bacteria and viruses, and it can be boosted by taking supplements. Nutrient consumption from natural resources, particularly those in their natural form, is one way to boost the immune system. Hence, microalgae are utilized as a supplement as they could strengthen the immune system. Microalgae such as *chlorella*, *D. salina*, and *spirulina* have been reported to provide nutrients to activate the immune system [10]. The immune system has two arms: innate immune response and adaptive immune response, which work together to respond to and protect our bodies from harmful diseases like cancer [11].

Notably, the immune system has a wide range of protective mechanisms to defend the host from various pathogens, species, and toxic agents [12]. Following infection by a pathogen, the innate immune system reacts rapidly. On the contrary, the adaptive immune system, which consists of a few cells and is dependent on the antigen-specific receptor expressed by adaptive immune cells, reacts at a slower pace [11].

The immune system can respond to pathogens through innate immune cells called neutrophils and macrophages, which detect pathogens or foreign molecules, e.g., poisonous molecules in the case of insect bites. These cells effectively kill pathogens using high destruction

substances such as enzymes that can digest proteins and the active chemical portion that can destroy any pathogen [12]. The cells phagocytose the pathogen/foreign material via phagocytosis. However, the reaction is not pathogen-specific. Thus, any pathogen/foreign material that is not destroyed by this attack will attract the lymphocytes, a cell that functions to adapt and memorize any previous attack, allowing the immune system to develop an automatic response and remember any previous infection [13].

1.3 Improving human health condition

Microalgae discovery has improved human health and is widely used in the pharmaceutical industry due to its benefits and nutrient content. Because of advancements in biotechnology, the uses of microalgae have now expanded beyond the pharmaceutical industry to include aquaculture, the oil and gas sector, and waste management. Today, we are more concerned with the fortification of human health and well-being through the benefits of microalgae. Worldwide, there are over 200,000 confirmed microalgae species and over 800,000 unidentified species [14].

Humans have been using microalgae as food for several decades, and supplementation with microalgae has helped relieve the scarce resources of demanding terrestrial food crops [15]. Because of their active and modern lifestyles, the local populace of some developing countries consumes high-calorie diets, resulting in health concerns such as obesity, which often leads to ailments like high blood pressure, heart disease, and other problems. Therefore, a nutritious diet consisting of antioxidants, vitamins, and minerals is important for a healthier lifestyle. Proteins, carbohydrates, lipids, and other bioactive compounds have been reported in various microalgae species [16].

1.4 Commercial production and importance of microalgae in diet

Because of western diets, people in developing countries typically consume high-calorie foods, which lead to obesity and, in the worst-case scenario, diseases such as heart disease. Microalgae have become a better option as a supplement as they provide vitamins and minerals. However, microalgae are underutilized as a natural source for a nutritious diet [17,18]. A previous study has shown that microalgae contain vitamins and minerals such as vitamins A, B1, B2, C, E, and B3 [18].

Microalgae have fewer polysaccharides in their cell walls, causing them to be easily absorbed and suitable for human consumption [19]. Extracellular polysaccharides (EPSs) provided by microalgae are excellent sources of nutrients for the human body. By improving biological processes and increasing the organism's resistance to environmental stress through genetic modification, microalgae can create many more EPSs for human consumption [20].

Microalgae supplements are now available as pills, powders, and liquids in the health food markets. The most commonly used microalgae species for human consumption are D. salina and C. vulgaris, as they are protein-rich and high in nutrition [16]. The global business intelligence study says customer interests are one of the products containing microalgae protein. The global industry insight from the algae protein report 2020 to 2026 shows that the size of the algae protein market between 2020 and 2026 is above 700 million and that the Compound Annual Growth Rate (CAGR) is expected to rise above 6% [21].

Algae could be processed into consumable, refined, and healthy foods. It is estimated that the global market size for algae products could grow by more than three billion at the end of 2025. This is because microalgae are adapted to many different markets and different applications. Additionally, it provides energy and recycling options and can be utilized as a raw material for the production of petroleum and biodiesel [21].

Spirulina and *chlorella* are currently used on a large scale compared to others since they have high protein levels and great nutrients. In addition, it provides a rich natural supply of vitamins A, B1, B2, and B12 besides carotenoid and xanthophyll phytopigments [22].

The world's largest producer of spirulina is Hainan Simai Corporation, headquartered in the Hainan Province of China and producing an output of 200 tons of powdered algae annually. There are also other spirulina manufacturing companies in China and around the world. Besides China, other world producers of spirulina are the USA, Japan, Mexico, and Taiwan. Most of these manufacturers produce microalgae as tablets and powders [23].

1.5 Carotenoids as beneficial health metabolites from microalgae

Carotenoids are pigments synthesized by natural sources such as plants, algae, and bacteria, and are rich in compounds of color. Many of them have yellow, orange, red, and green pigments as the basis of many plants [24]. Fruits and vegetables provide more than 40–50 carotenoids in the human diet, such as carotene, β-carotene, b-cryptoxanthin, lutein, zeaxanthin, and lycopene [25].

The absorption of dietary carotenoids in the intestine could be enhanced by fat [20]. Additionally, taking 3–5 g of fat with each meal assists the absorption of carotenoids from food. However, the volume of dietary fat required for the absorption of carotenoids varies [26]. The type of fat, fiber, and carotenoids in a diet often influences the rate and degree of carotenoid uptake, but in addition, carotenoids are far more effective than carotenes in the absorption of supplements [25]. Although enterocytes and intrinsic membrane protein receivers, cluster determinant 36 (CD36), were linked to the absorption of carotenoids in the intestines [27], fat (fatty acid translocase) also facilitated the absorption of carotenoids in the intestines.

Provitamin A, a type of carotenoid, can be transformed into vitamin A, reinforcing the immune system. Carotenoids, a type of antioxidant, are essential in the diet because they improves the immune system and overall health, besides ensuring healthy eyes [28]. Nevertheless, excessive consumption of supplements is detrimental to health [29].

1.6 Antioxidants for human health

Antioxidants are a vital component of our bodies, and our bodies is responsible for producing antioxidants. Although antioxidants can be acquired from plants and animal products, most of them come from plants, particularly vitamins A, C, and E [30]. These nonessential antioxidants are included in food and play a crucial role in our general health, even if they are insufficient for the body [31].

Natural compounds are now being increasingly used to facilitate the treatment of human diseases by supplementing the action of existing drugs or treatment. Microalgae can produce antioxidants in the form of carotenoids, phenolic compounds, and polyunsaturated fatty acids under stress conditions [32].

Nowadays, many of the supplementary products, cosmetics, and food industries have found that photo-synthetic species can form secondary metabolites and, thus, produce some toxic effects inside the body. This might be the source of chronic diseases such as cancer and aging problems. Therefore, the use of carotenoids as an antioxidant has significantly increased [33]. Antioxidants protect our skin during ultraviolet exposure and treatment of skin conditions, while zeaxanthin, a type of carotenoid, assists in eye health and the action of β-carotene [34].

The antioxidant function of microalgae could be measured and compared to the antioxidant activity of other plants. Earlier analysis showed that the antioxidant level of green microalgae such as Chlorophyceae and red microalgae, Rhodophyta could be compared through the Trolox equivalent. For example, the Chlorophyceae class produces 5.50–214.34 g^{-1} DM antioxidants, while various Rhodophyta groups only contain 16.61–67.95 g^{-1} DM antioxidants, and the *Dinophyceae* and *Carthamus tinctorius* L. contain 2.20–6.30 and 1.80 g^{-1} DM, respectively. This is one of the reasons for the promising green microalgae utilization in human health [8].

1.7 Removal of excessive heavy metal and toxic

The human body requires limited quantities of heavy metals, such as zinc, copper, chromium, iron, and manganese [35]. These heavy metals are required for cellular and metabolic functions [36]. However, too much heavy metal can cause toxicity to the human body, leading to multiple metabolic dysfunctions. Among the consequences are a mental disorder and damaged blood cells and organs, e.g., the lungs, liver, kidneys, etc.

Excessive heavy metal exposure maybe harmful to the liver, neurons, and brain [35]. Microalgae could assist in eliminating excess heavy metals, including certain vital elements such as iron, zinc, and copper [37]. Chlorella has been reported to eliminate heavy metal toxicity in the liver, brain, and kidney. Dioxin is an example of a toxic compound that could interrupt hormone production and cause toxicity to humans and animals [38]. Hence, chlorella facilitates the removal of toxins and heavy metals from the body [39].

2 Research findings from great microalgae studies

Several studies have reported the various bioactive compounds that lead to the antibacterial, antioxidant, and antiinflammatory characteristics of C. vulgaris, D. salina, and spirulina [33]. In addition, the intracellular and extracellular metabolite production of algae and cyanobacteria (blue-green algae) and prokaryotic algae renders their antibacterial and antioxidant activities [38]. Microalgae can also improve the immune response to excessive metals and reduce glucose and lipids in treating chronic diseases [40,41].

2.1 Microalgae *chlorella*

Microalgae are common green algae, consumed and embraced by the Japanese as a food supplement. *Chlorella* is high in chlorophyll, protein, polysaccharides, minerals, vitamins, and significant amino acids [33]. *Chlorella* is high in protein, with up to 51%–58%, and is comprised of the necessary amino acid composition [42]. In addition, chlorella contains vitamin B,

such as B12, which is essential to blood cell development. The use of *chlorella* as a supplement should adhere to the following criteria: be certified safe by the U.S. Food and Drug Administration (USFDA), be a noninflammatory food, and undergo testing to provide evidence concerning antitumor, antibacterial, antioxidant, and antihyperlipidemic properties. Based on the criteria, *chlorella* is known as a safe food.

The antifungal properties of microalgae are responsible for preventing chronic diseases, brought about by compounds such as lutein, α-carotene, β-carotene, ascorbic acid, and α-tocopherol [43]. These chemical compounds play an important role reducing the effects that could cause cancer and preventing vision loss or macular degeneration [33]. It facilitates in reducing chronic diseases and blood cholesterol [15]. In addition, preventive actions caused by compounds like glycolipid, peptides, and nucleotides have been documented in chlorella, which protects against atherosclerosis, hypercholesterolemia, and antitumor activity [44].

2.2 Microalgae *D. salina*

D. salina is a green, unicellular microalga that has been extensively studied for its active biomass compounds with various biological activities, such as antioxidant and antiviral [33]. It contains carotenoids and lipids. The primary sources of carotenoid in D. salina is β-carotene, and under conditions of high salinity, light, temperature, and nutrients, it can produce up to 14% of its dry weight [45]. *D. salina* is rich in essential minerals, which can be consumed safely and generally recognized as healthy (GRAS) [32]. The biomass of D. salina can be used directly in food and dietary supplement products [46].

Several studies have revealed that D. salina contains antibacterial properties because the extract could inhibit bacterial growth, such as Staphylococcus aureus and Bacillus subtilis [47]. In addition, another study reported that *D. salina* exhibited antibacterial activity against other microorganisms like Escherichia coli and *Aspergillus niger*, demonstrating its value in the food industry and supplement products [48].

In optimal conditions, *D. salina* could produce around 400 mg beta-carotene/m^2. Many countries, including Australia, India, the USA, and China, have cultivated D. salina [16]. Pentapharm, a pharmaceutical company based in Basel, Switzerland, has released an ingredient to culture microalgae D. salina with the potential to stimulate cell growth and metabolic skin energy. In countries such as India, Chile, Mexico, Cuba, Iran, Taiwan, Japan, Spain, and Kuwait, new pilot plant production systems are being built to produce the mass culture of D. salina [40].

2.3 Microalgae *spirulina*

Spirulina, like other microalgae, is common as a food supplement as it grows readily in fresh or saltwater [49]. *Spirulina* contains several nutrients and antioxidants beneficial to humans, particularly the brain, due to its high vitamins, minerals, and fats [50]. The key active compound in *spirulina* is phycocyanin, which produces antioxidant and inflammatory agents and aids in preventing oxidative damage and the growth of cancer cells. It could also lower the bad LDL cholesterol level that causes heart disease and triglyceride levels while increasing the good HDL cholesterol [45]. Furthermore, the carotenoid is responsible for the green and blue colors of *spirulina* [51].

3 How algae can help solve some of the world's most severe health problems

Microalgae are a major source of nutrients. The most available microalgal products are high-nutrient dried algal biomass products, comprising high-quality compounds, like fatty acids, antioxidants, and polysaccharides. The compounds reported are vitamins, minerals, proteins, and antioxidants, obtained from *chlorella* and *Dunaliella* biomass. These compounds highlight the importance of microalgae in pharmaceuticals, cosmetics, nutraceuticals, and functional foods. In addition, microalgae can remove excess toxic and heavy metals from the body, which might damage the liver, brain, pulmonary artery, and kidney. Other studies in red algae reported exopolysaccharides and carbohydrates, which act as a source of biofuel and chemicals.

According to the 2019 World Health Organization (WHO) statistical report, the leading global cause of death is heart disease, with about 10 million people dying due to ischemic heart disease from 2016 to 2019. Death due to road accidents came as the second cause of death, while trachea, bronchus, and lung cancer represented the third cause. Finally, other chronic diseases make up the fourth cause of death. Microalgae have the potential to overcome these chronic diseases and facilitate treatment. The onset of the diseases could be minimized by taking algae supplements. Furthermore, microalgae are inexpensive, simple to grow, and easy to manufacture [52].

Not only does it help treat various diseases, but it could also help boost the immune system. Besides their roles in the health industry, they also contribute to the minimization of climate change. Microalgae produce oxygen, stabilizing the environment through photosynthetic processes and reducing carbon dioxide. Without a doubt, microalgae are vital in clearing the atmosphere and pharmaceutical, nutraceutical, and cosmetic industries.

4 Conclusions and future outlook

Microalgae contain metabolites that promote health and help combat diseases. These nutritional benefits are due to carotenoids, fatty acids, amino acids, antioxidants, and other secondary metabolites in microalgae. In recent decades, microalgae's mass culture and trade have grown tremendously. They are highly beneficial to the pharmaceutical and food industries, as well as for increasing productivity. Microalgae have also contributed to the pharmacological field in the treatment of several human and animal maladies. This review highlights the importance of bioactive microalgae components commonly used in the food and pharmaceutical industries. Other beneficial compounds in microalgae are lipids, pigments, carbohydrates, and polysaccharides. Due to their nutritional values, microalgae are widely utilized in industries, including cosmetics, food technology, energy generation, biodiesel, and many others.

Microalgae contribute more than 75% of the oxygen supply. The unappealing appearance of microalgae piques people's interest, camouflaging their vast benefits. Nonetheless, people are becoming more aware of the importance of this species. Besides contributing to the atmosphere, they could also be used for other things, like an alternative to gasoline, bio-crops, and even food. Genetic engineering approaches could help increase the nutritional content of microalgae by twice as much as usual development.

Furthermore, microalgae can be defined as the future energy source. They could be a potential substitute for traditional fossil fuels such as diesel as they could generate electricity. When the costs of conventional fossil fuels and microalgae are compared, microalgae comes out on top. Microalgae can survive in any environment as long as water is available. Thus, the potential of microalgae is endless. Among the applications of microalgae are the replacement of conventional drug medications with microalgal supplements, improved methods of sewage treatment plants, and the transformation of organic nutrients into usable biomass. In conclusion, microalgae have tremendous potential in various fields, yet, there are more possibilities to be explored.

Although human beings have long been aware that the current global environment is formed by microalgae, they have not focused their attention on microalgae from the perspective of actively utilizing them. However, problems connected to the existence of human beings came to the fore, such as diseases caused by people's poor eating habit which lead to various medical and health-related issues being increased [53]. Therefore, to cope with this situation, people have come to pay more attention to preventing diseases and maintaining good health, rather than taking medicine after suffering from diseases. This has prompted the idea that microalgae, which form the basis of the food chain, should be actively utilized as it has been found that various substances produced by microalgae also have bioactive and other useful functions. One of the most feasible biotechnology fields is red biotechnology, an area concerning medicine and health, including drugs, bioactive substances, and nutraceuticals. It focuses on functional substances produced by microalgae and aims at making use of them.

Microalgae come in various colors. Chlorophyll makes some microalgae green, red, orange, and yellow. These colors are all derived from carotenoids or natural pigments [54]. It has been proven that carotenoids have antioxidant and other bioactive effects. Studies are now underway to use them as functional food ingredients and cosmetics.

For instance, it has been reported that the astaxanthin produced by the orange-colored *Haematococcus lacustris* has a high antioxidant effect that protects human bodies from ultraviolet light and excessive oxidation of fat in the blood [1]. Therefore, astaxanthin has been drawing attention lately in such fields as the prevention of aging, easing of eye strain, relaxation of tired muscles, and prevention of arterial sclerosis [55]. Several corporations have started operating microalgae cultivation facilities to produce astaxanthin for their future studies.

Since microalgae are widely distributed in saline and fresh water and there are about 100,000 different kinds, it is believed there are many compounds yet to be discovered biological activities. Therefore, microalgae are promising treasure troves for researchers looking for candidate substances for medicines and functional ingredients.

Such an idea may have sounded far-fetched until recently. However, thanks to progress in biotechnology, it has become possible for us to draw on the capability of microalgae to address the various problems we are facing. The technology to assess, sort out, and make use of microalgae that are useful for specific products from among the various microalgae possessing biodiversity can be called a common fundamental technology. It is important to promote research activities that will form common bases for microalgae contributing to the various fields of medicine, health, and the environment, and energy. For example, it is necessary to develop a technology to sort out microalgae for respective objectives, a highly efficient culture technology for mass production, and a recombinant technology using microalgae as hosts [56]. In particular, it is necessary to promote the application of

cost-conscious cultural methods and other basic technologies. Therefore, it is necessary to draw up a roadmap from a broad standpoint going beyond the boundaries for the application of microalgae, so that the microalgal technologies that the current status already possesses can be fully utilized.

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CHAPTER

18

Potential applications of the low-molecular-weight metabolome of *Synechocystis aquatilis* Sauvageau, 1892 (Cyanophyceae: Merismopediaceae)

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1 Introduction

The diversity of cyanobacteria, a heterogeneous group of prokaryotic, principally photosynthetic organisms, is exceptionally high. Estimates of the number of described species of Cyanobacteria range from 2000 to 8000, but the best asymptotic Gompertz model approximates that this group must contain more than 6000 species [1]. Species of cyanobacteria differ both in their habitats and in their ecological functions and sizes, from 0.6 μm (*Prochlorococcus*, planktonic coccoid unicellular cyanobacteria) [2] to more than 180 μm (*Oscillatoria simplicissima* Gomont, filamentous cyanobacteria) [3]. Cyanobacteria are widely distributed in all environments (freshwater, marine, and terrestrial), and they are very attractive for the production of valuable bioactive natural compounds. The well-developed phototrophic apparatus of cyanobacteria has given rise to research into the utilization of cyanobacteria for generating chemicals, renewable biofuels, and using sunlight and the greenhouse gas CO_2 [4,5].

Cyanobacteria (and other microalgae) are very promising for creating various products in the food and chemical industries, pharmaceuticals, and cosmetics: biofuels, lipids and fatty acids, terpenes, pigments, sugars, proteins, etc. With the development of appropriate technologies, progress in this area is evident [6]. Cyanobacteria are generally characterized by a higher growth rate than eukaryotic plants (e.g., aquatic macrophytes) and microalgae. At the same time, it can be noted that, to date, extensive genetic information has been accumulated about many species of cyanobacteria, which objectively makes these organisms the best candidates for creating highly productive strains for the biosynthesis of biofuels and chemicals using genetic engineering [7].

Singh et al. [8] noted that cyanobacteria are undoubtedly an excellent source of vitamins and proteins, and they are also reported to be a source of valuable bioactive compounds and renewable fuel. Martínez-Francés and Escudero-Oñate [9] paid great attention to the generalization of information on the primary and secondary metabolites of cyanobacteria, showing that these microorganisms have been demonstrated to play a key role in the production of special chemicals: antioxidants to protect photosynthetic cells from oxidative stress; a variety of polyunsaturated and monounsaturated fatty acids with properties to improve human health; as well as medicinally used polysaccharides, glycerol, glycoproteins, and antibiotics. Among the species of cyanobacteria, representatives of the genus *Synechocystis* are among the most promising in terms of their use in biotechnological processes. They can be genetically programmed to synthesize various useful (e.g., from a commercial point of view) substances. Thus, the work [10] presents the results for the *Synechocystis* strain, which was endowed with the synthesis of the plant secondary metabolite geranyllinalool, a diterpene alcohol of commercial interest. The total average yield of geranyllinalool was 360 μg/g dry cell weight per a 48-h cultivation period.

A distinctive feature of the species *Sphingomonas aquatilis* is that it can be successfully cultivated since it is very unpretentious for cultivation, both in the laboratory and "outdoor" conditions in different types of bioreactors [11–13]. For example, the strain *Synechocystis* 6803 is characterized by a versatile carbon metabolism, which allows the species to grow in both photoautotrophic and mixotrophic and heterotrophic conditions [14]. Under appropriate conditions, the species has a fairly high rate of organic matter synthesis [11] and can ideally be

cultivated in a continuous photo-bioreactor for extraction of lipids, including saturated fatty acids (palmitic acid) as well as polyunsaturated fatty acids (linoleic and α-linolenic acid). *S. aquatilis* is a cyanobacterial species that can grow in extreme environmental conditions and has the ability to recycle large amounts of CO_2 [13]. It has been shown that *S. aquatilis* is an active producer of biopolymers, such as exopolysaccharides which are widely exploited by the industry as hydrocolloids (gelling, thickening agents) and biological agents (antiinflammatory, antiparasitic, antioxidant, etc.) [15,16]. This species is also known to contain valuable pigments (i.e., chlorophyll, phycocyanin), proteins, and lipids [17]. For example, *Synechocystis* strains, including the one we used, can be convenient tools for the investigation of rate-limiting factors for cell cultivation; for using molecular tools for genetic modifications; for high-throughput instruments of system biology for genome-wide analysis; for metabolic modeling for physiological prediction and rational metabolic engineering; for applications in producing diverse chemicals for medicine and the production of bioplastics; and to producing a new generation of algicides [12,18,19].

The model strain *Synechocystis* PCC6803 is the best-studied cyanobacterium, whose genome has been fully sequenced [20]. To date, significant progress has been made in using *Synechocystis* strains as "phototrophic cell factories." However, the authors in [19] note that the biotechnology for compounds from *Synechocystis* is still significantly lagging behind that for heterotrophic microbes (e.g., for *Escherichia coli*). Since cyanobacteria synthesize a considerable number of metabolites of various chemical natures and different properties, they are actively used in various fields of biotechnology. A review of substances (mainly macromolecular ones) and areas of their biotechnological use is given in the review [21]. A general overview of cyanobacterial metabolites of biotechnological interest is also presented in a review [22]. The use of cyanobacterial metabolites is currently being studied extensively, and many promising new compounds have been reported. Pigments, vitamins, lipids, proteins, polypeptides, antioxidant enzymes, polysaccharides, etc. obtained from cyanobacteria are included in the field of biotechnology. Because of their high productivity, cyanobacteria are ideal tiny microbial factories for the production of food ingredients, biofuels, cosmetics, pharmaceuticals, wastewater treatment, and other products. Further research can help identify more metabolites of cyanobacteria and new approaches to their widespread use in a variety of practice areas [23].

To date, it is generally accepted that cyanobacteria synthesize a wide range of secondary metabolites, including biological active compounds with antibacterial, antiviral, antifungal, and anticancer activity. Some other important metabolites of cyanobacteria include enzymes, toxins, UV-absorbing pigments, and some fluorescent dyes. In addition, the production of biofuels based on cyanobacterial cultivation technologies represents one of the most promising areas for biotechnological applications. In addition, the production of other compounds, for example, alcohols and isoprenoids, biopolymers, and proteins, using genetic engineering seems promising. In the field of agriculture, highly productive strains of cyanobacteria, including those using the metabolism of nitrogen-fixing cyanobacteria, can play the role of biofactories for the production of biofertilizers to increase soil fertility [21].

Phycobiliproteins of Cyanobacteria are used in the manufacture of immunofluorescence techniques, antibody labeling, food coloring (ice cream and sweets), cosmetics, immunofluorescence techniques; antibody labeling, carotenoids are used in the manufacture of antioxidants, anticancer, antiobesity, antiproliferative activity, antiproliferative, and antiinflammatory [24]. The use of photosynthetic cyanobacteria is promising for "green" energy since they are directly

capable of converting CO_2 into biofuel. The ability of cyanobacteria to decompose many environmental pollutants and remove heavy metals also opens prospects for their use as a promising tool for bioremediation and wastewater treatment. The biotechnological significance of cyanobacteria is due to the fact that they can serve as sources of valuable chemicals, such as pigments, vitamins, and enzymes. In addition, it is important that they possess the ability to produce a whole spectrum of valuable bioactive compounds with antibacterial, antifungal, antiviral, and antialgal properties, which are of pharmaceutical and agricultural importance [25]. Despite the good knowledge of biology and application of *S. aquatilis* strains, information about their complete low-molecular-weight metabolome is rather scarce. For example, there is information on the synthesis of such metabolites as exopolysaccharides by this species, and their composition is quite diverse [26]. Since metabolomics focuses on low-molecular-weight metabolites [19], in this regard, the task of our study was to study the component composition of low-molecular-weight organic compounds in *S. aquatilis* by gas chromatography–mass spectrometry and to assess the possibilities of practical use of the most promising of its discovered metabolites.

2 Material and methods

2.1 Investigated species and its cultivation

We investigated an axenic strain of *S. aquatilis* Sauvageau No. 1336 from the collection of living cultures of cyanobacteria, algae, and algal parasites (CALU, Collection of Algae of Leningrad University). This strain was provided by the Centre for Culture Collection of Microorganisms of the Research Park at St. Petersburg University. Initially, the species was isolated from water samples taken in the desalinated part of the Gulf of Finland in the Baltic Sea near Sosnovy Bor.

Cyanobacteria were cultured on medium No. 6 [27] with pH 8 in 0.5 L glass jars with a constant air purge. Syringe-Driven Filters Jet Biofil # FPE204030 were used to protect the culture from the ingress of foreign organisms. Cultivation of cyanobacteria was carried out in a special aquarium using a liquid circulation cryothermostat with cooling and heating (Baths WCR Circulation water bath WCR-MaXircu CR-P8 (Daihan (Witeg)). The unit maintained a constant temperature of 25°C during the cultivation. The lamp (Lamp Biodesign RIF 80/110/PANORAMA 80/100/DIARAMA 150/200) provided a luminous flux of 1500 lm. The day-night mode (16–8 h) was set using an adjustable timer (FERON TM50, 3500 W/16A230V).

2.2 Metabolites identification

S. aquatilis metabolites were extracted from the dry biomass of this cyanobacterium. The culture of cyanobacteria in the stationary growth phase was concentrated by evaporation at 40 °C to a constant weight in glass Petri dishes on a thermostat. For the steam distillation process on the Clevenger apparatus, weighed portions of 1.34–1.4 g of the dry biomass were used. A fraction of volatile low-molecular-weight organic compounds (LMWOCs) was obtained using the same Clevenger-type apparatus method that is commonly used to obtain essential oils from terrestrial and aquatic plants [28–30]. Hydrodistillation was performed for

7 h in three replicates. Then the samples were extracted with hexane (5 mL). The extraction into hexane was done directly during the hydrodistillation process. We consider the resulting fraction of LMWOCs as an essential oil in accordance with the Vocabulary of Natural Materials [31] of the International Organization for Standardization. The extracts were stored in a freezing chamber (−18°C) before GC-MS analysis.

The concentrations of LMWOCs and their composition were determined using a POLARIS Q gas chromatograph-mass spectrometer (Thermo Electron Corporation) equipped with a TRACETM TR-5MS GC Column (0.25 μm film, 0.25 mm I.D., 30 m length). Helium served as a carrier gas. The ionization voltage was 70 eV. The mass spectra were registered in scan mode for the whole mass range (30–580 amu) in a programmed temperature regime: the oven temperature was kept at 40°C for 3 min; then the temperature was increased to 80°C at a rate of 5°C/min and kept constant for 3 min; after that, the temperature was increased to 150°C at a rate of 10°C/min and kept constant for 3 min; then it was increased to 240°C at a rate of 15°C/min and kept constant for 10 min. The LMWOCs determined in the sample were identified by matching their mass spectra with those from the NIST_2014 and the Wiley mass spectral libraries. Identification of the compounds was confirmed by linear retention indices obtained from a series of straight-chain alkanes (C7–C30) [32]. Quantitative analysis was performed with the help of Merck's certified reference materials decafluorobenzophenone and benzophenone (CAS Numbers 119-61-9 and 853-30-4) as internal standards. The analyses were carried out at the Resource Centre of St. Petersburg University.

3 Component composition of low-molecular-weight metabolome of *S. aquatilis* Sauvageau, 1892

A large number of LMWOCs (85 in total, 10 compounds remained unidentified) belonging to different classes of chemicals (Tables 18.1 and 18.2) were found in the essential oil of *S. aquatilis*. The identified LMWOCs accounted for more than 99% of the total essential oil composition. The two most abundant groups of LMWOCs in the investigated strain of *S. aquatilis* were hydrocarbons (52.35% in content) and alcohol (26.13%). Ketones occupied third place in the relative content of the low-molecular-weight metabolome (LMWM) (10.71%). All the other groups together accounted for only 10.81%. A general view of the chromatogram of a sample of *S. aquatilis* essential oil, indicating the most abundant compounds, is shown in Fig. 18.1.

In the species *S. aquatilis* studied by us, the palmitic acid median content was 4.94% (115.4 μg/g DW) of the total amount of LMWOCs. This is not the highest value known in the literature. So, in the work [11] it is reported that C16:0, along with C18:2 and C18:3 fatty acids, were found to be predominant. At the same time, a lipid extraction procedure consisting of sonication and solvent extraction (chloroform: methanol as 1:1 by volume) resulted in the highest lipid yield of 18.58% [11]. The calculated palmitic acid content ranged from 4.07% to 4.79%, with various CO_2 concentrations in continuous experiments. These findings on the content of C16:0 in *S. aquatilis* are nearly identical to the value obtained in our study. According to Eungrasamee et al. [33], in various strains of *Synechocystis* PCC 6803 (including wild type), the maximum content (on the 5th day of cultivation) of free fatty acids

TABLE 18.1 Average (median) relative content (% of total essential oil) and concentration (C, μg/g DW (dry weight) of the main groups of compounds in *S. aquatilis*.

	%	*C*, μg/g DW
Aromatic hydrocarbons	0.65	15.19
Alcohols	26.13	610.52
Aldehydes	1.37	32.09
Hydrocarbons	52.35	1223.25
Carboxylic acids	4.98	116.27
Esters	0.63	14.68
Unidentified	0.92	21.56
Ketones	10.71	250.35
Diverse functional groups	2.02	47.17
Nitrogen-containing	0.14	3.20
Phenols	0.10	2.36
Total	100.00	2336.62

TABLE 18.2 The composition of the essential oil of *S. aquatilis*.

