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Macroalgae for biofuels production: Progress and perspectives



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ABSTRACT

Algae is a very promising source for renewable energy production since it can fix the greenhouse gas (CO₂) by photosynthesis and does not compete with the production of food. Compared to microalgae, researches on biofuel production from macroalgae in both academia and industry are at infancy for economically efficient and technological solutions. This review provides up to-date knowledge and information on macroalgae-based biofuels, such as biogas, bioethanol, biodiesel and bio-oils respectively obtained from anaerobic digestion, fermentation, transesterification, liquefaction and pyrolysis technique methods. It is concluded that bioethanol and bio-oils from wet macroalgae are more competitive while biodiesel production seems less attractive compared to high lipid content microalgae biomass. Finally, a biorefinery concept based on macroalgae is given.

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1. Introduction

Methods to convert biomass to competitive biofuels are increasingly attractive as fossil hydrocarbons are likely to become scarce and costly. Interest has now been diverted to the third-generation biomass

like algae, since the first-generation feedstock (edible crops, sugars and starches) are under serious controversy considering the competition between food and fuel feud [1] and the second-generation biomass (lignocellulosic biomass) are limited by the high cost for lignin removal. Algae is a very promising source for renewable energy production since it can fix the greenhouse gas (CO₂) by photosynthesis. The average photosynthetic efficiency is 6–8% [2] which is much higher than that of terrestrial biomass (1.8–2.2%). Algae can be cultivated on unproductive or abandoned land [3], and it is also very efficient in utilizing the nutrients from waste water including nitrogen

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Table 1
Macroalgae and microalgae composition (w/w% dry biomass) for a variety of species.

	Macroalgae			Microalgae
	Green algae	Red algae	Brown algae	
Polysaccharide	Mannan	Carrageenan	Laminarin (up to 35 [24])	Starch
	Ulvan	Agar (up to 52 [22])	Mannitol	Total carbohydrate
	Starch	Cellulose	Alginate (up to 40 [25])	Arabinose
	Cellulose (38–52)	Lignin	Glucan	Fucose
Monosaccharide	Glucose	Glucose	Cellulose	Galactose
	Mannose	Galactose	Glucose	Glucose
	Uronic acid	Agarose	Galactose	Mannose
			Uronic acid	Rhamnose
Representative	<i>Ulva lactuca</i> [21]	<i>Gelidium amansii</i> [23]	<i>Laminaria japonica</i> [21]	Ribose
Carbohydrates	54.3	83.6	59.5	Xylose
Lipid	6.2	0.9	1.5	
Protein	20.6	12.2	30.9	
Ash	18.9	3.3	8.1	

and phosphorus [4] due to its rapid growth rate and the nutrients can be recycled back to the soil by fertilizing the waste by-products.

Microalgae and macroalgae investigated as potential fuel sources are the two groups of algae [5]. Productivity, scalability and a continuous supply of biomass are critical factors in selecting biofuel feedstock. Anaerobic digestion, fermentation, transesterification, liquefaction and pyrolysis can convert algal biomass into biofuels, such as biogas, bioethanol, biodiesel and bio-oils. Researches on microalgae (a lipid-based biorefinery platform) biodiesel are dominated over the past years as they have higher per hectare yield (158 vs. 60–100 t of macroalgae) [6], and are easier to be degraded due to their low carbohydrate content [7] whilst the poor returns in biomethane production [8] and energy balance [9] partly limited it. According to a Life Cycle Assessment [10], macroalgae can generate a net energy of 11,000 MJ/t dry algae compared to 9500 MJ/t relevant to micro-algae gasification. Compared to microalgae, macroalgae is multicellular plant and possesses plant-like characteristics, making its harvesting more easily [11] and it mainly consists of carbohydrates which are good candidates for biofuel production such as biogas, bioethanol and bio-oils. Biofuel production from macroalgae in both academia and industry are at infancy for economically efficient technological solutions [12].

Macroalgae are classified into three major groups based on their photosynthetic pigmentation variations: red (*Rhodophyta*), brown (*Phaeophyta*) and green (*Chlorophyta*) [13,14]. Globally, red is the most species-rich group (6000) followed by green (4500) and brown (2000) [15]. Brown algae mainly grow in temperate to cold or very cold waters, and red algae grow especially in inter-tropical zones. The green algae grow in all type of water environment [16]. Macroalgae is cultivated at present for food production, fertilizers and hydrocolloid extraction in Asia with China, Korea, Philippines and Japan accounting for about 72% of global annual production [17]. The productivity for macroalgae ranges from 150 to 600 t per hectare per year fresh weight and the total worldwide production reaches 12 million tonnes dry matter/year [18]. However, production of biofuels from macroalgae biomass receives less attention. Currently, the only industrial product of significance from macroalgae is hydrocolloids extraction. However, there are many other opportunities for extraction of high-value niche products from macroalgae. There are several reviews on biofuel production from algae, but they focused on microalgae utilization [16] or only one technique like biogas production from macroalgae [20]. The multiple biofuels production from macroalgae in a biorefinery concept has not been documented. Therefore, the objective of this review is to provide up-to-date knowledge on macroalgae-based biofuels, such as biogas,

bioethanol, biodiesel and bio-oils from the anaerobic digestion, fermentation, transesterification, liquefaction and pyrolysis technique methods and give the perspectives on macroalgae-based biorefinery technologies.

2. Chemical composition of macroalgae

Macroalgae contains generally only 10–15% dry matter [17]. The dehydrated macroalgae consists of large amounts of carbohydrates (approximately 60%). Cell wall components of macroalgae are the major sources of carbohydrates. Some contains cellulose and/or starch (especially green and red). The biochemical composition and ash content have seasonal variations. For example, the content of storage carbohydrates reaches the maximum value in autumn [14]. Table 1 shows the main composition of macroalgae and microalgae. Mannan, ulvan, carrageenan, agar, laminarin, mannitol, alginate, fucoidin, fucose and uronic acid [15] make macroalgae different from microalgae and lignocellulosic biomass. Additionally, lignin content, as low as 0.03 g/kg dry matter [26], offers suitability for downstream processing such as anaerobic digestion or the fermentation process [27] without costly pre-treatment.

Red macroalgae is mainly composed of polymers of modified galactose: carrageenan and agar. *Palmaria palmata* [28] contains the highest carrageenan with the concentration of 354 mg/g by evaluating 20 macroalgae and such a high carrageenan content makes the high yield of bioethanol feasible. Brown algae, the first cultivated algae worldwide, on the other hand, is rich in alginate and contain large quantities of laminarin, important quantity of mannitol in autumn and few standard sugars [21]. The brown and red algae show a low lipid (less than 5% [29,30]) composition which percentage is too low to be converted to biodiesel. Some species of green algae are rich in cellulose (*Valoniopsis* up to 70% cellulose [16]) and most are rich in starch (20–30%). Besides, macroalgae have relatively higher alkali metals and the halogen contents (0.5–11%) than those of terrestrial biomass (1–1.5%) [24]. The nutrient content (N, P) depends primarily on macrophyta morphology and then on environmental nutrient pollution [22].

3. Biofuels production from macroalgae

The productions of biofuels from macroalgae by biochemical and thermochemical technologies are technically possible through

a variety of pathways. Biomethane, bioethanol, biodiesel and bio-oils can be obtained by different conversion methods including anaerobic digestion, fermentation, transesterification, liquefaction and pyrolysis processes which are outlined in Fig. 1.

3.1. Biogas

Biogas production is a long-established technology. The conventional feedstocks for biogas production are agricultural crops, animal wastes, sewage sludge, some house refuses and so on. There are numerous active biogas installations, from large-scale ones to small ones fed with various feedstocks [32]. The water content in macroalgae is higher than in terrestrial biomass (80–85%), making them more suitable for microbial conversion than for thermochemical conversion process. Biogas production from macroalgae is more technically-viable than for other fuels since all the organic components (carbohydrate, protein, etc.) in macroalgae can be converted into biogas by anaerobic digestion (AD), and also the low lignocellulose content make their biodegradation easier than their relatives microalgae to produce significant levels of biogas [33–35]. Methane yields are related to both ash content and the level of storage sugars and varies with biochemical composition. Table 2 summarizes the methane yields from different macroalgae, and the methane yields of macroalgae range from 0.12 to 0.48 m³ CH₄/kg VS.

