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A review on third generation bioethanol feedstock

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ABSTRACT

The current issues of the depletion of fossil fuels reserve and environmental changes have increased the concern for the hunt of sustainable renewable energy for the future generations. Biofuels emerged as a promising viable alternative to replace the existing fossil fuels. Among these, bioethanol outstands due to its ability to substitute gasoline. However, the major challenge in bioethanol industry is the need to discover a suitable feedstock together with an environmentally friendly approach and an economically feasible process of production. The first generation and second generation bioethanol appeared unsustainable due to its impact on food security as well as inflated production process. These problems and concerns have directed the search for the third generation bioethanol (TGB) feedstock from marine algae. The integration of algae (microalgae and macroalgae) as a sustainable feedstock for bioethanol has gained worldwide attention in terms of food security and environmental impact. The research on algal utilization in bioethanol has increased in recent years and is expected to become the major drives in bioethanol industry. Therefore, the potential and prospects of the third generation bioethanol feedstock are being highlighted in this review. An insight into the current hydrolysis and fermentation technologies on algal conversion together with the economics and viability of the process are also accounted. This review can be crucial in providing ideas for the future studies that can be implemented in the commercialization of bioethanol from the third generation feedstock.

Contents

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1. Introduction

The rapid industrialization and high population growth are the two major factors which contribute to the global energy crisis. The man was so dependent on non-renewable feedstock like fossil fuels for their day to day needs. Unfortunately, the massive use of fossil fuels led to the problems such as depletion of its reserves, price fluctuation, negative environmental impacts and climatic change [1,2]. The alarming rate of dependence on fossil fuel reserves can be affirmed by the fact that majority of energy is produced from fossil fuels whereas only about 10% of it is produced from renewable energy sources [3]. Hence, the key challenge for the present world is to discover new renewable energy resources which can attenuate these problems for sustainable development of energy in the future.

Biofuels emerged as a promising solution to alleviate the energy crisis to a greater extent as there is substantial reduction of fossil fuels supply for the past several decades [4,5]. The main attraction which give biofuels superior benefits compared to fossil fuels are due to its ability to reduce the greenhouse gases (GHGs) emission, continuous supply of feedstock throughout the year, ease of cultivation, harvesting and transportation as well as the unique properties which contributes to the improvement of engine efficiency [2,6]. It is estimated that by 2050, liquid biofuels such as bioethanol is predicted to be on top of the 'biofuel ladder' due to their effectiveness in replacing gasoline for the transportation sector [7]. In other words, bioethanol can be termed as a promising fuel alternative globally because of its easily biodegradable nature which paves a way to address the current environmental issues [8]. The ease of availability of feedstock with respect to its geographical distribution plays an important role in the development and commercialization of bioethanol [9]. The biofuels industry including bioethanol is expected to open up a lot of opportunities for socio-economic development in various sectors [10].

The industrial potential of ethanol has been tested early in 1800 to be used as an engine fuel after the invention of an internal combustion engine. According to Morris [11], during the end of the 1800s, the sale of ethanol exceeded 25 million gallons per year since it was used as lamp fuel in the United States. However, the occurrence of Civil War induced the government to place a tax on ethanol in order to fund the war in which the action almost destroyed the ethanol industry [12]. The highlighted concerns over the limited use of ethanol continued until the oil crisis in the 1970s and the use of ethanol as a fuel was reborn in the late 1970s [12].

Almost 85% of the global production of biofuels is contributed by bioethanol within the period of 2000 until 2007 [13]. A wide variety of potential feedstock from all around the world can be utilized for bioethanol production [14]. But the search of a suitable feedstock for bioethanol has led to the rise of three generations so far namely first generation derived from edible crops, second generation from non-edible crops and third generation from the algal feedstock. At present, the biofuels research is focused on the third generation feedstock due to its ease of availability and immense potential for commercialization [15].

Up to now, most of the reviews published have focused mainly on the sustainability of microalgae as the feedstock for biofuel [15–20]. At some point, the uniqueness in the characteristics of macroalgae also holds an immense potential to be emphasized further. Therefore, the novelty of this review is to present the detailed utilization of both micro- and macro-algae in biofuels application particularly for the third generation bioethanol production. The importance of third generation bioethanol including its feedstock, geographical distribution, conversion technologies, economics and financial aspects together with its commercial viability are highlighted in this review. Technically, this review attempts to suggest some ways for a better commercialization of the third generation bioethanol with respect to Asian perspective. A thorough understanding of the significance of bioethanol production paves a way for its use as a versatile transportable fuel with excellent performance [21].

2. Bioethanol generations

In comparison to the fossil fuels, production of bioethanol based on biomass are more sustainable and widely distributed. Currently, there are three generations of bioethanol that have been flourished based on different feedstock. First generation bioethanol is derived from fermentation of glucose contained in starch and sugar crops [22]. USA and Brazil are the main producers of bioethanol worldwide utilizing corn and sugarcane while potato, wheat and sugar beet are the common feedstock for bioethanol in Europe [23]. However, the main drawback of first generation bioethanol is the threat of limitation in food supply which may affect the human world population as the feedstock are derived from food sources [24]. Millions of people around the world are currently suffering from hunger as well as malnutrition and moreover utilization of food resources for fuel can lead to an increase in food prices [25,26]. Ritslaid et al. [27] specified that first generation bioethanol is economically unreasonable, since the carbon contents of the plants are mostly lost during the conversion process. Considering this limitation, the researchers have come out with an idea that was more technologically efficient and versatile which is second generation bioethanol [28].

The term 'second generation bioethanol' emerged as a boon to overcome the 'food versus fuel' feud faced by the first generation bioethanol [29]. Second generation bioethanol also referred to as 'advanced biofuels' are produced by innovative processes mainly using lignocellulosic feedstock and agricultural forest residues [24,30]. The advantages of these feedstocks are the ease of availability which does not compete with food and thus eventually has a much lesser impact on the environment. However, the industrial scale-up of second generation bioethanol experienced the main hurdle due to some technological issues [31]. This refers to the high cost and medium yield of bioethanol due to its lignin composition [32]. Other main problems that are related to the second generation bioethanol production are the requirement of advanced technologies and facilities to aid the conversion process [33]. Furthermore, for the collection of feedstock such as woody biomass, logging and forest clearance are needed in which the act can destroy the nature [34]. Hence, there is a demanding challenge to develop bioethanol from marine plants as they have high potential to produce large amounts of biomass.

The emergence of third generation bioethanol provides more benefits as compared to the first and second generation. The third generation bioethanol is focused on the use of marine organisms such as algae. The public acceptance on the ability of algae to

Table 1
Comparison of first, second and third generation bioethanol [27,38,39].

Comparison	First Generation	Second Generation	Third Generation
Feedstock sources	Edible crops	Non-edible crops (lignocellulosic, forest residues)	Algal biomass
Land usage for cultivation	Grows on arable land	Grows on arable and marginal land	Seawater, freshwater, wastewater
Conversion technologies	Sugar extraction, fermentation, distillation	Pretreatment, hydrolysis, fermentation, distillation	Hydrolysis, fermentation, distillation
Bioethanol yield	Low	Medium	High
Impact to the environment	Low contribution to the mitigation of CO_2	High contribution to the mitigation of CO_2	High contribution to the mitigation of CO_2
Main advantages	Relatively simple conversion process	No competition with food resources	High growth rate
Main disadvantages	"Food vs fuel" debate	Recalcitrant structures of the feedstock	Limited investments and difficulties in process design

provide biomass for bioethanol production is positive as this action can limit the feedstock competition from agriculture plants [35]. Algae represents as a promising alternative feedstock due to its high lipid and carbohydrate contents, high proton conversion, easy cultivation in a wide variety of water environment, relatively low land usage and high carbon dioxide (CO_2) absorption [36]. In terms of potential, Schenk et al. [37] reported that the maximum theoretical yield for algal biomass production has been calculated at 365 tonnes of dry biomass per hectare per year. Above all, algae have a low level of lignin and hemicellulose which makes it significant to be used in bioethanol production. Eventhough the research on the application of algal feedstock in bioethanol is still in its naive stage, but it holds immense potential as a promising feedstock for commercial bioethanol production in future. Table 1 shows the comparison of different generations of bioethanol in several aspects.

