doi: 10.2166/9781789063547_0229

Chapter 12

Processes and biorefinery approach for enhanced algal bioproduct recovery in the form of lipid and UV protectant

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ABSTRACT

This chapter discusses various methodologies for lipid extraction, including solvent extraction, enzymatic treatment, ultrasonic aided extraction, and supercritical carbon dioxide extraction, underscoring the need for further research and optimization for large-scale applications. The chapter further explores the potential symbiotic relationship between algal fuel production and waste treatment. This strategy effectively utilizes microalgae's natural ability to thrive in adverse conditions and sequester CO_2 and other pollutants. This approach can simultaneously reduce the environmental footprint while generating valuable biomass for biodiesel production. Another noteworthy point the chapter brings forward is the ability of microalgae to produce valuable compounds under environmental stress, particularly UV radiation. The UV-absorbing compounds such as mycosporine-like amino acids (MAAs) and scytonemin, present substantial potential for use in the cosmetic and pharmaceutical sectors due to their potent UV absorption and photoprotective properties.

12.1 INTRODUCTION

The idea of a 'biorefinery' has arisen as a set of integrated processes for turning microalgal biomass into fuel and other high-value products (Cherubini, 2010; Thomassen *et al.*, 2018). A more sustainable and cost-effective strategy that just concentrates on fuel production is made possible by the diverse and complementary outputs (Salama *et al.*, 2018). Based on current capital costs per unit of fuel production, the generation of biofuel from microalgae is not economically viable. As a result, producing high-value co-products is necessary to increase a microalgae biorefinery's profitability.

Microalgae are microbial factories that can produce a variety of substances besides lipids for biodiesel, having a lipid (7–23%), carbohydrate (5–23%), and protein (6–52%) composition (Chandra *et al.*, 2014). Microalgae can be an excellent source of raw materials for commercially significant value-added products utilized in the food, nutraceutical, cosmetic, and pharmaceutical industries (Haznedaroglu *et al.*, 2016). An integrated biorefinery can maximize product outputs from a single biological source, capturing the value of numerous components (Oh *et al.*, 2018).

The concept of a biorefinery was inspired by petroleum refineries, which provide fuels, oils, and other materials used in the chemical industry (Roux *et al.*, 2017). A biorefinery uses a series of interconnected processes to utilize all the components of the raw materials without causing any loss or harm to the finished goods. In an algae-based biorefinery, there are major hurdles to the sustainable extraction of these chemicals when taking green chemistry principles into account (Yellapu *et al.*, 2018). Maximizing microalgae biomass utilization requires a lot of energy, while utilizing the least amount of energy is still the key goal (Bakonyi *et al.*, 2018). For instance, the Department of Energy's (DOE) primary goal, as stated in the outlook provided in the U.S. multi-year program plan, is cost reduction to produce algal biofuel (Barry *et al.*, 2016).

In this chapter, the most recent research on how to effectively use algal biomass in a sustainable way by using biochemical processes and a bio-refinery technique is discussed. In addition, the framework enables an algal bio-refinery to effectively create value-added products like oil and UV protectant. This chapter also provides a thorough overview of current advancements in the processing of algal biomass utilizing various sustainable methods in an integrated biorefinery.

12.2 FERMENTATION

12.2.1 Selective fermentation

Despite its benefits, microalgal biofuel has not been commercially successful in part because of technical and financial difficulties with algae harvesting and lipid extraction. Pretreatment techniques including pulsed electric fields (PEFs), ultrasound, and acid/alkaline hydrolysis can be effective but are typically too energy-intensive and therefore expensive (Lai *et al.*, 2014; Laurens *et al.*, 2015; Sheng *et al.*, 2011a; Zbinden *et al.*, 2013). To lessen risks to the environment and workers, the present 'gold standards' for lipid extraction, Folch (1:1 chloroform:methanol) and Bligh & Dyer (1:1:0.5 chloroform:methanol:water), must be replaced by non-toxic 'green' solvents. Hexane and isopropanol mixed 1:1 (v/v) is an illustration of a non-toxic solvent (Lai *et al.*, 2014, 2016a, 2016b).

