Chapter 23
Microalgae: Cultivation, Biotechnological, Environmental, and Agricultural Applications

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Abstract This chapter presents general aspects regarding microalgae biology and growth under ex situ conditions. Emphasis is given on some aspects of microalgae responses to major environmental and nutritional factors, for example, temperature, light, nutrients, and pH. Then, management of photobioreactor systems where microalgae are grown to achieve the objectives of producing high biomass and bioactive compounds for biotechnological applications is addressed. The feasibility of producing multiproducts has led to more efficient production pathways and use of materials and energy. Most of the studies about microalgae are addressed in an interrelated way with environment and agricultural applications.

Keywords Biostimulants · Bioenergy · Chlorophyll · Lipid · Microalgae supply chain · Wastewater

23.1 Introduction

Microalgae are a noteworthy photosynthetic microbial group, which grow in a wide range of aquatic environment such as freshwater, seawater, and also wastewater; they also occur in soil and plant rhizosphere microhabitat. These microorganisms present higher photosynthetic efficiency, faster growth rates, and higher yields per unit of area than terrestrial plants. From the point of view of the environmental and biotechnological applications, microalgae have an essential role due to their versatile metabolism as they have broad potential for the production of biomass under different conditions.

Microalgae have been used as food, bioingredients, and by-products that are biologically active compounds, such as polyunsaturated fatty acids (PUFAs), carotenoids, phycobiliproteins, sterols, vitamins, and polysaccharides. These byproducts have proved to have many important biological functions, making them biomaterials and bioactive products of increasing importance for a wide range of applications, from industry to agricultural activities.

Most biotechnologically relevant microalgae are the green algae (Chlorophyceae), for example, *Chlorella vulgaris*, *Haematococcus pluvialis*, *Dunaliella salina*, and the Cyanobacteria *Spirulina maxima* (*Arthrospira*), which are already widely commercialized and used mainly as food supplements for humans, as animal feed additives (Gouveia et al. 2008; Nethravathy et al. 2019), as nutraceuticals compounds (Matos 2017), as feed in aquaculture industry (Hemaiswarya et al. 2011), for cosmetic industry, and as bioenergy feedstock production (Converti et al. 2009; Yin et al. 2019).

Microalgae can be used for bioremediation of wastewater (Tonhato Junior et al. 2019; Zhu et al. 2019), reducing the concentration of polluting compounds that alter the quality of natural resources. These microalgae biomass and by-products can be

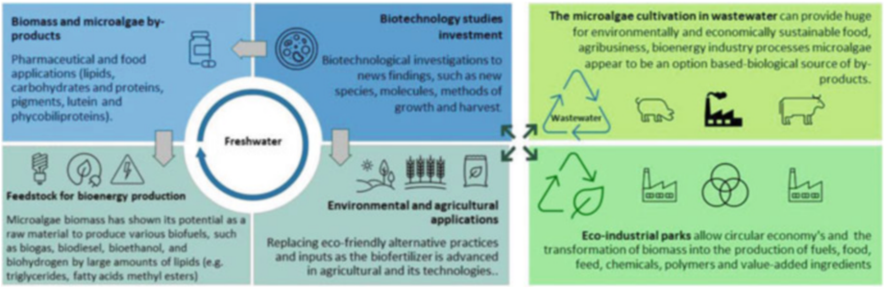


Fig. 23.1 An overview of microalgae biomass production in freshwater and wastewater

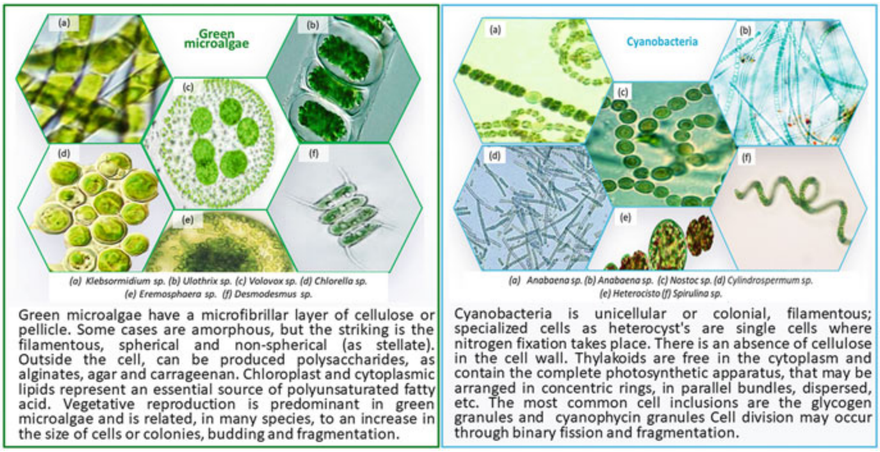
obtained from wastewater or renewable resources, reducing the environmental impact of anthropic activities (Fig. 23.1).

In this chapter, the following points are presented: (1) general aspects of microalgae biology and the nutritional and environmental factors affecting their growth; (2) production systems using photobioreactors and procedures of biomass harvesting; (3) applications of microalgae biomass as a feedstock for bioenergy and for other biotechnological purposes; and (4) microalgae cultivation in wastewater is addressed, since they reduce polluting compounds that alter the quality of natural resources and to the environmental function aiming to generate biomass that can be used for several purposes, mainly as biofertilizers in agriculture.

23.2 General Aspects of Microalgae

Microalgae are a diverse group of microscopic photosynthetic microorganisms (i.e., the prokaryotic cyanobacteria and the eukaryotic microalgae) that inhabit continental freshwater and seawater, and they are microhabitats of air, soil, and rhizosphere environments. As primary producers, they are important in food chain in these environments, and some of them are with symbiotic capacity. In applied phycology, the term microalgae refers to any microorganisms (prokaryotic or eukaryotic) with chlorophyll *a* and a thallus not differentiated into roots, stem, and leaves. The akinetes and heterocyst are the differential cellular structure that, respectively, are vegetative in chlorophyte and cyanophyte cells that accumulate oil, pigments, and other reserve substance, and also resistance spores (Fig. 23.2a, b).

These photosynthetic microorganisms include species from different phyla such as Cyanophyta (blue green algae, cyanoprokaryotes, and cyanobacteria), Chlorophyta (green algae), Rhodophyta (red algae), Cryptophyta, Haptophyta, Pyrrophyta, Streptophyta, and Heterokontophyta. In general, microalgae have different types of cell organization, for example, unicellular, colonial, and filamentous, and the main structure is thallus called "stalk," being able to be unicellular or multicellular, colonial, filamentous, or siphonaceous, and some species have centrioles, one or two flagella (Richmond 2004).



Green microalgae have a microfibrillar layer of cellulose or pellicle. Some cases are amorphous, but the striking is the filamentous, spherical and non-spherical (as stellate). Outside the cell, can be produced polysaccharides, as alginates, agar and carrageenan. Chloroplast and cytoplasmic lipids represent an essential source of polyunsaturated fatty acid. Vegetative reproduction is predominant in green microalgae and is related, in many species, to an increase in the size of cells or colonies, budding and fragmentation.

Cyanobacteria is unicellular or colonial, filamentous; specialized cells as heterocyst's are single cells where nitrogen fixation takes place. There is an absence of cellulose in the cell wall. Thylakoids are free in the cytoplasm and contain the complete photosynthetic apparatus, that may be arranged in concentric rings, in parallel bundles, dispersed, etc. The most common cell inclusions are the glycogen granules and cyanophycin granules Cell division may occur through binary fission and fragmentation.

Fig. 23.2 Diagram showing general aspects of cell morphology of (**a**) green microalgae and (**b**) cyanobacteria

The origins of applied phycology most probably date back to the establishment of a culture of *Chlorella* by Beijerinck in 1890. According to Richmond (2004), several species to genus *Chlorella* take up the first place in the commercial use of these microorganisms alike others genera belonging to Chlorophyta, green microalgae, which have great morphological variability (Fig. 23.2a).

Cyanobacteria were the first organisms to evolve oxygenic photosynthesis, and in addition, as prokaryotes, some species are N_2 fixing. In many cyanobacteria, single heterocyst develops at intervals of approximately 10–15 vegetative cells forming a one-dimensional pattern. Most filamentous cyanobacteria species present cells differentiated from vegetative cells called heterocyst, which has ability to fix atmospheric nitrogen even under N-limiting medium (Fig. 23.2b). Heterocyst development is repressed in the presence of a rich source of combined nitrogen, such as ammonium or nitrate (Adams 2000).

Biological nitrogen fixation (BNF) relies on CO_2 fixation as a source of carbon skeletons and reduced organic compounds as observed in the freshwater filamentous cyanobacterium *Anabaena oscillarioides* (Paerl and Bland 1982).

Microalgae displays a significant ecological plasticity by the ability to adapt to changing extreme environmental conditions such as temperature, light, pH, salinity, and moisture, which describes their worldwide distribution. They have different pathways to fix atmospheric carbon dioxide and to efficiently utilize the nutrients to convert it into biomass (Alwathnani and Johansen 2011; Sharma and Sharma 2017).

Microalgae belong to the fastest-growing photosynthetic organisms since their cell doubling time can be as little as a few hours. They are the highly efficient biological approach for converting CO_2 and nutrients to biomass (Sigamani et al. 2016).

23.3 Microalgal Growth

Like other microbial groups, microalgae growing is altered by the nutrients, pH, and temperature, and when under phototrophic conditions, they also depend on the light that is an essential factor for this particular group. These microorganisms are still capable of tolerating fluctuations in humidity, lighting, salinity, and nutrients (Tiwari et al. 2019), but for ex situ intensive growth, microalga depends on the balance of nutrients in the culture media according to needs for cell multiplication and by-product production and on the adjustment of light intensity and photoperiod cycles.

The lipid content of microalgae is strongly influenced by the variation of nutrients and temperature in the cultivation medium; under stress conditions, it results in high lipid accumulation but as also in low biomass productivity, overall lipid productivity is consequently lower. An increase in temperature from 20 to 25 °C resulted in significant increase in the lipid content of *Nannochloropsis oculata* (from 7.90% to 14.92%), while an increase in temperature from 25 to 30 °C brought about a decrease of the lipid content of *Chlorella vulgaris* (from 14.71% to 5.90%) (Converti et al. 2009).

23.3.1 Factors Affecting Microalgal Growth

An economical process of microalgae mass culture for oil production depends on both high biomass productivity and high lipid content in cells, which can increase or decrease under advantageous or disadvantageous nutrient such for N content in the cultivation medium. It should also be considered that variation in temperature seems to influence lipid content, and this effect depends on the microalgae species.

Several microalgae species have metabolic capacity to produce large amounts of lipids as a storage product using an inorganic carbon source and light energy, which makes microalgal biomass an attractive resource for biodiesel production and other biotechnological applications.

Microalgae biomass can be obtained through heterotrophic, autotrophic, or mixotrophic metabolism and growth conditions, but they are preferably derived from photosynthesis, and some species of Chlorophyceae, Volvocales, show an average biochemical composition: 30–50% proteins, 20–40% carbohydrate, and 8–15% of lipids under favorable environmental conditions, but under unfavorable conditions up to 80% of fatty acids, 80% of hydrocarbons, and 40% of glycerol on dry weight (Richmond 2004).

Among these environmental factors, we can point out that the light, temperature, nutrient status, and salinity not only affect photosynthesis and productivity of cell biomass but also change the cellular metabolic activity resulting broad biotechnological implications, as an example, lutein content in microalgae adapts according to pH, temperature, salinity, nitrogen availability, and mainly the specific growth rate of the cultured strain (Guedes et al. 2011) and light intensity (Coulombier et al. 2020).

As a photosynthetic microorganism, the photoperiod is an essential factor, especially in the photoautotrophic; both natural light (sunlight) and artificial light (lamps) are key areas of microorganism life development. Maintaining an adequate level of light throughout the life of cell culture or using it efficiently is a significant factor (Richmond 2004). At the high light intensity on microalgae, cells can cause photoinhibition, decreasing photosynthetic efficiency and biomass production (Borowitzka 2018; Richmond 2004); still, in the superficial part, they have a high luminosity incidence.

In the open cultivate, the sun is the light supplier for cultivation, and at night, additional artificial light can be provided to obtain better efficiency in the use of nutrients within the shortest time, besides. Light and chemical energies are vital to increasing biomass photoautotrophic and mixotrophic cultivation systems (Sipaúba-Tavares et al. 2019; Sirisansaneeyakul et al. 2011).

Photobioreactors illuminated with mixed light-emitting diode (LED) wavelength have been showing more efficient performance for microalgae growing (Che et al. 2019), mostly when the aims are to removal of pollutants such as carbonaceous organic matter, nitrogen, phosphorus, and other compounds from wastewater.

Microalgae do not distinguish between natural and artificial light, for instance, LED-illuminated photobioreactors with microalgae are a promising technology for wastewater treatment applications (Silva et al. 2020), but they are susceptible to high light intensities, as well as changes in the light/dark cycle.

Photoautotrophic microalgae productivity is limited mainly by the irregular light supply of which generates a low efficiency of energy conversion inside the cultures. Some enzymes as a photoenzyme acting on lipids show that light-driven catalysis is not restricted to the processes of light capture and use or to the repair of UV damages in DNA. Some microalgal enzymes involved in metabolic or signaling pathways are regulated by light, for instance, in *Chlorella variabilis*, a photoenzyme called fatty acid photodecarboxylase converts fatty acids to hydrocarbons (n-alkanes or n-alkenes) in response to blue light (Sorigué et al. 2017). Light intensity has a strong influence on production and activity of compounds with antioxidant capacity of microalgae as shown by 12 microalgae species that were cultivated at two light intensities (Coulombier et al. 2020).

Lipid accumulation and carbohydrate degradation of *Chlamydomonas* sp. were deferred under the light/dark when compared to the continuous light photoautotrophic cultivation condition, for instance, phosphoenolpyruvate accumulates and glycerol 3-phosphate decreases under the light/dark condition, suggesting that it was the imbalance of the metabolites which seems to be the cause of delay in the accumulation of lipids (Kato et al. 2019). Also, in this study, the metabolic dynamic profile showed higher levels of lipid/carbohydrate anabolism (as the production of 3-phosphoglycerate and acetyl-CoA) of CO_2 and the highest biomass yield in the light/dark, indicating a more significant fixation of CO_2 than in the light condition.

Temperature is a relevant factor for biomass and by-product production, independent if cultivation is in an open or closed system, although microalgae can easily withstand a wide range of temperatures; variation in their ideal temperature might result in total yield loss. Strain selection can be important for optimizing productivity, and some of the major characteristics that need to be considered are range of temperature tolerance, resistance to photoinhibition, harvest ability (trichome size), and quality (composition) of the biomass produced (Borowitzka 2018).

In a study that examined the hypothesis whether temperature alters biomass and metabolite production of microalgae according to species and even strain, Maroubo et al. (2018) concluded from long-term data collection that it is possible to choose a strain suitable for growing in each season depending on the temperature of a given region. The genetic of species has an ideal temperature range for cultivation and full crop development, depending on the location where the species was isolated. According to Metsoviti et al. (2019), both temperature and light intensity influence the growth rate, as well as the biomass production of five species *Chlorella vulgaris*, *Botryococcus braunii*, *Chlamydomonas reinhardtii*, *Euglena gracilis*, and *Nannochloropsis oculate*.