No.	Compound	Formula	RT	RI	%	*C*, μg/g DW
1	1,2,4,4-Tetramethylcyclopentene	C_9H_{16}	8.75	857	0.04	0.98
2	1,2-Dimethylbenzene	C_8H_{10}	9.1	867	0.15	3.56
3	1,3-Dimethylcyclohexene	C_8H_{14}	9.68	883	0.04	0.86
4	Heptanal	$C_7H_{14}O$	10.35	903	0.04	1.02
5	Benzaldehyde	C_7H_6O	12.41	961	0.07	1.70
6	Oct-1-en-3-one	$C_8H_{14}O$	13.05	979	0.04	1.02
7	2-Pentylfuran	$C_9H_{14}O$	13.41	989	0.15	3.43
8	Octan-2-one	$C_8H_{16}O$	13.53	992	0.03	0.62
9	Unidentified *m/z*? [M+], 67 (100)		13.92	1003	0.04	0.83
10	(*E*)-Oct-2-enal	$C_8H_{14}O$	15.13	1031	0.29	6.79
11	1-(4-Methylcyclohex-3-en-1-yl)ethanone	$C_9H_{14}O$	16.15	1056	0.06	1.31
12	(1*S*,2*R*,4*S*)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol; [*endo*-Borneol]	$C_{10}H_{18}O$	18.29	1111	0.17	3.94

TABLE 18.2 The composition of the essential oil of *S. aquatilis*—cont'd

No.	Compound	Formula	RT	RI	%	*C*, μg/g DW
13	1-Methyl-9-oxabicyclo[6.1.0]nonane	$C_9H_{16}O$	19.55	1160	0.11	2.49
14	3,3-Dimethyl-2-(propan-2-ylidene)cyclopentanone	$C_{10}H_{16}O$	19.6	1162	0.09	2.21
15	4-Ethylbenzaldehyde	$C_9H_{10}O$	20.05	1179	0.14	3.22
16	1-(4-Methylphenyl)ethanone	$C_9H_{10}O$	20.31	1189	0.06	1.41
17	2,6,6-Trimethylcyclohexa-1,3-diene-1-carbaldehyde; [Safranal]	$C_{10}H_{14}O$	20.63	1202	0.19	4.51
18	2,6,6-Trimethylcyclohexene-1-carbaldehyde; [beta-Cyclocitral]	$C_{10}H_{16}O$	21.06	1225	0.26	6.03
19	(5*R*)-5-Methyl-2-propan-2-ylcyclohex-2-en-1-one	$C_{10}H_{16}O$	21.66	1257	0.03	0.73
20	2-(2,6,6-Trimethylcyclohexen-1-yl)acetaldehyde	$C_{11}H_{18}O$	21.77	1262	0.13	3.14
21	(4a*R*,7*S*,7a*S*)-4,7-Dimethyl-5,6,7,7a-tetrahydro-4a*H*-cyclopenta[*c*]pyran-1-one; [Nepetalactone]	$C_{10}H_{14}O_2$	22.71	1313	0.03	0.78
22	2,2,6,8-Tetramethyl-7-oxatricyclo[4.3.0.01,8]nonane	$C_{12}H_{20}O$	22.87	1323	0.03	0.70
23	4,4,7-Trimethyl-2,3-dihydro-1H-naphthalene; [α-Ionene]	$C_{13}H_{18}$	23.48	1358	0.11	2.64
24	Hex-4-yn-3-yl furan-2-carboxylate	$C_{11}H_{12}O_3$	23.56	1363	0.10	2.23
25	3-Bromo-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one	$C_{10}H_{15}BrO$	23.85	1380	0.02	0.43
26	2,3,8,8-Tetramethyltricyclo[5.2.2.0(1,6)]undec-2-ene	$C_{15}H_{24}$	23.97	1387	0.03	0.65
27	(2*S*,8a*R*)-2,5,5,8a-tetramethyl-3,6-dihydro-2*H*-chromene	$C_{13}H_{20}O$	24.07	1392	0.02	0.50
28	(*E*)-4-(2-Methylcyclohexen-1-yl)but-2-enal	$C_{11}H_{16}O$	24.24	1402	0.16	3.66
29	(*Z*)-Dodec-8-en-1-ol	$C_{12}H_{24}O$	24.34	1406	0.03	0.76
30	(*E*)-4-(2-Methylcyclohexen-1-yl)but-3-en-2-one	$C_{11}H_{16}O$	24.55	1416	0.01	0.22
31	[(2*S*,7a*R*)-4,4,7a-Trimethyl-2,5,6,7-tetrahydro-1-benzofuran-2-yl]methanol	$C_{12}H_{20}O_2$	24.86	1430	0.08	1.77
32	4-(2,6,6-Trimethylcyclohexen-1-yl)butan-2-one; [dihydro-β-ionone]	$C_{13}H_{22}O$	25.1	1441	0.02	0.40
33	(4*Z*)-4-(2,2-Dimethyl-6-methylidenecyclohexylidene) butan-2-ol	$C_{13}H_{22}O$	25.3	1450	0.07	1.72
34	(*E*)-4-(2,4,4-Trimethylcyclohexa-1,5-dien-1-yl)but-3-en-2-one	$C_{13}H_{18}O$	25.69	1468	0.01	0.32
35	Methyl (2*S*,7a*R*)-4,4,7a-trimethyl-2,5,6,7-tetrahydro-1-benzofuran-2-carboxylate	$C_{13}H_{20}O_3$	25.78	1472	0.08	1.95

Continued

TABLE 18.2 The composition of the essential oil of *S. aquatilis*—cont'd

No.	Compound	Formula	RT	RI	%	*C*, µg/g DW
36	Tetradec-3-yne	$C_{14}H_{26}$	25.93	1479	0.02	0.37
37	***(E)-4-(2,6,6-Trimethylcyclohexen-1-yl)but-3-en-2-one; [β-Ionone]***	***$C_{13}H_{20}O$***	***26.25***	***1494***	***4.66***	***108.94***
38	Pentadec-1-yne	$C_{15}H_{28}$	26.58	1511	0.01	0.30
39	2,4-Ditert-butylphenol	$C_{14}H_{22}O$	26.66	1516	0.10	2.36
40	Methyl 3-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)propanoate	$C_{15}H_{21}NO_4$	26.87	1528	0.05	1.10
41	9*H*-Pyrido[3,4-*b*]indole; [Norharmane]	$C_{11}H_8N_2$	26.92	1531	0.06	1.46
42	5-(2,3-Dimethyl-3-tricyclo[2.2.1.02,6]heptanyl) pentan-2-one	$C_{14}H_{22}O$	27.02	1537	0.02	0.40
43	(1*R*,4*R*,6*R*,10*S*)-4,12,12-Trimethyl-9-methylidene-5-oxatricyclo[8.2.0.04,6]dodecane	$C_{15}H_{24}O$	27.17	1545	0.03	0.81
44	[(*Z*)-Dodec-8-enyl] acetate	$C_{14}H_{26}O_2$	27.3	1553	0.23	5.42
45	1-Cyclohexyl-2-methoxybenzene	$C_{13}H_{18}O$	27.43	1560	0.02	0.55
46	Unidentified *m/z* 222 [M+], 165 (100)		27.48	1563	0.03	0.68
47	2,5,5,8a-Tetramethyl-7,8-dihydro-6H-chromen-3-one	$C_{13}H_{20}O_2$	27.53	1566	0.04	1.04
48	Unidentified *m/z*? [M+], 81 (100)		27.6	1570	0.06	1.46
49	2-Methyl-6-(4-methylphenyl)heptan-4-one	$C_{15}H_{22}O$	27.69	1576	0.01	0.30
50	(*Z*)-Hexadec-7-ene	$C_{16}H_{32}$	27.72	1577	0.03	0.74
51	Unidentified *m/z*? [M+], 81 (100)		27.88	1587	0.02	0.44
52	2,6,10-Trimethyltetradecane	$C_{17}H_{36}$	28.01	1594	0.94	21.86
53	1-[(1*S*,3a*R*,7*S*,7a*S*)-4-Methylidene-7-propan-2-yl-1,2,3,3a,5,6,7,7a-octahydroinden-1-yl]ethanone; [β-Oplopenone]	$C_{15}H_{24}O$	28.12	1601	0.09	2.18
54	Undecylcyclopentane	$C_{16}H_{32}$	28.26	1612	0.13	3.14
55	2-Methylsulfanyl-1,3-benzothiazole	$C_8H_7NS_2$	28.32	1617	0.08	1.90
56	Unidentified *m/z*? [M+], 81 (100)		28.42	1625	0.03	0.78
57	*N*-Phenylaniline	$C_{12}H_{11}N$	28.55	1635	0.03	0.64
58	2-Methyl-*E*-7-hexadecene	$C_{17}H_{34}$	29.04	1674	0.66	15.36
59	Cyclotetradecane	$C_{14}H_{28}$	29.11	1680	0.07	1.73
60	***Heptadec-8-ene***	***$C_{17}H_{34}$***	***29.35***	***1699***	***39.54***	***923.82***
61	(*Z*)-Hexadec-9-enal	$C_{16}H_{30}O$	29.5	1714	0.09	2.02

TABLE 18.2 The composition of the essential oil of *S. aquatilis*—cont'd

No.	Compound	Formula	RT	RI	%	*C*, μg/g DW
62	2,6-Di(propan-2-yl)naphthalene	$C_{16}H_{20}$	29.73	1736	0.35	8.10
63	Pentadecan-1-ol	$C_{15}H_{32}O$	29.81	1744	0.18	4.24
64	Unidentified *m/z*? [M+], 135 (100)		29.99	1762	0.04	0.94
65	2-[[3,4-Dihydroxy-5-(hydroxymethyl)oxolan-2-yl] oxymethyl]-6-hexoxyoxane-3,4,5-triol	$C_{16}H_{16}$	30.05	1768	0.04	0.88
66	3,5-Ditert-butyl-4-hydroxybenzaldehyde	$C_{15}H_{22}O_2$	30.17	1779	0.07	1.68
67	Octadecane	$C_{18}H_{38}$	30.3	1792	0.08	1.79
68	1,4a-Dimethyl-7-prop-1-en-2-yl-3,4,5,6,7,8-hexahydronaphthalen-2-one	$C_{15}H_{22}O$	30.33	1795	0.18	4.30
69	2,3-Diphenylcycloprop-2-en-1-one	$C_{15}H_{10}O$	30.41	1803	0.03	0.73
70	(*E*)-3-[(4*S*,7*R*,7a*R*)-3,7-Dimethyl-2,4,5,6,7,7a-hexahydro-1*H*-inden-4-yl]-2-methylprop-2-enoic acid; [Valerenic acid]	$C_{15}H_{22}O_2$	30.48	1811	0.04	0.82
71	Unidentified *m/z*? [M+], 81 (100)		30.51	1815	0.07	1.58
72	Unidentified *m/z*? [M+], 95 (100)		30.59	1824	0.06	1.43
73	2-Methyloctadec-7-yne	$C_{19}H_{36}$	30.68	1834	0.22	5.07
74	***6,10,14-Trimethylpentadecan-2-one; [hexahydrofarnesyl acetone; phytone]***	***$C_{18}H_{36}O$***	***30.76***	***1844***	***5.36***	***125.25***
75	Cyclohexadecane	$C_{16}H_{32}$	31.08	1880	0.78	18.14
76	Methyl 2-hydroxypentadecanoate	$C_{16}H_{32}O_3$	31.19	1893	0.19	4.39
77	Unidentified *m/z*? [M+], 107 (100)		31.42	1922	0.44	10.30
78	3,7,11,15-Tetramethylhexadec-1-en-3-ol; [Isophytol]	$C_{20}H_{40}O$	31.59	1945	0.51	11.99
79	***Hexadecanoic acid; [palmitic acid]***	***$C_{16}H_{32}O_2$***	***31.76***	***1967***	***4.94***	***115.45***
80	***Icos-3-yne [3-eicosyne]***	***$C_{20}H_{38}$***	***32.72***	***2091***	***9.78***	***228.45***
81	***(E)-3,7,11,15-Tetramethylhexadec-2-en-1-ol [Phytol]***	***$C_{20}H_{40}O$***	***32.96***	***2119***	***25.16***	***587.88***
82	***6-Hydroxy-1,4a-dimethyl-2,3,4,9,10,10a-hexahydrophenanthrene-1-carbaldehyde; [Podocarpal]***	***$C_{17}H_{22}O_2$***	***33.3***	***2157***	***1.11***	***26.04***
83	(8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-Hydroxy-13-methyl-2,6,7,8,9,10,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[*a*]phenanthren-3-one; [Nandrolone]	$C_{18}H_{26}O_2$	33.55	2184	0.16	3.84
84	Unidentified *m/z* 300 [M+], 111 (100)		35.48	2352	0.13	3.13

Continued

TABLE 18.2 The composition of the essential oil of *S. aquatilis*—cont'd

No.	Compound	Formula	RT	RI	%	*C*, μg/g DW
85	(2*S*,3a*R*,5a*S*,9a*S*,9b*R*)-3a,4′,6,6,9a-Pentamethylspiro[1,4,5,5a,7,8,9,9b-octahydrobenzo[e][1]benzofuran-2,5′-furan]-2′-one; [α-Levantenolide]	$C_{20}H_{30}O_3$	35.99	2389	0.08	1.89
	Total, %				100.00	
	Total number of compounds				85	
	Total number of major compounds				7	
	Major compounds, %				90.55	
	Major compounds, μg/g DW					2115.81
	TOTAL, μg/g DW					2336.62

RT is the retention time, min; RI is the linear retention index; % is the percentage of the compound regarding the sum of all compounds of the essential oil (median); C is the absolute content of the compound, μg/g DW (median).
Note: Common or most frequently used names are given in square brackets (here and further away) after the semicolon; the major compounds (the proportion of which exceeds 1%) are highlighted in bold italics.

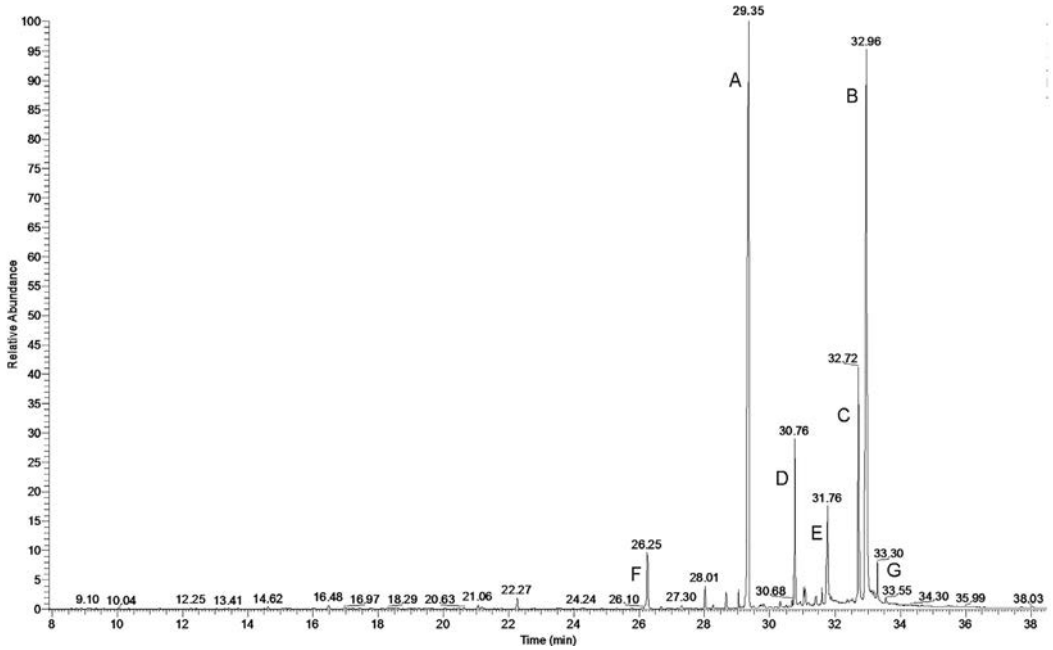

FIG. 18.1 Chromatogram of *S. aquatilis* essential oil with marked peaks of major components: (A) heptadec-8-ene, (B) 3,7,11,15-tetramethylhexadec-2-en-1-ol, (C) icos-3-yne, (D) 6,10,14-trimethylpentadecan-2-one, (E) hexadecanoic acid, (F) β-ionone, and (G) podocarpal.

was from 2% to 9.6%. At the same time, among all the studied strains, palmitic acid predominated among the extracellular free fatty acids, with the highest content in the wild strain (about 90% of the fatty acid fraction).

When studying the ability of 19 common strains of cyanobacteria from 13 genera to produce hydrocarbons, it was found that the content of hydrocarbons in 9 strains exceeded 1.0 mg/g DW of cells, and the maximum content of 1.8 mg/g DW of cells was observed in *Nostoc spongiaeforme* C. Agardh ex Bornet & Flahault [34]. In the mentioned research, it was reported that the content of hydrocarbons in *Synechocystis* sp. PCC 6803 was 1.3 ± 0.11 mg/g DCW, which practically coincides with the result obtained in our study. Also, our results on the content of hydrocarbons are almost identical to the results obtained in [35], where it was shown that alka(*e*)ne content in a mutant strain *Synechocystis* slr1556 gene loci (LX56) was 1.3% of cell dry weight, which was enhanced by 8.3 times compared with wild-type strain (0.14% of cell dry weight, or 1.4 mg/g DW).

Similar estimates were also presented in [36], where it was shown that in *Synechocystis* sp. PCC 6803, heptadecane, and heptadecene are the major constituents of alka(*e*)nes, and their total content does not exceed 0.15% of the cell dry weight. In Table 18.2 the discovered components are listed in the order of their elution from the column in the course of the GC-MS analysis. The major components (relative content >1%) in the composition of LMWM of *S. aquatilis* were only seven compounds: heptadec-8-ene (39.54%), phytol (25.16%), icos-3-yne (9.78%), 6,10,14-trimethylpentadecan-2-one (5.36%), hexadecanoic acid (4.94%), β-ionone (4.66%), and podocarpal (1.11%). These major components accounted for more than 90% of the total LMWOCs content in *S. aquatilis* (Table 18.2).

Current study identified heptadecene as 8-heptadecene (Fig. 18.2). However, it is possible that the *S. aquatilis* metabolome contains several isomers of heptadecene, for example, 7-heptadecene and 1-heptadecene. A similar possibility is indicated by the results obtained in the study of some microalgal species [37]. In particular, it was shown that the major isomer of heptadecene produced by *Chlamydomonas reinhardtii* P.A. Dangeard and *Chlorella variabilis* Shihira & Krauss was 7-heptadecene. The minor isomer was 8-heptadecene (around 20% of the major isomer). To establish the exact composition of heptadecene isomers and their ratio in *S. aquatilis*, it is necessary to carry out special studies related to the derivatization of the produced heptadecene, for example, using dimethyl disulfide [37].

It should be noted that 8-heptadecene was identified as one of the major compounds in *Synechocystis* sp. PCC6803 in the studies [34,38]. Icos-3-yne (3-eicosyne) was identified in the essential oil of *Centella asiatica* (L.) Urb. as a major component (8.069%) [39]. The presented estimate of the content of 3-eicosyne in *C. asiatica* is very close to the content of this component in *S. aquatilis* (9.78%) (Table 18.2). Also, 3-eicosyne was found in the essential oil of *Tamarindus indica* L. [40]. The content of this compound during extraction into chloroform using a Soxhlet apparatus for 12 h was 4.62%. An almost identical content of 3-eicosyne (4.75%) was found in the ethanol extract of the Malacca leaf (*Phyllanthus emblica* L., also known as Malacca tree) [41]. At the same time, among the phytocompounds present in the ethanolic leaf extract of *Erythrina variegata*, 3-eicosyne accounted for 54.93% [42]. 6,10,14-trimethylpentadecan-2-one was detected in isolates of *Botryococcus braunii* Kützing (Chlorophyta) collected from different Nile River sites in the Delta region, Egypt [43]. Its content in different isolates ranged from 0.216% to 13.86% LMWOCs in n-hexane extracts of *B. braunii*. In aquatic macrophytes, hexahydrofarnesyl acetone is often included in the major components. Its content (% of the total amount of LMWOCs) is: in *Ceratophyllum demersum* L.—from 2.38% to 4.47%; *Nuphar*

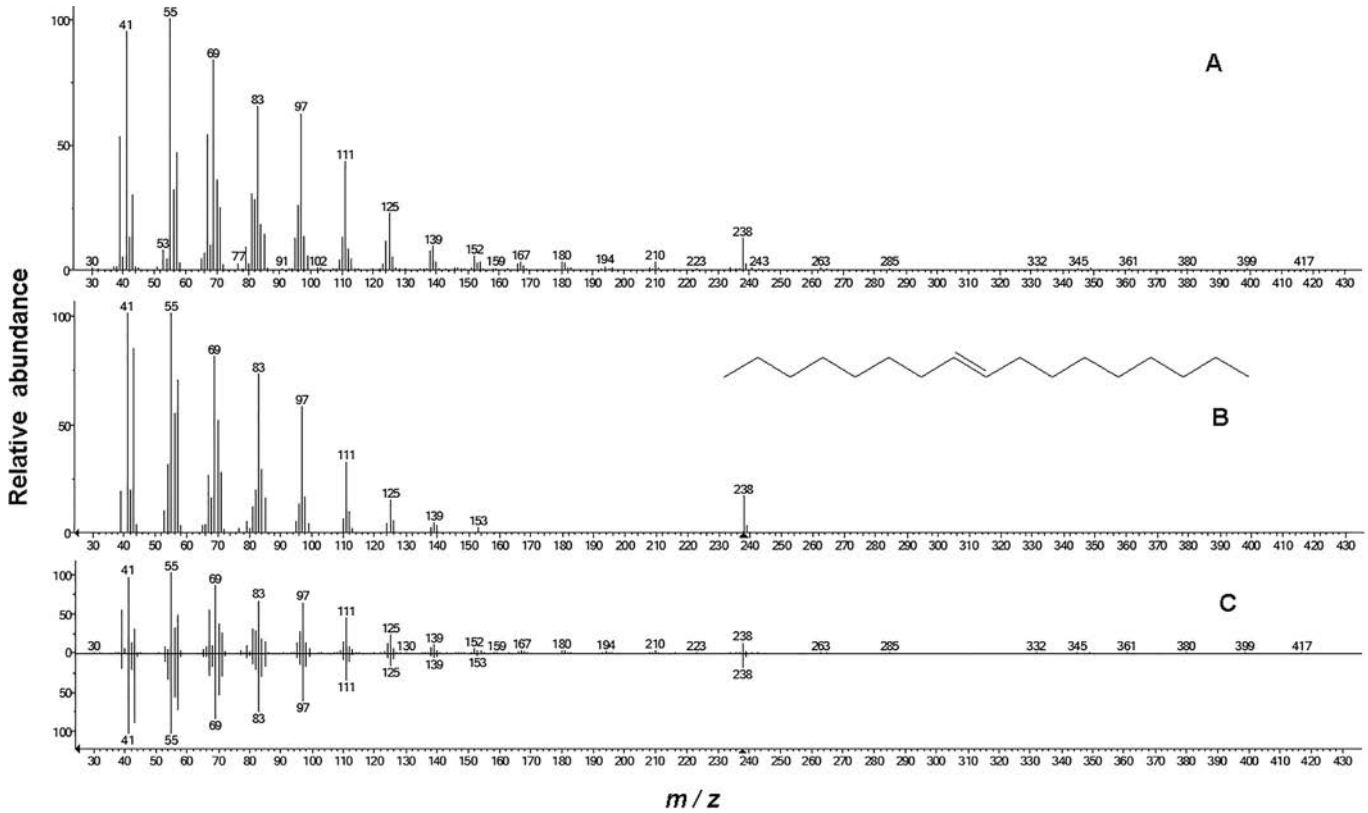

FIG. 18.2 Mass spectra of heptadec-8-ene: (A) an experimental spectrum from metabolome of *S. aquatilis*, (B) NIST-library spectrum, (C) the difference between experimental and library spectra.

lutea (L.) Sm.—3.27%; *Potamogeton perfoliatus* L.—2.6%; *Potamogeton pectinatus* L.—from 3.12% to 9.09% [30,44–46].

In *Iris pallida* Lam. from Ukraine, terpene 6,10,14-trimethylpentadecan-2-one (8%) was included in the major components in the essential oil of the leaves [47]. The phytone content in plant materials can be higher. So, among 19 detected compounds in the hexane extract of aromatic water from the flowers of *Salix caprea* L., hexahydrofarnesyl acetone was the predominant component, with a content of 38.3% [48]. Notable is the high concentration of LMWOCs in the dry biomass of *S. aquatilis*. Their content was 2336.62 μg/g DW, which is several times higher than the content of LMWOCs in aquatic macrophyte dry biomass [30,46], with the major components accounting for 2115.81 μg/g DW. The work [49] presents a list of 34 compounds identified in two *Synechocystis* strains (freshwater *Synechocystis* sp. PCC 6803 and marine *Synechocystis* sp. PCC 7338), obtained by gas chromatography–mass spectrometry (GC-MS) analysis. Of the 6 fatty acids (linoleic acid, linolenic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid) identified in this study, in our case, we found only palmitic acid, with a fairly high content (4.94% of the total content LMWOCs and 115.4 μg/g DW, Table 18.2).

β-Ionone is a cyclic terpenoid compound, and its structural nucleus forms molecules of such compounds as retinol, retinoic acid, b-carotene, and vitamin A [50,51]. This compound is present in many essential oils of both terrestrial and aquatic plants [45,52]. β-Ionone and its derivatives can be found in plant grains, vegetables, fruits, and, therefore, in various products of plant origin [53]. The β-cyclocitral and β-ionone identified by us in *S. aquatilis*, along with α-ionone and geranylacetone, are widespread among the metabolites of cyanobacteria and are also the main components in many species, for example, those of the genus *Microcystis* [54–56]. The reported content of β-ionone in *S. aquatilis* biomass is approximately 670 times higher than in cyanobacterial mats in eutrophic freshwater Lake (162.20 ng/g DW) Yangtze River delta, China [57] and 17–53 times higher than that freshwater macrophytes *P. pectinatus* and *Ceratophyllum demersum* (2.0–6.3 μg/g DW and 3.0–3.8 μg/g DW), respectively [30,46].

4 Prospects for the use of metabolites of *S. aquatilis*

Thus, by now it has become obvious that strains of the genus *Synechocystis*, including *S. aquatilis*, can be source-producers of several useful substances. Below, we will consider the possible fields of application of those compounds that are major in the species *S. aquatilis* studied by us, namely, in its wild strain. Today, we can speak of the fact that many primary and secondary metabolites of cyanobacteria can have commercial potential in the pharmaceutical industry and agriculture [58]. *S. aquatilis* has one of the highest rates of carbon dioxide fixation (CO_2 fixation rate) (138.9 g/(m^3 h)) among cyanobacteria [59], and making it one of the most promising for obtaining cyanobacterial biomass subsequent versatile applications.

Heptadec-8-ene. Heptadecene may be considered a compound that may be used widely in a range of applications of human activity. One such area is beekeeping. In this industry, great attention should be paid to the bee colony's health, which is a necessary basis for obtaining

high yields of honey. However, there are multiple risks of developing beekeeping. So, due to the widespread of various diseases and pests, the deterioration of the ecological situation in the regions, the destruction of local populations of aboriginal bees, the lack of fully effective drugs, and the use of pesticides, as a result, there are cases of honey bee colony losses [60]. In this regard, it seems relevant to research the search and application of environmentally friendly and affordable protective agents of plant origin.

Varroosis is a dangerous disease of bees that entails large losses in apiaries. The parasitic mite, *Varroa destructor* Anderson and Trueman, is the most important threat to apiculture in most bee-keeping areas of the world [61,62]. Also, Hristov et al. [60] highlight that the ectoparasitic mite *V. destructor* is a major factor in honey bee colony loss worldwide. Being an obligatory ectoparasite, the mite not only feeds on the hemolymph of bees but additionally is an active vector for pathogenic viruses, which have become more abundant and virulent since the invasion of the mite [61]. Experimental data from [63] uncovered an unrecognized mutualistic symbiosis between Varroa and the deformed wing virus. The discovered loop of reciprocal stimulation is the reason for the escalating negative effects on honey bee immunity and health.

Several substances have been identified in recent years that are repellents for parasite mite, particularly bee venom [64] and royal jelly with octanoic acid as the main active ingredient [65]. Another study revealed the presence of such a mite-specific repellent as Z-8-heptadecene in bee colonies [66]. This repellent from the group of alkenes is secreted by adult bees, which leads to the fact that mites, in general, prefer to parasitize on young intrahive individuals without adequate protection. At the Department of Plant Protection of the University of Udine (Italy), experiments on the use of certain doses of heptadecene biologically characteristic of bees were carried out in laboratory and field conditions. In experiments, it was shown that heptadecene stimulates the laying of eggs by the queen bee and is a repellent for the mite. The control group of families was more infected with the parasite *V. destructor*, while in the experimental group, heptadecene assimilated by bees had a repellent effect on mites [67]. It was established that a specific chemical communication exists between the *V. destructor* mite and the honey bee *Apis mellifera* L. [66,68]. The mite avoids individuals in which the content of heptadecene in the cuticle is increased, individuals with a decreased content of heptadecene being affected by *V. destructor* [61]. It was also reported that (Z)-8-heptadecene, caused a 30% reduction in the mean number of offspring of mites reared in cells treated with this compound [66]. In the work [69], the repellent effect of heptadecene, the cuticular substance of bees, on the ectoparasite *V. destructor* was also established, and a biological method for reducing the infestation of bee colonies was substantiated. Heptadecene, when administered weekly in the amount of 5 μL per colony in the diet of bees, stimulates the laying of eggs by the queen bee and has a repellent effect on the *V. destructor* mite.

In experiments by Trifonova [70], it was shown that the productive qualities of bee colonies in the experimental group fed with heptadecene increased in terms of the level of flight activity in the period of supporting honey collection by 1.41 times ($P < .01$), during the main honey harvest by 1.46 times ($P < .001$); by the level of commercial honey produced by 1.61 times (by 9.53 kg), wax—by 1.97 times (by 3.37 honeycomb frames) ($P < .001$). In studies [61,71] it was also shown that heptadecene is a bee forager-specific deterrent against *Varroa*. Thus, (Z)-8-heptadecene may play an important role in the host-parasite relationship, and the study of its influence on the biological and productive indicators of bee colonies gives

grounds for using an alternative method for reducing parasites to increase the yield of commercial honey. Heptadecene, which can be produced from the cultivated biomass of *S. aquatilis*, can be used to improve beekeeping conditions and therefore increase honey production.

(E)-3,7,11,15-Tetramethylhexadec-2-en-1-ol [Phytol]. Phytol can have a variety of biomedical applications. It has been investigated and has shown activity for the following biological effects: anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, antiinflammatory, immune-modulating, and antimicrobial effects [72]. Phytol can be used in cosmetology and perfumery, it is irreplaceable and improves the quality of shampoos, toilet soaps, household cleaners, and detergents [73]. Its global demand is estimated to be 0.1–1.0 metric tons per year [74]. Recent large reviews [72,75] provide extensive information on the practical use of phytol. In particular, it is noted that phytol is a potential candidate for a broad range of applications in the pharmaceutical and biotechnological industries. For example, phytol, a fragrance ingredient, is used in the fragrance industry and is used in decorative cosmetics, fine fragrances, shampoos, toilet soaps, and other toiletries as well as in noncosmetic products such as household cleaners and detergents [73]. This state of affairs is due to the fact that phytol demonstrated odorous, anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy-inducing, apoptosis-inducing, antinociceptive, antiinflammatory, immune-modulating, and antimicrobial effects [72,76] and do not show pronounced mutagenic properties [77], and also do not lead to malformations. Moreover, it greatly reduced retinol-induced teratogenic effects (ear anotia, taildefects, exencephaly) in mice, indicating that phytol may be useful for the prevention of vitamin A teratogenicity [78]. Earlier, when using the QSAR method for identifying the promising compounds in the essential oil of *N. lutea*, *C. demersum*, and *Potamogeton obtusifolius* for further experimental research designed for medical, pharmaceutical, cosmetic, and environmental purposes, it was shown that phytol is a promising compound with a high probability of manifestation of antineoplastic, antiinflammatory, antifungal, and antibacterial activities [44].

