Chapter 12
Bioethanol Production from Marine Algae: A Novel Approach to Curb Global Warming

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Abstract The diminution of fossil fuel and environmental pollution has amplified the hunting for renewable energy sources. Bioethanol is an essential class of biofuel that emerges as an alternative renewable energy source. First- and second-generation feedstock for bioethanol production are not considered useful and sustainable due to high production costs. On the contrary, the third-generation feedstock has been proven to be efficient for bioethanol yield. Marine algae possess high carbohydrate concentrations within themselves, proving them to be suitable substrates for ethanol generation. Marine biomass, i.e., yeast, has shown remarkable tolerance to salt and is ideal for seawater fermentation. The combination of marine biomass, algae, and yeast has become an essential resource for greener and sustainable ethanol production. Successful bioconversions of algal biomass to ethanol have been carried out, and ongoing research aims to optimize the process further.

Keywords Bioethanol · Biofuel · Fermentation · Bioconversion · Algae

12.1 Introduction

The gradual increase in population demanded a rapid production of all essential goods. This led to the industrial revolution in the early sixteenth century. The overall impact on the environment has already taken its toll, and with the threat of global warming, the dwindling reserves of these fossil fuels also raise numerous concerns. Fossil fuel depletion and climate change have become significant concerns regarding global warming (Kim 2015). This approach has become a field of intense study owing to its environmental and social implications. There are various fermentable sugars for implementing this approach, including glucose, etc. Bioethanol can be used as an essential renewable energy source. Properties of ethanol, such as low heat of combustion and higher heat of vaporization, make it an ideal choice as a

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transportation fuel (Datta et al. 2011). In addition to all these properties, bioethanol can easily be stored and distributed. Several blends of fossil fuels also enhance the characteristics of this fuel (Harun 2014). Presently, industrial production of ethanol is done using sugarcane juice and molasses as the primary substrate. Besides these, corns, barley, rye, and triticale are also used. Since all of these belong to the category of cereals, there is always a conflict between food and fuel. As a remedy, rice straw, wheat straw, and sugarcane bagasse were employed. Recent studies and evidence suggested that marine algae can produce the third generation of biofuels (Durbha et al. 2016; Swain and Natarajan 2017). It involves fermentation technology using land biomass. Biomass wastes contain a mixture of complex polysaccharides including cellulose, hemicellulose, lignin, etc. The mechanism generally includes the hydrolysis of these carbohydrates, leading to fermentable sugars' formation into bioethanol. Bioethanol is used with a combination of nonrenewable energy sources like petrol. The mixture is 10% ethanol with 90% petrol. Marine algae can be used as an efficient energy source for biofuel production due to its high carbohydrate content. The concept of biofuel has emerged from a sharp contrast between the increasing demand for energy and the decreasing of traditionally available energy sources (Lakatos and Ranglová 2019). Bioethanol is also considered as an alternative to gasoline. It has high financial and ecological profits. The feedstock of bioethanol is renewable plant material; therefore, there is no carbon release resulting in little environmental pollution (Arora and Behera 2015; Chowdhary et al. 2018; Chowdhary et al. 2020; Chowdhary and Raj 2020). Plant feedstock can be classified into three main categories:

I. Sugar syrups (e.g., sugarcane juice).
II. Starchy grains.
III. Cellulosic materials.

The usage of cellulosic feedstock like rice straw and wheat straw is relative to low cost. However, the conversion of these feedstocks into bioethanol is a time-consuming and costly process. To combat the present scenario, the concept of producing algal-based biofuels emerges. However, bioethanol production from algae is a multifaceted process. Alginic acid is a polyuronic acid. It consists of mannuronate and guluronate. The quantity of alginate (alginic acid) is different in various species of algae (Takeda and Yoneyama 2011). The conversion of lignocellulosic biomass to bioethanol production is an example of the saccharification process. The hydrolysis of cellulosic material or polysaccharides has proven to be an emerging method for bioethanol production or fermentation (Lee and Oh 2011).

This chapter highlights mainly the third generation of algal-based biofuel that is bioethanol production from marine algae (Fig. 12.1).