There are several challenges for biogas production from macroalgae: (a) the nitrogen content in some algae are high, resulting in inappropriate C:N for microbial growth and also the high levels of

ammonia are toxic to methanogens; (b) physical or chemical pre-treatment is needed to break down cell walls and make the organic matter in the cells more accessible; (c) alkaline metals inherent in macroalgae can inhibit the anaerobic digestion process [32]; To get higher yield of CH₄, the optimal C:N (20–30) ratio is necessary. When C:N is lower than 20, the imbalance ratio will lead to increased levels of ammonia in the bio-reactor which can eventually decrease the rate of methane production [47]. The C:N of macroalgae roughly equals to 10 and the C:N of straw is close to 33, while the C:N of wood is up to 244 [48]. Co-digestion of high protein content macroalgae biomass with a co-substrate that has high C:N ratio can balance carbon and nitrogen ratio. Blends of macroalgae and straw will have C:N ratios more conducive for biogas production [43]. Yen [47] found that co-digestion of macroalgae and waste paper resulted in higher biogas yields. Besides, the increase of methane production rate by 26% was reported by co-digesting macroalgae (*Ulva* sp.) with manure and waste activated sludge (ratio 15% U: 85% S) [49]. Furthermore, other empirical regularities can also be adopted from the AD process of sewage sludge [50] as both of the biogas sources have complex composition thus appropriate pre-treatment is necessary and some common inhibitors like ammonia, alkaline metals and volatile fatty acids during the biogas production should not be neglected.

Hydrolysis is the first and rate-limiting step [50] of macroalgae AD process for biogas production. The intracellular polysaccharides, celluloses and proteins are not freely accessible to the inoculum. The

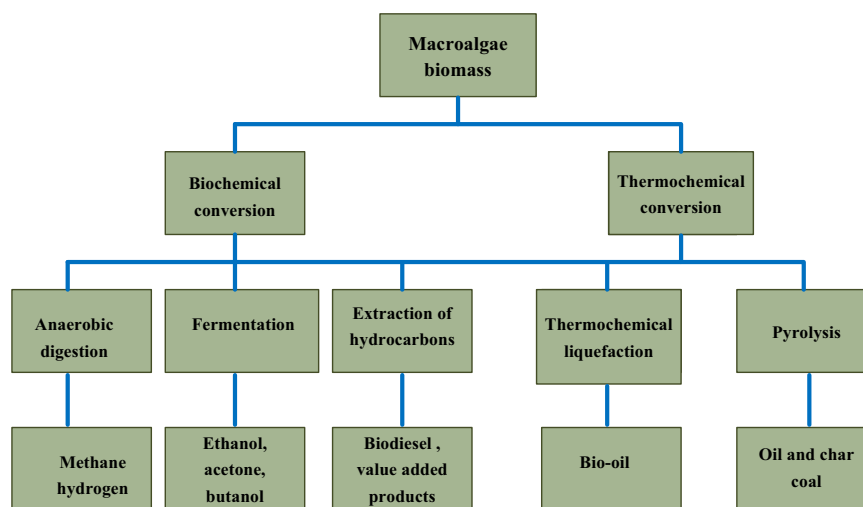


Fig. 1. Biochemical and thermochemical biofuel conversion processes from macroalgae, referred to [35].

Table 2

Effectiveness of biogas production with the use of macroalgae as a substrate in methane fermentation processes.

Groups	Macroalgae	Pretreatment	Quantity of methane	Reference
Green	<i>Ulva lactuca</i>	Macerated	271 L CH ₄ /kg VS	[36]
	<i>Ulva lactuca</i>	Macerated	250 L CH ₄ /kg VS dried 183 L CH ₄ /kg VS fresh	[37]
Red	<i>Ulva biomass</i>	Ground	0.43 L CH ₄ /g COD removed	[38]
	<i>G. vermiculophylla</i>	Maceration	481 ± 9 L CH ₄ /kg VS	[39]
Brown	<i>Laminaria</i> sp.	Mechanical	430 cc gTS ⁻¹	[40]
	<i>Laminaria</i> sp.		22 m ³ CH ₄ /t wet	[41]
	<i>S. latissima</i>	Ground	268 L CH ₄ /kg VS	[42]
	<i>Macrocystis pyrifera</i> <i>Durvillea antarctica</i>	Two-phase digestion system	117.3 CH ₄ /kg VS dried	[43]
Green, brown		Ground	256 CH ₄ /g VS green 179 CH ₄ /g VS brown	[44]
Brown red and green	Ten algae		Average 0.20 L CH ₄ /g VS	[45]

theoretical methane production potential of *Gracilaria* is $0.40 \text{ m}^3 \text{ CH}_4/\text{kg VS}$ [51] but experiment only determined $0.182 \pm 0.023 \text{ m}^3 \text{ CH}_4/\text{kg VS}$ due to the limitation of hydrolysis [52]. In order to break down cell walls and liberate the organic matter needed for microorganisms, pre-treatment processes (milling, maceration, thermal or extrusion) for efficient biogas production are necessary. Milling was reported to reduce the digestion time by 23–59% and cause an increase in biogas yield by 5–25% of most lignocelluloses [53]. Maceration pre-treatment of *Ulva* sp. can increase the surface area, which make the substrate more accessible and cause an increase of 22% methane production [52]. Vivekanand [43] showed that methane yield increased up to 20% after the *Saccharina latissima* biomass treated with steam explosion at 130°C for 10 min. The significant amount of saline (sodium, potassium, calcium and magnesium), halogens [54] and sulfur [55] may also restrict the growth and productivity of the anaerobic microorganisms

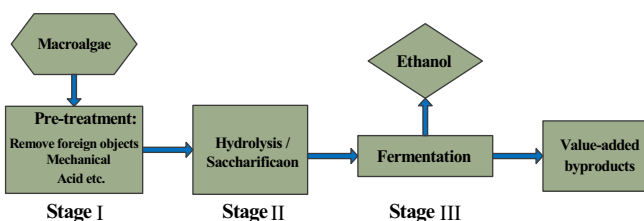


Fig. 2. The technological processes of bioethanol from macroalgae.

and are likely to cause fouling issues [29]. Pre-treatment in both water and weak acid can remove a significant proportion of the mineral matter. Pre-treatment of 10 g of algae in 50 cm^3 of 2.0 M HCl for 6 h at 60°C removed over 90% of Mg, K, Na, Ca, and also a large proportion of the trace mineral matter [55].

3.2. Bioethanol

Bioethanol and synthetic biodiesel from algal feedstock are two liquid algal transportation biofuels. Comparing to edible and lignocellulosic biomass materials, algal biomass is gaining wide attention as an alternative renewable feedstock for the production of bioethanol [12,21,28]. Previous researches on fuels and energy from algae only focused on the production of methane [33,51,52], and methanol [12]. Macroalgae has high content of carbohydrates and little lignin [56] and thus are suitable to be used as substrate in fermentation process for bioethanol production after hydrolysis process. Fermentation by *B. Custersii* produced 11.8 g/L ethanol from 90 g/L sugar in a batch reactor, and 27.6 g/L ethanol from 72.2 g/L sugar in a continuous reactor [57]. Also, results from Inn [58] concluded that *E. Cottonii* could be a potential feedstock for bioethanol production. The technological processes of bioethanol from macroalgae are similar to the common ethanol production as Fig. 2 shows.

Just as biogas production from macroalgae, pre-treatment in the first stage also play an important role in the following

Table 3
Prime polysaccharides hydrolyzed and suitable fermenting enzyme of three families of Macroalgae.

Class	Carbohydrate	Chemical construction	Degradation enzymes [17,70]
Red algae	κ -Carrageenan		<i>Saccharomyces cerevisiae</i>
	Cellulose		<i>Saccharomyces cerevisiae</i>
Brown algae	Alginate		<i>Escherichia coli</i>
	Laminarin		<i>Pichia angophorae</i> <i>Kluyveromyces marxianus</i> <i>Pachysolen tannophilus</i>
	Mannitol		<i>Zymobacter palmae</i> <i>Pichia angophorae</i> <i>Escherichia coli</i> <i>Escherichia coli</i> KO11
Green algae	Starch		<i>Saccharomyces cerevisiae</i>
	Cellulose		<i>Saccharomyces cerevisiae</i>
Common	Galactose		<i>Brettanomyces custersii</i> <i>Saccharomyces cerevisiae</i>
	Glucose		<i>Saccharomyces cerevisiae</i> <i>Brettanomyces custersii</i> <i>Clostridium</i> <i>Escherichia coli</i> KO11

saccharification and fermentation procedure thoroughly. The carbohydrates including laminarin [59], carrageenan and alginates as shown in Table 3 are not freely available to be hydrolyzed. Mechanical or acid pre-treatment can increase the reaction area and make locked sugars in the structural polysaccharides more accessible to hydrolytic enzymes [60], which results in the acceleration of the hydrolysis. Acid pretreatment is one of the shared methods to attain high sugar yields from lignocellulosic biomass. Red algae, *Palmaria palmata*, mainly containing carrageenan, released glucose, galactose and sugars by acid hydrolysis (0.4 M H₂SO₄ at 125 °C for 25 min) and then were fermented to ethanol [28]. *Kappaphycus alvarezii* [61] biomass was saccharified at 100 °C in 0.9 M H₂SO₄ and the best yields for saccharification were 26.2% and 30.6% (w/w) at laboratory (250 g) and bench (16 kg) scales, respectively. Horn et al. [62] reported that washing macroalgae in acidic water (0.09 M HCl in H₂O) at 65 °C enhanced hydrolysis of laminarin, while another report [63] showed the contrary results.

