# Handbook of Biofuels

Edited by **Sanjay Sahay**

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Edited by

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Chapter 15

# Third-generation bioethanol: status, scope, and challenges

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## 15.1 Introduction

Major commercial energy sources such as oil, coal, and natural gas are extracted from fossil fuels. The burning of fossil fuels results in the escalation of $CO_2$ in the atmosphere, which is a major cause of global warming, price volatility, air pollution, and environmental degradation (Adenle et al., 2013; Naik et al., 2010). Surging demand in these sectors has led to an increase in oil production from the finite source of fuel reserves. Continuous exploitation is depleting these reserves at a staggering speed, which will no longer suffice the world's energy demand (del Río et al., 2020; Goli et al., 2016; Hirsch et al., 2005; Raheem et al., 2018), leading to a global energy crisis. Hence fossil fuels are regarded as unsustainable and questionable from economic, ecological, and environmental points of view (Naik et al., 2010). Therefore the quest for an economical, renewable, sustainable, and environmentally benign source of energy is underway (Hahn-Hägerdal et al., 2006; Tripathi et al., 2016). Biomass energy in the form of cow dung cake, firewood, agriculture residue, and other natural feedstock for cooking and heating has been prevailing for ages and contributes to 80% of rural energy in developing countries like India (Kumar et al., 2015; Ramachandra, 2010; Ramachandra et al., 2000, 2004). Biofuels from biomass such as plants, algae, or organic waste are emerging as promising alternative renewable energy sources to liquid fuels (Jambo et al., 2016). Different technologies have evolved toward the conversion of biomass into fuels and other value-added products that have the advantage of mitigating global warming by cutting down carbon dioxide emissions, as $CO_2$ is fixed by the biomass via photosynthesis, making it a carbon-neutral emission (del Río et al., 2020) and also easing the dependency on oil reserve (Bhattacharyya, 2006; Kumar et al., 2015; McKendry, 2002; Naik et al., 2010). Biofuels are of two types, namely bioethanol and biodiesel; bioethanol is produced from carbohydrate-rich algal biomass (e.g., macroalgae), whereas biodiesel is produced from lipid-rich algal biomass (e.g., microalgae). The dependence on fossil fuels (gasoline) in the transport sector can be reduced by bioethanol, as it is effective in replacing or blending with gasoline. The development and commercialization of bioethanol are largely achievable due to the availability of feedstock in large quantities (Jambo et al., 2016). Bioethanol feedstocks are categorized into first, second, and third generations based on the feedstock's carbon source. Bioethanol from first-generation feedstock (1G) involves food crops like corn and sugarcane, which encounter resistance due to the arable land, freshwater source for its cultivation, and competition with food crops (Naik et al., 2010). The lacunae of 1G bioethanol in supplementing the growing energy demand led to the exploration of alternate feedstocks involving agricultural residues and woody biomass rich in lignocellulose [second-generation (2G) bioethanol feedstock]. However, 1G and 2G bioethanol production failed due to process technology involving the cost-intensive delignification process and difficulty in scaling up (Zhu and Pan, 2010). Bioethanol potential from 1G and 2G feedstock marginally complies with various other sustainability criteria, such as the conversion of ecologically vulnerable wetlands, extensive usage of fertilizers, soil erosion, rainforests, peatlands, savannas into energy croplands, and disruption of global food supply contributing to several magnitudes of $CO_2$ (Gasparatos et al., 2013; Maeda et al., 2015). Bioethanol production from third-generation feedstock (3G) involves algal biomass that is grown in freshwater, wastewater (Ramachandra et al., 2013), and marine waters with zero nutrient input and, more importantly, noninterference with the lands required for food production (Demirbas,

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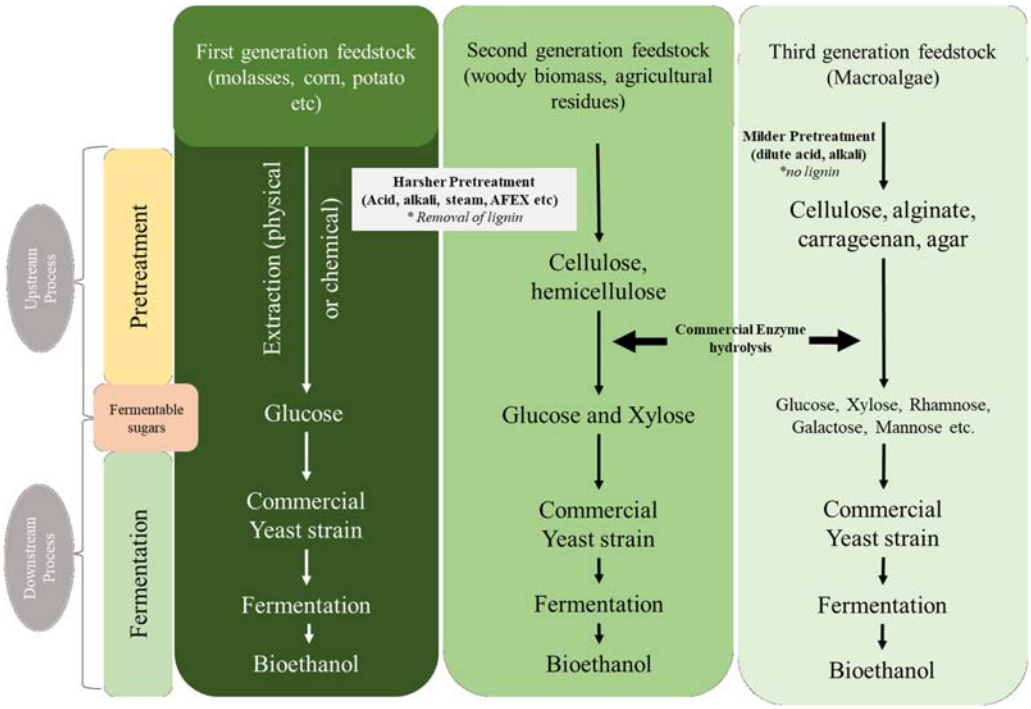



**FIGURE 15.1** Bioethanol production process from biomass.

2008; Odum and Heald, 1972). At present, the research focus is currently on bioethanol production from 3G feedstock due to higher photosynthetic efficiency (6%−8%), productivity (∼13.1 kg dry weight/$m^2$ over 7 months), ease of cultivation, low consumption of fertilizers, no alteration with food supply, and high absorption of $CO_2$ (8−10 tonnes $CO_2$ per hectare) (Kraan, 2013), potential to obtain high value-added products (pigments, cosmetics, food additives, etc.) Algal biomass has emerged as one of the ideal feedstock for achieving sustainable biorefinery having immense potential for commercialization (Jambo et al., 2016) (Fig. 15.1).

## 15.2 Bioethanol production from algal biomass

Production of bioethanol from algal biomass involves three steps, namely pretreatment, saccharification, and fermentation, which are discussed in detail in the subsequent sections. Algae are of two types: micro- and macroalgae. Microalgae are explored for the production of biodiesel (Ramachandra et al., 2009; Saranya et al., 2018), whereas macroalgae, rich in carbohydrate, are suitable for the production of bioethanol (Borines et al., 2013; John et al., 2011; Ramachandra and Hebbale, 2016, 2020; Roesijadi et al., 2010; Wei et al., 2013; Yanagisawa et al., 2013). Macroalgae (commonly known as seaweeds) are multicellular, photosynthetic algae growing in marine environments and, to a lesser extent, in brackish waters. Photosynthetic pigments in seaweeds impart a characteristic range of colors, for example, red (Rhodophyta), green (Chlorophyta), and brown (Phaeophyta) algae (Abbott et al., 1992; Smith, 1938; Van Den Hoek, 1984). Green seaweeds are euryhaline, that is, tolerating a wide variations in salinity levels, whereas red and brown seaweeds are strictly marine dwelling. Seaweeds have a wide distribution from tropics, temperate, and polar regions to tidal pools, estuaries, deep waters, and rocky shores, whereas brown seaweed species, belonging to the order Laminariales, occur mostly in temperate regions (<24°C) (Abbott et al., 1992). Seaweeds grow by attaching to a substrate (natural or artificial); due to the need for stable anchorage, large seaweed beds are restricted to rocky substrates (Abbott et al., 1992; Speight and Henderson, 2013). Macroalgal tissues lack specialized translocatory systems and the structure of the "higher plants" (Abbott et al., 1992). The macroalgal body is a rootless, stemless, and leafless entity called thallus, although many have superficially leaf-like blades and stem-like stipes and often have attaching organs called holdfast or haptera (Lobban et al., 1994; Smith, 1938). Most algae lack these structures, owing to their morphological adaptations and modifications (Abbott et al., 1992). Seaweeds reproduce either asexually or sexually (Lobban et al., 1994). Asexual reproduction is a common mode of reproduction in seaweeds.

