Chapter 12
Third Generation Biorefineries Using Micro- and Macro-Algae

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Abstract Algal biomass, which contains a range of biochemical components such as carbohydrates, lipids, and protein, has emerged as a possible alternative to traditional feedstocks for third-generation biofuel production and industrially high value-added bioproduct extraction. Micro- and macro-algae are gaining popularity as viable feedstock for biofuels such as biodiesel, biogas, bioethanol, and biohydrogen. Other high-value-added bioproducts must be extracted from algal biomass under the biorefinery concept to improve the economic feasibility of algal biofuel production. In this chapter, techniques for algal biofuel production are discussed, such as biochemical and chemical conversion routes, extraction of bioproducts, and advanced techniques in cultivation, extraction, and starch saccharification along with biofuel and bioenergy conversion schemes. Overall, micro-and macro- algae biorefineries open up new possibilities for many new products. The multiproduct biorefinery technique is expected to make micro-and macro-algal technology highly competitive and pave the way for large-scale applications.

Keywords Biorefinery · Extraction process · Biofuels · High value-added products · Biomass valorization · Bioethanol

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Nomenclature

CBP	Consolidated bioprocessing
GHG	Greenhouse gases
ORP	Open raceway pond
SHF	Separated hydrolysis and fermentation
SSCF	Simultaneous saccharification and co-fermentation
SSF	Simultaneous hydrolysis and fermentation
ppm	Part per million
dw	Dry weight
PLE	Pressurized liquid extraction
SFE	Supercritical fluid extraction

12.1 Introduction

Over the last few decades, unrestricted population growth, rapid industrialization, and economic development have resulted in an escalation of the global energy crisis and, as a result, exponential deterioration in non-renewable energy resources such as coal, natural gas, and oil. In addition to the energy crisis, the prolonged use of petroleum-based fuels has resulted in pollution and global climate change. Crude oil (34%), coal (28%), and natural gas (23%) have all contributed significantly to global energy generation [1]. Furthermore, the overabundance of plentiful non-renewable resources has resulted in excess greenhouse gases (GHG) such as CO_2, CH_4, and others, resulting in global climate health being disrupted. Global temperature has been reported to be rising at an alarming rate of 0.07 °C per year, with CO_2 levels increasing at a rate of 3 ppm per year, with the maximum level being 410 ppm [1]. Researchers are seeking alternative resources that are less destructive to the environment and economically affordable. Renewable energy options have been on the experts' radar for the past decade [2–4].

In this chapter, extraction of energy products in a usable form from natural sources is referred to as primary energy production, for example, in coal mines, crude oil fields, and hydropower facilities [5]. Aside from that, renewable energy resources are receiving much attention in developed countries. For example, the European Union has maintained its 2030 mandatory objective of 27%, which was pushed backward in 2014 to 32% in June 2018 [4, 6]. At the same time, the US is working to improve renewable energy resources.

One of the critical motivations for using renewable energy resources is to consider ecologically favorable energy sources. Environmental awareness is high for the world population at this time; it is believed that previous reliance on fossil fuels has resulted in carbon dioxide (CO_2) emissions, greenhouse gas (GHG) concerns, and pollution [4].

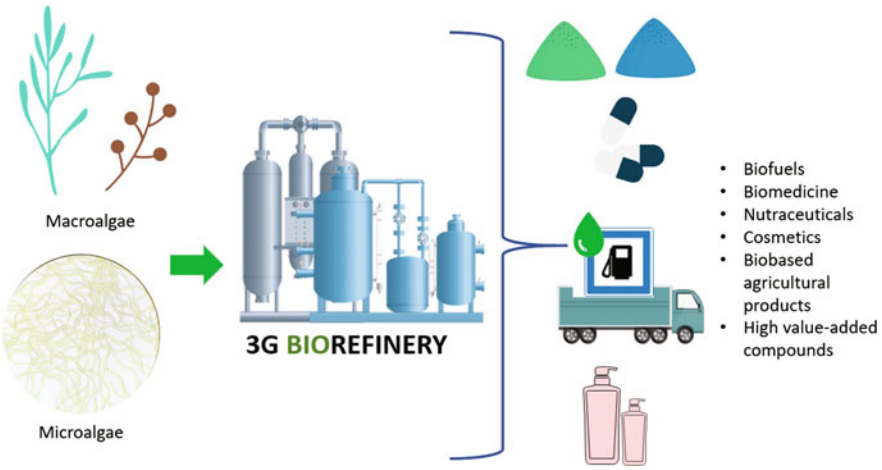


Fig. 12.1 Third generation biorefinery with biofuels and other high value-added compounds

Nowadays, research has investigated alternate sources of clean biofuels derived from renewable sources that are referred to as first-generation, second generation, and third generation. Biological biofuels are produced by biological routes like pretreatment, harvesting, and biochemical conversion processes under the biorefinery concept.

Biofuels like bioethanol, biodiesel, and biogas are considered clean and renewable. Each has massive advantages over other fuels like environmentally friendly, low toxicity, and low burn pollutant environments for replacing fossil fuels [3]. They can be produced from sugarcane, corn starch, and other cellulosic feedstocks. However, although these feedstocks are less expensive than fossil fuels, their use can influence food costs [7]. Therefore, researchers are examining alternative sources, which do not affect the food chain and agriculture.

Micro- and macro-algal biofuels are considered to be renewable and sustainable energy sources. Micro- and macro-algae are recognized as superior biomass as compared to terrestrial plants—in terms of solar energy storage, nutrient assimilation, and potential for biofuel production—due to significant advantages such as higher photosynthetic efficiency, higher biomass yield and rates, and reduced toxic gas emissions in the environment [8]. Micro- and macro-algae provide a new path to biomass production as a sustainable material for bioethanol and other high value-added bioactive compounds production under the biorefinery concept, shown in Fig. 12.1 [7, 9, 10]. For example, microalgae are tiny photosynthetic microorganisms, primarily existing as small cells of about 2–200 μm and inhabitants of freshwater, seawater, and even wastewater [11]. Microalgae efficiently convert solar light and atmospheric carbon dioxide to produce biomass by photosynthetic process [10, 12]. Microalgae are one of the favorable possibilities for eliminating CO_2 from the atmosphere by CO_2 bio-fixation. Microalgae can consume CO_2 in three ways: CO_2 from soluble carbonates, atmospheric CO_2, and CO_2 present in the

stack and discharge gases from industries. Microalgae are described as unicellular/multicellular photosynthetic microscopic cyanobacteria used to produce renewable fuels [10]. Micro- and macro-algae has significant oil content that allows biodiesel production and energy-containing polysaccharides like starch which can be degraded chemically or enzymatically that allows bioethanol production via fermentation [12].

This chapter intends to provide an overview of micro-and macro-algae biomass conversion into biofuels and other high value-added compounds in terms of the biorefinery concept. This chapter also covers cultivation, the extraction process, enzymatic hydrolysis, and fermentation strategies.

12.2 Biorefinery of Microalgae

12.2.1 Microalgae Overview and Growth Culture in the Accumulation of Starch

Microalgae and cyanobacteria are photosynthetic microorganisms with a cell size of 2–200 μm [12]; they can convert solar energy into chemical energy by CO_2 fixation primary carbon source [13]. There are four significant modes for microalgae cultivation: photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic cultivation [13]. Therefore, they may use another carbon source, different CO_2, to produce a large amount of biomass, containing carbohydrates, lipids, proteins [12], high-value-added compounds such as vitamin pigments, and some organic acids [14].

Microalgae are assimilating inorganic nitrogen and phosphorus during all their growth phases. Nitrogen source and concentration have been reported as parameters that significantly affect lipid yields to the inside of the microalgae. Various nitrogen sources, such as ammonia (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), and urea (CH_4N_2O), can be used for the culturing microalgae, and the choice of nitrogen source will strongly depend on the type of microalgae [15, 16]. On the other hand, the limitation of phosphorus (PO_4^{3-}) source within culture medium has negatively impacted the formation of carbohydrates and growth rate in several microalgae strains compared with other macronutrients [17]. Environmental parameters such as light intensity, nitrogen, carbon nutrient levels, salinity, temperature, and others significantly impact microalgae'biomass and chemical composition. In general, microalgae'growth rate and biomass production rely primarily on nitrogen availability in culture ingredients [18]. Under nitrogen-sufficient circumstances, the majority of oleaginous microalgae grow faster and produce less lipid. Instead, nitrogen loss or famine causes increased lipid accumulation in microalgae, which is most likely related to the movement of metabolic carbon from carbohydrate and protein production to lipid production. Thus, understanding the trade-off connection between

microalgae biomass, lipid, and nitrogen levels in a system during the culture phase is critical for optimizing lipid and protein synthesis, among other bioproducts [19].

Microalgae are currently contributing to the global bioeconomy by providing significant biomass for human-related uses like pharmaceuticals, cosmetics, food, and feed [20]. Microalgae biomass is considered potential biomass for biofuel production, such as bioethanol, biodiesel, biohydrogen, and biomethane. Therefore, they will play a significant role in the renewable energy sector and in the uptake of inorganic matter [21].