A revolutionary biological strategy for simplifying and improving the economics of lipid extraction is called selective fermentation (SF) (Lai *et al.*, 2016a, 2016b). SF takes advantage of the fact that, under anaerobic conditions, lipids typically biodegrade more slowly than do carbohydrates and proteins. Because they grow slowly, lipid-fermenting bacteria (Christ *et al.*, 2000) can be removed from a reactor with a short solids retention time (SRT) by their washout (Lai *et al.*, 2016a, 2016b). As a result, SF permits the fermentation of carbohydrates and protein in microalgae cells while leaving lipids unaltered. Yet this results in a condition that is much easier to extract because the 'protection' provided by the carbohydrates and proteins has been removed (Lai *et al.*, 2016a, 2016b). Protein fermentation may be a bottleneck in SF since it proceeds more slowly than carbohydrate fermentation (Lu *et al.*, 2012).

The process of biohydrogenation, which transforms long-chain fatty acids (LCFAs) into saturated forms like C18:0, C16:0, and C14:0, is another advantage of SF. Because they have a higher energy content, a higher-octane number for improved combustion efficiency, and a stronger resistance to oxidation, saturated fatty acids are advantageous for the production of transportation fuel (Knothe, 2011). There are two main ways that biohydrogenation can take place. One method is the direct conversion of unsaturated bonds to saturated bonds. In this process, H_2 serves as the electron donor and the LCFA molecule's carbon content remains constant (Lai *et al.*, 2016a, 2016b). An example of direct biohydrogenation is the reduction of C18:1 to C18:0.

Low H_2 concentrations can thermodynamically limit direct biohydrogenation, whereas high H_2 concentrations can accelerate direct biohydrogenation (Cavaleiro *et al.*, 2016; Lai *et al.*, 2016a, 2016b). Strains of the genera *Butyrivibrio* and *Pseudobutyrivibrio* (both in the order Clostridiales) can directly biohydrogenate C18:2 n-6 and C18:3 n-3 to C18:0 (John Wallace *et al.*, 2006; Van De Vossenberg & Joblin, 2003). The family Porphyromonodaceae (Order Bacteroides) and Ruminococcaceae (Order Clostridiales) are involved in direct biohydrogenation in ruminants, according to in vivo research (Castro-Carrera *et al.*, 2014; Huws *et al.*, 2011).

The second method involves the beta-oxidation process, which converts an unsaturated LCFA into a saturated LCFA with the loss of two C atoms as acetate (Cavaleiro *et al.*, 2016). An example of the second route is transformation of C18:1 to C16:0.

As H_2 is produced during beta-oxidation, this pathway does not require an external source of H_2. In theory, processes that utilize H_2, acetate, or both might give this method of biohydrogenation a thermodynamic boost (Cavaleiro *et al.*, 2016).

Lipid conservation is valuable and varies with the biohydrogenation route. β-Oxidation reduces saturated LCFA chain length by two C atoms per step, and the loss is more substantial if multiple steps of beta-oxidation occur. An example is the transformation from C16:0 to C14:0, which produces 2 moles of H_2 and 1 mole of acetate per 1 mole of C14:0 produced.

12.2.2 Electrofermentation

Anode respiring bacteria (ARB) establish a biofilm on the anode in microbial electrolysis cells (MECs), oxidize short-chain fatty acids (SCFAs), and then extracellularly transmit the extracted electrons to the anode (EET) (Reguera *et al.*, 2005; Torres *et al.*, 2009b, 2009a; Yang *et al.*, 2012). Through the external circuit, electrons move to the cathode, where they are absorbed by water molecules to create H_2, which emerges from the cathode as a gas. The MEC is a potential technique for accelerating protein biodegradation in substrates made of complex organic molecules in case of microalgae (Lu *et al.*, 2012).

