Renewable and Sustainable Energy Reviews 65 (2016) 235–249

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Recent developments on biofuels production from microalgae and macroalgae

Kanhaiya Kumar [a], Supratim Ghosh [a], Irini Angelidaki [b], Susan L. Holdt [b], Dimitar B. Karakashev [b], Merlin Alvarado Morales [b], Debabrata Das [a,*]

[a] Department of Biotechnology, Indian Institute of Technology Kharagpur, 721301 West Bengal, India
[b] Department of Environmental Engineering, Technical University of Denmark, Denmark

ARTICLE INFO

Article history:
Received 23 September 2015
Received in revised form
27 February 2016
Accepted 26 June 2016
Available online 16 July 2016

Keywords:
Biodiesel
Bioethanol
Biohydrogen
Biogas
Hydrothermal liquefaction

ABSTRACT

Biofuels from algae are considered as promising alternatives of conventional fossil fuels, as they can eliminate most of the environmental problems. The present study focuses on all the possible avenues of biofuels production through biochemical and thermochemical conversion methods in one place, bringing together both microalgae and macroalgae on the same platform. It provides a brief overview on the mechanism of different biofuel production from algae. Factors affecting the biofuel process and the associated challenges have been highlighted alongwith analysis of techno-economic study available in literature. Undoubtly, biodiesel is the center of attraction among other biofuels. However, their routes and process need to be optimized in order to bring the minimum fuel selling price (MFSP) of biodiesel competitive. Technological challenges have not been overcome to make biofuel production process energetically and commercially viable. Macroalgae are low in lipid content. Therefore, the use of macroalgae is restricted for gaseous fuels or fermentative methods of liquid biofuels production. Anaerobic digestion of algal biomass is easy and seems promising as the process is simple in terms of engineering and infrastructure requirement. Hydrogen production by microalgae through biophotolysis seems interesting as it directly converts the solar energy into hydrogen. However, the process has not been scaled-up till today. Hydrothermal liquefaction (HTL) is more promising due to handling of wet biomass at moderate temperature and pressure and conversion of whole biomass into high quality oil. However, HTL process is energy intensive.

Contents

* Corresponding author.
E-mail address: ddas.iitkgp@gmail.com (D. Das).

http://dx.doi.org/10.1016/j.rser.2016.06.055
1364-0321/

1. Introduction

The gap between world energy requirement and supply is growing wider. Fossil fuels resources are localized in few countries, making most of the other countries to be dependent upon them for their energy requirement. An increase in the energy demand, technology and regulatory policies such as fuel efficient cars and green house gas (GHG) reduction have great influence over the choice of quantity and quality of energy carriers. Bioenergy can play a significant role to meet the global challenges of clean, self dependent, and sustainable energy requirement. Research on algal biology is not completely explored; it has a great potential to support bioenergy requirement. Microalgae have higher nutrients and CO_2 uptake efficiency compared with terrestrial plants. This is because of high surface to volume ratio of algal cells [1]. Microalgae have high growth rate and do not compete directly with the food supply chain. It can be grown in non-arable land and can be cultivated throughout the year. The algal biodiesel productivity was estimated to be 10–23 times higher in comparison to the highest oil producing terrestrial oil crop – palm [2]. In addition, production of microalgae captures the CO_2 from the environment and thus reduces the global warming [3].

The cost of algal biomass production and energy recovery from the biomass is still too high to compete with the cost of fossil derived energy carrier. Therefore, it is imperative to look for biorefinery approach utilizing most of the algal biomass for energy recovery. Algal biomass can be used as an energy carrier in the form of solid (e.g. direct combustion of biomass), liquid (e.g. biodiesel, bioethanol), and gas (e.g. biohydrogen, biogas, syngas) (Fig. 1). The ratio of carbohydrates, proteins, and lipids determine the suitability of microalgae for a particular type of energy carrier [4].

In recent times, algae has been treated as a vital source for renewable biodiesel worldwide [5]. Synthesis of triglycerides (TAGs) containing favorable fatty acid chain length in algae makes them an ideal substrate to produce biodiesel [6]. The reported physicochemical characteristics of algal biodiesel such as pH, density, and viscosity were in the range of 6–7, 0.85–0.89 g cm^{-3}, and 3.8–4.4 $mm^2 s^{-1}$, respectively which is similar to fossil derived diesel [3]. Biodiesel has several advantages such as a renewable energy source of hydrocarbons derived from solar energy and has a high amount of oxygen that leads to reduced emission of CO, and particulate matters. Biodiesel has shown to emit nearly 70% less carbon dioxide compared to petroleum diesel [7]. In addition, the handling and storage are safer, because of higher flashpoint, faster biodegradation, and greater lubricity. The consumption of biodiesel instead of fossil diesel reduces the CO_2 emission by 3.3 kg L^{-1} [1]. However, increased emission of NO_x and unsuitability for cold countries because of poor cold temperature properties are some of the disadvantages of biodiesel.

The main cost of biodiesel currently includes land crop cultivation, constituting a load as high as 90% of the total costs for biodiesel production. High microalgal productivity with high lipid content are difficult to achieve, because of poor light penetration in the culture [8] and trigger of lipid synthesis only in stress conditions. The lipid content above 40% was found to trigger generally under certain stress condition. For example, *Nanochloropsis sp*. attains 60% lipid content in dry cell weight under nitrogen starvation stage in a flat alveolar photobioreactor. However, this stress condition compromises with the biomass productivity.

Hydrogen production by biophotolysis is another area, where microalgae can be harnessed for energy production. Gaffron and

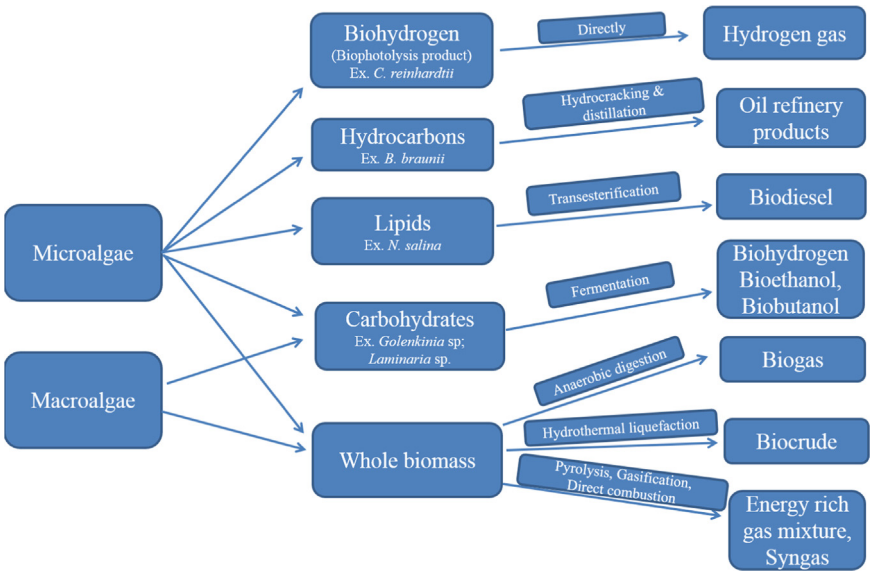

Fig. 1. Different routes of bioenergy generation from microalgae and macroalgae.