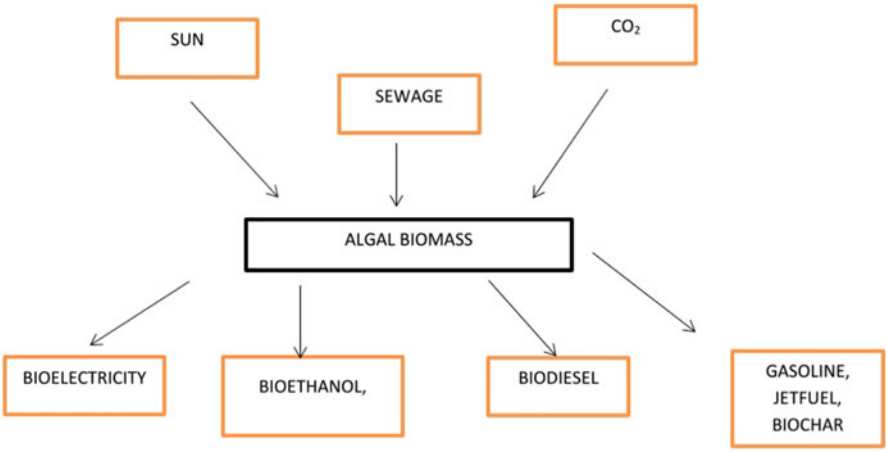

Fig. 12.1 Growth and utilization of algal biomass

12.2 Bioethanol as an Energy Source

Microalgae and marine algae are the photosynthetic microorganisms that are suitable for the application of this approach. They require a minimum amount of nutrients and minimal space to grow. Marine-derived microorganisms have high osmotic pressure, can utilize specific sugars, and produce particular enzymes necessary for bioethanol production (Zaky 2014). They are considered third-generation marine feedstock (Zaky 2014; Kim 2015). Marine seaweed is of three categories: brown algae, red algae, and green algae. The red algae (*Gelidium amansii*) is a potential basis for bioethanol production. The reason behind this is high carbohydrate content that can be effectively fermented into glucose and galactose (Kim 2015). However, there are distinctive classifications around the generation of biofuels.

The first-generation biofuels include biodiesel, bioethanol derived from crops, maze, etc. It has several disadvantages, such that it requires a large amount of cultivating fields. Besides, it has a notable amount of political, economic, and environmental concerns. Second-generation biofuels do not depend on food crops. Instead, they require advanced technology and highly skilled experts (Table 12.1, 12.2, 12.3 and 12.4).

The third-generation biofuel is microalgae based. They have the edge of being the most suitable source due to the high production capacity of storage compounds, low emission balance (almost zero), and waste nutrient use. The third-generation biofuels have no competition against the food resources and therefore are more suitable than them (Bellou and Baeshen 2014; Lakatos and Ranglová 2019). Approximately 20–30% of the marine algae consists of cellulose, xylulose, and glucose. There are different pathways to convert these sugars into bioethanol.

Table 12.1 Different types of biomass and their bioethanol yield

Generation	Biomass source	Bioethanol production	References
First generation	Sorghum Sweet potato	33.9%	Mojović and Nikolić (2006)
Second generation	Sugarcane	29%	Dave (2019)
Third generation	*Laminaria japonica* (microalgae) *Chlorella vulgaris* (microalgae)	29–40%	Ho (2013)

Table 12.2 Microalgae and their glucose yield

Classification of algae	Name of the biomass	Sugar yield	References
Red microalgae	*Gracilaria verrucosa*	Glucose (86%)	Kumar (2013)
Brown microalgae	*Alaria crassifolia*	Glucose (83.9%)	Yanagisawa (2011)
Green microalgae	*Ulva pertusa*	Glucose (18%)	Yanagisawa (2011)

Table 12.3 Comparison between acid hydrolysis and enzymatic hydrolysis

Enzymatic hydrolysis	Acid hydrolysis	References
Time consumption is long	The time consumption is short	Jambo and Abdulla (2016)
Sugar yield is high	Sugar yield is low	
The equipment corrosion is low	The equipment corrosion is high	
The conducting temperature is low	The conduction temperature is high	