Microalgae are also being studied as a viable biomass feedstock for biofuel production and play a valuable role in the renewable energy sector. However, cultivating microalgae to meet only world transportation fuel demands utilizing microalgal biomass as feedstock raises various practical concerns and substantial limits, such as high land usage, high energy, water, and fertilizer consumption. The use of wastewater streams and seawater for microalgae growth may reduce the consumption of inorganic fertilizer while treatment of the wastewater occurs. They are of enormous importance due to their rich content in nutrients, which can fulfill the microalgal cyanobacterial nutrient needs. Wastewater and seawater are characterized by containing several different nutrients like carbon, nitrogen, phosphorus, and potassium (macro-nutrients) such as Mg, S, Ca, Na, Cl, Fe, Zn, Cu, Mo, Mn, B, and Co (micro-nutrients) [21]. It should be highlighted that wastewater streams limit biomass applications because they may have various pollutants present in the wastewater. Therefore, microalgae produced in wastewater can be mainly used to make biofuels rather than food or feed applications [21]. For many years, microalgae cultivation systems have been investigated. The factors more critical to microalgae growth are; illumination, photoperiod, pH, carbon and nitrogen sources concentration, and temperature [22, 23].

These factors can be monitored in open raceway pond (ORP) and controlled in closed PBRs since these devices offer suitable conditions for its investigation. The open PBRs have been developed for large-scale microalgae cultivation because they are easy to make and relatively simple to operate. These ORP generally use outdoors, which permits microalgae to CO_2 uptake from the atmosphere with a poor mass transfer rate inside the culture medium, higher risk of contamination, and a high evaporation water rate. The closed PBRs are more complex systems because these do not allow direct mass transfer between culture media and atmosphere, and its use to pilot or large scale is usually considered nonviable by the enormous consumption amount of energy, despite allowing to attain a higher yield of microalgae biomass without risk of contamination, in comparison with open PBRs. When high-value-added chemicals are manufactured, such as biopharmaceuticals, top-grade cosmetics, and human health foods, closed PBRs are widely accessible [24]. Figure 12.2 shows photobioreactor technology used for microalgae culture.

The major challenge in PBRs design and scale-up is increasing the CO_2 transfer rate in the gas-liquid interface into the microalgae suspension because microalgae cannot directly use the CO_2 bubbles injected inside PBRs as the gas aerated into solution is sparingly soluble in the culture medium. The way of dissolving CO_2 bubbles in the culture medium is through decreasing the bubble diameter, which

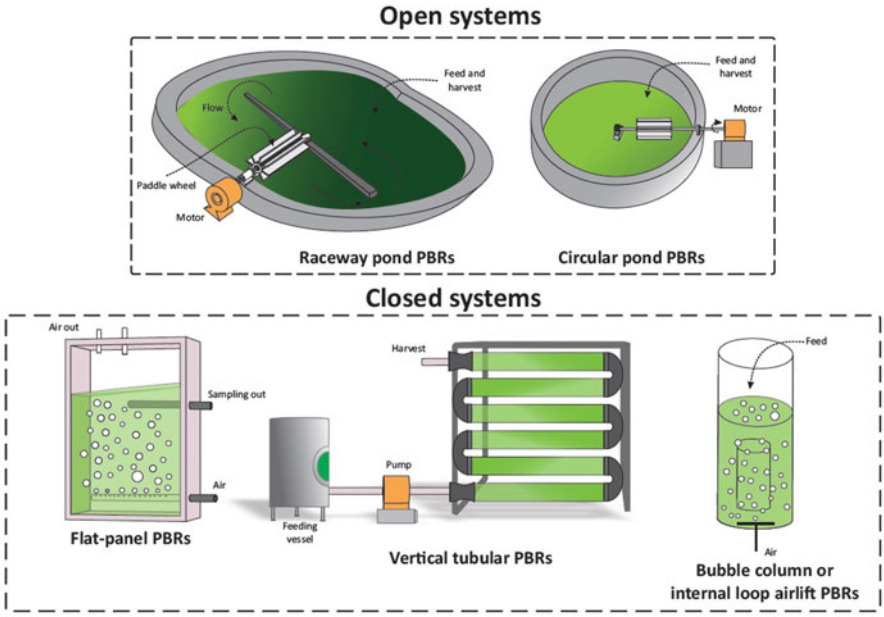


Fig. 12.2 Photobioreactors (PBRs) technology used for microalgae culture

increases the gas-liquid contacting area. It prolongs the retention time of the bubble in the microalgae suspension so that the dissolved CO_2 can be captured by the microalgae cells and converted into organic matter to form biomass through photosynthesis [25].

The culturing of some microalgae like *Chlorella*, *Dunaliella*, *Chlamydomonas*, *Scenedesmus*, and *Spirulina* in PBRs has massive carbohydrate amounts ($\geq$20% of dry weight), which is excellent biomass for bioethanol production [26, 27]. Compared with conventional crops, there are various advantages to employing microalgae for bioenergy production, including: (1) the capacity to be farmed on marginal areas without causing land-use change, (2) high exponential growth rates potential to utilize CO_2 from industrial flue gas (1 kg of dry algae biomass uses about 1.83 kg of CO_2) and nutrients (mainly nitrogen and phosphorus) from wastewater, (3) semi-continuous to continuous harvesting and (4) variable lipid content in the range of 5–50% dry weight of biomass [28, 29]. The accumulation of carbohydrates, fatty acids, and pigments inside microalgae happens in the chloroplast, and this organelle is in charge of the photosynthesis process [30]. The accumulated carbohydrate by microalgae can be converted directly to ethanol under anaerobic conditions and dark [31]. Table 12.1 shows the content of carbohydrates some microalgae cultivated in PBRs, which can be used for bioethanol production.

Table 12.1 Carbohydrate content in microalgae biomass for bioethanol production

Microalgae	% (g / dry weight)	PBRs type	Cultivation	References
Tribonema sp.	14.5	Bubbles column	–	[32]
Chlorella vulgaris FSP-E	51.0	Glass vessel	2% CO_2/air, 28 °C, pH 6.2, agitation 300 rpm, and a light intensity 60 μmol. m^{-2} s^{-1}	[33]
Synechococcus elongatus PCC7942 (transgenic cells)	90.0	Glass vessel	5% CO_2/air (0.2 vvm), 28 °C, and a light intensity 200 μmol. m^{-2} s^{-1}	[34]
Synechococcus PCC 7002	60.0	Bubbles column	5% CO_2/air, pH 8.0–8.5, 28 °C, and a light intensity 100 μmol. m^{-2} s^{-1}	[35]
Synechococcus sp. PCC 7002	60.0	Bubbles column	1% CO_2/air, 38 °C, and a light intensity 250 μmol. m^{-2} s^{-1}	[36]
Pseudochlorella sp.	36	Glass vessel	Air at 0.3 vvm, 27 °C, 150 rpm, and a light intensity 60 μmol. m^{-2} s^{-1}	[37]
Chlamydomonas mexicana	50			
Chlamydomonas pitschmannii	23			

12.3 Extraction of Starch from Microalgae

Starch is a polysaccharide that consists of numerous glucose units joined by glycosidic bonds, found naturally in green plants for energy storage. Starch content depends on plant species, environmental conditions, and biotic or abiotic factors of the aquatic ecosystem [38]. It is expected that third-generation biofuels produced from algae and aquatic plants will become carbon-neutral since they use atmospheric CO_2 for the energy acquiring process.

Most microalgae species contain around 37% of starch (Table 12.2); even some strains such as *Dunaliella, Scenedesmus, Spirulina,* and *Chlamydomonas* can have more than 50% starch [39].

Starch originates in the chloroplasts of microalgae as semi-crystalline granules (Fig. 12.3). Anhydrous starch granules of mainly consist of two major unbranched, and large polymers such as amylose, which is a linear polysaccharide composed entirely of D-glucose units, joined by α-1,4-glycosidic linkages polymer, and amylopectin, which is a branched-chain polysaccharide consisting of glucose units linked primarily by α-1,4-glycosidic bonds, but with few α-1,6-glycosidic bonds, that are responsible for the branching [48]. Starch in the microalgae cell requires disruption of the outer cell wall composed mainly of pectin, agar, and alginates; meanwhile, the inner cell wall comprises cellulose hemicellulose glycoprotein [49].