Rubin reported the use of *Scenedesmus obliquus* as a source for the hydrogen production under anaerobic conditions [9]. After a long dark period followed by illumination the microalgal cells acquire the ability to produce H_2 [10]. The hydrogenase enzyme catalyzes the hydrogen production, which gets suppressed during oxygenic photosynthesis due to oxygen evolution. Anaerobic conditions are necessary to activate hydrogenase enzyme. Now a days, most of the research is focused on creating the anaerobic environment and enhancing the catalytic activity of hydrogenase enzyme.

Microalgal biomass is rich in carbohydrates, protein and lipids, though the concentration varies depending upon the strain and on various physico-chemical parameters influencing their metabolic pathway during cultivation. Continuous supply of carbonaceous feedstock of biomass could be ensured by ease of cultivation and presence of a significant amount of these energy storage components. Some microbes under dark fermentation are considered as potent microorganisms for the biohydrogen production utilizing glucose and other starch based waste materials such as distillery effluents, rice spent water etc. In view of this, algal biomass as carbonaceous substrates can be fermented by microbes for the hydrogen or bioethanol production [11–13]. Bioethanol can be blended with diesel in different proportions to use in vehicles. Bioethanol is similar to biodiesel, however, having less energy density. Ethanol has an energy content of 29.7 MJ kg^{-1} [1]. Ethanol concentration greater than 8% is requisite in the fermentative medium as the energy required during distillation rises exponentially in the lower level of ethanol concentration [4].

Anaerobic digestion of microlagal biomass has been the subject of extensive research over the years [14,15]. It was started in fifties by Golueke et al., when they reported biogas productivity of 0.5 m^3 kg^{-1} [16]. Generation of methane rich biogas through anaerobic digestion can be considered for the conversion of algal residuals to generate additional energy source [17]. Microalgal biomass has a higher specific methane content (61–67%) compared to maize silage (54%) [18]. Microalgae do not contain lignin, which is the main component in many agricultural residues, and therefore, it has better prospects for efficient biodegadation for biogas production. Biogas can be directly used to produce heat or electricity through co-generation or it can be further purified to use as natural gas. The biogas production process has a high energy yield. It does not require drying step as anaerobic fermentation is carried out using wet fermentation. All microalgal biomass irrespective of lipid content can be used for anaerobic digestion to produce biogas. Co-digestion with other feedstocks is possible. In addition, microalgae can be further utilized to improve the biogas quality by sequestration of CO_2 from the biogas [19]. However, the biomethanation of microalgae depends on the microalgal chemical composition which again is varying with the cultivation conditions, seasonality, availability of nutrients etc. For example, *Arthrospira plantensis* cultivated under phosphorous limiting conditions accumulated a higher amount of carbohydrates which in turn led to increase bio-methane yields. The highest biomethane yield was 203 ± 10 mL CH_4 g $COD_{infl}{}^{-1}$ from biomass having 60% carbohydrates, while the lowest biomethane yield was 123 ± 910 mL CH_4 g $COD_{infl}{}^{-1}$, when the biomass contained only 20% carbohydrates [20].

Similar to microalgae, several research groups are assessing the suitability of macroalgal/seaweed biomass for biogas production since the 1970s [17]. Macroalgae synthesize very low quantities of oils, but are a good source of carbohydrate. Therefore, macroalgae can be utilized for the production of bioethanol and biogas by the process of fermentation and anaerobic digestion, respectively [21]. The US Marine Biomass Program of the 1970's and early 1980's [22] studied the anaerobic digestion of macroalgae and established the feasibility of converting *Macrocystis pyrifera* to methane. The biochemical methane potential of *M. pyrifera* was comparable to or exceeded that from all terrestrial biomass sources by over three-fold [23].

The present review provides information for different routes of biofuels production through biochemical and thermochemical conversion methods that can be applied in algal biorefinery. Pathways and mechanism of synthesis of different possible biofuels such as biodiesel, bioethanol, biohydrogen, biogas, and bio-oil from microalgae and macroalgae have been discussed in details. At the end, a brief discussion of techno-economic analysis is included to identify the cost effective routes of algal biomass utilization for biofuel production.

2. Biofuels production

2.1. Biodiesel production from microalgae

The total lipid (neutral and polar) content of algal biomass varies from 1% to 75%, depending upon microalgae strain and cultivation conditions, with values generally greater than 40% in nutrient stress condition [4] (Table 1). Total lipid content in microalgae is generally higher than the cyanobacteria [27]. Contrary to microalgae, macroalgae in general have very low lipid content (up to 4.5%w/w) which make them unsuitable for biodiesel production [28,29]. Biodiesel production can be summarized as a two stage process: lipid extraction followed by transesterification to produce biodiesel. Transesterification is a reaction between lipid/oil and a monohydric alcohol using homogenous or heterogeneous catalysts to produce mono alkyl esters (FAME) and glycerol as a byproduct [30,31]. Transesterification converts TAG to diglycerides followed by monoglycerides and then esters along with glycerol as a byproduct [32]. Transesterification of lipid reduces the lipid/oil viscosity near to the normal diesel oil. Generally methanol and lipid is mixed in 1–9 ratio, which produce biodiesel and glycerol in the ratio of 9–10:1, respectively [1]. Traditional two stage process of transesterification is costly and replaced by a single stage process. Direct or wet transesterification can significantly reduce the downstream processing by reducing the two steps of lipid extraction and transesterification into a single step [33].

Foam formation is a common bottleneck during the tranesterification process due to the presence of free fatty acids [1]. Cleaning of biodiesel is required to remove the foam and the organic acids. Further, microalgae have mostly polyunsaturated lipids having four or more than four double bonds [4]. Therefore, biodiesel obtained from microalgae is susceptible to oxidation in proportion to the extent of unsaturation [1]. The short life of biodiesel can be enhanced by partial hydrogenation.

Transesterification of 100 kg of lipid produces nearly 100 kg of biodiesel, which corresponds to 117.6 L of biodiesel assuming a density of biodiesel equal to 850 kg m^{-3} at 278–373 K [3]. This will require 200 kg of algal biomass to produce 117.6 L of biodiesel assuming algal biomass contains 50% (w/w) of lipid [34,35]. In order to have an economically competitive biodiesel production, algal biomass cost should be below approx. 0.5 \$ kg^{-1} algal biomass based on the current oil price. However, the current cost of algal biomass needs to decrease by at least 6–7 fold to make the process economically viable. The price of biodiesel extracted from microalgae seems to equate the cost of the diesel by the year 2050. Some other reports estimated an optimistic view of biodiesel cost in a range of US\$ 0.42–0.97 L^{-1}. Contrary to this, the cost calculated by Solazyme approximates US\$17 L^{-1} to produce biodiesel using heterotrophic cultivation methods [21]. The cost of algal biomass depends upon the type of algal species, growing system, climatic conditions, availability of labour etc.

Table 1
Some of the potential algae for high lipid production [24–26].