Although acid hydrolysis of biomass was reported to be the most cost-effective method to date, glucose decomposition occurred during the hydrolysis [64]. The by-products during acid hydrolysis including 5-hydroxy-methyl-furfural and levulinic acid, had a profound impact on the efficiency of ethanol production [65]. Compared to acid hydrolysis of carbohydrates, enzyme hydrolysis also faces several challenges such as difficulty in recovering the enzyme from the products and requiring long hydrolysis time [58]. Recently, saccharification is often enhanced by introduction of two-step process of biomass treatment: combination of acid and enzymatic pretreatment [66]. The maximum glucan content of 29.10%, which was four-fold higher than that of the raw *Saccharina japonica*, was obtained by pretreatment with sulfuric acid of 0.06% at the temperature of 170 °C for 15 min [67], and then followed by enzymatic pretreatment. The total biomass digestibility reached to 83.96%. Another study [21] reported that 7.0–9.8 g/L ethanol was produced from 50 g/L sugar in dilute-acid pre-treated biomass of brown algae *L. Japonica* by simultaneous saccharification and enzymatic fermentation. At present, it is necessary to get proper enzymes for high hydrolysis efficiency, and also recovery of enzyme [68] and re-use of enzymes in biorefining processes holds promise. Membrane separation has been demonstrated as an effective way for the recovery of enzyme.

After effective hydrolysis step, most of the polysaccharides and disaccharides become monosaccharides as shown in Eq. (1): taking sucrose as an example, invertase enzyme can catalyze sucrose into glucose and fructose and then *Saccharomyces cerevisiae*, converts glucose and fructose into ethanol [66] as shown in Eq. (2).



Appropriate enzyme and bacteria are often used to gain high efficiency fermentation of the hydrolyzed sugars. Table 3 also shows the main strategies of three kinds of macroalgae fermentation enzymes. A study [14] found that enzyme *Cocktail* from a marine fungus could degrade 90% of the polysaccharides of *L. Digitata* while *Saccharomyces cerevisiae* is thought to be the most common organism for bioethanol production from algae [62] because their capacity of bearing high concentration of ethanol. However, another study [14] found that *Pichia angophorae* was better than *S. cerevisiae* for ethanol production from brown algae as *S. cerevisiae* did not grow well in broths or on agars while *P. angophorae* can utilize mannitol of algae well and meanwhile produce laminarinase, generating higher ethanol yields. It was also found that only 0.45% v/v ethanol was produced after 40 h fermentation by *S. cerevisiae* from macroalgae hydrolysate [62] and the

value was less than 40% of the theoretical yield. The reason for the poor efficiency was that *S. cerevisiae* could only consume glucose in the hydrolysates but not mannitol. However, one study found that *Zymobacter palmae* can grow in a synthetic mannitol medium under oxygen limited conditions and produced ethanol with a yield of 0.38 g/g mannitol [69]. As Table 3 shows, most enzymes have the nature of selectivity while the complex and divers carbohydrate composition of macroalgae requires fermenting microorganisms to be able to metabolize mixed sugars. The coexistence of glucose and galactose in red algae hydrolysates significantly reduces overall ethanol productivity [71,72] and for brown algae, microorganisms cannot utilize various sugars concurrently because they lack the ability to use alginate and thus ethanol production can hardly reach its maximum level [17]. Therefore, it can be concluded that cost-effective hydrolysis of macroalgae polysaccharides into monosaccharide and efficient fermentation of various sugars into ethanol are the pivotal issues for bioethanol production from macroalgae.

3.3. Biodiesel

Biodiesel obtained by transesterification (alcoholysis) of triglycerides is derived commercially from vegetable, animal fats or other plant oils in the international market. Macroalgae is considered for making biogas and bioethanol rather than biodiesel as they do not generally contain triglycerides. To date, macroalgae biodiesel has been reported sparingly and yields are much lower than those of microalgae [73,74]. The first report on biodiesel production from macroalgae focused on the comparison of thermochemical liquefaction and supercritical carbon dioxide extraction techniques [75] and another report compared the biodiesel production from both macroalgae (*Cladophora fracta*) and microalgae (*Chlorella protothecoides*) and it demonstrated the weaknesses of the former for biodiesel.

Macroalgae is usually converted into bio-oil (lipids and free fatty acids), and then the lipids are separated for biodiesel production. The high content of free fatty acids (FFAs) in the oil can restrain the target transformation although the FFAs are also precursor of biodiesel. Tamilarasan [76] esterified the FFAs of *Enteromorpha compressa* algal oil from 6.3% to 0.34%, and then two steps are developed for biodiesel production. During the first step the FFAs were established with acid catalyst and then the oil are turned into biodiesel in the second step. Another attempt used *Cladophora glomerata* to produce glucose and then converted glucose to free fatty acids for biodiesel. Recently, Xu [77] attempted using macroalgae as carbon source of oleaginous yeast to produce biodiesel and the maximum lipid content was 48.30% meanwhile the by-product FFAs accompany mannitol can be used to culture the oleaginous yeast. Some new techniques like ultrasound irradiation are also used to assist the transesterification by forming fine emulsion between oil and alcohol and reaction rate was accelerated because of cavitation [76]. Besides, biodiesel yield obtained from wet biomass was almost ten times lower than from dry biomass which implies negative effect of water on transesterification experiments [66], and thus the dehydration procedure is necessary to achieve high efficiency.

3.4. Bio-oil

Possessing the major superiority of significantly faster rates than those of anaerobic digestion, thermochemical conversion techniques, including pyrolysis and liquefaction, can convert biomass to liquid bio-oil [78] quickly. Compared to microalgae bio-oil refine research, less attention has been devoted to thermochemical conversion of macroalgae.

Table 4
Yields and properties of bio-oils obtained from pyrolysis of different macroalgae at 500 °C.

Algae	Brown algae					Red algae	Green algae
Name	<i>Undaria pinnatifida</i>	<i>Laminaria japonica</i>	<i>Laminaria digitata</i>	<i>Fucus serratus</i>	<i>Sargassum natansa</i>	<i>Prophyra tenera</i>	<i>Enteromorpha clathrata</i>
Yield (%)	39.5	37.5	17	11.0	–	47.4	–
C (%)	56.5	73.5	55.6	68.4	53.8	65.7	57.8
H (%)	5.7	7.9	7.1	8.9	8.2	7.4	7.9
N (%)	7.2	5.7	2.2	2.7	6.55	9.6	9.3
S (%)	0.8	0.2	0.1	0.1	–	0.2	–
O (%)	29.8	12.9	–	–	31.46	17.3	25.0
H/C	0.10	0.11	0.13	0.13	0.15	0.11	0.14
O/C	0.53	0.18	–	–	0.58	0.26	0.43
HHV (MJ/kg)	23.33	33.57	23.08	32.46	–	29.74	–
Ref.	89	89	83	83	85	97	85

3.4.1. Pyrolysis

Pyrolysis of macroalgae for bio-oil, in recent years, has attracted increasing interest. On one hand, macroalgae can be easily harvested from water and dehydration; on the other hand, pyrolysis is likely to be the most tolerant method to the high ash content of the algae compared to other biochemical methods. Pyrolysis is accomplished at temperatures between 400 and 600 °C and atmospheric pressure but requires dry feedstock. During the pyrolysis process, organic structures are decomposed into vapor phase, gas compounds and a carbon-rich solid residue (char). The vapor phase then condensed into liquid product called bio-oil (or bio-crude).

Studies on the pyrolysis behaviors and products characteristics of several macroalgae including brown algae, red algae and green algae [54,79–83], have shown that the pyrolysis process of macroalgae for biofuels production, which is similar to the pyrolysis process of terrestrial plants and microalgae [84–88], undergoes three stages: moisture evaporation, primary devolatilization and residual decomposition [41], and the activation energy of macroalgae is higher compared to terrestrial biomass [82]. The yields and properties of bio-oils highly depend on several factors including algal composition, pyrolysis temperature, heating rate, inorganic material content and others. Pyrolysis of macroalgae at 500 °C has been demonstrated to be a suitable temperature to achieve the maximum yield of bio-oil [82–85,89,90]. The heating rate is also important during the formation of bio-oils. Low heating rate leads low gas evolution but result in low bio-oil yield with high char yield. The increased heating rate provides higher thermal energy to facilitate better heat transfer between the surroundings and the inside of the samples, leading to a higher bio-oil yield [84,90–92].