In the cultivation of microalgae, the process of homogenization and aeration is essential for a greater distribution of gases and nutrients, and with that, there is increase in the productivity of biomass and some compounds of interest. Aeration, injection of gases, into the culture medium allows movement and greater maintenance of the cells in suspension, improving the light efficiency and exchange of gases, preventing thermal stratification, aiding in the homogeneous distribution of nutrients, preventing the accumulation of organic matter at the bottom of the bioreactor, and reducing photooxidation, factors that influence the biomass productivity (Uggetti et al. 2018; Yin et al. 2019).

In order to provide microalgae cell growth ex situ, there is a need to properly balance the supply of essential nutrients, generally in liquid media in which must meet all the nutritional needs for cell synthesis and for the production of wanted metabolites of biotechnological interest. As an essential approach to reach higher microalgal biomass production, it is necessary to study the nutrient requirements to meet the specific needs of each species. The nutritional elements of more significant quantitative proportion are carbon (C), nitrogen (N), phosphorus (P), magnesium (Mg), potassium (K), and calcium (Ca). Already manganese (Mn) and cobalt (Co) favor their vital activities to a lesser extent, for example, the ratio of C:N:P in the cells of microalgae is 100:16:1 (Geider and La Roche 2002).

The hydrogen potential (pH) control can be done using buffer compounds. This factor is one of the most relevant and related to the solubility of CO_2 and availability of other chemical elements in the culture media, and, consequently, to optimal cell metabolism (Richmond 2004).

By controlling the pH for cultivation of *Nannochloropsis gaditana* at an optimum value, ranging from 8 to 9, exhibited higher CO_2 conversions in biomass, which suggests reduction in the cost of the microalgae production process using tubular photobioreactors under outdoor conditions (Moraes et al. 2020).

Microalgae as photoautotrophic microorganisms using light and CO_2 are responsible for large amount of global photosynthesis and CO_2 fixation, but these microorganisms also can grow under heterotrophic conditions by using organic compounds such as energy and C source, or also in mixotrophic metabolism, where light, CO_2, and organic substrate are simultaneously used (Sabia et al. 2015; Silva et al. 2016; Sipaúba-Tavares et al. 2019).

Overall, in microalgae culture for CO_2 sequestration, increasing CO_2 bubbling depth and keeping higher carbon concentration and higher pH can improve CO_2 absorption ratio, which will optimize the biofixation of CO_2 by microalgae furthermore (Yin et al. 2019).

Using untreated urban wastewater dominated by *Scenedesmus* sp. from the treatment plant, the addition of CO_2 resulted in an increase in biomass ranging from 66% to 100% (Uggetti et al. 2018), and for *Scenedesmus obliquus* in the culture medium, the injection of 10% CO_2 increased the biomass production, suggesting that microalgae grown at high CO_2 levels that are equivalent to those of power plant emissions can be nutritious and sustainable animal feed (Molitor et al. 2019).

Chlorella sp. cells growing in buffered medium showed that the stress of pH induces a shift in lipid metabolism from membrane lipid syntheses to storage, as in the alkaline pH had a greater accumulation of triglycerides with a decrease in the membrane lipids classes, glycolipid, and polar lipid, regardless of the content of N or carbon (Guckert and Cooksey 1990). These authors highlighted the use of suitable buffer in the growth media to avoid misinterpretation of results when studying changes of pH on biochemical and biomass production of microalgae. For instance, a great potential for the production of astaxanthin in *Haematococcus lacustris* was detected when there was the application of pH shock, which suggests some advantages, such as low cost, rapid induction, and wide applicability (Han et al. 2020).

Due to the cost of buffers when using scale-up production systems, other techniques are used to control the pH, such as pumping natural or CO_2-enriched air, contributing to stimulate growth and microalgae biomass production (Moraes et al. 2020). Also as pointed out by Galès et al. (2020) using polycultures of microalgae cultivated in outdoor raceways at high rate algal ponds, when by using control at pH 8.0, it was observed that higher biomass productivities and CO_2 use efficiencies were reducing carbon losses to the atmosphere and overall process costs. A key point for CO_2 fixation improvement in intensive cultures is control of pH that can eliminate contaminants and unwanted organisms since they are not tolerant to pH below 6.0 or 9.0, and this is a variable that can help and be useful on a large scale. At pH 8.0, using wastewater from dairy was considered as an optimum value for chemical oxygen demand (COD) removal by *C. vulgaris* (Valizadeh and Davarpanah 2020).

The key to obtain high biomass productivity and to reduce costs is recycling autotrophic and mixotrophic growth media of the microalgae, which provides a more sustainable impact on water resources; however, the presence of free fatty acids and metabolites from the oxidation of unsaturated fatty acids has inhibitory effects on microalgae cells, reducing the production of biomass (Sabia et al. 2015).

Photosynthetic carbon fixation in microalgae cell suspension can been measured in solutions with special electrodes in which is the partial pressure of carbon dioxide (pCO_2) (Richmond 2004). Increasing CO_2 bubbling depth and keeping higher carbon concentration and higher pH when growing *Scenedesmus* sp., *Porphyridium* sp., and *Dunaliella salina*, an increase in CO_2 absorption ratio was detected (Yin et al. 2019); overall, there is an interaction between environmental and nutritional factors when growing microalgae, which will optimize the biofixation of CO_2.

23.3.2 Microalgal Bioreactor Systems and Biomass Harvest

There are many systems to cultivate microalgae, including raceway pond and photobioreactors. There are a variety of open cultivation systems and different designs about the size, material, type of construction, agitation, and inclination. One more widely used photobioreactors is made up of transparent plastic bags supported by a metal structure, which can be supported or hung in this structure (Patent BR1020140215670) (Silva et al. 2014), which seems to be suitable to grow microalgae for inoculum for scale-up system cultivation as shown in Fig. 23.3a.

The open systems called as raceway pond are extensive circular ponds with the presence of a rotating arm, the lake in agitated track with rotating paddles and inclined systems forming a thin layer of cascade culture medium mirror, which has lower construction and operating costs when compared to closed photobioreactor systems. For this reason, most microalgal producers are still using this system of cultivation despite the concerns regarding the difficulties to keep an extensive system contamination-free by microorganisms and by other animals.

In general, most of the open raceways run at a depth of 20–40 cm, as the light energy must cover the entire cell mass and also allowing the aeration system and homogenization of the medium (Fig. 23.3b).

Most open systems are homogenized, especially in large-scale production, promoting the rapid circulation of microalgae cells from the dark to the light zone of the bioreactor, for example, rotating blades, as the cultivation must be mixed by moving the cell from the bottom of the tank to the top to avoid a decline in productivity because light and aeration are essential to microalgae growth (Richmond 2004).

Photobioreactors are classified as the closed tower, plate, tubes, bags, or tank reactors. There are different shapes of closed photobioreactor systems in the form of plates that are built from glass or acrylic to metal structures that have a thin layer of plastic bags or honeycomb panels with internal partitions. Constructed of glass, transparent Teflon tubes, or transparent PVC tubes, they are organized in parallel lines or helically wound around central support. The tower-shaped system consists of vertical cylinders, usually constructed of acrylic or fiberglass.

For these photobioreactors, the culture medium circulation has been carried out of air injection by a compressor and the temperature control is done by heat exchangers or evaporative cooling by spraying water on the reactor surface. Large tubular photobioreactors had the tubes floating in a large pool for temperature control; microalgae advantages cultivation system in “closed” photobioreactors which eliminates contamination. Among the techniques for sterilizing photobioreactors are the use of water vapor and the use of chemical compounds (Richmond 2004). Outdoor pilot-scale tubular photobioreactors have been used or located inside a greenhouse which controls temperature and light (Moraes et al. 2020).

Fig. 23.3 (**a**) Transparent tubular photobioreactor for the cultivation of microalgae aiming inoculum production and (**b**) open tank

Overall, microalgae grow in systems and environments that are aqueous with nutrients and aeration with O_2 or CO_2 in the appropriate proportions for each species; therefore, one of the important aspects of large-scale cultivation is the harvest that is the concentration of cells when the biomass should be removed. From the point of view of microalgae farming, harvest comprises the separation of the solid–liquid phases of the cultures, the solid part being composed of cells and the liquid part being composed of water and the other compounds, including nutrients.

For food applications, the harvested and concentrated algal biomass is to be further utilized, a product with a water content of less than 10% is required. Moisture affects the spoilage of the dried algal product by supporting the growth of bacteria, mold, and fungi (Becker 2013). By physical, chemical, or biological stages, the main stage can be performed by centrifugation, filtration, flotation, sedimentation by gravity, flocculation, and coagulation. Harvesting, which consists of separating the microalgae cells from the liquid part of the culture medium and drying process, represents a significant proportion of the production cost due to the general low concentration of biomass. Overall, the cost for harvesting and drying processes has been reported as ranging from 20% to 30% of the total value of the production cost, which presents great challenges for the commercial use of microalgae, mainly for the production of biofuels.

To select harvesting method, whether filtration, centrifugation, flocculation, or sedimentation, some factors must be considered, such as cell morphology, for example, the shape (spherical cells, in chains or filaments), the size (usually between 2 and 30μm), specific weight, area of charge surface (typically negative), and in which system and how microalgae are growing; and the cost and efficiency of the process will depend on the final application of biomass (Richmond 2004).

The recovery for harvesting biomass is crucial in the cost–benefit of producing microalgae, since the cells are relatively small varying between 2 and 30 μm in diameter, with few exceptions (e.g., *Arthrospira*), and with very low concentration of 0.1–5 g L^{-1} of dry biomass. There is no single harvesting method recognized as the best or the most suitable for all microalgae species; flocculation is a more convenient harvesting method, such as centrifugation and filtration, as allowing the treatment of large volumes of microalgae culture.

Filtration is a technique that allows to accurately determine the equivalent volume of the culture with high efficiency in separating the biomass from the culture medium. The filtration method is the operation in which a solid is separated from a liquid employing a porous medium, which retains the solid fraction and allows the liquid fraction to pass (Richmond 2004). The filter medium can be composed of paper, fabric, or other porous solid, such as ceramic or a thin layer of sand. However, in large-scale cultivation, the separation of large quantities of microalgae is only viable if the species has large cells or filamentous structure since small cells cause the rapid clogging of any significant volume filtration system.

Centrifugation is a practical and straightforward method of harvesting cells and can be performed without adding chemicals, preserving the original characteristics of the biomass. However, on a commercial scale, sometimes this is not feasible due to the high energy expenditure for operating the system, the difficulty in processing large volumes of cultivation, and the need for high investments in the acquisition of large equipment.

To address this challenge, flocculation has been identified as a low cost and promising technique. To reduce harvesting costs, some flocculation methods are being explored, including auto-flocculation with titanium dioxide (TiO_2) plus intense pulsed light as reported for *Tribonema* sp. and *Synechocystis* sp. cultivated together in swine wastewater (Cheng et al. 2020) by bio-flocculation with bacteria and filamentous fungi for *Chlorella pyrenoidosa* (Jiang et al. 2020). There is no single harvesting method recognized as the best or the most suitable for all microalgae species; flocculation is a more convenient harvesting method, such as centrifugation and filtration, as allowing the treatment of large volumes of microalgae culture. A wide variety of chemicals are studied as a flocculating agent for microalgae as it was emphasized in a review on flocculation methodologies (Li et al. 2020). Flocculation consists of removing the cells' ability to remain in suspension or stimulating aggregation to form flakes that can settle or float (Fig. 23.4).

By using nonstarch-based cationic polymer as flocculant for harvesting *Chlorella* sp., *Micractinium* sp., and *Scenedesmus* sp., the obtained efficiency ranged from 96% to 97% at an optimized dosage (Kumar et al. 2019b). Also, there are other studies comparing different bio-based organic polymers flocculants and also doses, for example, for *N. oculata*, the biomass harvesting efficiency of flocculation when using cationic cellulose nanocrystals was 90% and when using chitosan was >95% (Verfaillie et al. 2020), and the values range from 85% to 95% according to doses of two cationic polymers (Vu et al. 2020), and there is the coagulation–flocculation by alkaline pH induction (Ajala and Alexander 2020).

The advantages of flocculation methods using organic polymers are related to the low cost of operation and high efficiency (>90%) because chemicals are not used to

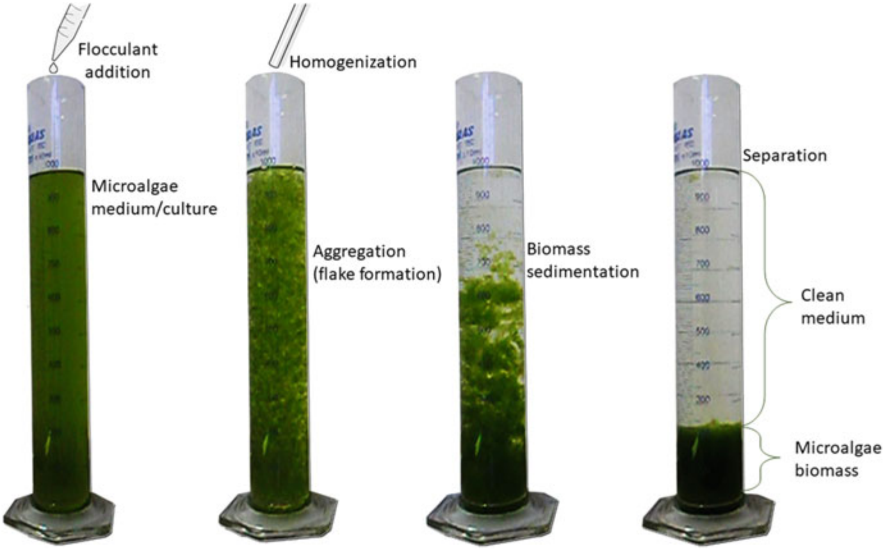


Fig. 23.4 Illustrations on flocculation of microalgal cells and sedimentation process using flocculants. Modified from Silva et al. (2014)

concentrate the biomass, which avoids the use of anions based on chlorine or sulfate (Mubarak et al. 2019).

In a study of coagulation/flocculation treatment of brewery wastewater using flocculant based of vegetable tannin showed to be efficient (Tonhato Junior et al. 2019). These authors point out that the flocculation adds the cost 0.335 per kg of dry biomass recovered and cost of flocculant per volume of treated effluent of U of US 0.13 m^{-3}. It achieved approximately 98% efficiency in nutrient removal from a municipal wastewater, when *Chlorella vulgaris* was cultivated for 30 days in a laboratory scale hybrid process by combining an aerobic membrane bioreactor with a membrane microalgal reactor and using flocculation cationic polyacrylamide polymers to harvest (Nguyen et al. 2020).

23.4 Microalgal Biomass and By-Products: Pharmaceuticals and Food Applications

One of the major current challenges for the planet is to provide enough food for its population. As predictions of the world population will have increased by another 2 billion by 2050, current estimations have indicated that sufficient water and arable land are not available to support such demand (Smithers 2016). Microalgae (including cyanobacteria) are promising organisms for sustainable products for use as feedstocks for food, feed, fine chemicals, biofuels, and agro-industrial. They can synthesize a broad range of products with high-value market price such as polysaccharide, single-cell protein (SCP), carotenoids and phycobilin pigments, and long-chain polyunsaturated fatty acids. These products are commercialized in the food industry as dietary supplements and functional foods, in the pharmaceutical and chemical industries as cosmeceuticals and flavorings, and in the therapeutic field as nutraceutical compounds (Matos 2017).