3. Third generation bioethanol (TGB) feedstock

Algae is considered as the potential feedstock for the production of third generation bioethanol as the biomass can be converted directly into energy. Generally, utilization of this feedstock for bioethanol production depends on factors such as technology and marine environment [40].

3.1. Algae

Algae comes from a large group of photosynthetic organisms. The classification of algae is still under controversy particularly on the status of *Cyanobacteria* [41]. Algae can be unicellular (microalgae) or multicellular (macroalgae). Microalgae commonly floats on the water surface (phytoplankton) due to their lipid content, while for macroalgae (seaweeds) are normally seen attached to rocks or other structures [42]. Out of these, microalgae have already caught the attention of biofuel researchers from all over the world. Recently 'biofuel world' has also understood the potential of macroalgae as the third generation feedstock and this initiated the research on macroalgae to a greater extent [29].

3.1.1. Microalgae

Dinoflagellates, green algae (*chlorophyceae*), golden algae (*chryosophyceae*) and diatoms (*bacillariophyceae*) are several types of microalgae [43]. The content of protein, carbohydrate and lipid vary in different species. Most of the microalgae can store highly concentrated lipid which can exceed 70% by weight of dry biomass [44,45]. The carbohydrate content was also found to be relatively high which is up to 50% of dry weight for some species such as *Scenedesmus*, *Chlorella* and *Chlamydomona* [46,47]. Factors such as light, temperature, nutrient content, pH, O_2 and CO_2 level, salinity and toxic chemicals influence the lipid and carbohydrate contents of microalgae [48]. Some of the common components in the cell wall of microalgae are cellulose, protein, lignin, pectin, hemicelluloses and other carbohydrates which can be converted to monomers through an acid or enzymatic hydrolysis to produce bioethanol [16,49].

The research on liquid fuel from microalgae started as early as 1980s [50]. Since then, many types of microalgae species has been studied in producing liquid transportation fuels under certain growth conditions [51,52]. As a source of energy, microalgae are able to offer considerable amounts of fuel from small crop areas and its high photosynthetic efficiency can help in the mitigation of global warming [53]. The potential of microalgal extracts not only can be applied in bioethanol sector, but also in large to small sectors, including food, pharmaceutical, fertilizer, lubricants and even cosmetics [27,36,54,55].

3.1.2. Macroalgae (seaweed)

Macroalgae usually known as seaweeds are widespread and since centuries have been used as a marine vegetable in Asian countries like China, Japan and Korea [56]. Eventhough it was found that seaweeds have high proteins, vitamins, amino acids, growth hormones, minerals and considered suitable for a meal, but the consumers do not make it as a major source of energy in day to day life [57]. Western countries utilized seaweeds widely in the food industry, production of agar, alginate, carrageenan and as a gelling agent [58,59]. The availability of seaweeds throughout the year made it as one of the most important cultivated marine biomass. With respect to it, seaweeds also provide nutrition and habitat for the other marine living organisms.

Seaweeds are classified into three main groups which are brown (*Phaeophyceae*), red (*Rhodophyceae*), and green (*Chlorophyceae*) [60]. There are three main compositions of seaweeds including carbohydrates, proteins and lipids where the content of each composition differs greatly from one another. The structural cell wall of seaweeds usually consists of a matrix, which is made up of linear sulphated galactan polymers. Several researchers proposed seaweeds as one of the most promising feedstock that can be easily converted to bioethanol, since these are known to have low or free lignin composition [61,62]. Laminaran, mannitol, fucoidan, cellulose and alginates are the carbohydrates in brown seaweeds [63]. The red seaweed cell wall consists of polysaccharides such as agar, cellulose, xylene, mannan and carrageenan while for the green seaweed, it is made up of cellulose, mannose, and xylene [64]. As a comparison, red seaweed contains the highest carbohydrate compositions among the three different types of seaweeds [65].

Recently, numerous studies have been conducted on seaweeds especially on the potential of its composition. The carbohydrates represent a large ratio content in seaweeds and subsequently, the conversion of this composition into bioethanol is very crucial during the production phase [66]. On the other side, its ability to

Table 2
Compositions of different species of algae [18,54,64,66–69].

Algal species	Compositions (%)		
	Protein	Carbohydrate	Lipid
Microalgae			
Chlamydomonas rheinhardii	48	17	21
Chlorella vulgaris	41–58	12–17	10–22
Porphyridium creuntum	28–39	40–57	9–14
Prymnesium parvum	28–45	25–33	22–39
Scenedesmus dimorphus	8–18	21–52	16–40
Scenedesmus obliquus	50–56	10–17	12–14
Macroalgae			
Eucheuma cottonii	9–10	26	1
Gelidium amansii	20	66	0.2
Laminaria japonica	8	51	1
Sargassum ilicifolium	8–9	32–33	2
Ulva lactuca	17	59	3–4
Undaria pinnitifida	24	43	3–4

Note: Compositions expressed on a dry matter basis.

Table 3
Algal species exploited in bioethanol production [70].

Types of algae	Species in bioethanol production
Microalgae	*Chlorococcum infusionum, Chlamydomonas reinhardtii UTEX 90, Chlorella vulgaris*
Macroalgae	**Green:** *Ulva lactuca, Ulva pertusa* **Red:** *Kapphaphycus alvarezii, Gelidium amansii, Gelidium elegans, Gracilaria salicornia* **Brown:** *Laminaria japonica, Laminaria hyperborean, Saccharina latissima, Sargassum fulvellum, Undaria pinnitifida, Alaria crassifolia*

store sufficient carbon sources is also needed in the process of producing bioethanol. Table 2 summarizes the compositions of protein, carbohydrate and lipid of some species of microalgae and macroalgae while Table 3 depicts the species of algae that are currently exploited for bioethanol production.

4. Habitat and distribution of TGB feedstock

Algae are widely known to grow in almost every habitat and in a wide range of conditions. They are common to terrestrial water, freshwater and saltwater environments. Table 4 shows the distribution of algal species in three different water environments.

Microalgae are commonly known with the ability to adapt the different environmental conditions. *Diatoms, Cyanoprocaryota, Euglenophycota, Cryptophycophyta* and *Chlorophycophyta* are some examples of major groups of microalgae that are able to live in extreme habitats. The species such as *Chlorella* sp. and *Scenedesmus*

Table 4
Distributions of algae in different water environments [71].

Algae	Habitat		
	Marine	Freshwater	Terrestrial
Dinoflagellates	√	√	n.d
Diatoms	√	√	√
Blue-green algae	√	√	√
Golden algae	√	√	√
Brown algae	√	√	√
Red algae	√	√	√
Green algae	√	√	√

Note: n.d (not detected).