The bio-oil obtained from pyrolysis of macroalgae is a mixture of water and various organic compounds. Due to different algal feedstocks, analysis methods of bio-oil, processing devices or methods, for example, applying microwave-mediated pyrolysis [93], the main compounds in bio-oils vary between macroalgae and are greatly different from those of terrestrial biomass, but similar to bio-oils obtained from pyrolysis of microalgae [84]. By performing GC/MS, compounds identified in the bio-oil mainly include groups of carboxyl acids, furans, ketones, sugars, phenols, N-containing compounds, hydrocarbons, fatty acids and their derivatives. Totally, the bio-oil has a high content of oxygenates with a very small amount of hydrocarbons, thus, resulting in a relatively low heating value. The high heat values (HHVs) are around 20–30 MJ/kg. Table 4 shows a summary of yields, properties and compositions of bio-oils obtained from pyrolysis of different macroalgae at 500 °C. It can be seen that for different species of macroalgae, the properties and compositions of bio-oils could be very different; meanwhile, the high content of N-containing compounds (refer to element N content) in bio-oils presents a challenge to its direct use as fuels. What's more, the macroalgae contains a high mineral content (Ca,

K, Na and Mg) which may be potentially problematic for pyrolysis, needing pre-treatment, e.g. using acid to remove the most proportion of the mineral matter [54,89]. Another inevitable issue is that due to the high energy penalty of the drying process of the wet macroalgae, pyrolysis is found to produce less energy than it consumed [94]. Based on this constraint, thermochemical pathways using pyrolysis to transform algal biomass to bio-oil needs further verification [95].

3.4.2. Liquefaction

Liquefaction is a process by which biomass undergoes complicated thermochemical reactions in a solvent medium to form mainly liquid products. Hydrothermal liquefaction (HTL) is a process using water as reaction medium, carried out in sub-/supercritical water (200–400 °C) under sufficient pressure to liquefy biomass for bio-oil production [96,97]. The liquid bio-oil is usually separated by extraction of the reaction mixture with organic solvents such as dichloromethane, trichloromethane and acetone.

liquefaction of high moisture content algal biomass requires no feedstock drying and no use of organic solvents, which is more feasible from both economical and energetic points of view. However, macroalgae has been mostly overlooked as a feedstock for bio-oil by HTL because microalgae are thought to be candidate with higher lipid content inherently. Elliott et al. [98] published the first report of HTL of macroalgae (*Macrocystis* sp.) using a batch reactor fed with kelp dry mass at 10 wt% in water. After 4 h at 350 °C, they reported an oil yield of 19.2 wt% based on solvent separation of oil product. Zhou et al. [99] studied the HTL of the green marine macroalgae *Enteromorpha prolifera* and obtained a maximum yield of bio-oil 23.0% dw with an energy density of 29.89 MJ/kg at 300 °C, 30 min, and using Na₂CO₃ as catalyst. Anastasakis and Ross [100] investigated the influence of reaction parameters on the liquefaction behavior of a typical brown macroalga *Laminaria saccharina*, and the highest bio-crude yield of 19.3% af dw was obtained with a 1:10 biomass: water ratio at 350 °C and a residence time of 15 min without catalyst. Their bio-crude yield is much lower than the result of Li et al. [101] obtained by HTL of another brown macroalgae at 340 °C, while the HHV of the bio-crude (36.5 MJ/kg) is much higher than that of Zhou et al. Neveux et al. [102] converted six species of marine and freshwater green macroalgae into bio-crude through HTL in a batch reactor, and found the bio-crude yields were similar to those produced from HTL of green macroalgae *E. prolifera* and brown macroalga *L. saccharina*. The ash content in macroalgae is much higher than that of microalgae, which resulted in lower yields of bio-oils than that obtained from HTL of a range of microalgae (26–57% dw) [95]. So far, reports of the HTL of macroalgae have been limited to batch

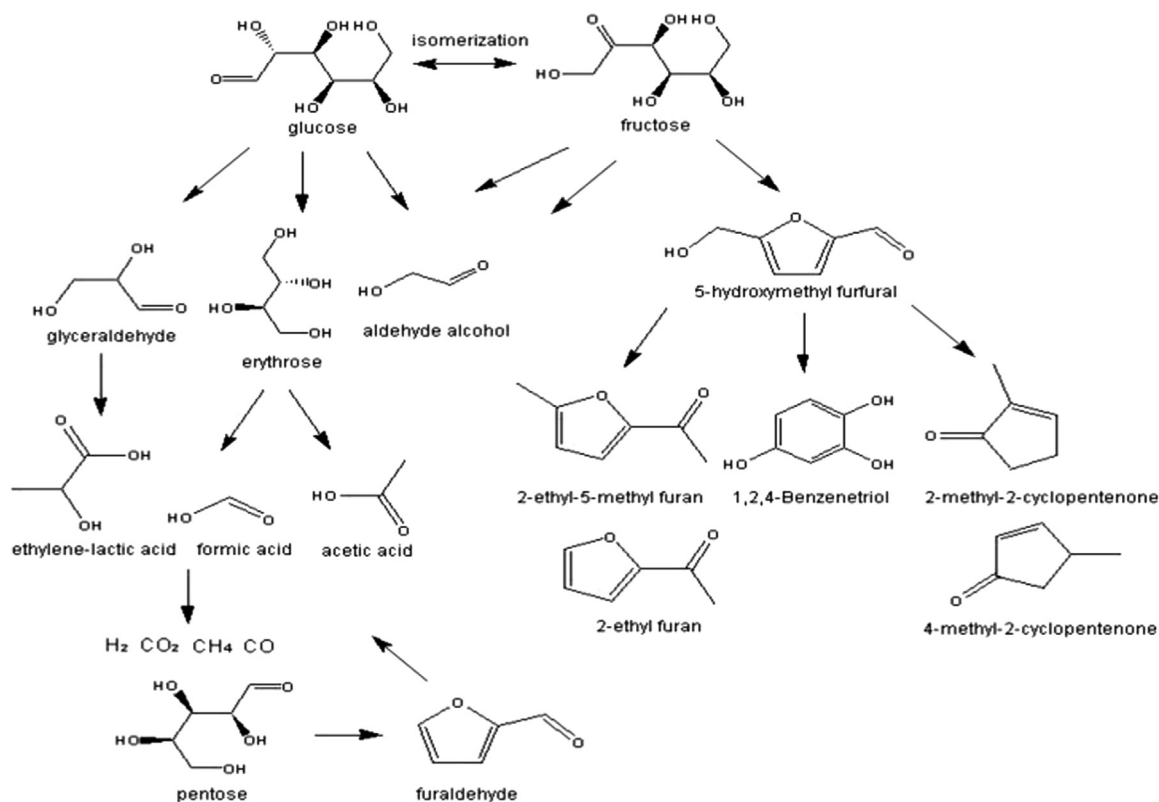


Fig. 3. Probable transform mechanisms of monosaccharides in the HTL process.

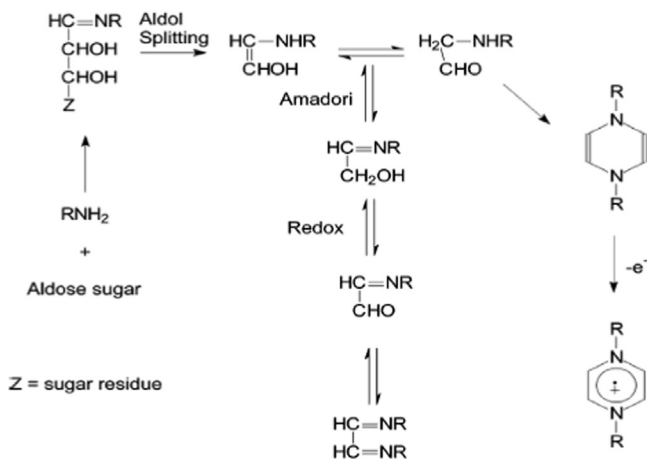


Fig. 4. Maillard reaction of amines and sugars [110].

reactor testing. Recently, Elliott et al. [103] designed a bench-scale continuous-flow reactor system and converted wet macroalgae slurry the kelp, *Saccharina* spp. with concentrations at 5–22 wt% dry solids into bio-crude at 350 °C, 20 MPa. The bio-crude product was recoverable by gravity separation, but the oil yield is low, in the range of 8.7–27.7% af dw for six HTL tests. The biochemical compositions of algal biomass significantly influence the liquefaction yields. The higher yields of bio-oils achieved by microalgae are generally attributed to higher lipid content, compared to macroalgae that have commonly higher carbohydrate content. A recent study on the co-liquefaction of *S. platensis* and *E. prolifera*, has shown that the yield of bio-oil increases with the ratio of microalgae to macroalgae [7]. Besides, for the formation of char, Biller and Ross [104] explained the greater proportions of carbohydrates in macroalgae have a positive impact on char formation by liquefying model compounds glucose and starch.