Dilute acid/alkali processes and enzymatic hydrolysis are traditional algae cell disrupter methods; nevertheless, pressurized liquid extraction, supercritical fluid extraction, ultrasonication, bead beating, microwave, and pulse electric fields have

Table 12.2 Starch content in microalgae

Microalgae	Starch content (% weight)	References
Dunaliella, *Scenedesmus*, *Spirulina* and *Chlamydomonas*	~50	[39]
Tetraselmis subcordiformis	62.1	[39]
Chlorococcum sp.	26	[40]
Chlorella vulgaris	60	[41]
Chlamydomonas reinhardtii	49	[42]
Chlorella sorokiniana	40	[43]
Neochloris oleoabundans	27	[44]
Tetraselmis subcordiformis	44.1	[45]
Chlorella sp.	19.3–38.2%	[46]
Oscillatoria sp.	63.85	[47]

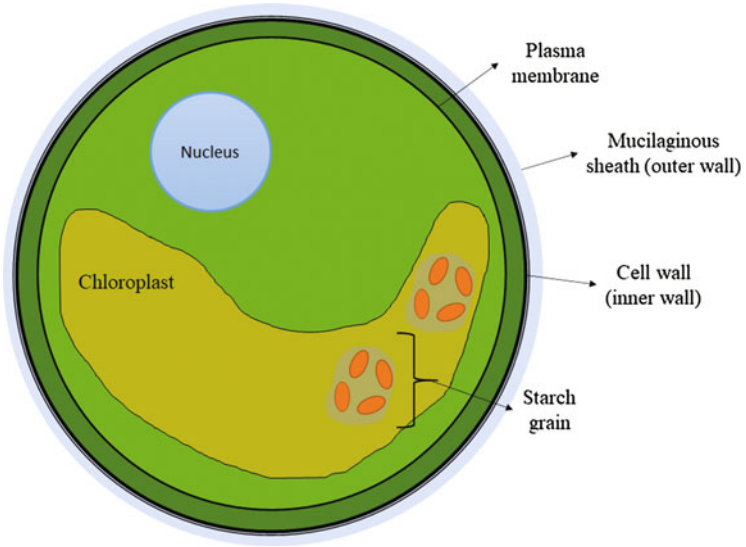


Fig. 12.3 Microalgae cell basic structure for starch localization

been evaluated as novel methods to achieve algal cell hydrolysis [12, 50]. After cell wall hydrolysis, the soluble fraction needs to be separated from the solid fraction, which conserves the starch content, usually by centrifugation. Water washes and the centrifugation process should be repeated using a Percoll gradient to isolate pure starch. Figure 12.4 summarizes the starch extraction process from microalgae.

Pressurized liquid extraction (PLE): Compared to conventional procedures, PLE uses fewer solvents and delivers quicker extractions due to the fast mass transfer rate. Solvents have enhanced solubility and lower viscosity due to the higher temperatures, which helps boost mass transfer rates and penetration into the matrix.

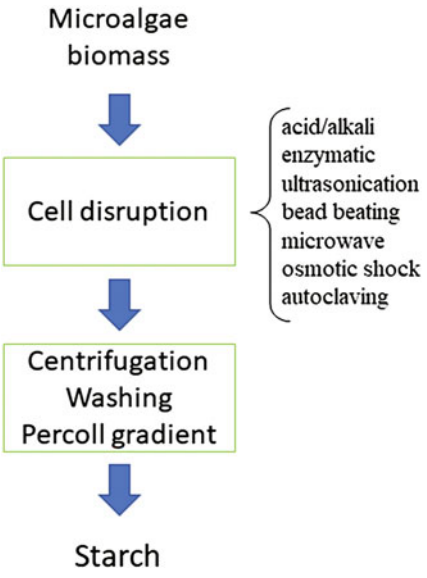


Fig. 12.4 Process stages for starch obtention from microalgae

Furthermore, while water is kept in its liquid state, a rise in temperature causes a significant drop in the dielectric constant (ε). This number is typically used to determine the polarity of a solvent. In this way, though water has a dielectric constant of around 80 at room temperature when heated to 250 °C under appropriate pressure to keep it liquid, it drops to approximately 30, equivalent to some dielectric constants organic solvents like ethanol or methanol [51].

Supercritical fluid extraction (SFE): Carbon dioxide is the most often used supercritical fluid for extracting natural sources, including microalgae. Its low critical temperature and pressure (31.1 °C and 73.8 bar) are easily attained, and it is GRAS for the food sector, inexpensive and safe. Another unique feature of this method is that supercritical CO_2 (sc-CO_2) is a very selective solvent. The most significant factors during extraction are temperature and pressure, which together govern the density of the sc-CO_2. Hence, it is the capacity to selectively remove particular compounds from the natural matrix [51].

Diluted acid/alkali hydrothermal process: This is a chemical, non-mechanical, cheap, and fast method for microalgae cell wall disruption. Nevertheless, it uses the breakdown of essential compounds and produces toxic elements that usually inhibit fermentation [52]. Acidic or alkali hydrolysis is a non-specific reaction, generally performed with concentrations between 1 and 10% w/v and temperatures of 100–160 °C [39, 50]. These chemicals limit used in more significant amounts during hydrolysis; then, pH adjustment before the fermentation process is needed that releases more salt, inhibiting yeast activity [50].

Enzymatic hydrolysis: Classified as the most efficient biological and non-mechanical pretreatment, particularly for microalgae [53], hydrolysis made by enzymes is a costly and slow procedure but environmental-friendly. This biological

hydrolysis often requires expensive pretreatment processes to enhance efficiency [52]. Apart from the pretreatment and enzyme costs, enzymatic hydrolysis provides a more specific disruption with low heating cost and no degradative effects derived from the mild temperature and pressure used [50].

Ultrasonic treatment: Ultrasonic pretreatment is a mechanical technology that produces alternating low- and high-pressure waves (20–100 MHz) in the aqueous phase, causing the formation and vigorous collapse of microbubbles [52, 54]. The microbubbles' violent failure occurs within a few microseconds inducing the occurrence of cavitation. All processes generate theoretical temperatures and pressures of up to 5000 K and 500 bar and initiate powerful hydro-mechanical shear forces and highly reactive radicals [55].

Bead beating: Another mechanical method is the bead-beating method, which involves applying glass or steel beads into a vessel where the high-speed agitating movement of beads can disrupt the algal cell wall. Bead beating is used for both disruption and extraction [56]. This disruptive mechanical method is considered an efficient technique [57].

Microwave: Microwave method is based on the perpendicular mixture of electric and magnetic waves that fluctuate at defined frequencies ranging from 0.3 to 300 GHz [58]. Microwaves use high-frequency waves to create water molecule vibrations inside microalgae biomass, increasing the humidity and pressure caused by water evaporation, causing cell wall rupture [12, 57]. Microwaves have various advantages like fast heating, uni-directional heat flow and mass, selective energy dissipation, more rapid, increase purity and yield capacity of the anticipated product [52].

Pulsed electric field lysis: In this technique, cells in a liquid media are subjected to pulses of a strong electric field ranging from 100 V/cm to 300 kV/cm within a short period of nanoseconds or milliseconds, which principally affects the formation of pores in the cell wall [12, 52]. The pores formed in the cell wall allow biochemical components to leach out from the cell. Pretreatment methods for microalgae used as feedstock for biofuels are summarized in Table 12.3.

12.4 Enzymatic Hydrolysis of Microalgae Starch

Enzymatic hydrolysis (saccharification) is the critical step for converting polysaccharides into monosaccharides that requires the action of cellulolytic enzymes sequentially and synergistically for subsequent fermentation and bioethanol production [12, 65]. Enzymatic saccharification of starch is performed at high temperatures, and it is separated into three parts: gelatinization of starch, liquefaction, and saccharification.

Gelatinization of starch and liquefaction involves breaking starch granules into a gelatinized suspension at 105 °C followed by converting oligosaccharides from gelatinized starch at 95 °C by using an α-amylase enzyme that has thermostable properties as shown in Fig. 12.5. The saccharification process converts saccharide

Table 12.3 Pretreatment processes for starch extraction from microalgae sources

Source	Pretreatment	Operational conditions	Yield (%)	References
Chlorella Salina	Physiochemical	Megazyme total starch analysis kit (90 °C, 30 min)	323.1 ± 32.03 (increment) 96.60 ± 2.73 (starch recovery)	[59]
Chlorella sorokiniana Nannochloropsis gaditana Scenedesmus almeriensis	Enzymatic	15 FPU for Celluclast 1.5 L and 15 IU for Novozyme 188 per g of DW	6.7[a] ~1.4[a] ~2.7[a]	[60]
Chlorella sorokiniana Nannochloropsis gaditana Scenedesmus almeriensis	Enzymatic	240 α-amylase units and 750 amyloglucosidase units for Liquozyme SC DS and Spirizyme fuel	10.1[a] ~6.0[a] ~4.0[a]	[60]
Chlamydomonas fasciata	Ultrasonic	30 W and 20 kHz for 0–40 min	93.8	[61]
Scenedesmus obliquus		30 W for 25 min	91.0	[50]
Chlorella Salina		30 W and 25 kHz for 5 min	35.7	[59]
Chlorella Salina	Bead beating	950 mg of glass beads (15.8 g of glass beads/1 g of biomass) at 5 min	65.4	[59]
Chlorella sp.	Microwave	Irradiation power of 530 W at 2450 MHz frequency, for 45 s	82[b]	[62]
Nannochloropsis oculata		Irradiation power of 943 W at 2450 MHz frequency, for 5 min	~70[b]	[63]
Ulva ohnoi	Pulse electric field	Field strength of 1 kV cm^{-1}, pulse duration of 50 μs, and pulse repetition rate of 3 Hz	59.4	[64]

[a]Yield % referred to a total carbohydrate
[b]% of cell rupture

polymer to monomers like glucose with additional disaccharides like maltose and isomaltose at significantly lower concentrations. Glucoamylase and isoamylase enzymes are added during the process to break down α-(1 → 4) glycosidic bonds as well as α-(1 → 6) glycosidic bonds at 65 °C [66–69].