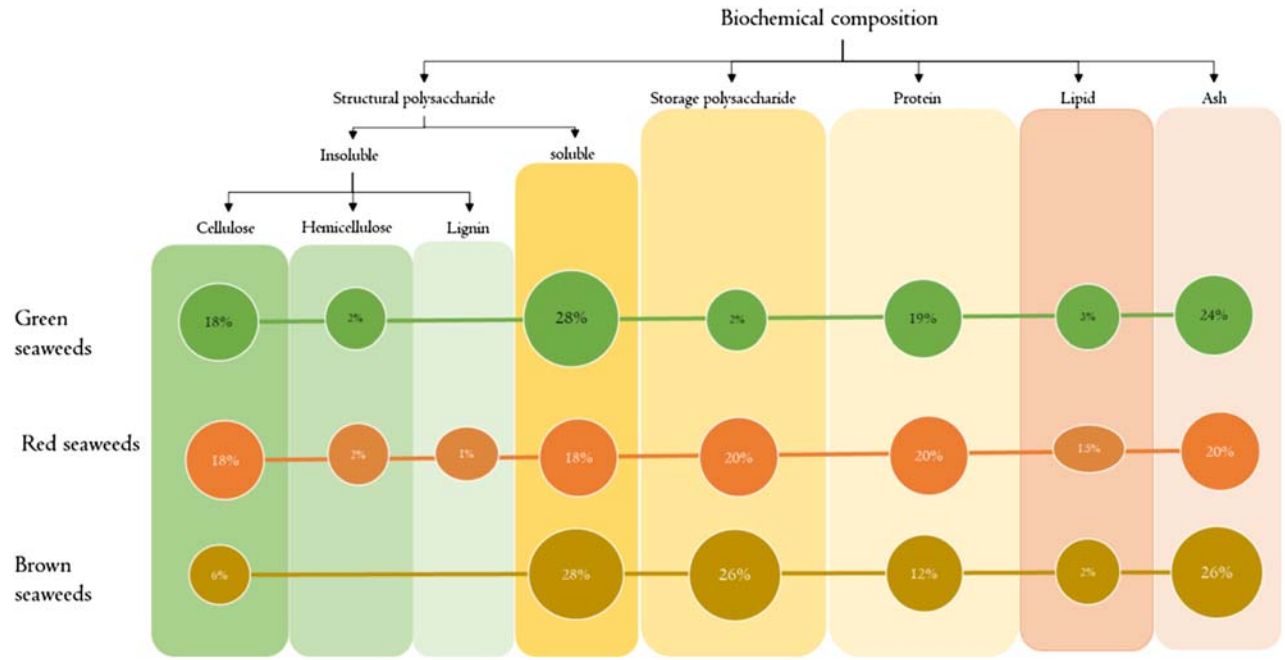



**FIGURE 15.2** Macroalgal biochemical composition profile.

Generally, the biochemical composition of seaweeds is as follows: carbohydrates: 25%−77% dry weight, proteins: 5%−43% dry weight, lipid: 1%−5%, and ash content: 9%−50% dry weight followed by higher water content of 70%−90% fresh weight (Jung et al., 2013; Praveen et al., 2019). Seaweeds consist of varied profiles of structural and storage carbohydrates (Daroch et al., 2013; Kostas et al., 2016a,b) based on the respective intercellular spaces and cell wall (Pereira and Neto, 2014) (Fig. 15.2).

Seaweed polysaccharides show a range of structures and fulfill a variety of functions similar to neutral sugars and sugar acids of terrestrial plants. Certain seaweeds also contain acidic half-ester sulfated groups attached to hydroxyl groups of sugars. Hexose sugars such as glucose, galactose, and mannose found in these polysaccharides have identical chemical compositions. Carbohydrate reserves of red algae are usually stored in the form of small grains that lie in the cytoplasm outside the algal plastids, the chromatophores. The insoluble carbohydrate reserve of red algae has been called *Floridean starch* (intermediate between true starch and dextrin) (Yu et al., 2002). Polysaccharides such as starch and cellulose in green algae are similar to those of terrestrial plants. Macroalgal biomass lacks lignin in its composition (Jung et al., 2013), except in a few red seaweed species. Apart from the higher content of carbohydrates in seaweeds, protein and ash contents are also relatively higher; however, lipid fraction is considerably low.

An effective biorefinery process is achieved by the characterization of the feedstock employed, such as a large variety of carbohydrates (mono-, di-, polysaccharides) that serve as raw materials for bioethanol production. Quantification of carbohydrate content (Table 15.1) in the biomass is an essential step in the biorefinery process as it is directly proportional to ethanol yields in the biochemical conversion process and facilitates overall process efficiency calculations as well as mass balance (Aden et al., 2002; Kostas et al., 2016a,b). Seaweeds accumulate large concentrations of carbohydrates (polysaccharides) made up of various monosaccharides such as xylose, glucose, galactose, and fructose. These sugars are converted to bioethanol through fermentation via THE appropriate microorganisms.

### 15.2.1 Availability of macroalgal feedstock

Macroalgae occur along the nutrient-rich coastal zones by attaching to hard substrata. Global seaweed distribution is highest between 60°N and 60°S latitude with 900−1100 species; the least number of species are recorded >60 degrees in both hemispheres. In these regions, mostly, cold water-desiring macroalgae are recorded, such as *Laminaria* and *Undaria*. (Hurd et al., 2014). The most prominent macroalgal genera along the coastal regions of India explored for bioethanol potential are indicated in Fig. 15.3.

**TABLE 15.1 Biochemical composition and monosaccharide profile of potential macroalgal genera for bioethanol production.**

| Biochemical composition (%)/ 100 g dry biomass | *Ulva* sp. | *Gracilaria* sp. | *Gelidium* sp. | *Sargassum* sp. | *Kappaphycus* sp. | *Laminaria* sp. |
|---|---|---|---|---|---|---|
| Ash | 18–49.6 | 22.9–26 | 2.5–4.8 | 40.0–46.0 | 18.0–19.7 | 8.7–41.2 |
| Lipid | 1–3.5 | 0.7–6 | 0.7–7.4 | 0.75–2.5 | 0.2–0.75 | 0.6–3.4 |
| Protein | 10.7–25.9 | 4.3–16 | 10.2–18.7 | 10.25–15.42 | 2.3–5.74 | 1.1–19.8 |
| Carbohydrate | 53–69.9 | 30.4–76.67 | 53.2–75.8 | 23.5–41.81 | 51.6–59.58 | 33.9–76 |
| Monosaccharides composition/100 g carbohydrate | | | | | | |
| 3,6-Anhydrogalactose | | | 28.9–43.5 | | | |
| Arabinose | 0.0–0.8 | | | | | |
| Fucose | 0.2–0.4 | | 33.6–57.0 | 0.2–7.4 | | 4.2–8.5 |
| Galactose | 7.2–8.5 | 30.6–42.8 | 21.8–40.6 | 0.0–4.0 | 20.3–22.39 | |
| Glucose | 0.2–25.4 | 20.5–24 | | 22.5–65.6 | 0.4–0.78 | 24.5–62.2 |
| Mannitol | | | 0.0–0.8 | 0.2–5.04 | | 11.2–37.9 |
| Mannose | 0.0–4.2 | 0.0–0.07 | 0.0–0.4 | 0.0–4.0 | | |
| Rhamnose | 3.3–12.7 | | | | | |
| Ribose | 0.1–2.7 | | | | | |
| Uronic acids | 25.9–28.8 | | | | | 20.8–41.6 |
| Xylose | 5.6–14.4 | 0.0–0.3 | 0.0–1.3 | 0.5–19.4 | | 6.0–12.0 |
| Bioethanol (L/100 kg dw) | 5.58–11.7 | 2.6–4.7 | 1.62 | 4.4 | 1.7–2.4 | 5.4–26.2 |

(Abd-Rahim et al., 2014; Borines et al., 2013; Chennubhotla et al., 1990; Masarin et al., 2016; Parthiban et al., 2013; Sung-Soo, 2012; Wu et al., 2014; Yeon et al., 2011).