Due to the need for pretreatment and the use of harmful solvents, extracting lipids from microalgae has been shown to be both commercially and environmentally unfeasible. By selectively biodegrading proteins and carbohydrates while preserving lipids, SF aids in the resolution of these issues. Electro-selective fermentation (ESF) enhances the fermentation performance through anode respiration in a microbial electrolysis cell (MEC) (Liu *et al.*, 2019). ESF was assessed and compared to SF using biomass from *Scenedesmus acutus*. Even though anode respiration only accounted for 1% of the total electrons supplied, ESF boosted protein breakdown three times more than SF did. Although ESF increased the total lipid loss, it tripled the effectiveness of lipid wet extraction with a non-toxic solvent.

The long-chain fatty acid (LCFA) profile changed from C18:1 to C16:0 and C14:0 as a result of lipid loss caused by beta-oxidation associated with biohydrogenation. Anode-respiring bacteria (ARB) on the ESF anode and protein-degrading bacteria and biohydrogenaters in the ESF suspension were highlighted by microbial community analysis. Overall, ESF enhanced the quality of biofuel and lipid extractability.

12.2.3 Coupling SF and electrofermentation

A combination ESF is created with the aid of the MEC and SF. It aids in enhancing protein and carbohydrate conversions while protecting lipids for extraction. A strong biofilm of ARB oxidizes SCFAs quickly in the ESF (Ki *et al.*, 2015; Torres *et al.*, 2007), leading to a low concentration of SCFAs in the anode liquid. By reducing a thermodynamic barrier, a lower concentration of SCFAs should encourage upstream fermentation reactions (Fukuzaki *et al.*, 1990; Jones *et al.*, 2015; Pratt *et al.*, 2012). Unfortunately, this method could potentially speed up beta-oxidation as well, which would lead to a loss of all LCFAs.

H_2 is also an ARB substrate, either directly or indirectly through its homo-acetogenic conversion to acetate (Parameswaran *et al.*, 2009). A well-known strategy for overcoming thermodynamic obstacles to fermentation is scavenging H_2 (Cavaleiro *et al.*, 2016; Parameswaran *et al.*, 2010). ESF may therefore hasten the fermentation of proteins and carbohydrates. The loss of protein could cause the cell membrane to rupture and release intracellular lipids for easy extraction by interfering with the hydrogen bonding between membrane proteins and lipids (Cooney *et al.*, 2009; Sheng *et al.*, 2011b).

12.3 BIODIESEL EXTRACTION FROM MICROALGAE

12.3.1 Pretreatment

Several techniques can be used to algae biomass in order to extract intracellular substances. There are various conversion techniques, but the mechanical-based techniques are among the most significant (Cherubini *et al.*, 2009). The biomass is concentrated once microalgae cultures reach the stationary growth phase, and the desired products can be recovered using either dry or wet biomass. To break down the cellular walls and encourage the release of microalgae components that are not released outside the cell, the initial biomass can be dewatered by centrifugation, which is then followed by a cell disruption technique. The approaches employed typically involve a disturbance, break, or breakdown (Dong *et al.*, 2016). The biomass is then thermally dried to obtain a dried form after the dewatering process, which typically results in a paste-like biomass with a dry weight above 85% (Xu *et al.*, 2011).

12.3.2 Extraction

12.3.2.1 Principle of solvent extraction

One of the primary methods for recovering valuable compounds from microalgae is organic solvent extraction. Based on the polarity of the target chemicals, solvents should be selected. Because TAGs, the primary lipid target for the manufacture of biodiesel, are non-polar molecules, a non-polar solvent is an appropriate option for extraction. The majority of solvent-based extraction methods used to extract lipids from microalgae are based on conventional procedures for extracting plant oils, including organic solvent extraction, the Folch method, and the Soxhlet method. Organic solvents penetrate the cell wall of the microalgae, where they promote swelling and cell rupture, releasing the contents of the cell for further separation steps (Grima *et al.*, 2003). When selecting a solvent to extract lipids from microalgae, the primary factors to take into account are polarity or extractability, lipid solubility, water miscibility (ability to operate in two-phase systems), and low toxicity (Bensalem *et al.*, 2018).