Bio-oils from HTL of macroalgae deliver a high-energy (30–40 MJ/kg) and lower oxygen and moisture content compared to pyrolysis bio-oil [97,105]. Compounds including ketones, phenols, alcohols, fatty acids, esters, aromatics and N-containing compounds are the common main components of bio-oil [99,102], which is similar to the main composition of bio-oil from HTL of microalgae [106,107]. Actually, much or most bio-oil from HTL of macroalgae dissolved in the water byproduct such as some carboxyl acids, typically, the acetic acid [101]. In the HTL process of macroalgae, the main components (carbohydrates, proteins, lipids) of the algae are decomposed mainly by hydrolysis into fragments of light molecules: carbohydrates i.e. polysaccharides decompose to monosaccharides including hexoses and pentoses; proteins decompose to various amino acids, and lipids to fatty acids and glycerin. At the same time, these fragments, which are unstable and reactive, rearrange through condensation, cyclization, and polymerization, leading to oily compounds and having appropriate molecular weights [108,109]. Fig. 3 shows the probable transform mechanisms of monosaccharides in the HTL process. The N-containing compounds such as indole, pyrroles, and pyridines are formed by Maillard reaction of amines and sugars, as shown in Fig. 4 [110]. In addition, using alcohols (e.g. methanol, ethanol [111] or ethylene glycol, etc. [112]) as medium for the liquefaction of macroalgae were also studied. The bio-oils obtained are mainly composed of fatty acids and esters, but the heating values of bio-oils are still low for the presence of large quantities of O, S, and N [112].

3.5. Others

Ethanol production from low-cost fermentation substrates is the most obvious one as it has a direct application in the replacement of fossil fuels. However, other products such as biobutanol and biohydrogen can be produced as well [113,114]. Biobutanol has higher heating and additional value inherent than ethanol (butanol 29.2 MJ/L, ethanol 19.6 MJ/L). It can be used to

Table 5
The main points of economic evaluation of biofuel production from macroalgae [119–121].

	Input	Main use	Output
Cultivation and harvest	Labor force Diesel Petrol Electricity	Transportation Transportation Lighting	Macroalgae
Pretreatment	Labor force Electricity	Illumination, pumping, grinding, milling, stirring	Feedstock
Technologies	Labor force Electricity and fuel Chemicals and enzymes	Machine running and heating	Biofuels CH ₄ and bioethanol for heat, electricity and chemical industry
Further use	Labor force Diesel	Transportation	Fertilizer and recovery
Potential impacts	CO ₂ emissions NOx and SOx emissions	Direct and indirect power release	CO ₂ : macroalgae uptake N and S: nutrients from waste water

Table 6
Strengths, weaknesses and the main products of different biofuel techniques from macroalgae.

		Strengths	Weaknesses	Primary product	Yield
Biochemical	Anaerobic digestion	No dewatering Maturity	High sodium and nitrogen inhibition	Methane	0.12–0.48 m ³ CH ₄ /kg VS
	Fermentation	High carbohydrates	Low efficiency, mixed sugars	Bioethanol	0.14–0.38 g ethanol /g sugar [21,57]
Chemical	Transesterification	No dewatering	Poor yield	FAME or FAEE	90.6–99.9% of free fatty acids [122]
Thermochemical	Pyrolysis	No caustic chemicals needed, Fast rates;	High temperature and energy consumption;	Aromatics, ketones, pentosans, phenols, hydrocarbons, nitrogen containing organics, palmitic acid, furanes	11.0–47.4% dw. [83,89]
	Hydrotherml liquefaction	No drying More effective	Viscous, high temperature and pressure	Aromatics, phenols, alkanes, fatty acids, ketones, heterocyclic compounds	19.2–44.69% dw [96,102]

supplement both gasoline and diesel fuels and can be handled by existing infrastructures [115]. There are only several literatures investigating the biobutanol production from macroalgae. Macroalgae, particularly the brown algae, is yet to be investigated in terms of their potential for biochemical conversion by *Clostridium* spp. to butanol and other solvents [116] through the acetone–butanol (AB) fermentation. Michael [116] successfully demonstrated the feasibility of acetone–butanol fermentation of brown macroalgae biomass by *C. acetobutylicum* and the butanol concentration

reached about 0.26 g butanol/g sugar in the hydrolysate, and meanwhile, the pilot study recovered 0.29 g butanol/g sugar [117]. Butanol, ethanol, acetone, butyric acid and acetic acid can be produced by *Clostridium* spp. but glucose-based polysaccharides such as mannitol cannot be effectively utilized [116]. The study also found that the fermentation of macroalgae extract exhibited triaxial growth, with glucose being utilized first, mannitol second, and polysaccharide-bound sugars last. To summarize, little is presently known about the bio-conversion of macroalgae to butanol and the poor productivity and limited known fermentative bacterium all need significant improvements.

4. Prospect on the utilization of macroalgae for biofuels production

Macroalgae is an abundant but under-utilized biomass resource. Currently, less than 1% of available macroalgae is utilized [118]. Although, the notion of aquatic biomass production for energy applications is environmentally better than the fossil fuels but still suffer from cost effectiveness and technological barriers [1]. The technologies still require considerable research and development.

Taking bioethanol production as an example, the estimated cost of macroalgae is ca. \$0.50/kg (dw) (\$0.16 from corn) [119]. There are limited economic assessments on macroalgae biofuel production as the research just started and it is not possible to make full-scale and regularly life cycle assessment based on current knowledge. Table 5 shows the main starting points of economic evaluation of biofuel production from macroalgae. The biofuel technologies still require considerable research and development. The technologies selected should be evaluated not only from the standpoint of technical feasibility, economic efficiency but also from the environmental point of view and the byproducts should be recycled. Table 6 outlines the strengths and weaknesses of different biofuel techniques from macroalgae.

As shown in Table 6, it can be concluded that fermentation for bioethanol and hydrothermal refinement from wet macroalgae are more competitive while transesterification for biodiesel, anaerobic digestion for biogas and pyrolysis for bio-oil are limited for low lipid content, mineral inhibition or the high energy consumption. Besides, the pyrolytic process as a fuel production needs further assessment as it accompanies nitrogen content in the oil. The commercially viable of macroalgae biofuel production is a key current issue. The cost of energy production from macroalgae is typically 5–60\$/GJ on the basis of macroalgal biofuels life cycle assessment [123] and it is well over those of coal, oil, and liquified natural gas. To decrease the cost associated with feedstock collection networks, finding applicable pretreatment, making hydrolysis and fermentation reactions efficient and scaling-up of the processing equipment and, commercializing of the systems and giving scientific life cycle assessments of variable production technologies are the emphasis for future macroalgae biofuel research. More work need to be done to develop this area to its full potential. Step-wise research challenges linked to this development are

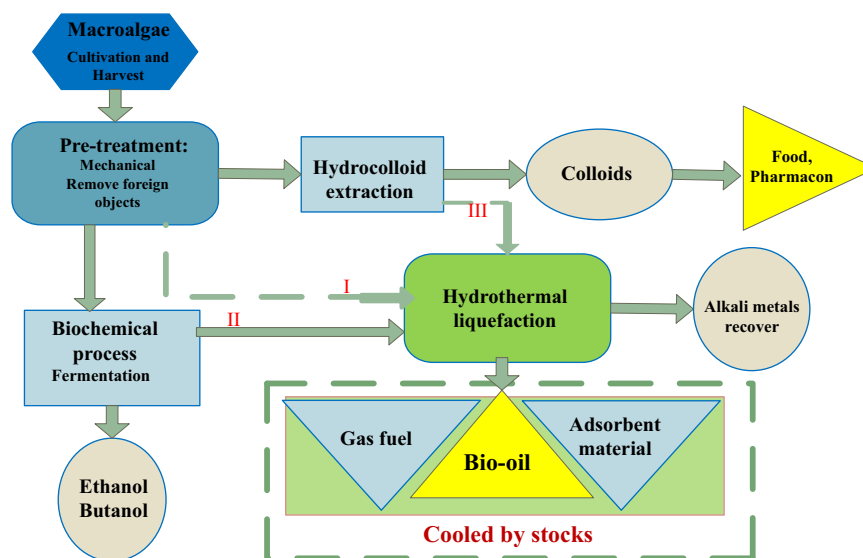


Fig. 5. The biorefinery conception based on macroalgae through biochemical and hydrothermal process.

inevitable. On one hand, macroalgae has high mineral or ash content, mainly comprised of K, Na, Ca and Mg [124] which are not beneficial for the use of macroalgae as a fuel. On the other hand, the alkali metal can be potential catalyst of hydro-thermal process. It is prudent to get high quality and quantities biofuel. Eventually, macroalgae integration into a biorefinery is a challenge.

Fig. 5 shows the biorefinery conception based on the summary of macroalgae conversion techniques. Mature macroalgae are harvested firstly under collection networks and mechanical pre-treatments are needed to remove foreign objects before biochemical and/or hydrothermal treatments. In the first transform route, treated macroalgae is used as the feedstock directly. Biogas, bio-oil and residue are acquired and during the process, products are cooled by the stuff. For the second route, residual after bioethanol fermentation is filtrated and then act as part of the hydrothermal feedstock. To stabilize and cycle the current hydrocolloids extraction, route third is designed. Extraction residue stock is also added to the hydrothermal liquefaction device meanwhile the high alkali metals are easily recovered after the hydrothermal process.