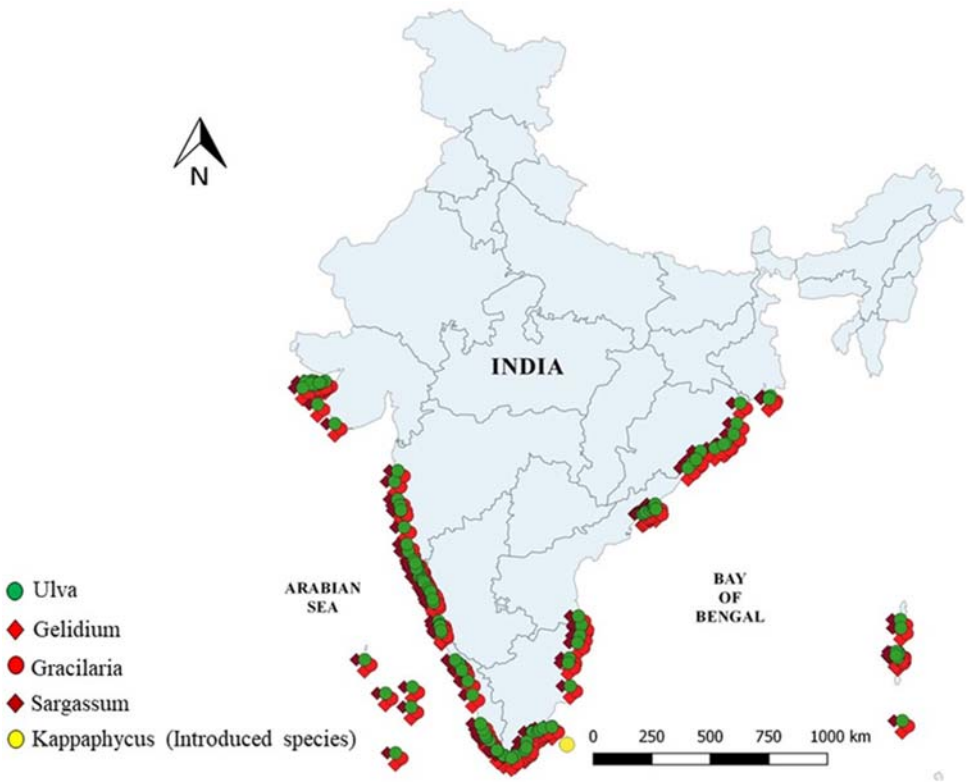



**FIGURE 15.3** Distribution of macroalgal species along the coastal regions across India, explored for bioethanol production potential (Ramachandra and Hebbale, 2020).

### 15.2.2 Pretreatment

Bioethanol production from macroalgae requires extraction of fermentable sugars; several studies have reported (Table 15.2) different pretreatment techniques (Wooley et al., 1999; Hendriks and Zeeman, 2009), including chemical, physical, or biological, or combinations of these techniques, through which higher sugar concentration can be obtained (Feng et al., 2011; Kim et al., 2014; Meinita et al., 2012b; Park et al., 2012; Yoon et al., 2010). Pretreatment of biomass is carried out to reduce the size and alter or remove structural and compositional impediments prior to subsequent enzyme hydrolysis. Pretreatment needs to be cost effective and release a high quantity of sugar with minimal inhibitor formation.

The most commonly used chemical pretreatment method for obtaining higher fermentable sugars from macroalgal biomass is the dilute acid pretreatment method, which employs mineral acids such as $H_2SO_4$ and HCl at milder concentrations of 0.3−0.9 N (Meinita et al., 2012a; Park et al., 2012). During the dilute acid pretreatment process, reaction parameters such as reaction time, acid concentration, and substrate concentration are involved for efficient sugar release from algal feedstock (Table 15.3). Pretreatment with dilute $H_2SO_4$ at optimal concentration and temperature is reported to be effective for cell wall depolymerization. The advantage of the dilute acid pretreatment method is lower energy consumption as compared to other pretreatments. However, a disadvantage of dilute acid pretreatment is the formation of fermentation inhibitors such as 5-hydroxymethyl furfural (HMF) and levulinic acid (LA) with the degradation of hexose sugars and furfurals from pentose sugar degradation. Hence enzyme hydrolysis has been determined to be a sustainable option for hydrolysis as it does not involve the formation of any inhibitors because enzymes do not cause the degradation of monosaccharides (Yanagisawa et al., 2013).

**TABLE 15.2** Assessment of selected pretreatment processes.

| | Pretreatment process | Yield of fermentable sugars |
|---|---|---|
| Physical or physicochemical | Mechanical | Low |
| | Steam explosion | High |
| Pretreatments | Ammonia fiber explosion (AFEX) | Moderate |
| | Carbonic acid | Very high |
| Chemical pretreatments | Dilute acid | Very high |
| | Concentrated acid | Very high |
| | Alkaline extraction | Very high |
| | Wet oxidation | High |
| | Organosolvent | Very high |
| Biological pretreatments | Commercial enzymes or bacterial/fungal enzymes | Very high |

Source: Hendriks, A.T.W.M., Zeeman, G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource Technology100, 10−18; Wooley, R., Ruth, M., Sheehan, J., Ibsen, K., Majdeski, H., Galvez, A., 1999. Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis current and futuristic scenarios.

**TABLE 15.3** Reducing sugar yield reported from macroalgal feedstock at different dilute $H_2SO_4$ concentrations.

| Macroalgal species | Dilute $H_2SO_4$ concentration | Reducing sugar yield | References |
|---|---|---|---|
| *Gracillaria verrucosa* | 1.5% | 430 mg/g | (Kumar et al., 2013) |
| | 373 mM | 7 g/L | (Nguyen et al., 2017) |
| | 0.1 N | 7.47 g/L | (Kim et al., 2015a,b) |
| *Kappaphycus alvarezii* | 0.9 N | 300 mg/g | (Khambhaty et al., 2012) |
| | 1% v/v | 81.62 g/L | (Hargreaves et al., 2013) |
| | 0.2 M | 30.5 g/L | (Meinita et al., 2012a) |
| *Laminaria japonica* | 0.06% | 29.09% | (Lee et al., 2013) |
| *Gelidium amansii* | 3% | 33.7% | (Park et al., 2012) |

### 15.2.3 Enzyme saccharification

The biological pretreatment method employs substrate-specific enzymes (Fig. 15.4). A major portion of the macroalgal cell wall is composed of cellulose, which is made up of glucose subunits. In order to break the cellulose structure, the cellulase enzyme is commonly used. Similarly, agarases are used for agar, carrageenase for carrageenan, alginase for alginate, and laminarases for laminarin. Pretreatment is a prerequisite prior to enzyme saccharification, as it opens up the cellulose fibrils and maximizes the enzymatic conversion of cellulose (Harun, 2011; Jeong et al., 2013; Kang et al., 2013; Kim et al., 2014). Commercial enzymes, as well as enzymes extracted from bacteria or fungi, have been reported for enzyme saccharification of macroalgal biomass (Table 15.4).

Enzyme saccharification of cellulose to glucose is considered an environmentally friendly pretreatment process. However, this research is at a nascent stage, orientated toward isolating efficient enzyme systems (Swain et al., 2017) from microorganisms that produce cellulolytic enzymes in their metabolic processes (Bhat and Bhat, 1997; Niehaus et al., 1999; Zhang and Kim, 2010). Higher concentrations of extracellular cellulase enzymes have been reported from bacteria and fungi that are feasible for large-scale production. Terrestrial sources for cellulase enzyme have been extensively explored and investigated; however, studies related to cellulase extraction from marine source is still an unexplored platform. A large reservoir of microbes thrives in the marine ecosystem at extreme conditions of salt, temperature, and high pressure (Trivedi et al., 2016), which imparts well-developed cellular machinery and stable enzymes, offering novel biocatalysts with unusual properties which can be explored for bioethanol production (Gao et al., 2010; Zhang and Kim, 2010).