12.3.2.2 Solvent extraction methods

12.3.2.2.1 Folch method

The Folch method, which is the foundation of many solvent extraction techniques currently in use, uses a 2:1 chloroform–methanol mixture to extract intracellular lipids. A cell homogenate is first stirred to equilibrate with 25% volume of saline solution. The lipids are allowed to settle on the top layer of this mixture until biphasic separation (Ranjith Kumar *et al.*, 2015). This procedure requires the breaking of microalgae cell walls as a preliminary step. It was initially intended for animal cells and tissues (Grima *et al.*, 2003).

12.3.2.2.2 Soxhlet extraction

In the Soxhlet extraction (SE) procedure, components of a solid sample that are only partially soluble are transported to a liquid phase (solvent) using a Soxhlet extractor. This method uses hexane and other non-polar solvents to produce neutral lipids. The extraction process involves inserting the solid sample into the Soxhlet apparatus's main chamber in a filter paper thimble. The solvent is then heated to reflux and forced into the main chamber, where the less soluble chemicals are recovered. Due to the recovery of complex lipids and pigments, a greater extraction yield from microalgae can be attained when the extraction solvent polarity increases (Baumgardt *et al.*, 2016). This is a crucial factor to take into account because whole lipid extracts using polar solvents are complicated and contain other metabolites besides lipids. The characteristics of a Soxhlet extraction are the solvent of choice, sample particle size, and extraction time (Sharif *et al.*, 2014). SE is typically done on a small scale in the lab and requires a lot of solvent and a long extraction period.

12.3.2.3 Bligh and Dyer method

The Bligh and Dyer method involves partitioning and extracting lipids simultaneously, with protein precipitation occurring at the interface of two liquid phases. This extraction method is comparable to

the Folch method, but with a different solvent combination composition and ratio. A cell homogenate's lipids are first extracted with a 1:2 solution of chloroform and methanol, and the chloroform phase – which is rich in lipids – is then recovered. Lipids from microalgae are removed and quantified using gravimetry. Both pilot and large-scale operations use this approach (Ranjith Kumar *et al.*, 2015). Instead of using water, this approach can be improved by adding 1 M NaCl to prevent the binding of acidic lipids to denatured lipids. The addition of 0.2 M phosphoric acid and HCl has resulted in shorter separation times. By adding 0.5% acetic acid (v/v), acidic phospholipid recovery has been improved (Ranjith Kumar *et al.*, 2015).

12.3.3 Mechanical methods

Solid shear, cavitation and collapse, PEFs, chemical hydrolysis, enzymatic digestion, subcritical water extraction, high-pressure homogenization, and bead milling are a few techniques used to destroy cells and thus release their content.

12.3.3.1 Milling

Bead milling is the process of breaking down the walls of microalgae cells by agitating and grinding the cells over a surface made of glass beads (Ghasemi Naghdi *et al.*, 2016). A disruption needs beads that are between 0.3 and 0.5 mm in size. Typically, zirconia–silica or zirconium oxide can be used to create the beads. The temperature, biomass concentration, flow rate, agitator movement type, and speed all affect how effectively the process works.

Milling can be carried out using agitated beads or shaken vessels. In the shaking vessel method, a vibrating platform is used to shake the culture vessel, which causes the microalgae cells to migrate and crash into one another. When Ryckebosch *et al.* (2012) used this technique, they were able to recover 40% of the lipids from a culture of *Phaeodactylum tricronutum,* which was the highest lipid recovery achieved. On the other hand, Zheng *et al.* (2012) used a bead milling vessel to extract 11% of lipids from a culture of *Chlorella vulgaris*. According to Lee *et al.* (2010) agitated beads use a method in which the beads and the culture are stirred around by a rotating agitator inside the culture vessel while also being heated to aid in the disruption process. Using cultures of *Botryococcus* sp., *Chlorella vulgaris*, and *Scenedesmus* sp., the authors employed this methodology and obtained an oil yield between 7.9 and 8.1 g/L.