Table 12.4 Various species of *Sargassum* used for bioethanol extraction

Seaweed	Part used for hydrolysis and fermentation	Conditions	References
Sargassum vulgare	Whole biomass	Dilute acid hydrolysis +121 °C	Durbha et al. (2016)
Sargassum sp.	Whole biomass	Dilute acid + enzyme	
Sargassum fulvellum	Whole thallus	Acid + enzyme	
Sargassum sagamianum	Whole thallus	200 °C and 15 MPa for 15 min	

12.3 Mechanism of Bioethanol Production Using Microalgae

Microalgae are considered to be an ideal substrate for bioethanol production as it has high biomass productivity. Various microalgal strains can vary due to their different marine habitats, climatic variation, and other factors. So it is necessary to effectively identify the suitable strain capable of producing the desired amount of bioethanol

with optimal growth (Dave 2019). There are several in situ and ex situ procedures to collect specific indigenous marine microalgal strains. *Ulva lactuca* and *Cystoseira amentacea* are the two strains cultivated using ex situ technologies (Dave 2019). The whole process is conducted using a photobioreactor or dark, sterile chambers. The onshore cultivation technique or in situ approach is used for the commercial production of microalgae. It involves the three-dimensional propagation of microalgae on nets under seawater. One major disadvantage of this method is that it is a costly and time-consuming process.

12.3.1 Microalgae as a Feedstock

The scarcity of conventional fuel has increased the need for bioenergy from the third-generation feedstock. The bioethanol production from the third-generation feedstock has the highest utility among all three generations. The following table shows the comparative study of bioethanol production from the three generations.

The first generation biofuel was obtained mainly from corn and sugarcane juice. However, due to several issues, this was abandoned. The second-generation biofuel came from the lignin-cellulose-rich parts of plants. However, this was also challenging due to land and livestock feed availability. The process also generates a low yield and higher cost. The third generation of biofuel proposed the usage of algae as a suitable substrate. Both macro- and microalgae are good examples of third-generation biofuel substrates (Sirajunnisa and Surendhiran 2016; Swain and Natarajan 2017).

The bioethanol yield depends on the biomass of microalgae. Recent studies show that marine algae's summer and winter season biomass productivity is different, affecting bioethanol yield. It is due to the variation of carbohydrate content. Bioethanol production occurs from the algal biomass and can be described by the equation. It involves the degradation of the carbohydrates to yield ethanol.

$$C_nH_{2n}O_n \rightarrow C_2H_5OH + CO_2 + \text{Heat}$$

12.3.1.1 Culturing Condition of Algae

Algae are considered the only alternative to food crops in biofuel production because of their morphological properties and unique characteristics. Macroalgae are marine algae and thus can be grown over a large portion of the area. They are some of the fastest-growing plants globally, and their cell walls are rich in lipids and carbohydrates. Depending upon the culturing conditions, algae can be classified as phototrophic, heterotrophic, and mixotrophic. As the name implies, phototrophic algae derive their nutrition from sunlight and absorb carbon dioxide as their inorganic carbon source. Heterotrophic algae, on the other hand, can utilize organic

substrates as their energy source. Mixotrophic cultivation can grow using both pathways and generally chooses one depending upon the carbon source availability (Harun 2014).

12.3.1.2 Classification of Microalgae and their Effectiveness in Bioethanol Production

Algae have a large quantity of biomass and have greater sustainability, so more excellent harvests are possible (Sirajunnisa and Surendhiran 2016; Swain and Natarajan 2017). The use of seawater as a source of algal farming was also a novel pathway. It was done to reduce freshwater consumption. Typically, it was estimated that, during the first and second generation of biofuel production about, twice the amount of water was taken to produce a single unit of ethanol.

Macroalgae or as they are, commonly known as seaweeds, can grow over a length of 60 meters. They can be subdivided into three major categories: red algae (*Rhodophyta*), brown algae (*Phaeophyta*), and green algae (*Chlorophyta*) (Greetham and Zaky 2018). The green algae grow in the upper littoral zone of seawater. The red algae grow in the middle, and the brown algae grow in the lower site. The diversity assessment determines the collection of the microalgal biomass. The carbohydrate content of microalgae varies, and they can be divided into two parts: easily hydrolyzable glucan part and non-glucan part (Dave 2019).