The antibacterial properties of phytol and its derivatives are also confirmed by the data presented in publications [79,80]. Taking into account all the available information on phytol, summarized in [72,75] phytol from a pharmaceutical viewpoint may be considered as a new drug candidate. Phytol can also be used as an element of chemical protection against unwanted insects. The study [81] provides experimental evidence that herbivorous beetles form a chemical defense using both primary and secondary host-derived compounds, including phytol and its derivatives. Investigated beetle species carried out modifications to host-derived precursors before incorporating the metabolites into fecal shields. Fecal shields, containing phytol-like compounds (phytol derivatives), protected herbivorous beetles against ant attack [82]. Thus, the cultivated biomass of *S. aquatilis* (its various strains) can serve as a good source of the natural form of phytol in order to cover its needs for various uses.

Icos-3-yne [3-eicosyne]. According to information from [40], compound 3-eicosyne belongs to that class of metabolites that have been cited as potent antimicrobial and cytotoxic substances. According to [39], the essential oil of *C. asiatica*, which contains 3-eicosyne as a major component (8.069%), can be used for inhibiting MAO-A (Monoamine oxidase A) activity in the brain. It should be noted that *C. asiatica* (also known as Gotu kola) is a medicinal plant, and it is widely used for food and medical purposes in the form of various types (natural raw

materials, extracts, powders, decoctions, etc.). It is used as a universal remedy for 3000 years in folk medicine as well as now in scientifically oriented medicine and cosmetology [82–86]. 3-Eicosyne is also a major component of the Malacca tree (*P. emblica*) which is one of the plants that is often used by the population of Indonesia as a traditional medicine for the treatment of various diseases such as antimicrobial, antibacterial, antifungals, antivirals, antimutagenic, antimalaria, and antiallergic [41]. Certain beneficial properties of products from *C. asiatica* and *P. emblica* may be provided due to 3-eicosyne, which makes it relevant to research to identify the beneficial properties of this compound, as well as the use of various sources to obtain its "natural" form. *S. aquatilis*, namely its cultivated biomass, can serve as such a source. If 3-eicosyne possesses a wide range of phytochemical and therapeutic characteristics, its active principle molecule can be used individually or in combination with other components to treat patients with various diseases. At the same time, the high content of 3-eicosyne found in some plants (e.g., 54.93% in the ethanolic leaf extract of *Erythrina variegate* L.) allows us to consider this compound not only as a medical component but also as a nutrient [42,87]. As shown in [88] hexahydrofarnesyl acetone has been found to be a major component in tibial fragrances and is a pheromone of the species of genus *Euglossa* for males of orchid bees.

Phytone has been found to serve as a Bermuda grass phagostimulant, and it determines the host plant preference for insects [89]. It is a fact that this widespread ketone occurrs in plants and insects and some species (e.g., the Large Cabbage White butterfly, *Pieris brassicae* (L.)) are used as part of their pheromone bouquet [90]. The leaf oil *Ecballium elaterium* (L.) A. Rich. was found to contain 19.1% of hexahydrofarnesyl acetone, which was the main component among 21 constituents detected in the leaf oil. At the same time, this oil exhibited strong allelopathic effects against lettuce [91]. Ahmed et al. [48] provided information about the volatile constituents of *S. caprea* to assess the quality profile of this product. traditionally used as a cordial tonic. The findings indicate that the hydrodistillate has significant antioxidant and antiinflammatory properties. At the same time, hexahydrofarnesyl acetone prevailed among the components of aromatic water from the flowers of *S. caprea* (38.3%). Results presented in [48] may also suggest that the hydrodistillate of the flowers of *S. caprea* can be used as a natural preservative ingredient in the food and/or pharmaceutical industry as well.

In studies assessing the composition of essential oils and phytotoxicity carried out on species such as *Launaea nudicaulis* (L.) Hook. f., *Heliotropium curassavicum* L., and *Ailanthus altissima* (Mill.) Swingle [92–94], it was demonstrated that hexahydrofarnesyl acetone, as one of the main components, plays a leading role in the formation of phytotoxicity and allelopathic effects of essential oils of these plants. Thus, hexahydrofarnesyl acetone as an active pheromone and a phytotoxic and allelopathic agent can be used in systems for controlling the development of insects and plants, and highly productive cultures of *Synechocystis* strains can serve as its source.

Hexadecanoic acid. In the species *S. aquatilis* studied by us, palmitic acid was detected, with a rather high content (Table 18.2) of 4.94% or 115.4 μg/g DW. This fact speaks in favor of the fact that the cultivated biomass of *Synechocystis* can be a source of this acid with its subsequent use for various purposes. Furthermore, fatty acids produced in *Synechocystis* 6803 after several rounds of genetic modification and optimization may be used as biofuel precursors and constituents [36,95]. At the same time, genetically modified strains can significantly increase the production of fatty acids [19]. Palmitic acid (and its salts) can be used in a wide variety of fields of human activity from the production of soaps and cosmetics to civil and military

use, for example as a thickening agent. Palmitic acid and its salts (e.g., sodium palmitate) are of great importance in the food industry. Palmitic acid and palmitates are of great importance in the fields of medicine and human health, for the production of biofuels and other industrial sectors. Information on these issues is extensive and, in particular, is collected on the page [96–105]. We will focus here on providing information on the use of palmitic acid as an allelochemical, which can be used as a component for producing a new generation of algicides to control and suppress such a dangerous phenomenon as cyanobacterial "bloom" in water bodies. Many authors reported [106–119] that some fatty acids (including C16:0) inhibited the growth of cyanobacteria.

Standard compound addition tests indicated that palmitic acid and alpha-linolenic acid might play important roles in the induction of colony formation and growth inhibition of *Chlorella vulgaris* Beijerinck [120,121]. Moreover, our investigation of potential biological activities of major LMWOCs of aquatic macrophytes using the QSAR method has discovered that fatty acids possess various types of bioactivities with the highest probability of manifestation ($P_a > 0.9$) that can induce inhibition of cyanobacteria growth [122]. Earlier, in our laboratory experiments with fatty acids for the study of their effect on the cyanobacteria *S. aquatilis* and *Aphanizomenon flos-aquae* (L.) Ralfs ex Bornet & Flahault, and which are described in detail in [18] it was found that linoleic, heptanoic, octanoic, tetradecanoic, and hexadecanoic acids exhibit an inhibitory allelopathic effect in relation to cyanobacteria. However, their inhibitory effect were different. It should be noted that hexadecanoic acid had some of the highest values of the suppression index (SI, defined as the cyanobacterial density in control divided by the cyanobacterial density in an experiment with allelochemicals: SI = 10.4 at a concentration of 0.1 mg/L on the 13th day of the experiment with maximum SI) [18]. These results, as well as the results of our previous studies [18,30,122], show that hexadecanoic acid is a very promising component for the creation of a new generation of algicides [123] against cyanobacteria based on such allelochemicals as fatty acids.

β-Ionone. β-Ionone, a cyclic terpenoid compound, is a precursor for the synthesis of carotenoids, which are one of the most important groups of pigments in nature. In addition, β-ionone is a metabolite of β-carotene and α-carotene [52,124]. Natural β-ionone has obvious advantages in a variety of applications, as it allows, for example, the production of flavoring and aromatic products bearing the desired label "natural" according to the U.S. and European food and safety regulations [51]. The high level of β-ionone synthesis in *S. aquatilis*, therefore, makes it possible to use this species for cultivation in order to obtain (including) natural β-ionone. It has long been known that β-ionone and its derivatives have antifungal and antibacterial activity [125]. All the tested compounds possessed antifungal activity against all the phytopathogens used in this work (*Staphylococcus aureus* 209 P, hemolytic *Streptococcus pyogenes* FF-38, *Streptococcus* sp. TOM-1606, *Micrococcus luteus* 2665, and *S. aureus* Rosenbach).

According to results received in [126–128], β-ionone and its derivatives possess pronounced activity against the Gram-positive, Gram-negative bacterial strains, against *Candida albicans* (C.-P. Robin) Berkhout, and against *Streptococcus mutans* Clarke, which is the primary bacterium causing dental caries in humans [129]. Ikawa et al. [54] investigated the ability of volatile organic compounds produced by cyanobacteria in freshwater lakes to inhibit the growth of the green alga *Chlorella pyrenoidosa* Chick. According to this study, geosmin, β-cyclocitral, α- and β-ionones, and geranylacetone exhibited inhibitory activity by diffusion in the 2–5 mg/mL range. Among the studied LMWOCs α- and β-ionones and geranylacetone

showed the best inhibition through the vapor phase at 10 mg/mL. The work [130] also showed that β-ionone, limonene, and longifolene may poison other algae by inducing programmed cell death. The information available in the literature shows that β-ionone can be considered as the most typical terpenoid-allelochemical in HABs (harmful algal blooms) control [131,132]. In particular, it was found in the extraction of *Elodea nuttallii* (Planch.) H.St.John, a macrophyte with strong allelopathic properties. On exposure to β-ionone, *Microcystis aeruginosa* Kützing was inhibited via the distortion of thylakoids with EC50 of 25.3 mg/L [131]. Another study [132] showed that not only β-ionone but also its derivatives (4-oxo-β-ionone, 3-hydroxy-5,6-epoxy-β-ionone, and 6- hydroxy-3-oxo-α-ionone) from *Vallisneria spiralis* L. exhibited antialgal activities against *M. aeruginosa* cells.

The use of β-ionone in household chemicals, perfumes, fragrances, decorative cosmetics, shampoos, and soaps is also noteworthy [133]. According to the findings of studies [134,135] have shown that β-ionone can be classified as valuable chemopreventive and chemotherapeutic compounds. In particular, β-ionone is characterized by the ability to prevent the formation of metastases and to induce apoptosis for the selective destruction of tumor cells (breast cancer, hepatic cancer, osteosarcoma, gastric cancer, prostate cancer) [136–145]. Moreover, information was obtained [146] that β-ionone derivatives also exhibit a broad spectrum of biological activities, and the results showed that most of the β-ionone derivatives were more active than β-ionone. So, the β-ionone derivatives with ortho-substituents on the aromatic ring exhibited much stronger cytotoxicity than their corresponding meta- and para-substituted compounds [146].

Ansari and Emami [52], conducted a literature review on b-ionone and its derivatives and concluded that b-ionone, its derivatives, and analogs are versatile frameworks with high potential for the development of new anticancer agents with greater activity than the parent compounds. According to research, the antiinflammatory effects of b-ionone make it a viable therapeutic agent for treating lipopolysaccharide-induced diseases [147]. Thus, several investigations have elucidated unique biological properties and activities of β-ionone that indicate its great potential, in particular, in chemopreventive and chemotherapeutic strategies for overcoming different kinds of cancer. This makes it relevant to use natural, highly productive sources of "natural" β-ionone, to which *S. aquatilis* strains can be attributed.

Podocarpal. The use of another major compound found in *S. aquatilis*, namely podocarpal, has not been developed and is not obvious currently. Given the chemical structure of this compound and the known fields of use of structurally related compounds (e.g., diquat, benzo[c]cinnoline), it should be tested for the possibility of use as a herbicide, in medicine and pharmacology, as a drug product in veterinary medicine, and in the production of new polymers [148–150]. *S. aquatilis* is reported to be the source of antimicrobial agents against five Gram-positive bacteria, three Gram-negative bacteria, and two fungi [151]. The antimicrobial activity (assessed by the disc diffusion method) of the methanol extract of *S. aquatilis* was the highest of all 7 tested thermophilic species of cyanobacteria (*Oscillatoria subbrevis* Schmidle, *Oscillatoria tenuis* C.Agardh ex Gomont, *Oscillatoria limnetica* Lemmermann, *Oscillatoria angusta* Koppe, *Oscillatoria articulata* N.L.Gardner, *S. aquatilis*, and *Synechococcus cedrorum* Sauvageau). The work [152] also showed that ethanol extract has pronounced antibacterial activity.

In a study [153] the antimicrobial activity of seven alkaliphilic cyanobacterial cultures that were isolated from the alkaline saline Lake Lonar (India) was tested. Among the cyanobacterial cultures under test, the unicellular cyanobacterium, *S. aquatilis* showed the highest antibacterial activity against the test bacterial cultures (*E. coli* (Migula), *S. aureus*, *Proteus vulgaris* Hauser, *Pseudomonas aeruginosa* (Schröter 1872) Migula) [153]. Some minor components found in the composition of LMWM of the *S. aquatilis* strain studied by us also deserve attention. Thus, in [154] it was shown that *S. aquatilis* produced the indole alkaloid norharmane with strongly pronounced allelopathic properties against two cyanobacteria species (*M. aeruginosa* and *O. limnetica*) and two green algae species (*C. vulgaris* and *Ulothrix sp.*). It was found that the concentration of norharmane in the culture medium reached rather high values (86 μg/L). The author also concludes that norharmane can be used as a natural algicide to prevent the formation of HABs, by controlling or mitigating [154]. According to [155] norharmane was found to be cytotoxic against the seven investigated cyanobacterial test organisms in low quantities (0.5–18.0 μg). It should also be noted that the metabolite norharmane can suppress the development of the species itself that produces it, apparently, based on the principle of autotoxicity. Thus, in [156] it was shown that the time-dependent minimum cytotoxic concentration of norharmane against *S. aquatilis* was 32 μg/mL.

However, in our study, the concentration of this compound in the LMWM of the investigated *S. aquatilis* strain was rather low—only 1.46 μg/g DW, which was 0.06% of the total amount of LMWOCs. In the studied culture fluid, this substance was not found by us at all. In the work [157], in the process of screening of culture media obtained from 33 selected microalgal species to identify the most active producers of norharmane, it was obtained that *S. aquatilis* was not included in cyanobacteria (*Anabaena cylindrica* Lemmermann, *Anabaena inaequalis* Bornet & Flahault, *Anabaenopsis siamensis* Komárek & Anagnostidis, *Chroococcus minutus* (Kützing) Nägeli, *Nostoc carneum* Agardh ex Bornet et Flahault, *Nostoc commune* Vaucher ex Bornet & Flahault, and *Phormidium foveolarum* Gomont) which were found to excrete norharmane in different amounts into their environment. The production of one or another allelochemical agent strongly depends on the conditions of existence of the species. A similar effect of a strong change in LMWM depending on conditions was also demonstrated for aquatic macrophytes [45,46]. In our case, under the conditions of pure monoculture, the level of norharmane synthesis in *S. aquatilis* was extremely low due to the absence of competitors from the phytoplankton composition, which suppressed the development of the norharmane that can be synthesized by the species in significant quantities under appropriate conditions. The antimicrobial metabolite norharmane is of special interest in the development of new environmentally harmless and tributyltin-free antifouling paints for ships as well [156,158]. The authors note that further active research is needed for the application of norharmane in this field, focused, in particular, on the study of the biodegradability of norharmane, its compatibility with the carrier material, as well as the release rate of the substances from the appropriate carrier. Another interesting minor component found in *S. aquatilis* is 2,6,6-trimethylcyclohexene-1-carbaldehyde or β-cyclocitral (Table 18.2). It has been shown [159] that β-cyclocitral is a compound that only produces a characteristic color change of culture broth from green to blue. This color change is similar to the observed when a sudden decline in the growth of cyanobacteria and their withering away begin in a natural environment. The β-cyclocitral concentration in *S. aquatilis* is not very high—only

6.03 μg/g DW or 0.26% of the total amount of LMWOCs. However, β-cyclocitral produced by *S. aquatilis* can be used in a complex of compounds to suppress the development of cyanobacteria while preventing the "bloom" of water bodies.

The study [160] identified allelochemicals produced by *Microcystis*, and it was shown that β-cyclocitral is one of the compounds that helped *Microcystis* to take dominance in water bodies. Thus, β-cyclocitral at a concentration of $\geq$0.05 mM showed toxic effects on *C. reinhardtii* cells, and at a concentration of 0.4 mM, complete death of *C. reinhardtii* cells was observed during 24 h [160]. In cyanobacteria, β-cyclocitral functions not only as an inhibitor of competing microalgae but also as a repellent against grazers [161]. The ability of cyanobacteria to synthesize certain valuable compounds can be enhanced by certain manipulations. For example, they can be engineered to produce fatty alcohols, fatty acid ethylesters, and hydrocarbons by altering the metabolic route that synthesizes fatty acids. The resulting compounds can be used as chemical precursors, or directly, as biofuels [22,38]. Cyanobacteria are those organisms that can be called the best producers of hydrocarbons due to their high rate of biosynthesis of organic compounds. They are capable of synthesizing various classes of hydrocarbons such as alkanes, alkenes, and terpenes, which can be used as valuable chemicals in the fields of food, fuels, pharmaceuticals, nutrition, and cosmetics [7].

Unlike plants and eukaryotic algae, cyanobacteria, being prokaryotic microorganisms, are able to grow a lot faster. Genetic engineering platforms for cyanobacteria are well developed, making them an essential foundation for improving the biosynthesis of valuable compounds. Currently, it can be argued that genetic engineering is a powerful and realistic tool for the efficient production of biofuels from cyanobacteria [38,162,163]. The obtained results [164,165] using mutant strains of *Synechocystis* and *Synechococcus* provide promising prerequisites for the use of metabolic and genetic engineering of cyanobacteria to improve the photosynthetic production of biofuels based on hydrocarbons and fatty acids of cyanobacteria.

Currently, significant progress has been made in the development of potential methods to improve the productivity of fatty acid derivatives through genetic modification, physiological regulation, and environmental stress induction, as demonstrated in [38]. However, the authors note the still insufficient efficiency of industrial biosynthesis but emphasize the high potential of this approach for the synthesis of fatty acids, fatty alcohols, and hydrocarbons based on cyanobacteria. This potential is primarily due to the conversion of carbon dioxide into solar energy-driven to high-value chemicals and high-energy fuels through genetic engineering strategies involving photosynthetic cyanobacteria [38].

An example of realizing this potential can be found in the results obtained by Hu et al. [166], when it was shown that the *Synechocystis* 6803 strain with additional copies of the FAR and FAD genes encoding, respectively, the enzymes of fatty acyl-ACP reductase and fatty aldehyde decarbonylase in the pathway of alkane biosynthesis, demonstrated an increase in the intracellular level of heptadecane content by five times, a threefold increase in 9-heptadecene content, as well as a significant increase in the secretion of 16:0 and 18:0 free fatty acids. When assessing the production of certain hydrocarbons, one should also take into account the fact of the influence of temperature as one of the important factors, since at different temperatures of growth of a culture of microalgae, the composition and amount of certain hydrocarbons can differ significantly [167].

An important issue in the use of cyanobacteria for the synthesis of certain target components (compounds) is the choice of light conditions for the cultivation of cyanobacteria.

Thus, photobioreactors with light-emitting diode (LED) illuminating systems produce significantly more biomass than photobioreactors with solar illumination and open ponds [168]. However, one should take into account the significantly different production costs per unit of biomass. The cost of biomass production is lowest in open ponds—only 3 USD per kilogram, while in photobioreactors with LED lighting it is 8–10 times higher (25–30 USD). When growing transgenic cyanobacteria with high productivity at low cost in open ponds, the risk of GMOs leaking into the environment should be considered, which could create additional environmental problems [169].

5 Conclusion and future outlook

It is now well established that several cyanobacteria species, including *S. aquatilis*, can function as biofactories to synthesize "natural" valuable chemicals. Our findings provide essential information for selecting *S. aquatilis* for various practical applications and knowledge for the future use of *S. aquatilis* for novel *Synechocystis* strains to produce specific metabolites in high concentrations via appropriate metabolic pathways. Despite the fact that the cost-effectiveness of producing valuable chemicals from *Synechocystis* biomass has been called into question [12], such production is promising and appealing because it allows for the production of valuable products using sunlight and carbon dioxide from an inexhaustible source. Currently, the insufficient economic efficiency of the production of valuable compounds from the biomass of cyanobacteria is one of the main obstacles to the large-scale development of various biotechnological industries based on the production of cyanobacterial biomass and its further multifaceted use [22]. The use of appropriate strains, the introduction of synthetic biology discoveries, the improvement of bioreactor designs, and improved extraction and purification techniques for target biocomponents would help tackle these challenges. In this regard, an important task is to search for the corresponding strains of *S. aquatilis* and their genetic modification for the production of certain substances.

In addition, the synthesis of many valuable compounds can largely depend on the conditions in which the cultivation of *Synechocystis* takes place. This makes it relevant to research the influence of various environmental factors on the production of certain compounds. Success should be facilitated both by the development of traditional methods for extracting valuable substances from the biomass of *Synechocystis*, and novel techniques, like supercritical fluid extraction, microwave-assisted extraction, and ultrasound-assisted extraction [6]. In addition, environmental protection strategies and legal frameworks need to be developed to prevent environmental risks due to the inevitable leakage of genetically modified cyanobacteria into the environment [169]. The use of the studied *S. aquatilis* strain (as well as other strains of this genus) for the production of "natural" compounds seems promising and beneficial due to increased consumer demand for products with the labels "natural," "organic," or "bio" [170]. For example, "natural" β-ionone has a market value of about 10–100 times higher than the synthetic form of this compound [51]. In this regard, the development and improvement of technologies that enable the extraction and purification of required valuable chemicals from the cultivated biomass of Synechocystis strains and the creation of novel high-yielding strains have become critical.

Conflict of interest

The authors declare that there is no conflict of interest.

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CHAPTER

19

Microalgae potentials as bioactive phytochemicals for human's health: Novel highlights on their production, applications, and emerging analytical technologies

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1 Introduction

The international community's attention has lately turned in a major way to the emerging market for dietary phytochemical supplements, which is considered to be safe. Microalgae are considered future superfoods, as mentioned by Torres-Tiji et al. [1]. It has several important nutritional phytochemicals such as phycocyanin, a novel protein in *Spirulina*, polyphenols, and flavonoids. The nutritional content varies significantly among microalgal species. Furthermore, only a few microalgae have been designated as "Generally Recognized As Safe" (GRAS) by the Food and Drug Administration [1]. These microalgae include *Arthrospira platensis*, *Chlorella vulgaris*, and *Scenedesmus obliquus*. They are popular in the United States, China, India, and Japan [2,3]. Several studies have reported that microalgae extracts have potent radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) [2]. On the other hand, scientists are trying to find instant and noninvasive techniques to explore the scientific principles of microalgae phytochemicals' functional principles, especially bioactive proteins, phenolic and flavonoid compounds [4]. These phytochemicals increase the medium antioxidant, anticancer, and other physicochemical potentials [5–10].

Important microalgae nutritional components like lipids and proteins are indispensable components of these microalgae cells and are precursors of many essential molecules and are a novel source of these bioactive molecules. For example, *Phaeodactylum tricornutum* EPA, which is generated as an eicosapentaenoic acid in its dry biomass, accumulated up to 30%–40% of the overall fatty acids. These lipids also include important fatty acids such as linolenic acid, linoleic acid, and docosahexaenoic acid (DHA), which are all known as omega-3 fatty acids [1]. Also, microalgae are considered as a new source of novel proteins. These proteins include commercially available phycocyanin novel food protein supplements produced by *Spirulina* (*Arthrospira platensis*; or Cyanobacteria) [11].

On the other hand, edible films, which are biopolymer-based materials produced from microalgae as a natural, safe source of polysaccharides, provide many intriguing aspects in the food science and technology area as a viable alternative to nonbiodegradable plastics. One of these biopolymers, alginate, is one of the most versatile biodegradable polymers, algal-extracted polysaccharides [12]. Yet, these authorized microalgae have a long history of safety. However, significant concerns have been raised regarding certain products produced from these microalgae owing to contamination with cyanotoxins, hazardous metals, or inorganic arsenic.

Thus, it is highly recommended to establish a comprehensive overview of microalgae organic composition through advanced instant measurements applicable during the cultivation process [10,13,14]. Moreover, the physical and chemical effects of the different industrial processes on the functional phytochemicals could change their complicated chemical composition and the desired complex mechanism [15]. Thus, many beneficial impacts could be achieved by enhancing this field of biochemical investigation. Several analytical methods

could be combined, like spectroscopic, chromatographic, electrochemical, immune, and molecular techniques, to discuss and improve this field. The potential use of microalgae extracts for treating different health diseases will add to this field. Moreover, several sources, phytochemistry, and the risks of the different alternative and edible microalgae are very important to be further investigated. Currently, chemical and chromatographic methods are used for the evaluation and analysis of microalgae chemical composition. However, these methods are chemical and time-consuming, and cannot obtain detailed information about the single-cell without affecting its structure [16]. On the other hand, the microalgae electrochemical charge is a very important parameter for identifying and establishing a reference method for its characterization based on electronegativity fingerprint [17–19]. This chapter discusses the micro and macromolecules in microalgae as alternative novel sources for human consumption and the rapid detection methods for them. Furthermore, analytical methods applied to their safety were summarized and recent advances in the methodology were highlighted.

2 General applications of microalgae in biotechnology field

2.1 Microalgae and bioremediation

Several large-scale wastewater treatment plants have developed and used microalgal technologies for detoxifying organic and inorganic pollutants with emerging contaminants (ECs) inclusive [20]. Some advantages of microalgal-based wastewater treatment systems, such as lower initial capital and operational costs, the availability of natural disinfection and rapid growth, and the ability to be reused when the nutrients absorbed are recovered, have made it one of the best systems for effective and efficient remediation of ECs and other pollutants when compared to other currently available systems [20]. In general, microalgae can bioremediate ECs via three major pathways: bioadsorption, biouptake, and biodegradation [21]. The bioadsorption of ECs into the cell wall components or onto organic substances excreted by the cells is what is termed microalgal bioadsorption, while the active transport of the ECs into the algal cells, which binds itself to intracellular proteins and other compounds, is termed biouptake. Both the nutrients absorbed and uptaken could be recovered and reused via biorefining [20]. Microalgal biodegradation refers to the conversion of complex chemicals into simpler molecules via catalytic metabolic degradation of ECs [20].

Furthermore, [22] reported on the biosorption of a heavy metal, Mn (II), by the algae species *Thalassiosira pseudonana*, as well as membrane performance in a hybrid algae-membrane system. The Mn (II) removal was mainly attributed to algal mechanisms. The Mn (II) removal was mainly attributed to algal mechanisms. The higher removal efficiency (>66%) was achieved at a higher algal inoculation ratio. Although increasing the Mn (II) concentration could reduce the algal uptake ratio of Mn (II), the membrane performance was improved. The optical coherence tomography (OCT) analysis revealed that the specific volume of the cake layer increased gradually over time. However, irreversible fouling was predominant and associated with the presence of building blocks in soluble organics [22]. Fig. 19.1 schematic diagram of a cross-flow membrane filtration setup with optical coherence tomography (OCT), adapted from [22].

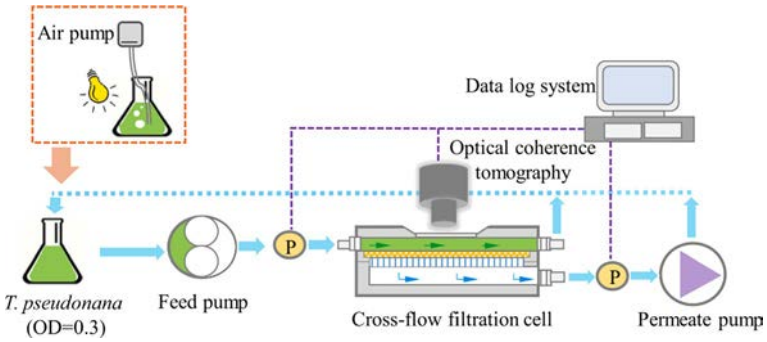

FIG. 19.1 Schematic diagram of a cross-flow membrane filtration setup with optical coherence tomography (OCT). *From L.N. Sim, J.S. Ho, N.B. Khaswan, B. Wu, T.H. Chong, Membrane filtration of manganese (II) remediated-microalgae: manganese (II) removal, extracellular organic matter, and membrane fouling, Algal Res. 55 (2021) 102279 (Copyright permission: 5040071072175).*

2.2 Microalgae and biodiesel

Microalgae are regarded as one of the most promising alternative biofuel sources due to their increased lipid production capacity when compared to other oleaginous agricultural crops consumed by humans and animals [23,24]. Researchers have classified feedstocks for the purpose of biodiesel production into first-generation feedstocks (e.g., edible crops like soybean, palm-oil, etc., which compete with humans and animals for food, water, and fertile land), second-and third-generation feedstocks (e.g., nonedible resources like organic municipal waste, lignocellulosic wastes, and microalgae), although some species of algae are edible [21,25]. As a result, microalgae fall into the second-generation category and, of course, have the greatest potential to generate the required biodiesel, as they can grow in both wastewater and seawater, ensuring the availability and sustainability of the edible crops in the first-generation category [21]. Abomohra et al. [21] reported that transesterification of lipids from the chlorophyte *Nannochloropsis* sp. using $Ca(OCH_3)_2$ nanoparticles as catalyst at 3% loading yielded 99% biodiesel, which was the highest value compared to other biodiesel sources. Besides, biodiesel produced from microalgae contains higher energy content (41 MJ/kg) compared to biodiesel from other sources, such as jatropha oil, castor oil, and rubber seed oil, although some have low-energy content from *Cladophora fracta* and *Chlorella protothecoides* species [25]. Wijffels and Barbosa [23] reported the development of low-cost photobioreactors specifically for the harvesting of microalgae with flat-panel reactors and green wall-panel reactors (Fig. 19.2). Fig. 19.3 shows the suggested approach to harvesting microalgae for enhanced biodiesel production using fat, oil, and grease [26] which could enhance the microalgal growth and hence more lipid accumulation [21].

2.3 New technologies used microalgae

Intracellular spectral recompositioning (ISR) of light is a newly developed approach to achieving higher production of sustainable biofuels by diatoms through absorption of excess blue light and its intracellular emission in the green spectral band, which improves light

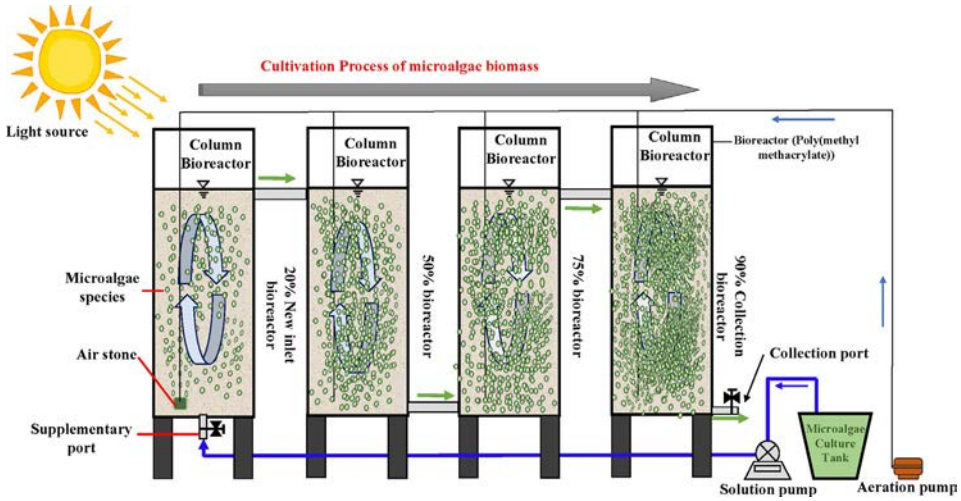

FIG. 19.2 Development of low-cost photobioreactors (Green Wall Panel reactors) [23].