Enzymatic hydrolysis efficiency depends on enzymes, substrate loading, pH, temperature, and incubation time, such as *Synechococcus* sp. PCC 7002, a marine cyanobacterium with a rich source of carbohydrates, was used for bioethanol production as feedstock when boosted accumulation was induced by nitrogen

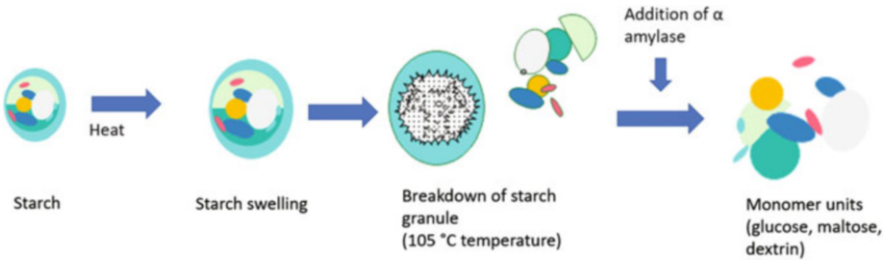


Fig. 12.5 Starch gelatinization, liquefaction, and saccharification

sources like nitrate [36, 70]. Optimizing the enzymatic hydrolysis process is essential in developing a cost-effective and efficient saccharification strategy for increased sugar concentration. The optimal enzymatic hydrolysis process conditions vary depending on the configuration of carbohydrates between the green, brown, and red algae [71]. Enzymatic saccharification structures use mild temperatures and have lesser ruin risks. Enzymes, typically amylases, cellulases, and pectinases (separately or together), are used to saccharify microalgae biomass [72].

Enzymatic hydrolysis is an eco-friendly process for the environment due to the low energy consumption and fermentable sugars produced from the feedstocks under light operational conditions, absence of corrosive problems, and excellent yields of free and limited byproducts [73]. Enzymatic hydrolysis uses mild operating conditions, gives high sugar yields, has high selectivity, and generates minimal byproducts formation [74]. Enzymatic hydrolysis has other advantages like procedure conditions with ensuing low energy requirements, high selectivity and biological specificity, and straightforward scale-up [75, 76]. However, enzymatic hydrolysis has disadvantages like the capital cost of enzymes and problematic recovery, making the process uneconomical. Enzymatic hydrolysis primary effectiveness depends on operation limits like temperature, pH, time, enzyme type and concentration, and parameter optimization for obtaining high yields and reducing capital costs [75].

Amylase enzyme is one of the most popular enzymes because it catalyzes starch to glucose precisely and effectively, as shown in Table 12.4. For example, α-amylase can randomly cut α-1-4-glucoside bonds of amylose or amylopectin, resulting in short-chain dextrin and maltose [76, 77]. In contrast, glucoamylase can cut α-1-6-glucoside bonds in amylopectin, which α-amylase cannot attack [76, 78]. The α-amylase and glucoamylase enzymes coordinate to complete the hydrolysis process for ethanol production from starch converted into glucose by fermentation. α-Amylases (EC 3.2.1.1) are endo-acting enzymes used to arbitrarily cut of α-1,4 glycosidic bonds present inside the starch and quickly break down the starch completely and release non-reducing ends for glucoamylase. Glucoamylases (EC 3.2.1.3) is an exo-acting enzyme that cut α-1,4 glycosidic bond and α-1,6 glycosidic bond to produce monomers sugar, the non-reducing ends that released from the starch degradation [79, 80].

Table 12.4 Bioethanol production from microalgae using amylolytic enzymes with optimal operating conditions

Algae species	Enzymes and operational condition	Concentration	Product	References
Chlorella sorokiniana	α-Amylase Amyloglucosidase	0.464 ± 0.013 g/g reducing sugar	Bioethanol	[83]
Chlorella sorokiniana	Cellulase, amylase (150 rpm, 72 h, pH 5.5–6.5)	58.78% total reducing sugar 0.504 $g_{ethanol}/g_{glucose}$	Bioethanol	[84]
Chlorella vulgaris	α-Amylase Amyloglucosidase CTec2 (50 °C, 200 rpm, 72 h)	54.5% Reducing sugar	Bioethanol	[85]
Mixed microalgae	Cellulase (50 °C, pH 4.5)	96.3% Maximum sugar yield	Bioethanol	[86]
Mixed microalgae *Neochloris* sp., *Scendesmus* sp., *chlorella* sp.	Cellclast, β-Glucosidase, α-Amylase, Amyloglucosidase (pH 5, 60 °C, 150 rpm)	0.126 $g_{ethanol}/g_{dried\ algae}$	Bioethanol	[87]
Rhizoclonium sp.	Mixed enzyme Cellulase Amylase Xylanase Pectinase (45 °C, 48 h)	140.72 mg/g reducing-sugar 195.84 mg/g reducing sugar	Bioethanol	[71]
Spirulina platensis	Amylase	6.5 g/L ethanol	Bioethanol	[88]
Synechococcus sp.	Lysozyme (100 mg/L, 37 °C for 3 h), α-Amylase 240 U/g (85 °C for 1.5 h), Amyloglucosidase 750 U pH 5.5–6	0.27 $g_{ethanol}/g_{cell\ dry\ weight}$	Bioethanol	[36]
Tetraselmis subcordiformis	α-Amylase (AmyP) with calcium (40 °C, 2 h)	74.4% from 4% or 53% from 8% raw microalgae starch hydrolysate	Biofuel	[89]
Arthrospira platensis	Amylolytic enzyme (-α-amylase 0.3 U/L, glucoamylase 0.1 U/L) 168 h	43 g/L glucose concentration (without lysozyme or $CaCl_2$) 67 g/L glucose concentration (with lysozyme or $CaCl_2$)	Bioethanol	[90]

For cellulose, endo β-(1–4)-glucanase arbitrarily hydrolyzed amorphous areas of cellulose β-(1–4)-glycosidic bond and creating an innovative chain end. The exo β-(1–4)-glucanase enzyme performances on non-reducing ends of cellulose

molecule and cellodextrins and redeeming cello-oligomers and cellobiose units (each unit has two β-(1–4) bonded glucose molecules). Hydrolysis is the final step to produce glucose monomers using β-glucosidase of these β-linkages of cellobiose molecules [81, 82]. Hemicellulose is like xylose, galactose, mannose, and other sugars with β-(1–4) and β-(1–3) linkages. These linkages are cut by enzymes like xylanases, α-L-arabinofuranosidase, and β-glucosidase and change into glucose monomer's sugars. Starch and glycogen have α-(1–4) D-glucosidic bonds that are hydrolyzed in a liquefaction process using α-amylase. Maltodextrin is a mixture of polymers of glucose having three or more α-(1–4)-linked D-glucose units. By the saccharification process, maltodextrin transforms into glucose oligomers by using amyloglucosidase. Saccharification process performance depends on both α-(1–4) and α-(1–6) D-glucosidic bonds [82].

Many authors have worked on enzymatic hydrolysis and its strategies on microalgae biomass. For example, Choi et al. [75] showed that hydrolysis efficiency improves to around 94% with a fermentation yield of approximately 60% for *S. cerevisiae* S288C in enzymatic hydrolysis of *Chlamydomonas reinhardtii* (initially carbohydrate content 59.7%), treated by SHF with amylases enzymes, in which α-amylase (0.005% v/w) from *Bacillus licheniformis* was used at 90 °C for 30 min, and with pH 6 to liquefaction and amyloglucosidase (0.2% v/w) from *Aspergillus niger* at 55 °C for 45 min, and pH 4.5 to saccharification [75].

Ho et al. [33] used a mixture of enzymes that contained endoglucanase (0.65 U mL^{-1}), β-glucosidase (1.50 U mL^{-1}), and amylase (0.09 U mL^{-1}) for enzymatic hydrolysis on *C. vulgaris* biomass. This biomass had initial carbohydrates 51% and glucose 93.1%. Feedstock and enzyme ratio was 10 g mL^{-1}, at 200 rpm for shaking on 45 °C with 20 g L^{-1} and reported results as 0:461 $g_{glucose}/g_{algae}$ dw (~97%) after 48 h. Furthermore, those authors compared results with dilute acid hydrolysis biomass performed at 1% H_2SO_4, 121 °C, 20 min, and 50 g/L of biomass. Lastly, 23.6 g/L (~100%) glucose concentration yields a similar yield by enzymatic hydrolysis [33].

Kim et al. [91] studied two enzymes separately for analyzed the enzymatic hydrolysis effect of microalgae, 1% (w/v): cellulase (Celluclast 1.5 L) and pectinase (Pectinex SP-L). The activities of these enzymes were 0:122 FPU/mg of protein and 240 UI/mg of protein. These enzymes were added (1.88 mg protein/g) on *C. vulgaris* biomass (22.4% of total carbohydrates) for bioethanol production, at 50 °C, 200 rpm, pH 4.8, 72 h. After enzymatic hydrolysis, sugar released from cellulase and pectinase 10% and 45%, respectively, liberating 0:1 g glucose/g algae dw. Various methods for cell lysis applied on *C. vulgaris* with bead beating combined with pectinase enzyme that extracts from *Aspergillus aculeatus*. After that, sugar extraction improved between 45% to 70%, and 89% ensuing fermentation yield after 12 h with *S. cerevisiae* KCTC 7906. The pectinase enzyme seems more practical than cellulases, amylases, and xylanases [91].