For example, *K. alvarezii* has 88.6% carbohydrate content and 60.0% for *G. amansii* (Mushlihah and Husain 2020).

12.3.1.2.1 Red Microalgae

Red microalgae consist of cellulose, galactan, and glucose. Their cell wall is approximately 65% of total biomass. It has three domains: fibrillar division, amorphous matrix, and glycoprotein domain (Cian and Drago 2015). The main components of the amorphous matrix are agar and carrageenans. Carrageenans are sulfated polyglactin. The monomers of carrageenans are D-galactose, and 3,6-anhydro-D-galactose (Wei 2013). Carrageenans are classified into two types – lambda (λ) and kappa (κ) (Campo 2009). Agarose has a gelling property and can be converted into galactose monomers. Bioethanol yield depends on the carrageen content. The microalgae *P. palmate* has shown the best bioethanol yield due to having high carrageen content.

12.3.1.2.2 Green Microalgae

Green microalgae have high cellulose content. Apart from cellulose, their cell wall also has pectin. They have high carbohydrate content in the form of starch. Ulvans and sucrose are the components found in green algae (Lahaye and Robic 2007). The

polysaccharides consist of ulvanobiouronic acid 3-sulfate types containing glucuronic or iduronic acid. An example of green microalgae is *Valonia sp.*

12.3.1.2.3 Brown Microalgae

The dominating carbohydrates of brown microalgae are laminarin and mannitol carbohydrates. Laminarin is a β-1, 3-linked glucan. A glucose chain (G-chain) is generally attached to its reducing end. Sometimes a mannitol chain (M-chain) can also be observed in the reducing end. As mannitol is a sugar alcohol, it can be easily converted into bioethanol. It is a hydrolysis reaction catalyzed by laminarase (M 2014).

12.4 Pretreatment Process

The microalgal cell wall is composed of the primary and secondary layers. Pretreatment is an essential process in the bioprocessing industry. In this step, the outer cell wall is broken down, and the biomolecules like cellulose, etc. come out. Cellulose, glucan, and glucanase are products easily transformable into bioethanol. The microalgal cell wall has a crystalline structure and thus has higher stability. Hence, it requires specific hydrolysis to break down this cell wall. The structural change via physical, chemical, and biological pretreatment processes maximizes the yield of sugar, leading to a high amount of bioethanol production. However, by the only pretreatment, complete hydrolysis of the microalgal biomass is not conducted. A small number of hemicelluloses should be retained unhydrolyzed as recommended by scientists for an effective pretreatment process (Maurya and Singla 2015).

12.5 Hydrolysis of Microalgae

Hydrolysis of microalgal biomass is an essential step before bioethanol production. It is also known as saccharification. There are several types of hydrolysis mechanisms, which are as follows.

12.5.1 Acid Hydrolysis

Microalgal biomass such as *Gelidium amansii* and *Kappaphycus alvarezii* undergo this type of hydrolysis process. Generally, sulfuric acid is used for this purpose. H_2SO_4 breaks the glycosidic bonds. The optimum molarity of sulfuric acid is

0.1–0.94 (M). There is no requirement for any particular catalyst. However, only a small concentration of dilute sulfuric acid is required to increase the rate of reaction. It is recommended to execute a two-step semi-hydrolysis process instead of conducting a single step. *K. alvarezii* was first hydrolyzed with 0.45 (M) H_2SO_4 followed by five times recycle without the decrease in efficiency (Khambhaty 2012). Acid hydrolysis was carried out on *Undaria pinnatifida* by using 0.94 (M) H_2SO_4 at 120 °C. It is a high-energy-requiring process. Some reducing sugars are produced, including sucrose, glucose, and fructose. The higher energy requirement is due to the long duration time for completing the reaction. The high concentration of acid can cause reactor corrosion and generates a considerable amount of acid waste (Lee and Oh 2011).