### 15.2.4 Fermentation

Sugars obtained from dilute acid hydrolysis, enzyme saccharification, or a combination of both are subjected to fermentation, where microorganisms consume the sugar as their sole source of carbon and metabolize it for their growth and reproduction and yield ethanol as a by-product. Fermentation is dependent on the simple sugars; seaweeds consist of both $C_6$ and $C_5$ sugars, but not all the microorganisms can metabolize both the sugars simultaneously. Hence the choice of the organism for fermentation plays a pivotal role. The most widely used microorganism for ethanol fermentation is *Saccharomyces cerevisiae*, which metabolizes hexose ($C_6$) sugars. Fermentation of glucose alone will not produce high yields of ethanol. *Pichia stipitis and Pichia angophorae* can metabolize pentose ($C_5$) sugars. Other than yeast microorganisms, bacteria such as *Pacchysolan tannophilus* and *Escherichia coli* have also been studied for ethanol production from hexose and pentose sugars. Macroalgae are also composed of sugar alcohols that are not metabolized by yeast microorganisms; *Zymobacter palmae* isolated from palm was observed to convert mannitol present in brown algae into ethanol (Horn et al., 2000a,b).

Glucose is metabolized in a series of enzyme-catalyzed reaction processes called glycolysis to yield two molecules of three-carbon compound pyruvate. Under hypoxic or anaerobic conditions, pyruvate is decarboxylated, and acetaldehyde is reduced to ethanol through alcohol dehydrogenase (Nelson and Michael, 2008). Xylose is converted to xylulose and phosphorylated to xylulose-5-phosphate and further metabolized to glyceraldehyde-3-phosphate and fructose-6-phosphate, which then enters the glycolysis pathway for subsequent pyruvate and ethanol production (McMillan, 1993), as illustrated in Fig. 15.5.

*S. cerevisiae* is the predominant microorganism utilized in ethanol fermentation in industrial bioethanol production processes. Ethanol is produced via homoethanol pathways, by Embden−Meyerhof−Parnas (EMP) glycolytic pathway,

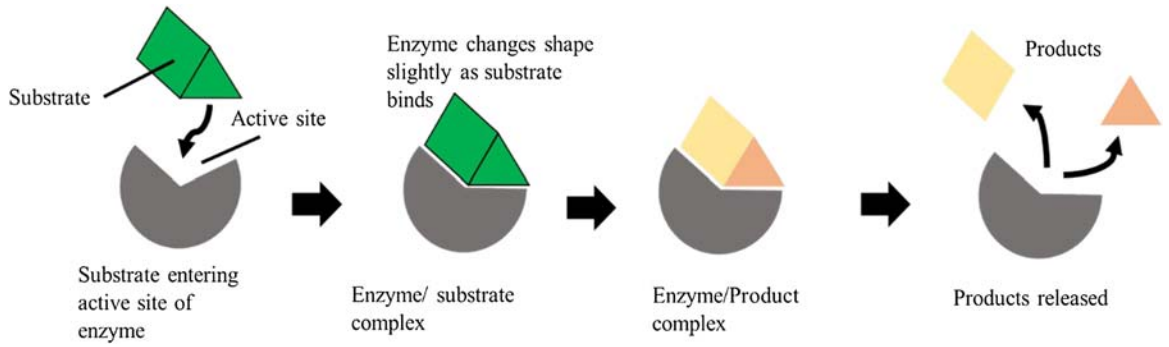



**FIGURE 15.4** Schematic representation of enzyme action on substrates.

**TABLE 15.4** Reducing sugar yield reported from macroalgal feedstock using enzymes.

| Macroalgal feedstock | Enzymes hydrolysis | Sugar yield | References |
|---|---|---|---|
| *Enteromorpha intestinalis* | Viscozyme L and Cellic CTec2 | 20.1 g/L | (Kim et al., 2014) |
| | Celluclast 1.5 L and Viscozyme L | 40 g/L | (Cho et al., 2013) |
| *Ulva fasciata* | Cellulase 22119 | 215 mg/g | (Trivedi et al., 2013) |
| | Viscozyme L | 206 mg/g | |
| | Cellulase isolated from *Cladosporium sphaerospermum* | 112 mg/g | ( Trivedi et al., 2015) |
| *Ulva pertusa* | Meicelase-simple saccharification | 43 g/L | (Yanagisawa et al., 2011) |
| | Meicelase | 78.8 g/L | |
| | Meicelase | 59.1 g/L | |
| | Cellulase and amyloglucosidase | 26 | (Choi et al., 2012) |
| *Gelidium elegans* | Meicelase | 70.9 g/L glucose | (Yanagisawa et al., 2011) |
| | | 53.2 g/L galactose | |
| *Gelidium amnasii* | Cellulase 0.98 FPU/g β-glucosidase 10.4 U/g | 43.7% glucose | (Kim et al., 2015) |
| | | 12% galactose | |
| *Kappaphycus alvarezii* | Celluloclast 1.5 L and Novozyme | 11 g/L | (Tan and Lee, 2014) |
| | Multifect | 81 g/L | (Hargreaves et al., 2013) |
| *G. amansii* | Enzyme viscozyme L | 2.4 g/L | (Ra et al., 2013) |
| *G. amansii* | Celluclast (0.168 EGU/mL) | 10.5 g/L | |
| *Gracillaria verrucosa* | 10% enzyme extract | 7.47 g/L | (Kim et al., 2015) |
| *Acleisanthes crassifolia* | Meicelase | 66.3 g/L | (Yanagisawa et al., 2011) |
| *Saccharina japonica* | Enzyme cellulase- 45 FPU/g cellobiase- 55 CBU/g | 268.5 mg/g | (Ge et al., 2011) |
| *Undaria pinnatifida* | Celluclast 1.5 (4 mL/100 g of cellulose) Novozyme 188 | 65 mg/g | (Lee et al., 2011) |
| *Sargassum* sp. | 10 FPU cellulase /g, 250 CBU cellobiase/g | 120 mg/g reducing sugar | (Borines et al., 2013) |
| *S. japonica* | Novozyme (Termamyl 120 L) | 20.6 ± 1.9 g/L | (Jang et al., 2012) |

which is summarized below (Walker and Walker, 2011):

$$Glucose + 2ADP + 2Pi + 2NAD^{+} \rightarrow 2Pyruvate + 2ATP + 2NADPH + 2H^{+}$$

*S. cerevisiae* reoxidizes the reduced coenzyme NADH to $NAD^{+}$ in terminal fermentative step reactions emanating from pyruvate:

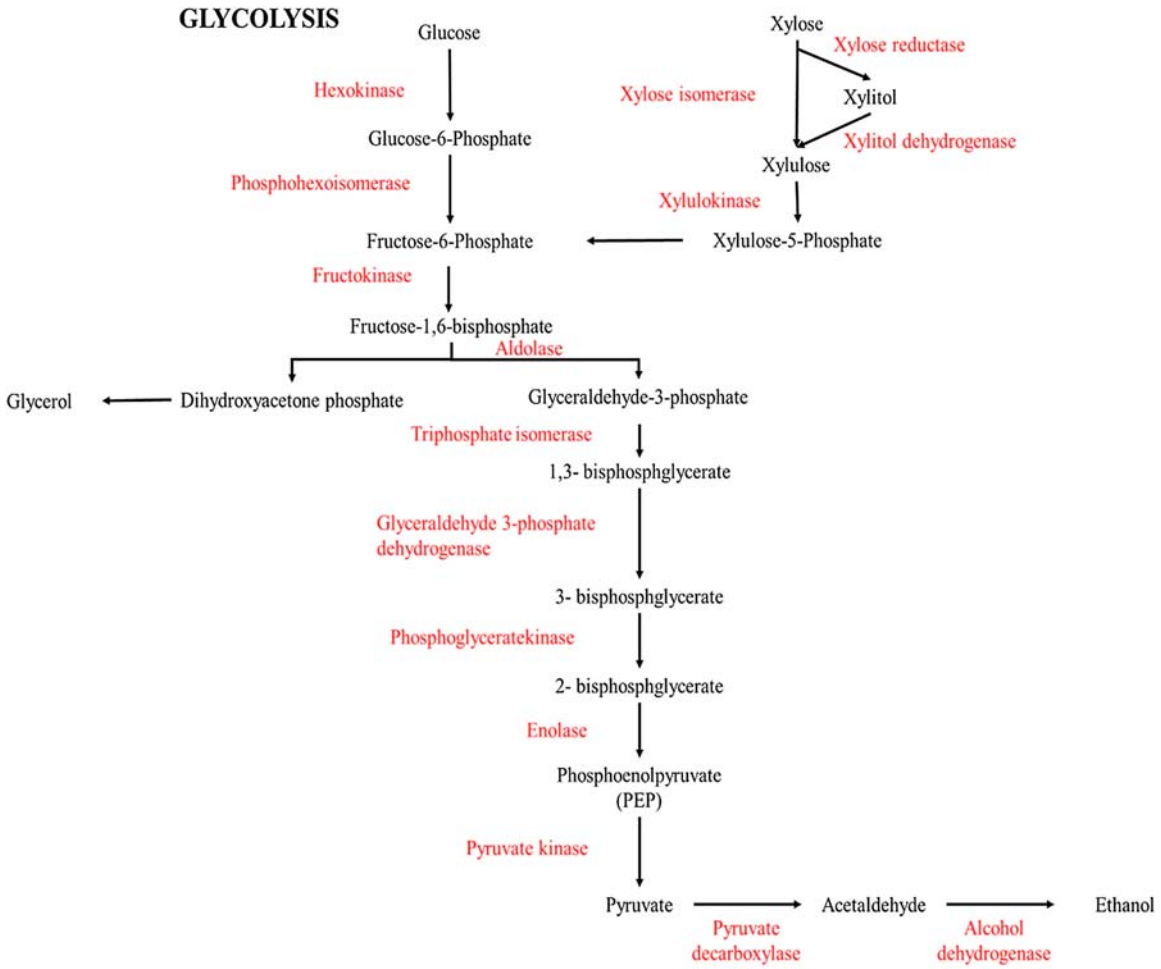
$$2Pyruvate + 2NADH + 2H^{+} \rightarrow 2NAD^{+} + 2Ethanol + 2CO_2$$