Microorganisms	Biomass productivity (g/L d)	Lipid content (%, w/w)	Lipid productivity (mg/L d)
Botryococcus braunii	0.02	25.0–75.0	5–15
Chlorella emersonii	0.036–0.041	25.0–63.0	10.3–50.0
Chlorella protothecoides	2.00–7.70	14.6–57.8	1214
Chlorella pyrenoidosa	2.90–3.64	2.0	58–72.8
Chlorella sorokiniana	0.23–1.47	19.0–22.0	43.7–323.4
Chlorella sp.	0.02–2.5	10.0–48.0	2–1200
Chlorococcum sp.	0.28	19.3	54
Dunaliella salina	0.22–0.34	6.0–25.0	13.2–85
Euglena gracilis	7.70	14.0–20.0	1078–1540
Nannochloropsis sp.	0.17–1.43	12.0–53.0	20.4–757.9
Phaeodactylum tricornutum	0.003–1.9	18.0–57.0	0.54–1083
Scenedesmus obliquus	0.004–0.74	11.0–55.0	0.44–407
Scenedesmus sp.	0.03–0.26	19.6–21.1	5.88–54.6
Spirulina platensis	0.06–4.3	4.0–16.6	2.4–713.8
Spirulina maxima	0.21–0.25	4.0–9.0	0.84–2.25
Tetraselmis sp.	0.30	12.6–14.7	43.4

2.1.1. Lipid distribution and importance in algal cells

Neutral lipids (4–50% of total dry biomass, depending upon algal strains) are distributed in the cytoplasm as free fatty acids, sterols, waxes, tri-, di-, monoglycerides, isoprenoids such as carotenoids, etc. Polar lipids such as glycolipids and phospholipids generally constitute cell membranes. Hydrophilic polar sugars, phosphate moieties and degree of saturation of fatty acyl chains determine the membrane fluidity [27]. Glycolipids are the esters of fatty acids and glycerol in which one of the hydroxyl groups is used to bind sugars like galactose [30]. Saturated and monounsaturated fatty acids are most common in algal samples with palmitic acid (C16:0), oleic acid (C18:1) as the major fatty acids (Table 2). However, algae are able to synthesize significant amount of very long chain-poly unsaturated fatty acids (VLC-PUFAs) which probably helps them to adapt to abrupt changes in the environment, temperature, pH, UV radiation [8].

TAGs are highly reduced form of carbon consisting of three fatty acids bound to a glycerol backbone which accumulate in the cytoplasm. Algae mainly synthesis glycerol based membrane lipids to support the growing needs of membranes under normal conditions. In stress conditions, the metabolic pathway shifts towards synthesis and accumulation of neutral lipids in the form of TAGs [8,41]. TAGs are synthesized in the algal cells when energy input exceeds the energy consumption of the cells. Energy is stored in TAGs that it can be readily utilized under favorable environmental conditions [42]. Free fatty acids (FFAs) present in the cell's cytoplasm cause cell lipotoxicity. TAGs serve as a sink for FFAs and thus save from cell lipotoxicity. In addition, TAGs save the cell from oxidation during stress condition by removing excess energy and electrons [33,41].

2.1.2. Different factors affecting TAGs synthesis

TAGs synthesis can be enhanced by various conditions causing stress to the microalgae such as temperature, pH, salinity, nutrient (ex. nitrogen, sulfur, phosphorous, zinc, iron, salt etc.) starvation and algal culture age [21]. These factors also influence the fatty acid composition. For example, a lower temperature encourages the synthesis of unsaturated fatty acids in order to maintain membrane fluidity for sustaining cellular processes [43]. Lower temperature significantly increased the yields of EPA and PUFAs in *P. tricornutum* [38]. Nitrogen starvation was found to divert the flow of fixed carbon towards the formation of lipid and sugar instead of protein [44]. More than fourfold increase in lipid synthesis was observed in *Chlorella vulgaris* and accumulation of significant amounts of C18:1 was favored during nitrogen deprivation, but the amount of PUFAs such as C16:2, C18:2, C18:3 decreased [45]. In case of *C. reinhardtii*, there was a significant increase in C18:1 and C16:0 content, at the cost of linoleic acid (C18:2) [46]. Low light intensity was found to support the formation of PUFAs, whereas the higher light intensity induced saturated and mono-unsaturated fatty acids synthesis [41]. An increase in PUFAs was observed as the cell transfers from logarithmic phase to stationary phase of the cell life cycle [47].

Higher amount of TAGs increases the yield and efficiency of biodiesel, though all forms of the lipids can be converted into biodiesel. The presence of saturated and unsaturated fatty acids in the TAGs determines the quality of biodiesel. Ignition quality, oxidative stability and cold storage properties are three important parameters determining the characteristics of biodiesel. Higher cetane number ensures quicker ignition and complete combustion whereas oxidative stability prevents biodiesel from hydrolytic degradation by water and helps in long term storage of biodiesel. Higher concentration of unsaturated to saturated fatty acid composition improves the quality of cold flow properties of the biodiesel and prevents it from freezing at lower temperature. However, this compromises with the oxidative stability and the cetane

Table 2
Fatty acid composition of some algal lipid.

Fatty acids	*N. salina* (%)	*P. tricornutum* (%)	*B. braunii* (%)	*S. obliquus* (%)	*C. pitschmannii* (%)	*C. vulgaris* (%)	*C. Mexicana* (%)
C12:0	5	0.0	0.7	11	10	5	34
C14:0	0.0	4.5	0.8	–	–	–	–
C15:0	0.5	0.0	0.5	–	–	–	–
C16:0	37.5	25.8	21	29	26	22	50
C16:1	23.3	37.5	2.0	–	–	–	–
C16:3	0.0	0.0	15.2	–	–	–	–
C17:0	0.4	0.0	0.1	–	–	–	–
C18:0	0.9	1.3	2.9	17	20	5	6
C18:1	11.9	0.0	3.2	20	13	53	0.0
C18:2	1.5	5.1	13.6	–	–	–	–
C18:3	0.0	2	33	23	23	8	0.0
C20:0	0.1	0.0	0.2	–	–	–	–
C20:4	2.2	1.6		–	–	–	–
C22:0	0.0	0.0	0.1	–	–	–	–
C20:5	15.3	13.1	0.0	–	–	–	–
C22:0	0.4	0.0	0.0	–	–	–	–
C24:0	0.0	0.0	0.2	–	–	–	–
Others	0.0	0.0	0.0	0.0	8	7	10
References	[36,37]	[36,38]	[37,39]	[40]	[40]	[40]	[40]

number [42]. In this regard, it was proposed to channel the metabolic pathways for enhancing the oleic acid and lowering the saturated and polyunsaturated fatty acids in lipid [48]. Lower acyl chain length (C8–C14) was shown to help in the transesterification and purification of FAME [42,48].