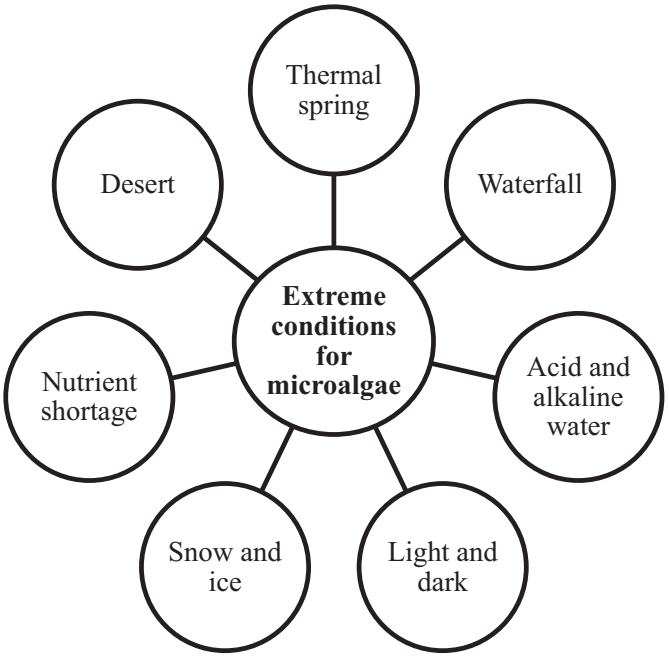

Fig. 1. The extreme conditions for microalgae.

obliquus sp. can be found in the alkaline water environment [72]. Moreover, microalgae also have been successfully grown in industrial liquid wastes since it provides a source of nutrients [73]. Fig. 1 shows several extreme conditions for microalgae such as heat, cold and even stress. These microalgae do not compete with conventional agriculture plants since they can survive extreme conditions and can be cultivated in different water environments [18].

On the contrary to plant-based crops, the ability to double the biomass in less than one day is one of the benefits offered by microalgae. As long as the conditions are favourable, microalgae can be harvested throughout the year [74]. According to Lam & Lee [75], production of microalgal biomass can be achieved between 15 and 25 tonnes per year. Chemical, physical and biological properties play an important role in conveying the potential of microalgae. Production of biomass from microalgae involves the use of solar energy to combine water with CO_2 and the biomass can be considered almost carbon neutral [76,77]. There are various methods applied in the cultivation of microalgae such as open or covered ponds or closed photobioreactors. On the other hand, methods such as centrifugation, foam fractionation, flocculation, membrane filtration and ultrasonic separation are employed for its harvesting [52]. The biomass of microalgae are concentrated in the upper zone, rather than the lower zone, thus making the harvest process more efficient and economically feasible [78]. Conditions of light intensity, photoperiod, temperature and nutrient usually determine the rate of microalgal growth in the culture system [79].

As for macroalgae (seaweed), it grows both intertidally and subtidally. Seaweeds are always attached to other structures such as seabed. Their lifespan is reduced when they are detached from the seabed and live as free-floating seaweed drift [80]. The seaweed distribution can be divided into four different regions which are Eastern Atlantic, Western Atlantic, Indo-West Pacific and Eastern Pacific [81]. Seaweed diversity was found to vary in different regions of the world as depicted in Fig. 2. The global production of seaweed involves 25 producing countries, where the seaweed utilized in a number of industries such as food and phycocolloid production [82]. The statistics published by FAO in 2012 [83] illustrated the production of seaweed increased from 16.83 in 2008–19.9 million mt in 2010. Fig. 3 shows the worldwide production of seaweed aquaculture, where the majority of

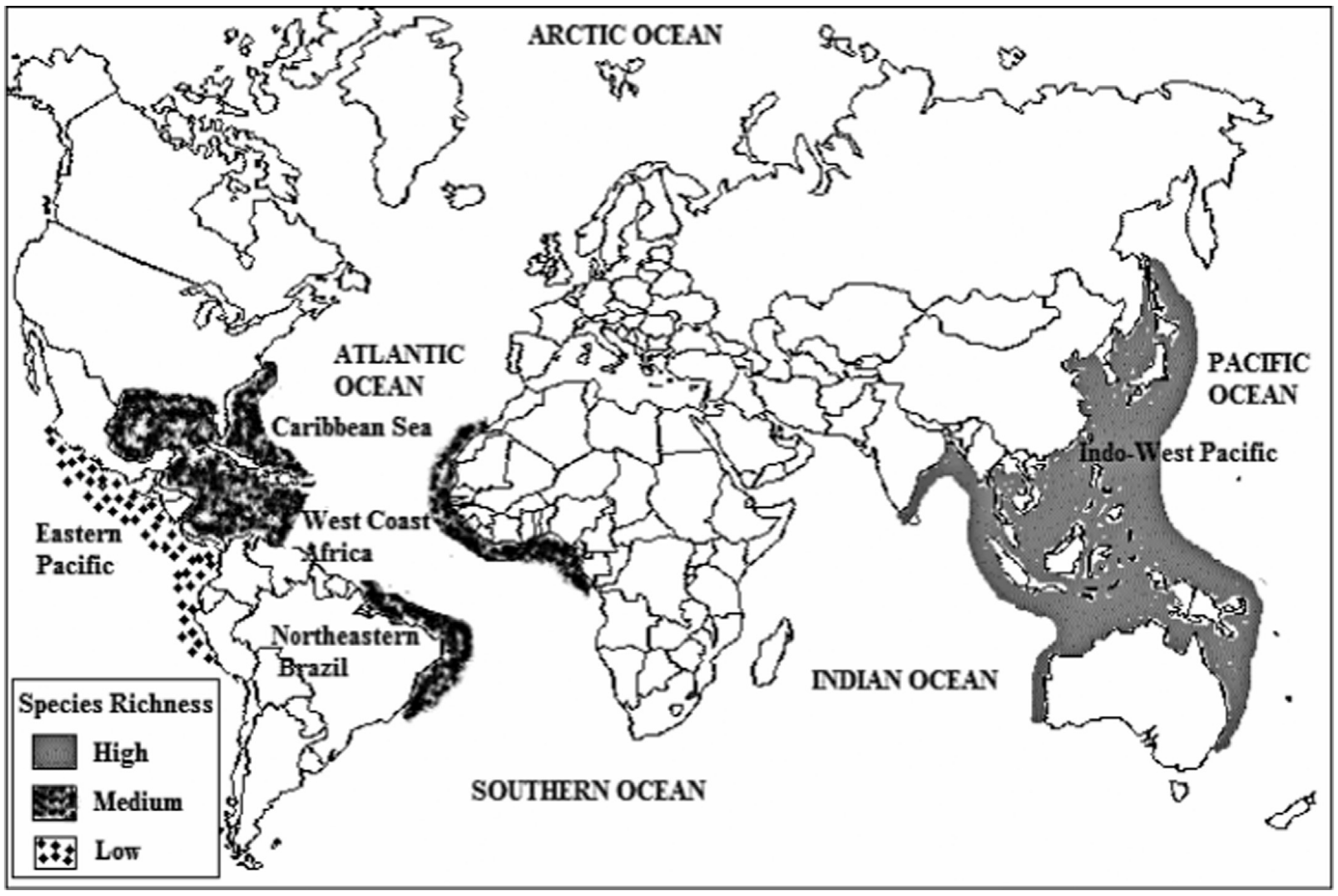

Fig. 2. Global seaweed distributions across the different regions of the world.

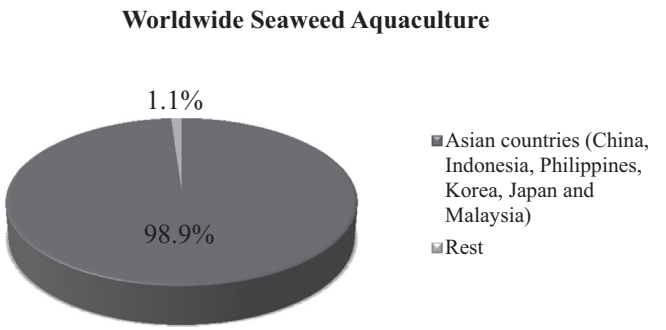

Fig. 3. The worldwide production of seaweed aquaculture [87].

productions are concentrated in Asian countries such as China, Indonesia, Philippines, Korea (North and South), Japan and Malaysia. China accounts for the highest production of seaweed, followed by the Republic of Korea and Japan. As for the Southeast Asia region, seaweed farming in the Philippines contributed to 69% of the total aquaculture production followed by Indonesia which is a maritime country having the world's second longest coastal line [84].