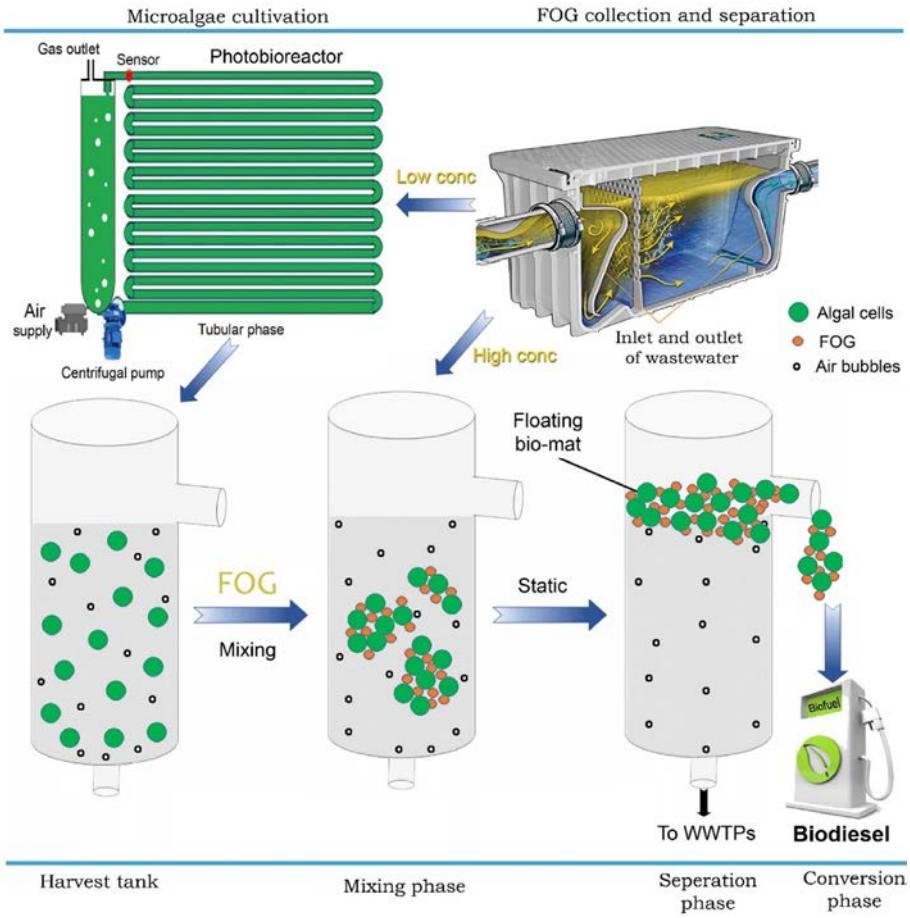

FIG. 19.3 Suggested approach to harvest microalgae for enhanced biodiesel production using fat, oil, and grease. *From A.E.-F. Abomohra, M. Elsayed, S. Esakkimuthu, M. El-Sheekh, D. Hanelt, Potential of fat, oil and grease (FOG) for biodiesel production: a critical review on the recent progress and future perspectives, Prog. Energy Combust. Sci. 81 (2020) 100868 (Copyright permission: 5101470765934).*

utilization and saves energy and cost [27]. The discovery of a novel bZIP1 transcription factor, NobZIP1, was reported by Li et al. [28] where a notable increase in lipid secretion and accumulation was witnessed using the *Nannochloropsis oceanica* microalgal specie without causing any damage to any of the specie's physiological setup. It was further observed that the up- and downregulated genes by NobZIP1 were involved in the synthesis of lipogenesis and cell wall polymers (using chromatin immunoprecipitation-qPCR), which happened to play a vital role in the secretion and overproduction of lipid from the *Nannochloropsis oceanica* [28].

In addition, microalgae have lately been used in the production of biodegradable bioplastic polyhydroxybutyrate (PHB) natural plastic, which aids in the protection of the world from environmental hazards. PHB can be produced intracellularly by photosynthetic cyanobacteria. Furthermore, its PHB is of a higher grade than that of other prokaryotes. Fig. 19.4 depicts the process of PHB production from microalgae [29].

3 Microalgae healthy macromolecules and phytochemicals

Microalgae contain important nutritional phytochemicals such as phycocyanin, a novel protein in Spirulina, polyphenols, flavonoids, and vitamins, all of which have been shown to benefit human health. Its importance in the food industry is growing due to the importance of its macro and micro-molecules. For instance, Table 19.1 presents the edible marine microalgae oil, protein, and carbohydrate contents, in which it can be observed that *Phaeodactylum tricornutum* contains 18%–57% of oil. On the other hand, *Spirulina maxima* contains 60%–71% protein. Thus, microalgae in general could be the perfect sustainable alternative to the commonly used animal sources [30].

Microalgae could be used as a healthy food. These naturally existing and very fast-growing unicellular microorganisms' phytochemicals have been applied in the medical area to increase oxygen local level in tumor regions [31]. Also, microalgae have the potential to scavenge a wild range of reactive oxygen species (ROS) through their high phenolic and flavonoid content (Table 19.2). For instance, our recent study of four micro algae species confirmed their high potential as antioxidant agents (Table 19.3).

An innovative way of in situ oxygen-generation by an engineered *Chlorella vulgaris* (CV) to overcome hypoxia in wounds was reported by Qiao et al. [31], whereby the engineered live CV added to hypoxic tumor areas has both increased local oxygen levels and resensitized resistant cancer cells via microalgae-mediated photosynthesis under red-light beams. This engineered-CV was achieved by adding an engineered red blood cell membrane (RBCM) to the CV-surface, which reduces macrophage uptake and systemic clearance of the CV and was termed "RBCM-Algae." Meanwhile, the increased oxygen level in the tumor areas by the RBCM-Algae resulted in remarkably high radiotherapeutic efficacy. Besides, the chlorophyll from the CV was observed to produce reactive oxygen species during laser irradiation, adding to tumor cells' apoptosis [31]. Furthermore, it was demonstrated that the engineered-CV when injected into the body via either intravenous or intratumoral, could efficiently reach the tumor spot and improve oxyhemoglobin, as it shows an extended life span with increased stability during in vivo circulation, which exhibits excellent biocompatibility with tissue cells [31]. Furthermore, when compared to most normal tissues that were equally

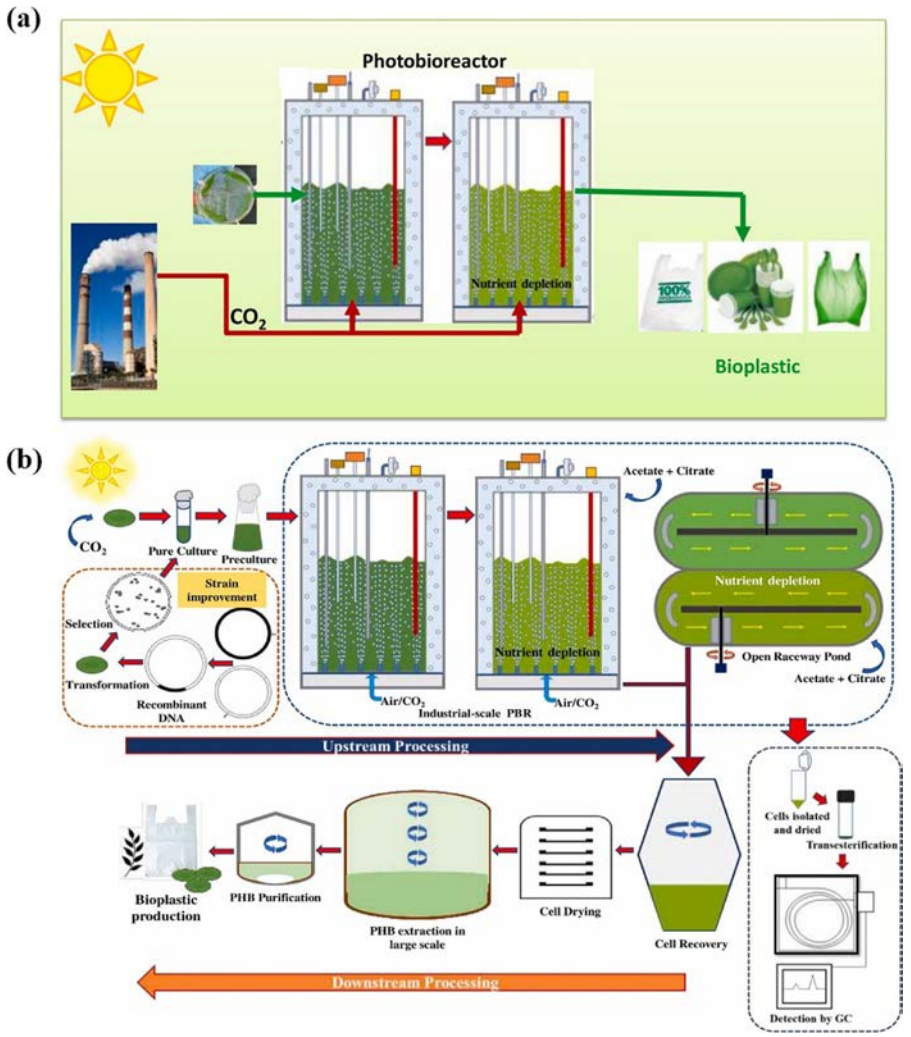

FIG. 19.4 Polyhydroxybutyrate (PHB) production process from cyanobacteria in which it could be established in closed and open systems. (A) Schematic diagram of its potential benefits related to the environment. (B) Flow chart of PHB industrial process. *From P.R. Yashavanth, M. Das, S.K. Maiti, Recent progress and challenges in cyanobacterial autotrophic production of polyhydroxybutyrate (PHB), a bioplastic, J. Environ. Chem. Eng. 9 (2021) 105379 (Copyright permission: 5100101221007).*

inoculated with the RBCM-Algae, such as the brain, bone, heart, kidney tissues, and so on, the tumor tissues showed the highest uptake of the RBCM-Algae, as evidenced by fluorescence signals of those major organs' tissues over a range of timings (Fig. 19.5) [31].

In another study by Chen et al. [32], an oxygen-producing-patch filled with gel beads containing active *Synechococcus elongatus* was reported to have efficiently supplied more than 100 times sufficient oxygen than the available conventional ways of supplying oxygen, such as

TABLE 19.1 Oil, protein, and carbohydrate contents of marine microalgae are expressed on a dry matter.

Class	Edible vegetable oil	Oil content (%)	Protein (%)	Carbohydrates (%)
Bacillariophyceae	*Phaeodactylum tricornutum*	18–57	30	8.4
	Thalassiosira weissflogii	5–20	43	12
	Skeletonema costatum[a]	13–51	25	4.6
	Chaetoceros muelleri	13–24	31–43	7–28
Chlorophyceae	*Dunaliella primolecta*	23	<64%	11–23
	Dunaliella tertiolecta	11–16	20–29	12.2–14
	Dunaliella salina	6–25	57	32
	Dunaliella bioculata	8	49	4
	Nannochloris sp.	20–56	16.69	–
	Nannochloropsis oculata	22–29	35	7.8
	Scenedesmus obliquus	30–50	10–45	20–40
	Scenedesmus quadricauda	1.9	40–47	12
	Scenedesmus dimorphos	16–40	8–18	21–52
	Scenedesmus sp.	17–24	29–37	32.7–41
	Ankistrodesmus sp.	11.48–31	16.24–18.66	4.48–5.97
	Ankistrodesmus fusiformis			
	Chlamydomonas reinhardtii	21	48	17
	Chlamydomonas sp.	22.7	58.8	18.5
	Parietochloris incisa	62	–	–
	Tetraselmis tetrathele[b]	25–30	–	–
	Neochloris oleoabundans	35–65	10–27	17–27
	Scenedesmus falcatus	6.41–9.6	3.37–7.83	2.73–6.83
	Scenedesmus protuberans	17.53–29.30	25.4–45.05	20.95–29.21
Eustigmatophyceae	*Chlorella* sp.	28–53	25–45	24–30
	Chlorella vulgaris	41–58	51–58	12–17
	Chlorella pyrenoidosa	2	57	26
	Chlorella protothecoides	40–60	10–28	11–15
	Chlorella emersonii	23–63	36	41
	Chlorella sorokiana	22–24	40.5	26.8
	Chlorella minutissima	14–57	47.89	8.06
Cyanophyceae	*Nostoc commune*	22	20.3–43	34–56.4
	Synechocystis sp.[d]	11	63	15

TABLE 19.1 Oil, protein, and carbohydrate contents of marine microalgae are expressed on a dry matter—cont'd

Class	Edible vegetable oil	Oil content (%)	Protein (%)	Carbohydrates (%)
	Spirulina platensis	4–11	46–63	8–14
	Spirulina maxima	6–7	60–71	13–16
	Spirulina sp.	7.72	57.47	23.9
Haptophyceae	*Pavlova lutheri*[e]	35	29	9
	Pavlova salina[f]	12–30	26	7.4
Prymnesiophyceae	*Emiliana huxleyi*	43.8	39.2	17.2
Raphidophyceae	*Heterosigma akashiwo*	43	50	7
Cryptophyceae	*Chroomonas salina*	12–14.5	29–35.5	9–11
Rhodophyceae	*Porphyridium cruentum*[c]	9–14	28–39	40–57
Conjugatophyceae	*Mesotaenium* sp.	19–35	53	27
Labyrinthulomycetes	*Schizochytrium limacinum*	43	39	5
	Schizochytrium sp.	50–77	15	12
	Aurantiochytrium sp.			

a: Phylum: Bacillariophyceae, Class: Mediophyceae; b: Phylum: Cholorophyta, Class: Chlorodendrophyceae; c: Phylum:Rhodophyta, Class: Porphyridiophyceae; d: Phylum: Cyanobacteria; Class: Porphyridiophyceae; e: Phylum: Haptophyta; Class: Pavlovophyceae; f: Phylum: Haptophyta, Class: Pavlovophyceae, g: Phylum: Haptophyta, Class: Prymnesiophyceae.

From B. Sajjadi, W.-Y. Chen, A.A.A. Raman, S. Ibrahim, Microalgae lipid and biomass for biofuel production: a comprehensive review on lipid enhancement strategies and their effects on fatty acid composition, Renew. Sustain. Energy Rev. 97 (2018) 200–232 (Copyright permission: 5042910970300).

TABLE 19.2 Total phenolics, total flavonoids and phenolic compounds separation by HPLC by using 9 standards for *Arthrospira platensis* (AP), *Chlorella vulgaris* (CV), *Phaeodactylum tricornutum* (PT), *Scenedesmus obliquus* (SO).

	Rt.	Unit	AP	CV	PT	SO
Total phenolics	–	mg/gDB*	7.59 ± 0.32^{c}	5.87 ± 0.61^{b}	10.46 ± 0.77^{d}	3.55 ± 0.37^{a}
Total flavonoids	–	mg/gDB**	9.26 ± 0.95^{c}	5.47 ± 0.44^{b}	15.29 ± 0.90^{d}	4.42 ± 0.68^{a}
Gallic acid	7.10	mg/100 gDB	0.16 ± 0.05	–	–	–
Catechin	18.84	mg/100 gDB	2.24 ± 0.22^{b}	–	0.24 ± 0.00^{a}	0.13 ± 0.07^{a}
Caffeine	19.84	mg/100 gDB	0.52 ± 0.05	–	–	–
Epigallocatechin gallate	24.76	mg/100 gDB	0.33 ± 0.03^{b}	–	0.05 ± 0.02^{a}	–
Vanillin	26.36	mg/100 gDB	–	–	0.02 ± 0.01	–
Epicatechin gallate	32.57	mg/100 gDB	0.54 ± 0.10^{a}	–	1.51 ± 0.33^{b}	–
Quercetin	49.90	mg/100 gDB	0.27 ± 0.18	0.73 ± 0.60	0.38 ± 0.26	0.21 ± 0.19

**Gallic equivalent*

*** quercetin equivalent.*

Mean ± SD with different alphabet superscript within the same row indicate that values differ significantly. Rt., retention time of HPLC peak; DB, dry weight biomass.

From M. Gouda, K. Chen, X. Li, Y. Liu, Y. He, Detection of microalgae single-cell antioxidant and electrochemical potentials by gold microelectrode and Raman micro-spectroscopy combined with chemometrics, Sens. Actuators B 329 (2021) 129229 (Copyright permission: 5042920470955).

TABLE 19.3 The antioxidant activity of *Arthrospira platensis* (AP), *Chlorella vulgaris* (CV), *Phaeodactylum tricornutum* (PT), *Scenedesmus obliquus* (SO) by three different radical scavenging methods.

	DPPH				ABTS			NO	
Group	INH %*	EC_{50} (μg/mL)	Gallic equivalent (μg/g*)	Quercetin equivalent (μg/g*)	INH %*	EC_{50} (μg/mL)	Quercetin equivalent (μg/g*)	Trolox equivalent (μg/g*)	Sodium nitrite equivalent %*
AP	48.21 ± 2.85^{c}	4.53 ± 0.11^{a}	26.02 ± 1.00^{c}	39.00 ± 1.97^{c}	77.95 ± 4.66^{c}	3.55 ± 0.04^{a}	106.30 ± 8.83^{c}	194.55 ± 17.74^{c}	21.92 ± 0.90^{c}
CV	35.06 ± 1.64^{b}	7.24 ± 0.44^{c}	15.10 ± 1.81^{b}	24.91 ± 0.96^{b}	58.93 ± 3.80^{b}	4.92 ± 0.05^{c}	65.88 ± 6.25^{b}	119.81 ± 11.51^{b}	11.38 ± 0.57^{b}
PT	96.71 ± 6.00^{d}	6.54 ± 0.23^{b}	54.02 ± 2.31^{d}	82.31 ± 3.23^{d}	99.71 ± 0.61^{d}	4.55 ± 0.16^{b}	205.29 ± 12.58^{d}	375.61 ± 23.98^{d}	28.73 ± 3.05^{d}
SO	17.71 ± 1.14^{a}	12.75 ± 0.31^{d}	6.58 ± 0.80^{a}	11.54 ± 0.64^{a}	33.62 ± 1.67^{a}	6.75 ± 0.09^{d}	45.98 ± 1.91^{a}	84.25 ± 3.81^{a}	3.84 ± 0.62^{a}

**The unit is equivalent to dry weight of microalgae biomass.*

Mean ± SD with different alphabet superscript within the same column indicate that values differ significantly. INH is the inhibition percentage. EC_{50} is the concentration which are causing 50% inhibition against the used radicals.

From M. Gouda, K. Chen, X. Li, Y. Liu, Y. He, Detection of microalgae single-cell antioxidant and electrochemical potentials by gold microelectrode and Raman micro-spectroscopy combined with chemometrics, Sens. Actuators B 329 (2021) 129229 (Copyright permission: 5042920470955).

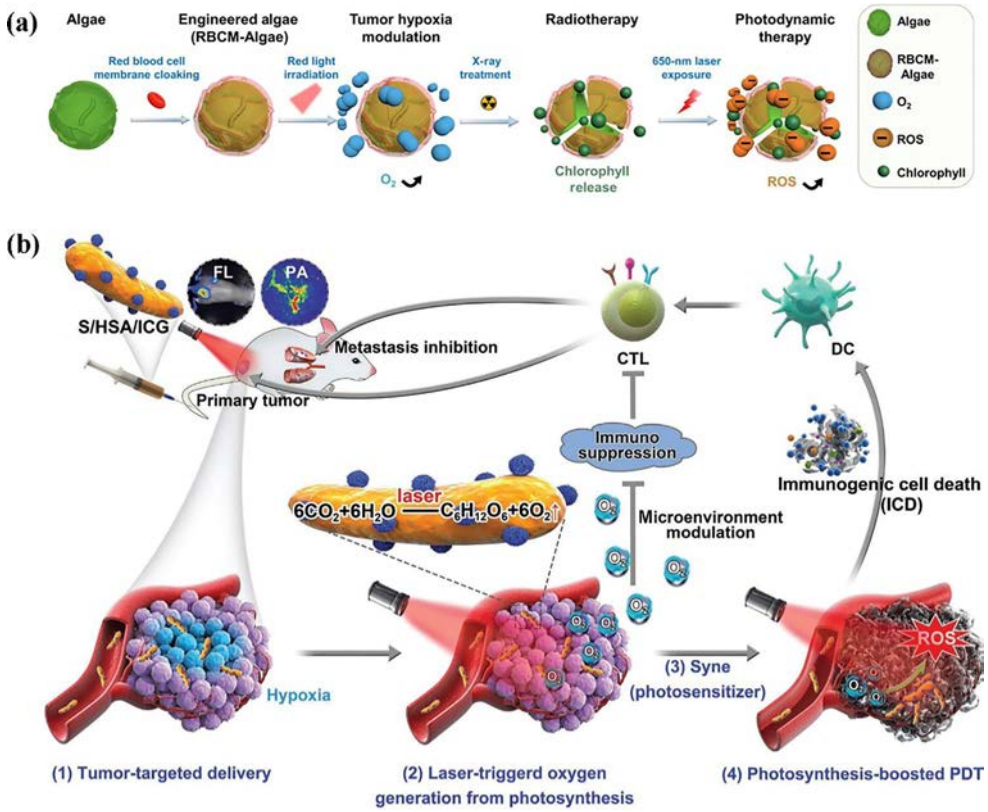

FIG. 19.5 (A) Illustrative description of engineered processes and treatments. (B) Human serum albumin (HSA)/indocyanine green (ICG) conjugated Synechococcus 7942 and in situ generated O_2 through photosynthesis to enhance photodynamic therapy (PDT) efficacy and evoke immunogenic cell death (ICD)-mediated antitumor immune responses. *From H. Hu, X. Qian, Y. Chen, Microalgae-enabled photosynthetic alleviation of tumor hypoxia for enhanced nanotherapies, Sci. Bull. 65 (2020) 1869–1871 (Copyright permission: 5100031007029).*

hyperbaric oxygen (HBO), topical dissolved oxygen (TDO), and topical gaseous oxygen (TGO) (Fig. 19.6). For example, based on the level of oxygen penetration into the dermis, TDO and TGO have recorded >700 and only 300 μm of human skin, respectively, demonstrating TDO as the best available method to supply oxygen to chronic diabetic wounds. Based on these excellent qualities demonstrated by *S. elongatus* gel-wound dressing, the hypothesized microalga-hydrogel patch could be applied to the diabetic chronic wounds as it showed increased wound oxygenation, fibroblast proliferation, and angiogenesis during their research. Because microalgae were discovered to be nontoxic and do not elicit an immune response, the hypothesized microalga-hydrogel patch could be applied on diabetic chronic wounds as it demonstrated increased wound oxygenation, fibroblast proliferation, and angiogenesis. This microalgae was discovered to be nontoxic and did not elicit an immune response, making it a novel medical innovation for the treatment of diabetic foot ulcers (DFU) (Fig. 19.6) [32].

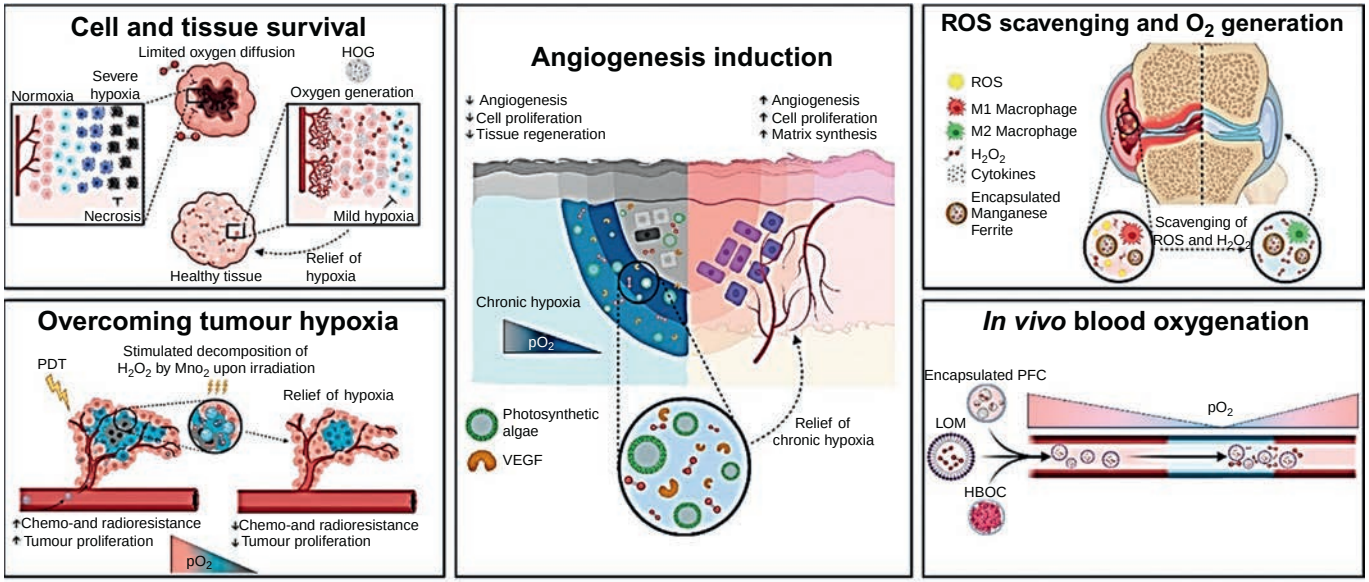

FIG. 19.6 Research applications of oxygen-carrying and oxygen-generating biomaterials (OCBs and OGBs) include the microalgae. *HBOC*, hemoglobin-based oxygen carrier; *HOG*, hydrophobic oxygen generator; *LOM*, lipid-based oxygen microbubble; MnO_2, manganese (IV) oxide; *PDT*, photodynamic therapy; *PFC*, perfluorocarbon; pO_2, partial pressure of oxygen; *ROS*, reactive oxygen species; *VEGF*, vascular endothelial growth factor. *From N.G.A. Willemen, S. Hassan, M. Gurian, J. Li, I.E. Allijn, S.R. Shin, J. Leijten, Oxygen-releasing biomaterials: current challenges and future applications, Trends Biotechnol. 39 (2021) 1144–1159 (Copyright permission: 5100120391285).*

4 Microalgae as source of bioactive and novel macro- and micro-molecules

4.1 Microalgae as a novel polyunsaturated fatty acids (PUFAs) source

Polyunsaturated fatty acids (PUFAs), which are widely spread in microorganisms, plants and aquatic animals, are a category of fatty acids containing more than one double bond in the backbone [33]. Despite the efforts made by many researchers to improve the extraction and use of PUFAs from microalgae in animal feed, some problems need to be addressed. Problems such as the low ratio of ω-3/ω-6 (2.0/1–3.0); the presence of heavy metals (e.g., Cd^{2+}, Cd^{3+}, Cu^{2+}, Hg^{2+}, Ni^{2+}, and Pb^{2+}) in the algal biomass which may pose potential risk to the animals when consumed in their feed; and high cost of extracting the algal biomass. For example, the prices for extracting PUFAs for feedstock from *Crypthecodinium cohnii* and *Chlorella* sp. algae species were around €36/kg and €43/g, respectively, while around 400–1500 € per kg EPA/DHA for algae-oil, and about €5500 per kg DHA for algae-DHA supplements [33]. Table 19.4 presents the polyunsaturated fatty acids (PUFAs) in microalgae as a source for human and animal feed.

TABLE 19.4 Screening of algal strains with the potential for PUFAs production.

Algal strain	Source	Algae growth condition	Biomass yield or productivity	Percentages of PUFAs
Arcocellulus cornucervis	Norwegian fjord waters	Marine and cold environment	8.67 mg/L/day	EPA: 1.36% of DW and 12.0% of TFA; DHA: 0.23% of DW and 2.02% of TFA.
Phaeodactylum tricornutum			60.00 mg/L/day	EPA: 3.14% of DW and 7.33% of TFA; DHA: 0.25% of DW and 0.58% of TFA.
Attheya septentrionalis	Atlantic waters around Spitsbergen		22.22 mg/L/day	EPA: 4.58% of DW and 24.1% of TFA; DHA: 0.60% of DW and 3.17% of TFA.
Thalassiosira hispida			12.86 mg/L/day	EPA: 4.10% of DW and 11.5% of TFA; DHA: 0.47% of DW and 1.33% of TFA.
Scenedesmus dimorphus	Himachal Pradesh of India	Area with high altitude	14.0 mg/L/day	PUFA: 39.03% of TFA
Monodus subterraneus	NA[a]	NA	0.45–1.42 g/L/day	GLA: 0.9%–1.1% of DW and 20.2%–27.5% of TFA
Parietochloris incisa	NA	NA	NA	DHGLA: 16.7%–31.5% of TFA

Continued

TABLE 19.4 Screening of algal strains with the potential for PUFAs production—cont'd

Algal strain	Source	Algae growth condition	Biomass yield or productivity	Percentages of PUFAs
Ankistrodesmus fusiformis	Eutrophic lagoon located at Salvador City, Bahia, Brazil	NA	0.24 g/L/day (lipid content: 20.66% of dry weight)	PUFA: 40.24% of TFA ALA: 26.28% of TFA LA: 12.23% of TFA
Botryococcus terribilis			0.20 g/L/day (lipid content: 49.00% of dry weight)	PUFA: 12.56% of TFA ALA: 7.22% of TFA LA: 5.02% of TFA
Chlorella vulgaris			0.73 g/L/day (lipid content: 28.07% of dry weight)	PUFA: 10.33% of TFA ALA: 1.57% of TFA LA: 8.54% of TFA
Kirchneriella lunaris			0.14 g/L/day (lipid content: 17.30% of dry weight)	PUFA: 44.83% of TFA ALA: 39.66% of TFA LA: 4.50% of TFA
Attheya septentrionalis	Arctic Waters Marine	Marine and cold environment	0.54–0.57 day^{-1}	EPA: 7.1% of dry weight
Botryococcus braunii	Open drainages situated in Penang Island, Malaysia	Trophic regions with low latitude	158.9 mg/L/day (lipid content: 41.98% of dry weight)	PUFA: 11.49% of TFA ALA: 8.41% of TFA
Chlorella sp.			249.2 mg/L/day (lipid content: 21.54% of dry weight)	PUFA: 55.52% of TFA ALA: 28.25% of TFA LA: 22.19% of TFA
Chlorococcum humicola			198.3 mg/L/day (lipid content: 29.16% of dry weight)	PUFA: 14.07% of TFA LA: 10.45% of TFA
Chlamydomonas sp.			236.8 mg/L/day (lipid content: 21.92% of dry weight)	PUFA: 36.27% of TFA ALA: 14.16% of TFA LA: 19.35% of TFA
Fistulifera sp.	The junction of the Sumiyo and Yakugachi Rivers, Japan	Marine diatom	NA	EPA: 17.1% of total FAMEs
Synedra fragilaroides	NA	Marine diatom	0.43 g/L (lipid content: 3.6% of dry weight)	PUFA: 36.8% of TFA EPA: 11.0% of TFA DHA: 2.1% of TFA

TABLE 19.4 Screening of algal strains with the potential for PUFAs production—cont'd

Algal strain	Source	Algae growth condition	Biomass yield or productivity	Percentages of PUFAs
Nitzschia closterium			0.10 g/L (lipid content: 13.5% of dry weight)	PUFA: 33.0% of TFA EPA: 19.5% of TFA DHA: 1.1% of TFA
Phaeodactylum tricomutum			0.22 g/L (lipid content: 21.3% of dry weight)	PUFA: 14.9% of TFA EPA: 8.8% of TFA DHA: 0.5% of TFA

[a]*"NA" refers to "Not available."*

Individual fatty acids are a percentage of TFA. ALA, *α-linolenic acid;* DHA, *docosahexaenoic acid;* DHGLA, *dihomo-γ-linolenic acid;* DW, *dry weight;* EPA, *eicosapentaenoic acid;* GLA, *γ-linolenic acid;* LA, *linoleic acid;* PUFA, *polyunsaturated fatty acid.*

From Q. Lu, H. Li, Y. Xiao, H. Liu, A state-of-the-art review on the synthetic mechanisms, production technologies, and practical application of polyunsaturated fatty acids from microalgae, Algal Res. 55 (2021) 102281 (Copyright permission: 5042940041838).

4.2 Microalgae as a novel protein source and its risk factors

Commercially available food supplements based on microalgae, such as Spirulina (Cyanobacteria), are gaining popularity among the world's scientists [34]. Microalgae are rich in important nutritious components such as phytochemicals, vitamins, and minerals, all of which positively enhance human health [1]. Several microalgae have been approved for human consumption, with no toxic metabolites [35]. However, serious concerns have been raised about some products manufactured from these microalgae due to their contamination with cyanotoxins [36], toxic metals [37], or inorganic arsenic [38]. Also, a case report of allergic reactions to *Spirulina* microalgae as a nutritional supplement was reported and considered as a primary de novo sensitization [39]. Some other natural hazardous compounds could exist, as shown by a recent study that reported two cases of human poisoning following the simultaneous use of *Spirulina* and *Chlorella* products [34].