Moller et al. [36] reported *Synechococcus* sp. PCC 7002 biomass for enzymatic hydrolysis. They used 3 g/L of biomass concentration to afford 60% carbohydrate content efficiency for enzymatic hydrolysis and achieved 80% sugars with hydrolyzed after enzymatic treatment. These enzymes are lysozyme and α-glucanases

Liquozyme SC DS, and Spirizyme for biofuel. Ethanol yields reached 86% of the theoretical maximum rate with the help of *S. cerevisiae* [36].

Mahdy et al. [92] used urban wastewater to cultivate *C. vulgaris* have carbohydrate 39.6% and protein 33.3%. They used two enzymes separately, like 2.5 L alcalase (0:585 AU/g dw), and viscozyme (36:3 FBG/g dw), to solubilize protein-carbohydrate. These two enzymes alcalase with pH 8 (3.2% w/v), and 5.5% viscozyme, were carried out in enzymatic hydrolysis at 50 °C, for 3 h, in which pH was maintained during the process. The authors reported that the hydrolysis efficiency of organic matter was 54.7% for proteins (alcalase) and 28.4% for carbohydrates (Viscozyme) [92].

12.5 Conversion of Microalgae starch into Monomers for Ethanol

Starch is the principal polysaccharide formed in microalgae and can be converted into bioethanol using enzymes and microorganisms. Enzymes such as α-amylase and glucoamylase break the glycosidic bonds present in starch, then *S. cerevisiae* yeast is used in fermentation to reduce sugars [46]. Fermentation is a metabolic process, principally converting monosaccharide sugars into bioethanol and other value-added products using fermentative microorganisms [82, 93]. In the fermentation process, yeast and bacteria are commonly used as fermentative microorganisms. Some fermentative organisms play an essential role in fermentation, like *S. cerevisiae, Z. mobilis, E. coli, P. stipitis, Kluyveromyces fragilis, K. marxianus*, and *Klebsiella oxytoca*; the result is microalgal photosynthesis and intracellular anaerobic fermentation-derived bioethanol [93]. *Saccharomyces* and *Zymomonas* fermentative microorganisms are frequently used for bioethanol production, such as molasses, starch-based substrate (like algae), sweet sorghum cane extract, lignocellulose, and other wastes. *Z. mobilis* is a natural ethanologenic microorganism that has many advantageous properties, such as higher ethanol tolerance efficiency up to 16% and ethanol yield in a varied pH between 3.5 and 7.5. *Z. mobilis* does not need controlled aeration during fermentation time, which reduces the product capital cost. *Z. mobilis* is an appropriate industrial microbial biocatalyst used for the commercial production of bioproducts through metabolic engineering [94]. *Zymomonas* is a gram-negative bacteria with several advantages, including a higher specific rate of sugar uptake, a higher ethanol yield, lower biomass production, and the absence of the need for controlled oxygen addition during fermentation [95], and it is used for bioethanol production from starch and glycogen in fermentation [70, 96]. Theoretically, ethanol yields (0.49 to 0.50) g/g, or ethanol yields of up to 97% of theoretical values, can be obtained [97].

S. cerevisiae may play a critical role in the industrial biotechnology sector to develop a green replacement for petrochemical products due to its outstanding productivity to convert monomer sugars like glucose into ethanol and its high

tolerance. In addition, *Saccharomyces* is generally recognized as a harmless microorganism according to generally recognized as safe (GRAS) criteria. While growing, it produces flocs in the fermentation media that quickly settle down and separate. *S. cerevisiae* has a higher tolerance for alcohol, higher glucose uptake, and higher bioethanol yield than *Zymomonas* microorganism [70, 98]. Theoretically, 1 kg of glucose and xylose produce 0.51 kg ethanol with 0.49 kg of CO_2 [82, 93, 99].

One of the main complications of effective fermentation is the incapability of commonly used microorganisms that convert pentose sugars into bioethanol. Therefore, economic bioethanol production must use all potential feedstocks (i.e., cellulose and hemicellulose). Naturally occurring microorganisms that convert primary pentose sugar from hemicellulose like xylose into bioethanol exist, for example, specific bacteria, fungi, and yeasts [74]. Fermentation processes are represented by the following strategies [74, 93, 100]:

1. Separated hydrolysis and fermentation (SHF)
2. Simultaneous hydrolysis and fermentation (SSF)
3. Simultaneous saccharification and co-fermentation (SSCF)
4. Consolidated bioprocessing (CBP)

SSF and SHF are primarily used to produce bioethanol from microalgae using different fermentation strategies with various fermentative microorganisms (Table 12.5). The total valuation of the fermentation process is usually based on cell growth, consumption of reducing sugar, and bioethanol production. Environmental and operational factors greatly influence bioethanol production from algal biomass, like (i) nutrient levels; (ii) alkalinity; (iii) concentration of toxic substances; (iv) temperature; and (v) optimum pH of the fermenting microorganism [74].

12.5.1 Separated Hydrolysis and Fermentation (SHF)

Enzymatic saccharification of starchy biomass is carried out first in a SHF process at the optimum temperature using a saccharifying enzyme. The saccharified solution is then fermented using suitable microorganisms [93]. These advantages of SHF are the low capital cost of chemicals, short residence time, and simple equipment systems, which inspire its large-scale processing [93, 100]. The SHF process is usually active in research studies to enhance the operative conditions such as pH, temperature, and time of both stages, which help determine the diverse mechanisms involved in the process and the effect as displayed by several parameters and continuous fermentation with cell recycling. Nevertheless, the operation procedure of SHF has some drawbacks. When compared with SSF (Sect. 12.5.2 below), the SHF process has disadvantages such as higher capital cost due to the large mechanical setup for separation steps, and elevated enzyme concentrations and low solids loading required to achieve good ethanol yields.

Moreover, the longtime running of the process may lead to contamination of the substrate by microorganisms [108]. The main advantage of the SHF process is that

Table 12.5 Comparison of bioethanol yields from microalgae by fermentation processes with fermentative microorganisms

Microalgae species	Hydrolysis	Fermentation	Fermentative microorganism	Fermentation condition	Bioethanol yields	References
Chlorococcum infusionum	Chemical (NaOH)	SHF	*S. cerevisiae*	200 rpm, 72 h	0.26 $g_{ethanol}/g_{algae}$	[101]
Chamydomonas reinhardtii UTEX 90	Enzymatic	SSF	*S. cerevisiae* S288C	160 rpm, 30 °C, 40 h	0.235 $g_{ethanol}/g_{algae}$	[75]
Chlorella vulgaris	Chemical (H_2SO_4)	SHF	*E. coli* SJL2526	170 rpm, 37 °C, pH 7	0.4 $g_{ethanol}/g_{algae}$	[102]
Porphyridium cruentum	Enzymatic	SSF	*S. cerevisiae* KCTC 7906	37 °C, 9 h, pH 4.8	2.77 mg/mL (seawater) and 2.98 mg/mL (freshwater)	[103]
Scenedesmus obliquus CNW-N	Chemical (H_2SO_4)	SHF	*Z. mobilis* ATCC29191	30 °C within 4 h, pH 6	8.55 g/L	[33]
Chlamydomonas fasciata	Enzymatic	SSF	*S. cerevisiae*	100 rpm, 40 °C, 30 h	0.194 $g_{ethanol}/g_{algae}$	[61]
C. vulgaris	Enzymatic	SHF	*Z. mobilis*	30 °C in desktop fermentation	0.178 $g_{ethanol}/g_{algae}$	[33]
C. vulgaris	Enzymatic	SSF	*Z. mobilis*	30 °C in desktop fermentation	0.214 $g_{ethanol}/g_{algae}$	[33]
C. vulgaris	Chemical (H_2SO_4)	SHF	*Z. mobilis*	30 °C in desktop fermentation	0.233 $g_{ethanol}/g_{algae}$	[33]
Scenedesmus abundans	Enzymatic	SHF	*S. cerevisiae*	200 rpm, 30 °C for 48 h	0.103 $g_{ethanol}/g_{dry\ weight\ algae}$	[104]
Chlorella sorokiniana	Enzymatic	SSF	*S. cerevisiae*	150 rpm, 72 h, pH 5.5–6.5	0.292 $g_{ethanol}/g_{algae}$	[84]
Spirulina platensis LEB 18	Enzymatic	SSF	*S. cerevisiae*	60 h	73 g/L	[105]
Chlorella sp.	Enzymatic	SHF/SSF	*S. cerevisiae*	30 °C, 20 h	0.4/0.16 g/g	[23]
Chlorococum sp.	Enzymatic	SHF	*S. cerevisiae*	50 h	0.48 g/g	[101]
T. suecica	Chemical (NaOH)	SHF	*S. cerevisiae*	30 °C, 48 h	0.073 g/g	[106]
Chlamydomonas Mexicana	Combined (sonication and enzymatic)	SSF SHF	Yeast cells	30 °C, pH 5 50 °C, pH 5, 24 h	10.5 g/L 8.48 g/L	[107]

enzymatic hydrolysis and fermentation work at their optimum conditions. However, the operational disadvantage of the SHF process is an accumulation of sugars that inhibit enzyme activity [100, 109].