12.3.3.2 Pressing

One of the traditional techniques for obtaining value-added goods from a variety of sources is the use of presses (Kumar *et al.*, 2020a, 2020b). The mechanical crushing of materials with a very low moisture content is the foundation of this technique. Dried biomass is first put under intense mechanical pressure to shatter and crush the cells, and then it is squeezed to extract the oil. Variations in the pressure force, algae strain, and press and piston arrangement can all increase the extraction efficiency (Kumar *et al.*, 2020a, 2020b). With the gel-press approach, algae are first rinsed before employing diluted alkali to extract the carbohydrates. Centrifugation is used to separate the residues, then they are filtered through porous silica, and finally concentrated using evaporation. The recovered material is extruded into a cold potassium chloride solution using spinnerets, and the threads that have gelled are then compressed to remove water (Amin, 2009).

High pressures are used by shear-based machines like the French press and Hughes press to push a biomass solution through a tiny aperture. The average oil recovery is between 70% and 75% (Kumar *et al.*, 2020a, 2020b). Mechanical crushing is occasionally employed in addition to chemical procedures for better oil recovery. The primary limitations of this technology are that it requires expensive maintenance and is less effective than other mechanical extraction methods (Ranjith Kumar *et al.*, 2015).

12.3.3.3 Freeze–thaw method

Since the loss of volatile lipids owing to evaporation is reduced to a minimum with the freeze–thaw process, lipid extraction from microalgae biomass is favored. By freezing the wet biomass at a temperature of −80°C, the intracellular water crystallizes in this process. The samples are then

thawed, causing the ice crystals to expand and lyse the frozen cells. To maximize yield efficiency, this process is typically used in conjunction with another technique, such as ultrasonication, microwave-assisted extraction (MAE), or bead milling (Esquivel-Hernández *et al.*, 2017; Parfati *et al.*, 2018). Cycles of freezing and thawing must be carefully controlled, though. Unfrozen samples showed a 10% decrease in reproducibility after the first cycle and a further 7% decrease after the second, according to a study of the metabolic profile of marine microalgae after freeze-thawing under standard freeze-storage temperatures (–20°C and –78°C) for 1 and 2 cycles of 7 days each (Chr. Eilertsen *et al.*, 2014).

12.3.4 Enzymatic methods

A mixture of enzymes is used in enzymatic extraction procedures to dissolve the algal cell wall, expel lipid bodies from the cell, and separate the lipid fraction from the lipid/protein matrix. An alternative to mechanical cell destruction is enzymatic lysis. Due to the presence of polysaccharides like cellulose and hemicellulose in algal cell walls and lipids, packed in a sac surrounded by phospholipids, in algal cell walls, the lytic enzymes must be specific for the microalgae species, with cellulase and lipase being the most prevalent (Parfati *et al.*, 2018).

Microalgae lipids can be extracted using the cell disruption method known as aqueous enzymatic aided extraction (AEAE). High selectivity, gentle reaction conditions (neutral pH, incubation from 25°C to 37°C), and the lack of labor-intensive drying processes are noteworthy characteristics (Sierra *et al.*, 2017). The best extraction parameters were determined to be 37°C, pH 5.0, 1.3% cellulase, liquid/solid ratio 15 mL/g, and 5 h. An improved approach for enzymatic lysis combined with thermal treatment for extracting lipids from *N. oceanica*. Up to 28.8% of lipids were produced under these circumstances (Amin, 2009).

Biomass collection, enzyme conditioning and addition, stirring incubation to break down algal cell walls, solvent addition (if necessary), centrifugation, and lipid fraction recovery are the primary steps in the enzymatic extraction of lipids from microalgae (Lee *et al.*, 2010). Moreover, after the removal of lipids, the carbohydrate biomass can be saccharified via enzymatic digestion to produce bioethanol (Parfati *et al.*, 2018).

12.3.5 Physical extraction methods

12.3.5.1 Supercritical fluid extraction

By exerting pressure and temperature above a compound's or mixture's critical point, supercritical fluid extraction (SFE) makes use of a supercritical fluid's solvating capability. Solvent, temperature, pressure, solvent flow rate, extraction time, sample size, usage of a modifier, and particle size are some of the adjustable parameters to take into account for SFE (Sharif *et al.*, 2014).