One of the most common causes of serious contamination in microalgal products is improper culture media purity, which contains toxic contaminants. Microcystins, for example, are the most common cyclic protein peptide toxins that primarily affect the safety of microalgae production. They are considered powerful hepatotoxins that cause extremely dangerous liver cancer in mammals because they inhibit acetylcholinesterase activity, which is required for cell regulation [40]. Despite all of the benefits associated with microalgae proteins, there are concerns about their safety, such as microcystin bioaccumulation, toxic protein contaminants, and allergenicity [41,42]. These concerns have been acknowledged, and it has been recommended that a comprehensive risk assessment be performed on microalgae novel proteins in accordance with EC regulation No. 258/97 and EU recommendation 97/618 EC. Furthermore, the potential risks of microalgae being safe for consumed proteins stem from some consumers' allergies to their consumption. In most cases, consuming this protein induces immunological tolerance, allowing consumers to consume these foods safely. However, in some people, insufficient tolerance may result in reactivity upon reexposure to the specific protein, a process known as "sensitization" [43].

A multidisciplinary approach was used to assess their safety. This included cytotoxic tests using human whole-blood in vitro and fluorescent-based flow cytometry [34], analytical screening of 30 elements, and determination of cyanotoxins like anatoxin-A, cylindrospermopsin, and microcystin. They used two models to calculate metal toxicity: the Food Supplement Metal Index (FSMI) and the Toxic Elements Contamination Index (TECI) (TECI). The aqueous extract of *Chlorella* with high levels of FSMI and TECI (69.8, 2.9) showed a median share of necrotic cells in samples (5%). However, chlorella with lower FSMI and TECI (38.2, 1.8) did not show any cytotoxicity in the studied model because neutrophil viability was not altered. The authors came to the conclusion that the source of microalgae is the most important factor in its harmful effect on human health [34].

5 Methods for identification of microalgae macromolecules

Microalgae macromolecule analysis has been done for decades. Lipids and carbohydrates derived from insects and plants are of particular interest for specific applications. A promising strategy for microalgae chemical assessment is using bioinformatics to provide a fast prediction tool for its lipids, proteins, and even carbohydrates. Nowadays, a potential limitation is the lack of reference structures of some microalgae proteins, and thus a wide range of analytical methods should be used for building a strong database based on these different analytical techniques, either destructive or nondestructive, combined with chemometrics and other informatics methods.

5.1 Chemical free nondestructive methods

Spectroscopic methods for determining the chemical composition of microalgae have grown in popularity. Raman micro-spectroscopy, for example, is a quick, chemical-free, and noninvasive tool for characterization of single-cell molecules and their activities by detecting functional groups frequency vibration by these molecules, as well as laser light inelastic scattering [2,16]. Also, Raman spectroscopy has been used in many aspects of single microalgae research, such as chemical imaging of microalgae biochemical molecules like antioxidants and phytochemicals [2] (Fig. 19.1), astaxanthin [44], and carotenoids [45].

5.1.1 Spectroscopic methods for microalgae physicochemical structure

5.1.1.1 Raman micro-spectroscopy of single-cell microalgae

Raman micro-spectroscopy is a quick, label-free, and nondestructive technology that is used for the characterization of single-cell chemical construction and their bioactivities through information frequency vibration of the functional molecules by laser light inelastic scattering [16]. Also, Raman spectroscopy has been used in many fields of single microalgae research, such as the study of their nutritional status [46], chemical imaging of biochemical contents like carotenoids [45]. However, Raman frequently interferes with fluorescence caused by chromophores in microalgae like chlorophyll green pigment [47]. Thus, more-reliable information should be obtained by correlating it with the chemical and chemometric analyses for confirmation of the bands' relationship with the standard chemical methods [48].

Fig. 19.7 shows a practical example of using micro-Raman in the field of single cell microalgae detection. In this study, four microalgae species were evaluated and characterized as follows: *Arthrospira platensis* (AP), *Chlorella vulgaris* (CV), *Phaeodactylum tricornutum* (PT), and *Scenedesmus obliquus* (SO). The full scan (400–1800 cm^{-1}) of the four studied microalgae species confirmed the differences among them by Raman microspectroscopy. In which, by principle component (PC)1,2 and clustering heatmap, it was found that CV and SO were clustered as the same group compared to AP and PT for the wavenumbers (1522, and 1160 cm^{-1}) (Fig. 19.7B and C). Li et al. [16] and Wei et al. [49] mentioned that (1518–1525 cm^{-1}) are matched to β-carotene C═C stretches which is mainly present in the nonpolar phase of microalgae. By VIP top scores, 1514 cm^{-1} (C═C stretches) was the top significant wavenumber, in which, SO was the highest, and PT was the lowest (Fig. 19.7D). Besides, Huang et al. [45] mentioned the positive correlation between microalgal carotene and the nonpolar triglycerides measured by Raman spectroscopy.

5.1.1.2 Surface-enhanced Raman scattering technique

Using Raman spectroscopy scattering, Raman spectroscopy [50] technique plots the relationship between light intensity expressed in arbitrary units versus scattered light frequency expressed in frequency units. The technique has attracted considerable interest in biodetection of microalgae organic molecules [49] due to its nondestructive and ultrasensitive features. Also, SERS technology has also been applied to detect biotoxins, such as poisonous proteins and microcystin [51]. This technique provides a "molecular fingerprint" that can be used to identify a molecule or verify its presence in a sample using its intrinsic signals. The technique has been found to have significant advantages, such as high affinity for molecules, high sensitivity, and fingerprint resolution, which can effectively enhance the signal strength up to 10 orders of magnitude [52]. Due to these excellent features, SERS has been commonly applied in immunoassays for the detection of microalgae protein allergens [53–55]. In this application, glass coated slides with nanoparticles such as silver nanoparticles are used due to their wide utilization as a matrix in immunoassays and their good adsorption and fixation capacity for antibodies [56] (Fig. 19.8).

Furthermore, [57] presented a Raman-activated droplet sorting (pDEP-RADS) based on positive dielectrophoresis, in which fast-moving cells are trapped by a periodic pDEP force, followed by simultaneous microdroplet encapsulation and cell sorting.

Behrendt et al. [58] developed a "PhenoChip" that enhances the phenotyping of cyanobacteria and microalgae on the basis of multiparametric photophysiological characterization and selection of unicellular phenotypes to be controlled by the user. They used the PhenoChip to detect single cells of the coral symbiont Symbiodinium to thermal and chemical treatments, as well as to monitor single-cell photophysiology using chlorophyll fluorometry [58]. He et al. [59] developed a method based on nanosilver particles for the detection of allergenic proteins by Raman spectroscopy. They focused on the detection of Ricin toxin protein, which is naturally present in the castor bean plant (*Ricinus communis*) by using aptamer (artificial ssDNA antibody that is generated from randomized nucleic acid) and nanosilver. The authors established a "two-step" aptamer-based surface-enhanced Raman scattering [50] detection assay for ricin in liquid foods. The Ricin B chain was first captured out of food matrices by aptamer conjugated silver dendrites and then the spectrum was directly read on the silver dendrites (Fig. 19.9). Also, they mentioned that SERS can detect very low concentrations

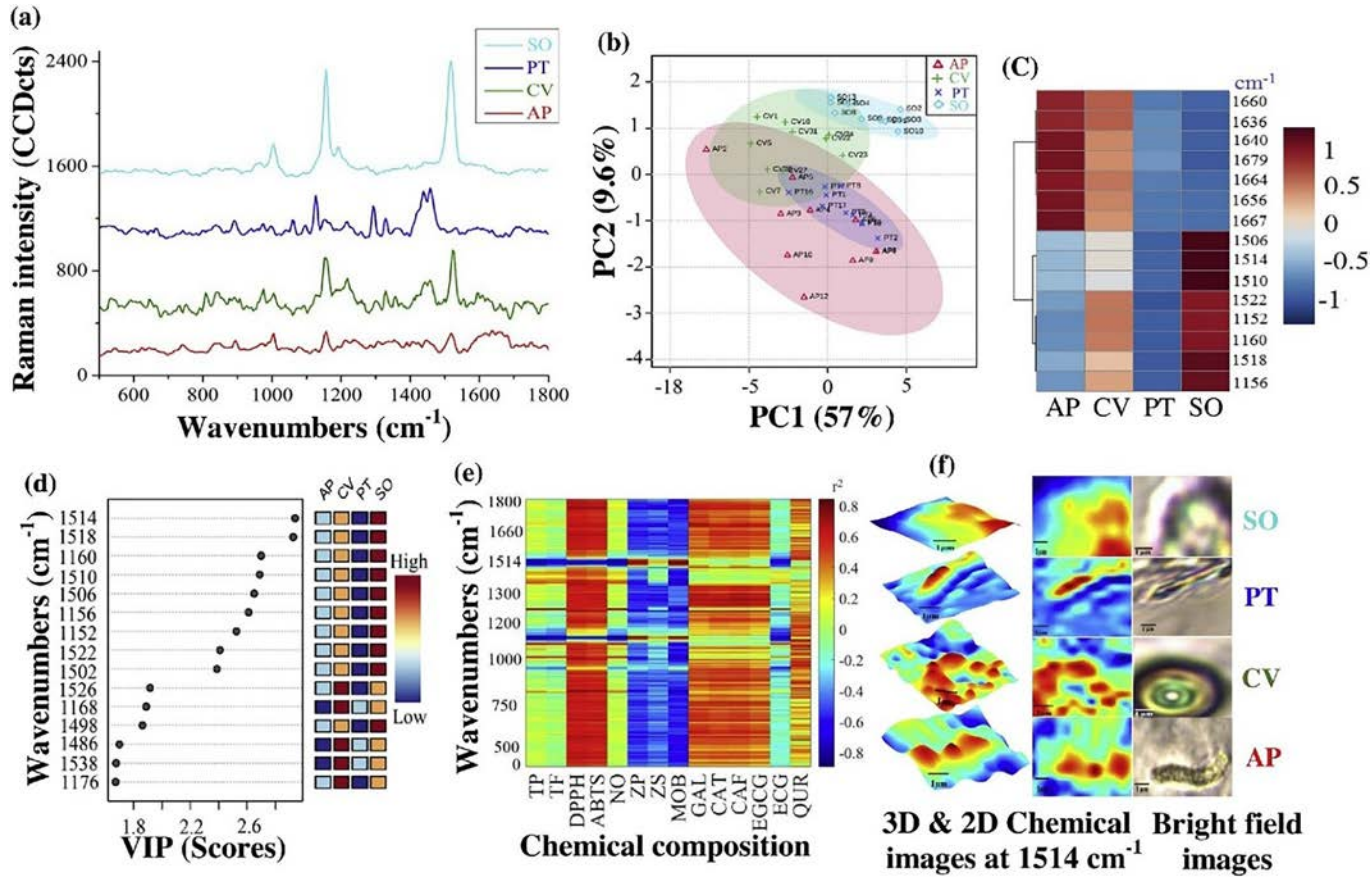

FIG. 19.7 Single-cell Raman microspectrometry (SCMR) chemical composition of *Arthrospira platensis* (AP), *Chlorella vulgaris* (CV), *Phaeodactylum tricornutum* (PT), *Scenedesmus obliquus* (SO) microalgae species; (A) full spectra of the four studied species; (B) principle component analysis; (C) clustering heatmap; (D) important features identity classification; (E) correlation (r^2) summarization between the spectral bands and the chemical composition; (F) 3D and 2D chemical images at wavenumber of 1514 cm^{-1} (1 μm), and the bright-field images. *From M. Gouda, K. Chen, X. Li, Y. Liu, Y. He, Detection of microalgae single-cell antioxidant and electrochemical potentials by gold microelectrode and Raman micro-spectroscopy combined with chemometrics, Sens. Actuators B 329 (2021) 129229 (Copyright permission: 5042920470955).*

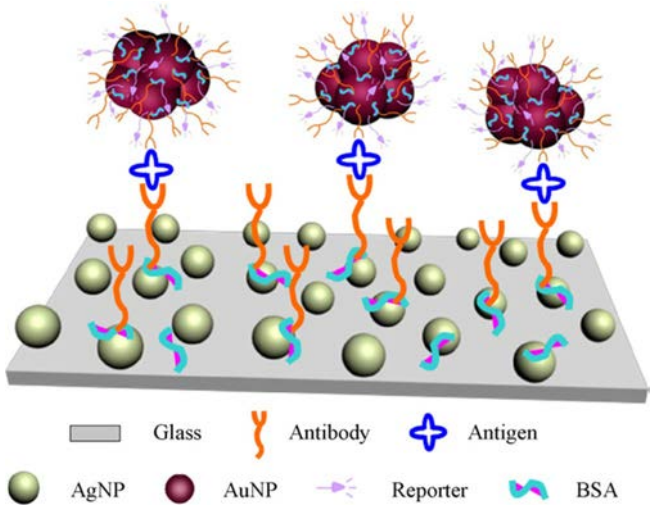

FIG. 19.8 Sketch map of the standard sandwich protocol of SERS-based immunoassay. *From C. Song, Z. Wang, R. Zhang, J. Yang, X. Tan, Y. Cui, Highly sensitive immunoassay based on Raman reporter-labeled immuno-Au aggregates and SERS-active immune substrate, Biosens. Bioelectron. 25 (2009) 826–831 (Copyright permission: 5043000864329).*

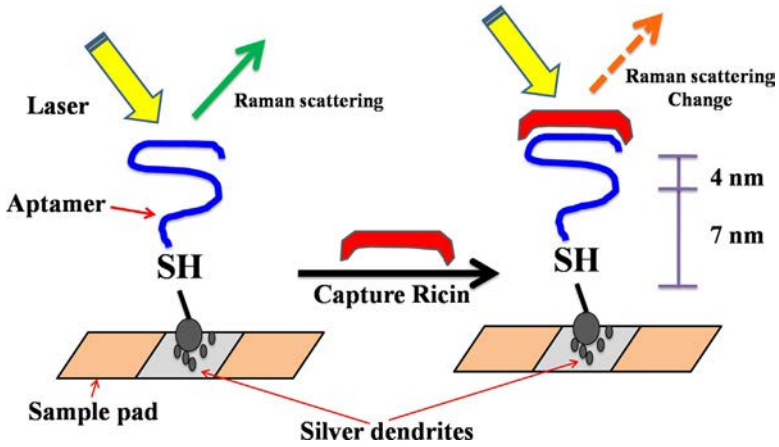

FIG. 19.9 Illustration of the "two-step" aptamer-based SERS assay. *Modified from L.L. He, E. Lamont, B. Veeregowda, S. Sreevatsan, C.L. Haynes, F. Diez-Gonzalez, T.P. Labuza, Aptamer-based surface-enhanced Raman scattering detection of ricin in liquid foods, Chem. Sci. 2 (2011) 1579–1582 (Copyright permission is confirmed by Royal Society of Chemistry).*

of this protein at a concentration level as low as 100 ng/mL. Zhu et al. [60] determined the feasibility of using the SERS immunoassay to rapid detect microcystin heptapeptide. In that study, gold nanoparticles were assembled into nanorod chains to obtain ultra-sensitive detection of microcystin with a limit of detection as low as 5 pg/mL and an assay time as short as 15 min. Moreover, A double antibody sandwich method for rapidly detection of

β-conglycinin by Raman enhanced immunoassay with colloidal gold as the active substrate was established [61]. In that study, the authors developed a flow immunoassay strip test based on surface-enhanced Raman spectroscopy coupled with gold nanoparticles for the rapid detection of β-conglycinin allergen [61].

5.1.1.3 Fourier transform infrared (FT-IR) spectroscopy of microalgae

Fourier transform infrared (FT-IR) is a molecular vibrational spectroscopic method that dissects chemical functional groups in different absorbance regions between 4000 and 400 cm^{-1}. For instance, the three main cell compositions, for instance, lipid, protein, and carbohydrate, have characteristic absorbance in different frequency regions of the mid-infrared spectrum, giving FT-IR the potential to be an effective tool for microalgal biochemical composition monitoring [62]. Gouda et al. [2] conducted research on the use of FT-IR for microalgae characterization. Fig. 19.10 showed the full scan of 400–4000 cm^{-1} for the four studied species and confirmed the differences among them (Fig. 19.10A). By clustering heatmap and VIP top 10 wavenumbers, AP was found to be the most significant ($P < .05$) compared to SO at 1118, and 1576 cm^{-1} (Fig. 19.10C and D). The significant changes in (1118, 1403, and 1576 cm^{-1}) are attributed to the significant differences in the secondary protein structures, especially amide bonds (1400 cm^{-1}) and α-helix (1574 cm^{-1}) [63], in which AP (60%) compared to SO (25%) [64,65]. Challagulla et al. [66] reported that FT-IR multivariate analysis with PLS model could be the best way for the characterization of microalgae biochemical molecules.

Also, FTIR spectroscopy is a nondestructive technique for proteins and polypeptide structural characterization [9,13,67]. The FTIR spectra of proteins are interpreted as vibrations of repeating structural elements. The protein unit scan defines nine characteristic absorption bands (amides A, B, and I–VII). Amide I bands (1700–1600 cm^{-1}) are related to the protein secondary structural components. This method is commonly used for the assessment of conformational changes, secondary structure, and structural dynamics, proteins stability, and aggregation, all of which have a direct influence on the microalgae proteins allergenicity [68]. For instance, it has been shown that increasing the temperature affects the shape of the FTIR amide I band, which indicates the formation of β-structures of Ara h 1 protein allergen. This change in the Ara h 1 structure indicates the protein's aggregation by heat, which subsequently will be affect its solubility and quality of measurement by other methods like CD spectra. FTIR is regarded as one of the best nondestructive techniques for confirming protein structural features of aggregated or insoluble proteins [50,69,70].

5.1.2 Recent innovations of using gold nanosensors in microalgae single cell phytochemical studies

Single-cell electrochemical current by microelectrode has developed as an important technique for major studies of single-cell functionality [71]. It is known that the combination of biomolecules with nanoparticles like gold nanoparticles creates interesting features for the development of nanosensors [72]. Gouda et al. [2] fabricated a new method for detecting microalgae single cell antioxidants through their single cell current (SCC) by using a gold nanoprobe. For which, the most straight forward pathway is using metal nanoparticles like gold or silver for the detection process [73].

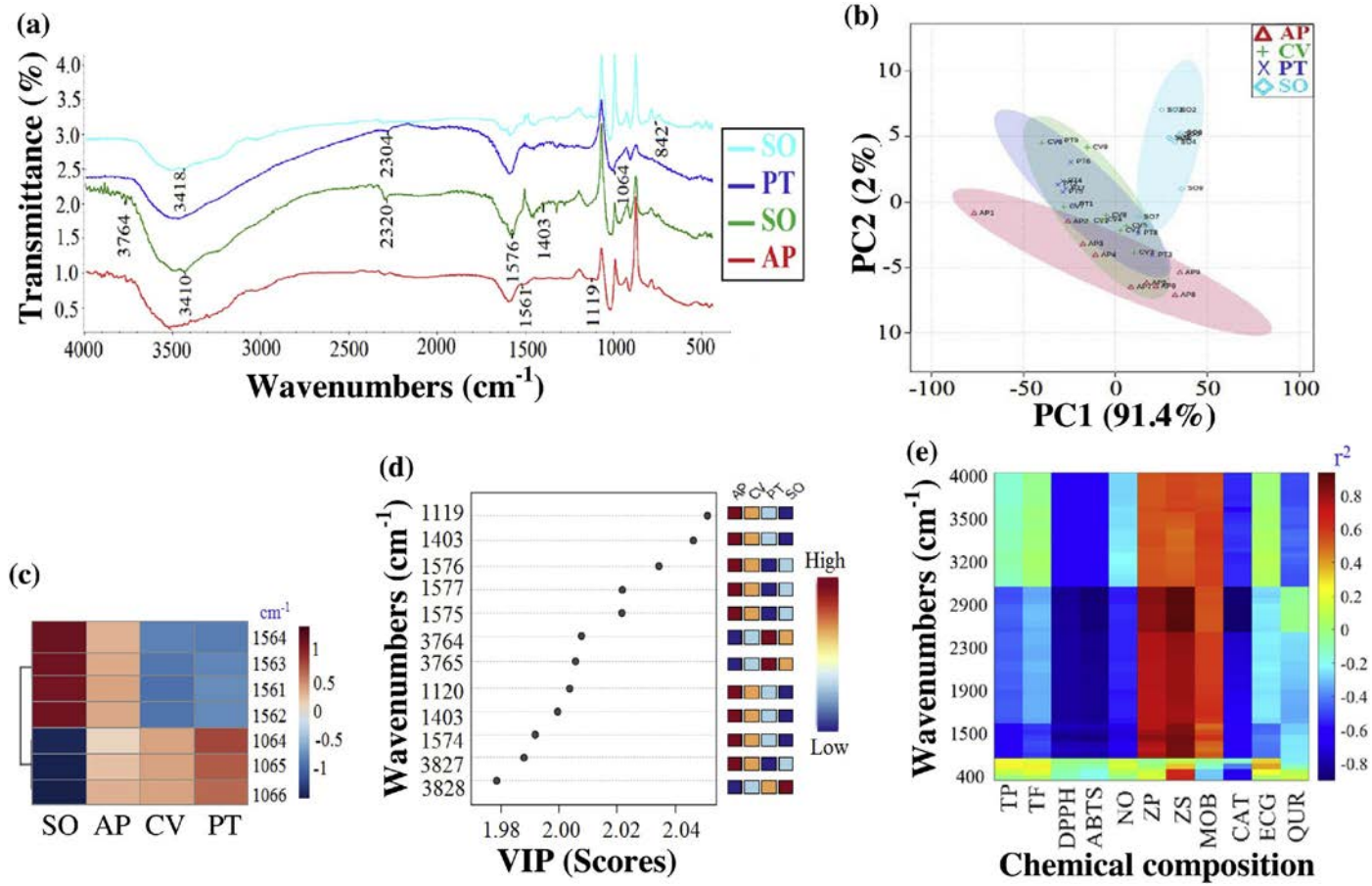

FIG. 19.10 FT-IR characterization, differentiation, and correlation with the chemical composition of *Arthrospira platensis* (AP), *Chlorella vulgaris* (CV), *Phaeodactylum tricornutum* (PT), *Scenedesmus obliquus* (SO); (A) full spectra of the four studied species; (B) principle component analysis (PCA) scores for 800–1600 wavenumbers; (C) clustering results for (800–1600 nm region), shown as heatmap; (D) important features identity (VIP) classification by PLS-DA for the full spectrum, the *colored boxes* on the right indicate the relative intensity for the species; (E) correlation (r^2) summarization between the full spectral wavenumbers and the chemical composition, zeta potential (ZP), zeta size (ZS), and mobility (MOB). *From M. Gouda, K. Chen, X. Li, Y. Liu, Y. He, Detection of microalgae single-cell antioxidant and electrochemical potentials by gold microelectrode and Raman micro-spectroscopy combined with chemometrics, Sens. Actuators B 329 (2021) 129229 (Copyright permission: 5042920470955).*

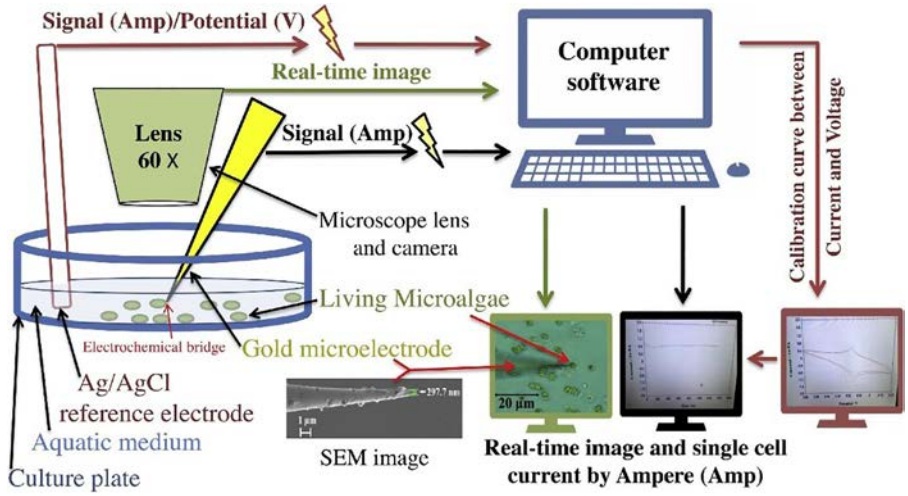

FIG. 19.11 Illustration of microalgae single-cell electric current analytical detection by gold microelectrode. *From M. Gouda, K. Chen, X. Li, Y. Liu, Y. He, Detection of microalgae single-cell antioxidant and electrochemical potentials by gold microelectrode and Raman micro-spectroscopy combined with chemometrics, Sens. Actuators B 329 (2021) 129229 (Copyright permission: 5042920470955).*

The critical dimension of these kinds of microelectrodes is so small in nanodiameters that it could be used to detect cellular biomolecule activities [74]. Gold nanoprobe biosensors can also be used in micro space electrochemistry, and their pulled end can be precisely controlled down to 50 nm of attachment with a single cell [75,76]. Recently, nanometer-sized electrodes were used to record intracellular reactive oxidative markers like hydrogen peroxide and nitrogen monoxide [72]. A single cell current (SCC) study was established by using a gold nanoprobe. As shown in Fig. 19.11, SCC full-time scan of 2000 s of the four studied species confirmed the differences among them (Fig. 19.11A). By PCA and clustering heatmap, it has been found that SO had the highest current level compared to AP and PT with their high antioxidant activities. In terms of VIP top scores, 1120 s demonstrated the most significant time duration. He et al. [77] reported that gold nanosensors have a high sensitivity to the surface charge of the microorganisms, like microalgae. Furthermore, it has been established that various electrochemical sensors can be used to assess the antioxidant capacity of various plant species [78,79].

However, specific care is needed to maintain a very small current response (10^{-10} A) with the actual biochemistry of cells. Therefore, the continuous development of intracellular electrochemical detection and its relationship with chemical invasive methods and other noninvasive methods like micro-Raman spectroscopy should be more solidified.

5.1.3 Acoustic-based sensors and biosensors

The use of the US for measuring the chemical composition of microalgae, drawing chemical images of their tissues, and visualizing their biomolecules has emerged as a hot scientific research topic. An acoustic wave sensor typically consists of a piezoelectric

substrate (e.g., quartz crystal), coated with sensing material (polymeric film), and two interdigital transducers (one input and one output) that are commonly used for chemical composition purposes [80]. There are three different types of these kinds of sensors (surface acoustic wave sensors (SAW), bulk acoustic wave sensors (BAW), and micro/nanoacoustic biosensors) [26,81]. The acoustic wave that propagates on the surface of the substrate is called SAW, while the wave that propagates through the substrate is called BAW (Fig. 19.12) [82].

For example, Tekaya et al. [83] used SAW for real-time tracking of *Arthrospira plantensis* microalgae biofilm on microfluidic chip. They mentioned that SAW allowed the optimization of the deposition process of microalgae biofilm for sensitive detection of microalgae heavy metals (HM). In that study, they characterized the toxicity of *Arthrospira plantensis* microalgae heavy metal (Cd^{2+} and Hg^{2+}) with detection limit 10^{-12} M (Fig. 19.13). On the other hand, there are still some limitations to these kinds of sensors. For example, QCM sensors have complex circuitry, poor signal-to-noise ratio, and can be influenced by humidity [80].

Nanoacoustic biosensors have been established to enhance the bioactivity of specific biomolecules, like enzymes, to increase detection sensitivity. These biosensors use air-filled protein nanostructures called gas vesicles that vibrate in response to ultrasound vibrational waves [84]. The principle of using acoustic-based biosensors is based on linking the

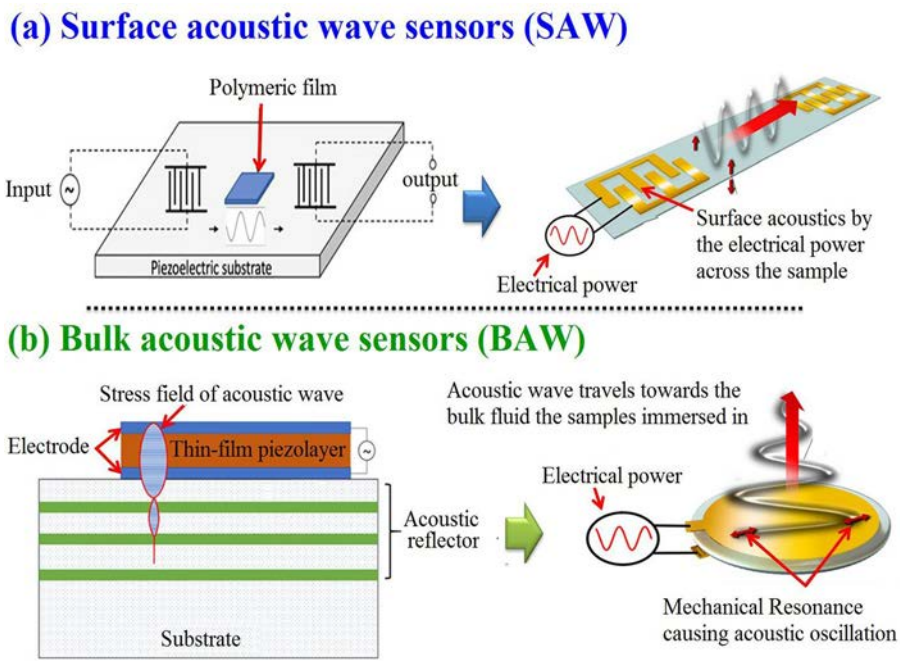

FIG. 19.12 Graphic depicting in general terms the processes for the generation of surface and bulk acoustic waves. *From A.E.D. Bekhit, M. Gouda, Y. Tang, Y. Huang, L. Huang, X. Li, Y. He, Recent innovations of ultrasound green technology in herbal phytochemistry: a review, Ultrason. Sonochem. 73 (2021) 105538 (Open access and no requirement of Copyright permission).*

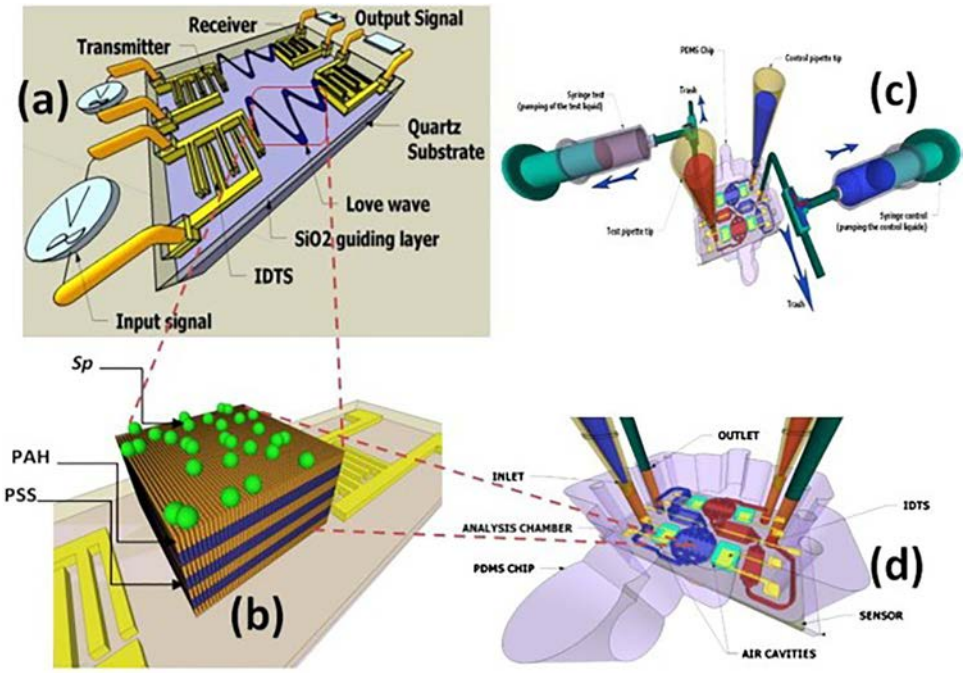

FIG. 19.13 Schematic of a SAW sensor with hybrid biofilm of polyelectrolyte microalgae. (A) Scheme of SAW (surface acoustic wave), (B) Spirulina immobilization on a polyelectrolyte multilayer (PEM) coated with a layer by layer (LBL) method and (C and D) hydrodynamic chip with microfluidic network, aligned on SAW. *From N. Tekaya, I. Gammoudi, M. Braiek, H. Tarbague, F. Moroté, V. Raimbault, N. Sakly, D. Rebière, H. Ben Ouada, F. Lagarde, H. Ben Ouada, T. Cohen-Bouhacina, C. Dejous, N. Jaffrezic Renault, Acoustic, electrochemical and microscopic characterization of interaction of Arthrospira platensis biofilm and heavy metal ions, J. Environ. Chem. Eng. 1 (2013) 609–619 (Copyright permission: 5042980431030).*

measurement criteria (like adsorption) as a modulation of the physical characteristics of ultrasound waves (like US frequency and velocity), which is correlated with the concentration of analyte in the samples [26]. Gouda [81] mentioned that US-assisted solvothermal reaction could be used for bioimaging of plant elements like zinc-ion by using quantum dot technology. The authors suggested that the viability of the technique could be used for in vitro cell imaging and in vivo imaging of natural plants.