As can be seen from the Fig. 2, it clearly conveys the potential of Asian countries to invest in the development of algal bioethanol [85]. According to Khan & Satam [86], from approximately 99% of worldwide seaweed production, about 90% of it comes from cultivation practices. *Laminaria japonica* accounted for 60% of the total production of cultivated seaweed followed by the other species such as *Porphyra*, *Kappaphycus*, *Undaria*, *Eucheuma* and *Gracilaria*.

Sabah and Sarawak are the two states in Malaysia that mainly involved in the seaweed cultivation. In 1978, a researcher from the University of Hawaii highlighted the importance of seaweed growth in Semporna district of Sabah and the commercialization was started in 1989 [88,89]. There are several seaweed species found growing naturally on the reefs of Semporna, south of Sabah and on Banggi Island in Kudat [90]. Department of Fisheries (DOF) Sabah is the authority that initiates the involvement of Semporna's residents in seaweed cultivation [91]. According to Sade et al. [92], the areas around the East coast of Sabah are actively used for cultivation of seaweed. The production of seaweed in Sabah is mainly dominated by Semporna district and followed by Kunak [93]. In terms of commercialization, Sabah is the only state in Malaysia that cultivates seaweed types of *Kappaphycus alvarezii* (*cottonii*) and *Eucheuma denticulatum* (*spinosum*). Currently, there are many types of cultivation techniques applied for seaweed which include conventional long-line, improved long-line and basket method.

The seaweed industry of Malaysia is expecting to generate about 150,000 metric tons of seaweed by the year of 2020. Suhaimi Yasir, a professor at Universiti Malaysia Sabah have said in the interview that "Seaweed farming is a high-growth sector with tremendous potential, as processed seaweed is gaining widespread use as additives in food industries, cosmetics, health products, pharmaceuticals, horticulture and biofuels" [94]. From this statement, the potential of seaweed in biofuels application specifically for bioethanol are very crucial in Malaysia. This vision is supported by the Malaysia's government under the seaweed miniestate project in which a total of 11,000 hectares zone have been opened for seaweed cultivation particularly in Sabah.

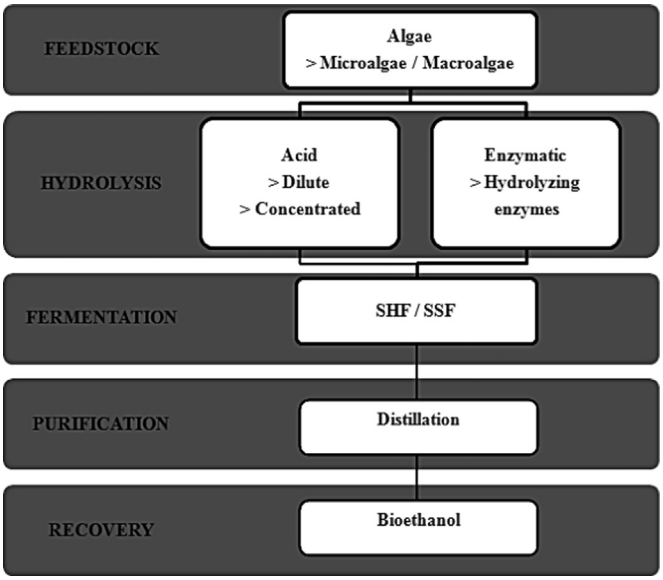

Fig. 4. The general description of processes in bioethanol production.

5. Hydrolysis, fermentation and purification of TGB feedstock

The ease of cultivation and abundance have led to the worldwide utilization of algae for bioethanol production. Algal feedstock has the potential to be converted into bioethanol by using the method of extractions such as thermo-chemical or biological [95]. The production of bioethanol involves an extensive process and varies depending on the type of feedstock used. In general, drying process is the first step in handling the fresh algae collected from the sea, which is important in order to preserve the crude extract and prevent the algae from gelling [96,97]. The size reduction of the feedstock is important to increase surface area for subsequent analysis. The powdered form and slurry of algae are usually used for the next step in the process which involves the hydrolysis followed by fermentation [98]. Fig. 4 shows the general steps in bioethanol production using the third generation feedstock.

5.1. Hydrolysis

The exposure of the intracellular components of algae by using hydrolysis is crucial for bioethanol production. Cell walls are the main structures in algae that need to be depolymerized in order to extract the polysaccharide contents such as alginates, fucans, laminaran, agarans, carrageenans and ulvans [99]. During the conversion, the polysaccharide will be hydrolyzed into free monomer molecules which can be readily fermented to bioethanol [100]. Generally, the chemical approach of hydrolysis is the most commonly used method to hydrolyze the polysaccharide, while the enzymatic hydrolysis is the recent approach which already gained its spotlight from researchers all over the world [101].

5.1.1. Acid hydrolysis

The release of simple sugars from the polysaccharides component can be significantly improved by using chemicals such as acid. In this type of hydrolysis, a wide range of acids have been used in which sulfuric acid (H_2SO_4) is the most preferred one [102]. It was found out that polysaccharides of the three classes of macroalgae (brown, red, and green) can be effectively hydrolyzed to monosaccharides by dilute H_2SO_4 treatment at high temperature [103]. The acid role in hydrolysis can be seen in its ability to break the bonds which connect the long chains of polysaccharides. Binod et al. [104] explained that in the initial step, there is the occurrence of hydrogen bonds destruction in order to rupture the polysaccharide chains turning it into a completely amorphous state. The polysaccharide is extremely susceptible to hydrolysis at this point. Then, the acid will serve as catalyzer where it will cleave polysaccharide by hydrolyzing the glycosidic bonds. At the end of the process, any addition or dilution with water at moderate temperature will provide complete and rapid hydrolysis of the hydrolysate into monosaccharide.

5.1.2. Enzymatic hydrolysis

The conversion of complex sugars to its simple form by the employment of enzymatic hydrolysis is a definite way to reduce the negative environmental impacts to some extent [105]. The ability to achieve more than 80% conversion rate also makes the enzymatic approach seemingly more attractive for the application in bioethanol production [106]. In enzymatic hydrolysis, *cellulases* are the enzymes that mostly employed to degrade the polysaccharides and it can be categorized into three main types including *endo-glucanases*, *exo-glucanases* and *β-glucosidase*. Carere et al. [107] have stated that the mechanisms possessed by *endo-glucanases* are the ability to hydrolyze the complex sugars of the feedstock by attacking the interior parts of the amorphous region of cellulose. As for *exo-glucanases*, they degrade cellulose by cleaving cellobiose units from the non-reducing end of a cellulose fibre to enable the enzyme attack. With the combined efforts from *β-glucosidase*, the cellobiase residues finally split into two units of glucose [108].