2.1.3. Mechanisms of lipid synthesis

TAGs can be synthesized in the cell using two pathways, one acyl CoA dependent pathway (*de novo* fatty acid biosynthesis pathway) and another is an acyl CoA independent pathway. The chloroplast of algal cell is the site for *de novo* synthesis of fatty acids. The *de novo* pathway starts with carboxylation reaction with formation of 3 carbon compound, malonyl CoA (Fig. 2). In this step, acetyl CoA irreversibly combines with CO_2 with the help of acetyl CoA carboxylase (ACCs) using ATP. Acetyl-CoA used in the reaction can be obtained from either plastidial or cytosolic glycolysis, or directly from dihydroxyacetone phosphate [30]. In green algae, acetyl CoA is formed in both cytosol and chloroplast [33]. Over expression of ACCs was proposed to push the substrate malonyl CoA for the synthesis of fatty acids, because this is the first assigned step in fatty acid biosynthesis pathway. However, this only increased lipid synthesis by 5% in seeds of higher plants [41]. Similarly, in the diatom *Cyclotella cryptic,* ACCs was overexpressed two to three fold with no increase in the oil content [49]. Further, malonyl-CoA: ACP transferase (MAT) helps in the transfer of malonyl moiety to acyl carrier protein (ACP) forming malonyl-ACP. Acetyl CoA combines with malonyl-ACP and undergoes condensation, reduction, dehydration and again reduction reaction by enzymes 3-ketoacyl-ACP synthase (KAS), reductase (KAR), dehydrase (HD) and enoyl-ACP reductase (ENR), respectively. The four-step of repeating cycles result in successive elongation of two carbon per cycle to the precursor acyl-ACP moiety chain [30]. Saturated 16 and 18 carbon chain fatty acid are form at the end of the cycle. The conversion of acetyl CoA to 16 carbon compound palmitic acid requires 7 cycles utilizing 7 ATP and 14 NADPH as shown in Eq. (1) [30].

$$8\ \text{acetyl CoA} + 7\ \text{ATP} + 14\ \text{NADPH} + 6\ H^{+} \rightarrow CH_3(CH_2)_{14}COOH + 14\ NADP^{+} + 7\ \text{ADP} + 8\ \text{CoA} + 6\ H_2O + 7\ P_i \quad (1)$$

Double bonds are introduced in saturated fatty acid chain by stearoyl ACP desaturase. The elongation reaction terminates by either of the following: the removal of acyl group from ACP by the action of acyl-ACP thioesterases (FATs) or direct transfer of the ACP to glycerol-3-phosphate (G3P) backbone by acyltransferases in Kennedy pathway. FATs hydrolyze the thioester bond of the acyl-ACP and release free fatty acids (FFAs) to the cytosol by acyl-CoA *syn*thetase [50]. Kennedy pathway involves stepwise addition of FFAs, adding to each hydroxyl group of glycerol beginning with G3P. Over expression of G3P resulted in a 40% increase in oil

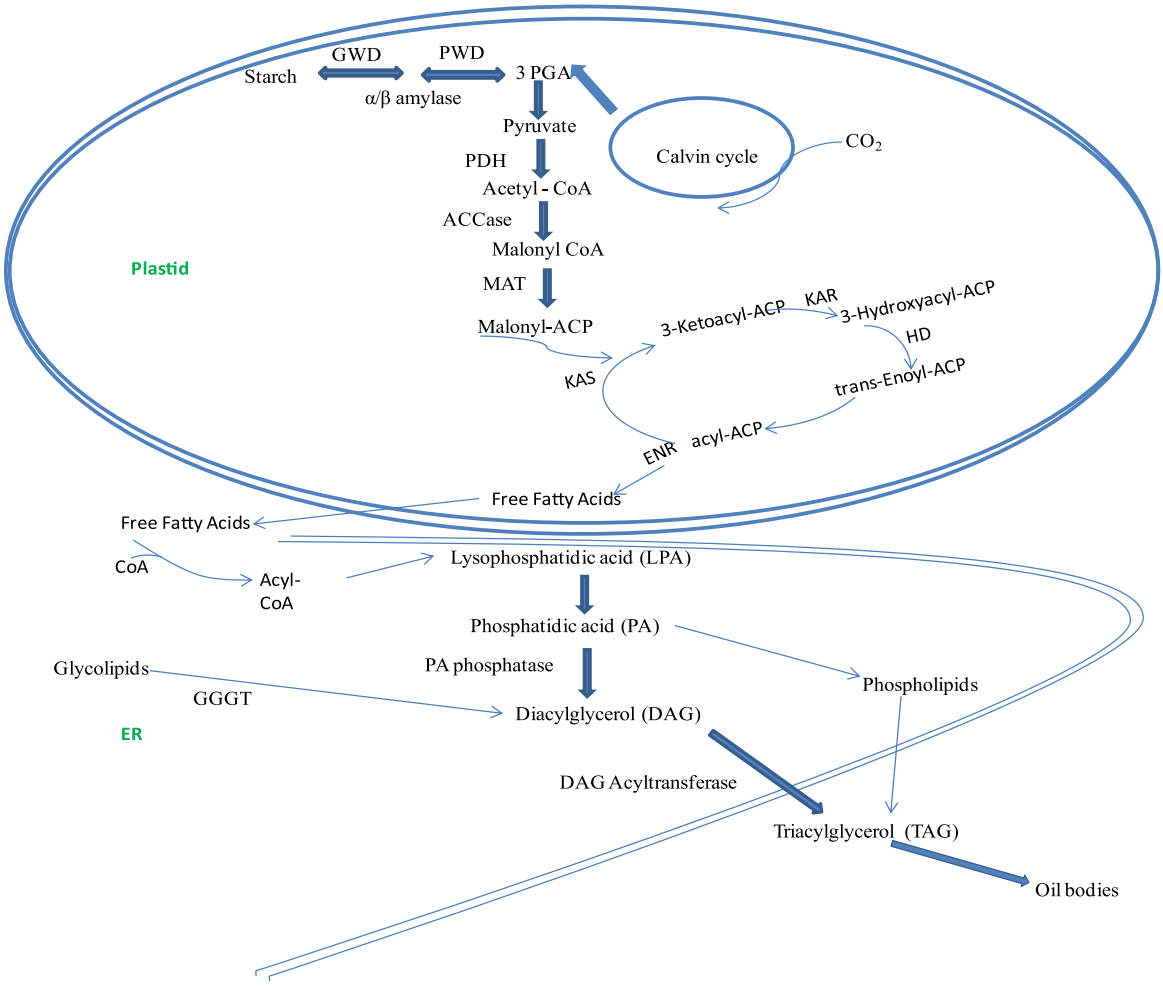

Fig. 2. Schematic diagram of metabolites and fatty acid synthesis pathway in green algae. Synthesis of free fatty acids take place in chloroplast whereas TAG may assemble in endoplasmic reticulum (ER). GWD: glucan-water dikinases; PWD: phosphoglucan water dikinases; 3 PGA: Phosphoglyceric acid; PDH: pyruvate dehydrogenase complex; ACCase: acetyl-CoA carboxylase; KAS: 3-ketoacyl-ACP synthase; GGGT: galactolipid:galactolipid galactosyltransferase; MAT: malonyl-CoA:ACP transacylase; KAR: 3-ketoacyl-ACP reductase; HD: 3-hydroxyacyl-ACP dehydratase; ENR: enoyl-ACP reductase; FAT: fatty acyl-ACP thioesterase (adapted and modified from Hu et al. [41] and Radakovits et al. [117]).

content of the algae [42]. Transfer of first FFA chain to position one of G3P is catalyzed by glycerol-3-phosphate acyltransferase (GPAT) to form lyso-phosphatidic acid (LPA). Second FFA transfers to LPA forming phosphatidic acid (PA) using lyso-phosphatidic acid acyltransferase (LPAAT). The dephosphorylation of PA forms DAG using enzyme phosphatidic acid phosphatase (PAP). Lastly TAG forms, when position three of DAG is occupied by another FFA with the help of diacylglycerol acyltransferase (DGAT) [41,50].