12.5.2 Enzymatic Hydrolysis

Enzymatic hydrolysis is essential for producing sugars from cellulosic material. It generates a lesser amount of wastes compared to acid hydrolysis (Meinita 2012). The two main enzymes that catalyze this enzymatic hydrolysis are cellulase and cellobiase. These enzymes hydrolyze lignocellulosic materials. After the hydrolysis, this hydrolyzed biomass is converted into bioethanol via fermentation. The main three steps are adsorption, biodegradation, and fermentation.

Another enzyme meicelase is responsible for converting *Ulva pertusa* and *Alaria crassifolia* into bioethanol. This enzyme can degrade glucan efficiently. *Ulva pertusa* has a carbohydrate content of 43 ± 4.5% dry weight which efficiently can produce ethanol with an 18.4% yield as reported by Kumar et al. (Kumar 2013). *K. alvarezii* after carrageenan extraction shows more than 80% efficiency of enzymatic hydrolysis. However, it was a time-consuming process due to the high concentrations of the enzyme leading to increased waste generation and caused diffusional limitation (Hargreaves 2013).

12.5.2.1 Factors Limiting the Enzymatic Hydrolysis

pH, temperature, and substrate loading are the crucial factors for determining the enzymatic hydrolysis efficiency. Some enzymes such as laminarinase and agarose can also effectively catalyze enzymatic hydrolysis reactions. But these enzymes require pretreatment of the microalgal biomass before fermentation.

12.5.3 Catalyst-Dependent Hydrolysis

Hydrolysis of polysaccharides is a sequential process. It includes the diffusion of polysaccharide molecules onto the acidic site of catalyst, cleaving of β-1,4

glycosidic bonds, and hydrolysis of polysaccharide sugars into glucose and galactose. Some of the solid acids having high catalytic efficiency indicate that this pretreatment process may not require acid hydrolysis. Therefore, it can be a great alternative to liquid acid hydrolysis and biomass pretreatment. This method's efficiency solely depends on the Brönsted acid sites, a good affinity for the reactant substrates, surface area, etc.

12.6 Chemical Pretreatment of Microalgae

The fundamental goal of pretreatment process is to reduce cellulose crystallinity and increase the membrane porosity; thus membrane-bound cellulose material or carbohydrate becomes accessible to the hydrolysis process. It consequently increases the bioethanol yield. Like any other bioprocess, the pretreatment process also needs to be optimized. It depends on several factors, such as treatment time, temperature, biomass loading, etc. The unoptimized pretreatment process may lead to undesirable by-products such as formic acid, acetic acid, furanic acid, etc. The microalgae floating residue wastes from *Laminaria japonica* is effectively converted into fermentable sugars. Dilute H_2SO_4 is used to catalyze this process following enzyme hydrolysis as stated earlier. Although H_2SO_4 is a powerful agent for cellulose pretreatment, it is corrosive and hazardous. So, a corrosive-resistant reactor is recommended.

12.7 Pretreatment of Biomass with Acid Catalyst

It is a rigorous process involving the occurrence of few side reactions. In this process, sugar does not form as it is a mild reaction. The conditions for conducting this reaction include low temperature, short processing time, and low acid concentration (Feng 2013). The advantage of this method is that a part of cellulose is converted into oligosaccharides and the structural conformation of biomass changes resulting in a more irregular structure. It helps effective enzyme hydrolysis after the pretreatment.

12.8 Recent Studies and Research

Recent research carried out in Bhubaneshwar used *Enteromorpha* species to serve as the experimental subject. Both dry and fresh algae were used in this study. Firstly, estimation for the cellulose content was carried out for the algae. The dry algae were ground in a mortar and pestle. The fresh algae were first dehydrated. After removing

the pigments, it was centrifuged, and the pellet was collected for estimation. After that, the carbohydrate estimation for algal biomass was performed. Pretreatment of algae was performed either by physical or chemical methods. In the physical pretreatment method, the algal biomass was subjected to intense pressure and high temperatures of about 120 °C. The chemical pretreatment was a complex series of steps. Fermentable sugar present in the biomass was measured. The pretreated samples were taken in a test tube in respective amounts and diluted with distilled water. The glucose standard curve was then plotted by treating the samples with appropriate DNS reagent quantities and Rochelle salt. The O.D. was measured accordingly. Then the alcoholic fermentation was carried out. A fixed amount of the pretreated sample was taken and incubated with commercial yeast at 37 °C in a conical flask. After 3–4 days, it was then subjected to anaerobic fermentation. The results were then analyzed. The cellulose content and carbohydrate contents were estimated accordingly. It was found that the yield of fermentable sugar which was obtained was more significant in the case of dry algae. The results revealed a sufficient quantity of the sugar converted to ethanol by fermentation (Nahak et al. 2011).