5.2 Chemical dependent destructive methods

The analysis of microalgae macromolecules such as proteins, carbohydrates, and lipids using mass spectrometry is a very promising technique for multidetection and in-depth characterization of these molecules' physicochemical properties [9,85]. Also, chromatography-dependent methods enable researchers to separate these important molecules, identify their properties, and determine their amounts [86].

5.2.1 Mass spectrometry methods

The analysis of microalgae macro and micro hydrocarbon molecules using mass spectrometry can determine the primary sequence, postprocessing modifications, molecular interactions, and structural studies of molecules like proteins. In which it may help in identifying and modifying their composition due to treatments (e.g., thermal and nonthermal treatments, processing storage under a modified environment, and so on).

Mass spectrometers are basically composed of an ion source, a mass analyzer, and a detector, and various designs and modes of action are available for different applications. Electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) are the most common mass spectrometers for proteomic studies [87]. The MALDI technology observes singly charged ions, whereas the ESI generally induces multiply charged states. Surface-enhanced laser desorption ionization (SELDI) is a modified technique of the MALDI technology that introduces a further purification step on the probe surface before the MS analysis [88]. There are five kinds of mass analyzers commonly used in studying biofuel macromolecules. These include time-of-flight (TOF), ion traps (IT), quadrupoles (Q), Orbitraps, and Fourier transform ion cyclotron resonance (FTICR) analyzers [89–91]. The mass analyzers can be combined into tandem MS (MS-MS) such as Q-TOF, Q-IT, TOF-TOF, and IT-FTICR [92]. Advanced sequencing methods combine sequencing experiments of digested proteins that were treated with proteases such as trypsin to generate peptides and with database searches of the obtained sequences. Recent development of multiple reaction monitoring mass spectrometry (MRM-MS) has made significant progress in the assessment and quantification of biofuel macromolecules. Specific peptide signatures can be quantified by MRM and the use of internal labeled peptide standards [93]. Relative and absolute quantitation were achieved by using multiple reaction monitoring (MRM) with isotope-labeled peptides as internal standards.

5.2.2 Chromatographic approaches

Chromatography enables researchers to separate components of a mixture, identifying their properties, and determining their amounts [6,14,94]. Preparation of the proteins through elimination of contaminants and separation of complex mixtures before mass spectrometer analysis is required to reduce the matrix complexity. Over the last decade, there have been an increasing number of publications describing the use of LC-MS methods in the microalgae field for the characterization of their bioactive phytochemicals [95]. Also, the GC-MS technique is a powerful approach for the analysis of microalgal functional processes like pyrolysis. Fig. 19.14 presents the setup of GC-MS of microalgae in single and double-shot pyrolysis.

Other chromatographic examples include ion exchange chromatography, which allows separation based on the charge of molecules. Elution is carried out by changing the ionic strength of the mobile phase, either by modifying pH or increasing salt concentration. On the other hand, reverse phase chromatography is based on repulsive hydrophobic forces from interactions between a polar carrier solvent, a nonpolar separated compound, and a nonpolar stationary phase. Another chromatographic example is affinity chromatography, which is used when specific antibodies are available, and the target protein is only available at a low concentration [96].

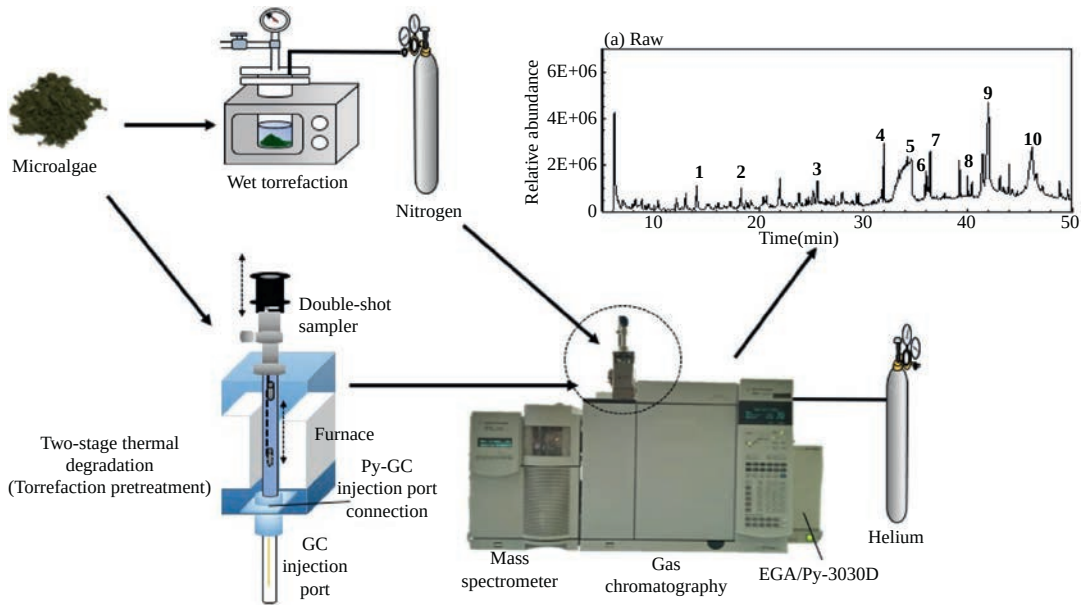

FIG. 19.14 The experiment procedures of GC-MS analysis on wet microalgae and two-stage thermal degradation of microalgae. *From Y.Y. Gan, W.-H. Chen, H.C. Ong, H.-K. Sheen, J.-S. Chang, T.-H. Hsieh, T.C. Ling, Effects of dry and wet torrefaction pretreatment on microalgae pyrolysis analyzed by TG-FTIR and double-shot Py-GC/MS, Energy 210 (2020) 118579 (Copyright permission: 5042960988033).*

Alsenani et al. [97] combined chromatographic and mass spectrometry with antimicrobial analyses for the evaluation of microalgae components' antimicrobial activity to construct new bioactive phytochemical compounds for the food and pharmaceutical industries (Fig. 19.15). In that study, the authors evaluated a microalgae species for its antimicrobial activity against pathogens using various extraction techniques and antimicrobial assays. By using a mixture of different chromatography techniques, like gas chromatography-mass spectrometry (GC-MS) and ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF-MS). They concluded that these methods had a high potential to separate bioactive compounds from microalgae species. These separated compounds were dominant and responsible for the inhibitory activity against pathogenic bacteria.

6 Conclusion and future remarks

In conclusion, the chemical composition of microalgae plays an important role in its novel applications as alternative nutrient sources. The advancement of commonly used analytical techniques for quantifying microalgae species can increase their applicability and potential uses in pharmaceuticals, food, and all relevant scientific fields, including the environmental and chemical industries. These advances could include the identification of new microalgae strains, as well as the investigation of the real-time functional activity of microalgae bioactive

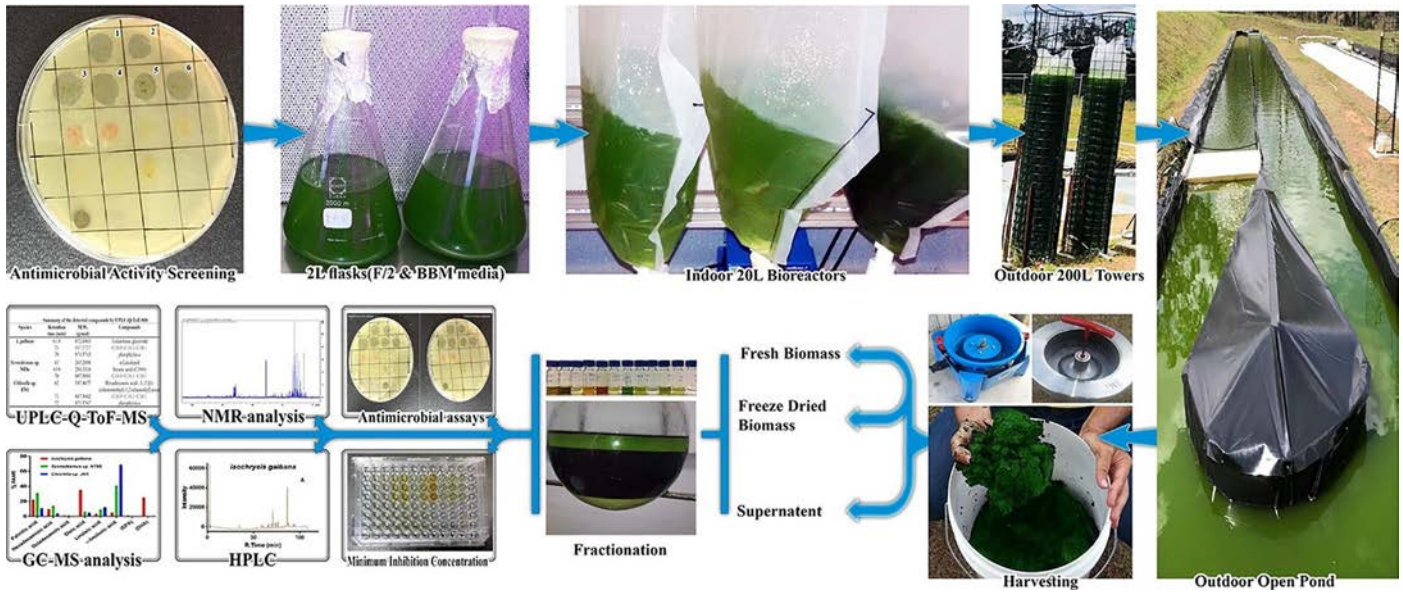

FIG. 19.15 Using of chromatographic and mass spectroscopy combined methods for the evaluation of microalgae antimicrobial compounds. *From F. Alsenani, K.R. Tupally, E.T. Chua, E. Eltanahy, H. Alsufyani, H.S. Parekh, P.M. Schenk, Evaluation of microalgae and cyanobacteria as potential sources of antimicrobial compounds, Saudi Pharm. J. 28 (2020) 1834–1841 (Copyright permission: 5042961405345).*

phytochemicals during microalgal cultivation, as well as the assessment of their physiological status, as a critical approach to be developed for microalgae biotechnology and related fields. Furthermore, nanoprobe microelectrodes with gold and other nanoparticles such as silver have emerged as one of the most important techniques to confirming the functioning of various microalgae (e.g., antioxidant activity) as well as analyzing their physiological status. As a result, traditional as well as spectroscopic approaches might be employed to validate the health advantages of microalgae and their bioactive components.

Authors contributions

Mostafa Gouda: Conceptualization, studied the literature, drafted, and edited this manuscript; **Musa Tadda:** Helped in writing process. The final edited manuscript was proofread and accepted by all the authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CHAPTER

20

Microalgae carotenoids: An overview of biomedical applications

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OUTLINE

1 Introduction

Carotenoids are a diverse group of natural lipophilic structures with a comprehensive spectrum that are responsible for the color of many yellow, orange, or red foods. They are widespread and have multiple functions and actions [1]. These pigments have exceptional bioactivity, which allow them to channel and orchestrate critical biomedical applications [2–4]. These compounds play a notable role as antioxidants, as precursors of vitamin A, and in eye health [5–7]. In addition, positive effects on reducing cardiovascular disease, obesity, diabetes, cancer, and the protection of neurons have been described [7–9]. A global trend in the pursuit of healthiness has considerably boosted the search for natural health promotion

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alternatives [10]. In this circumstance, microalgae are considered a promising alternative since they present a range of compounds capable of positively modulating human health, especially carotenoids [3,11]. It is estimated that more than 1200 natural carotenoids have been characterized from different sources [12]. About 200 carotenoids were found in microalgae [13]. These microorganisms incorporate a diverse group, with about 72,500 species cataloged. Among the species of commercial relevance are *Spirulina* (*Arthrospira*) sp., *Chlorella* sp., *Crypthecodinium cohnii*, *Porphyridium cruentum*, *Haematococcus pluvialis*, *Phaeodactylum tricornutum*, *Nannochloropsis* sp., and *Dunaliella* sp. [3,14,15]. However, *Haematococcus pluvialis*, and *Dunaliella*, stand out for their industrial output of astaxanthin, and β-carotene, respectively [16–18].

Also, several other carotenoids have been isolated from microalgae biomass, such as zeaxanthin, α-carotene, lutein, myxoxanthophyll, violaxanthin, canthaxanthin, and echinenone [14,19,20]. Canthaxanthin and echinenone are two examples of compounds that are not found in higher plants but have potentiated bioactive activities [20,21]. In general, the chemical structural characteristics of carotenoids determine their actions and properties, including from color, to their bioactivities at the biological level [22]. Although microalgae are considered ideal cell factories for obtaining natural carotenoids, developing a sustainable and profitable production model is still a challenge for using these compounds [23,24]. In this sense, this chapter brings together a description of the biomedical applications of microalgal carotenoids, including aspects related to production, chemical structure, relationships between structure and bioactivity, and challenges for use. Thus, this chapter was designed for scientists and researchers to deepen their knowledge in this area of expertise.

2 Microalgae-based carotenoids production

Due to the special bioactive functions of carotenoids, there is a global interest in exploring the production of this compound from microalgal biomass [3,20,25]. These microorganisms are considered the best commercial sources of natural carotenoids [26]. Biotechnological strategies such as high-performance photobioreactors and better development conditions are used to achieve this production, despite the fact that different forms of cultivation have different configurations and, as a result, can interfere both in the increase of microalgal cells and in the gain of carotenoids [27,28]. Although photoautotrophic cultivation is the most common, research with different strains of microalgae indicates that mixotrophic cultivation is an attractive alternative, as it promotes higher levels of biomass and progressively faster and, consequently, higher productivity of total carotenoids in dry biomass when compared to other means of cultivation [29]. In addition, to obtain high productivity of compounds, it is necessary to guarantee the high growth rate and high productivity of biomass, and mixotrophic cultivation is considered a favorable model because it presents a high index of development and biomass gain, reducing the evolution time beyond biomass waste [30].

Among the microalgae species that produce carotenoids, *Haematococcus pluvialis*, *Chlorella* sp., and *Dunaliella* sp. are reported as potential raw materials for commercial production of astaxanthin. Astaxanthin accumulation within the cells of *Haematococcus pluvialis* already

exceeds that of any other known species (4% of dry biomass); thus, it has been chosen for large-scale production [14,31–33]. Other carotenoids are also synthesized by *Haematococcus pluvialis*, such as violaxanthin, lutein, zeaxanthin, α-carotene, and β-carotene [23]. Carotenoids are also extracted from the biomass of the microalgae *Dunaliella*, in addition to astaxanthin, such as β-carotene [34], echinenone, and zeaxanthin [35]. Among the natural sources studied so far, *Dunaliella* has the highest β-carotene concentration, and it is the world's leader in production. This product represents about 10%–14% of its dry mass [31]. The isoprenoid fraction of *Spirulina*, the most commercially produced microalgae worldwide, includes β-carotene, zeaxanthin, and β-cryptoxanthin [23,34]. The microalgae *Porphyridium cruentum* also produces zeaxanthin and β-carotene [36].

Other microalgae used for zeaxanthin production are *Chlorella* sp., *Phaeodactylum tricornutum*, and *Nannochloropsis oculata* [15,37,38]. *Nannocloropsis*, in addition to zeaxanthin, is also known to produce astaxanthin and canthaxanthin [23]. *Chlorella* sp. is a type of microalgae that contains lutein as the primary carotenoid, in addition to α-carotene and β-carotene [15,23]. *Chlorella vulgaris*, in addition to lutein and β-carotene, contains violaxanthin and neoxanthin [38]. The microalgae *Nitzschia* sp. is already known to produce fucoxanthin [39]. According to carotenoid productivity data, the global market is estimated at $1.7 billion in 2022. Carotenoids like β-carotene, astaxanthin, and lutein have the most significant market share. Projections show that in 2022, astaxanthin will reach its value of $426.9 million, β-carotene US$572.78 million, and lutein US$357.7 million [19]. Thus, many multinational companies have started to produce diversified carotenoids for different applications, an profitable business for biomedical and industrial applications [39].

3 Chemical structure and relationship of structure-biological activity

The microalgae have carotenoids structurally comparable to conventional sources and some with specific structural characteristics [21]. The chemical structure knowledge of many compounds has been essential to predict their bioactivities [22,40]. Although it is not entirely clear, some structure-activity relationships were previously established for some properties, mainly antioxidant and pro-vitamin A activity, as described below [41,42]. Concerning chemical structure, carotenoids are classified according to the number of constituents of carbon, e.g.,C30, C40, C45, and C50. However, most carotenoids have C40 skeletons (i.e., tetraterpenes/tetraterpenoids). In turn, these structures are found in nature more abundantly and, consequently, more approached in the literature [12]. They are formed from eight C5 isoprenoid units joined head-to-tail, except at the center where a tail-to-tail linkage reverses the order, with the basic skeleton being linear and symmetrical. They are characterized by an extended network of conjugated double bonds (CDBs) centrally located in their structures [43]. This series of CDBs generates an electron resonance system π and corresponds to the chromophore, moving along the entire polyenic chain. Due to this structural feature, these compounds absorb light in the visible region of the electromagnetic spectrum, with strong absorption in the region of 400–500 nm [44]. Also, this system is primarily responsible for the functions and activities of carotenoids, such as chemical reactivity, molecular form, and activity in energy transfer processes [45].

Carotenoids can be classified as acyclic (e.g., lycopene, ζ-carotene) or cyclic when they have at their ends one (monocyclic) or two (dicyclic) terminal groups in the form of rings (e.g., γ-carotene, δ-carotene) [46]. Regarding their geometric configuration, carotenoid molecules can exist in two forms, called *trans* or *cis*, usually equivalent to *E* and *Z*, respectively. This characteristic depend exclusively on the disposition of substituent groups, specifically those that constitute a continuation of the polyene chain, about that double bond. In general, isomers (all-*E*)- usually have long, linear, and rigid molecules, while their *Z*-counterparts have bent structures [43]. Carotenoids' basic skeletons can be modified in extremities via hydrogenation reactions, cyclization, dehydrogenation, double bond migration, rearrangement, the introduction of oxygen-containing groups, chain extension or shortening, or combinations thereof, resulting in a variety of structures [47]. Still referring to the patterns of chemical modifications, the polyene chain can be modified by the presence of one or two acetylenic (—C≡C) or allenic (—C=C=C–) groups, present exclusively in microorganisms, such as microalgae and cyanobacteria, e.g., peridinin and diadinoxanthin; fucoxanthin and neoxanthin, respectively [48].

Another important classification of these isoprenoids is based on their chemical composition. According to the chemical elements present in their structure, they are classified into carotenes and xanthophylls subfamilies [43]. Carotenes are compounds that contain only hydrocarbons in their structure (e.g., lycopene, β-carotene), while xanthophylls are oxygenated carotenes, which contain different functional groups such as epoxy (neoxanthin, violaxanthin, fucoxanthin), keto (astaxanthin, canthaxanthin), hydroxy (zeaxanthin and lutein), glycosylated (myxoxanthophyll), and methoxy (spirilloxanthin) functional groups [49,50]. As can be seen in Fig. 20.1, in microalgae, many of these structures have already been isolated and identified, including mainly compounds such as α-carotene, β-carotene, zeaxanthin, lutein, violaxanthin, neoxanthin, astaxanthin, crocoxanthin, canthaxanthin, fucoxanthin, myxoxanthophyll, and echinenone, some of which are produced exclusively by microalgae [21,48,51,52].

In short, the chemical structural characteristics of carotenoids determine their actions and properties, including color, hydrophobicity, stability, aggregation, crystallization, solubility, and can determine their possible bioactivities at the biological level [22]. In fact, the biomedical contribution of carotenoids is made possible in part by their structural-chemical properties that channel and orchestrate essential biological activities, mainly exceptional antioxidant properties, and pro-vitamin A. The antioxidant capacity of these isoprenoid compounds is closely related to their structural characteristics, such as the type of structural end-groups, the extension of the chromophore, and the oxygen containing substituents [53]. In turn, it is considered that the size of the chromophore is the most influential parameter in the scavenging ROS. In parallel, the influence of the inclusion of oxygenated functional groups, such as keto (CO) and hydroxyl (OH) groups, on the terminal ring on the antioxidant capacity of carotenoids depends on the type of functional groups, their number, and whether or not they are part of the chromophore [41].

The action mechanisms of carotenoids as antioxidants are directly related to their ability to deactivate reactive oxygen (ROS) and nitrogen (RNS) species, minimizing or preserving oxidative damage in biological systems [41]. In general, three mechanisms are suggested for the deactivation of radicals (such as $ROO^{\bullet}$ and $HO^{\bullet}$) by carotenoids, namely, allylic hydrogen abstraction, electron transfer, and the addition of the radical to CDBs [54]. These reaction

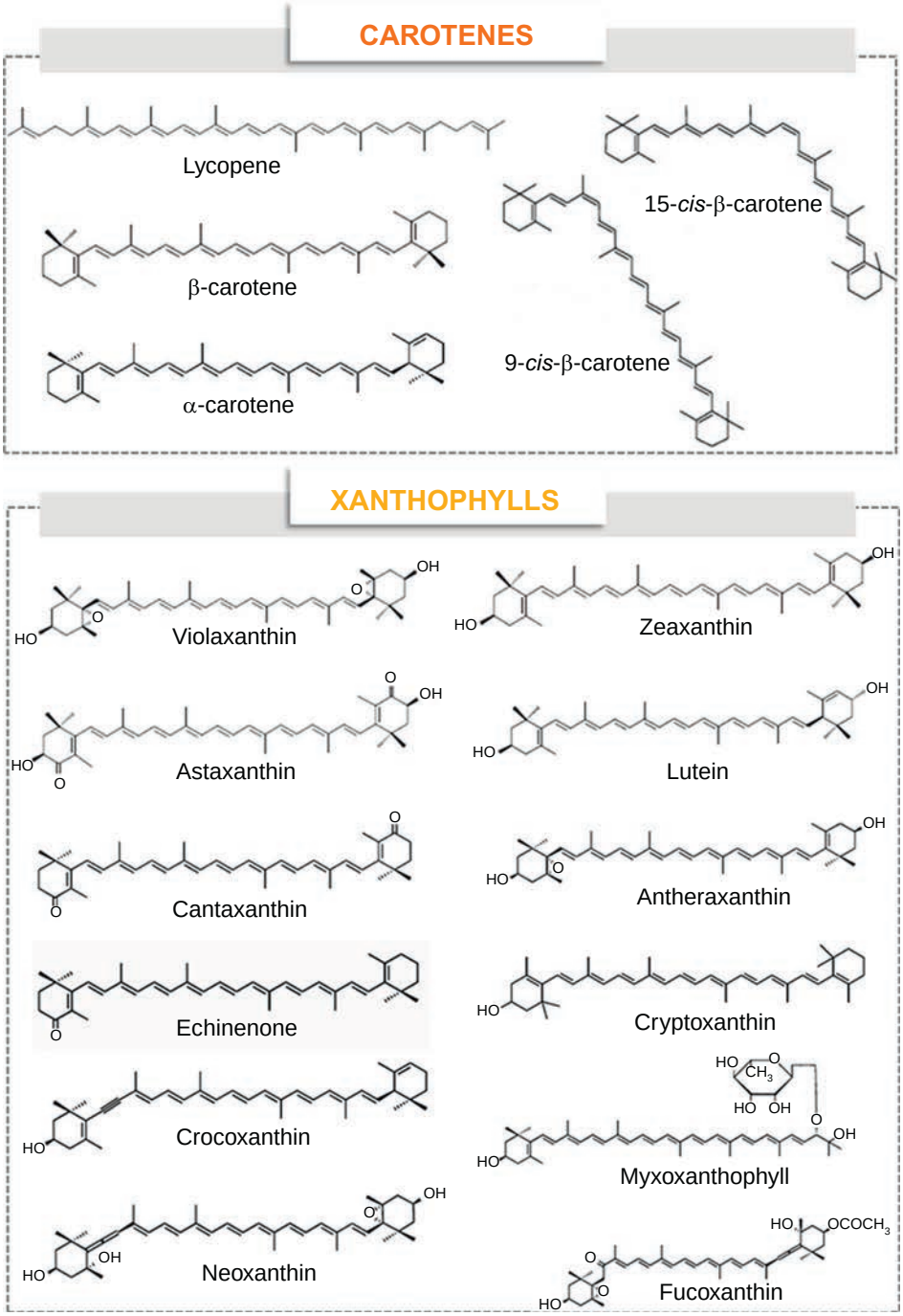

FIG. 20.1 Chemical structure of carotenes and xanthophylls.

mechanisms of carotenoids with free radicals, $R^{\bullet}$, are illustrated in the Eqs. (20.1)–(20.3) below:

$$[CAR] + R^{\cdot} \rightarrow [CAR^{+}] + R^{-} \text{ (Electron transfer)} \quad (20.1)$$

$$[CAR] + R^{\cdot} \rightarrow [CAR^{\cdot}] + RH \text{ (Hydrogen abstraction)} \quad (20.2)$$

$$[CAR] + R^{\cdot} \rightarrow [RCAR^{\cdot}] \text{ (Addition)} \quad (20.3)$$

These mechanisms and rates of scavenging are less dependent on the carotenoid structure and strongly dependent on the nature of the free radical species [46]. However, the preferential antioxidant mechanism of carotenoids involve the donation of polyenic chain electrons (Eq. 20.1). In this reaction, the carotenoids [CAR] donate electrons to the reactive species ($R^{\bullet}$), resulting in the formation of the radical cation [$CAR^{\bullet+}$] [40,53]. In general, the pioneering study by Rodrigues et al. [41] demonstrated that structural characteristics such as linearity and the extension of CDBs in carotenoid molecules seem to facilitate the antioxidant mechanism. In this study, the opening of the β-ionone ring (lycopene, 11 CDBs) and the increase in the length of the chromophore (astaxanthin, 13 CDBs) were the main factors that increased the peroxyl elimination capacity of carotenoids. At the same time, *cis* isomers showed less antioxidant capacity than their corresponding *trans*-isomers.

Concerning terminal groups, it has been shown that for carotenoids with the same chromophore (11 CDBs), the 1 OH group in all-*trans*-β-cryptoxanthin does not affect the ability to eliminate peroxyl radicals like that of all-*trans*-β-carotene (no oxygen substituent). However, the presence of 2 OH groups in all-*trans*-zeaxanthin increases the elimination capacity. In addition, all-*trans*-astaxanthin (13 CDBs, 2OH, 2CO) was 100% more effective than all-*trans*-β-carotene (11 CDBs). When comparing astaxanthin and zeaxanthin (11 CDBs, 2OH), it can be seen that the presence of 2 CO groups at the ends of the chromophore increases its antioxidant capacity by about 85%. In microalgae, the presence of carotenoids with extended chromophores in *Chlorella vulgaris*, such as canthaxanthin (13 CDBs), myxoxanthophyll (12 CDBs), and echinenone (12 CDBs), possibly contributed to a more significant antioxidant potential when compared to carotenoid extracts from other microalgae without the presence of these compounds [52].

Carotenoids also have excellent physical deactivation capabilities for singlet oxygen ($^1O_2$) through physical or chemical quenching [53]. In turn, chemical quenching is much more effective than physical quenching, which transfers excitation energy from the singlet oxygen to the carotenoid, resulting in ground state oxygen and excited triplet-state carotenoid [55]. Consequently, this excitation energy of the carotenoid is dissipated, resulting in ground state carotenoid and thermal energy [46]. This mechanism occurs by energy transfer and, therefore, does not alter the carotenoid structure that can undergo further cycles of $^1O_2$ quenching [56]. In contrast, carotenoids undergo structural modifications, such as oxidation or oxygenation, when they act as chemical quenchers of $^1O_2$ [54].

The efficacy of the physical extinction of $^1O_2$ by carotenoids is directly related to the number of CDBs, which determines their lowest triplet energy level. Carotenoids with 11 CDBs have especially been shown to possess a powerful extinguishing ability against singlet oxygen quenching [57]. Among the carotenoids already studied for this purpose, lycopene, an open-ring carotene, is considered the most efficient. Similarly, other compounds like

β-carotene, α-carotene, zeaxanthin, and cryptoxanthin are also considered highly active oxygen singlet suppressors, as they have triplet energy levels close to $^1O_2$, enabling energy transfer [58]. However, there is some reduction in singlet oxygen quenching efficiency for compounds with 10 conjugated double bonds, such as lutein [59].

Specifically, lutein and its structural isomer zeaxanthin are known as retinal xanthophylls, as they act as potent antioxidant molecules in eye health against oxidative stress [60]. In general, these macular pigments can reduce these potentially pathologic effects in two ways: a protection mechanism against oxidative damage to photoreceptors by their ability to extinguish reactive oxygen species and free radicals. Second, its spectral absorption capacity ($\lambda_{max} = 460$ nm) makes it an ideal prereceptor absorber of blue light (400–550 nm) known to cause severe damage to photoreceptors. When present in significant concentrations, these molecules are estimated to absorb 40%–90% of the incidence of light [61]. A significant property of carotenoids for human nutrition is their use as precursors of vitamin A (i.e., retinol, retinal and retinoic acid). However, the ability of carotenoids to exert pro-vitamin A activity is restricted due to related to their molecular structures [49]. The pro-vitamin A activity capacity of carotenoids is limited to structures with at least one unsubstituted β ring, and an 11-carbon polyene chain. At the biological level, these structures undergo enzymatic cleavage to produce at least one retinol molecule [42].

The main precursor of vitamin A is β-carotene, considered the most potent, as it shows two unsubstituted β rings), to which 100% activity is assigned [62]. In addition to β-carotene, other carotenoids, such as γ-carotene, β-cryptoxanthin, α-carotene, β-carotene-5,6-epoxide, α-cryptoxanthin, and some β-apo-carotenals, can also be converted into vitamin A [63]. However, since they only contain one unsubstituted ring, they exhibit about half the bioactivity of β-carotene [62]. In summary, the chemical structure of carotenoids orchestrates a series of activities at the biological level that enable these isoprenoids to act as important bioactive compounds in several biomedical applications.