The addition of microalgal biomass to food products is an interesting tool for providing nutritional supplementation with biologically active compounds (e.g., antioxidants, PUFA-ω3) besides coloring purposes. Accordingly, the selection of microalgae species with balanced nutritional profiles is fundamental for successful novel food development. A detailed physicochemical characterization of the microalgae is an essential stage that will allow determining which algae are best suited for different applications and purposes (Batista et al. 2013).

Some eukaryotic microalgae species produce a hug diversity of compounds that are widely studied for their bioactivities in the fields of cosmetics and nutrition especially to prevent overweight, including two molecular families, omega-3 long-chain polyunsaturated fatty acids (PUFAs) and carotenoids that comprise two major subfamilies, carotenes and xanthophylls (Delbrut et al. 2018; Sathasivam and Ki 2018).

Microalgae by-products are significant source of fine chemicals, such as natural pigments, carotenoids, vitamins, proteins, fatty acids, sterols, among other biologically active compounds, presenting potential benefits for human and animal health (Gouveia et al. 2008; Soares et al. 2019) and polysaccharides (Vishwakarma and Sirisha 2020).

23.4.1 Enzymes, Polysaccharides, and Proteins

Enzymes are essential components of biological reactions and play important roles in the scaling and optimization of many industrial processes. Due to the growing commercial demand for new and more efficient enzymes to help further optimize these processes, many studies are now focusing their attention on more renewable and environmentally sustainable sources for the production of these enzymes. Microalgae are very promising from this perspective since they can be cultivated in photobioreactors, allowing the production of high biomass levels in a cost-efficient manner. This is reflected in the increased number of publications in this area, especially in the use of microalgae as a source of novel enzymes (Vingiani et al. 2019).

Enzymes for healthcare applications can include L-asparaginase. Paul (1982) first purified the L-asparaginase in *Chlamydomonas* spp. with limited anticancer activity and tested it in an in vivo anti-lymphoma assay. Ebrahiminezhad et al. (2014) screened 40 microalgal isolates via activity assays and reported that *C. vulgaris* was a potential feedstock for L-asparaginase production. There are other microalgal enzymes involved in the synthesis of bioactive compounds; some studies have focused on polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPSs). PKSs produce polyketides, while NRPSs produce nonribosomal peptides. Polyketides and nonribosomal peptides have been reported to have antipredator, allelopathic, anticancer, and antifungal activities (Kohli et al. 2016). PKS can be multidomain enzymes (Type I PKS), large enzyme complexes (Type II), or homodimeric complexes (Type III). Genes potentially encoding these first two types of PKSs have been identified in several microalgae (e.g., *Amphidinium carterae*, *Gambierdiscus* spp., *Karenia brevis* in cyanobacteria, for instance, *Anabaena* sp. PCC 7120, *Nostoc punctiforme*, *Gloeobacter violaceus*, *Crocosphaera watsonii*, and *Anabaena variabilis*) (Jenke-Kodama et al. 2005), also in *Azadinium spinosum* (Meyer et al. 2015), in *Gambierdiscus excentricus* and *Gambierdiscus polynesiensis* (Kohli et al. 2017), and in *Amphidinium carterae* (Lauritano et al. 2017).

Enzymes for bioremediation can be as follows: (a) enzymes directly used for the degradation of toxicant compounds to less or nontoxic versions (e.g., the hexavalent chromium is converted to the less toxic trivalent chromium due to the activity of chromium reductase); and (b) enzymes involved in cellular stress response mechanisms such as peroxidases (Px), superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR). SOD, Px, and CAT typically function in helping detoxify the cell from oxygen reactive species, while GR replenishes bioavailable glutathione, catalyzing the reduction of glutathione disulfide (GSSG) to the sulfhydryl form (GSH) (Vingiani et al. 2019).

Reactive oxygen species (ROSs) are generated in aerobic organism as result of respiration and substrate oxidation. Environmental stresses such as intense light, heavy metals, herbicides, UV radiation, high salt concentrations, and extreme temperatures stimulate ROS production. Consequently, microalgae possess antioxidant defense mechanisms that combat ROS cell damage. Enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione reductase, catalase, and peroxidase (Mallick and Mohn 2000). Superoxide dismutase (EC 1.15.1.1) is a metalloenzyme that converts superoxide radicals (O_2) into oxygen (O_2) and hydrogen peroxide (H_2O_2). The applications of SOD include therapeutic and prophylactic applications in humans, in the preservation of biological materials (organs for transplantation and sperm), in the preservation of perishable materials such as foodstuffs and vaccination agents, and as an antigenic agent for the serodiagnosis of pathogens (Bafana et al. 2011). By comparing SOD activities in three cyanobacteria, Gunes et al. (2015) found that the maximum specific activities in *Synechococcus nidulans*, *Arthrospira platensis*, and *Pseudanabaena* sp. were 50.4, 30.0, and 18.4 U mg^{-1} of protein, respectively. Because SOD is a promising and potent antioxidant enzyme, future studies should evaluate SOD synthesis in microalgae.

The presence of the enzymes in microalgae has important biological properties. In a study in silico, it was described in different microalgal classes that the enzymes, such as monogalactosyldiacylglycerols and sulfoquinovosyldiacylglycerols, maintaining in conserved domains, could be effectively involved in the synthesis of compounds with recognized anticancer and immune-modulatory activities (Riccio et al. 2020). Other compounds with antioxidant activity are also produced by microalgae, for example, *Pediastrum boryanum* that showed ability to produce free phenolic compounds with potential antioxidant activity in vitro (Corrêa da Silva et al. 2020).

Polysaccharides are widely used in the food industry primarily as gelling and/or thickening agents. Beta-1,3-glucan, a natural soluble fiber active as immune-stimulator, antioxidant, and reducer of blood cholesterol, has to be mentioned, which is accessible from the cultivation of *Chlorella* strains (Spolaore et al. 2006). In addition to the therapeutic use, this carbohydrate can be implemented in food and beverage manufacturing, mainly as fat substitute for texturizing. It is possible to add beta-1,3-glucan to novel food products such as functional beverage, functional bread, ready-to-serve soups, functional snack foods and a variety of sauces, creamers, bakery products, and additional food products (Ahmad et al. 2012). It has to be emphasized that beta-1,3-glucan displays a considerably higher market value if compared with other algal carbohydrates that are of importance for technical applications, such as the gelling or thickening compound agar (produced by macroalgae belonging to the Rhodophyta group), alginates, cellulose, or carrageenan that is used as emulsifier and stabilizer in various food products. Carrageenan, also known as food-additive E407, can similarly be implemented for pharmaceutical applications (Koller et al. 2014).

Comprehensive analyses and nutritional studies have shown that microalgae proteins are of high quality and comparable to conventional vegetable proteins. The protein content of microalgae can be in the range between 6% and 71% depending on the species (Becker 2007; Nethravathy et al. 2019). The content of standard amino acids in almost all microalgae is favorable compared to that of the WHO/FAO reference and other food proteins such as soy and egg (Richmond 2004). Microalgae can synthesize high protein content, for example, *Spirulina platensis* (60–65%) and *C. vulgaris* (51–58%) of dry matter, and this outstanding capacity has been one of the main reasons to consider these organism as a source of proteins (Henrikson 2010).

Since protein is one of the most valuable algal components, four important parameters of protein quality are used to determine the appropriate nutritive value of algal protein, that is, protein efficiency ratio (PER), biological value (BV), digestibility coefficient (DC) or true digestibility, and net protein utilization (NPU). The nutritive value of the alga-protein depends on the type of postharvesting process, and most of the microalgae have relatively thick cell wall, which makes improperly treated algal biomass indigestible for humans (Becker 2013).

23.4.2 Chlorophylls, Carotenoids, Lutein, and Phycobiliproteins

There is a growing interest in the field of biotechnology for obtaining nonvegetable sources of dyes. The use of natural pigment production by biotechnology involving microalgae has advantages such as continuous cultivation and rapid multiplication of these microorganisms, which can guarantee such productivity for the process. A large number of pigments associated with light occurrence are found in microalgae. Expect chlorophyll as primary photosynthetic compound, the important ones are carotenoids and phycobiliproteins. Similar to what occurs in other organisms, each class of microalgae has its own combination of natural pigments and, consequently, different coloring. Carotenoids extracted from microalgae have various applications in market: β-carotene from *Dunaliella* as vitamin supplement in health foods; lutein, zeaxanthin, and canthaxanthin for pharmaceutical uses and chicken skin coloration; and astaxanthin for aquaculture to provide natural red color for some fishes like salmon, extracted from *Haematococcus*. The phycobiliproteins like phycocyanin and phycoerythrin which are unique in algae are already in use as food and cosmetic applications (Pulz and Gross 2004).

According to Borowitzka (2013), it is possible to increase the synthesis of these bioactive compounds through the manipulation of cultivation conditions, usually due to some environmental stress. New microalgal bio-products from microalgae are being produced, and they are being developed for the scale-up production of health foods from *Chlorella* and *Arthrospira* (*Spirulina*), valuable fine chemicals such as β-carotene from *Dunaliella salina*, astaxanthin from *Haematococcus pluvialis*, and long-chain polyunsaturated fatty acids from *Crypthecodinium cohnii* and *Schizochytrium* (Borowitzka 2018).

Chlorophylls stand out among the most well-known pigments being responsible for capturing sunlight and producing oxygen and sugar through photosynthesis. Chlorophyll is registered and approved as a colorant additive (E140) and is mostly used in the food pigmentation and dietary supplement industries. Famous “chefs de cuisine” use chlorophyll to provide a green coloring to foodstuffs and beverages, such as pasta, pesto, and absinthe (Koller et al. 2014).

In general, most chlorophylls available on the market are in the form of derivative sodium copper chlorophyllin, which makes these structural changes favorable to convert fat-soluble chlorophyll into a water-soluble compound, and this derivative molecule (chlorophyllin) has shown antimutagenic effects to various polycyclic procarcinogens such as aflatoxin-B1, polycyclic aromatic hydrocarbons, and some heterocyclic amines, demonstrating potential chemopreventive agent (Coates et al. 2013).

Carotenoids are primarily a major class of fat-soluble pigments and antioxidants, and the intake of some carotenoids is associated with lowered risk of diseases through their involvement in cell signaling pathways (Stahl and Sies 2005). In the case of β-carotene, due to its antioxidant activity and the nutritional value as pro-vitamin A (Grune et al. 2010), it has been widely applied in food products and cosmetics (Edge et al. 1997). Several microalgal species can accumulate a high concentration of β-carotene, astaxanthin, or canthaxanthin, for example, which have a wide application as natural dyes and antioxidants (Pulz and Gross 2004).

Carotenoids display so-called secondary light harvesting pigments, supporting the "primary pigment" chlorophyll in capturing light energy. They also act as antioxidants that inactivate reactive oxygen species (ROS) formed by exposure to excessive solar radiation. However, in a review, Gong and Bassi (2016) argued that a better understanding of suitable and economically feasible biotechnological strategies for carotenoids from microalgae is needed.

The industrial interest in these natural pigments can be explained by the ability attributed to them to prevent degenerative diseases: combating free radicals and functioning as anticancer agents and stimulators of the immune system (Orosa et al. 2000; Pangestuti and Kim 2011). Compared to synthetic dyes, they are more resistant to the presence of ascorbic acid, to heat, and to freezing processes, and they are efficient even when applied to food in small quantities (Skulberg 2004). The strict regulation for the application of synthetic dyes in the food industry stimulates research aimed at the productive development and the use of microalgal carotenoids as a food additive (Del Campo et al. 2000).

Microalgal species like *Chlorella zofingiensis*, *Spirulina platensis*, and *Caulerpa taxifolia* are known to synthesize β-carotene at an average yield of 0.1% and 2% of their dry biomass weight (Rammuni et al. 2019). However, the halophilic green biflagellate *Dunaliella salina*, which accumulates up to 13% of β-carotene on its dry biomass, is the predominant source for commercial production of natural β-carotene (Rammuni et al. 2019). In fact, the first high-value product commercially produced from microalgae was β-carotene from *D. salina*. In contrast to synthetic β-carotene, which is limited to its all-trans isomer, natural β-carotene consists of a mixture of cis-trans isomers (9-cis-β-carotene isomer) which shows higher bioavailability, thus considered as a superior product (Raja et al. 2007). Natural β-carotene finds application as a food colorant to enhance appearance and consumer acceptability of products like margarine, cheese, fruit juices, baked goods, dairy products, canned foods, and others (Begum et al. 2016). Global market for carotenoids such as overall is a reality in some countries, but still it is being a potential field in demand in most of them.

Lutein (b,ε-carotene-3,3′-diol) is a naturally occurring pigment belonging to the xanthophyll division of carotenoids. The role of this compound in human health and in particular visual function (lutein is accumulated in the macula) is well established from epidemiological, clinical, and interventional studies (Abdel-Aal et al. 2013). Astaxanthin and β-carotene have been well recognized in prevention and treatment of various diseases. Also, there is an evidence that lutein may have biological effects that include anti-inflammatory and antioxidant properties and play a role in cognitive function (Johnson 2014).

Studies on astaxanthin esters, *cis* and *trans* forms of carotenoids, lutein, and fucoxanthin in vitro and in vivo models are essential for the development in biotechnological applications. *Dunaliella*, *Muriellopsis*, *Scenedesmus*, and *Chlorella* accumulate high lutein content, which varies between 3.4 and 7.6 mg g^{-1}

dry weight of biomass (Fernández-Sevilla et al. 2010). Ambati et al. (2019) discussed some studies that reported the major carotenoid pigments from microalgae with commercial values, such as astaxanthin and astaxanthin esters in *H. pluvialis*, *Chlorococcum* spp., and *Chlorella* spp.; β-carotene in *D. salina*, *S. platensis*, and *Scenedesmus* spp.; lutein in *B. braunii*; canthaxanthin in *Nannochloropsis* spp.; and fucoxanthin from diatoms. These carotenoids have high demand in the global market for health food applications. In the European Union, plant origin lutein is allowed as a food and feed additive and finds applications as a color enhancer of poultry products. In 2015, the global market of lutein was estimated at 135 million US$, with a predicted annual growth rate of 5.3% until 2024 (Hu et al. 2018).

Most of the light energy used by any photosynthetic organism is absorbed by a collection of accessory pigments, since chlorophyll absorbs light energy only in a limited region of the solar spectrum. Phycobiliproteins are a hydrophilic family of pigments of a protein nature, which is soluble in water and functions as accessory pigments of the photosynthetic apparatus in cyanobacteria and in various groups of eukaryotic algae. More specifically, they have antennas of light-collecting pigments and have chromophores called bilins. Phycobiliproteins are classified by the three main pigments or chromophores depending on the color and the absorbance properties: phycoerythrin (red), phycocyanin (bright blue), and allophycocyanin (green-blue) (Matos 2017).

Natural pigments, among their various functions in the food, pharmaceutical, and biochemical areas, have antioxidant activity. Microalgae are photoautotrophic organisms that are exposed to high rates of oxygen and radical stress and, consequently, have developed several efficient protection systems against reactive oxygen species and free radicals. The content and type of antioxidant compounds depend on the microalgae species and their growing conditions (Pulz and Gross 2004).

In addition to carotenoids and other bioactive compounds, microalgae lipids have gained attention not only due to their potential applications in many areas but also as great source of essential polyunsaturated fatty acids, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In addition to the interest in the production of biofuels, long chains of omega-3 polyunsaturated fatty acids (PUFAs) are valuable lipids produced from microalgae, which cannot be synthesized by higher plants or animals, and they are widely used as nutritional supplements (Matos 2017), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have attention due to their bioactivities. The production of DHA from microalgae has already been commercially exploited (Vingiani et al. 2019), contributing for the feasibility of the microalgae supply chain (Andrade et al. 2020b).