The physical structure of the feedstock or substrate and its interaction with the enzymes are some factors that need to be addressed properly during the process [109]. Adsorption of enzymes and the formation of enzyme-substrate complexes are considered to be critical steps in the enzymatic hydrolysis [110]. The enzymes will break down the cell wall of the algae in order to release more monosaccharide of the feedstock [28]. In the reaction, the binding of the enzymes to the algal feedstock will rupture the bonding of polysaccharides, consequently, enzyme concentration decreases and conversion proceeds into bioethanol in the fermenting step. Meng and Ragauskas [111] appointed that the fundamental barrier to effective enzymatic hydrolysis is the accessibility of a reactive cellulose surface. However, algal-based feedstock have superior features in terms of its porosity which can enhance the contact of the enzyme during the hydrolysis [112]. It has been shown that the accessibility of enzyme into the substrate during hydrolysis is through the pores in the cell wall which is the major contributor for the efficient hydrolysis process. Table 5 shows the comparison of acid and enzymatic hydrolysis methods.

5.2. Fermentation

The simple sugars released in hydrolysis step can be easily converted to bioethanol with the help of few microorganisms [114]. Bioethanol is the main product of fermentation together with few by-products such as CO_2 and water [115]. The expression of microorganism used in the fermentation is one of the major

Table 5
Comparison of acid and enzymatic hydrolysis [113].

Parameters	Acid	Enzymatic
Time	Short	Long
Cost	Low	High
Temperature	High	Mild
Product inhibition	No	Yes
Sugar yield	Low	High
Equipment corrosion	Yes	No
Undesirable by-products	Yes	No

factors that affect the results of other metabolic steps are not rate limiting. Various microorganisms, such as bacteria, yeast and fungi are regularly employed for fermentation. *Saccharomyces cerevisiae* (yeast) is the most commonly employed strain in fermentation of bioethanol due to its characteristics such as high selectivity, low accumulation of by-products, high ethanol yield as well as high rate of fermentation.

Fig. 5 shows the two examples of sugars produced after the hydrolysis stage and its pathway to the production of ethanol. The conversion of glucose and galactose into ethanol involves Embden-Meyerhof pathway of glycolysis and Leloir pathway respectively [116]. In the Embden-Meyerhof pathway, there are two major stages encountered by the glucose. The first one is the conversion of the sugar to a common intermediate, glucose-6-phosphate followed by the second stage, which is the conversion of the intermediate into pyruvate [117]. The end product of this pathway varies depending upon the microorganism used. In the case when yeast is utilized, it reduces the pyruvate to alcohol (ethanol) and CO_2 by an enzyme-catalyzed two-step process, termed as alcoholic fermentation [118].

In the Leloir pathway process, galactose-1-phosphate is converted to glucose-1-phosphate followed by its conversion to glucose-6-phosphate. The resulted molecules then may enter both the Embden-Meyerhof pathway and the pentose phosphate pathway (PPP) which proceed to the production of ethanol [119]. Leloir pathway in galactose metabolism is more complex than glycolysis, which leads to the slow consumption of galactose when compared with the consumption of glucose during the fermentation [120]. The distinction of these sugar's metabolism mainly affects the bioethanol yield especially during the fermentation of mixed sugars substrate using *Saccharomyces cerevisiae* [121]. However, Park et al. [65] has reported that other than *Saccharomyces cerevisiae*, *Brettanomyces custersii* also have the ability to ferment galactose in the presence of mixed sugars.

In advanced bioethanol production, the assimilation of new fermentation technologies has been progressing annually. Instead of a simple fermentation process, more viable steps are being invented in order to increase the production rate in an economically feasible way. Separated hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) are very well known in bioethanol production industry.

5.2.1. Separated hydrolysis and fermentation (SHF)

The basic mechanism of SHF is based on separation of hydrolysis and fermentation into two distinct processes. As in normal bioethanol production process, hydrolysis will be conducted first to degrade the feedstock into monomer sugars by utilization of enzyme. This process is followed by the fermentation reaction which will utilize the sugars formed in hydrolysis stage. However, one major problem associated with the SHF process is the end-product inhibition by sugars which are formed during the hydrolysis [122].

5.2.2. Simultaneous saccharification and fermentation (SSF)

In SSF, the hydrolysis and fermentation process is conducted simultaneously in a single step which involves a single reactor. During the reaction, the feedstock, enzyme and yeast are put together in an orderly manner so that the sugars released are rapidly converted into bioethanol. SSF can limit the end-product inhibition by removing the residual sugar [123]. Higher bioethanol yield can be obtained if the conditions are appropriate during the SSF reaction.

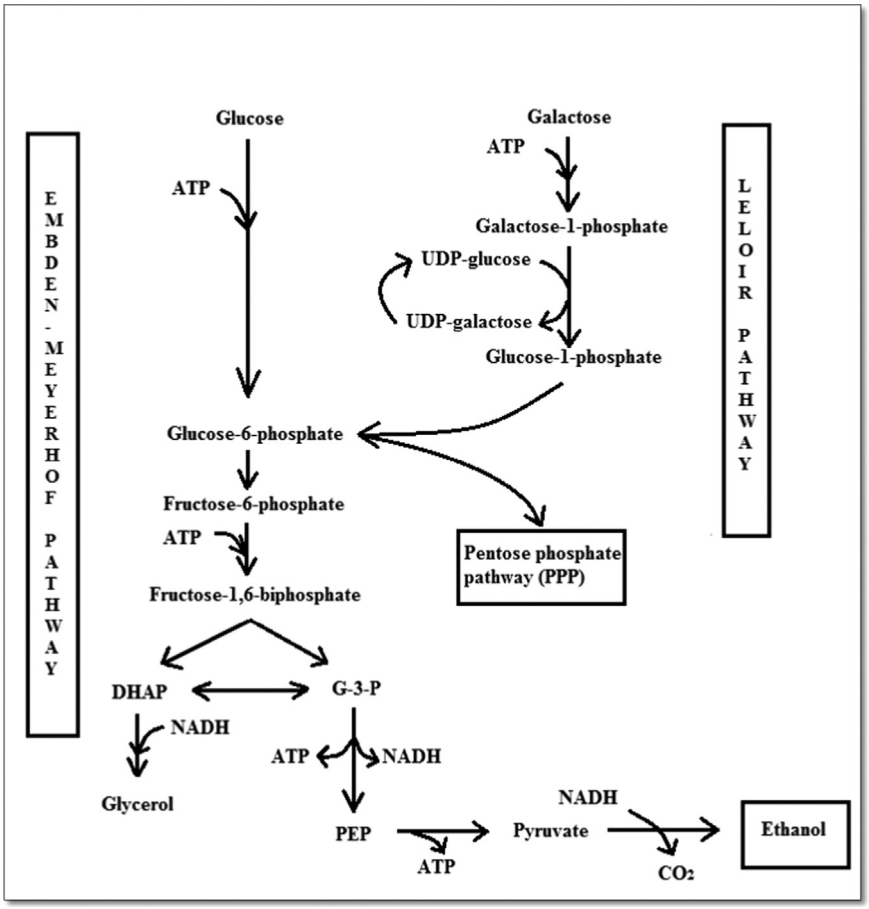

Fig. 5. The Embden-Meyerhof pathway of glycolysis and Leloir pathway for ethanol synthesis from glucose and galactose.

Table 6
Acid hydrolysis and algal fermentation under different conditions for bioethanol production.