Our previous work for the biorefinery was conducted firstly with macroalgae *E. prolifera*. The macroalgae was converted to bio-oil by hydrothermal liquefaction in a batch reactor. The effects of temperature (varied from 220 to 320 °C) and time (varied from 5 to 60 min) on the bio-oil production were studied. The highest yield (23.0 wt%) of bio-oil with HHV about 30 MJ/kg was obtained at 300 °C [99]. The liquefaction solid residue (hydrochar, 15–20 wt %) could be prepared as adsorbent material based on our previous study [125], where magnetic carbon composite was prepared via thermal pyrolysis of hydrochar, obtained from the hydrothermal liquefaction of *Salix psammophila*, and the activated hydrochar had high surface area and pore volume (up to 1351 m²/g and 0.549 cm³/g, respectively) which can be used for pollutant removal from aqueous solutions. The liquid fraction (35–50 wt%) which contains large amount of organic acids, is favorable for biogas (biomethane) production by anaerobic digestion, and the relevant work is underway.

5. Conclusions

Macroalgae has inherent advantages that make them environmentally sustainable compared to first and second generation biofuel mass but researches on biofuel production from the

macroalgae in both academia and industry are at infancy. Anaerobic digestion technology has sufficiently been matured to offer a range of possibilities to further optimize biogas (methane) yields. Bioethanol and hydrothermal refinement from wet macroalgae are more competitive while transesterification for biodiesel, anaerobic digestion for biogas and pyrolysis for bio-oil are limited for low lipid content, mineral inhibition or the high energy consumption. Macroalgae does not contain as much phenolic material in the biofuels owing to the absence of lignin type materials but the fate of nitrogen and low quality are potentially problematic in using these as fuels. Macroalgae integration into a biorefinery is promising for its efficient conversion to biofuels.

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References

- [1] Singh A, Olsen SI. A critical review of biochemical conversion, sustainability and life cycle assessment of algal biofuels. *Appl Energy* 2011;88:3548–55.
- [2] Aresta M, Dibenedetto A, Barberio G. Utilization of macro-algae for enhanced CO₂ fixation and biofuels production: development of a computing software for an LCA study. *Fuel Process Technol* 2005;86:1679–93.
- [3] Levine RB, Pinnarat T, Savage PE. Biodiesel production from wet algal biomass through in situ lipid hydrolysis and supercritical transesterification. *Energy Fuels* 2010;24:5235–43.
- [4] Singh A, Nigam PS, Murphy JD. Renewable fuels from algae: an answer to debatable land based fuels. *Bioresour Technol* 2011;102:10–6.
- [5] Sheehan J, Dunahay T, Benemann J, Roessler P. A look back at the U.S. Department of Energy's Aquatic Species Program: biodiesel from algae. National Renewable Energy Laboratory; 1998.
- [6] Chisti Y. Biodiesel from microalgae. *Biotechnology* 2007;25:294–306.
- [7] Jin BB, Duan PG, Xu YP, Wang F, Fan YC. Co-liquefaction of micro- and macroalgae in subcritical water. *Bioresour Technol* 2013;149:103–10.
- [8] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. *Renew Sustain Energy Rev* 2009;14:217–32.
- [9] European Academies Science Advisory Council (EASAC). The current status of biofuels in the European Union, their environmental impacts and future prospects; 2012.