As for the health benefits of EPA and docosahexaenoic acid DHA, both compounds have been well recognized for the prevention of cardiovascular diseases by alleviating adipose tissue inflammation and insulin resistance (Kalupahana et al. 2011). Also, EPA- and DHA-derived lipids such as oxylipins have played an extremely important role in the resolution of inflammation. For instance, PUFAs produced in microalgae can relieve inflammatory bowel disease (IBD) symptoms when consumed in diet (de los Reyes et al. 2014).

23.5 Feedstock for Bioenergy Production

In the past few decades, the use of fossil fuels has significantly increased greenhouse gas emissions. These problems have aroused interest in the search for cleaner energy production to help environmental conservation, with biofuels being a great alternative in solving these problems (Gavilanes et al. 2017). The most important advantages of biofuels lie in the fact that their physical properties and combustion characteristics are very similar to those of fossil fuels and, therefore, could be used without any significant modification of the existing infrastructure for storage, transport, and combustion. Also, all forms of renewable energy have the exceptional merit of being sustainable, potentially CO_2 neutral, and of low or zero air pollution (Peng et al. 2020).

First-generation biofuels are derived from edible raw materials such as wheat, palm, corn, soybeans, sugar cane, rapeseed, oilseeds, beets, and corn. In contrast, second-generation biofuels use dedicated lignocellulosic materials and waste, such as raw materials: jatropha and grass. One of the main disadvantages of first- and second-generation biofuels is that the cultivation of these food or nonfood crops for the production of biofuels will compete for limited arable land, which should be used to grow crops for food production. Microalgae biofuels, known as third-generation biofuels, are treated as a technically viable alternative energy solution that overcomes the main disadvantages related to the first and second generations (Noraini et al. 2014).

Compared to first- and second-generation biofuels, microalgae biofuels offer many more advantages in addition to lipid yields, such as high growth rate, high-efficiency CO_2 mitigation, do not compete with land-based food crops, less water demand than terrestrial crops, tolerance to wastewater during cultivation, use of low-quality land and water, integration with point sources of carbon dioxide, such as coal plants, and more economical agriculture (Bennion et al. 2015; Bharadwaj et al. 2020; Noraini et al. 2014).

Microalgae biomass has shown its potential as a raw material for the production of various biofuels, such as biogas, biodiesel, bioethanol, and biohydrogen. At the current stage of biofuel development, it is still too early to indicate which would be the most beneficial route for the production of biofuels from algae biomass. However, anaerobic digestion appears to be the least complex of all; besides, it can play an important source of energy combined with other biofuel production. In this context, the specific characteristic of the strain of the selected microalgae is essential (González-Fernández et al. 2012).

23.5.1 Biogas, Biodiesel, Biohydrogen, and Bioethanol

Biogas

The generation of biogas is a biochemical process with cooperative action by multiple microorganisms, involving several mandatory or optional anaerobic microorganisms. Microorganisms play a decisive role in the efficiency of biogas production (Deng et al. 2020). The composition of biogas can vary according to the type of raw material, and the operating conditions of the digester contain from 50% to 75% CH_4 and 25% to 50% CO_2, together with other components, such as water vapor (H_2O), hydrogen sulfide (H_2S), and ammonia (NH_3) (Andrade et al. 2020a)

In comparison to the production of biodiesel, relatively few studies have been published on the anaerobic digestion of microalgae; although it was already studied in the 1960s, the hype of microalgae in recent years has revived the research on methane production. It is essential to mention that anaerobic digestion is a more direct process of energy production, as it does not need an intense concentration of cells, drying, and oil extraction that increases production costs (Ayala-Parra et al. 2017), making more financially feasible its use in biogas generation.

The methane yield of anaerobic digestion of microalgae can be achieved in several stages, including cultivation, harvesting, pretreatment, and, finally, some pretreatment techniques for microalgae before biodigestion. However, due to the wide variation in the composition of several microalgae species, the potential of methane also varies significantly between species (Table 23.1), which must be considered before selecting any strain as a methane producer. In addition, several other factors, mainly process parameters, significantly affect the throughput and efficiency of the overall process (Chu and Phang 2019).

Factors that must be taken into account in the process of anaerobic digestion are the pH and temperature of the substrate, which play a crucial role in the production of methane gas. Alkaline conditions are more suitable for the generation of biogas by microalgae since alkaline conditions can increase the solubility of the biogas CO_2 remaining in the form of dissolved carbonate generating a highly pure biogas (Chu and Phang 2019).

From different species of microalgae found in Table 23.1, it is shown that the methane gas content varied from 40% to 78.6%, with the majority of the research being carried out with a batch-type bioreactor with retention varying between 2.2 and 64 days.

Microalgae, in addition to the isolated effect for the generation of biogas, may have an effect accompanied by other microorganisms such as endophytic bacteria. In one study, it was demonstrated that the cocultivation of the microalgae *Chlorella vulgaris* with endophytic bacteria resulted in higher removal of nutrients and CO_2 than the monoculture of microalgae, besides efficiency in removing the chemical oxygen demand showing essential implications for improving wastewater purification and biogas (Xu et al. 2020).

Another alternative is the cocultivation of microalgae with fungi, with excellent results in the generation of methane gas and the treatment of wastewater (Muradov

Table 23.1 Microalgae species used in anaerobic biodigestion of biomass

Microalgae species	Study domain/emphasis	Reactor type	Temp.	HRT	CH_4 %	References
Chlamydomonas reinhardtii and *Chlorella vulgaris*	Enhanced methane production of *Chlorella vulgaris* and *Chlamydomonas reinhardtii* by hydrolytic enzymes addition	Batch	35	22	72–75	Mahdy et al. (2014)
Chlamydomonas reinhardtii, *Chlorella kessleri*, *Dunaliella salina*, *Euglena gracilis*, and *Scenedesmus obliquus*	Microalgae as substrates for fermentative biogas production in a combined biorefinery concept	Batch	38	32	66–67	Mussgnug et al. (2010)
Chlamydomonas reinhardtii and *Pseudokirchneriella subcapitata*	Sunlight to biogas energy conversion system	Batch, semicontinuous	34–41	2.5	40–65	De Schamphelaire and Verstraete (2009)
Scenedesmus sp. and *Chlorella* sp.	The superiority of co-digestion as a step toward maximizing methane production from microalgae	Batch	35	40	–	Zhen et al. (2016)
Chlorella sorokiniana	Anaerobic digestion of residual algal biomass	Batch	30	42	–	Ayala-Parra et al. (2017)
Chlorella vulgaris	Anaerobic digestion of *Chlorella vulgaris* for energy production	Batch	28–31	64	67.8–75.3	Sánchez and Travieso (1993)
Dunaliella tertiolecta	Methane production	Batch	37	49		Lakaniemi et al. (2011)
Nannochloropsis sp., *Nanofrustulum* sp., and *Phaeodactylum tricornutum*	Efficient anaerobic digestion of whole microalgae biomass and lipid-	Batch	35	30	64.81–69.56	Zhao et al. (2014)

(continued)

Table 23.1 (continued)

Microalgae species	Study domain/emphasis	Reactor type	Temp.	HRT	CH_4 %	References
	extracted microalgae residues for methane energy production					
Nannochloropsis salina (lipid-extracted biomass)	Thermal pretreatment on anaerobic digestion of *biomass*	Batch	40	49	–	Schwede et al. (2013)
Scenedesmus obliquus	Anaerobic digestibility of *Scenedesmus obliquus* under mesophilic and thermophilic conditions	Hybrid flowthrough anaerobic reactor	54	2.2–22.3	77.1–78.6	Zamalloa et al. (2012)
Tetraselmis	Biomethanation of *Tetraselmis*	Completely stirred tank reactor	35	14	72–74	Di et al. (2007)
Isochrysis galbana	Biogas production from dry and wet lipid extraction	Batch	38	30	–	Sánchez-Bayo et al. (2020)

et al. 2015). Cocultivation between *Chlorella vulgaris* and the fungus *Ganoderma lucidum* resulted in 64.92% CH_4 and 35.08% CO_2, with the removal of the chemical oxygen demand, total nitrogen, and total phosphorus of 86% (Wang et al. 2017).

The integration of the microalgae cultivation process to remove CO_2 from biogas and treat wastewater is a promising strategy for the economic viability of producing microalgae-based biofuels (Srinuanpan et al. 2020). The cocultivation of microalgae with other microorganisms and their applications have great potential in the generation of biogas and wastewater treatment to reduce contamination.

Biodiesel

All biodiesels have the same renewable and primary origin; they are produced from the photosynthetic conversion of solar energy into chemical energy, which makes them isolated from the initial photosynthesis. According to the American Society of Testing and Materials (ASTM), the term biodiesel is attributed to monoalkyl esters of long-chain fatty acids resulting from edible oils, nonedible oils, and used oils, produced from the process of transesterification of triglycerides using methanol and catalyst (Singh et al. 2020a) thus producing biodiesel and glycerin (soap) (Chua et al. 2020).

Microalgae have been identified as the most promising third-generation raw material with great potential for the production of biodiesel since its cultivation requires less cultivated land compared to conventional oilseeds and the high growth rate of microalgae (Goh et al. 2019; Yin et al. 2020). Its lipid content varies according to the different species of algae and growth periods, generally between 20% and 50% of the biomass, and reaches 70% under certain culture conditions. Lipid productivity instead of lipid content is generally accepted as an indicator for assessing the performance of microalgae in oil production. Lipid content is the concentration of lipids in the microalgae cells, regardless of biomass production, and lipid productivity depends on the production of biomass and refers to the accumulation of lipids in the cells in the total biomass produced (Xue et al. 2020). Under normal cultivation conditions, it can reach from 1.9% to 54% by weight of the lipid content, and in species under conditions of lack of nitrogen, it reaches between 18.42% and 64% (Goh et al. 2019).

The lipid content of microalgae biomass can vary between 2% and 41% of dry weight (Gouveia et al. 2008). It represents a very diverse group of compounds that have essential biological functions, such as the formation of structural components of cell membranes, serving as a source of energy and storage, and participation in metabolic pathways.

Microalgae cells are known to accumulate large amounts of lipids, with triglycerides (TAGs) which can be converted into fatty acids methyl esters (FAME) that can be used as feedstock for biodiesel production (Soares et al. 2019) and polyunsaturated fatty acids (PUFA) being the most studied from a biotechnological application (Bellou et al. 2014; Khan et al. 2018). Algae lipids are composed of polar and nonpolar lipids. Polar lipids are produced in the growth phase and are enriched in the chloroplast and cell membrane system (Guckert and Cooksey 1990).

The most frequently studied enzyme involved in lipid synthesis is acyl-CoA diacylglycerol acyltransferase (DGAT), which is involved in the final reaction of the TAG biosynthetic pathway (Merchant et al. 2012; Xu et al. 2018). Three independent groups of enzymes, referred to as acyl-CoA diacylglycerol acyltransferases types 1, 2, and 3 (DGATs 1-2-3), take part in the acyl-CoA-dependent formation of TAGs, which has been analyzed in different microalgae, for instance, in *Ostreococcus tauri* (Wagner et al. 2010), *Chlorella ellipsoidea* (Guo et al. 2017), and *Nannochloropsis oceanica* (Wei et al. 2017).

Different isoforms of DGAT2 (NoDGAT2A, 2C, 2D) have successively been identified in *N. oceanica*, and different combinations of either overexpression or under expression have been analyzed. These combinations gave different fatty acid production profiles, with some optimized for nutritional applications and others for biofuel purposes (Xin et al. 2017). In *Chlorella variabilis* lipid metabolism, an enzyme named fatty acid photodecarboxylase was identified, which belongs to a microalgae-specific clade of the glucose–methanol–choline oxidoreductase family and which catalyzes the decarboxylation of free fatty acids to hydrocarbons (n-alkanes or n-alkenes) important for biofuel production (Sorigué et al. 2017).

Microalgal biomass pretreatment is essential for further processing, which depends on microalgae cell structure and composition and energy demands during the process. According to de Carvalho et al. (2020), high-pressure homogenization and acid hydrolysis remain economically competitive, and that those could be upgraded to increase the viability. These authors argued that operations reliable at a small scale, such as sonication and lyophilization, may prove impractical or too expensive on an industrial scale. In contrast, uncommon steps, such as freeze-thawing and pulsed electric fields, can end up having a positive energy balance.

The high content of free fatty acids in the lipids of microalgae biomass is an important topic, which must be addressed when considering the production of biodiesel from microalgae biomass (Krohn et al. 2011). The oils extracted from the microalgae biomass are characterized by having a high content of free fatty acids that can reach up to 85% of the total lipids, depending on the cultivated microalgae strain and the cultivation conditions (Chen et al. 2012; Krohn et al. 2011).

Lipids extracted from microalgae biomass, which have a high content of free fatty acids, are unsuitable for the synthesis of biodiesel when transesterified with primary catalysts since the high content of free fatty acids decreases the catalytic activity due to saponification. An alternative to overcome this limitation is the use of two steps in the crude oil treatment process, which involves the esterification reaction of microalgae lipids with a high content of free fatty acids with methanol to convert free fatty acids into ester fatty acid methyl and then followed by transesterification (Dong et al. 2013). When the level of free fatty acids in oils is higher than 5%, saponification will inhibit the separation of methyl and glycerol esters, which causes the formation of an emulsion during washing with water; therefore, it is necessary to convert free fatty acids into methyl esters (Huang et al. 2010).

The two-step proposal for catalytic conversion was also proposed by Chen et al. (2012) because it had a high potential for the production of biodiesel from microalgae oil rich in free fatty acid. After optimized esterification–

transesterification procedures, the conversion rate of triacylglycerols and free fatty acids to methyl esters reached 100%.

Regarding the extraction of lipids and proteins from wet microalgal biomass in a 3G biorefinery by comparing supercritical fluid extraction (SFE) and low-pressure solvent extraction (LPSE), it was showed that supercritical fluid extraction for wet microalgae processing is not economically attractive, as it increases the total investment by 71% (Albarelli et al. 2018).

For microalgal biomass, the extraction of the crude hexane lipid fraction, using mechanical stirred associated with ultrasound technique, allowed greater extraction of the crude hexane lipid fraction (Gomes et al. 2019). These authors argued that the ester profile with relatively elevated concentration of polyunsaturated fatty acids (C18:3) is unfeasible in their application for biodiesel production.

After obtaining the biomass of microalgae depending on the type of microalgae, it is necessary to carry out the cell rupture process, which is a pretreatment for the extraction, to facilitate the extraction of the metabolites of interest, in the case of biodiesel production, lipids (Fig. 23.5). It is not a mandatory step, and the decision for its use depends on the extraction method to be used. According to Mata et al. (2010), rupture and extraction can occur in two ways:

- By mechanical action: through the high-pressure homogenizer, ball mill, ultrasound, autoclaves or freeze-drying, microwave or
- By nonmechanical action: by freezing, using organic solvents, osmotic shock, or reactions of acids, bases, or enzymes, for example.

These pretreatment steps use energy intensively and, therefore, can only be carried out by increasing the efficiency of lipid extraction from microalgae. Most cell disruption pretreatments require water, and therefore must be performed before the drying process.