To avoid using hazardous solvents, supercritical fluid extraction with carbon dioxide (SFE-CO_2) has been used as an alternative green extraction technique (Hernández *et al.*, 2014). SFE-CO_2 has several benefits, including being generally recognized as safe (GRAS) by the Food and Drug Administration (FDA), having a low critical point of CO_2 at near room temperature and relatively low pressure (30.9°C and 73.9 bar), and being ecologically benign (Reverchon & de Marco, 2006). Moreover, CO_2 is converted to gas after depressurization, which allows it to be removed from the sample without leaving any traces of solvent behind. This allows it to be recycled for additional extraction cycles, which has both financial and environmental advantages. Supercritical CO_2, which is especially helpful for the extraction of biodiesel, is very selective for non-polar lipids like triglycerides and does not solubilize phospholipids (Hernández *et al.*, 2014). Hydrocarbons (hexane, pentane, and butane), nitrous oxide, sulfur hexafluoride, and fluorinated hydrocarbons are some of the additional solvents employed in SFE (Reverchon & de Marco, 2006).

12.3.5.2 Microwave-assisted extraction

MAE depends on the interaction of a dielectric polar substance (such as water) and a rapidly oscillating electric field created by microwaves (Esquivel-Hernández *et al.*, 2017; Moretto *et al.*,

2022). This electric field generates heat as a result of the friction created by the movement of the molecules within and between it. The cell begins to produce water vapor as a result of the heat, which finally ruptures the cell and leads to increased intracellular component leakage and release, driven by the electroporation action (Ghasemi Naghdi *et al.*, 2016). As a result, MAE is recognized as a quick, easy, safe, efficient, and affordable approach for the extraction of lipids that does not necessitate sample dewatering beforehand (Bensalem *et al.*, 2018). Moreover, microalgae processed with microwaves have numerous microfissures in the cell wall, which increases the amount of bio-oil recovered (Šoštarič *et al.*, 2012).

In addition to oil extraction, microwaves can be used to transesterify oils into biodiesel, which is a desirable alternative due to its quick reaction time (15–20 min), low operating costs, and effective extraction of algal oils. The substantial maintenance costs associated with using this technology on a commercial scale are a significant downside (Kumar *et al.*, 2015). The primary factors to be considered for MAE are extraction time, temperature, the process mixture's dielectric characteristics, the solid/liquid ratio, and the kind and concentration of the solvent (Ghasemi Naghdi *et al.*, 2016).

12.3.5.3 Ultrasound-assisted extraction

Using cavitation, ultrasonic-assisted extraction (UAE) can recover oils from microalgae cells (Harun *et al.*, 2010). Little vacuum bubbles with a high intensity are produced in the liquid during the low-pressure cycle. A high-pressure cycle occurs when the bubbles violently collapse once they reach a particular size. Locally, extremely high pressures and fast-moving liquid jets are created during the implosion, and the ensuing shear stresses cause the mechanical breakdown of the cell structure. The extraction of lipids from algae is supported by this outcome (Wei *et al.*, 2008). Solvent diffusion into the cell structure is supported by the high-pressure cycles of the ultrasonic waves. Lipids are more easily transferred from the cell into the solvent when using ultrasound because it mechanically breaches the cell membrane through cavitation shear pressures (Cravotto *et al.*, 2008).

By extending the exposure period and combining polar and non-polar solvents, lipid recovery can be improved. UAE also supports mass transfer and solvent penetration inside the cell to release the contents of the cells into the solvent. UAE may be carried out at low temperatures, which is ideal when dealing with the extraction of compounds that are thermally sensitive (Ghasemi Naghdi *et al.*, 2016).

12.3.5.4 Pressurized liquid extraction

Wet algal biomass is used in the wet lipid extraction procedure along with a corresponding amount of solvent (Al-Jabri *et al.*, 2022; Sathish & Sims, 2012). Although it differs depending on the biomass type, this technique is similar to the wet solvent extraction procedure (see Section 3.2). Biomass is transformed into liquid biocrude through the process of hydrothermal liquefaction in hot, compressed water (Biller *et al.*, 2012; Zhang *et al.*, 2022). Because the water must remain in the subcritical area to prevent the latent heat of vaporization, processing temperatures vary from 200°C to 350°C with pressures of around 15–20 MPa (Biller *et al.*, 2012). Complex molecules are disassembled and repolymerized to oily substances under these circumstances. This process eliminates the need to dry the feedstock, making it suitable for converting high-moisture biomass like microalgae.