The intermediate compound, acetaldehyde, acts as the electron acceptor:

$$CH_3COCOOH(Pyruvate) \xrightarrow{Pyruvatedecarboxylase} CH_3CHO + CO_2(Acetaldehyde + CO_2)$$
$$\xrightarrow{Alcoholdehydrogenase} CH_3CH_2OH(Ethanol)$$

$NAD^{+}$ is regenerated by alcohol dehydrogenase, which requires zinc as an essential cofactor for its activity. Fermentation thus maintains the redox balance by regenerating NAD and keeps glycolysis proceeding. In doing so, yeast gets energy for its own maintenance by generating 2ATP. The theoretical (stoichiometric) conversion to ethanol from glucose is as follows:

$$C_6H_{12}O_6(Glucose, 180kg) \rightarrow 2C_2H_5OH(Ethanol, 92\ \ kg) + 2CO_2(Carbon\ \ dioxide, 88\ \ kg)$$




$$Glucose + 2ADP + 2Pi \rightarrow 2\,Ethanol + 2CO_2 + 2ATP + 2H_2O$$

$$3Xylose + 5ADP + 5Pi \rightarrow 5Ethanol + 5CO_2 + 5ATP + 5H_2O$$

**FIGURE 15.5** Glucose and xylose metabolism and conversion to ethanol.

For each kilogram of glucose fermented, around 470 g of ethanol can be produced (i.e., <50%), representing a yield of 92% of the theoretical maximum. In industrial fermentation practice, however, the best yields are only around 90% of this theoretical conversion due to the diversion of fermentable carbon to new yeast biomass and minor fermentation metabolites (organic acids, esters, aldehydes, fuel oils, etc.). Bioethanol production from macroalgal biomass is carried out either by separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF) processes. In the SHF process, dilute acid hydrolysis/enzyme saccharification and fermentation are carried out separately. This process involves higher operating costs, higher energy consumption, and more reaction time. Not all the sugars in the medium are utilized at the end of this process. In the SSF process, enzymatic saccharification and fermentation are achieved in the same reactor. This process is favorable as it requires slower process time and less energy and yields more ethanol. However, the process times required for both the enzyme and yeast microorganisms are different, which results in the slower release and consumption of sugar. Lower concentrations of inhibitors are formed in the SSF process.

### 15.2.5 Current status

*Kappaphycus, Gelidium, Gracilaria, Sargassum, Laminaria*, and *Ulva* are the most cultivated macroalgal genera for hydrocolloid extraction and human food usage in China, the Philippines, and Indonesia. However, in recent years, these genera have been regarded as potential feedstocks for biofuel production in addition to the value-added products for phycocolloids extraction, human food, cosmetics, fertilizer, and other chemicals (Harun, 2011; Jang et al., 2012,

**TABLE 15.5** Current status of seaweed utilization.

| Species | Food | Feed | Industrial | Medicine | Fertilizer | Biofuel feedstock |
|---|---|---|---|---|---|---|
| *Ulva fasciata*[a] | + | + | − | + | − | + |
| *Enteromorpha compressa*[a] | + | + | − | + | − | − |
| *Enteromorpha intestinalis*[a] | + | + | − | + | + | + |
| *Monostroma oxyspermum* | + | + | − | − | − | − |
| *Cladophora fascicularis*[a] | + | + | − | − | − | − |
| *Chaetomorpha media*[a] | + | + | − | − | + | − |
| *Codium fragile* | + | + | − | + | − | − |
| *Caulerpa sertularioides* | + | + | − | − | − | − |
| *Dictyota dichotoma*[a] | + | + | + | − | − | + |
| *Spatoglossum asperum*[a] | − | − | + | − | + | + |
| *Hydroclathrus clathratus* | − | − | + | − | + | − |
| *Stoechospermum marginatum* | − | − | + | − | + | − |
| *Colpomenia sinuosa* | − | − | + | − | + | − |
| *Dictyopteris australis* | − | − | + | − | + | − |
| *Padina tetrastromatica* | − | − | + | − | + | + |
| *Sargassum cinereum*[a] | − | − | + | + | + | + |
| *Sargassum ilicifolium*[a] | − | + | + | + | + | + |
| *Laminaria digitata* | − | − | + | + | + | + |
| *Macrocystis pyrifera* | − | − | + | + | − | + |
| *Porphyra vietnamensis*[a] | + | + | − | − | − | + |
| *Amphiroa fragilissima*[a] | + | − | − | − | − | − |
| *Jania adhaerens*[a] | − | − | − | + | − | − |
| *Gracillaria corticata*[a] | + | + | + | − | − | + |
| *Hypnea musciformis*[a] | + | + | + | − | − | + |
| *Centroceros clavulatum* | + | − | + | − | + | − |
| *Laurencia papillosa*[a] | + | + | + | − | − | − |
| *Chondrus crispus*[a] | + | − | + | − | − | + |
| *Eucheuma uncinatum* | + | + | + | − | − | + |
| *Gelidiella acerosa*[a] | − | − | + | − | − | + |

[a]*Seaweeds distributed along the Indian coast.*

Dhargalkar and Pereira, 2005; McHugh, 2003; Yanagisawa et al., 2013). Species from these genera have been chosen considering the availability and assessment of resources around the globe, ease of cultivation, and harvesting. The shorter life cycles of seaweed are taken as an advantage for large-scale cultivation, which is cost effective and involves environmentally friendly methods, zero input of fertilizers, and no changes in land use as they are exclusively grown in marine waters. *Laminaria* is the most cultivated seaweed with an average production of 5.14 million tonnes (Alaswad et al., 2015) (Table 15.5).

### 15.2.6 Enzyme saccharification

Bioethanol of 40 g/L has been reported from green seaweed by the glucose subunits alone, whereas other sulfated polysaccharides, such as ulvan, are yet to be explored. In brown seaweeds, mannitol is fermented to produce 40 g/L of bioethanol, whereas techniques for the conversion of alginate sugar to ethanol are still underway. Whereas, in red seaweeds, 3,6-anhydrogalactose (composed of glucose and galactose) poses a hindrance for conversion to ethanol (Yanagisawa et al., 2013). A higher concentration of bioethanol is obtained by the conversion of all the sugars present in the seaweed, which can be achieved by developing methods appropriate to each seaweed species.

### 15.2.7 Challenges in bioethanol production

Following are the challenges to be addressed for successful bioethanol production from macroalgal biomass:

- Major cost reductions need to be achieved by suitable biocatalysts and optimal processes.
- Microorganisms possessing enzymes, which have the ability to convert polysaccharides to fermentable sugars, need to be screened or constructed.