Several approaches of genetic engineering have been reported to enhance the lipid content and alter the fatty acid composition such as increasing the supply of reducing sugar, overexpression of thioesterase to decrease feedback inhibition due to increased acyl-ACP concentration, elimination of enzymes responsible for β-oxidation of fatty acid and optimizing fatty acid chain length by introducing the plant derived thioesterase [24]. It is important to identify the limiting factor controlling the lipid synthesis pathway as previously, several attempts of over expression of genes involved in fatty acid synthesis were not successful. For example, malic enzyme is an NADPH-generating enzyme, which has been found to play controlling role in lipid enhancement is fungi [24,51]. It indicates an adequate supply of reducing equivalents may be more important compared to supply of carbon for fatty acid synthesis [24]. Similar strategies need to be implemented in algal system also.

2.2. Bioethanol

Microalgae and macroalgae synthesize large amounts of carbohydrates that can be fermented to produce bioethanol or biohydrogen. Carbohydrates are the main components of algae and found in different combinations across different species. Besides lipids, algae store energy in the form of carbohydrates. The single carbohydrate unit (CH_2O) has a 1/6 of the glucose ($C_6H_{12}O_6$) energy content i.e. 467 kJ mol^{-1}. The energy content in the photosynthetic active radiation (PAR) region (400–700 nm) can be generalized to 217 KJ mol^{-1}, which is 43% of the incident light. Therefore, the quantum limit of the energy storage efficiency in the form of carbohydrate is 11.6% as 8 photons in the PAR region is required to produce one carbohydrate unit [4].

2.2.1. Bioethanol from microalgae

2.2.1.1. Fermentable route. Algal biomass need to be pretreated to extract fermentable sugars before its use as a substrate for microorganisms. The method applied for hydrolysis must be cost effective, easy to handle, energy efficient, and give maximum yield of fermentable sugars [52]. The pretreatment process can be broadly divided into physical, biological and chemical methods. Enzymes, concentrated and diluted acids are generally used for algal biomass hydrolysis. The purpose of using concentrated H_2SO_4 is to breakdown intra and inter chain hydrogen bonds of biomass followed by dilution of acids to release fermentable sugars. Diluted acid method reported by Zhou et al. was essentially a two stage process to ensure the release of structurally different sugars like hemicelluloses and cellulose. The addition of 2.5% $MgCl_2$ in 2% HCl for algal biomass hydrolysis resulted in recovery of more than 83% of total sugar consisting mostly xylose, glucose and arabinose [53]. This was a synergistic effect, because the effect was higher than the sum of the sugar released individually by each of the components, according to the author. Laurens et al. demonstrated an integrated technology based on acid-catalyzed algal biomass pretreatment at moderate temperatures and low pH for simultaneous extraction of soluble sugars and lipids, leaving behind a protein-enriched fraction [54]. In enzymatic hydrolysis, starch is converted into fermentable sugars by using two enzymes in sequence: alpha amylase and gluco-amylase [1]. Being a cellulosic based material, cellulase is often used in the hydrolysis of microalgal biomass. Cellulose-hydrolysing enzymes mainly consist of 1,4-β-D-glucan glucanohydrolases (*endo*glucanases); 1,4-β-D-glucan cellobiohydrolases and 1,4-β-D-glucan glucanohydrolases (*exo*glucanases); β-D-glucoside glucohydrolases (β-glucosidases). Temperature, acid concentration and algal loading were found to be the most important parameters in their order for the release of glucose yielding 64.2% (w/w) at pH 4.5 and 40 °C using 10 g L^{-1} of biomass [55].

Ethanol from microalgae can be produced by two processes: i) yeast fermentation of microalgal biomass and ii) direct production by genetically engineered microalgae (Rojan et al. [56]). Starch extracted from algae, can be fermented into ethanol through a process technology similar to other starch-based feedstocks [56]. Bush and Hall reported a process for the production of ethanol by harvesting starch-accumulating algal biomass selected from *Zygnemataceae, Cladophoraceae, Oedogoniales*, or a combination of different microalgal biomasses [57]. The harvested biomasses were subjected to decay through an anaerobic aqua environment. The digested biomass were fermented by the yeasts *Saccharomyces cerevisiae* and *S. uvarum*. The process described by Bush and Hall (2006) claimed to be superior to another patented technology [58] which express a unique source of fermentable sugars. Single celled and free floating microalgae are cultured by exposure to sunlight in the presence of nutrients such as nitrogen and phosphorous containing minerals. The main drawback of single cell free floating approach is the lack of industrial applicability due to the need to supply a large amount of carbon dioxide, thereby implying high energy consumption and operating cost.

The residual lipid extracted algal (LEA) biomass are gaining attention as a substrate for ethanol production. Harun et al. studied ethanol fermentation using *Chlorococum* sp. as a feedstock [59]. Lipids were extracted from the microalgal cells through supercritical extraction and the LEA was dried and used for further bioethanol production. Results showed a maximum ethanol concentration of 3.83 g L^{-1} obtained from 10 g L^{-1} of lipid-extracted microalgal debris (equivalent to ~76% of the maximum theoretical yield based on the starch content of microalgae).

2.2.1.2. Direct biosynthesis by genetic modification. Several attempts have been made to redirect 3-PGA (Phosphoglycerate) to ethanol by introducing ethanol producing genes [56]. Deng and Coleman (1999) genetically engineered a cyanobacterium, *Synechococcus* sp. to produce very small amounts of ethanol [60]. The cyanobacterium *Synechococcus* sp. strain PCC 7942 was transformed by introducing the coding sequences of pyruvate decarboxylase and alcohol dehydrogenase II from the bacterium *Zymomonas mobilis* through a shuttle vector. This ethanol concentration is much lower than the recently reported benchmark ethanol derived from lignocellulosic fermentation using *S. cerevisiae* [61]. However, the technology for the commercial production of microalgae-based bioethanol is yet under development and is being further investigated. Some private companies such as Algenol Biofuels Inc. have used the above described ability of microalgae to photosynthetically produce ethanol, and have launched a photosynthetic ethanol production process [62]. The company has developed a technology that employs genetically modified blue-green algae (cyanobacteria) to convert pyruvate made from carbon dioxide into ethanol using horizontal plastic film photobioreactors system. However, they have optimized the process by shifting from a horizontal reactor system to a vertical one, which pushed its annual production per acre to approximately 8000 gallons for the company´s four most important fuels –ethanol, biogasoline, biojet fuel and biodiesel –at a cost of approximately $1.27 per gallon.

2.2.2. Bioethanol from macroalgae

2.2.2.1. Fermentative route. Macroalgae such as kelp-species are more promising for ethanol production compared to microalgae as the former has high quantity of fermentable sugars. These sugars can be utilized for the synthesis of biogas, bioethanol and butanol. Macroalgae, in contrary to plants lack lignin, crosslinking molecules in their cellulose structures, which is beneficial in pretreatment [63]. In addition, depending on type of species, environmental conditions, maturity, gender and season, the polysaccharide/carbon content of macroalgae can account for up to 60% of dry matter [29,64]. Further, the ethanol production process is well established with yeast as a fermentative microorganism and glucose as a carbon source [65].