12.3.5.5 Osmotic pressure

A quick shift in the solute concentration around a cell results in a rapid alteration in the transport of water across the cell membrane, which is known as osmotic shock or osmotic stress (Fajardo *et al.*, 2007). The microalgae's cellular contents are released as a result of this shock. The technique works better with strains grown in maritime conditions (e.g. *Nannochloropsis* sp.). To release cellular components for biochemical examination, osmotic stress is also generated (Alami *et al.*, 2021; Shen *et al.*, 2010). *Halorubrum* sp. isolated from saltern ponds can likewise be treated using this technique. Increased lipid productivities and different lipid compositions were observed (Lopalco *et al.*, 2004).

12.3.5.6 PEF technologies

A technique called pulsed electric field (PEF) processing uses brief bursts of a powerful electric field to process cells (Chittapun *et al.*, 2020). Between two electrodes, algae biomass is positioned, and a PEF is applied (Käferböck *et al.*, 2020). The holes in cell membranes are made larger and release their contents when exposed to an electric field (Wang *et al.*, 2023).

12.4 BIOREFINERY

12.4.1 Cyanobacterial biorefineries

The most basic type of photosynthetic microorganisms, cyanobacteria, have a significant potential for the generation of bioenergy as well as high-value food and pharmaceutical items (Thajuddin & Subramanian, 2005). Since it remains a difficult task, extensive research is being done to turn cyanobacterial lipid into a significant industrial process (Patnaik & Mallick, 2015). In recent years, the process of scaling up and commercializing cyanobacterial or microalgal products has begun, but cautious and methodical development is required to make it a sustainable industrial process.

At an industrial scale, cyanobacteria have enormous promise for recovering value-added products and biofuels. They are rich in lipids, susceptible to metabolic engineering, and contain value-added components like antioxidants (Esquivel-Hernández *et al.*, 2017), phycobiliproteins (Chandra *et al.*, 2017), UV protectants (Rastogi & Incharoensakdi, 2014), and vitamins (Esquivel-Hernández *et al.*, 2017). This makes it a potential feed stock for biorefinery (Sheng *et al.*, 2011b; Vermaas, 1996). Despite the fact that cyanobacteria are ideally suited for biorefining due to the diverse composition of their biomass, recovering co-products from cyanobacteria remains a difficult problem. Therefore, it is necessary to investigate moderate and sequential extraction techniques that maintain the usefulness of different cell compounds such as UV protectant, protein, vitamins, lipid, and carbohydrates.

12.4.2 Cyanobacterial biorefinery products

12.4.2.1 Biodiesel

A possible renewable feedstock for the manufacture of value-added goods and ethanol appears to be cyanobacteria. A comprehensive assessment of multiple product recovery is required to guarantee the economic and sustainability of biofuel production. Chandra *et al.* (2019, 2020) provided strong evidence in favor of producing mycosporine-like amino acids (MAAs) and high-quality biodiesel in succession. A procedure in which *Lyngbya* biomass was sequentially collected from all experimental variations after UV irradiation and treated with 100% HPLC-grade methanol for 12 h at 4°C before being centrifuged at 4000 rpm for 15 min at 4°C. The supernatant was gathered, dried at 38°C, and combined with the pellet for lipid extraction. It was determined that the dried methanolic residue is MAAs. To remove the photosynthetic pigment, this MAA was washed with chloroform and water. Together with the residue from the previous stage, the aqueous phase used to collect MAAs and the chloroform phase were both treated for lipid recovery. According to this method, recovering UV protectant after UV exposure with biodiesel is a more sustainable solution for high fuel productivity. The manufacture of algae biodiesel gains value as a result of this procedure. The problem of heterotrophic bacterial contamination is lessened by UV exposure, and the lipids content and saturation index of biodiesel are increased. This results in a biorefinery that is both affordable and sustainable (Chandra *et al.*, 2020).