- [10] Aitken D, Bulboa C, Faundez AG, Gomez JLT, Antizar-Ladislao BA. Life cycle assessment of macroalgae cultivation and processing for biofuel production. *J Clean Prod* 2014;75:45–56.
- [11] Maceiras R, Rodríguez M, Cancela A, Urrejola S, Sanchez A. Macroalgae: raw material for biodiesel production. *Appl Energy* 2011;88:3318–23.
- [12] Bastianoni S, Coppola F, Tiezzi E, Colacevich A, Borghini F, Focardi S. Biofuel potential production from the Orbetello lagoon macroalgae: a comparison with sunflower feedstock. *Biomass Bioenergy* 2008;32:619–28.
- [13] John RP. Micro and macro algal biomass: a renewable source for bioethanol. *Bioresour Technol* 2011;102:186–93.
- [14] Kraan S. Mass-cultivation of carbohydrate rich macroalgae, a possible solution for sustainable biofuel production. *Mitig Adapt Strateg Glob Change* 2013;18:27–46.
- [15] Jung AK, Lim SR, Kim Y, Park JM. Potentials of macroalgae as feedstocks for biorefinery. *Bioresour Technol* 2013;135:182–90.
- [16] Bucholc K, Szymczak-Zyła M, Lubecki L, Zamojska A, Hapter P, Tjernström E, et al. Nutrient content in macrophyta collected from southern Baltic Sea beaches in relation to eutrophication and biogas production. *Sci Total Environ* 2014;473:298–307.
- [17] Roesijadi G. Macroalgae as a biomass feedstock: a preliminary analysis. U.S. Department of Energy under contract, Pacific Northwest National Laboratory; 2010.
- [18] FAO Statistics (2008data) from IFREMER website: (http://aquaculture.ifremer.fr/statistiques_mondiales/presentation) general production aquacole; 2010.
- [20] Dębowski M, Zieliński M, Grala A, Dudek M. Algae biomass as an alternative substrate in biogas production technologies—review. *Renew Sustain Energy Rev* 2013;27:596–604.
- [21] Kim NJ, Li H, Jung K, Chang HN, Lee PC. Ethanol production from marine algal hydrolysates using *Escherichia coli* K011. *Bioresour Technol* 2011;102:7466–9.
- [22] McHugh DJ. A guide to the seaweed industry. Rome: Food and Agriculture Organization of the United Nations; 2003.
- [23] Jang SS. Production of mono sugar from acid hydrolysis of seaweed. *Afr J Biotechnol* 2012;11:1953–61.
- [24] Mautner H. The chemistry of brown algae. *Econ Bot* 1954;8:174–92.
- [25] Draget KI, Smidsrød O, Skjåk-Bræk G. Alginates from algae. *Biopolymers Online*. Wiley-VCH Verlag GmbH & Co. KGaA; 2005.
- [26] Ventura MR, Castanon JIR. The nutritive value of seaweed (*Ulva lactuca*) for goats. *Small Ruminant Res* 1998;29:325–7.
- [27] Horn SJ. Bioenergy from brown seaweeds (Ph.D. thesis). NTNU; 2000.
- [28] Mutripah S, Meinita MDN, Kang JY, Jeong GT, Susanto AB, Prabowo RE, et al. Bioethanol production from the hydrolysate of Palmariapalmata using sulfuric acid and fermentation with brewer's yeast. *J Appl Phycol* 2014;26:687–93.
- [29] Ross AB, Jones JM, Kubacki ML, Bridgeman T. Classification of macroalgae as fuel and its thermochemical behaviour. *Bioresour Technol* 2008;99:6494–504.
- [30] Bucholc K, Szymczak-Zyła M, Lubecki L, Zamojska A, Hapter P, Tjernström E, et al. Nutrient content in macrophyta collected from southern Baltic Sea beaches in relation to eutrophication and biogas production. *Sci Total Environ* 2014;473:298–307.
- [32] Demirbas A. Use of algae as biofuel sources. *Energy Convers Manag* 2010;51:2738–49.
- [33] Ostgaard K, Indergaard M, Markussen S, Knusten SV, Jensen A. Carbohydrate degradation and methane production during fermentation of *Laminaria saccharina* (Laminariales, Phaeophyceae). *J Appl Phycol* 1993;5:333–42.
- [34] Peu P, Sassi JF, Girault R, Picard S, Saint-Cast P, Béline F, et al. Sulphur fate and anaerobic biodegradation potential during co-digestion of seaweed biomass (*Ulva* sp.) with pig slurry. *Bioresour Technol* 2011;102:10794–802.
- [35] Gunaseelan VN. Anaerobic digestion of biomass for methane production: a review. *Biomass Bioenergy* 1997;13:83–114.
- [36] Bruhn A, Dahl J, Nielsen HB, Nikolaisen L, Rasmussen MB, Markager S, et al. Bioenergy potential of *Ulva lactuca*: biomass yield, methane production and combustion. *Bioresour Technol* 2011;102:2595–604.
- [37] Allen E, Browne J, Hynes J, Murphy JD. The potential of algae blooms to produce renewable gaseous fuel. *Waste Manag* 2013;33:2425–33.
- [38] Oliveira JV, Alves MM, Costa JC. Design of experiments to assess pre-treatment and co-digestion strategies that optimize biogas production from macroalgae *Gracilaria vermiculophylla*. *Bioresour Technol* 2014;162:323–30.
- [39] Tedesco S, Barroso TM, Olabi AG. Optimization of mechanic al pre-treatment of *Laminaria* spp. biomass-derived biogas. *Renew Energy* 2014;62:527–34.
- [40] Kim J, Jung H, Lee C. Shifts in bacterial and archaeal community structures during the batch biomethanation of *Ulva* biomass under mesophilic conditions. *Bioresour Technol* 2014;169:502–9.
- [41] Vergara-Fernández A, Vargasa G, Alarcón N, Velasco A. Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. *Biomass Bioenergy* 2008;32:33–44.
- [42] Gurung A, Ginkel SWV, Kang WC, Qambrani NA, Oh SE. Evaluation of marine biomass as a source of methane in batch tests: a lab-scale study. *Energy* 2012;43:396–401.
- [43] Vivekanand V, Eijssink VGH, Horn SJ. Biogas production from the brown seaweed *Saccharina latissima*: thermal pretreatment and co-digestion with wheat straw. *J Appl Phycol* 2012;24:1295–301.
- [44] Matsui JT, Amano T, Koike Y, Saiganji A, Saito H. Methane fermentation of seaweed biomass. San Francisco: American Institute of Chemical Engineers; 2006.
- [45] Jard G, Marfaing H, Carrère H, Delgenes JP, Steyer JP, Dumas C. French Brittany macroalgae screening: composition and methane potential for potential alternative sources of energy and products. *Bioresour Technol* 2013;144:492–8.
- [47] Yen HW, Brune DE. Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresour Technol* 2007;98:130–4.
- [48] Trinh TN, Jensen PA, Johansen KD, Knudsen NO, Sørensen HR, Hvilsted S. Comparison of lignin, macroalgae, wood, and straw fast pyrolysis. *Energy Fuels* 2013;27:1399–409.
- [49] Costa JC, Gonçalves PR, Nobre A, Alves MM. Biomethanation potential of macroalgae *Ulva* spp. and *Gracilaria* spp. and in co-digestion with waste activated sludge. *Bioresour Technol* 2012;114:320–6.
- [50] Appels L, Baeyens J, Degre've J, Dewil R. Principles and potential of the anaerobic digestion of waste-activated sludge. *Prog Energy Combust Sci* 2008;34:755–81.
- [51] Bird KT, Chynoweth DP, Jerger DE. Effects of marine algal proximate composition on methane yields. *J Appl Phycol* 1990;2:207–13.
- [52] Briand X, Morand P. Anaerobic digestion of *Ulva* sp. 1. Relationship between *Ulva* composition and methanisation. *J Appl Phycol* 1997;9:511–24.
- [53] Hendriks ATWM, Zeeman G. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresour Technol* 2009;100:10–8.
- [54] Ross AB, Anastasakis K, Kubacki M, Jones JM. Investigation of the pyrolysis behaviour of brown algae before and after pre-treatment using PY-GC/MS and TGA. *J Anal Appl Pyrolysis* 2009;85:3–10.
- [55] McCarty PL, McKinney RE. Salt toxicity in anaerobic digestion. *Water Pollut Control Fed* 1961;33:399–415.
- [56] John RP, Anisha GS, Nampoothiri KM, Pandey A. Micro and macroalgal biomass: a renewable source for bioethanol. *Bioresour Technol* 2011;102:186–93.
- [57] Park JH. Use of *Gelidium amansii* as a promising resource for bioethanol: a practical approach for continuous dilute acid hydrolysis and fermentation. *Bioresour Technol* 2012;108:83–8.
- [58] Tan IS, Lam ML, Lee KT. Hydrolysis of macroalgae using heterogeneous catalyst for bioethanol production. *Carbohydr Polym* 2013;94:561–6.
- [59] Wei N, Quarterman J, Jin YS. Marine macroalgae: an untapped resource for producing fuels and chemicals. *Trends Biotechnol* 2013;31:71–7.
- [60] Han KH, Ko JH, Yang SH. Optimizing lignocellulosic feedstock for improved biofuel productivity and processing. *Biofuels Bioprod Biorefin* 2007;1:135–46.
- [61] Khambhaty Y, Mody K, Gandhi M, Thampy S, Maiti P, Brahmabhatt H, et al. *Kappaphycus alvarezii* as a source of bioethanol. *Bioresour Technol* 2012;103:180–5.
- [62] Adams JM, Gallagher JA, Donnison IS. Fermentation study on *Saccharina latissima* for bioethanol production considering variable pre-treatments. *J Appl Phycol* 2009;21:569–74.
- [63] Nguyen MT, Choi SP, Lee JH, Sim SJ. Hydrothermal acid pretreatment of *Chlamydomonas reinhardtii* biomass for ethanol production. *J Microbiol Biotechnol* 2009;19:161–6.
- [64] Jeong ST, Choi CH, Lee JY, Oh KK. Behaviors of glucose decomposition during acid-catalyzed hydrothermal hydrolysis of pretreated *Gelidium amansii*. *Bioresour Technol* 2012;116:435–40.
- [65] Klinke HB, Thomsen AB, Ahring BK. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Appl Microbiol Biotechnol* 2004;66:10–26.
- [66] Darocha M, Geng S, Wang GY. Recent advances in liquid biofuel production from algal feedstocks. *Appl Energy* 2013;102:1371–81.
- [67] Lee JY, Li P, Lee J, Ryu HJ, Oh KK. Ethanol production from *Saccharina japonica* using an optimized extremely low acid pretreatment followed by simultaneous saccharification and fermentation. *Bioresour Technol* 2013;127:119–25.
- [68] He Y, David M, Bagley DM, Leung KT, Liss SN, Liao BQ. Recent advances in membrane technologies for biorefining and bioenergy production. *Biotechnol Adv* 2012;30:817–58.
- [69] Horn SJ. Production of ethanol from mannitol by *Zymobacter palmae*. *J Ind Microbiol Biotechnol* 2000;24:51–7.
- [70] Jung KA, Lim SR, Kim Y, Park JM. Potentials of macroalgae as feedstocks for biorefinery. *Bioresour Technol* 2013;135:182–90.
- [71] Johnston M. Multiple mechanisms provide rapid and stringent glucose repression of gal gene-expression in *Saccharomyces cerevisiae*. *Mol Cell Biol* 1994;14:3834–41.
- [72] Ostergaard S. The impact of GAL6, GAL80, and MIG1 on glucose control of the GAL system in *Saccharomyces cerevisiae*. *FEMS Yeast Res* 2001;1:47–55.
- [73] Afify AEMR, Shalaby EA, Shanab SMM. Enhancement of biodiesel production from different species of algae. *Grasas Aceites* 2010;61:416–22.
- [74] Maceiras R, Rodríguez M, Cancela A, Urréjola S, Sánchez A. Macroalgae: raw material for biodiesel production. *Appl Energy* 2011;88:3318–23.
- [75] Aresta M, Dibenedetto A, Carone M, Colonna T, Fragale C. Production of biodiesel from macroalgae by supercritical CO₂ extraction and thermochemical liquefaction. *Environ Chem Lett* 2005;3:136–9.
- [76] Tamilarasan S, Sahadevan R. Ultrasonic assisted acid base transesterification of algal oil from marine macroalgae *Caulerpa peltata*: optimization and characterization studies. *Fuel* 2014;128:347–55.
- [77] Xu X, Kim JY, Oh YR, Park JM. Production of biodiesel from carbon sources of macroalgae, *Laminaria japonica*. *Bioresour Technol* 2014;169:455–61.
- [78] Rowbotham J, Dyer P, Greenwell H, Theodorou M. Thermochemical processing of macroalgae: a late bloomer in the development of third generation biofuels. *Biofuel* 2012;3:441–61.