The macroalgal sugars/polysaccharides are very different to the land plant polysaccharides, which are mainly dominated by C6 plant polysaccharides such as starch and cellulose. Depending on species and season, macroalgae composition involves some unique polysaccharides such as ulvan, fucoidan, alginate, laminaran, floridean starch; mannitol (a sugar alcohol), and monosaccharides such as rhamnose, fucose, uronic acids. In some marine algae, sugar is trapped in the form of agar, a polymer of galactose and galactopyranose. Therefore, saccharification of biomass is necessary to release galactose and glucose from the agar and cellulose, respectively [63].

Horn et al. investigated the fermentation of liquid extracts from the brown macroalga *Laminaria hyperborea* to produce ethanol by conversion of mannitol and laminaran fractions [66]. Both mannitol and laminaran are by-products of alginate extraction. *Pichia angophorae* was used as fermentative microorganism and was able to ferment both laminaran and mannitol simultaneously, but with different uptake rates. The final yield of fermentation attained corresponds to 0.43 g ethanol g^{-1} substrate in batch culture. Industrial implementation of this process implies not only process optimization, but also a successful utilization of all carbohydrate fractions (alginate, laminarin and mannitol) to be converted to ethanol at high yields and productivities. This may be achieved in a two-step fermentation process in which both laminarin and mannitol would be utilized in a first-step by *P. angophorae*; then in a second step the alginate fraction may be converted into ethanol by a single or different microorganism(s). Ethanol production may also be performed by a single microorganism that can utilize all substrates (alginate, laminarin and mannitol) simultaneously in a single process-step. This necessarily implies that the single microorganism should be able to produce the enzymes to breakdown both alginate and laminarin into their monomeric units [66].

Laminarin can be hydrolyzed into glucose by enzymes such as laminarinase [67] and/or cellulose [68] while alginate can be partially hydrolyzed into uronic acids by alginate lyases [69]. Conversion of one molecule of glucose to two molecules of pyruvate has a net yield of two molecules of NADH in the net reaction for glucose glycolysis (Eq. (2)).

$$Glucose + 2NAD^{+} \rightarrow 2Pyruvate + 2NADH \rightarrow 2Ethanol + 2CO_2 + 2NAD^{+} \quad (2)$$

In ethanol fermentation, the pyruvate is converted first to acetaldehyde and carbon dioxide, then into ethanol. Pyruvate to NADH ratio of 1:1 is required for ethanol production (Eq. (2)).

However, mannitol is not readily fermentable to ethanol due to the fact that one extra reducing equivalent (NADH) is produced changing the ratio pyruvate to NADH and resulting in an imbalance redox conditions (Eq. (3)).

$$Mannitol + 3NAD^{+} \rightarrow 2Pyruvate + 3NADH \quad (3)$$

The regeneration of all the NAD^{+} requires either oxygen or transhydrogenase [69]. Hence, many microorganisms are not able to carry out this reaction in anaerobic environment.

In contrast to mannitol glycolysis, (Eq. (3)), the metabolism of one molecule of uronic acid to two molecules of pyruvate has a net yield of one molecule of NADH (Eq. (4)). This results in the deficit of one reducing equivalent (NADH) to allow the pyruvate reduction to ethanol to proceed further.

$$Uronic\ acid + NAD^{+} \rightarrow 2Pyruvate + NADH \quad (4)$$

Therefore, in the catabolic pathway alginate provides both an additional source of sugars (uronic acids) and a counter balance to the excess-reducing equivalents produced by mannitol catabolism, enabling the ethanol fermentation of all three substrates (glucose, mannitol and uronic acids) in macroalgae simultaneously.

A project called “Ocean sunrise” presents a concept of an off shore farm using the water face of less than 1% of an exclusive economic zone of 4.48 million km^2 to cultivate *Sargassum fulvellum* for bioethanol production in Japan [70]. This macroalgal biomass would be processed using highly efficient fermentation technologies aiming to convert alginate, mannitol and fibers contained in macroalgae into ethanol. The fermentation technology for ethanol conversion of alginates, relies on finding a fermentative microorganism that can survive the salty environment.

2.2.2.2. Genetic modification. Recently, Wargacki et al. have developed a microbial platform for direct bioethanol production from brown algae [61]. The microbial platform consists of an engineered *Escherichia coli* strain (which was named BAL1611) capable of metabolizing alginate, mannitol and laminarin simultaneously. Wargacki et al. [61] selected this microorganism due to its usual aptitude to metabolize mannitol and glucose. This platform enables bioethanol production directly from macroalgae via a Consolidated BioProcessing (CBP) [71]. In CBP a microbial community produces all the required enzymes and ethanol in a single reactor. The microbial platform was tested with *Saccharina japonica* as feedstock achieving an ethanol concentration titer of 4.7% v/v (37 g L^{-1}), which is comparable with the minimal required ethanol concentration (4.0% w/v) for an economical distillation process [72]. This ethanol titer corresponds to a bioconversion value of 0.28 g ethanol/g dry macroalgae and a yield of 0.41 g ethanol/g total sugars (alginate, mannitol and glucan) (equivalent to ~80% of the maximum theoretical yield from the sugar composition in macroalgae).

2.3. Biohydrogen production

2.3.1. Fermentative route

Similar to bioethanol production, fermentable sugars extracted from microalgae and macroalgae can be used as a substrate for biohydrogen production by dark fermentation using *meso*philic and thermophilic microbes. The carbohydrate content may be increased by applying stress conditions during algal cultivation. Simple technology for conversion of biomass to hydrogen makes the use of algal biomass economically viable [7]. Biohydrogen was produced using algal biomass from the range of 6–14 g L^{-1} [11]. Pretreatment of algal biomass is a prerequisite to release fermentable sugars. Studies on pretreatment methods emphasized that HCl–heat method was the most suitable for maximization of carbohydrate extraction [55]. The differences of structure between cellulose and hemicelluloses were eliminated by the use of two stage pretreatment method [51]. The morphological characteristics of the algal cells also changed due to pretreatment. The intact, separated and spherical cells of *Chlorella sorokiniana* turned brown due to HCl-heat pretreatment. The cells ruptured and clumped together released the internally stored sugars. The average COD observed was about 1.83 g COD per g algal biomass, which stood

proportional to algal biomass concentration. The highest cumulative hydrogen production was 0.1 mol L^{-1} corresponding to yield of 5.78 mol H_2 (kg COD reduced)$^{-1}$ at initial algal biomass of 10 g L^{-1} [11].

2.3.2. Biophotolysis route

2.3.2.1. Microalgae. Microalgae can be utilized to produce hydrogen by biophotolysis of water. Hydrogen formation is not limited by the slow CO_2 fixation rates and increases proportionally with faster light harvesting complexes [4]. Water molecules split into protons and electrons at PSII during oxygenic photosynthesis. It is desired to reduce the PSII activity to such an extent so that respiration by algal cells producing CO_2 exceed the oxygen evolution activity by microalgae. This is necessary in order to create anaerobic conditions in the algal culture as the hydrogenase enzyme is oxygen sensitive. The residual amount of protons produced at PSII is further utilized for hydrogen production. Sulfur deprivation reduces the PSII activity to a great extent and is considered as a suitable approach for a sustainable hydrogen production. In this condition, simultaneous supply of protons and electrons is possible in anaerobic condition [73]. Protons are stored in the lumen of the thylakoid membrane, whereas electrons travel through several electron carriers, and PSI to reach to ferredoxin [74]. The reduced ferredoxin further transfers electron to *ferredoxin* $NADP^+$ reductase (FNR) for the generation of reductants, which is further utilized in the Calvin cycle for CO_2 fixation. However, in anaerobic condition, reduced ferredoxin channels electrons towards hydrogenase enzyme instead of FNR. Protons come out into the stroma during ATP synthesis by ATP synthase, which is further catalysed to produce hydrogen by reduced hydrogenase.