12.5.2 Simultaneous Saccharification and Fermentation (SSF)

SSF process uses both saccharification (enzyme hydrolysis) and fermentation processes in a single reactor or vessel, unlike SHF. In this process, feedstocks, enzymes, and yeast are added in an organized and orderly way to release fermentable (monomer) sugars, and then monomer sugars are converted into bioethanol [93, 100]. SSF is an effective process over the dilute acid or high-temperature water pretreated biomass, providing more exposure to the hydrolase enzymes. Saccharides are converted into fermentable sugars using cellulases and xylanases enzymes in SSF [93, 110]. SSF process required compatible conditions with similar pH, temperature, and optimum substrate concentration [93, 111].

Many studies specify that SSF provides better processing than other methods due to reduction in capital cost, due to the requirement of a small number of enzymes, processing time, lower risk of contamination, minor inhibitory effects, and higher production of ethanol [93, 99, 108, 112, 113].

12.5.3 Simultaneous Saccharification and Co-Fermentation (SSCF)

Fermentative microorganisms like *Saccharomyces cerevisiae* are used in fermentation for bioethanol production. Still, these fermentative microorganisms are not able to convert carbohydrates like pentose sugars into bioethanol under mild conditions, which leads to impurities in biomass and decreases bioethanol production. Genetically engineered yeasts can be used to convert leftover pentose sugars into bioethanol. Genetically modified yeasts and cellulase enzyme complex are used in the same vessel or equipment for ethanol production from feedstock in SSCF. SSCF process is usually the same as the SSF process [114]. SSCF process has many advantages like eliminating end products of enzymatic saccharification that inhibit cellulases or β-glucosidases enzymes and higher yield of ethanol and efficiency than separate hydrolysis and fermentation (SHF), and reduced capital cost [115].

SSCF is a capable process for bioethanol production from both pentose sugars (hemicellulose) and hexose sugars (cellulose) in which saccharification and fermentation coincide in a single vessel and reactor [74, 93]. SSCF is a recommended process when a significant contribution of the pentoses sugars (C5) originates after hydrolysis. Genetically modified microorganisms like *S. cerevisiae* and *Z. mobilis*

are primarily used in the SSCF to break down glucose and xylose. To reach the higher ethanol yield route, Peralta-Ruíz et al. [116] did the handling of simulated technological paths by ASPEN PLUS 7.1 software which was based on experimental information; simulation results showed the advancement of ethanol yield by 23.6% in the SSCF pathway, 20.1% enhancement by SSF pathway as well as 18.5% advancement by the SHF pathway also. Therefore, SSCF can achieve the hydrolysis and co-fermentation of pentose and hexose sugars in the same vessel or reactor without restrictive ethanol made from cellulosic biomass [93, 117]. SSCF process can break down glucose and pentoses in the same vessel or reactor. Simultaneously, SSF is separated from pentoses in fermentation, but both approaches have a quick enzymatic hydrolysis process, low capital cost, and higher ethanol yield than SHF [93, 118].

12.5.4 Consolidated Bioprocessing (CBP)

CBP integrates hydrolysis (saccharification) and fermentation of feedstock to the desired bioproduct, requiring fewer energy inputs and fewer equipment requirements than the conventional multi-step fermentation process [119]. Microorganisms, which have been modified to enhance the production of ethanol as well as tolerance of ethanol. Instead of this, there is no single commercially available consolidated bioprocessing (CBP) organism reported. One single genetically engineered microorganism is used for hydrolysis and fermentation steps in the biological approach to CBP. A consortium consists of an enzyme-producing strain that can hydrolyze the biomass and another two different strains that can ferment C5 and C6 sugars into ethanol. Brethauer and Studer [120] proposed a model utilizing *Trichoderma reesei*, which necessitates aerobic conditions for resourceful enzyme secretions; *Saccharomyces cerevisiae* breakdown hexoses sugar to ethanol. *Scheffersomyces stipitis* is one of the best natural yeasts that uses pentose sugars and capably produces ethanol under microaerophilic conditions. In a biofilm membrane reactor, all of these microbes convert lignocellulosic biomass into ethanol, and the approach seems reasonable. Still, the primary obstacle of CBP is controlling the consortium. It is also challenging to find microorganisms with identical fermentation conditions [100], potentially reducing capital costs and increasing process efficiency. However, microorganisms producing enzymes for hydrolysis of biomass and fermentation of released sugars are still in the early stage of development [121].

12.6 Macroalgae Biorefinery

Macroalgae can constitute the raw materials for third-generation biorefineries as these are composed of fermentable carbohydrates and have the advantage of not having lignin in their structure. This section will review the chemical and structural characteristics of macroalgae that can be used in a biorefinery.

According to their photosynthetic pigment, macroalgae, also known as "seaweed," are photosynthetic aquatic organisms divided into red, green, and brown varieties. Thus, these are *Chlorophyta* (green algae), *Rhodophyta* (red algae), and *Phaeophyta* (brown algae). Macroalgae do not compete for space in farmed areas since they are aquatic plants. Water makes up 90–85% of its content, in addition to collecting CO_2 from the atmosphere [122, 123].

Macroalgae have structures similar to land plants since they have leaves, stems, and some roots, as shown in Fig. 12.6, and are listed as:

- The Thallus: which is a body-like structure that can perform photosynthesis.
- Lamina or blades: lamina is a leaf-like structure, having great property to absorb sunlight, and it is one of the keys of photosynthetic systems.
- Stripe: it a stem-like structure that provides support and exists only in some species. It can be long and challenging that transports sugars from the blades and acts as an attachment.
- Floats: floating structures filled with a kind of gas that is located on the lamina and stipe. They hold mainly carbon monoxide, and the primary function is to maintain the edges in shallow waters where light is easily captured.
- Holdfast: it is a root-like structure that assists in holding the plant on the surface of rocks and does not penetrate in the sand. It does not support gathering nutrients from the surroundings.
- Frond, commonly referred to as the combination of the blade and stipe [124]

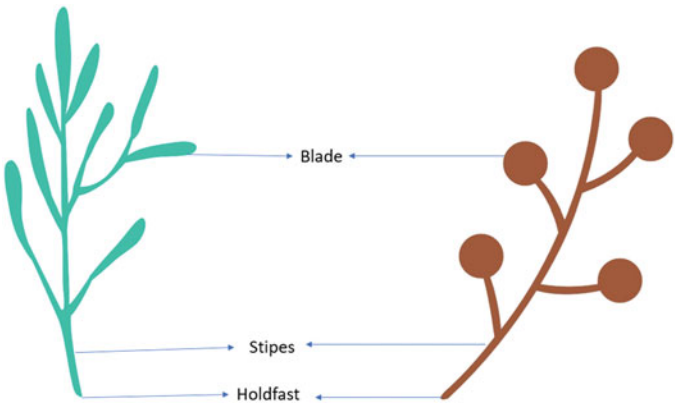


Fig. 12.6 Morphology characteristics of macroalgae

The required components for growth are frequently available in the coastal environment; therefore, seaweed production does not require arable land or fertilizer. Furthermore, macroalgae biomass outputs can be higher than most terrestrial crops throughout a growing season [125]. In this regard, using seaweed biomass to make biofuels seems to be a potential approach for supplementing and securing energy supply while also reducing reliance on fossil fuels, which is in line with the EU's goal [8].

Macroalgae are extremely important, since they can control pollution, eutrophication, and increase biomass in water bodies due to increased nutrients such as nitrogen and phosphorus. They also have characteristics that make them good candidates for application in the biorefinery. Macroalgae have higher efficiency in photon conversion than terrestrial plants and accumulate large amounts of carbohydrate biomass from inexpensive nutrient sources. Because they are buoyant, they do not produce structural polysaccharides like hemicellulose and lignin, so the process for ethanol production, in the pretreatment part, is much more straightforward [26]. Biomass production from red algae produces more energy than other biomass sources. Like terrestrial plants, macroalgae contain high value-added chemicals like carbohydrates, lipids, proteins, and other compounds, such as chlorophyll or carotenoid pigments. Carbohydrates are divided into polysaccharides and monosaccharides. These carbohydrates are in the cell walls and are generally alginates, agar, carrageenan, cellulose, fucoidan, and hemicellulose [124]. Macroalgae have advantages over terrestrial plants because several of these carbohydrates are different from glucose polysaccharides. These compounds can be used in various processes, almost always stabilizing thickening or gelling agents [126]. Also, macroalgae contain sulfur carbohydrates (sulfated carbohydrates) such as fucoidan, which has immunomodulatory and anti-inflammatory activities, lower blood lipid levels, and anticoagulant, antithrombotic antivirus antitumor, and antioxidant activity and activity against hepatopathy and renal disease, among others [127]. In the same way, mannitol, sugar alcohol, has hydrating and antioxidant activity and has a sweet taste, so it is used as a sweetener and reduces the crystallization of sugars.