Another study carried out in Egypt used macroalgae native to the Red Sea. *Sargassum latifolia* was collected during the summer season and *Jania rubens* and *Ulva lactuca* in the winter season. After collection, the algal samples were washed thoroughly with distilled water and then allowed to dry for 3 days. After drying, the algal biomass was then milled to powder, and it was then stored in a place out of direct sunlight. Next, the moisture content of the algae was determined. The samples were weighed before and after subjecting them to a high-temperature oven at 105 °C for 4 h. The algal mass was subjected to a higher temperature of 550 °C, and the ash was then subjected to chemical analysis. The total reducing sugars (TRSs) present in the sample were determined accordingly. Alkaline hydrolysis of the algal model was carried out, therefore. The dried algal biomass was dissolved in a solution of H_2SO_4, NaOH, and HCl in several containers at different concentrations. Then, they were all autoclaved and filtered using a cheesecloth. The filtrate was further subjected to centrifugation at about 10,000 RPM for 10 mins, and then the total reducing sugar (TRS) was determined using the DNS method (Harun and Cherrington 2011).

The determination of the algal species' biochemical composition showed the variation of carbohydrate content among the species. However, the number of carbohydrates was revealed to be relatively high in *Sargassum* and *Ulva*. Also, the algal species displayed a variety in the concentration of the protein content present in them. Thermochemical hydrolysis of the algal biomass revealed that the brown *Sargassum* upon treatment with sulfuric and hydrochloric acids yielded high TRS content. The yield was affected by the variation in pressure, time, and temperature. When *Ulva was* treated under the same conditions, the yield content of TRS decreased with the increase in the concentration of sulfuric and hydrochloric acids. Statistical analysis was carried out to devise the ideal experimental condition required for performing the thermochemical hydrolysis. After hydrothermal hydrolysis, fungal saccharification was carried out. Then batch fermentation was done, and

the results were analyzed. The study revealed that the alga is a good source of carbohydrates and sugars and lipids (Soliman and Younis 2018).

Studies were also carried out upon *P. tetrastromatica* and *S. vulgare.* In a vegetative state, these macroalgae were collected from the shorelines adjacent to the Bay of Bengal in the coastline of Visakhapatnam. After collection, the algal biomass was washed carefully to remove all the debris. Then they were dried in a hot air oven and ground to fine dust. The algal biomass was then subjected to pretreatment with different concentrations of sulfuric acid and hydrochloric acid. This mixture was then autoclaved and performed under alkaline conditions. Here, NaOH is recommended in an appropriate concentration, and the combination is to be autoclaved after neutralizing with H_2SO_4. During the treatment process, a small amount of mixture was collected and tested by DNS test, and the reducing sugar content was determined. After that, the samples, detoxified with ethyl acetate and CaO_2, were incubated along with *Saccharomyces cerevisiae* at 30 °C with continuous agitation. Simultaneously, a chemical analysis was done to measure the quantities of holocellulose, lignin, ash, etc. *S. vulgare* contained more lignin than *P. tetrastromatica.* The holocellulose content was more in *S. vulgare* itself.

Further analysis revealed that the pretreatment conditions could be optimized. The reducing sugar yield was higher when diluting sulfuric acid was employed. The detailed statistical analysis shows that the two algal species are potential bioethanol sources (Durbha et al. 2016).

Another study was conducted upon the species *Chlorococum littorale* by researchers in Japan in which the primary focus was on the performance of dark fermentation. The algal biomass was grown in the laboratory in a 5-liter jar with the necessary ingredients. Seawater was used as a nutrient source, and the setup was exposed to a continuous source of light and air. After 7 to 10 days, all the cells present in the linear phase of growth were extracted using centrifugation. A fixed amount of the cell suspension was taken in a vial, saturated with oxygen. It was sealed adequately and wrapped with aluminum foil and placed in a gyrating shaker at a controlled temperature. After a fixed amount of time, a vial of the cell suspension was taken out and subjected to analysis. The cell suspension was passed through a filter before the examination and was subjected to drying at 105 °C for 3 h.