4 Biomedical application

The biological actions of carotenoids that underlie their biomedical applications are described in Table 20.1 and are often explained by their antioxidant properties. Although specific carotenoids can also act through other mechanisms; for example, β-carotene can serve as a precursor to vitamin A, while zeaxanthin or lutein constitute the macular pigment of the eye [5–7]. Moreover, there is evidence that these molecules have effects on oxidative stress, mainly benefiting cardiovascular health. Also, they can help prevent obesity, diabetes, some types of cancer, and neurological sequelae [7–9].

4.1 Protection against oxidative stress

Oxidative stress is closely related to the increase in various chronic diseases. This happens when there is a cellular physiological imbalance between the free radicals and the endogenous antioxidant system; consequently, it triggers the oxidation of biologically relevant molecules like lipids, proteins, and nucleic acids, leading to such damages as lipid peroxidation,

TABLE 20.1 Microalgae bioproduct and their physiological properties.

Microalgae species	Bioproduct	Effects	References
Arthrospira platensis	Whole dried biomass	↓ MDA and TBA in liver, plasma, heart and kidney ↑ GSH level in liver and blood ↑ GR, GPx, and GST activities in liver and blood ↓Oxidative damage of DNA in lymphocytes	[64]
Scenedesmus obliquus	Whole dried biomass	↓ MDA, SOD and CAT in heart, liver, kidneys and spleen ↑ GPx in kidneys, ↑ GR in liver	[25]
Scenedesmus obliquus	Isolated carotenoid extract	↑ MDA in liver ↓ MDA, GPx and GR in spleen ↓ GR and CAT in liver ↑ GR and GPx in heart ↑ GPx in kidneys	[20]
Dunaliella salina	Isolated carotenoid extract	↑ SOD, CAT, Peroxidase in liver ↓ MDA	[65]
Haematococcus pluvialis	Isolated carotenoid extract	↑ SOD, CAT and GPx activities in liver ↓ MDA	[66]
Dunaliella salina	Whole dried biomass	↓ C-reactive protein, adhesion molecules and LOX activity in blood ↓ cardiac tissue damage	[67]
Dunaliella salina	Whole dried biomass	↓ TNF-α and IL-1β in jejunal tissue ↓ jejunal tissue damage	[68]
Scenedesmus obliquus	Whole dried biomass	↓ fasting glucose ↑ total cholesterol/HDL-cholesterol ratio ↓ total triglycerides	[69]
Haematococcus pluvialis	Isolated carotenoid extract	↓ weight gain and epididymal adipose tissue ↓ glucose, insulin, TNF-α and IL-6 levels in serum ↑ insulin signaling in skeletal muscle	[70]
Dunaliella bardawil	Whole dried biomass	↓ plasma cholesterol (high-fat diet + whole microalgae) ↑ plasma triglycerides (low-fat diet and *Dunaliela* β-carotene deficient powder) ↓ atherosclerotic lesion area	[71]

mitochondrial swelling, mutagenic actions, and posttranslational protein modifications [72,73]. The stabilization of pro-oxidants chemical species occurs through the action of the endogenous antioxidant system represented by enzymes as superoxide-dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione transferase (GST), and glutathione reduced (GSH) and oxidized (GSSG) [20,74].

Also, the body counts on the aid of dietary antioxidants, such as carotenoids, that can act as antioxidants, especially by donating electrons [41], or still, can stimulate the mechanisms of the endogenous antioxidant system [20,75]. To this end, microalgae are considered sources of

molecules with high antioxidant capacity. Thus, β-carotene and astaxanthin demonstrated effects as endogenous antioxidant defense stimulators, preventing oxidative stress by eliminating free radicals and lowering lipid peroxidation [76]. Finally, most studies evaluating the microalgae antioxidant properties have this trend mainly for lipid peroxidation reduction [20,25,64–66].

4.2 Supply of vitamin A

Vitamin A status involves various physiological mechanism, including vision, energy metabolism, immune systems, and embryonic development [77,78]. Thus, the contribution of carotenoid to provitamin A is significant if an animal-derived diet is absent [79]. In order to exhibit a provitamin A activity, the carotenoid structure must have at least one unsubstituted β-ionone ring [2]. In that case, the β-Carotene molecule is the main vitamin A precursor [7,77]. Based on this understanding, microalgal products have considerable potential to meet dietary vitamin A deficiency as a sustainable strategy to compensate for the dearth of animal products in food [38,80]. This potential is due to the fact that these microorganisms are a success model for carotene production and have been used as dietary supplements [3,81].

However, the impact of the microalgae matrix on carotenoid bioaccessibility and bioavailability is a significant problem. In general, the bioaccessibility of β-carotene from microalgae is low (from 0% to 27%). A possible explanation might be the composition of the cell walls, which hampers release and subsequent micellarization, and is increased by processing operations and consuming fat-rich food (from 5% to 81%) [82–84]. However, no impact on the retinol level measurable in mice was observed from the diet of *Phaeodactylum tricornutum*, which showed a bioaccessibility of 19% for β-carotene [38].

4.3 Eye disorders and diseases

Eye conditions affect all age groups, but as society ages, poor eye health and impaired vision have risen to the forefront as a global health concern. About, 33 million people with vision impairment are aged 50 years or older. The most common causes include cataracts, glaucoma, age-related macular degeneration, diabetic retinopathy, and presbyopia, which prevalence increases with age [85,86]. Carotenoids, in particular, play an important role in maintaining ocular health [7]. Especially, lutein (36%), zeaxanthin (18%), and mesozeaxanthin (18%) are the main carotenoids in the eye and are collectively denominated as macular pigment [6]. Macular carotenoids as antioxidants and blue light filters protect the macula from light-induced oxidative stress [87,88].

The chemical structures of the macular pigments are characterized by the presence of hydroxyl groups attached to the terminal ionone rings, and their pattern of conjugated double bond is closely related to their light-absorbing properties, influences the antioxidant action (described in Section 4.1), and anatomical location [88]. Lutein differs from other macular pigments (zeaxanthin and mesozeaxanthin) because it has one fewer conjugated double bond. Thus, the maximum absorption of lutein is about 445 nm, while that zeaxanthin is around 450 nm. Therefore, the absorbance spectrum of macular pigment peaks at 460 nm, matching the "blue light hazard" wavelength of 450–500 nm [89].

Thus, the macular pigments absorb 40%–90% of the incident short-wavelength, high-energy visible blue light, protecting the retina from photochemical damage. Moreover, some evidence reports an association between zeaxanthin and lutein status and the incidence of nuclear cataracts [7,86,89–91]. However, the macular carotenoids cannot be synthesized by humans and must be obtained through diet or supplementation [7]. In such cases, microalgae species represent a potential dietary source for these xanthophylls [19,52]. In the line with this, studies reported the lutein accumulation in the eyes of rats after gavage with *Botryococcus braunii* microalgae for 15 days [92].

4.4 Obesity and comorbidities

The COVID-19 pandemic has had widespread health impacts, revealing the particular vulnerability of those with comorbidities, and the most common underlying health conditions include diabetes, cardiovascular diseases, being overweight, and obesity [93–95]. There is evidence that carotenoids may benefit some of these factors, decreasing the severity of comorbidities and their fatal consequences. Thus, carotenoids can reduce cardiovascular disease risk because of their antioxidant action (described in Section 4.1) and low-density lipoprotein (LDL) oxidation, preventing plaque formation. Additionally, there is increasing evidence that endogenous carotenoids and their cleavage product's metabolism may modulate physiological mechanisms connected to body fat regulation [8,96]. Also, studies have shown that carotenoids have an antidiabetic effect by modulating the insulin signaling pathway [97–99].

A study by El-Baky et al. [100] demonstrated that carotenoids extracted from *Dunaliella salina* were fed to rats as a dietary supplement (500 mg/kg diet), exerted potent hypolipidemic effects that may prevent cardiovascular diseases. Furthermore, in rats and humans, *Chlorella* and *Arthrospira*-promoted serum cholesterol reduction and increases in high-density lipoprotein to low-density lipoprotein ratios [101,102]. Also, El-Baz et al. [67] showed that the carotenoid-rich fraction of microalgae *Dunaliella salina* could improve various disorders related to cardiac dysfunction in obese rats. Studies have shown that astaxanthin is effective and safe in controlling body weight, being considered an antiobesity agent. The administration of astaxanthin significantly reduces body weight and adipose tissue induced by a high-fat diet and may reduce hepatic, plasma, and total cholesterol triglycerides [103]. Finally, [104] investigated the association of a carotenoid-rich diet with the incidence of type 2 diabetes. According to this study, the intake of β-carotene and α-carotene was favorable for reducing diabetes, both in men and women.

4.5 Cancer and neuroprotection

Cancer is projected to surpass mortality from cardiovascular disease to become the leading cause of death by 2030. Carotenoids help reduce the incidence of some cancers by suppressing the growth of cancer cells through the induction of apoptosis, in addition to their antioxidant potential [4,9]. Due to structural features, carotenoids produced exclusively by microalgae, such as canthaxanthin and astaxanthin, are associated with high antioxidant potential [20]. According to studies, the effects of canthaxanthin on breast tissue chemically induced

carcinogenesis in mice show that ingesting canthaxanthin for 3 weeks, before cancer induction with dimethylbenzanthracene, can reduce the occurrence of cancer by 65% [39]. Furthermore, a study in Wistar rats concluded that astaxanthin was efficient in treating colon cancer [105].

Several carotenoids appear to have effects on cognitive functioning. The underlying mechanism is not precise, but it may relate to antioxidant action [106]. One example is the decline in cognitive ability accompanying Alzheimer's disease, apparently caused by persistent oxidative stress in the brain. Supplementation with *Chlorella* sp. (containing β-carotene and lutein) significantly reduce cognitive impairment in mice [107]. Furthermore, astaxanthin can cross the blood-brain barrier and thus extend its antioxidant effect. Therefore, astaxanthin can work by reducing the effects of Alzheimer's disease and other neurological dysfunction [108].

5 Challenges and future research

Although microalgae are a source of natural carotenoids with excellent biological potential, several challenges to their practical utilization must be overcome. According to Ambati et al. [23], the synthesis of these microalgal chemicals is not yet economically feasible to compete with synthetics. Generally speaking, carotenoid-producing microorganisms are characterized by the timid growth rate and productivity of the target compound, in addition to being recalcitrant, making extraction even more complex [24]. As a result, new technologies for cell harvesting, disruption, and downstream processing are required [23]. Among the strategies to achieve sustainable and profitable processes are the advancement of enhanced cultivation systems with intelligent screening of potential species and improvement of strains, more efficient and nontoxic extraction protocols, as well as downstream processing methods with an emphasis on circular economy and sustainability (biorefinery systems) [24,109].

In parallel, biomass use in therapeutical applications may become a future trend to minimize costs and environmental impacts [81], as it eliminates the carotenoid extraction stage. However, aspects related to bioaccessibility must be considered to guide the application [29,82,84]. Research on microalgae carotenoids and their biomedical applications is expected to focus on bioavailability, metabolism, and consumption and effect correlations in the near future.

6 Conclusions

Finally, microalgae are prospective sources of natural carotenoids with important bioactive activities and consequently, biomedical applications. In part, its bioactivity stems from its chemical structure. These compounds act in the biological system as notable antioxidants, precursors of vitamin A, and the eyes' health, and show positive effects in heart diseases, obesity, diabetes, cancer, and neuroprotection. Strategies that integrate production maximization, cost reduction, and sustainability are fundamental for establishing the use of these microalgal isoprenes. Bioavailability, metabolism, and the complete establishment of consumption-effect relationships are crucial for future perspectives in this field of application.

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CHAPTER

21

Diatomite-based nanoparticles: Fabrication strategies for medical applications

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OUTLINE

1 Introduction

Researchers with expertise in the fields of chemistry, biology, physics, materials science, and engineering have the ambitious aim to solve medical issues through the application of nanotechnology to medicine, an approach known as nanomedicine [1]. Research in nanomedicine includes a multitude of applications, including drug delivery, vaccine development, antibacterial, diagnosis, and imaging tools [2–4]. In particular, nanomaterials have the potential to deliver chemotherapeutics to specific tissues and improve the drug efficacy, as well as its transport and fate in the blood [5]. For this reason, nanomaterials offer some

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unique advantages for cancer treatment. Conventional anticancer compounds lack selectivity against tumors since they also attack normal tissues, causing excessive systemic toxicity that limits the application of the correct drug dosage and, consequently, the effectiveness of the treatment. Targeted therapy based on nano-drug delivery systems able to increase the intracellular concentration of drugs in cancer cells while avoiding normal cells is considered the most promising strategy to overcome the limits of standard therapeutic approaches [5]. Biocompatible nanoparticles (NPs) are the key components of nanomedicine. They can be extraordinary "smart" devices able to load the drug, recognize cancer cells, and release the drug into the tumors, increasing its therapeutic index, protecting the payload from degradation, and properly modulating the pharmacokinetic and pharmacodynamic profiles. The employment of NPs for the treatment of diseases is promoted by their ability to cross biological barriers and target cancer cells by both passive and active targeting strategies [6,7]. Passive targeting is based on the enhanced permeability and retention (EPR) effect, which promotes the accumulation of macromolecules, including NPs, in the tumor interstitial space. This phenomenon is related to cancer cells' rapid growth, which necessitates an intricate vasculature network to supply additional oxygen and nutrients; the new vessels that are growing disorganized and dilated are leaky junctions through which NPs can penetrate and be internalized [8]. Examples of nano-based chemotherapy drugs using passive targeting are Doxil (i.e., doxorubicin encapsulated in a liposome) and Abraxane (i.e., albumin-bound Paclitaxel), whose success in clinics is just due to the EPR effect [9]. The specificity of NPs using the EPR effect is about 25% higher in tumors than in normal tissue. Active targeting can favor the internalization of cancer cells by receptor-mediated endocytosis based on the specific interaction between ligands or antibodies immobilized on NP surface and receptors/antigens expressed on cancer cells [10]. There is an unresolved debate about the efficacy of receptor-mediated targeting versus the passive approach, but the selective recognition and binding of NPs to cell surface receptors is likely to ameliorate the therapeutic outcomes of the treatment [11]. NPs explored in biomedicine can be realized using several materials such as organic, inorganic, and biological, as well as their combinations. Among them, inorganic NPs offer many advantages, including well-defined size and shape, long half-life, thermal and chemical stability in physiological conditions.

In recent years, inorganic NPs obtained from the amorphous silica of diatomite powder have been explored as nano-based drug delivery systems [12]. Diatomite is a material of sedimentary origin abundant in many areas of the world and mainly formed by fragments of diatom skeletons, known as frustules [13]. The frustule morphological structure is characterized by micro/nanopores, conferring to the material a large specific surface area (about $100\ m^2\ g^{-1}$) with high absorption capability [14]. The Food and Drug Administration (FDA) agency recently approved diatom biosilica as "Generally Recognized as Safe (GRAS)" for food and pharmaceutical production. Moreover, silica from diatoms was classified in the third group of "Not classifiable as to its carcinogenicity to humans" by the International Agency for Research on Cancer (IARC) [15]. Diatomite-based NPs (DNPs), having sizes ranging between 100 and 400 nm, can be easily prepared by macroscopic diatomite fragments applying high-amplitude ultrasonic waves without losing the porous morphology useful to load a variety of drugs, including aptamers, chemotherapeutics, and small molecules. The surface of DNPs is highly hydrophilic due to the presence of silanol groups that can be used to perform several functionalization strategies. Through surface modification approaches, the biocompatibility, intracellular uptake, and drug-loading efficiency can be

improved/modified according to the goal of the biomedical application [16]. The internalization kinetic studies of DNPs revealed that they penetrate cancer cells embedded in a lipid vesicle by endocytosis [17]. In this work, Raman imaging studies performed on label-free DNPs demonstrated that the cell uptake occurred after 6 h of incubation, and that it achieved the saturation point after 18 h with a homogeneous distribution of DNPs in the cytoplasm.

Unfortunately, the main drawback of diatom biosilica is its poor biodegradability in the human body, which causes severe accumulation in diverse organs, hindering the medicinal use of DNPs. To date, only one work investigating the biodistribution of biosilica into mice has been published. In this study, the authors demonstrated that, 8 days after the intravenous injection, the presence of diatom biosilica was still observed in sections of the liver and kidney but not in other vital organs [18]. To address this issue, a magnesiothermic reduction process was proposed to convert DNPs into biodegradable silicon nanoparticles [19]. Taking into consideration the biocompatibility of the material and the promising results obtained both in vivo and in vitro, the use of DNPs can be considered as an effective and cheap weapon in nanomedicine, and especially as nanocarriers for drug delivery. Moreover, the ultimate goal of nanomedicine is the development of multifunctional nanodevices able to perform both the monitoring of diseased tissue (i.e., diagnosis) and the site-specific delivery of therapeutic compounds (i.e., therapy); these revolutionary nanomachines, called nanotheranostic devices, are the last frontier of nanomedicine. Recently, a multifunctional hybrid platform constituted by gold nanoparticles (AuNPs) synthesized on the surface of DNPs was realized to gain advantages from the porous structure of DNPs for drug loading and the contrast properties of AuNPs for imaging. The biocompatibility of the hybrid system and its ability to improve the therapeutic effect of the delivered drug without causing toxic effects were demonstrated in colorectal cancer cells [20].

This chapter explores the use of DNPs in nanomedicine, reporting their main fabrication strategies, surface modifications, and drug-loading techniques. Biocompatibility and uptake of DNPs in living systems are also described.

2 Fabrication and surface modifications of DNPs

Diatomite is an ancient fossil remnant naturally formed by the siliceous skeletons of diatoms over the centuries. This powder is constituted of 70%–90% silica derived from diatom frustules and by a fraction of clay and metallic oxides, such as aluminum oxide (Al_2O_3) and iron(III) oxide (Fe_2O_3) [21]. Diatomite-derived biosilica is frequently a blend of several species with varying dimensions (from 2 m to nearly 1 mm) and, in most cases, fractured due to time erosion or mining and grinding [22]. This low-cost material is well-suited for biomedical applications thanks to the possibility to obtain uniform in size-NPs with a top-down approach followed by a separation process. The reduction of diatomite powder in NPs can be obtained through ultrasound sonication approaches. In powder dispersions, sonication causes microjet, shock-wave impacts on the surface of particles, and interparticle collisions, that result in particle-size reduction [23]. Rea et al. proposed the application of ultrasounds and settling for the feasible reduction of diatomite to NPs [12]. The treatment of the diatomite powder with ultrasounds disrupts large aggregates and reduces the average size of NPs,

which are collected through sedimentation and filtration processes afterward. According to Stoke's law, NP settling time in suspension is influenced by the density of the NPs and medium, the medium viscosity, and NP radium. Therefore, the size uniformity of diatomite nanoparticles (DNPs) can be improved by collecting them after repeated cycles of settling in ethanol [24]. A crucial step for the application of DNPs to nanomedicine is the purification process to remove organic contaminants and metallic impurities. Purification treatments with strong acids have proven to be effective for removing both undesired organic and inorganic impurities. The estrangement of organic compounds can be achieved by dispersing DNPs in Piranha solution for 30 min, whereas the inorganic contamination can be removed by treating DNPs in 5 M chloride acid solution (HCl) for 16 h [12,25]. After the reduction and purification treatment, DNP with a 100–400 nm size range can be obtained, as shown in Fig. 21.1A. The Fourier-Transform InfraRed (FTIR) analysis in Fig. 21.1B shows that the characteristic peaks of contaminants disappeared after the acid treatment, whereas silica content remained evident. The Energy Dispersive X-Ray Spectroscopy (EDS) analysis in Fig. 21.1C and D confirmed that, after purification, the percentage of calcium, iron, and aluminum significantly decreased, whereas the silica percentage increased to 95% [26].

The removal of impurities from DNPs allows the exposure of surface reactive silanol groups (—SiOH) suitable for binding various biomolecules to the surface of DNPs [27–29]. Indeed, Si—OH groups can be used to make siloxane linkages with silane coupling agents constituting the interface between the inorganic materials and the organic molecules [30]. Organosilane agents suitable for inorganic material have the general formula $(RO)_3Si—(CH_2)_n—X$, where R is a methyl or propyl group, and X is a functional group (e.g., mercapto, amino, phenyl, etc.) that can be used for subsequent immobilization reactions [31]. Among all the organosilanes, (3-aminopropyl)triethoxysilane (APTES) has been widely used for the functionalization of DNPs. The aminosilanization of DNPs can be easily performed by dispersing the NPs in a 5% APTES solution in ethanol for 1 h and carrying out a curing process at 100°C for an additional hour [29,32,33]. The as-prepared DNPs present amine groups (NH_2) on the surface that make the surface charge turn from negative to positive due to the protonated NH_3^+ form [12]. The NH_2 groups can be used for the covalent immobilization by click-chemistry of different biomolecules or chemical moieties to the surface of DNPs, including drugs, enzymes, proteins, antibodies, aptamers, DNA, and sensing probes (Fig. 21.2A) [34,35]. In 2015, Rea et al. proposed the immobilization of small interfering ribonucleic acid (siRNA) on the DNP surface for drug delivery applications to human epidermoid cancer cells (H1355) [12]. —The study's goal was to show that DNPs could transport siRNA inside cancer cells, overcoming siRNA's inability to penetrate the cell membrane through systemic administration. The NH_2-modified DNP surface acted as a nucleophile against a heterobifunctional crosslinker, N-(-maleimidobutyryloxy) succinimide ester (sulfo-GMBS), forming an amide bond (CN) to conjugate the siRNA to the DNP surface. On the other side, the maleimide ring of sulfo-GMBS reacted with the cysteine sulfhydryl group of a peptide complex bound to the siRNA, and a thioether bond was formed. The strategy of immobilizing siRNA in a complex with a positively charged peptide is highly functional for the delivery of siRNA inside cancer cells; the complex peptide/siRNA was obtained by modulating the nitrogen/phosphate ratio to optimize electrostatic interaction between the positive charge of the peptide (NH_3^+) and negative charge of the siRNA phosphodiester backbone (PO_4^-). The electrostatic attraction between the DNP surface and siRNA was demonstrated by the fact that

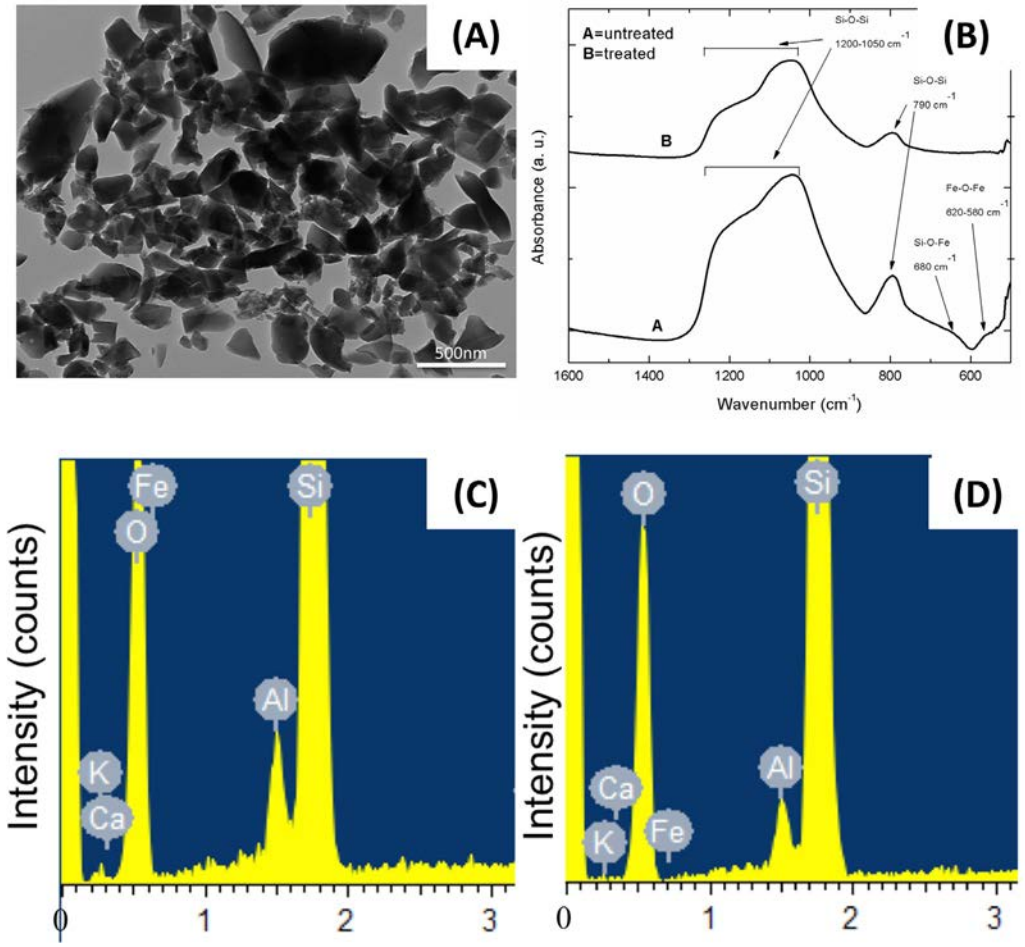

FIG. 21.1 (A) TEM images of DNPs after reduction by ultrasonic treatment. (B) FTIR analysis before and after the purification treatment; (C, D) EDS spectra of untreated and purified DNPs. *Reproduced and adapted from I. Ruggiero, M. Terracciano, N.M. Martucci, L. De Stefano, N. Migliaccio, R. Tatè, et al., Diatomite silica nanoparticles for drug delivery, Nanoscale Res. Lett. 9 (2014) 1–7. https://doi.org/10.1186/1556-276X-9-329 with the permission of Springer Nature and from I. Rea, N.M. Martucci, L. De Stefano, I. Ruggiero, M. Terracciano, P. Dardano, et al., Diatomite biosilica nanocarriers for siRNA transport inside cancer cells, Biochim. Biophys. Acta Gen. Subj. 1840 (2014) 3393–403. https://doi.org/10.1016/j.bbagen.2014.09.009 with the permission of Elsevier.*

a part of the molecules were released rapidly, with the rest of the nucleic acid released in 48–72 h due to the progressive weakening of interactions between the DNP surface and siRNA.

Alternative modifications of the DNP surface with biomolecules by click-chemistry were presented by Terracciano et al. for improving biocompatibility [16]. Carbodiimide chemistry was used to conjugate the carboxyl (COOH) groups of polyethylene glycol molecules to the DNP surface for improving their stability. The PEG COOH groups were further conjugated

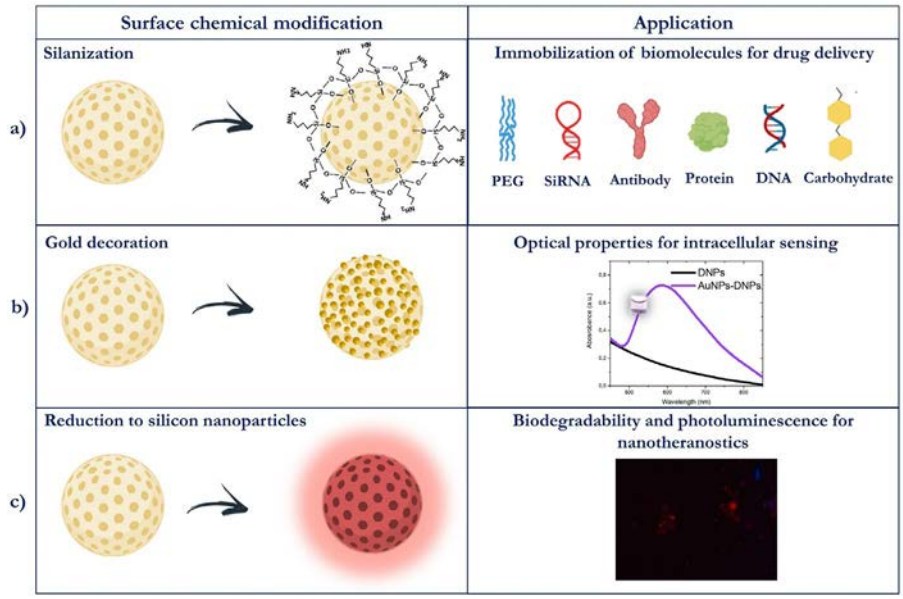

FIG. 21.2 Surface chemical modifications (on the left) and their potentiality in biomedical applications (on the right): (A) silanization of the surface to enable the immobilization of biomolecules, (B) gold decoration to enhance optical properties, and (C) reduction to silicon nanoparticles to improving the material biodegradability and confer intrinsic photoluminescence properties for nanotheranostic applications.

via carbodiimide chemistry to the NH_2 group of a cell-penetrating-peptide that allowed NPs to cross the cell membrane. This functionalization and the obtained outcomes will be better described in Section 3.

In recent years, a further step has been taken in the application of different inorganic NPs as a platform for nanotheranostic applications combining imaging and drug delivery [36–41]. The large surface area of silica, its porous structure, and biocompatibility also represent a good opportunity for the combination of imaging and drug delivery purposes [42,43]. The development of nanotheranostic devices made of DNPs is a fascinating challenge that could encourage the application of this material in nanomedicine. In 2018, Rea et al. demonstrated that it is possible to combine DNPs with metal nanostructures such as gold nanoparticles (AuNPs) to obtain a hybrid nanovector for imaging and drug delivery applications (Fig. 21.2B) [20]. The property of AuNPs to absorb the laser light and generate electrons oscillation results in a phenomenon called localized surface plasmon resonance, which can be used for imaging applications [44,45]. To build the hybrid nanovector, Rea's group amino-modified the surface of DNPs to guarantee the electrostatic interaction between the negative ions of gold precursor ($AuCl_4^-$) and the DNP surface, thus promoting an in-situ synthesis of the AuNPs. Specifically, amino-modified DNPs were dipped in chloroauric acid solution ($HAuCl_4$) and, in the presence of diacid PEG as a stabilizer, sodium borohydride was added to reduce Au^{3+} to nanostructured Au^0. The formation of AuNPs on the biosilica surface was confirmed to the naked eye by a change in the color dispersion from pale yellow to deep

purple. Thanks to this simple approach, the surface of DNPs was covered by a uniform carpet of AuNPs of about 14 nm. Analysis before and after the decoration with AuNPs highlighted that the metal nanostructures reduced the DNP surface area filling the smaller pores of DNPs, whereas pores bigger than 20 nm were still able to accommodate biomolecules for drug delivery purposes. While bare or amino-modified DNPs do not absorb light in the visible range, the complex DNP-AuNPs showed an absorption peak in the visible region at 550 nm, which could be useful for the analysis of further steps of functionalization by spectrophotometry. Moreover, since noble metal particles enhance the Raman signal of molecules in their proximity, this study shows the potential of hybrid nanoplatforms for intracellular tracing of biomolecules by Surface Enhanced Raman Spectroscopy (SERS). In the recent work of Rea's group, the DNP-AuNPs complex served as a SERS platform to trace the drug release of the anticancer agent Galunisertib to colorectal cancer cells [46]. The AuNPs grown in situ on the DNP surface provided the platform with optical properties and a localized surface plasmon resonance (LSPR) at 576 nm. Furthermore, AuNPs (25 nm in size) provided near-field optical amplification to the hybrid complex, allowing label-free sensing of Galunisertib loaded in the complex via SERS analysis. The proximity of AuNPs to the encapsulated anticancer agent produced an enhancement factor of the Galunisertib SERS spectrum of 4.5×10^5, which allowed monitoring the drug release with attogram-scale resolution in colorectal cancer cells.