- Commercial enzymes such as amylases, cellulases, and proteases are available, but they are more efficient in depolymerizing polysaccharides from terrestrial sources. To produce these enzymes for commercial use, microbial bioreactors are utilized by exploiting the microalgal strains to accumulate carbohydrates and directly utilize their enzymatic or anaerobic digestion system to produce ethanol, resulting in a cost effective bioethanol production process. In order to proceed with this procedure, screening of high-carbohydrate-accumulating seaweeds from natural water bodies based on their growth cycle is to be done.
- Large-scale production, to be economical, needs to utilize all sugars present in macroalgal biomass to achieve 100% efficiency.
- Mannitol is a nonfermentable sugar alcohol produced from brown algae; most of the anaerobic bacteria are unable to carry out fermentation of mannitol as there is a requirement of oxygen for the regeneration of $NAD^+$ for the conversion of NADH to NADPH, which is obtained from mannitol dehydrogenase during oxidation of mannitol to fructose and NADH. A facultative anaerobic bacterium, *Z. palmae*, ferments sugar alcohols, including mannitol from *Laminaria hyperborea* extracts. *P. angophorae* is also seen to consume both mannitol and laminarin and yield ethanol. Similar investigations are to be carried out for ulvan, alginate, and 3,6-anhydrogalactose conversions to bioethanol.
- Bioethanol is an intermediate product obtained during the digestion of organic materials and is produced by specific microbial strains only, which makes it an obvious practical constraint of keeping the microbial culture from getting contaminated by other microbes (Horn et al., 2000a,b; Nguyen et al., 2017). Hence a controlled condition needs to be maintained.
- Setting up decentralized biorefinery systems in coastal areas with supporting infrastructure (e.g., roads, utilities).
- Economically feasible algal bioethanol can be turned into reality only through breakthrough technological innovations. Getting algae to produce bioethanol in very large volumes and at a very low cost is the grand challenge that young biotech firms have to shoulder.

## 15.3 Case study: bioethanol from *Enteromorpha intestinalis*

The abovementioned challenges are addressed in this case study, which involves bioethanol production from green macroalgae; *E. (Ulva) intestinalis* of the *Ulvaceae* family. They grow profusely and occupy intertidal zones under favorable nutrient, salinity, light, and temperature conditions. *E. intestinalis* is composed of 40.1% total carbohydrate, 20.4% protein, and 2.8% lipid. Elemental analyses including carbon 33%, nitrogen 4.36%, and hydrogen 6.44% were recorded. The biochemical composition of *E. intestinalis* is comparable to those found in earlier studies. Cho et al. (2013) recorded 42.8% carbohydrate, 31.6% crude protein, and 1.3% crude lipid. Bioethanol prospects from *E. intestinalis* are elucidated in this section (Fig. 15.6).

Dilute acid hydrolysis of *E. intestinalis* at 0.7 N $H_2SO_4$, 5% substrate concentration, and 121°C for 45 min produced 239.94 ± 1.3 mg/g of reducing sugar. Enzyme is extracted from marine bacteria *Vibrio parahaemolyticus* (Hebbale et al., 2019). Pretreated biomass of *E. intestinalis* subjected to enzyme saccharification at pH 6 and 50°C for 24 yielded 289.89 ± 2.4 mg/g of reducing sugar. Acid-pretreated macroalgal biomass was subjected to enzyme hydrolysis using an enzyme and was incubated for 24 h, and a 1.2-fold increase in reducing sugar was observed in *E. intestinalis* when compared to dilute acid pretreatment. Scanning electron micrographs of hydrolyzed biomass indicates that the dilute acid pretreatment prior to enzyme saccharification is a prerequisite as it loosens the rugged surface of the biomass, increasing the surface area and exposing more of internal cellulose, as seen in Fig. 15.7.

The acid hydrolysate obtained was subjected to SHF using the *Pichia kudriavzevii* yeast strain isolated from toddy juice at 35°C and 100 rpm for 24 h. Ethanol of 0.16 g with 51.8% efficiency was obtained. Pretreated biomass subjected to the SSF process using enzymes from *V. parahaemolyticus* and *P. kudriavzevii* yeasts at 55°C and 100 rpm for 24 h. Ethanol of 0.10 g was obtained with 65.1% efficiency. SSF exhibited higher efficiency than the SHF process.

Mass-energy balance was carried for analyzing ethanol production from *P. kudriavzevii* (TY) and the sugars obtained from both SHF and SSF processes. Results obtained were extrapolated to 1 kg to make the study more comprehensive. Fermentation of *E. intestinalis* in the SHF process produced 23.9 g/kg (30.4 mL/kg) of ethanol with a 55.9% conversion efficiency, whereas in the SSF process, 28.9 g/kg (35.8 mL/kg) of ethanol with an 83.9% conversion efficiency was obtained (Table 15.6). Ethanol from SSF was estimated to be 1.18-fold higher than the ethanol obtained from the SHF process, indicating better efficiency. Similar results were obtained for the fermentation of *E. intestinalis* using *S. cerevisiae*. The SSF process achieved 30.5% efficiency when compared to the SHF process (26.9%), indicating better performance regarding fermentation yield and a faster process. A similar mass-energy balance study was reported with various feedstocks; 1 kg of *Saccharina japonica* biomass yielded 23.1 g (29.2 mL) of ethanol using the SSF