Further study was carried out to determine the dry weight and starch content. The protein content was analyzed using the Lowry method. It was observed that *C. littorale* accumulated a stable amount of starch within them during the linear growth phase. However, during the dark incubation, starch was decomposed. A tabular version of the data was presented depicting the amount of starch consumed during this fermentation process concerning time and a constant temperature of 25 °C. It was seen that *C. littorale* possessed a unique metabolism that allowed it to perform in dark fermentation and could be a suitable source for ethanol (Ueno and Kurano 1998).

12.9 Bioethanol Conversion Pathway

Bioethanol production from various microalgae depends on the physical and chemical characteristics of the biomass. Bioethanol is the end product in this method, and CO_2 is the by-product. The bioethanol yield depends on the types of biomass, the conditions used, and the metabolic steps involved.

The frequently used microbial species for bioethanol production is *Saccharomyces cerevisiae*. On the contrary, algae are carbohydrate-rich species having a high amount of reducing sugars, making them ideal for bioethanol production. The enzymatic pretreatment using cellulose and amylase is conducted followed by physical-chemical or biological pretreatment of algal biomass. *Chlorococum humicola* is a microalgae that is pretreated with enzymes resulting approximately 64.2% bioethanol yield as reported by Harun et al. (Harun 2014). Various components like proteins and carbohydrates present in biomass dictates the efficiency of the enzyme.

There are two significant bioethanol conversion pathways: the Embden-Meyerhof pathway and Leloir pathway. In the Embden-Meyerhof pathway, there are two main stages. First, the sugar is converted into glucose-6-phosphate. And the second stage consists of the conversion of glucose-6-phosphate into pyruvate. Glucose-6-phosphate is also produced as an intermediate in Leloir pathway. However, this pathway is a complex, galactose metabolism pathway. Here the starting sugar is galactose. These two pathways are observed in yeast. For microalgae, there are several conversion processes.

12.10 Bioethanol Conversion Technology

12.10.1 Separate Hydrolysis and Fermentation (SHF)

Separate hydrolysis and fermentation is a critical process to convert the sugars into bioethanol. This approach dwells on two distinct processes, i.e., hydrolysis of the biomass followed by fermentation. The first process converts the reducing sugars into monomers. Various enzymes are responsible for this conversion. The main disadvantage of this process is the end product inhibition by sugar produced during hydrolysis (Jambo and Abdulla 2016). The bioethanol production from acid-hydrolyzed *Gracilaria* sp. resulted in 0.236 gm bioethanol production from 1 gm dry weight of microalgae as reported by Wu et al. (Wu and Technology 2014). Other red microalgae *Gracilaria* sp. gives 81% theoretical bioethanol yield in enzymatic hydrolysis. Generally, dilute H_2SO_4 is used in this process (Wu and Technology 2014). The pH is around 4.5–5 and static fermentation is for 48 h. *Kappaphycus alvarezii* also gives a yield of 4.6 of bioethanol provided 10% % v/v *S. cerevisiae*, 28–30 °C for 168 hr. in acid hydrolysis (Candra 2011).

12.10.2 Simultaneous Saccharification and Fermentation (SSF)

Here, hydrolysis and fermentation are conducted in a single step. It is widespread and recommended than SHF due to the higher rate of bioethanol production and comparative less end product inhibition by removing the reducing sugars. It is conducted in a single reactor (Jambo and Abdulla 2016). There are several advantages of the SSF process, such as a low chance of contamination, high energy efficiency, etc. The potentiality of cellulosic residue from *K. alvarezii* was evaluated, and it was found that 53% yield was obtained via SSF. There are also several factors to carry out this process, depending on the biomass. Approximately 67% bioethanol yield was obtained from *S. japonica* (Vickers 2017). Kim et al. reported that SSF is more efficient than SHF for bioethanol production from *Gelidium amansii* (Kim 2015). The yield was approximately 77% compared to 67% during 24 h fermentation. Recent studies showed that green seaweed *Ulva rigida* also gives a 6.2% bioethanol yield.