12.4.2.2 UV protectant

Due to its great market demand and value, UV protectant could be a significant product of an algal biorefinery. For instance, it is predicted that by 2024, the global demand for this kind of goods will increase to $13.2 billion from more than $7.6 billion in 2012 (Oilgae, 2014). By doing so, it is possible to enhance the value of the process, the number of products with added value, and the environmental impact all at once. Because of their special makeup, microalgae can contain a variety of pigments, such as UV filters and carotenoids, astaxanthin, lutein, zeaxantin, phycocyanin, and phycoerythrin.

Oil accumulation in microalgae is known to be significantly affected by exposing cells to environmental stress (ultraviolet radiation) (Arakaki *et al.*, 2017), nutrient depletion (Pancha *et al.*, 2014), salinity (BenMoussa-Dahmen *et al.*, 2016), oxidative stress (Yilancioglu *et al.*, 2014), temperature or pH changes. In order to reduce the stress condition, these parameters also have an impact on the other cell components and trigger the production of new molecules. Ultraviolet radiations (UVR) are a key method in this regard to generate UV inhibitors like mycosporin-like amino acids (MAAs) and investigate their impact on lipid productivity (Chandra *et al.*, 2019). By manufacturing MAAs and scytonemin, cyanobacteria are known to defend themselves against photochemical harm (Chandra *et al.*, 2020). They reside in the sheath of cyanobacteria and are lipophilic. Because the growth medium is not changed and heterotrophic contamination may be minimized, UVR provides various benefits for oil production.

A range of defense mechanisms are used by the cyanobacteria and marine algae group to endure and thrive under high UV fluxes. These organisms synthesize UV-absorbing substances like mycosporine-like amino acids (MAAs) and scytonemin as a mitigation mechanism (Chandra *et al.*, 2019, 2020). By scavenging significant amounts of reactive oxygen produced by supersaturated oxygen in deep, light-exposed water, MAAs display substantial antioxidant activity. The 3-dehydroquinate and 4-deoxygadusol precursors of the shikimate pathway are the sources of the main MAA, mycosporine-glycine (Chandra *et al.*, 2019). Mycosporine-glycine is converted into secondary MAAs via the addition or subtraction of amino acids as well as metabolic processes. However, it has been discovered that the cyanobacterium *A. variabilis* has an MAA biosynthetic gene cluster that transforms sedoheptulose-7-phosphate to shinorine via 4-deoxygadusol and switches the precursor 3-dehydroquinite (Balskus & Walsh, 2010). Tryptophan and tyrosine, two aromatic amino acids that are byproducts of the shikimate pathway, are thought to be the source of scytonemin. Moreover, a cluster of genes for scytonemin production has been found, and UV-A activation of these genes has been demonstrated (Rastogi *et al.*, 2015). MAAs and scytonemin can be used as active ingredients in the cosmetic and pharmaceutical sectors due to their potent UV absorption and photoprotective qualities.

12.5 CONCLUSION

Establishing a sustainable algal biomass-based biorefinery requires multidisciplinary research on biorefinery methodologies. A multi-product, integrated, and sustainable approach is crucial for efficient product recovery and process development. Microalgae biomass serves as a flexible feedstock for biodiesel production, utilizing techniques such as photobioreactors, fermenters, and open raceway ponds. Various lipid extraction techniques have been explored, including solvent extraction, enzymatic treatment, ultrasonic-aided extraction, and supercritical carbon dioxide extraction. Optimization work is needed for large-scale applications, particularly for supercritical carbon dioxide extraction (SFE-CO_2), PEFs, osmotic shock, hydrothermal liquefaction, and wet lipid extraction. Integrating algal fuel production with wastewater and waste treatment enhances economic viability. UV exposure stimulates UV defense synthesis and lipid productivity in *Lyngbya*, with UVB favoring fuel qualities and UVA benefiting food properties. The recovery of lipids and UV protectants from the same feedstock promotes cost-effective and environmentally responsible options in a sustainable biorefinery.

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