Hydrogenase enzymes need to compete with many other metabolic processes for acquiring electrons. In addition, all the hydrogenase do not function equally. Therefore, understanding the interaction of the hydrogenase enzyme with ferredoxin and other metabolic processes is necessary in order to genetically modify these interactions for the enhancement of biohydrogen production [33]. Dasgupta et al. reported simultaneous enhancement of lipid content and biodiesel quality in the sulfur deprived biomass of *Scenedesmus* sp. after the end of the H_2 production by biophotolysis [75].

2.3.2.2. Blue green algae (Cyanobacteria). Both unicellular non-nitrogen fixing and filamentous nitrogen fixing cyanobacteria have potential to evolve hydrogen similar to green algae. Bidirectional hydrogenase (NiFe) and nitrogenase present in the vegetative cells and heterocysts, respectively, catalyse the hydrogen production reaction. These enzymes are also sensitive to oxygen similar to unidirectional hydrogenase (FeFe hydrogenase) of green algae. NiFe hydrogenase causes hydrogen evolution or hydrogen consumption depending upon the partial pressure of hydrogen in the atmosphere. Nitrogenase enzyme is originally meant for nitrogen reduction and hydrogen production is only by product of the reaction. Hydrogen production using nitrogenase is energy intensive as it requires large amounts of ATP [74]. The advantage of heterocysts based hydrogen production is in the spatial separation of oxygen and hydrogen producing compartment. Lack of PSII causes anoxygenic photosynthesis resulting into the anaerobic environment in heterocysts. Vegetative cells supply the carbohydrates requirement of heterocysts in exchange of fixed nitrogen transported from heterocysts.

2.4. Hydrocarbons from microalgae

Triterpenes are long chain hydrocarbons, different from lipid synthesized by common microalgae. Triterpenes have the formula of $C_nH_{2\ n-10}$ where n is greater than 23. It can not be transesterified to produce biodiesel due to lack of free oxygen atom. The feedstock of triterpenes can undergo hydrocracking and distillation to produce different higher quality biofuels such as gasoline, kerosene, and diesel.

Botryococcus braunii is known to produce large amount of unusual long carbon chain triterpenes hydrocarbons (up to 86% of dry cell weight) along with lipid synthesis [21,76,77]. The interesting thing with this microorganism is in secretion of hydrocarbons outside the cell into the liquid culture, thus minimizing the cost of lipid extraction. In addition, hydrocarbon synthesis takes place during the growth without the need of nitrogen starvation. This microalga grows in large size colonies and hence easier for harvesting. However, this microalga has a slow growth rate and low biomass productivity. The ability to synthesize different length of hydrocarbon by *B. braunii* depends upon the different races such as A (C_{23}–C_{33}), B (C_{30}–C_{37}), and L (C_{40}–C_{78}) [77]. Among different races, race B accumulates triterpenes, predominately botryococcene, squalene, and their methylated derivatives and therefore considered as a promising microalga for renewable petrochemicals and biofuels [76]. In one report at optimum condition, *B. braunii* has a biomass concentration of 0.65 g L^{-1} with a hydrocarbon content of 50.6% (w/w) after four weeks of growth [77]. Khatri et al. (2014) reported maximum botryococcene (C_{30}) concentration of 4500 mg L^{-1}, equivalent to 225 mg g^{-1} with its productivity of 4 mg g^{-1} h^{-1} in a continuous culture [78]. Genetic approaches are being carried out to install the triterpene hydrocarbon biosynthesis pathway into other robust and flexible microorganisms such as *Rhodobacter capsulatus*. In the continuous culture, maximum concentration and productivity of botryococcene (C_{30}) were 30 mg g^{-1} and 0.5 mg g^{-1} h^{-1} respectively using genetically engineered *R. capsulatus* [79].

2.5. Biogas from algal biomass

The biogas is produced under anaerobic condition by methanogenic bacteria from the wide number of feedstocks having high content of organic compounds. Biogas mostly contain methane (55–75%), and CO_2 (20–40%) along with small quantities of H_2, N_2, O_2, H_2S, and water vapor [4]. A higher quantity of methane, and lower quantity of toxic gases indicate good quality of biogas. Otherwise, purification of gas is required before its use in large scale production process. The biogas production process is slow and takes weeks to complete, but it is considered as a robust process. Algal biomass being rich in organic compounds is considered as a suitable feedstock for the biogas production. Algal biomass obtained from algal blooms of lakes, ponds, oceans can be utilized for biogas production that will help in bioremediation as well as in energy generation [33]. However, the algal biomass contain significant amounts of sulfur, which produces corrosive H_2S gas. The leftover of the biogas production process contains liquid fraction containing salts, nitrogen and fiber fraction containing indigestible biomass [4]. The liquid fraction can be utilized as a fertilizer, but the fiber fraction is a challenging task as it is not suitable for combustion due to high sulfur and nitrogen content [4]. Some researchers utilized the residual algal residues obtained during energy extraction in AD process, in order to make the overall process economically and energetically favorable. For example, Prajapati et al. reported utilization of diluted liquid digestate (30% concentration) of AD process for algae cultivation and thus making a closed loop biogeneration process [80]. Tommaso et al. conducted study on the use of aqueous products from hydrothermal liquefaction for methane production in AD. The highest cumulative methane production was observed at 320 °C with moderate lag phase [81].

From the energy point of view, energy content in biogas varies from 16 to 30 MJ m^{-3} depending upon its quality. The reported

biogas production from algal biomass was in the range of 0.15 – 0.65 m^3 kg^{-1}, which is equivalent to 2.40–19.50 MJ kg^{-1} of algal biomass. The use of algal biomass as a feedstock for biogas production was considered as a most suitable technology for the energy recovery with an average net energy yield of 10 GJ ton^{-1} algal biomass after purification [4].

2.5.1. Biogas from microalgae

Similar to other carbon rich feedstocks, microalgae can be utilized for biogas production. The microalgal biomass can be used along with other carbonaceous feedstocks. The process involves high energy yields, direct use of microalgae biomass without drying, and all types of microalgae can be utilized in the anaerobic digesters [82]. A detail literature survey of the potential of microalgae and macroalgae has been shown in Table 3. Some of the bottlenecks of biogas production from microalgal species are the energy expenditure for heating the digesters and high investment costs including land cost and infrastructure. Biogas production from microalgae was found more energy intensive compared to biodiesel production from microalgae [33,86].