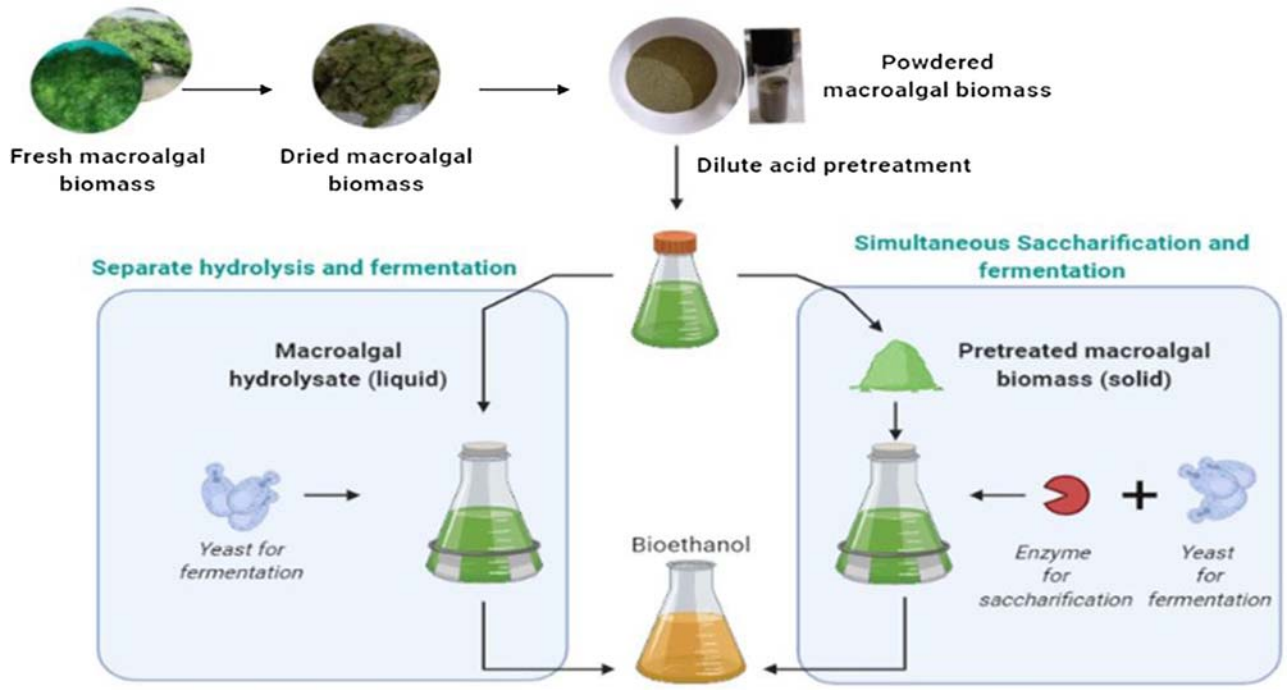



**FIGURE 15.6** Bioethanol production from green macroalgae *Enteromorpha intestinalis*.

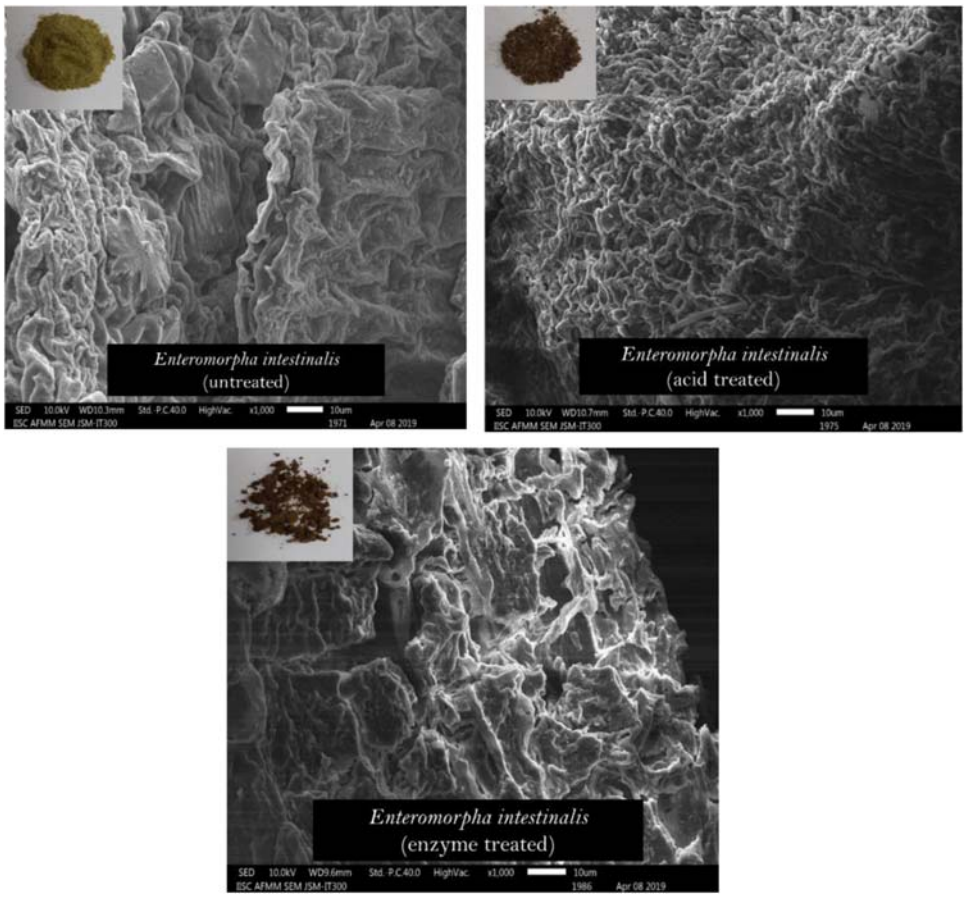



**FIGURE 15.7** Scanning electron micrograph of *E. intestinalis* illustrating ultrastructural variations in the feedstock after pretreatment and saccharification.

**TABLE 15.6 Ethanol production from different macroalgal feedstocks (expressed in L/100 kg of biomass).**

| Feedstock | | Ethanol L/100 kg |
|---|---|---|
| First-generation feedstock | Corn grain | 35.6 |
| | Sorghum | 35 |
| | Cassava | 17 |
| Second-generation feedstock | Rice | 43 |
| | Wheat | 34 |
| | Grapes | 13 |
| | Sugarcane bagasse | 76 |
| | Switchgrass | 22.6 |
| Third-generation feedstock | *Gracilaria verrucosa* | 4.7 |
| | *Ulva fasciata* | 11.7 |
| | *Kappaphycus alvarezii* | 1.7–2.4 |
| | *Gracilaria corticata* | 2.6 |
| | *Laminaria digitata* | 26.2 |
| | *Gelidium amnasii* | 1.62 |
| | *E. intestinalis*[a] | 3.55 |
| | *Ulva lactuca*[a] | 5.58 |
| | *Palmaria palmata* | 1.6 |
| | *Ulva pertusa* | 11.6 |
| | *Laminaria japonica* | 36.8 |
| | *Gelidium elegans* | 23.2 |
| | *Gelidium amansii* | 8.8 |
| | *Sargassum* sp. | 28.70 |

[a]*Present study.*

process, achieving a conversion efficiency of 67.41% (Lee et al., 2013). Fermentation of 1 kg *Gracillaria verrucosa* produced 38 g (48.1 mL) of ethanol from the SSF process, achieving a fermentation efficiency of 86% (Kumar et al., 2013). Acid pretreatment of 1 kg *Kappaphycus alvarezii* followed by detoxification produced 80 g of galactose, which was fermented (SSF) to produce 43.7 g (55.3 mL) of ethanol, achieving a 78.5% conversion efficiency (Hargreaves et al., 2013). Fermentation of 1 kg of switchgrass (2G feedstock) produced 178.4 g (226.1 mL) of ethanol using the SHF process, while the SSF process produced 183.5 g (232.5 mL) of ethanol but achieved lower conversion efficiency, which is attributed to the presence of insoluble lignin in the biomass, which was treated using ammonia fiber expansion (AFEX) (Jin et al., 2010). The higher ethanol in the SSF process is due to the rapid consumption of glucose by yeast as they were produced during enzyme hydrolysis (Xiao et al., 2004). Acid hydrolysis of 1 kg of *Lantana camara* followed by delignification, enzymatic hydrolysis of the biomass, and fermentation yielded 148.14 g (187.7 mL) of ethanol, whereas fermentation of pentose-rich hydrolysate yielded 51.6 g (65.3 mL) of ethanol (Kuhad et al., 2010). Bagasse pith (1 kg) (2G feedstock) produced 46.2 g (58.5 mL) and 66.4 g (84.1 mL) of ethanol through the SHF and SSF processes, respectively. In this study, the commercial enzyme cellulase and β-glucosidase were employed for enzyme hydrolysis, and fermentation was carried using *P. stipitis* JCM 10742 (Sritrakul et al., 2017). Notable advantages were observed from the SSF over the SHF process, as the SSF process is amenable to enzyme hydrolysis with the rapid ethanol production and occurs in a single reactor, thereby reducing the operation and investment costs for setting up a biorefinery.

Ethanol production from macroalgal biomass results in large quantities of spent biomass or waste products that are generally disposed. High-value products are created from these wastes through the concept of biorefinery, which aims to achieve no waste flow, resulting in economic and environmental benefits (Balina et al., 2017).

## 15.4 Economic prospects of macroalgae biorefinery

Seaweeds were mostly restricted to domestic purposes such as food and feed; preparation of industrial gels; and medicinal uses such as *Laminaria* sp. being used for dilation of cervix in difficult childbirth and *Gelidium* sp. used for intestinal afflictions. In recent times, macroalgal biomass is cultivated on a large scale for the production of more valuable commodities than food and feeds. These include the extraction of polysaccharides for agronomic applications,

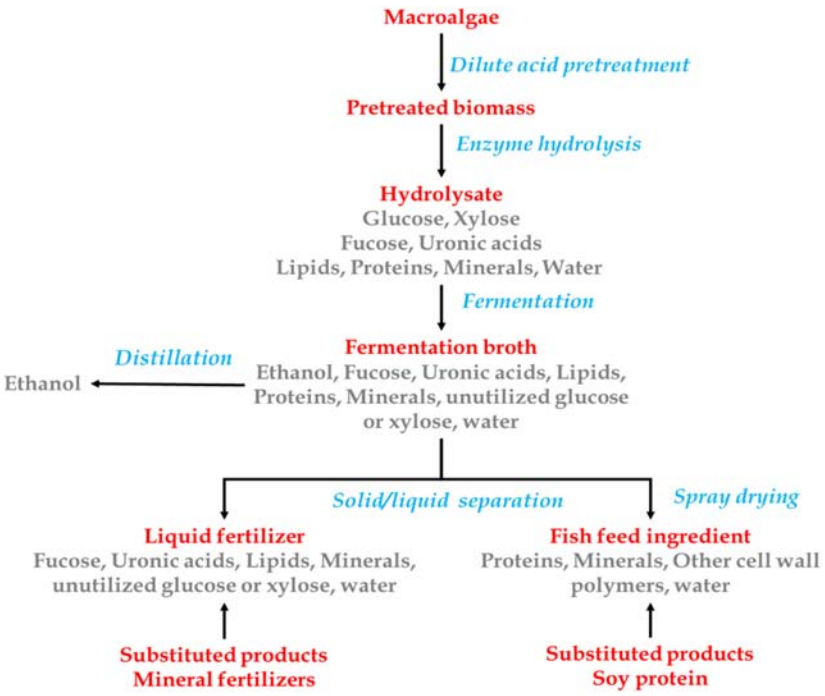



**FIGURE 15.8** Seaweed biorefinery with probable constituents.

cosmeceuticals, nutraceuticals, pharmaceuticals, and bioenergy. The seaweed biorefinery approach extracts the most valuable components from the macroalgal biomass without altering the residue for commodity purposes such as food, feed, and fertilizers (Balina et al., 2017; Buschmann et al., 2017).