Once the pretreatment is carried out, oil extraction follows. Peralta-Ruiz et al. (2013) mentioned that there are several methods of oil extraction used in microalgae; these methods can be divided into as follows:

- Methods assisted by mechanical interruption using homogenizing cells, ball mills, pressing systems, among others. These methods are not suitable for the extraction of oil on a laboratory scale, as they present high biomass losses and low selectivity for lipids.
- Enzyme-assisted extraction methods, in which the microalgae cell wall is degraded by enzymes that allow the release of lipids. However, enzyme activity is affected by several variables, including concentration and ratio of system compounds, acid profile greases, microalgae composition, temperature, among others. These issues make it challenging to maintain this route at this time for large-scale biomass processing.

Other methods are also used for the extraction of microalgae oil. One of the most used method is the extraction with an organic solvent. Currently, hexane and ethanol have been widely used in the extraction of edible oil, but chloroform, methane, benzene, and other organic solvents are toxic and therefore are not applicable.

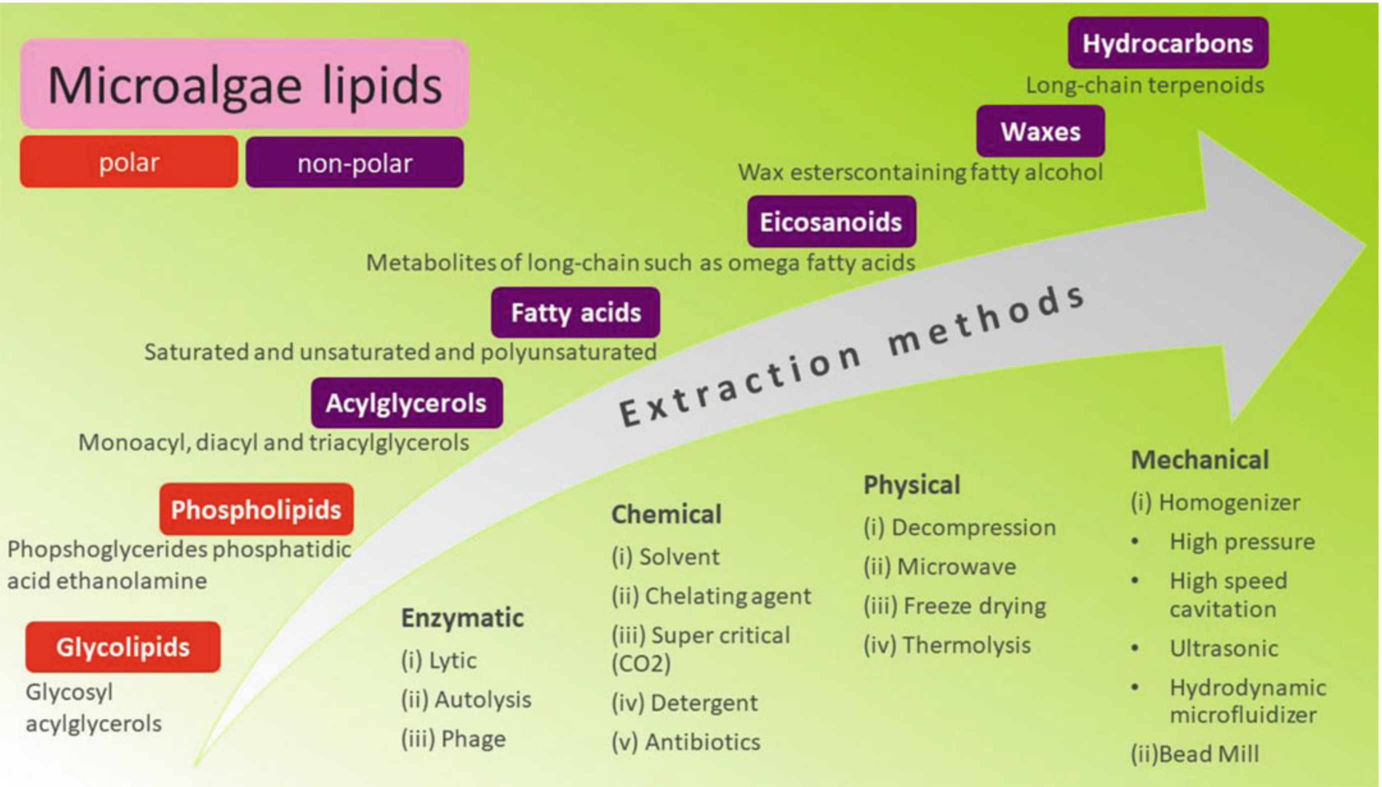


Fig. 23.5 Examples of methodologies for lipid extraction. Source: Modified from Kumar et al. (2015)

According to the similarity compatibility principle, nonpolar solvents dissolve and destroy nonpolar lipids in the cell membrane of microalgae to extract the oil. Because organic solvents are toxic, volatile, and difficult to recycle, some green solvents are also used, such as bio-based solvents, ionic liquids, convertible solvents, supercritical fluids, subcritical water, and pressurized solvents (Xue et al. 2020).

A widely used technology is supercritical fluid extraction (SFE), which allows the preservation of the natural qualities of bioactive compounds, reducing the environmental impact and minimizing energy costs at the same time (da Silva et al. 2016). Also, SFE allows us to prevent the presence of traces of solvent in the final extracts, with the possibility on a large scale of recovery of CO_2 in a closed circuit with an economic advantage with the use of other solvents (Molino et al. 2020).

Supercritical fluid technology is an analytical process in which the extraction and separation of organic compounds from a matrix can be carried out effectively. A pure substance is in a supercritical state when it is above its critical temperature and pressure (Akalın et al. 2017). Carbon dioxide (CO_2) and water are the most used supercritical fluids, which can potentially be used in the production of biofuels; supercritical CO_2 has several advantages, especially for the extraction of low polarity chemicals, such as biomass lipids (Li et al. 2019).

Transesterification is a multistep reaction, including three reversible steps in series: triglycerides are converted to diglycerides, then diglycerides are converted to monoglycerides, and monoglycerides are converted to esters (biodiesel) and glycerol (by-product). The transesterification reaction is where the radicals R1, R2, R3 represent long-chain hydrocarbons, known as fatty acids (Mata et al. 2010). For the conventional transesterification reaction, oil or fat and short-chain alcohol (the alcohols commonly used are methanol, ethanol, propanol, butanol, and amyl alcohol, but methanol is applied more widely due to its physical advantages and low cost) (Huang et al. 2010) are used as reagents in the presence of a catalyst (usually NaOH). Although the theoretical molar ratio of alcohol:oil is 3:1, the molar ratio of 6:1 is generally used to complete the reaction accurately. The ratio between the mass input of raw material and the mass production of biodiesel is about 1:1, which means that, theoretically, 1 kg of oil results in about 1 kg of biodiesel (Mata et al. 2010).

An alternative for obtaining a higher biodiesel content is the use of heterogeneous catalysts in addition to the use of ultrasound and microwave techniques and supercritical alcohols that generally improve biodiesel production (Goh et al. 2019).

In research carried out by Levine et al. (2010), wet *Chlorella vulgaris* biomass was directly processed, eliminating the use of organic solvents during lipid extraction, recovering nutrients and glycerol. They developed a catalyst-free technique for the production of biodiesel. First, wet biomass (about 80% humidity) reacted in subcritical water to hydrolyze intracellular lipids. In another step, solids rich in moist, fatty acids underwent supercritical transesterification in situ with ethanol to produce biodiesel in the form of ethyl esters of fatty acids. They examined hydrolysis at 250 °C for 15–60 min; the solids recovered by filtration contained 77–90% of the lipid initially present in algae biomass, mainly in the form of fatty acids. They determined that the higher time and temperature and higher ethanol load tended to increase the gross yields of biodiesel and fatty acid ethyl esters, which ranged about 56–100% and 34–66%, respectively, based on lipids in hydrolysis solids.

Another study with *Chlorella* sp. (Chauhan et al. 2020) reported the development of an efficient method of direct conversion to biodiesel via supercritical transesterification of methanol. The method involved the evaluation and optimization of the neutral lipid content and water content of the biomass as two critical attributes of the biomass quality to maximize the yield of fatty acid methyl esters (FAME). They obtained the highest FAME yield of 96.9% reaching an ideal value of lipid content, the water content of the biomass and methanol load of 52% (w/w), 5.75 mL g^{-1} and 115 mL g^{-1}, respectively. In general, the use of microalgae as raw material for biodiesel is technically viable, but not economically viable.

Biohydrogen

Biohydrogen is a natural and transitory by-product of several biochemical reactions of microbial origin; generation of H_2 gas either by biological machinery or by thermochemical treatment of biomass can be defined as "biohydrogen." The thermochemically produced H_2 is also being called bio-hydrogen due to the use of biomass as a substrate/raw material. On the contrary, several biological routes are available for the production of bio-hydrogen belonging to anaerobic/fermentation, photobiological, enzymatic, and electrogenic mechanisms (Mohan and Pandey 2013).

Biohydrogen can be generated by various biological forms and classified into two main categories (Aslam et al. 2018): light-dependent and dark fermentation processes. The primary light-independent process is dark fermentation, while light-dependent processes include photofermentation and photolysis. All bio-hydrogen production pathways depend on nitrogenase or hydrogenase for the evolution of hydrogen. These technologies derive energy directly from light energy or indirectly through the consumption of photosynthetically derived carbon compounds.

Some species of microalgae have potential indirect biophotolysis, especially *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Dunaliella tertiolecta*, *Nannochloropsis* sp., *Scenedesmus obliquus*, *Cosmarium* sp., *Thalassiosira weissflogii*, among others (Eroglu and Melis 2016). Their hydrogen can be obtained by different methods as follows:

- Chu and Phang (2019) reported that in direct biophotolysis, the photosynthetic apparatus chlorophyll and other pigments of eukaryotic green microalgae can retain light and energy from the sun. In addition, it is being improved with water separation to produce a low potential reducer or ferredoxin, which can moderate a hydrogenase or nitrogenase enzyme directly without temporary CO_2 fixation. The hydrogen ions generated are transformed into hydrogen gas in the medium with electrons donated by reduced ferredoxin in the presence of the enzyme hydrogenase. Naturally, direct bio-photolysis is a desirable method due to the use of solar energy to alter an easily obtained substrate, water, oxygen, and hydrogen, but, in practice, it is restricted by other problems such as enzyme hydrogenase activity affected by O_2, because it is related during the process of direct bio-photolysis and, therefore, inhibits the H_2 yield.

- In indirect bio-photolysis, the sensitivity problems of the hydrogen evolution process are potentially circumvented by the temporal and spatial separation of the evolution of oxygen and the evolution of hydrogen. Indirect bio-photolysis processes involve the separation of H_2 and O_2 evolution reactions in separate stages, coupled through CO_2 fixation/evolution; in this case, cyanobacteria have the unique characteristics of using CO_2 in the air as a source of carbon and energy solar as a source of energy. The cells absorb CO_2 first to produce cellular substances, which are later used in the production of hydrogen (Pareek et al. 2020).
- In dark fermentation, hydrogen is produced in the absence of sunlight, oxygen, and water. Fermentative microorganisms convert complex organic matter into a mixture of alcohol and organic acid, together with the production of hydrogen. Various carbon-rich waste resources can be processed by dark fermentation, producing hydrogen, and other significant by-products like volatile fatty acids, acetic acid, and butyric acid (Ren et al. 2019).
- Photo-fermentation is a fermentative conversion of organic substrates into hydrogen and carbon dioxide by using sunlight as an energy source. Using the light as a power source, organic acid substrates are oxidized using the tricarboxylic acid cycle, producing electrons, protons, and carbon dioxide. Its advantages are the removal of environmental pollutants, the use of industrial residues, and the use of organic acids produced from dark fermentation. The disadvantages are the need to limit the nitrogen condition and the pretreatment of the industrial effluent, as it can be toxic (Sharma and Arya 2017).
- When the process is carried out in two stages (integration of dark and photo-fermentation), during the first stage of dark fermentation, the substrate containing carbohydrates is converted into organic acids, CO_2, and hydrogen by mesophilic and thermophilic bacteria. In the second stage, dark fermentation residues containing organic acids, such as acetic and lactic bacteria, used in photosynthetic photofermentation or without purple sulfur, are transformed for later production of hydrogen (Mohan and Pandey 2013).
- Biocatalysis electrolysis is a technology that is related to the microbial fuel cell and overcomes thermodynamic barrier utilizing a small electrical energy input, making the process independent of the reactor's surface area. Biocatalyzed electrolysis achieves this by using electrochemically active microorganisms, which convert dissolved organic material into bicarbonate, protons, and electrons. By direct contact with the electrode surface or aided by redox mediators (excreted), these microorganisms release the electrons produced to the electrode surface, in order to generate current. When coupling this biological anode to a proton reducing cathode through a power source, direct conversion of dissolved organic material into hydrogen is carried out. The complete process takes place in an electrochemical cell in which the oxidation of dissolved organic material and the reduction of protons are separated into two chambers. The separation between these chambers is established through a cation exchange membrane (e.g.,

Nafion). Externally, the anode and cathode are connected to the power supply using an electrical circuit. As the power supply conducts electrons released from the anode to the cathode, an equal number of protons permeates through the membrane. At the cathode, protons and electrons combine to form pure hydrogen gas (Eroglu and Melis 2016).

Bioethanol

Ethanol is the most widely used liquid biofuel. It is an alcohol and is fermented from sugars, starches, or cellulosic biomass. Most commercial ethanol production is from sugarcane or beet, as starches and cellulosic biomass generally require expensive pretreatment. Usually, it is used as a source of renewable fuel as well as in the manufacture of cosmetics, pharmaceuticals, and also in the production of alcoholic beverages (Demirbaş 2005).

The production of bioethanol involves different stages of the process, including pretreatment of biomass, hydrolysis, fermentation, and product recovery. Pretreatment of biomass is one of the most important and expensive stages of the process. The pretreatment step is necessary to reduce the crystallinity of the biomass and increase the surface area to improve the digestibility of the substrate (Harun et al. 2011; Sarkar et al. 2012).

Certain microalgae species can produce high levels of carbohydrates instead of lipids as reserve polymers. These species are ideal candidates for the production of bioethanol since the microalgae carbohydrates can be extracted to produce fermentable sugars (Mussatto et al. 2010), among which stands out *Laminaria japonica*, *Sargassum fulvellum*, *Hizikia fusiformis* (Lee et al. 2009), *Chlorococum* sp. (Harun et al. 2010), *Gelidium corneum* (Yoon et al. 2012), *Schizocytrium* sp. (Kim et al. 2012), *Scenedesmus obliquus* (Miranda et al. 2012), *Chlorella vulgaris* (Ho et al. 2013), *Chlorella* sp. (Ngamsirisomsakul et al. 2019), *Chlorella sorokiniana* (Tatel and Madrazo 2020), *Scenedesmus acuminatus* (Chandra et al. 2020), *Chlamydomonas* sp. (Kim et al. 2020), *Ulva intestinalis*, *Amphiroa compressa* (Osman et al. 2020) among other species.

Some microalgae have great potential for the generation of different biofuels. Different technologies are ready for their development on a large scale, but there are still several obstacles that need to be overcome, such as the high costs of cultivation, harvesting, and processing, which consequently causes the price of microalgae biofuels higher than fossil fuels. Anaerobic biodigestion for the generation of biogas seems to be most financially viable due to its less complex processing compared with other biofuels.