Red algae, also called Rhodophyta, have agar and carrageenans in their cell wall, composed of sulfated galactan [128]. Green algae or Chlorophyta have three heteropolysaccharides in their cell wall: glucuronoxylorhamnans, glucuronoxylorhamnogalactans, or xyloarabinogalactans. Finally, the brown algae or Phaeophyta's cell wall comprises alginate, a uronide polymer comprising mannuronate and guluronate residues, and laminarin, a pillar of β-1,3-linked glucose moieties with β-1,6-linked branches [129].

As can be inferred, the composition of the different types of algae varies. Of the 10–15% of the dry matter that makes up algae, 60–65% is carbohydrates, and like all plants, this composition is influenced by the growing conditions and the climate [26]. In general, the carbohydrate composition is as follows:

- Green algae. Polysaccharides: mannan, ulvan, starch, cellullose. Monosaccharides: glucose, mannose, uronic acid.

- Red algae. Polysaccharides: carrageenan, agar, cellulose, lignin. Monosaccharides: glucose, galactose, agarose,
- Brown algae. Polysaccharides: laminarin, mannitol, alginate, glucan, cellulose. Monosaccharides: glucose, galactose, uronic acid.

Compared to other compounds, brown and red algae have less lipid content than green algae. In contrast, green algae species have higher cellulose content than red and brown algae and may contain starch. Furthermore, macroalgae have a higher range of alkali metals and halogen content [122].

Enzymatic hydrolysis research is focused on producing high-value products from seaweed biomass since the product yields could be more profitable in focused markets than biofuels. Seaweed is known to contain a wide array of naturally occurring bioactive compounds; carotenoids, fatty acids, phycocolloids, sterols, and an extensive range of secondary metabolites [130]. Compared with terrestrial biomass sources, algal biomass is composed mainly of lipids and proteins and has a faster growth rate, thus increasing photosynthetic efficiency [131]. This hydrolysis could imply a reliable source for biofuels and high added-value products. Table 12.6 lists some of the research reported for producing higher value-added compounds from seaweed biomass.

Considering the growing markets worldwide, such as the surge in some populational sectors demanding healthy products for consumption and some species of seaweed have been consumed historically in Asian cultures for millennia [132], opportunities exist for using edible seaweed biomass food formulations. Several studies propose the implementation of bioactive extracts in meat and meat derived products since the current overview of meat have been dwindling and is no longer considered essential in the human diet; polysaccharides, protein, omega-3 fatty acids, carotenoids, phenolic compounds, vitamins, and minerals could transform meat into a functional food since some formulations can improve the "bad" nutritional aspects but the most significant drawback encountered is the organoleptic modification of the meat, that impact negatively in consumer acceptance [133].

Biologically active compounds could become the backbone of some biorefinery processes. *Laminaria japonica* is a reliable source of alginate oligosaccharides that possesses a wide assortment of exploitable qualities: antioxidant, prebiotic activity, cytokine-inducing activity in mononuclear blood cells, and plant rooting enhancers, which are usually obtained with environmentally harsh procedures. It has been confirmed that a combination of commercial cellulases for the saccharification process and an engineered yeast (*Yarrowia lipolytica*) obtain a yield of 91.7% [16] and an oligosaccharide purity of 92.6%, with the added benefits of being an environmentally friendly procedure. Bioactive peptides with pharmaceutical activities are also obtainable since seaweed can be utilized as another alternative protein source, peptides are a given, and some peptides available from macroalgae present antioxidant, antihypertensive, anti-inflammatory, and antidiabetic activities, this, however, is limited to the variation of the protein content influenced by several factors, and the obtention can be difficult since the complex constitution of seaweed hinders the obtention of bioactive peptides. Also, there is a lack of proteomic studies to reduce the scope of peptide utilization and identification [134].

Table 12.6 High value-added bioproducts obtainable from macroalgal biomass using specific enzymes

Algae	Enzyme utilized/ methodology	Bioproduct obtained	Purposed outlook	References
Hizikia fusiforme	Commercial cellulases	Fucoidan	Antioxidant for food or cosmetic application	[136]
Sargassum horneri	Recombinant fucoidanase FFA1	Fucoidan	Anticancer and radiosensitizer action	[137]
Macrocystis pyrifera	Commercial cellulases	Bioactive proteins	Antioxidant, potential antihypertensive	[138]
Chondracanthus chamissoi	Commercial cellulases	Bioactive proteins	Antioxidant	[138]
Palmaria palmata	Cellulases/ alkaline extraction	Protein	Protein-rich feed for poultry or fish	[139]
Laminaria japonica	Alginate lyase/ thermo—acid pretreatment	Low-molecular-weight polysaccharides rich in uronic acid	Anti-obesity agent	[140]
Sargassum fulvellum	Commercial cellulases	Bioactive carbohydrates	Antioxidant	[141]
Porphyra dioica	Prolyve®1000 and Flavourzyme®	Bioactive proteins	Antioxidant	[142]
Gracilaria lemaneiformis	H_2O_2-assisted enzymatic method	Sulfated rich agar	Improved gel strength	[143]
Laminaria japonica	Cellulase and recombinant alginate lyase	Alginate oligosaccharides	Prebiotic, immunomodulating, antioxidant and plant rooting agent	[16]

Macroalgal biomass is predominantly used for high value-added byproducts and food production around the world. The biorefinery approximation for biofuels, bioactive compounds, and biomaterials production is currently under development [135]. The number of algal fuel producer companies is increasing globally, and there is undeniable potential for the utilization of enzymes for the marine biomass transformation industry.

12.6.1 Enzymatic Hydrolysis of Macroalgal Biomass

The more widespread utilization of enzymes in biorefinery is the hydrolysis of the structural polysaccharides to promote a more effective saccharification process to

widen the availability of assimilable sugars for posteriors biotransformation via microorganism's metabolism. Since the financial implications regarding the cost of the whole saccharification process do not allow the sole utilization of enzymes [7, 144], some methodologies have been coupled to synergize and lower the targeted production costs of biofuel or high added value products. All costs can provide seaweed biomass even in countries with cold weather; Nordic countries have limited light levels and low temperatures that hinder first-generation biofuels, but the vast coastlines are rich in marine biomass. For example, *Saccharina latissima* known for its high carbohydrate content, is widely available in the warm cost and studies to have been made for its utilization in methane production; an enzyme complex of β-1-3/1–4-glucanase, cellulase, xylanase, β-glucosidase, β-xylosidase, α-L-arabinofuranosidase was utilized to improve the reducing sugar release of alkaline treated pulp for anaerobic digestion. Enzymatic hydrolysis of macroalgal biomass can potentially harness 1760 m^3 per hectare of the productive seafloor for *S. latissima* [145].

Industries revolving around marine biomass residues can be a good source for biofuels and high added-value products. An estimated 57,500 tons of carrageenan are annually produced, and as long the hydrocolloid industry is growing, its waste will increment accordingly. The waste obtained from the carrageenan extraction of *Kappaphycus alvarezii* can be transformed with an acid pretreatment and later enzymatically hydrolyzed to enhance the saccharification of galactose and glucose 13.8 g/L of ethanol yield after a fermentation process utilizing a modified *Saccharomyces cerevisiae* (ATCC 200062) [146]. Agar is another phycocolloid obtained from red algae, and the agar extraction industry for *Gelidium* and *Gracilaria* seaweeds produces around 100,000 tons of carbohydrate-rich residues each year; this residue still has potential for the extraction of valuable compounds, according to a study [147] that hydrolyzed the residues using a sulfamic acid pretreatment and enzymatic hydrolysis.

12.6.2 Conversion of Sugars into Ethanol from Macroalgae

Bioethanol can be produced from macroalgae by converting sugars released in the enzymatic saccharification process [148] by fermentation using various microorganisms [149], as shown in Table 12.7. Fermentation is a process in which alcohol and CO_2 (carbon dioxide) are converted from glucose; stoichiometrically, 1 g of glucose produces 0.51 g of ethanol along with 0.49 g of CO_2 after fermentation. Bioethanol yields are highly dependent on temperature, pH level, growth rate, alcohol tolerance, osmotic resistance, and genetic stability of the fermenting microorganism. Among the organisms that can be employed in bioethanol production, the mainly used *Saccharomyces cerevisiae*, *Pichia angophorae*, *Pichia stipitis* [150, 151], *Kluyveromyces marxianus* [152], *Zymomonas mobilis* [153], among others shown in Table 12.6.