Algal feedstock	Hydrolysis parameters	Fermenting microorganism	Fermentation parameters	Bioethanol yield (g/g)	References
Macroalgae					
Eucheuma cottonii	5% (w/v) H_2SO_4, 100 °C, 30–120 min, pH 5.0	*Saccharomyces cerevisiae*	10% (v/v) *Saccharomyces cerevisiae*, 28–30 °C, 36–168 h,[a]	0.046	[124]
Kappaphycus alvarezii	5% (w/v) H_2SO_4, 100 °C, 60 min, pH 5.5	*Saccharomyces cerevisiae* (NCIM 3523)	5% (v/v) *Saccharomyces cerevisiae*, 30 °C, 150 rpm, 48 h, pH 6.4–6.8	0.390	[125]
Gelidium amansii	2% (w/v) H_2SO_4, 150 °C, 240 min,[a]	*Brettanomyces custersii* (KCCM 11,490)	8% (v/v) *Brettanomyces custersii*, 30 °C, 39 h, 150 rpm, pH 4.8–5.5	0.380	[65]
Undaria pinnatifida	10–20% (w/v) H_2SO_4, 121° C, 15–60 min,[a]	*Pichia angophorae* (KCTC 17,574)	10% (v/v) *Pichia angophorae*, 30 ± 2 °C, 72 h, 220 rpm, pH 7.0	0.330	[126]
Eucheuma cottonii	3% (w/v) H_2SO_4, 121 °C, 30 min, pH 5.0	*Saccharomyces cerevisiae*	Room temperature, 144 h,[a]	0.025	[127]
Microalgae					
Chlorococcum humicola	3% (w/v) H_2SO_4, 160 °C, 15 min, pH 7.0	*Saccharomyces cerevisiae*	3% (v/v) *Saccharomyces cerevisiae*, 30 °C, 50 h, 200 rpm, pH 4.8	0.520	[128]
Chlorella vulgaris FSP-E	1.0% (w/v) H_2SO_4, 121 °C, 20 min, pH 6	*Zymomonas mobilis*	30 °C in a desktop fermentor	SHF: 0.233	[129]
Scenedesmus obliquus CNW-N	0.5–5% (w/v) H_2SO_4, 121 °C, 20 min, pH 6.0	*Zymomonas mobilis*	30 °C, 4 h, pH 6.0,[a]	0.213	[130]

[a] Some data of the parameters were not reported.

Table 7
Enzymatic hydrolysis and algal fermentation under different conditions for bioethanol production.

Algal feedstock	Hydrolysis parameters	Fermenting microorganism	Fermentation parameters	Bioethanol yield (g/g)	References
Macroalgae					
Gracilaria salicornia	Amount of enzyme (0.5% (w/v) *cellulase*), 40 °C, 26 h, pH 5.0	*Eschericia coli* (KO11)	30 °C, 50 h,[a]	0.079	[131]
Gracilaria verrucosa	Enzyme activity (20 FPU/g dry substrate of *cellulase*, 60 CBU/g dry substrate of *β-glucosidase*), 50 °C, 150 rpm, 36 h, pH 5.0	*Saccharomyces cerevisiae* (HAU strain)	6% (v/v) *Saccharomyces cerevisiae*, 30 °C, 16 h, pH 6.0,[a]	0.430	[132]
Sargassum spp.	Enzyme activity (10 FPU/g substrate of *cellulase*, 250 CBU/g substrate of *β-glucosidase*, 50 °C, 100 rpm, 96 h, pH 4.8	*Saccharomyces cerevisiae*	5% (v/v) *Saccharomyces cerevisiae*, 40 °C, 48 h, pH 4.5,[a]	0.170	[133]
Ulva fasciata Delile	Amount of enzyme (2% (w/v) of *cellulase*), 45 °C, 150 rpm, 36 h, pH 4.8	*Saccharomyces cerevisiae* (MTCC No. 180)	28 °C, 120 rpm, 48 h, pH 6.8,[a]	0.450	[134]
Gelidium amansii	Enzyme activity (8.4 EGU/ml substrate of *cellulase*, 1.2 FGB/ml substrate of *β-gluccanase*), 45 °C, 30 rpm, 60 h, pH 4.5	*Acclimated Pichia stipitis and acclimated Saccharomyces cerevisiae*	30 °C, 30 rpm, 96 h, pH 6.4,[a]	AP: 0.500 AS: 0.440	[135]
Gracilaria sp.	Amount of enzyme: (1% (w/v) *cellulase*), 50 °C, 100 rpm, 6 h, pH 4.8	*Saccharomyces cerevisiae* (Wu-Y2 strain)	10% (v/v) *Saccharomyces cerevisiae*, 30 °C, 48 h, pH 4.5,[a]	0.470	[136]
Eucheuma cottonii	Enzyme activity (15 FPU/g substrate of *cellulase*, 52 CBU/g substrate of *β-glucosidase*), 30–60 °C, 150 rpm, 72 h, pH 7.8	*Saccharomyces cerevisiae* (YSC2, type II)	35 °C, 130 rpm, 6 h, pH 5.0,[a]	SHF: 0.559 SSF: 0.909	[137]
Microalgae					
Chlorella vulgaris FSP-E	Amount of enzyme (2% (w/v) *cellulase*+*amylase*, 45 °C, 200 rpm, pH 6.0	*Zymomonas mobilis*	30 °C,[a]	SHF: 0.178 SSF: 0.214	[129]
Chlorella vulgaris	Enzyme activity (240 IU/ mg substrate of *pectinase*, 50 °C, 200 rpm, 72 h, pH 4.8	*Saccharomyces cerevisiae* (KCTC 7906)	30 °C, 48 h,[a]	0.890	[138]

Note: - AP (acclimated *Pichia stipitis*), AS (acclimated *Saccharomyces cerevisiae*).

[a] Some data of the parameters were not reported.

Tables 6 and 7 presents some parameters in acid and enzymatic hydrolysis as well as fermentation of algae under different conditions for bioethanol production. Most of the recent research in acid hydrolysis has used varieties of seaweed species as the feedstocks such as *Eucheuma* sp., *Gelidium* sp. and *Undaria* sp. There are also some species of microalgae including *Chlorococcum* sp., *Chlorella* sp. and *Scenedesmus* sp. Sulfuric acid was commonly employed in the concentration of 1–5% (w/v) as the hydrolyzing agent. The reaction was conducted under a high temperature within the range of 100–160 °C for 15–60 min duration and *Saccharomyces cerevisiae*, *Brettanomyces custersii*, *Pichia angophorae* and *Zymomonas mobilis* have beenused as the fermenting microorganisms.

Khambathy et al. [125] used red seaweed species (*Kappaphycus alvarezii*) and reported a bioethanol yield of 0.390 g/g. Their work basically used 5% (w/v) sulfuric acid at 100 °C, hydrolysis duration of 60 min, fermented by *Saccharomyces cerevisiae* for 48 h. The research was able to obtain a high yield of bioethanol due to 80% conversion of the reducing sugar. Moreover, there was a repetitive treatment of the hydrolysate using additional granules to increase the reducing sugar concentration. The research provided a new insight in transportation fuel technologies as they produced E10 fuel (mixture of 10% bioethanol and 90% gasoline) and tested it on petrol vehicle. Park et al. [65] also used red seaweed *Gelidium amansii* as the feedstock which yields 0.380 g/g of bioethanol. The concentration of sulfuric acid used was 2% (w/v), in 150 °C, 240 min of hydrolysis, *Brettanomyces custersii* as the fermenting microorganism and 39 h fermentation. Practically in this work, they reported a high sugar and low inhibitor concentration due to the employment of continuous dilute-acid hydrolysis which was conducted in Plug and Flow reactor system.