23.6 Environmental and Agricultural Applications

The microalgae have the capability to remove more than 90% of nutrients and some extend of toxic chemicals and heavy metals from the industrial effluent, and it can be further increased by using growth stimulators or by developing growth. In addition, from the perspective of improvement of soil environments, microalgae, mainly cyanobacteria, are thought-out as a potential producer of exopolysaccharide and biomass production in large scale, aiming dispersion of inoculum in the field as efficient, eco-friendly method (Tiwari et al. 2019). Evidence reveals a higher amount of lipids, proteins, and pigments in biomass of these microorganisms plus to recycling water, generating applications in bioenergy (Zhu et al. 2019) in agriculture as biofertilizers (Castro et al. 2020).

23.6.1 Environmental Bioremediation Using Microalgae

For environmentally and economically sustainable food, agribusiness, and bioenergy industry processes, microalgae appears to be an option-based biological source of by-products. The microalgae cultivation can provide recovery of recyclable nutrients from secondary sources, which has an enormous role to global sustainable demands. The wastewaters have different origins, and most of them are rich in energy and nutrient sources that can be recovered and reused in a circular bioeconomy viewpoint (Nagarajan et al. 2020). Concomitantly, photosynthetic microorganisms when integrated with existing facilities to intensive cultivation that can be in different agro-industrial types of wastewater provide biomass production, environmental bioremediation, and reasonable return.

Both, cyanobacteria and microalgae in a mixotrophic or heterotrophic system, can utilize carbon, N, P, and other nutrients from different types of wastewater. Using industrial effluents through cultivation of microalgae is an alternative to synthetic media and viable to the increase of its biomass generated, with effects on both in the investments in the agriculture and in the reuse of wastewater from agro-industries by remediation and/or energy production recovery (Umamaheswari and Shanthakumar 2016).

23.6.2 Agro-Industrial Wastewater Treatments

Besides phototrophic growing using CO_2 as energy source, microalgae can use other carbon sources to increase biomass production in heterotrophic or mixotrophic cultivation systems, including organic carbon. The high availability of wastewater or effluents with high organic content, especially those derived from agro-industries, and the ability of microalgae to thrive in unsuitable waters benefit the generation of biomass for the production of biofuels. For instance, effluents from the brewing industry that generates a large amount of wastewater (Tonhato Junior et al. 2019) and unsterilized dairy-derived liquid digestate can be used for simultaneous biofuel feedstock production and contaminant removal (Zhu et al. 2019).

Studies have highlighted the significant potential and economic value of combining biorefinery treatment to recover wastewater with a high organic load. For sugarcane, Sydney et al. (2019) proposed an efficient process of growing microalgae and cyanobacteria for reusing vinasse from the bioethanol industries of bioethanol production. For food and agro-industrial activities, Vu et al. (2020) projected a hybrid system to collect energy, nutrients and microalgal biomass from highly organic and nutritious wastewater, which comprises an anaerobic membrane bioreactor to produce biogas and a microalgal membrane reactor.

Wastewater is a resource for the recovery of clean water, energy, and nutrients (Kehrein et al. 2020). Table 23.2 shows the wastewater types, microalgal genus or species used, the cultivation system, and the main contributions or relevant findings that were found in each study mentioned. The reviews were chosen to present how the microalgae cultivation in wastewater or agro-industrial waste reduced environmental impacts and to produce biomass as raw material for bioenergy and also as biofertilizers for agriculture.

In these works, chlorophytes and cyanobacteria were evaluated using growth media that included dairy residues; animal residues as pig-slurry, poultry, cattle, and fish; processing of cassava, maize, potatoes, coffee, sugar cane, grapes, palm oil, and soybean; animal feed production and yeast production; and brewery, tannery, and sewage (Table 23.2).

An option to bioremediation of swine/piggery wastewater is fermentation for biogas and after microalgae cultivation, mainly with green microalgae that are strong candidate for biomass production by using piggery wastewater. Addressing the treatment of swine/pig wastewater, some studies were highlighted, mainly those that evaluated the participation of microalgae-based processes in the removal of phosphorus, nitrogen, and organic matter, avoiding soil and surface water contamination. *C. vulgaris* and *Scenedesmus dimorphus* have the ability to remove P and N from pig slurry and dairy residues (González et al. 1997), while *C. zofingiensis* was able to adapt and grow well outdoors using pig slurry sterilized, which can expand the potential biomass production for biodiesel with a cost–benefit advantage (Zhu et al. 2013).

Anaerobic digestion and subsequent microalgal cultivation with the digestate under a circular economy concept might help improve the economic feasibility of in-farm biogas plants with net positive values (Nagarajan et al. 2020). Cultivation of *C. sorokiniana* on thin stillage digestate that was pretreated with struvite was able to remove chemical oxygen demand, ammonia–nitrogen, and total phosphorus with biomass production containing high protein, starch, and lipid contents (Sayedin et al. 2020), which is a value-added product for application as fertilizer. There are some biological processes (e.g., aerobic processes and anaerobic digestion) that are beneficial in nitrogen removal, but they are relatively expensive. To replace these processes, an option to remove organic carbon and nutrients from a wastewater purification perspective, a diluted soybean wastewater as a cultivation medium for *Chlorella* sp. promoted removal rate of 50–65% chemical oxygen demand (COD), 70–80% NH_3–N, and 95–100% total phosphorus (Song et al. 2019).

When growing in heterotrophic medium from untreated dairy wastewater or dairy farm effluent, green microalgae have been identified as a good candidate for biomass production for bioenergy and simultaneously for nutrients recycling, for instance,

Table 23.2 Example of agro-industrial waste and microalgae species used in cultivation system as nutrient removal and biomass production

Agro-industrial waste types	Microalgae Genus/species	System of cultivation	Highlight applications	Ref.
Dairy residues and pig-slurry	*Chlorella vulgaris* *Scenedesmus dimorphus*	Cylindrical and triangular bioreactors of glass	*C. vulgaris* and *S. dimorphus* have been shown to reduce pollutant elements in effluents in different ways *S. dimorphus* was more efficient in removing ammonia, at the end of the cultivation cycle, both removed the same amount of P from the residue *C. vulgaris*, the triangular bioreactor, was adequate to remove ammonia and cylindrical for phosphorus	González et al. (1997)
Pig-slurry	*Chlorella zofingiensis*	Photobioreactors bubble column	The combination of *C. zofingiensis* cultivation in swine wastewater can improve the expansion of algae biodiesel production and improve the cost–benefit ratio. Wastewater can replace the use of fertilizers The expansion will depend on the policy of governments to reduce carbon emissions, in addition to future research, through investments and subsidies	Zhu et al. (2013)
Municipal and piggery slurry	*C. sorokiniana*	UASB reactor + flat panel photobioreactors	UASB's high efficiency in removing organic matter (>90%) and biomass production (1 g L^{-1}), with average removal of dissolved inorganic carbon, phosphate,	Leite et al. (2019)

(continued)

Table 23.2 (continued)

Agro-industrial waste types	Microalgae Genus/species	System of cultivation	Highlight applications	Ref.
			and ammonia around 46–56%, 40–60%, and 100%, respectively	
Swine/piggery wastewater	*C. zofingiensis*, *Synechocystis* sp., *Tribonema* sp., and *Botryococcus braunii*	Glass bubbling bottles	Cultivation wastewater from anaerobic digestion of swine wastewater which was pretreated by sterilizing swine. pH 7.0. Microalgae grown in the pretreated wastewater were higher than that in the nontreated wastewater, but the protein content was lower	Cheng et al. (2020)
Processing of potato, fish, coffee, animal feed production, and yeast production	Consortium of *Phormidium* sp., and green microalgae *Oocystis* sp. and *Microspora* sp.	Glass bottles	The biodegradable total organic carbon was the limiting component during wastewater treatment in most of the evaluated agro-industrial effluents and dilutions The results highlight the need for an external carbon source (CO_2) supply, pH control strategies, and the dilution of the high ammonia concentrations	Posadas et al. (2014)
Meat-processing industry	*Scenedesmus* sp.	Photobioreactor	In a meat-processing industry after flotation treatment (PE) and after an activated sludge unit (SE) The dominance of the genus *Scenedesmus* (mixotrophic) in all	Tango et al. (2018)

(continued)

Table 23.2 (continued)

Agro-industrial waste types	Microalgae Genus/species	System of cultivation	Highlight applications	Ref.
			the operations showed the ability of that genus to survive in extreme environments	
Vinasse	*Scenedesmus* sp.	Air-lift photobioreactors	Light intensity and percentage of vinasse (up to 40%) influenced the amount of biomass to be produced by *Scenedesmus* sp.	Ramirez et al. (2014)
Vinasse	*Chlorella vulgaris*	Tubular reactors	Reduction in the concentration of most metabolites in the first days of microalgae growth in the dark under continuous air flow, due to the transition from autotrophic to heterotrophic metabolism	Quintero-Dallos et al. (2019)
Sugarcane	*Chlorella vulgaris*		The treatment of conventional filtration and bio-digested of sugarcane (vinasse) resulted in cleaner residues that supported the *C. vulgaris* growth put on 32× more cell density and higher final biomass	Candido and Lombardi (2017)
Soybean	*Chlorella vulgaris*	Conical bottles	The ammonia escape rate could be reduced to 15.8% and the carbon conversion capacity and efficiency of the hybrid process was around 44.3 mg/L/day and 60.8% with efficient purification of soy effluents Associated with	Song et al. (2019)

(continued)

Table 23.2 (continued)

Agro-industrial waste types	Microalgae Genus/species	System of cultivation	Highlight applications	Ref.
			nitrogen and carbon biotransformation, 78.8 mg/L/day of microalgae biomass could be grown to produce value-added ingredients to enhance the techno-economic viability of the hybrid microalgae absorption process	
Tofu whey wastewater	*C. pyrenoidosa*	Filtrated and sterilized	Growing at heterotrophic and mixotrophic conditions using 100% Tofu whey wastewater The biomass productivity was improved when using TWW, which resulted in higher lipid and protein productivity	Wang et al. (2018)
Grape processing	*Auxenochlorella protothecoides* and *Chlorella sorokiniana*	Photobioreactors	Two microalgae analyzed removed >90% nitrogen, >50% phosphate, and 100% acetic acid in the residual water of the winery Organic carbon did not play a limiting role in the growth of microalgae Bacteria and algae provided benefits to synergistic growth, contributing to wastewater treatments	Higgins et al. (2018)
Palm oil mill effluent	*Tetraselmis suecica*	Bottles	Cocultivation of microalgae with oil palm empty fruit bunch and palm oil mill effluent	Ahmad et al. (2014)

(continued)

Table 23.2 (continued)

Agro-industrial waste types	Microalgae Genus/species	System of cultivation	Highlight applications	Ref.
			presented the highest specific biogas production and biomethane yield was achieved with microalgae for anaerobic biomethane production	
Olive mill wastewater	A mix of *Spirulina platensis*, *Nostoc muscorum,* and *Anabaena oryzae*	–	Cyanobacteria biomass from the growth on olive mill wastewater was s applied as biofertilizers for celery in sandy soil	Rashad et al. (2019)
Fish farming	*Spirulina platensis*	Two boxes for raising fish and a swimming pool (pilot scale)	Two boxes for raising fish and a swimming pool (pilot scale). Two boxes for fish cultivation and a swimming pool (pilot scale) Carry out was successful to the consortium *S. platensis* with other organisms in polyculture systems, or integrated agriculture, with or without water recirculation	Nogueira et al. (2018)
Manure wastewaters (poultry, pig, and cattle), brewery, dairy residues, and sewage	*Scenedesmus oblíquus*	Bubble photobioreactors and flat plate	The biomass productivity achieved using the different wastewater was higher than the synthetic medium, except for birds, with a higher volume obtained in brewery wastewater The reduction of environmental impacts, in addition to indicating strategies for the future of	

(continued)

Table 23.2 (continued)

Agro-industrial waste types	Microalgae Genus/species	System of cultivation	Highlight applications	Ref.
			bioenergy production and circular economy	
Brewery effluent	*Chlorella* sp.	Flask	The SL Tanfloc tannin proved to be efficient in flocculating the brewery effluent, allowing the reuse of water and recovered biomass containing nutrients The sludge generated and accumulated in the brewery's effluent treatment process can be applied as a biofertilizer, after predrying, since it has considerable amounts of nitrogen and phosphate in its composition	Tonhato Junior et al. (2019)
Tannery (animal leather processing)	*Arthrospira* (Spirulina)	Open lane ponds	The odor emission was reduced with the cultivation of microalgae compared to the other lagoons. Thus, with treatment based on microalgae, the disposal of wastewater from tanneries can be compatible with the environmental and social acceptability. For the local community, the odor has decreased substantially	Dunn and Rose (2013)
Sewage and tannery	*C. vulgaris* and *Pseudochlorella pringsheimii*	Conical flask	Both species are apparent to treat tannery effluent in three dilutions, with the substantial removal of polluting	Saranya and Shanthakumar (2019)

(continued)

Table 23.2 (continued)

Agro-industrial waste types	Microalgae Genus/species	System of cultivation	Highlight applications	Ref.
			compounds, like NH_3, PO_4, and heavy metal chromium *P. pringsheimii* has higher lipid accumulation potential than *C. vulgaris* irrespective of the saline stress	
Dairy residues	*Chlorella* sp.	Suspended solid supports and polyethene foam	By using polyethene foam, it allows the cultivation of *Chlorella* sp. easily with large accumulated biomass, and for a relatively long period	Johnson and Wen (2010)
Dairy residues	*Neochloris oleoabundans*	Horizontal photobioreactors	Increase lipids in its biomass, and this is useful in biodiesel. In the same way, it demonstrates the potential capacity of larger photobioreactors and low cost for biomass production Microalgae monocultures have increased the possibility to reuse wastewater and produce high-quality biodiesel during wastewater treatment	Levine et al. (2011)
Dairy residues	*Chlorella pyrenoidosa*	Conical glass balloon	*C. pyrenoidosa* can remove up to 85% phosphorus and 80% nitrogen, and excellent lipid (oil) conversion	Kothari et al. (2012)
Dairy residues	*Chlorella* sp. and *Scenedesmus* sp.	Bottles in vitro and in situ	Microalgae grew in different dairy effluents, and *Chlorella* became best in high	Labbé et al. (2017)

(continued)

Table 23.2 (continued)

Agro-industrial waste types	Microalgae Genus/species	System of cultivation	Highlight applications	Ref.
			organic content and high ammonium loads, like the effluents from the cattle yard. *Scenedesmus* grew best with a high chemical and detergent load, like the waters of the milking parlor The potential use of dairy effluents does not produce microalgae for purposes and treatment and improves the finances of small- and medium-sized dairy farms	
Dairy residues	*Chlorella sorokiniana* (DS6)	Conical vials	Green unicellular microalgae *C. sorokiniana* isolated from the holding tanks of farm wastewater treatment plant using multistep screening and acclimation procedures was found high-lipid producing facultative heterotrophic microalgae strain capable of growing on dairy farm effluent (DFE) for biodiesel feedstock and wastewater treatment	Hena et al. (2015)
Dairy residues	*Chlorella vulgaris*	Micro-photo bioreactor	The maximum percent of chemical oxygen (COD) removal efficiency was 42.57% after 52 h, and the optimum conditions of COD were equal to flow rate =	Valizadeh and Davarpanah (2020)

(continued)

Table 23.2 (continued)