Even though silica has many intriguing features for biomedical applications, its use in therapy is still hindered by its poor biodegradability and consequent accumulation in the human body. Delalat et al. investigated the in vivo biodistribution of diatom silica microparticles after 8 days of intravenous injection [18]. It was shown that, although diatom silica did not cause structural alterations of the main organs (brain, kidney, heart, lung, liver, and tail), it accumulates in the kidney and liver. To solve the slow clearance and poor biodegradability in the body, Losic's group proposed the conversion of diatom silica into fully biodegradable porous silicon (PSi) through a magnesiothermic reduction process (Fig. 21.2C) [19,47]. Using a magnesium source to initiate a redox reaction at 650°C, Losic et al. maintained the original morphology and porous structure of diatoms and increased the biocompatibility of the material. In vitro degradation study of the as-produced "silicon diatom replicas," it was found that 60% of silicon diatoms dissolved in 30 days, whereas less than 20% of diatom silica degraded simultaneously. The dissolution behavior of the silicon replicas prevented the NP accumulation into the body since PSiNPs can be degraded into the nontoxic orthosilicic acid (H_4-O_4-Si) in the body [48].

The possibility to make diatoms biodegradable highlights the potential of this low-cost and abundant material for the creation of natural, environmentally friendly, and cost-effective nanoplatforms for biomedical applications.

3 Biocompatibility and uptake of DNPs in vitro and in vivo systems

Despite the numerous benefits of using NPs in drug delivery, some fundamental challenges continue to impede their effective use in clinical applications [49,50]. These challenges include promoting the active uptake of NPs by diseased cells rather than nontargeted or nondiseased ones; providing NPs with the ability to elude immune system mechanisms, such

as the reticuloendothelial system (RES), that rapidly shuttle the NPs out of circulation, inhibiting their therapeutic action; avoiding the interaction and aggregation of NPs with plasmatic proteins, causing the entrapment of NPs in the liver and consequent elimination; and preventing the toxicity and side effects of NP administration in the host (e.g., capillarity occlusions, deep vein thrombosis) [51–53]. Several in vivo and in vitro studies have established that the physicochemical properties of NPs can influence the NP-cell interactions as well as their uptake [54–56]. Thus, the optimization of NP properties through proper surface modification approaches seems to be necessary for improving the therapeutic effects of nanocarriers as well as their applications in nanomedicine [57,58].

The proper surface manipulation of NPs is fundamental in the development of efficient drug delivery carriers since it confers new or advanced physical, chemical, or biological properties to the one originally presented by the nanocarrier [59]. Diatom biosilica obtained by reduction and purification processes can be easily modified with different chemical procedures for the realization of sophisticated systems with ideal properties for drug delivery applications [60]. The most commonly used chemical strategy to develop advanced diatomite-based nanocarriers is the well-established silica chemistry. This approach exploits the ability of the silane compound APTES or mercapto propyltrimethoxysilane-(MPTMS) to form siloxane bonds (Si—O—Si) with the silanol hydroxyl groups (—OH) on the surface, thus introducing surface reactive groups (—NH_2, —SH, —COOH) useful for the conjugation of biomolecules to DNPs.

Terracciano et al. developed a dual-functionalization strategy based on the conjugation of PEG and a cell-penetrating peptide (CPP) to DNPs to increase their aqueous stability, biocompatibility, and cellular uptake (Fig. 21.3) [16]. To grant chemical functionality to the DNPs, silica was first silanized with APTES molecules (as described in Section 2), then a bifunctional PEG (NH_2—PEG—COOH, MW 5000) was bound to the DNP surface (PEGylation) via EDC/NHS chemistry. The PEGylation process can be a valid means to reduce some of the aforementioned challenges and promote the therapeutic application of NPs. PEG is an inexpensive, versatile polymer made of a repetition of ethylene ether units with dynamic conformations. The Food and Drug Administration (FDA) Agency approved it

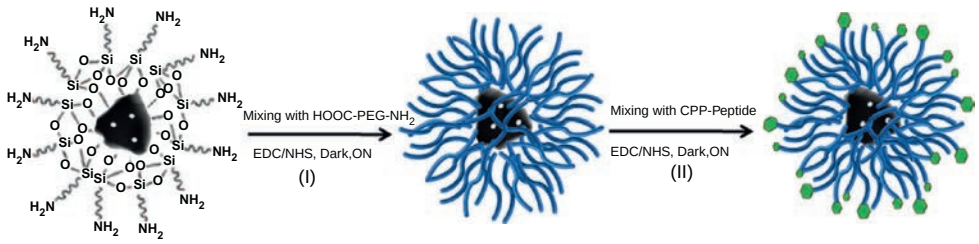

FIG. 21.3 Schematic representation of the DNP functionalization. Reaction I, PEGylation of the DNP-APT (I) via EDC/NHS, under stirring overnight (ON) at room temperature. Reaction II, CPP-peptide bioconjugation of the DNP-APT-PEG via EDC/NHS, under stirring ON at RT. The dual biofunctionalization is based on a covalent binding between the NP surface and biomolecules promoted by the EDC/NHS chemistry. *Reproduced with permission of The Royal Society of Chemistry from M. Terracciano, M.-A.A. Shahbazi, A. Correia, I. Rea, A. Lamberti, L. De Stefano, et al., Surface bioengineering of diatomite based nanovectors for efficient intracellular uptake and drug delivery, Nanoscale 7 (2015) 20063–20074. https://doi.org/10.1039/c5nr05173h.*

for many clinical applications [61], including drug delivery, because PEGylation of NPs decreases RES uptake and increases NP circulation time [62]. The PEG chains reduce the charge-based association of NPs with serum and tissue proteins, lowering aggregation phenomena. The solubility of PEG-coated NPs in buffer and serum increases due to the hydrophilic nature of PEG resulting from ethylene glycol repeats.

Moreover, due to the so-called "stealth" effect, pegylated NPs are protected from uptake by macrophages, and their circulation time is improved consequently [63]. The amine groups of PEG-modified DNPs allow anchoring carboxyl-containing biomolecules to the DNP surface through carbodiimide chemistry. Terracciano et al. conjugated the PEGylated DNPs with the CPP-peptide by carbodiimide chemistry to improve DNP cellular uptake. The CPP protein family constitutes a very heterogeneous and large group of polycationic or amphipathic peptides [64]. The CPP used in Terracciano and coworkers' study is a short peptide sequence of 16 amino acids rich in basic residues (Arg, Lys) able to interact with the negatively charged cell membrane, facilitating the internalization of cargos via constitutive endocytosis.

The hydrodynamic size (Z-average) of the obtained DNPs was 340 (8) nm with a polydispersity index (PDI) of 0.15 (0.5) and zeta ()-potential value of 40 (2) mV, which provided DNP suspension with monodispersity and stability. It was also demonstrated by nitrogen adsorption/desorption isotherm analysis that the chemical modification of the DNPs did not affect the material porosity, resulting in a specific surface area (SSA) of 23.6 (0.1) m^2/g after functionalization, which means that the DNPs could still be used for drug loading/delivery purposes [20]. Terracciano et al. tested the biocompatibility and hemocompatibility of the CPP-conjugated DNPs on breast cancer cell lines (MCF-7 and MDA-MB-231) and red blood cells (RBCs), confirming a great enhancement of DNP biocompatibility as a result of the double functionalization strategy [16]. Moreover, this study proved that PEGylation and CPP bioconjugation improved the loading/release kinetics of the poorly soluble anticancer drug Sorafenib, as well as their cellular uptake, making these nanocarriers suitable for intracellular drug delivery (Fig. 21.4).

Although the cytotoxicity of diatomite-based NPs has been demonstrated in vitro on several cancer cell lines, including colon (Caco-2, HT-29, HCT-116), human epidermoid (H1355), human breast (MCF-7, MDA-MB-231), murine A20 lymphoma, and human cervix epithelioid (HeLa) cell lines, these results represent only the first step in understanding whether diatomite can be considered a safe material for drug delivery applications. Although in vitro experimentation is the most prevalent scientific analysis used to verify NPs toxicity effects [12,65,66], positive in vitro results are not predictive of confident in vivo assays.

Recently, Terracciano and coworkers used the in vivo model cnidarian *Hydra Vulgaris* polyp, a simple water invertebrate, to understand the interaction and possible toxic effects of CPP-PEG-functionalized DNPs on a living organism [67]. *Hydra Vulgaris* represents a valuable system to study the impact and toxicological effects of nanomaterials at the level of a living organism [68]. The toxicity of a substance is conventionally measured in *Hydra* by observing the changes in the animal morphology according to Wilby's classification, in which a score of 10 corresponds to healthy polyps and decreasing scores indicate morphological alterations (Fig. 21.5A, upper panel). The morphological alterations of *Hydra* and the population growth rate, cells apoptosis, and the genotoxic effects were evaluated by Terracciano's group after exposing the polyp to 3.5 mg/mL of CPP-PEG modified DNPs for 3 days [67]. A representative in vivo image of *Hydra* after 72 h of exposure to the DNPs is reported in

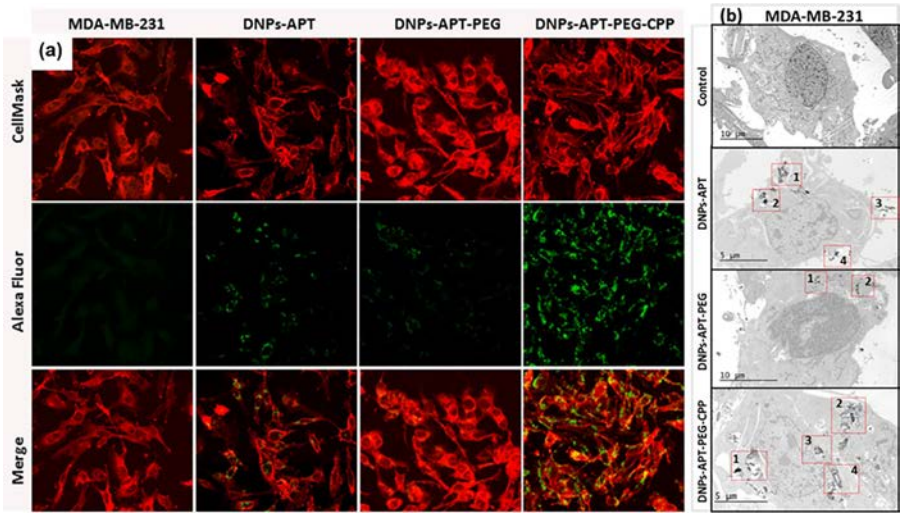

FIG. 21.4 (A) Confocal fluorescence microscopy of MDA-MB-231 cells treated with 50 μg/mL of DNPs-APT, DNPs-APT-PEG, DNPs-APT-PEG-CPP samples for 12 h at 37°C. CellMask (*red—dark gray in print version*) and Alexa Fluor-488 (green—*gray in print version*) were used to label the cell membrane and the DNPs, respectively. The merge figures are obtained by overlapping DNPs and cell membrane images, allowing to determine whether the DNPs were located outside (green color) or inside (*yellow color—light gray in print version*) the cells. (B) TEM images of MDA-MB-231 cells treated with 50 μg/mL of DNPs-APT, DNPs-APT-PEG, and DNPs-APT-PEG-CPP for 12 h at 37°C. A very small amount of APT- and PEG-modified DNPs (in dotted boxes) was found inside the cells. In the case of DNPs-APT-PEG-CPP (in dotted boxes), a considerable amount of the DNPs was observed inside the cells. Scale bars are 10 μm. *Reproduced with permission of The Royal Society of Chemistry from M. Terracciano, M.-A.A. Shahbazi, A. Correia, I. Rea, A. Lamberti, L. De Stefano, et al., Surface bioengineering of diatomite based nanovectors for efficient intracellular uptake and drug delivery, Nanoscale 7 (2015) 20063–20074. https://doi.org/10.1039/c5nr05173h.*

the bottom panel of Fig. 21.5A. The exposure of *Hydra* to bare and modified-DNPs did not induce any change in polyp morphology (score of 10 in Wilby's classification), thus confirming the DNP safety. The long-term effects of bare and modified DNPs on *Hydra* reproductive capabilities, quantified as population growth rates, were calculated and compared to untreated polyps until 14 days under a regular feeding regime. *Hydra* polyps can reproduce both sexually and asexually, and external factors, such as the presence of toxicants, pollution, or the feeding regime, can affect their reproduction rate. As shown in Fig. 21.5B, the growth rate of polyps treated with both bare and modified DNPs (3.5 mg/mL) was similar to that of untreated animals, excluding any long-term toxic effects. Moreover, the *ratio* n/n_0 (number of individuals/number of founders) at days 4 and 14 (Fig. 21.5C) did not show significant differences, suggesting that DNPs did not affect the reproductive capability of *Hydra*.

In Hydra polyps, the effect of chemical modifications to the DNP surface on cell uptake was investigated [67]. Living animals were treated with Alexa Fluor-488 labeled DNPs (modified-DNPs*) and unmodified labeled DNPs (DNPs*) for up to 48 h (Fig. 21.6). After 48 h of continuous exposure to the modified-DNPs, the strong fluorescence distribution in

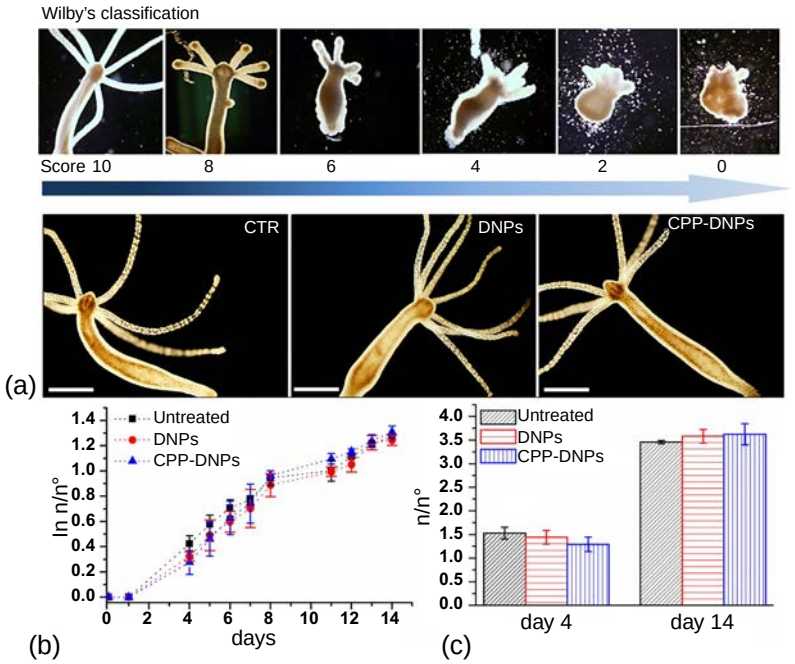

FIG. 21.5 In vivo effects of DNP on *Hydra* morphology and growth rate: (A) Upper panel: Wilby's classification of *Hydra* morphological alterations due to the exposure to a toxic environment. Lower panel: representative images of living *Hydra* polyps, untreated (CTR) and treated with bare DNPs and CPP-PEG-DNPs up to 72 h. Scale bar 500 μm. (B) The graph shows the n/n_0 values at each time point, where n is the total number of polyps and n_0 is the number of founder ones. (C) The graph shows the n/n_0 ratio (s.d.) obtained from growth curves on days 4 and 14. Error bars represent s.d. ($n = 3$). *Reproduced with permission of John Wiley and Sons from M. Terracciano, L. De Stefano, C. Tortiglione, A. Tino, I. Rea, In vivo toxicity assessment of hybrid diatomite nanovectors using hydra vulgaris as a model system, Adv. Biosyst. 3 (2019) 1–8. https://doi.org/10.1002/adbi.201800247.*

the whole body, from tentacle tips to the foot region, confirmed the nanostructure internalization in the living organism (Fig. 21.6A, 48 h). In the case of unmodified DNPs*, however, no significant cellular uptake was observed up to 48 h after treatment because DNPs accumulated on the animal's feet. Moreover, the investigations into the apoptotic rates of macerated *Hydra* cells and the gene expression analysis demonstrated the safety of this naturally derived material that is easily modifiable by low-cost chemical approaches for applications in drug delivery.

4 Drug loading and release from DNPs

The most intriguing feature of DNPs is the porous ultrastructure and biocompatibility of the silica they are made of, which make diatomite-based systems ideal carriers for drug delivery purposes [69]. Thanks to the expanding field of nanomedicine, a wide range of diatomite-based carriers have been developed for the controlled delivery of drugs, most of

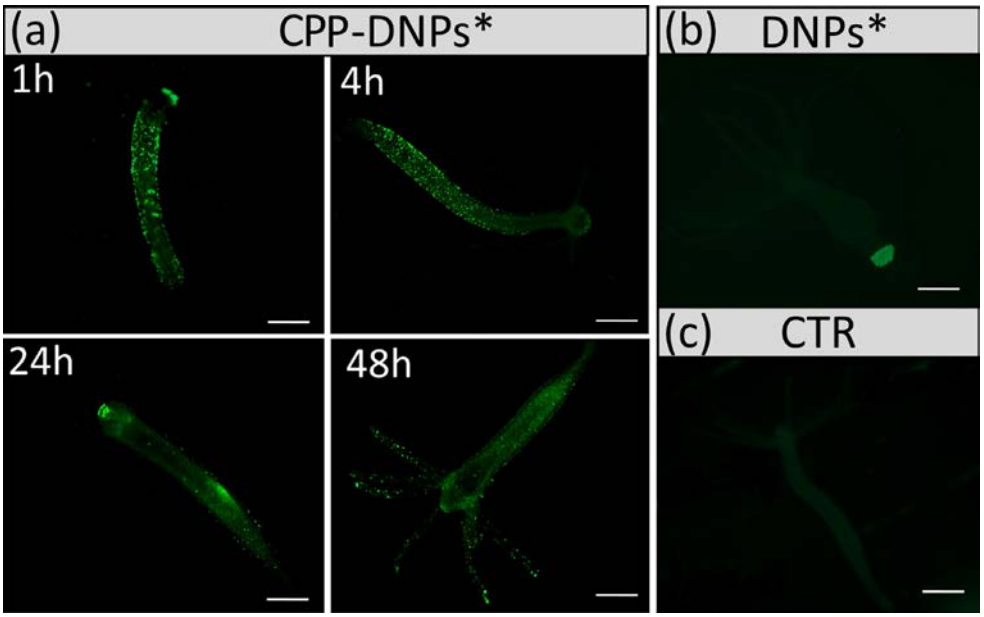

FIG. 21.6 In vivo fluorescence imaging of *Hydra* polyps treated with Alexa Fluor 488-labeled DNPs: (A) *Hydra* treated with labeled CPP-PEG-modified DNPs (modified-DNPs*) after 1, 4, 24, and 48 h of incubation; (B) *Hydra* after 48 h of treatment with Alexa Fluor 488-DNPs (DNPs*); (C) untreated *Hydra* as control (CTR). Scale bars 1 mm. *Reproduced with permission of John Wiley and Sons from M. Terracciano, L. De Stefano, C. Tortiglione, A. Tino, I. Rea, In vivo toxicity assessment of hybrid diatomite nanovectors using hydra vulgaris as a model system, Adv. Biosyst. 3 (2019) 1–8. https://doi.org/10.1002/adbi.201800247.*

which are toxic and dangerous at high concentrations. The treatment of some diseases requires long-term drug circulation in the plasma, whereas others call for a single high dose of pharmaceutical preparation [70]. Therefore, according to the urgency of the disease, the drug can be loaded into diatomite-based carriers with different methods and diverse drug release profiles can be achieved as well. The most common strategies for loading drug molecules in diatomite-based carriers are: (i) infiltration of drug molecules into the porous ultrastructure [71,72]; (ii) electrostatic interaction between the drug molecules and the carrier surface [12]; (iii) entrapment of the physisorbed drug in a polymeric matrix embedding the carrier (Fig. 21.7) [73]. Each of these methods presents both advantages and disadvantages that will be taken into consideration in this section to determine the most suitable methods for the fabrication of diatom-based drug delivery systems.

Two main factors influence the adsorption of molecules in the porous structure of NPs: the surface-to-volume *ratio* and the physicochemical properties of the NP surface. A higher surface-to-volume *ratio* means that the available area for drug adsorption is greater, and, in turn, more drug molecules can be loaded in the NP. In general, for porous drug carriers - such as diatom-based systems - smaller pores have a higher surface-to-volume *ratio*, meaning that more molecules can be accommodated in the carrier [74]. Regarding the physicochemical properties of the drug delivery system, the surface of NPs can be modified via functionalization strategies to favor either the adsorption of hydrophilic or hydrophobic

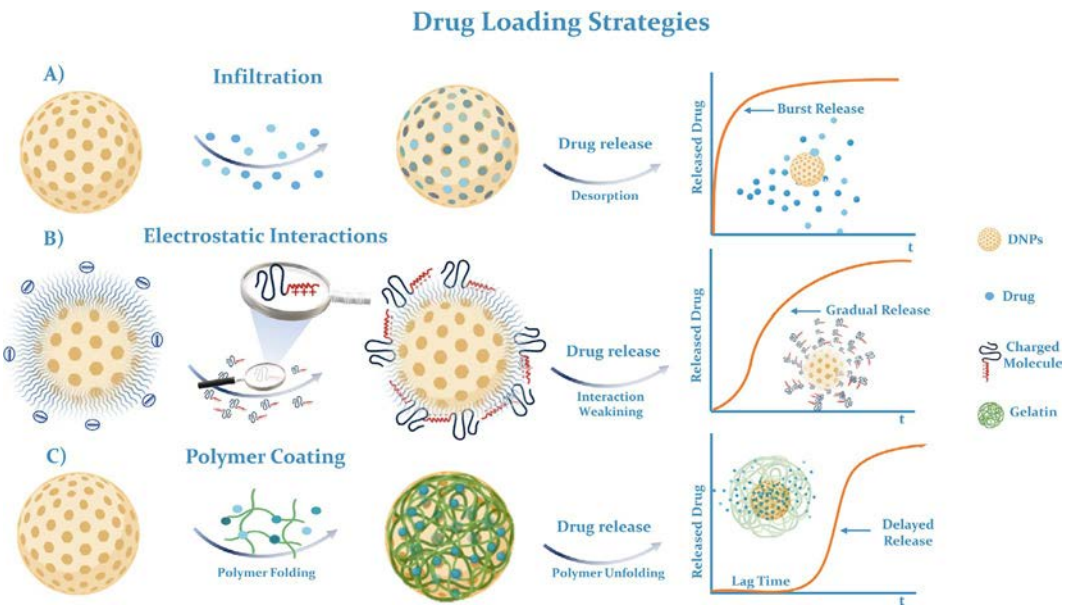

FIG. 21.7 Schematization of drug-loading strategies for diatom-based nanovectors. (A) Drug loading by infiltration method and relative drug release profile. (B) Loading of charged molecules in diatom-based carriers via electrostatic interactions and relative drug release profile. (C) Entrapment of drug molecules via the polymer folding on the nanoparticles and the expected drug release profile.

molecules [75]. The infiltration technique is preferentially performed to encapsulate drugs in diatomite frustules or microparticles (MPs) since the porous ultrastructure in the frustules is preserved better than in nanostructured particles (Fig. 21.7A) [18,76,77]. Zobi's group loaded diverse anticancer agents into diatom MPs such as the well-known Cisplatin, 5-Fluorouracil (5-FU), and in-house Ruthenium (III) complexes using the vacuum infiltration method [71,72]. This procedure presented by Vasani et al. allows the drug to penetrate inside the pores of the diatom frustules by soaking diatom MPs for days in a high-concentrated solution of the drug and then freezing and degassing the suspension to evaporate the solvent and obtain the drug-loaded MPs [78]. Although the technique can be considered time-consuming since it requires at least 1 day for molecule infiltration inside the pores, it has the advantage of allowing a successful loading degree of Cisplatin and 5-FU inside the MPs, about 6.9 and 7.3 wt% respectively. In most cases, the drug infiltration into the porous structure is promoted by the physicochemical properties of the carrier surface, including polarity and chemical reactivity [79]. For example, Delasoie et al. reported that the functionalization of diatom MPs with Vitamin B_{12} improved the drug-loading degree of 5-FU from 7.3 to 9.9 wt% thanks to the attraction between Vitamin B_{12} and drug molecules [72]. In this case, the structure of Vitamin B_{12} determined the hydrophobicity and stacking properties of the MP surface, and it promoted interaction with the aromatic ring of 5-FU. Therefore, to improve the infiltration degree of drugs with aromatic rings in porous systems, the carrier surface can be modified with hydrophobic moieties. However, even though successful drug infiltration has already been reported in diatomite-based carriers, applications of these materials have been hampered

by the burst release of the drug in solution (generally within 1 h). For molecules loaded through infiltration methods, the drug release is achieved by simply desorption in solution, due to the weak interactions between the drug and the carrier. The diffusion rate only depends on the drug concentration gradient and the surface-to-volume *ratio* of the carrier. The release is generally very fast in the early stage, and 100% of the total encapsulated drug is released within 1–2 h (burst release) (Fig. 21.7A). The initial burst release is explained by the predominant loading of the outer surface of the carrier that causes a rapid diffusion of molecules in the medium. The release of the drug from the inner cavity occurs in the second stage, and it is faster for small drugs that easily pass through the pores of diatom frustules.

Another approach for loading drugs in DNPs relies on the electrostatic attraction between the carrier surface and drug molecules (Fig. 21.7B). This loading method is generally preferred for charged molecules (i.e., DNA, RNA, miRNA, etc.) and requires modifying the carrier surface with negative or positive moieties to attract the charged drug molecules [80]. Rea et al. loaded a small interfering RNA (siRNA) into DNPs by exploiting the attractive forces between the poly-D-arginine (Arg) peptide (NH_3^+) immobilized on the surface and the siRNA (PO_4^-) negative molecules [12]. In this study, the surface of DNPs was functionalized with poly D-Arg then the nucleic acid was loaded in the carrier by electrostatic attraction between the negatively charged molecules of siRNA and the positive surface of Poly-D-Arg-modified DNPs. Differently from the infiltration method described above, the drug loading in NPs through electrostatic interactions is very fast and requires only 2 and 3 h to obtain the drug-loaded carrier. However, it can be considered more expensive than the infiltration method since it demands to modification of the DNP surface with biocompatible polymers that can be quite costly. Moreover, for molecules loaded on the outer surface of NPs through noncovalent binding, the release is often associated with a change in the solvent or environmental pH. Thus, the electrostatic forces attracting the drug and the carrier surface have the characteristics of poor selectivity and weak adsorption strength, leading to a quick and nonspecific drug release at the desired site of action. For example, when the drug is bound to the surface via hydrogen bonding, the linkage can be easily interrupted by polar molecules such as protic solvent, causing drug dispersion phenomena [81]. Although the noncovalent strategy can be considered poor-selective, it is worth mentioning that diverse approaches have been performed to strengthen the attraction between the carrier surface and drug molecules, avoiding burst release effects. Indeed, Rea et al. showed that by grafting the positive poly D-Arg peptide on the surface of DNPs, the interaction between the drug and the carrier was reinforced and, in turn, the drug release was delayed by up to 12 h [12]. Furthermore, Rea's group also showed that surface functionalization can not only improve the NP drug loading and release capacity but can also affect the carrier fate and cell uptake. To this aim, Managò et al. studied the internalization kinetics of siRNA-modified DNPs in cancer cells by Raman imaging, revealing that the modified carrier was predominantly internalized in cancer cells via endosomal vesicles within 18 h [17].

To avoid the unspecific release of chemotherapeutics, drugs can also be linked to DNPs via covalent linkages, such as ester, amide, hydrazone, or disulfide bonds. The breakage of these linkages is the first step toward drug release and may provide a solution to avoid therapeutic compound burst release [82]. Nevertheless, covalent linkages are very strong, and it is a challenge to break a covalent bond under physiological conditions. Moreover, the covalent binding of drug molecules to DNPs can also cause unintended modifications of the therapeutic

compound, compromising the drug's pharmacokinetics and pharmacodynamics [83]. For these reasons, the covalent binding of the drug on the surface of a diatom-based nanocarrier is generally not preferred.

Conversely, various intermediates, such as coating materials- can be used to mediate the loading of drugs in DNPs, avoiding burst release phenomena and providing the carrier with controlled delivery (Fig. 21.7C). The polymer-mediated drug-loading strategy does not require modifying the drug's chemical structure and can provide the carrier with external *stimuli*-responsive properties. In this regard, various approaches have been developed to coat diatom-based carriers with biocompatible polymers, including poly- or oligo-ethylene glycol [78], chitosan [84], gelatin [73,85], and polyacrylic acid matrices [86]. Among these polymers, gelatin has been extensively studied for cancer treatments since its pH-responsive feature is of specific interest for the delivery of anticancer agents to cancer cells because of the tumor extracellular acid environment [87]. Managò et al. showed that the gelatin coating on DNPs loaded with the anticancer agent Galunisertib provided the carrier with pH-responsive drug release features suitable for colorectal cancer treatments [46]. In this study, the drug was first loaded into DNPs by the physisorption method for 2 h, and then a solution of gelatin was mixed with the drug-loaded DNPs and in situ crosslinked via carbodiimide chemistry. The loading capacity of the gelatin-capped DNPs was reported to be about $2.0 \pm 0.4\%$. The cross-linked gelatin matrix on the drug-loaded DNPs acted as a gatekeeper, preventing the rapid release of Galunisertib from the carrier to the medium and allowing a sustained release over 48 h. Generally, in the case of drug-loaded DNPs coated with the polymeric matrix, the volume of the outer coating represents the main barrier to the diffusion of drug molecules in the medium: once the coating is dissolved in response to a change in the environment such as pH or temperature, the loaded drug is gradually released. This means that the drug release profile of polymer-coated DNPs exhibits a lag period (Fig. 21.4A) followed by a delayed-release with a typical sigmoidal behavior. The lag time corresponds to the time required for the matrix degradation or digestion under external *stimuli* and can be tuned according to the thickness of the polymer coating [88].

Therefore, three primary techniques for loading therapeutic compounds onto DNP-based carriers can be utilized, depending on the necessity of the treated disease. Although the infiltration method can cause uncontrolled burst release, it can be helpful in wound healing treatments, in which the burst release can offer immediate pain relief [89]. This burst release can be partially mitigated by using the electrostatic drug-loading approach, in which the release of molecules from DNPs is triggered by local changes weakening the attraction forces between the drug and carrier [90]. Finally, sustainable delivery via the polymer-coated approach can help get high concentrations of drugs at the target location and minimize the adverse effects of unspecific drug releases, which is thus of utmost importance in cancer treatment [91].

5 Conclusions and future trends

Diatomite, the fossil remains of dead diatoms, is a cheap material largely available in many areas of the world. The main properties of diatomite, such as the possibility to produce small

micro/nanoparticles, high porosity, low thermal conductivity, and chemical inertness, allowed the application of this intriguing material in several fields. To date, diatomite powder has found application in water treatment and filtration, pharmaceutic, and cosmetic fields. The biocompatibility of silica from diatoms, recently recognized as safe by the Food and Drug Administration agency, has also driven the use of diatoms as food and pharmaceutical additive products. In recent years, NPs obtained from diatomite (DNPs) have demonstrated their potential as innovative carriers for drug delivery purposes since their porous ultrastructure can load and deliver a wide range of chemotherapeutic agents. Pioneering studies have compared the advantages of DNPs obtained from natural silica with those of synthetic porous silica NPs, whose characteristics are described mainly in the scientific literature for biomedical applications. The results of these investigations revealed that DNPs, made of biosilica, could be a valid and cheap alternative to the expensive synthetic NPs, especially in developing countries where costly facilities for micro/nanofabrication are not available. The development of diatomite-based devices with multifunctional properties in both diagnostic and therapeutic fields is another aspect of paramount interest because it paves the way to personalized medicine for more efficient disease treatments. Unfortunately, diatomite-based nanodelivery systems are still embryonic devices, and their employment in nanomedicine is still hindered by the lack of knowledge of the long-term effects of diatom administration. Several issues regarding the elimination routes of DNPs, their clinical translation, and poor biodegradability are still open and need additional in vivo studies to encourage the application of diatomite in medicine. The results summarized in this chapter highlight the extraordinary potential of diatomite in nanomedicine and support the choice to focus research efforts on this field.

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