- [79] Li DM, Chen LM, Zhao JS, Zhang XW, Wang QY, Wang HX, et al. Evaluation of the pyrolytic and kinetic characteristics of *Enteromorpha prolifera* as a source of renewable bio-fuel from the Yellow Sea of China. *Chem Eng Res Des* 2010;88:647–52.
- [80] Zhao H, Yan H, Liu M, Zhang C, Qin S. Pyrolytic characteristics and kinetics of the marine green tide macroalgae. *Chin J Oceanol Limnol* 2011;29:996–1001.
- [81] Li DM, Chen LM, Zhang XW, Ye NH, Xing F. Pyrolytic characteristics and kinetic studies of three kinds of red algae. *Biomass Bioenergy* 2011;35:1765–72.
- [82] Li DM, Chen LM, Chen SL, Zhang XW, Chen FJ, Ye NH. Comparative evaluation of the pyrolytic and kinetic characteristics of a macroalga (*Sargassum thunbergii*) and a freshwater plant (*Potamogeton crispus*). *Fuel* 2012;96:185–91.
- [83] Yanik J, Stahl R, Troeger N, Sinag A. Pyrolysis of algal biomass. *J Anal Appl Pyrolysis* 2013;103:134–41.
- [84] Kan T, Grierson, Nys R, Strezov V. Comparative assessment of the thermochemical conversion of freshwater and marine micro- and macroalgae. *Energy Fuels* 2014;28:104–14.
- [85] Wang S, Wang Q, Jiang XM, Han XX, Ji HS. Compositional analysis of bio-oil derived from pyrolysis of seaweed. *Energy Convers Manage* 2013;68:273–80.
- [86] Peng WM, Wu QY, Tu PG, Zhao NM. Pyrolytic characteristics of microalgae as renewable energy source determined by thermo gravimetric analysis. *Bioresour Technol* 2001;80:1–7.
- [87] Miao XL, Wu QY, Yang CY. Fast pyrolysis of microalgae to produce renewable fuels. *J Anal Appl Pyrolysis* 2004;71:855–63.
- [88] Miao X, Wu QY. High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*. *J Biotechnol* 2004;110:85–93.
- [89] Bae YJ, Ryu C, Jeon JK, Park J, Suh DJ, Suh YW, et al. The characteristics of bio-oil produced from the pyrolysis of three marine macroalgae. *Bioresour Technol* 2011;102:3512–20.
- [90] Li R, Zhong ZP, Jin BS, Zheng AJ. Selection of temperature for bio-oil production from pyrolysis of algae from lake blooms. *Energy Fuels* 2012;26:2996–3002.
- [91] Caballero JA, Font R, Marcilla A, Conesa JA. Characterization of sewage sludge by primary and secondary pyrolysis. *J Anal Appl Pyrolysis* 1997;41:433–50.
- [92] Haykiri-Acma H, Yaman S, Kucukbayrak S. Effect of heating rate on the pyrolysis yields of rapeseed. *Renew Energy* 2006;31:803–10.
- [93] Budarin VL, Zhao YZ, Gronnow MJ, Shuttleworth PS, Breeden SW, Macquarrie DJ, et al. Microwave-mediated pyrolysis of macro-algae. *Green Chem* 2011;13:2330–3.
- [94] Jena U, Das KC. Comparative evaluation of thermochemical liquefaction and pyrolysis for bio-oil production from microalgae. *Energy Fuels* 2011;25:5472–82.
- [95] López BD, Prins W, Ronsse F, Brilman W. Hydrothermal liquefaction (HTL) of microalgae for biofuel production: state of the art review and future prospects. *Biomass Bioenergy* 2013;53:113–27.
- [96] Toor SS, Rosendahl L, Rudolf A. Hydrothermal liquefaction of biomass: a review of subcritical water technologies. *Energy* 2011;36:2328–42.
- [97] Peterson AA, Vogel F, Lachance RP, Fröling M, Antal Jr MJ, Tester JW. Thermochemical biofuel production in hydrothermal media: a review of sub and supercritical water technologies. *Energy Environ Sci* 2008;1:32–65.
- [98] Elliott DC, Butner RS, Sealock LJ. Product analysis from direct liquefaction of several high-moisture biomass feedstocks. In: *Pyrolysis oils from biomass: producing, analyzing, and upgrading*. Symposium series, vol. 376; 1988. p. 179–88.
- [99] Zhou D, Zhang L, Zhang SC, Fu HB, Chen JM. Hydrothermal liquefaction of macroalgae *Enteromorpha prolifera* to bio-oil. *Energy Fuels* 2010;24:4054–61.
- [100] Anastasakis K, Ross AB. Hydrothermal liquefaction of the brown macro-alga *Laminaria Saccharina*: effect of reaction conditions on product distribution and composition. *Bioresour Technol* 2011;102:4876–83.
- [101] Li DM, Chen LM, Xu D, Zhang XW, Ye NH, Chen FJ, et al. Preparation and characteristics of bio-oil from the marine brown alga *Sargassum patens* C. Agardh. *Bioresour Technol* 2012;104:737–42.
- [102] Neveux N, Yuen AKL, Jazrawi C, Magnusson M, Haynes BS, Masters AF, et al. Biocrude yield and productivity from the hydrothermal liquefaction of marine and freshwater green macroalgae. *Bioresour Technol* 2014;155:334–41.
- [103] Elliott DC, Hart TR, Neuenschwander GG, Rotness LJ, Roesijadi G, Zacher AH, et al. Hydrothermal processing of macroalgal feedstocks in continuous-flow reactors. *Sustain Chem Eng* 2014;2:207–15.
- [104] Biller P, Ross AB. Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. *Bioresour Technol* 2011;102:215–25.
- [105] Bridgewater AV. Review of fast pyrolysis of biomass and product upgrading. *Biomass Bioenergy* 2012;38:68–94.
- [106] Ross AB, Biller P, Kubacki ML, Li H, Lea-Langton A, Jones JM. Hydrothermal processing of microalgae using alkali and organic acids. *Fuel* 2010;89:2234–43.
- [107] Brown TM, Duan PG, Savage PE. Hydrothermal liquefaction and gasification of *Nannochloropsis* sp. *Energy Fuels* 2010;24:3639–46.
- [108] Balat M. Mechanisms of thermochemical biomass conversion processes. Part 3: reactions of liquefaction. *Energy Sources Part A: Recov Utilizat Environ Effects* 2008;30:649–59.
- [109] Zou X, Qin T, Huang L, Zhang X, Yang Z, Wang Y. Mechanisms and main regularities of biomass liquefaction with alcoholic solvents. *Energy Fuels* 2009;23:5213–8.
- [110] Kruse A, Maniam P, Spieler F. Influence of proteins on the hydrothermal gasification and liquefaction of biomass. 2. Model compounds. *Ind Eng Chem Res* 2007;46:87–96.
- [111] Zhou D, Zhang SC, Chen JM. Liquefaction of macroalgae *Enteromorpha prolifera* in sub-/supercritical alcohols: direct production of ester compounds. *Energy Fuels* 2012;26:2342–51.
- [112] Zhuang YB, Guo JX, Chen LM, Li DM, Liu JH, Ye NH. Microwave assisted direct liquefaction of *Ulva prolifera* for bio-oil production by acid catalysis. *Bioresour Technol* 2012;116:133–9.
- [113] Qureshi N, Saha BC, Dien B, Hector HE, Cotta MA. Production of butanol (a biofuel) from agricultural residues: Part I—use of barley straw hydrolysate. *Biomass and Bioenergy* 2010;34:559–65.
- [114] Survase SA, Sklavounos E, Jurgens G, Heiningen A, Granström T. Continuous acetone–butanol–ethanol fermentation using SO₂–ethanol–water spent liquor from spruce. *Bioresour Technol* 2011;102:10996–1002.
- [115] Fortman JL, Chhabra S, Mukhopadhyay A, Chou H, Lee TS, Steen E, et al. Biofuels alternatives to ethanol: pumping the microbial well. *Trends Biotechnol* 2008;26:375–81.
- [116] Huesemann MH, Kuo LJ, Urquhart L, Gill GA, Roesijadi G. Acetone–butanol fermentation of marine macroalgae. *Bioresour Technol* 2012;108:305–9.
- [117] Potts T. The production of butanol from Jamaica bay macro algae. *Environ Prog Sustain Energy* 2012;31:29–36.
- [118] Budarin VL, Zhao YZ, Gronnow MJ, Shuttleworth PS, Breeden SW, Macquarrie D, et al. Microwave-mediated pyrolysis of macro-algae. *Green Chem* 2011;13:2330–3.
- [119] Aitken D, Bulboa C, Godoy-Faundez A, Turrión-Gómez GL, Antizar-Ladislao B. Life cycle assessment of macroalgae cultivation and processing for biofuel production. *J Clean Prod* 2014;75:45–56.
- [120] Alvarado-Morales M, Boldrin A, Karakashev DB, Holdt SL, Angelidaki I, Astrup T. Life cycle assessment of biofuel production from brown seaweed in Nordic conditions. *Bioresour Technol* 2013;129:92–9.
- [121] Dave A, Huang Y, Rezvani S, McIlveen-Wright D, Novaes M, Hewitt N. Techno-economic assessment of biofuel development by anaerobic digestion of European marine cold-water seaweeds. *Bioresour Technol* 2013;135:120–7.
- [122] Suganya T, Gandhi NN, Renganathan S. Production of algal biodiesel from marine macroalgae *Enteromorpha compressa* by two step process: optimization and kinetic study. *Bioresour Technol* 2013;128:392–400.
- [123] Aitken D, Bulboa C, Godoy-Faundez A, Juan L, Antizar-Ladislao T-GB. Life cycle assessment of macroalgae cultivation and processing for biofuel production. *J Clean Prod* 2014;75:45–56.
- [124] Repérez P. Mineral content of edible marine seaweeds. *Food Chem* 2002;79:23–6.
- [125] Zhu XD, Liu YC, Luo G, Qian F, Zhang SC, Chen JM. Facile fabrication of magnetic carbon composites from hydrochar via simultaneous activation and magnetization for triclosan adsorption. *Environ Sci Technol* 2014;48:5840–8.