Agro-industrial waste types	Microalgae Genus/species	System of cultivation	Highlight applications	Ref.
			0.0125 Cm^3 min^{-1}, length of photo micro-bioreactor = 16 m, temperature 30 °C, and at initial pH 8.00	
Dairy residues	*C. vulgaris*	Discontinuous photobioreactors	To cultivate *C. vulgaris* at 25% was ideal for the complete removal of ammonium and phosphorus in addition to achieving high lipid yield that favors the production of biofuels. Bacteria do not influence microalgae growth but decreased microbial diversity. These findings contribute to a mix of bacteria in the cultivation of large-scale microalgae	Zhu et al. (2019)
Dairy residues	*Ascochloris* sp.	Column and flat plate photobioreactor	The cultivation of microalgae in photobioreactors outdoors generates yields and bioremediation of wastewater from untreated dairy products was used to produce biomass, lipids, other value-added by-products with a reduced organic compound. There is 100% use of raw effluent from untreated dairy products for the production of biomass, lipids, other value-added by-products, and	Kumar et al. (2019a, b)

(continued)

Table 23.2 (continued)

Agro-industrial waste types	Microalgae Genus/species	System of cultivation	Highlight applications	Ref.
			clean, odorless water for recycling and reuse	
Dairy wastewater	*Ascochloris* sp.	Ponds	Production of 504-ton biomass per year at \$0.482/kg with ~240,000 m^3 of treated clean water and high-volume V-shape ponds was one of the cost-effective and area-efficient microalgal cultivation systems for mass production	Kumar et al. (2020)
Cassava	*Chlorella pyrenoidosa*	Tubular photobioreactor	*C. pyrenoidosa* significantly reduces the organic amount of the residue, without altering the production of ethanol, and this residue can be reused up to four times	Yang et al. (2008)
Cassava	*Acutodesmus obliquus*	Open tank	*A. obliquus* cultivated biomass improved by the addition of cassava than the control, and the quantity of lipids and carbohydrates increased by 96.8% bioethanol and 98.7% biodiesel	Selvan et al. (2019)
Cassava	*Spirulina platensis*	Microbial fuel cell and microalgae-assisted cathode	A combination of two biocatalysts with indigenous microorganisms from wastewater at the anode and microalgae grown in situ at the cathode was able to reduce 67% of the initial organic quantity, to generate renewable bioelectricity and produce microalgae biomass	Hadiyanto et al. (2019)

(continued)

Table 23.2 (continued)

Agro-industrial waste types	Microalgae Genus/species	System of cultivation	Highlight applications	Ref.
Tapioca wastewater	*Scenedesmus* sp.	100 mL filtered in 250 mL flask	*Scenedesmus* sp. which was cultured in 50% of tapioca wastewater gives highest lipid production	Romaidi et al. (2018)
Maize	*Chlorella vulgaris*	Erlenmeyer and balloon bottles	The cultivation of *C. vulgaris* in vinasse removed 84–86% of total carbon with substrate degradation of 76–79% and high biomass production New phytoremediation strategy to treat effluents generated from the corn industry to ethanol and simultaneous production of value-added coproducts	Beigbeder et al. (2019)
Corn/maize stillage digestate	*C. sorokiniana*; *Scenedesmus obliquus*; *C. saccharophila*	Glass bottle	Pretreatment (centrifugation, chemical add) *C. sorokiniana* removed chemical oxygen demand, ammonia-N, and total P and produced biomass with high content of protein (37.8 ± 3.4%), starch (17.8 ± 0.8%), and lipid (8.9 ± 0.3%) Potential to integrate into an existing corn ethanol plant to reduce the corn consumption, increase the protein content of the dried distiller's grain and corn-oil yield	Sayedin et al. (2020)

Chlorella sp. (Johnson and Wen 2010), *C. pyrenoidosa* (Kothari et al. 2012), *C. sorokiniana* (Hena et al. 2015), and *C. vulgaris* (Valizadeh and Davarpanah 2020).

Levine et al. (2011) showed that using anaerobically digested dairy manure wastewater for growth Neochloris oleoabundans approximately 90–95% of the initial nitrate and ammonium was assimilated and yielded 10–30% fatty acid methyl esters of dry biomass after 6 d. These authors concluded that this microalgae species is an excellent green microalga for combined biodiesel feedstock production.

The selection of species and strains with the best performance to cultivation aiming for chemical removal and bioenergy feedstock production must be thoughtful, due to the high complexity in terms of nutritional composition and adaptability of the microorganisms, including isolation and screening for adaptation in media similar to wastewater (Chu 2017; Moreno Osorio et al. 2020).

The chemical, microbiological composition, and organic load of the wastewater also interfere with the growth of microalgae. Consequently, not only residues must be carefully characterized but also the growth of the species/strain must be evaluated in screening to better choose the wastewater cleaning technology based on microalgae cultivation. In a study using polluted effluents from the dairy industry for biomass production and phytoremediation, it was observed that *Chlorella* sp. grew better in effluents from the cattle yard with high organic and ammonium contents, while *Scenedesmus* sp. presented better growth in the milking parlous effluents with higher inorganic compounds and detergent cargo (Labbé et al. 2017). Biomass produced by *Ascochloris* sp., a strain isolated from dairy industrial effluent, exhibited a relevant lipid increase, showing potential for bioenergy production and for simultaneous bioremediation of raw dairy wastewater (Kumar et al. 2019a).

The feasibility of *C. vulgaris* cultivation in unsterilized dairy-derived liquid digestate diluted to 25% was observed by Zhu et al. (2019), who also estimated that for each ton of biomass produced, approximately 102 tons of wastewater can be treated with removal of N and P, allowing coproduction of bioenergy feedstock and chemical removal. In this study, also it was observed that bacteria do not influence microalgae growth but microbial diversity was decreased, inferring that the presence of bacteria does not affect the cultivation of large-scale microalgae using wastewater.

The use of effluents to generate biomass from microalgae is an approach that benefits both bioremediation and the production of biofuels. Regarding wastewater from leather industries, an effort has been made to treat them using phycoremediation, aiming to produce biomass for bioenergy, as it was showed with the cultivation of *C. vulgaris* and *Pseudochlorella pringsheimii* for treating the tannery effluent in dilutions <30%, which resulted in significant removal of polluting compounds like NH_3, PO_4, and heavy metal chromium (Saranya and Shanthakumar 2019).

Considering that wastewater is used to replace freshwater in the microalgal cultivation, according to Chu (2017), it is possible to reduce by 94%, which is indeed a strategic approach to use agro-industrial wastewater to enhance biomass productivity for biofuel production because such combine system also reduces the pollutants in the effluents before discharge.

Cyanobacteria species thrive well in wastewater from different effluents, for example, *Arthrospira* sp. (*Spirulina*) growing in tannery effluent and *Spirulina platensis* lessened odor characteristic of perfume activity (Dunn and Rose 2013) in pisciculture wastewater. There was a reduction of 19.8% in ammonia, 100% in nitrite, 98.7% in nitrate, and 94.8% in phosphate content, with nutrient levels within the standards those required by Brazilian environmental standards to release (Nogueira et al. 2018).

High organic and nutritional amount in the wastewater and effluents, especially from agro-industries, is synergistic to the ability of microalgae to thrive in such wastewater, and for example, biomass production for biofuels and conservation of natural resources (Zhu et al. 2019). For industrial and versatile scale, microalgae are aimed at different purposes, especially for the ability to convert the contents in the growth medium to a high content of lipids and carbohydrates and for being considered promising as a raw material in the production of biofuels (Ramirez et al. 2014; Zhu et al. 2019).

Effluents may contain growth inhibitors such as high concentrations of toxic compounds and high turbidity that reduces the availability of light that induce anaerobic conditions and hinder treatments of aiming biological degradation of organic matter (Panchangam and Janakiraman 2015). Additionally, photosynthetic microorganisms (e.g., photoautotrophic microalgae) when in medium with high turbidity that reduces the availability of light tend to have their growth limited. To overcome these limitations, some strategies can be considered: the choice of effluents to be treated and the adaptation or selection of microalgae species or strains to be used. Overall, biodegradable total organic carbon was the main factor limiting to remove nutrients and other compounds in effluents from the processing potato, fish, coffee, animal feed production, and yeast production; when growing a microalgae consortium (*Phormidium* sp., *Oocystis* sp., and *Microspora* sp.), also, it was observed that the initial C/N/P ratio of these wastewaters was correlated with its biodegradability (Posadas et al. 2014).

By using vinasse from ethanol industry, the amount of biomass produced by *Scenedesmus* sp. is altered with light intensity and percentage of vinasse added in the medium culture, showing that this effluent can be used as a nutrient source for microalgae production (Ramirez et al. 2014). Growing microalgae in a thin stillage effluent (vinasse) generated by a starch-based (maize) ethanol production industry, it was found that *C. vulgaris* were able to degrade both organic and inorganic compounds during mixotrophic growth producing biomass at a rate of 0.9 g^{-1} L^{-1} day^{-1} (Beigbeder et al. 2019). In addition, biomass had a high protein and carbohydrate contents, and natural photosynthetic pigments were generated at a rate of 0.98 mg^{-1} L^{-1} day^{-1} (total chlorophylls) and 0.19 mg^{-1} L^{-1} day^{-1} (carotenoids). This work highlights the potential of a novel microalgae-based thin stillage phytoremediation process with simultaneous cogeneration of high value-added metabolites as a source for bio-commodities, for instance, the high protein content as supplement to animal feed or fertilizer.

Cassava residue proved to be an excellent alternative as raw material for growing several species of microalgae as described in some studies, developing sustainable efficient and ecological by-products at low cost in the remediation of effluents as the technology for wastewater biotreatment developed. Using a undiluted effluents from ethanol fermentation of cassava powder, cultivation of *C. pyrenoidosa* significantly reduced the organic amount of the residue, without altering ethanol production, and can be reused up to four times, suggesting that the treated wastewater could be recycled in the process of ethanol production entirely and directly (Yang et al. 2008). Whereas by growing *Acutodesmus obliquus* in effluent from cassava industry as a nutrient source for the biomass production had two main roles: first, it removed the nutrients (NO3, PO_4, SO_4, Cl_2, Ca, K, Mg, Na, P, NH_4, and C) for the sustained growth and then produced lipid and carbohydrate for biodiesel and bioethanol production (Selvan et al. 2019).

By using *Spirulina platensis* as cathode biocatalyst for the bio-production of oxygen and the tapioca wastewater containing native microorganisms used as a substrate in the anode chamber, 67% of organic initial amount was reduced with an electrical output generated in the same system with an affordable microalgae biomass production (Hadiyanto et al. 2019).

Conventional filtration and bio-digestion of vinasse, a residue from sugarcane industry, resulted in cleaner residues in which *C. vulgaris* grew better with higher biomass production as a potential strategy to reduce the costs of microalgae production industry (Candido and Lombardi 2017).

Auxenochlorella protothecoides and *C. sorokiniana* grew significantly faster on winery effluents than on minimal media, showing that bacteria and green microalgae provided synergistic growth benefits, which contribute to higher levels of wastewater treatment (Higgins et al. 2018).

Microalgal biomass characteristically has a high protein content which contributes toward high total ammonia concentration in the effluent. Cocultivation of *Tetraselmis suecica* microalgae with oil palm empty fruit bunch and palm oil mill effluent presented the highest specific biogas production, and biomethane yield was achieved with microalgae for anaerobic biomethane production (Ahmad et al. 2014). Growing cyanobacteria in wastewater from olive oil, the produced biofertilizers were applied on a sandy soil to grow celery plant to replace chemical fertilizers (Rashad et al. 2019).

Although the results are still limited, the studies highlight the significant potential and economic merit of recovering energy and nutrients especially from wastewater from high-strength agro-industry because they have a high organic load (Vu et al. 2020). To overcome problems of recovering wastewater from the food industry and agroindustries, it may be necessary to replace chemical treatments with sustainable technologies that use microorganisms for bioremediation.

23.6.3 Agricultural Applications

Aiming to increase the productivity of crops, intensive agriculture has caused increases in production costs due to the need to recover areas degraded by successive monocropping, unsuitable soil management, and the increasing use of fertilizers and chemicals compounds to protect plants against insects and diseases. This strategy for food production has been replaced somewhat by environmentally friendly alternatives that are taking place in agricultural practices for the production of healthy foods, comprising sustainable agriculture advances in agricultural management practices and technologies (Singh et al. 2011).

23.6.3.1 Soil Restoration

Although naturally formed or artificial cyanobacterial crusts are known as colonizers and protectors of soil surfaces in biological soil crusts, which play a key role in hydrological processes on soil restoration or soil bioremediation, they are not able to prevent desertification process as quickly (Tiwari et al. 2019). However, it has been highlighted that these photosynthetic microorganisms can be used as a tool to change unused lands in cultivated soils for sustainable agriculture, because in addition to fixing N_2, cyanobacteria in soil surface consortia might also act to immobilize and retain nutrients, for example, nitrogen, helping to reduce runoff and increase N use efficiency (Peng and Bruns 2019). The exopolysaccharides produced by cyanobacteria are important constituents for the development of biofilm that are formed on the solid surface, allowing association of microbial communities that act as a reservoir of water and nutrients. Cyanobacterial inoculation into soil to induce protective coating is an alternative approach to be addressed to prevent soil degradation or for soil restoration by improving soil aggregate stability. For instance, inoculation of a cyanobacterium *Schizothrix* cf. *delicatissima* in a sandy soil induced a colonization with the establishment of a thick crust in a very short period of time, without any change in the hydrological properties, suggesting heterogenous distribution of the trichomes and exopolysaccharides on the surface and on the bulk of the crust (Mugnai et al. 2018).

For the development of this sustainable agriculture, the understanding of microbial and plant interactions is mandatory to achieve the goals of having a healthy environment, including soil, water, and air. Most of microbial and plant interactions that are called symbioses are effective as plant growth promoting (PGP), which include microalgae, representing a potentially sustainable alternative for the improvement and protection of crops.

In agriculture, biostimulants are resources that encompass both microorganisms and substances that are applied in seeds or rhizosphere whose function is to start or to accelerate mechanisms or metabolism aiming to enhance plant growth, nutrient use efficiency, tolerance to abiotic factors, and crop quality. The use of both green microalgae and cyanobacteria brings benefits to crops, increasing yields with higher nutritional values, due to metabolites such as phytohormones, polysaccharides, amino acids, and antimicrobial compounds, which are produced by these microorganisms (Rachidi et al. 2020).

These biostimulants, besides plant protection mechanism, play an important role in the colonization of plants and growth of microbial communities in soil. In a review paper, Chanda et al. (2019) described how microalgae polysaccharides are produced, their biological activities, and their possible application in agriculture as a potential sustainable alternative for enhanced crop performance, nutrient uptake, and resilience to environmental stress.

Stimulating effects of probable active compounds contained in wheat seed-coating formulation with *Enteromorpha* sp. and *Cladophora* sp. and enrichment with mineral stimulated seed germination and the initial plant growth phase (Dmytryk et al. 2015). Microalgae contribute significantly to agricultural activity due to the ability to produce metabolites, for example, phytohormones or bioactive compounds, such as the auxins, indole-3-acetic acid (IAA), and indole-3-acetamide (IAM) from the Chlorophyceae, Trebouxiophyceae, Ulvophyceae, and Charophyceae species (Stirk et al. 2013) and from cyanobacterium *Aphanothece* sp. (Gayathri et al. 2015). Polysaccharides and antimicrobial compounds can promote plant growth directly or indirectly and, thus, become suitable for inoculation and bio-fertilization.

For sustainable horticulture and agriculture, the use of microalgae-based products might help to ensure production of food to meet the needs of human with quality and without harm to the environment. For radish (*Raphanus sativus*) plants, filtrates and homogenates of *S. platensis* that were applied for seed soaking and for foliar spray increased the length of plants in comparison to control and commercial product, showing potential as biostimulant products to be used (Godlewska et al. 2019).