Acid hydrolysis was also attempted using microalgae species that produced a good bioethanol yield. Harun & Danquah [128] obtained 0.520 g/g of bioethanol eventhough the acid hydrolysis was only considered as pre-treatment on microalgal biomass. They tested the effect of number of parameters on acid pre-treatment and their results revealed that temperature was the crucial parameter for bioethanol production. Almost 88% of maximum theoretical bioethanol yield was achieved which used separate hydrolysis fermentation (SHF) process in Hoet al. research [129]. *Chlorella vulgaris* FSP-E was used as the feedstock which obtained 0.233 g/g of bioethanol under conditions of 1% (w/v) sulfuric acid, 121 °C, 20 min of hydrolysis and *Zymomonas mobilis* as the fermenting microorganism. The application of *Zymomonas mobilis* strain in the fermentation step also help to inhibit the formation of the unwanted compound that usually resulted from acid hydrolysis. Acid hydrolysis have its own advantages and disadvantages as depicted in Table 4. However, the needs of a cleaner production process in bioethanol are crucial since its usage will be reflected on the environment. For example, the employment of acids such as HCl is toxic to the environment since it is non-degradable in nature. The toxicity of acids can be overcome by employing enzymes for hydrolysis using “eco-friendly” approach.

Cellulase and *β-glucosidase*or combinations of both are the most favoured enzymes used in enzymatic hydrolysis due to their nature of being able to hydrolyze polysaccharides. Other than that, the reaction also was conducted under mild conditions which were optimum for the enzyme such as temperature in the range of 40–50° C, hydrolysis duration of 6–72 h, agitation of 100–150 rpm and *Saccharomyces cerevisiae*, *Eschericiae coli* and *Zymomonas mobilis* as the fermenting microorganisms. The research on *Gracilaria verrucosa* pulp by Kumar et al. [132] obtained 0.430 g/g bioethanol under conditions of 50 °C, 150 rpm, 16 h of hydrolysis time and using *cellulase* with the combination of *β-glucosidase*. This study found that the hydrolysis efficiency of 88% was comparatively higher than other species of seaweed due to more availability of fermentable carbohydrates in the *Gracilaria* pulp. Green seaweed *Ulva fasciata Delile* also employed in enzymatic hydrolysis by Trivedi et al. [134] and obtained 0.450 g/g of bioethanol. The research employed *cellulase* enzyme, at 45 °C, 36 h of hydrolysis, *Saccharomyces cerevisiae* as the fermenting microorganism and 48 h of fermentation. In this study, one of the factors that help to obtain a high bioethanol yield was the pre-heat treatment method in an aqueous medium.

Bioethanol produced by separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) from *Eucheuma cottonii* was studied by Tan and Lee [137]. Their work used enzyme *β-glucosidase* under 30–60 °C, 150 rpm, 72 h for hydrolysis and fermented by *Saccharomyces cerevisiae* for 6 h. They were able to obtain 0.559 g/g and 0.909 g/g of bioethanol respectively using the processes. They found that SSF was more effective as compared to SHF for bioethanol production. The employment of nutrient stress-induced microalgae *Chlorella vulgaris* was reported to obtain 0.890 g/g of bioethanol in the study by Kim et al. [138]. *Pectinase* is used under the temperature of 50 °C, 200 rpm agitation for 72 h, *Saccharomyces cerevisiae* (immobilized yeast) as the fermenting microorganism and 48 h in batch and continuous type of fermentation. Their study showed that microalgae which cultivated under nutrient stress were able to increase the carbohydrates content by 16–22.3% with hydrolysis efficiency of 79% and reported a yield of 89% of bioethanol.

The bioethanol production using enzymatic hydrolysis paves a more promising route as compared to acid hydrolysis in the coming years. The highest bioethanol yield from acid hydrolysis in macroalgae was reported to be only 0.390 g/g [125] while for microalgae it was 0.520 g/g [128]. On the other hand, the enzymatic hydrolysis in macroalgae gave a bioethanol yield of 0.909 g/g by using SSF technology [137] and for microalgae, it was 0.890 g/g [138]. The implementation of SHF and SSF technologies in hydrolysis and fermentation also played a significant role in bioethanol production to a greater extent [139]. According to Danquah et al. [140], each of the processes has their own pros and cons. SHF can be performed at optimum temperature independently, however, the occurrence of end-product inhibition as well as high contamination cannot be avoided in this process. As for SSF, the production cost can be lowered since the process needs only a small amount of enzyme, less contamination and low inhibitory effects. The only problem related to SSF is the higher optimum temperature for enzymatic hydrolysis which leads to the difficulty in process control. Based on literature [137,141,142], SSF is usually preferred over the SHF process due to its ability to reduce the costs and as well as high production rate.

The ability of different species of algae to produce bioethanol is another important aspect to be considered. Since microalgae and macroalgae differ in their cell wall compositions, the bioethanol yield depends on the sugars produced from that particular species. In producing bioethanol, the capability of macroalgae has gained a lot of attention due to its favourable traits. This is supported by the fact that macroalgae have carbohydrate as its main component which can be categorized into various types of sugars that can be fermented to bioethanol [143]. Apart from it, a low lignin composition also offers great advantages for macroalgae as it is suitable to be employed in fermentation with high yields and greater conversion rates.

Thus, enzymatic hydrolysis has a high potential to be applied in bioethanol production. A vast amount of research in enzymatic hydrolysis has proven its efficiency for bioethanol production [110]. However, the biggest obstacles that the bioethanol industries need to cope are the high cost of enzymes, time-consuming and labour intensive processes. In order to increase the process performance and reduce the major operating cost of bioethanol production, the research in developing an active enzyme and immobilization techniques are essential. The realization

of an optimum condition to obtain a high concentration of bioethanol from third generation feedstock is the best solution that can be done to deal with the problems. By assessing several parameters such as temperature, pH, incubation time, hydrolysates concentration, enzyme concentration and inoculum concentration in hydrolysis and fermentation processes may help in a better understanding and improvement for bioethanol production. In conjunction to this matter, more time and efforts are needed in order to generate a significant and reliable process for bioethanol production from third generation bioethanol feedstock.

5.3. Purification

Purification step in bioethanol involve several types of techniques such as rectification, distillation and dehydration which greatly influence the end products [144,145]. Among these techniques, distillation is the most widely used in purification stage despite its high energy consumption [146]. As the basic principle of distillation is to separate mixtures based on component volatilities, the resultant concentration of the content must be well observed. In order to be accepted for its commercialization, the bioethanol must fulfil all the required standards that have been set by the international standards such as ASTM and ANP.

A distillation unit normally consists of 1) feed (ethanol to be purified), 2) energy source (usually steam), 3) overhead, 4) bottom product, and 5) condenser [147]. However, in the case of obtaining the high purity of bioethanol, the system generally undergoes some modifications. This is in accordance with the advancement in the engineering technology which devotes to the production of high-grade bioethanol together with less energy usage. The distillation process facilitates mass transfer between different components moving in a counter-current fashion [148]. Two different zones will be formed based on the volatility of the components where more volatile components will be in vapor rich region while the less volatile components can be found in the liquid rich region. At the end of this stage, the end product will be drawn off from the system and can be blended with gasoline fuel or directly used as fuel on its own [149].

6. Economics, commercial viability and future prospects of TGB

The third generation bioethanol from algal feedstock currently have attracted many biofuels investors to provide substantial funds for research projects. For a full economic assessment of bioethanol production from algae, the selection of production scale must be appropriate or relevant [150]. The basic aim of economic research especially in biofuels is to minimize the cost of production [151–153]. As the supply chain accounts for the major cost in the production of bioethanol, more efforts should be focused on to it. According to Miretet al. [154], the cost covered by the supply chain includes the cost of feedstock, transportation, storage and also the conversion technologies. These days, researchers are engaged on finding an economically feasible way for sustainable bioethanol production. Thus, in this way, the viability of algae as a feedstock for bioethanol is not argued [155].