Macroalgae are subjected to dilute acid pretreatment, and the pretreated biomass is hydrolyzed using enzymes. Enzyme hydrolysate is fermented to produce ethanol. Solid/liquid separation is carried out for the fermentation broth. The liquid fractions are rich in lipids, minerals, and other unutilized sugars and are used as liquid fertilizers, which substitutes the conventional mineral fertilizers. The solid fraction is spray dried and rich in protein and minerals and is used as fish feed, which serves as a substitute for soy protein (Fig. 15.8). The selection of appropriate macroalgal feedstocks accumulating higher carbohydrate fractions and nutrients can lower the $CO_2$ level and provide climate change and marine eutrophication mitigation services (Seghetta et al., 2016).

Fatty acids content in dried and canned macroalgae are of linear structures and are major sources of essential fatty acids such as palmitic acid and ω-3, -6, and -9 fatty acids. Agar from *Gracilaria edulis, Gelidiella acerosa*, and *Gracilaria* sp. are extracted by boiling the seaweed, and the extract is filtered, freeze thawed and dried in the sun, and marketed as powder (Kaladharan and Kaliaperumal, 1999). *Macrocystis pyrifera* was harvested for the production of acetone and potash (Roesijadi et al., 2010). Macroalgal biomass is composed of high amounts of water-soluble potash, which is readily absorbed by the plants. Composting of seaweed along with shark liver sediments and fish offal (15:4:3 by weight) fetched high manure value with 2.4% N, 0.7% P, and 3.5% potash (Chennubhotla et al., 1981). Macroalgal biomass is regarded as a "superfood" for being rich in vitamins B12 and A and iodine. Seaweed meal incorporated in poultry and animal feed was found to increase the iodine content of the eggs and milk production in dairy cows (Hebbale et al., 2017; Holdt and Kraan, 2011; Torres et al., 2019). Discarded waste of algin-extracted macroalgal biomass is estimated to contain 93%−94% of iodine (Torres et al., 2019). Extracted protein fraction from *Ulva* increases ileal digestibility and rumen fermentation (Baeyens et al., 2015; Bikker et al., 2016). Apart from whole seaweed, the residue obtained from industries, floating residues, and spent biomass serves as feedstock for bioethanol production (Sudhakar et al., 2016). Therefore the biorefinery approach is sustainable and environmentally friendly as it reduces the burden on the environment.

## 15.5 Scope for further research

Marine macroalgae have been explored worldwide for various applications, owing to their ability to accumulate large concentrations of biomolecules (especially carbohydrates), which serve as raw material for bioethanol production and

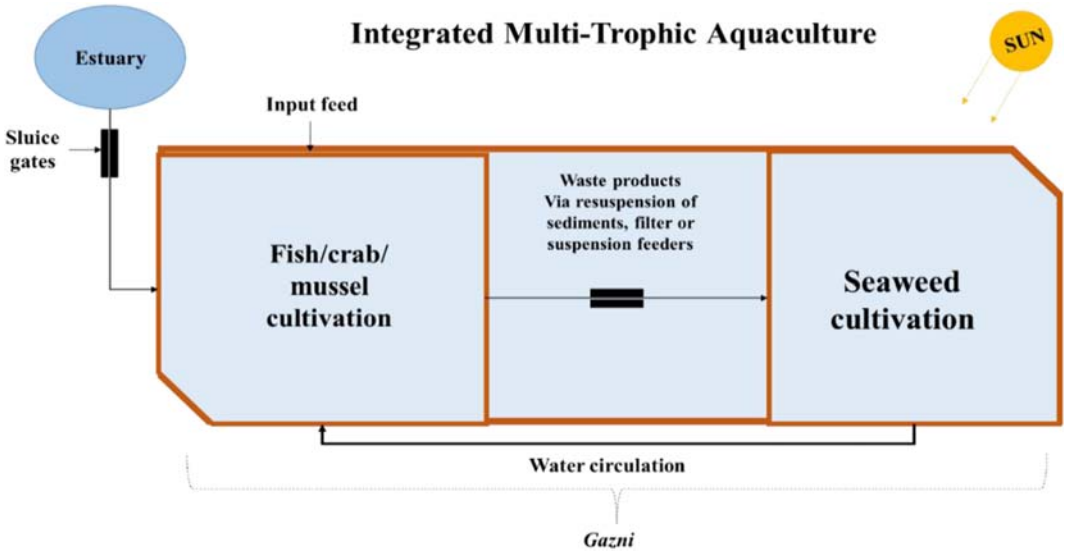



**FIGURE 15.9** Integrated multitrophic aquaculture model. Gazni: abandoned paddy cultivation land.

other value-added products. Bioprocess of bioethanol production involves three major steps: dilute acid pretreatment, enzyme saccharification, and fermentation. The major future prospects for bioethanol production from macroalgal biomass include (1) exploring enzymes having higher catalytic activity and stability at extreme conditions; (2) yeast microorganisms able to ferment a broad range of sugars; (3) improved ethanol yield by process optimization; and (4) a consolidated bioprocess involving cellulolytic yeast to hydrolyze cellulose as well as ferment subsequent glucose released during hydrolysis to ethanol. The biorefinery approach can be realized only with sufficient quantities of biomass. Large-scale cultivation of macroalgae in the open ocean results in disease outbreaks and destruction of habitat (killing endemic corals) (Bindu and Levine, 2011; Patterson Edward et al., 2008). In order to overcome this, the integrated multitrophic aquaculture (IMTA) (Fig. 15.9) concept is introduced, which involves farming macroalgae in close proximity to other species at different trophic levels on land. Land-based seaweed cultivation with adaptation to a much wider range of macroalgal genera offers raw materials for higher-value product development. Intertidal species like *Ulva* sp. and *Enteromorpha* sp. have a high tolerance to temperature and irradiance ranges, which can be cultivated in the IMTA system. The cultivation of seaweeds for biofuel production needs to be encouraged to meet the future fuel demand as seaweeds have high potential as feedstock for biofuel production as part of the nation's strategic energy security program. This would also empower rural women with job opportunities. The development of seaweed-based industries at decentralized levels along coastal areas, where resources are abundantly available, would enhance the job opportunities for the rural youth. Seaweed cultivation as a notable future enterprise can open up platforms for establishing seed hatcheries, seeding units, and processing units and enhance employment opportunities in rural coastal areas.

## 15.6 Conclusion

Macroalgal species with a higher carbohydrate content are vital for bioethanol production. Algal biomass consists of carbohydrates in the form of structural (cellulose) and storage (starch) polysaccharides, and hydrolysis of these polysaccharides results in monosaccharides (fermentable sugars), which serve as substrates for fermentation. Pretreatment using chemical and biological methods is a prerequisite for ethanol production. Wild bacterial/fungal strains are explored for enzyme production with the higher catalytic activity. The fermentative efficiency of the wild yeast strain *P. kudriavzevii* in fermenting macroalgal biomass was elucidated with a case study using the green macroalgae *E. intestinalis*. For macroalgal biomass, in addition to being a viable feedstock for bioethanol production, there is scope for the utilization of different by-products as well as high value-added products. Bioethanol production would address the growing needs of the transportation sector, help in mitigating the greenhouse gas footprint in the transportation sector, and ensure the strategic energy security of the nation. Judicious use of feedstock (macroalgae, agricultural residues) would aid in lowering import burdens while empowering rural women with a sustainable livelihood through integrated approaches in fishery, etc.

## Acknowledgment

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