In addition, this group has a role in soil nutrient cycle processes, such as mineralization of organic matter and inorganic material, immobilization, and availability of nutrients for plant and microbial community growth (Prasanna et al. 2016).

Some species of cyanobacteria and green microalgae also have the ability to solubilize chemical elements through co-coagulation processes that result in enrichment in food crops, mainly in grains with micronutrients such as iron, manganese, copper, and zinc that are essential for human and animal nutrition (Renuka et al. 2018).

Microalgae have intracellular hormones, though some can produce or excrete hormones in the surrounding environment (Prasanna et al. 2015a, 2016). It has been identified in several genera of microalgae, most all known phytohormones (e.g., auxins, cytokinins, abscisic acid, and gibberellins), jasmonic acid, and ethylene and as well their physiological activities stimulate crops (Ahmed et al. 2010; Gayathri et al. 2015; Hashtroudi et al. 2012; Mazur et al. 2001; Shevchenko et al. 2014; Stirk et al. 2013, 2002).

The growth of microalgae is controlled by the hormonal regulatory system, which might be on growth and biochemical compounds production. For *Desmodesmus* sp., when auxin and cytokine were added to the culture medium, it was observed an increase in biomass production, lipid content with higher levels of palmitic and oleic acids which are preferable constituents for achieving high-quality biofuel (Singh et al. 2020b).

For *Brassica oleracea*, the inoculation of cyanobacterial phytohormones, cytokinins, and indole-3-acetic acid (IAA) has shown to be the best for induction of adventitious roots and shoots on internodal and petiolar segments (Hussain and

Hasnain 2012). Cyanobacteria regulate the dormancy and germination of their own cells and/or other cells via phytohormone cytokinin as it was showed for *Nostoc* sp. germination dormancy cycle (Kimura et al. 2020). By combining two phases, the application of 2,4-dichlorophenoxyacetic acid with abscisic acid in culture of *Phaeodactylum tricornutum* enhanced the accumulation of biomass and lipid more than single phytohormone treatment (Zhang et al. 2020).

23.6.3.2 Biocontrol

The hydrolytic enzymes and biocidal compounds produced by microalgae have an antagonistic effect against many plant pathogens; unicyanobacterial isolates belonging to the genus *Anabaena* inhibit the growth of phytopathogenic fungi, such as *Fusarium moniliforme*, *Alternaria solani*, *Aspergillus candida*, *Drechslera oryzae*, and *Pythium aphanidermatum* (Prasanna et al. 2008).

The biocontrol of fungus and bacterial disease in plants might be due to indirect effects, which help to improve plant immunity after microalgae inoculation. *Anabaena laxa* and *Calothrix* sp. formulation applied in soils with high levels of *Rhizoctonia* spp. revealed significant reduction of cotton plant mortality, which also stimulated the activity of defense enzymes in the plants, such as β-1,3-endoglucanase activity, chitosanases, peroxidase, phenylalanine ammonia lyase, and dehydrogenase, in addition to higher levels of nitrogen and phosphorus available in rhizosphere soil (Babu et al. 2015).

With inoculation of *Calothrix* sp. in rice plants, there was an increase in the activity's peroxidase, polyphenol oxidase and phenylalanine ammonia lyase from root and shoot tissues, also, the activity of nitrogenase enzymes, CMCase, chitosanases, chlorophyll concentrations, growth, and biomass weight were higher in inoculated than non-inoculated plants (Priya et al. 2015). The activities of these enzymes are related to the quality of plants and their resistance. In addition, inoculation promoted growth and increased dry and fresh weight of the plant. The chlorophyll concentrations of rice seedlings increased 77% in the inoculated root tissues and 32% in the leaves compared to the control, and the production of EIA increased by 32% (Priya et al. 2015).

Some species of cyanobacteria that belong to the genus *Anabaena* have been described as producers of biocidal compounds that are secondary metabolites with antifungal action (Prasanna et al. 2008). Inoculation of *Anabaena* sp. in zucchini (*Cucurbita pepo*) against *Podosphaera xanthii* has shown to have both an inducer of systemic resistance and an active antifungal mean, which can be due to multiple mechanisms of enzymes, for example, chitinase with early activation and peroxidase and β-1,3-glucanase with direct antifungal activities in sporulation (Roberti et al. 2015).

23.6.3.3 Biofertilizers and Inoculants

Polysaccharides isolated from microalgae generally trigger a signaling cascade to activate the protection response of plants against salt stress, provide resistance against pathogens, represent a potential biological resource for the protection of agricultural crops, and act as biostimulants. In tomato, the use of polysaccharides from *A. platensis*, *D. salina,* and *Porphyridium* sp. improved significant development of plants compared to control. In addition, it increased the content of carotenoids, chlorophyll, proteins and nitrate reductase, NAD-glutamate dehydrogenase activities in plant leaves (Rachidi et al. 2020).

Applying foliar biofertilizer consisting of a mixture of intact cells of *Microcystis aeruginosa*, *Anabaena* sp., and *Chlorella* sp. under limited fertility conditions increased the activity of the enzymes dehydrogenases, ribonuclease, nitrate reductase, acid and alkaline phosphatase, the amount of nitrogen, phosphorus, and potassium and improved the growth of willow (*Salix viminalis*) plants (Grzesik et al. 2017).

Biofertilizer-based microalgae have shown potential for grain crops similar to a study with wheat. By using biofertilizers formulated with microalgae biomass consortia grown in agro-industry wastewater, nitrogen fertilizer dose can be reduced by 25% and the yields improved (Renuka et al. 2016). Application of cow manure combined with *Spirulina platensis* (*Arthrospira*) or *C. vulgaris* dry biomass in a sandy loam potted soil increased the development and yield of maize plants and resulted in higher content values of N and P in the shoot, and also, N, P, K, Fe, Mn, and Zn levels were improved in the seeds (Dineshkumar et al. 2019).

Application of an eco-friendly biofertilizer from biomass of cyanobacteria that were grown on olive milling wastewater significantly improved sandy soil properties and enhanced celery plant growth (Rashad et al. 2019). The produced biofertilizers were applied on a sandy soil to grow celery plant under different levels (25%, 50%, and 75%) of the recommended chemical fertilizers (Rashad et al. 2019).

Considering this background, both cyanobacteria and eukaryotic microalgae are considered as eligible for applications in the soil as biofertilizers and/or in crop seeds as simple inoculants or co-inoculations with other recommended beneficial microorganisms.

Garcia-Gonzalez and Sommerfeld (2016) showed that some applications of microalgae (e.g., *Acutodesmus dimorphus*) in vivo by cell extracts and by dry biomass can be as potential inoculants or biofertilizers in Roma tomato plants. The mix of microalgae species (*Nostoc commune* and *Nostoc carneum*) in rice contributed to promoting the growth of rice (*Oryza sativa*) seedlings by IAA and exopolysaccharide effects, suggesting that using the combined cyanobacteria biofertilizer with a half of the recommended dose of chemical fertilizer is to decrease production cost without any effects on rice quantity and quality (Chittapun et al. 2018).

The utilization of consortia/biofilms of green algae and cyanobacteria with different agriculturally beneficial microbes as biofertilizer has proved promising potential (Renuka et al. 2018) that gives an idea what the species concept of nontoxic cyanobacteria can help in most of inoculation strategies, aiming to increasing plant health and grain production. Cyanobacteria species that can fix atmospheric have been used in agriculture as a biofertilizer source, for instance, to increase biomass yield by reducing the use of fertilizer nitrogen and at the same time as conditioners to improve soil physical–chemical properties. Applying microalgae for crop production has shown results comparable to commercial treatments; besides, inoculations with these photosynthetic microorganisms have enhanced levels of carbohydrates and carotenoids in tomato fruits (Coppens et al. 2016).

Inoculants containing *Calothrix* sp. or *Anabaena* sp. in cotton improved N_2 fixation and phosphate solubilization and increased plant growth, possibly due to the release of enzymes by microalgae that degrade inorganic phosphate in the soil, increasing its bioavailability (Prasanna et al. 2015b).

Using *Chlorella* sp., *Anabaena* sp., and *Microcystis aeruginosa* as foliar biofertilizers for willow *Salix viminalis* increased the activity of enzymes assimilating nutrient dehydrogenase, nitrate reductase, acid, and alkaline phosphatase in the leaves which resulted in high shoot biomass similar to conventional fertilizers (Grzesik et al. 2017).

As biofertilizer for maize crop, *Spirulina platensis* and *Chlorella vulgaris* mixed with cow dung manure increased plant height growth, yield characters, biochemical and mineral components, and the germinability of the seeds produced (Dineshkumar et al. 2019).

In tomato, inoculation of *Acutodesmus dimorphus* as aqueous cell extracts in leaf spray and dry biomass as biofertilizer showed increased seed germination, plant growth, and vigor of seedlings, with higher effects when using living cells, and also dry biomass in earlier application had better results due to release of nutrients from biomass for plant uptake (Garcia-Gonzalez and Sommerfeld 2016). The growth of *Arachis hypogaea* and *Moringa oleifera* plants inoculated with an extra cellular products of a cyanobacterium *Aphanothece* sp. was higher than that when using commercial phytohormones, such as 6-benzylaminopurine and indole-3-butyric acid (Gayathri et al. 2015). By combining microalgae consortium, a wide range of horticultural plants have been inoculated, for example, *Anabaena laxa* and *Calothrix elenkinii* on coriander, cumin, and fennel plants (Kumar et al. 2013) and *Scenedesmus subspicatus* and humic acid on onion (Gemin et al. 2019), which in addition to promoting the growth exhibited antifungal activity.

By focusing on the biofortification of food crops to avoid problems of lack of healthy foods, microalgae-based inoculant in consortia or biofilm modes of cyanobacteria, bacteria, and green microalgae has been used as an approach to provide the enrichment of grains with micronutrients, particularly with iron, manganese, copper, and zinc, leading to improved grain quality and reduced production costs (Adak et al. 2016; Prasanna et al. 2015a, b). Inoculation of a consortium consisting of dry biomass of *Chlorella* sp., *Scenedesmus* sp., *Spirulina* sp., and *Synechocystis* sp. as pretreatment of tomato seeds as well as in foliar spray showed that, overall, seed treatment was found to be more effective than foliar spray (Supraja et al. 2020b).

23.7 Microalgae Supply Chain: Business Opportunity and Challenges

Worldwide, a significant increase in technologies for cultivation of microalgae has boosted the replacement of traditional crops and other raw material in many applications, mainly due to some advantages these microorganisms present, such as photosynthesis and fast biomass production. In a study aimed at prospecting markets for microalgae products, at least six potential major markets are found, for example, bioenergy production, bioplastics, biofertilizers, nutraceuticals, pharmaceuticals, and cosmetics (Rumin et al. 2020), although there are other consolidated opportunities, such as animal supplementation nutrition, biofibers, wastewater treatment, and soil remediation.

The growing market for products that use microalgae as a raw material, such as the dietetic and food, cosmetic, and pharmaceutical industries, has offers for business opportunity.

In the production chain, the main green microalgae (Chlorophyceae) produced aiming for biotechnological industries are *Tetraselmis*, *Chlorella*, *Dunaliella*, *Haematococcus*, *Nannochloropsis*, and cyanobacterium *Arthrospira* (*Spirulina*). With respect to the application of microalgae for the extraction of valuable bioproducts, they are used in different forms such as liquid, concentrates, extracts, powder, or flakes. Microalgal biomass has been used principally in food and feed industries as sources of important by-products, for example: (1) *Chlorella* and *Arthrospira* (*Spirulina*) are sources of functional foods, nutraceuticals, and health supplements (Osorio-Fierros et al. 2017); (2) *Dunaliella* is a rich source of natural β-carotene (Einali et al. 2017); (3) *Tetraselmis* is a source of protein and omega-3 (Riccio et al. 2020); (4) *Haematococcus pluvialis*, a source of astaxanthin produced and marketed in the form of powder, is available in orange- to red-colored powder or flakes for further extraction (Ahmed et al. 2015).

For biotechnological applications of microalgae, there is a huge opportunity for the discovery of novel bioactive metabolites, including the identification of compounds with potential antimicrobial, antifungal, and antitumorigenic, and already, there are many species for this purpose, such as *Tetraselmis chuii*, *C. sorokiniana*, and *Chondrus crispus* (Barkia et al. 2019).

Similar to human food and nutrition, microalgae applications for animals have a great challenge to animal production. Animal feed needs to become less dependent on expanding arable land and less impact on the environment; in this context, microalgae have been used as a source of protein, for example, Spirulina to poultry that benefits to color and flavor to kind of animal meat (Altmann et al. 2020).

The challenges for microalgae supply production chain have been on industrial integration with an analogous system to the concept of ecological symbiosis, where the interaction between different industries has higher advantages than operating alone which results in ecological industrial parks. In these conglomerates of enterprises, waste from industry A becomes the input for the production of microalgae in industry B; the raw material produced in industry B will be inputs for the production of coproducts in other industries. A good example of the environmental application of microalgae is bioremediation using the integrated agro-industry-biorefinery, as described by Kumar et al. (2020) for the dairy effluent treatment system based on the production of microalgae biomass in a large-scale plant to reuse water instead of the drinking water that is currently used for the growth of microalgae.

From the perspective of microalgae supply chain in a circular economy, the concepts are to reduce and to reuse resources for a longer period of time which can reduce greenhouse gas emissions and significantly reduce the volume of waste (Nagarajan et al. 2020). Feasibly, the most significant impact on the microalgae production chain is evidence of circular zero-residue process using these microorganisms for efficient water decontamination, biofuel production, and carbon dioxide fixation (Serrà et al. 2020). Take into consideration that there is significant interest in recycling water from hydroponic plant cultivation, shifting to a sustainable production system where residues become nutrients in new processes, products or materials can be repaired, reused, updated, or re-inserted in new cycles with the same or better quality instead of being discarded. In cocultivation mode, microalgae have been studied for growing the cyanobacterium and raising chrysanthemum nursery (Bharti et al. 2019) with tomato plants (Barone et al. 2019; Supraja et al. 2020a) and by utilizing coproduction of *Chlorella vulgaris* with arugula, purple kohlrabi, and Lettuce (Huo et al. 2020).

The option for a sustainable growth of the microalgae supply production chain, aiming to reduce the release of waste into the environment close to zero is the integration of industrial production plant units in shared areas in partnership with other agro-industry, the eco-friendly industrial parks.

Regarding the production of renewable energy from microalgae, biorefineries are the key to the integration of the state of the art in global scenarios; however, the intricate process design is triggered mainly by the lack of suitable technologies especially in cell disruption and extraction of specific compounds. One of the challenges for the microalgae production chain is still in large-scale biomass production, requiring investment in the line of research, development, and innovation.

23.8 Conclusions

The variety of products accessible from the primary and secondary metabolism of diverse algal species clearly demonstrates the importance of these versatile microorganisms as cellular factories. Microalgae have recently attracted considerable interest worldwide, due to their renewable, sustainable, and economical sources of extensive application potential in the renewable energy, biopharmaceutical, nutraceutical industries, biofuels, bioactive medicinal products, and food ingredients. Several microalgae species have been investigated for their potential as value-added products with remarkable pharmacological and biological qualities.

When the goal is to share microalgae production chains with another agro-industry production chain in eco-parks that generates nutrient-rich wastewater, growing microalgae in wastewater to replace conventional method of treatment of an industrial effluent requires only few investment and operating cost which is a promising future of microalgae cultivation.

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