Table 12.7 Ethanol yields from macroalgae biomass according to fermentation strategy and microorganism strain

Macroalgae biomass	Fermentation strategy	Strain	Ethanol concentration (g/L)	Ethanol yield (%)	References
Sargassum spp.	PSSF	*S. cerevisiae* PE-2	18.14 ± 1.11	76.23 ± 4.68	[160]
Saccharina japonica	SSF	Thermotolerant *S. cerevisiae* DK 410362	6.65	67.41	[161]
Laminaria digitata	SHF	Commercial yeast *S. cerevisiae*	20.7	70.6	[162]
Gelidium amansii	SHF	*S. cerevisiae* KCTC 7906	3.33	74.7	[163]
Gelidium amansii	SSF	*S. cerevisiae* KCTC 7906	3.78	84.9	[163]
Gelidium amansii	SSF	*S. cerevisiae* KCTC 7906	25.7	76.9	[163]
Ulva rigida	SHF	Adapted *Pachysolen tannophilus*	11.92	72.35	[158]
Sargassum muticum	SSF	*S. cerevisiae* CEN.PK 1137D	11.32	94.4	[164]
Sargassum muticum	SSF	*S. cerevisiae* PE-2	12.23	81	[164]
Sargassum muticum[a]	SSF	*S. cerevisiae* PE-2	14.10	81	[164]
Rhizoclonium sp.	SHF	Immobilized S. *cerevisiae* TISTR 5020	65.43 ± 18.13	–	[165]
Laminaria digitata	SHF	*S. cerevisiae* NCYC 2592	3.2	94.4	[166]

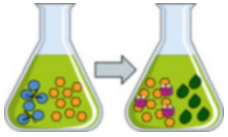
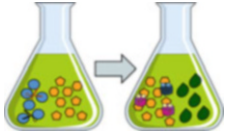
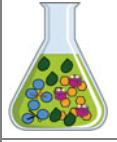
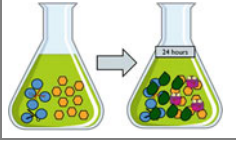
[a]Using hydrolysate from pretreatment as reaction medium

K. marxianus is a species of yeast that is thermotolerant with proficiency to ferment an extensive range of substrates. Some advantages involve the consumption of several sugars at elevated temperatures and weak glucose repression. *K. marxianus* can work at temperatures up to 47 °C with a solid affinity for xylose [152] and possesses high growth rates and less tendency to ferment when exposed to excess sugars [154]. *Z. mobilis* is a bacterium facultatively anaerobic and nonsporulating ethanologenic that converts sugars to ethanol through the Entner-Doudoroff pathway; this microorganism accumulates less biomass during fermentation more sugar can be converted to ethanol, increasing its observed yield. *Z. mobilis* metabolizes glucose, fructose, and sucrose. It can endure high sugar concentrations [155]. *P. stipitis*, also known as *Scheffersomyces stipites*, is a homothallic yeast that can ferment pentose sugar like xylose. The fermentation starting is not dependent on sugar concentration. However, it is regulated by a decrease in oxygen availability. It possesses a greater respiratory capacity owing to the existence of an alternate respiration system. It also includes the enzyme dihydroorotate dehydrogenase, which grants the ability to grow anaerobically [152]. *Pichia angophorae* showed that fermentation could occur with hydrolysates containing laminarin and mannitol present in brown macroalgae [151]. Other microorganisms have been used, like the marine yeast *Meyerozyma guilliermondii*, which can be a candidate for the marine bases substrates [156], non-adapted *Pachysolen tannophilus*, and the marine fungus *Cladosporium sphaerospermum* have also been studied on macroalgae feedstock for bioethanol production [157, 158]. However, *Saccharomyces cerevisiae* are the most employed microorganisms mainly due to their effectiveness, resistance to high ethanol and inhibitor concentrations, and high osmotic resistance [150, 151]. *S. cerevisiae* is the most exploited yeast in industrial for bioethanol production [157]. Besides that, *S. cerevisiae* has an exceptional function in high sugar concentrations that merge passive sugar transport with high glucose flux through glycolysis to ethanol production, despite the presence of oxygen, thereby having a strong positive Crabtree effect. These are an excellent advantage in the extensive industrial configuration where anaerobiosis has an additional level of difficulty, namely removing available oxygen in a closed batch bioreactor or fed-batch bioreactor using setting at the time of fermentation and avoiding the integration of ethanol at the final step of fermentation [159].

Another critical parameter is the fermentation strategy chosen. The primary users are SHF, Separate Hydrolysis and Co-Fermentation (SHCF), SSF, Simultaneous Saccharification and Co-Fermentation (SSCF), and Pre-Simultaneous Saccharification and Fermentation (PSSF), for bioethanol production based on first and second-generation. Table 12.8 shows all strategies in detail.

Studies have been reported for bioethanol production from macroalgae. Tan et al. [171], used *Saccharomyces cerevisiae* PE-2 under SSF strategy and reached 12.23 and 14.19 g/L of ethanol concentration employing water and hydrolysate from hydrothermal pretreatment as a medium, respectively, obtaining a conversion yield of 81%. Hou et al. [162] used *Laminaria digitate* as a feedstock for bioethanol production under SSF and SHF strategies using *S. cerevisiae* (Quick Yeast, Doves Farm Foods Ltd.), their results were 14.7 ± 0.3 g/L of ethanol equivalent to a

Table 12.8 Main strategies used in bioethanol production from macroalgae

Strategy	Schematic representation	Description	References
SHF		SHF is a process where hydrolysis of the polysaccharides from macroalgae and fermentation of hexoses are performed separately. Both operations can be carried out at optimal conditions (pH, temperature), thus maximizing general performance. The difference between SHF and SHCF is that pentoses and hexoses are simultaneously fermented in the second step. Advantages are that conditions are adequate for each procedure. Disadvantages are long process times, use of enzymes and microorganisms capable of assimilating pentoses, which entails increasing overall cost of the process	[167, 168]
SHCF			
SSF		SSF combines enzymatic hydrolysis of macroalgae and fermentation of the hexoses in a single stage. The process is faster, but inhibitors from pretreatment could affect with more intensity on yeast microorganisms. An in the above strategies, the difference between SSF and SSCF is that the second considers enzymes and microorganisms for hexoses and pentoses. SSF process has several advantages such as less enzyme requirement, low-risk contamination. Simultaneous approach is usually preferred over approaches that work separately due to the faster ethanol production rate resulting in the rapid metabolism by yeast of the sugars released. However, the difference between the optimal temperatures of hydrolysis and fermentation (50 °C for enzymatic hydrolysis and 30–35 °C for fermentation) comprises the primary deficiency	[169–171]
SSCF			
PSSF		PSSF consists of the first stage of enzymatic hydrolysis, generally between 4 and 24 h, at the optimal conditions of the enzymes, followed by the addition of the fermenting microorganism(s) to start the fermentation stage at the optimal conditions. This process allows the reduction of viscosity in the slurry, improving the mass transfer in the conversion to ethanol, thereby increasing the ethanol yields	[172, 173]

conversion yield of 50.5% under SSF strategy, and 20.7 ± 0.5 g/L of ethanol equivalent to a conversion yield of 70.6 ± 1.8 under SHF strategy. They concluded that the lesser ethanol produced is due to the low efficiency in the enzymatic hydrolysis stage (enzymes work at optimal conditions at 50 °C, and the experiment was carried out at 32 °C. Kim et al. [163] investigated bioethanol production from autoclave treated *Gelidium amansii* as biomass. The research study states that the comparative analysis of SHF and SSF for 2% (w/v) supports the SSF process for the highest bioethanol conversion yield corresponding to 90.7% with 3.33 mg/mL and 84.9% with 3.78 mg/mL, respectively. On proceeding for the SSF process at 15%, solid loading (w/v) gives a satisfactory result with an increment in bioethanol concentration 25.07 mg/mL with 76.9% conversion yield. Lee et al. [161] worked with thermotolerant yeast *S. cerevisiae* DK 410362 under SSF strategy, scaling from 3 to 6% (w/v) of solid loading. They achieved 3.84 and 6.65 g/L of maximum ethanol concentration for 3 and 6%, reaching 78.41 and 67.39% ethanol yield, respectively. Another study, El Harchi et al. [158], adapted *Pachysolen tannophilus* to ferment *Ulva rigida* biomass under SHF strategy; they reached 11.92 g/L of ethanol concentration 72.35% conversion yield.

The studies highlight that sugars from macroalgae could be a potential feedstock for bioethanol production. However, additional research is needed to achieve an eco-friendly and economically viable process. Further, more studies are required to fully comprehend the antiviral action mechanisms of algal chemicals and reap the benefits of their utilization as functional additives in the pharmaceutical and food sectors.

12.7 Conclusions and Future Outlook

Micro- and macro-algae biomass can produce novel bioproducts and are used as an indigenous biological source serving as a bridge between the environment and changing climatic conditions by creating eco-friendly energy products with extensive food, medicine, bioenergy, and cosmetics industries in terms of biorefinery. Micro- and macro-algae biofuel production under the biorefinery strategy is expected to significantly enhance algae biofuels' overall cost-effectiveness. However, integrating diverse biomass conversion methods in a whole algal biorefinery operation remains a fundamental problem. Before industrial use of algal technology and the commercialization of microalgal biofuels becomes realized, considerable technological breakthroughs and increased biomass production are required. In terms of biorefinery, technical advancements in extraction technique and enzymatic saccharification are necessary to improve the cost-effectiveness of end products such as micro-and macro-algae biofuels. Nonetheless, algal biorefinery processes can be implemented in the near future if the expense of biofuels is compensated by revenue from bioproducts for the circular bioeconomy.

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