The economic benefit of algae in bioethanol lies especially on its high energy content which is higher than the energy accumulated by edible crops and lignocellulosic biomass [156]. This is supported by Nguyen and Vu [97], from their estimation, microalgae are able to produce approximately 5000–15,000 gal of ethanol per acre annually which is more reliable than the first and second generation bioethanol feedstock. Despite its contribution in bioethanol industry, algal processing can result in varieties of high-value products such biochemicals, health care and cosmetics [157]. The productivity rate of algal feedstock such as seaweed is very high until it has become one of the sources of income for the coastal population. It helps the coastal population by increasing the employment opportunities through seaweed farming or cultivation which is one of the major contributors to the economic promotion in that area. The world average yield of seaweed of 730,000 kg/ha per year shows its sustainability as a feedstock source for renewable energy [158]. Table 8 shows the financial comparison of first, second and third generation bioethanol in terms of the production process.

From the financial comparison, it can be concluded that the production cost of third generation bioethanol is quite high. As a result, the commercial viability of algal bioethanol is still in the doubt. Lack of an efficient and reliable established technology contributes as a main factor in commercialization of algal bioethanol. The current inconsistent technologies clearly reduce the investor's interest to commercialize bioethanol as the revenues are too far to be achieved [162]. However, due to its promising prospects for the biofuel industry, researchers are still focused on the improvement of algal bioethanol technologies together with increased flow of investments from all over the world [163]. The ease of availability of feedstock together with conversion technologies should be significantly evaluated inorder to make algal bioethanol commercially viable [29]. Therefore, most of the research in bioethanol industry are mainly focused on the optimization of different factors to obtain reproducible results such as the feedstock, enzyme, microorganisms and also process parameters [39].

In terms of feedstock efficiency, the researchers need to evaluate the factors which induce the algal growth rates such as temperature, light intensity and nutrients for a sustainable productivity. Furthermore, the employment of genetic engineering for the production of transgenic algal strains is also one of the best measures to address the viability of algal fuels [164]. The shortage of water resources for algal cultivation can also contribute to the unsustainable bioethanol production. The water consumption for each liter of bioethanol production is estimated to be about 40–1600 L [165]. In the large scale production, the consumption may reach billions of gallons of water. Improvement in the water system design is the best option which can be done to avoid any difficulties during the cultivation process. According to Subhadra [166], the water system design must be equipped with the ability to recycle and evaporation control to reduce the utility cost. If all the conditions are met, significant reduction in cost of more than 50% may be achieved [167].

For the conversion technologies in bioethanol, hydrolysis and fermentation stages are the main focus for the evaluation of its commercial viability. In bioethanol production, an 'eco-friendly' approach is more preferred to give less impact to the environment. This increases the urge for researchers to put more effort in the establishment of efficient enzyme-based technologies which is very important in sustainable bioethanol production. The detailed study of the enzyme activity regulation is very crucial in order to analyze their optimum conditions during the process. On the other hand, researchers are also intensively involved in the development of an efficient microbial strain for bioethanol fermentation. The screening of wild-type strains of *Saccharomyces cerevisiae* which are able to ferment galactose is one of the initiatives that have been performed [168]. Additionally, lack of galactose metabolism also can be overcome with the modulation of target genes to enhance the consumption rate [169]. Although such ideas and research seemed easy to be achieved, but the success rate cannot be predicted especially when it involves the other external factors. However, this kinds of initiative is one of the benchmark that able to make the algal bioethanol more commercially viable in the future.

Table 8
The financial comparison of first, second and third generation bioethanol [159–161].

Financial Comparison			
Parameters	**First Generation**	**Second Generation**	**Third Generation**
Feedstock cost per unit of production	High	Low	High
Capital cost per unit of production	Low	High	High
Operating cost per unit of production	Low	High	High
Logistics	Low	High	Low
Total cost of production	Low	High	High

Similar to other liquid biofuels, algal bioethanol too confronts the issues on its commercialization. Although the research is still in the development phase, it is expected that its commercialization will face a lot of critics from around the world. One of the main hurdles is its high production cost [85]. In order to overcome this limitation, most of the new research has been diverted to implement new technical innovations and integrated scale-up in the processing routes [156,170]. Lack of well defined biofuel policies together with insufficient government support are also few of the obstacles in the process of commercialization [171]. Brazil and the United States can be set as leading countries which are almost near to commercialization of liquid biofuels like bioethanol. In Malaysia, the National Biofuel Policy is the main driven power that supports the bioethanol commercialization. However, as Malaysia is rich in palm oil biomass, the policy is still stuck with biodiesel commercialization as compared to bioethanol. Thus, for a successful biofuels policy implementation, a proper regulation, legislation and institutional framework need to be enhanced. Last but not least, the compatibility of the fuels to the vehicle's engine performance is also one of the hurdles in the penetration of bioethanol into the current market. Although bioethanol is a clean fuel which is able to reduce the maintenance cost of an engine, in certain cases, engine modifications are needed whether it is used as a blended fuel or on its own [172]. This problem arise lots of questions about the feasibility of bioethanol for transportation sector as there is no guarantee that the fuels can run on all types of vehicle's engine. In this matter, the collaboration of biofuels and automotive industry as well as government commitments are encouraged especially to attract the interest of investors and society.

Despite the economic challenges that constrain the algal bioethanol commercialization, its future prospects seem promising to a greater extent. The current technologies can be efficiently improved in the future. The cost barrier in bioethanol production can be efficiently managed using different life cycle analysis (LCA) techniques. Predominantly for the detailed study of cost of production, the employment of this kind of analysis can verify the reliability of third generation bioethanol over the complete period of its life cycle. By analysing using LCA study, it is expected able to build up new opportunities for discovering the most sustainable alternative to accommodate the difficulties encountered during the production [173]. Researchers are also focusing on manipulation of the genetic content of the algal species so that its potential can be utilized to the maximum [174]. This is in accordance with the advancement in the genome and transcriptome analysis in which the metabolic pathways in organism can be introduced, deleted or even changed [175]. At the same time, the employment of genetically engineered enzyme and yeast in hydrolysis and fermentation must be reviewed comprehensively so that it did not intervance the natural processes [19]. With the current technologies, it is unlikely that the scientific approaches alone will provide the means to allow full commercialization of third generation bioethanol production. Instead, there must be an integration of the scientific, human and environment cooperation to make the research become reality.

7. Conclusion

The potential of third generation feedstock for bioethanol production have been evaluated in this review together with its significant technological hurdles for viable commercialization in future. The sustainability of the algal feedstock and its susceptibility to energy conversion are the main desirable attributes that makes it suitable to be used in the production of bioethanol. The employment of micro- and macro-algae as the feedstock is an innovative move in bioethanol industry as a way to make the production more commercially viable. Many studies have proven the suitability of microalgae as feedstock for bioethanol especially due to its high production capacity of lipids. Currently, macroalgae (seaweeds) are also reported to be one of the best candidates that can be employed as the bioethanol feedstock. Despite having a significantly high level of carbohydrates, seaweeds also serve as a source of income which creating job opportunities to the coastline communities. Moreover, for the states that have a large amount of aquaculture activities such as Sabah, Malaysia, seaweeds can be a very promising alternative source of renewable energy. Enzymatic hydrolysis has a lot of potentials to be implemented in the production of bioethanol due to its reasonable economical cost and less negative impact on the environment. On the other hand, the microorganisms strain utilized in fermentation also play a great role in enhancing the conversion of sugars into bioethanol. Therefore, the detailed study of optimization in enzymatic hydrolysis and fermentation are required for the development of an efficient, advanced and significant bioethanol production process from third generation feedstock. But at some extent, the future prospects of algae are difficult to interpret as more time and effort are required to explore the ability of third generation feedstock for bioethanol world. However, what can be expected from algal bioethanol is its contribution to the decrease in the consumption of fossil fuels for a cleaner and sustainable earth in the future.

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