12.10.3 Simultaneous Saccharification and Co-Fermentation (SSCF)

Microalgae contain carrageenans, galactan, and cellulose which can be converted into bioethanol. One major factor in determining the bioethanol yield is the type of microalgal biomass used and its ability to consume total reducing sugar efficiently. Some microorganisms are known as carbon catabolite repression that helps maintain equilibrium between the microalgae's metabolic activity and the sugar uptake capability. It is better to use single reducing sugar as a carbon source rather than using a mixed sugar to obtain a higher bioethanol yield. The utilization of mixed reducing sugars sometimes causes a diauxic growth which causes low output. To combat these problems, mainly the SSCF concept has emerged. However, this process is generally useful for *Saccharomyces cerevisiae*.

Another emerging technology is known as direct microbial conversion (DMC). It is a method where microbes like algae and fungi are directly involved in carrying out both the hydrolysis and fermentation reactions. The marine fungus *Cladosporium sphaerospermum* is reported to produce a cellulase enzyme that can execute a hydrolysis process.

12.11 Factors Affecting Bioethanol Fermentation

A joint experimental study was carried out by the scientist of Shanghai, China, and Japan to find the factors affecting the fermentation process. *Saccharomyces cerevisiae* was used as the agent to induce fermentation. *S. cerevisiae* BY4742

was cultivated on agar slants at a temperature of 4 °C. The pre-cultures grown on these agar slants were then inoculated at the beginning of the fermentation process (Lin and Zhang 2012). Some influential factors are described below.

1. **Temperature:** The experimental studies were carried out multiple times to determine the optimum temperature range for maximum productivity. The data revealed that when temperature increased, fermentation reduces; however, the cell viability was compromised with excess temperature. Conversely, at a lower temperature, cells had a lower specific growth rate. It was also found that a temperature between 25 and 35 °C is ideal for fermentation (Lin and Zhang 2012).
2. **Substrate:** The substrate level also plays a crucial role in fermentation. Increasing levels of substrate concentration improve the yield but increase the incubation period. Thus, an optimum substrate level is necessary, assuming an incubation period of 48 h and a temperature of 30 °C approximately (Lin and Zhang 2012).
3. **pH:** In addition to temperature and substrate, it was seen that pH also serves as a crucial factor for fermentation. It was seen that at pH 5 the ethanol obtained was substantially low in quantity. Thus it was concluded that pH 4.0–5.0 was the lowest operational limit for a fermenter (Lin and Zhang 2012).

12.12 Future Aspect

Marine biomass has proven to be an essential feedstock for bioethanol production. It can produce a high yield of bioethanol (23.4 m^3/ha/y). It is higher than tenfold approximately over the conventional bioethanol production of corn and sugar feedstocks. It implies that bioethanol can be obtained with a higher yield if research continues on this. However, high efficiency, low cost, and dewatering technology are required for efficient bioethanol production. A novel approach is the replacement of freshwater with seawater that can reduce the bioethanol production water footprint.

12.13 Conclusion

In the past few years, much research has been implemented to tackle the fossil fuel problem. The development of different technologies to harness new resources, mainly algae, is being continuously discovered. Algae can be easily cultivated, and we can have launched a two-pronged attack against environmental pollution. At the current stage, microalgae and marine algae have become essential renewable energy sources. It has the potential to replace conventional fossil fuels that can cause global warming. Genetic engineering can also be applied in this approach whereby modification of the carbohydrate content of microalgal biomass can be increased. High substrate loading and efficient microbial utilization of sugars are required.

Marine algae production currently exceeds any other terrestrial plant production used as a substrate for ethanol production. The cultivation of other terrestrial planets have been researched thoroughly over the years; comparatively, algal production is a very new area. Thus there is an enormous hidden potential that can be reaped. In this book chapter, emphasis has been given to bioethanol production from marine algae on a lab scale. More detailed studies are being carried out; however, the bioconversion process needs to be carefully adjusted. But, it can be considered to be the finest technology and has a bright future ahead of human civilization.

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