2.5.1.1. Cell wall strength of microalgal species. Different species have different extent of cell degradation and a low amount of indigestible residues. This is because the efficiency of biogas production is species-dependent [18,33]. The microalgal cell wall is composed mainly of carbohydrates (30–75%) and proteins (1–37%). Algal biomass consisting of proteins, lipids, and carbohydrates are the substrates for biogas production. However, the kinetics of the process were hindered due to accessibility of substrates for biodegradability. Therefore, cell wall degradation to release intracellular components is the main rate limiting step for the biogas production process. The study of Mussgnug et al. found a correlation of biogas formation with cell wall digestibility. *C. reinhardtii, D. salina, A. platensis* and *E. gracilis* were found preferred species over *C. kessleri* and *S. obliquus*, mainly because of differences in the degree of degradation and lack of undigestable residue [18]. It was recommended to prefer cell wall laking algal species or having a protein based cell wall in the anaerobic digestion process. This will decrease the energy intensive pretreatment process and enhance the biogas formation [18]. Contrary to this, Zhong et al. found acetate and propionate degradation as the rate limiting step rather than hydrolysis [84].

Common pretreatments methods such as chemical (using acid, base), mechanical (using instruments such as autoclave, homogenizers, microwaves, sonication etc) and enzymatic methods (α-amilase, amylo-glucosidase, cellulose etc) can be applied prior to anaerobic digestion [82]. The enzymatic pretreatment methods was observed to be more suitable for CH_4 conversions than the mechanical size reduced samples [87]. Mechanical, chemical, and enzymatic methods enhance specifically the availability of total organic compounds, availability of organic compounds generally resistant to anaerobic hydrolysis, enhancing the cell wall hydrolysis, respectively. However, important things to consider during pretreatment process are energy consumption and increase in the biodegradability of cell wall rather than the solubility of organic matter [82].

Table 3
Macroalgal pretreatments overview for biogas production.

Macroalgae	Pretreatment	Anaerobic digestion conditions	mL CH_4 g VS^{-1}	Source
U. lactuca	Washed, macerated	Batch (lab), 55 °C, 34 d	255 ± 48	[95]
	Washed chopped		152 ± 19	
	Unwashed, macerated	Batch (lab), 55 °C, 42 d	271	[94]
	Washed, macerated		200	
	Washed, 130 °C/20 min		187	
	Dried, ground		176	
	Washed, roughly chopped		171	
	Washed, 110 °C/20 min		157	
	Unwashed, roughly chopped	Batch (lab), 37 °C, 58 d	162	
	Washed, macerated and dried, 80 °C	Batch (lab), 37 °C, 30 d	250.2	
	Dried		226	[96]
	Washed and wilted		221.1	
	Fresh		205	
	Fresh		183.2	
	Wilted and unwashed		165	
	Macerated		190.1	[97]
Ulva sp.	Ground	CSTR (lab), 35 °C, 15 d	203	[98]
	Ground	Batch (lab), 35 °C, 64 d	177	
	Non ground	Batch (lab), 35 °C, 42 d	145	
	Non-washed	Batch (lab), 35 °C, 23 d	110	
	Washed	Batch (lab), 35 °C, 44 d	94	
	Screw pressed (hydrolysate juice)	Continuous fixed bed reactor (lab), 35 °C, 10 d	290**	[99]
S. latissima	Chopped	Batch (lab), 36.5 °C, 30 days	230	[92]
		Semi continuous (lab), 35 °C, 24 d	230	
	Milled	Semi continuous (lab), 35 °C, 34–40 d	220–270	[100]
		Batch (lab), 35 °C, 32–35 days	25–200	
	Washed chopped	Batch (lab), 55 °C, 34 d	340 ± 48	[95]
	Washed, macerated		333 ± 64	
	Co-digestion with wheat straw	Batch (lab), 37 °C, 119 d	270	[93]
	Ground, steam explosion, 130 °C/10 min		268	
	Ground, steam explosion, 160 °C/10 min		260	
	Untreated		223	
	Macerated	Batch (lab), 37 °C, 30 d	341.7	[97]
L. digitata	Macerated	Batch (lab), 37 °C, 30 d	218	[97]
	Dried and milled	Batch (lab), 35 °C, 36 d	196–254	[101]

2.5.1.2. Carbon to nitrogen (C/N) ratio and ammonium inhibition. Anaerobic digestion of the nitrogen residue generates a high amount of ammonium ions (NH_4^+), which has an inhibitory effect on anaerobic microbial community, especially methanogenic bacteria [88,89]. Ammonium ions deprotonate into ammonia (NH_3) at high pH as shown in equation [84]. The feedstock loading into anaerobic digestor also influences the degree of inhibition by ammonia. It has been found that solid concentration greater than 2% starts posing inhibitory effect due to ammonia. The low carbon to nitrogen ratio (C/N ratio) inhibits the methane yield. Algal biomass has a low C/N ratio. Lipid extracted algal feedstock has further decreased amount of C/N ratio, undesirable for anaerobic digestion. The suitable C/N ratio in the feedstock was found to be 25–30:1 due to high requirement of carbon for bacterial metabolism [1,84].

$$NH_{3(aq)} + H_2O \leftrightarrow NH_4^+ + OH \ (pK_b = 4 \cdot 733 \text{ at } 35\ ^\circ C) \quad (5)$$

Co-digestion of algae and/or LEA biomass with carbon rich wastes having a high C/N ratio was successfully adopted to enhance the methane yield by reducing ammonia levels below their inhibitory levels [1,14,19,90]. Zhong et al. reported an enhanced methane production by anaerobic co-digestion of algae with corn straw in continuous feed digesters [84]. In a continuous feed digesters, methane yield and methane productivity were 234 mL CH_4 g VS^{-1} and 1404 mL CH_4 L^{-1} d^{-1} respectively with solid removal of 63% at OLR of 6.00 g VS L^{-1} d^{-1}. The addition of co-substrate can also enhance the enzymes synthesis and thus increasing the hydrolysis and degradability of feedstock [14]. Recently, Ras et al. investigated the feasibility of coupling algal production of *Chlorella vulgaris* to an anaerobic digestion unit [15]. Reported methane yield was 240 mL g VS^{-1} achieving 51% COD removal. The cost of anaerobic biogas from micro algae is relatively low and had high energy output.

2.5.1.3. Salt concentration. Marine microalgae poses problem in anaerobic digestion as it is associated with high salt concentration. Methanogenic bacteria require sodium at low concentration. This is probably because of participation of sodium ions in ATP formation and NADH oxidation. Sodium concentration in the range of 100–350 mg L^{-1} promotes the growth of *meso*philic bacterial growth. However, a high concentration of sodium has a severe inhibitory effect and causes dehydration in bacteria due to osmotic pressure [82]. This eventually reduces the growth rate of *meso*-philic methanogenic bacteria.

2.5.1.4. Microalgal cultivation conditions and nutritional composition. The composition of algal biomass / feedstock determines the methane yield. Being an energy rich component, higher lipid content in feedstock is more desirable. However, too much proportion of lipid in the the feedstock can reduce the pH in the digester leading to decreased lipid degradation due to increased long chain fatty acids and volatile fatty acid inhibition [83]. A balance in alkalinity is important to maintain lipolytic activity to enhance lipid conversion into methane. In comparison with carbohydrates, protein was found to play a more significant factor in the AD [83]. Increasing the protein content assists in favorable alkalinity levels for the lipid degradation stability. However, a higher proportion of protein in feedstock releases more ammonia in the AD, decreasing the methanogenic activity and methane production [83]. Based on the substrate composition, the theoretical methane yield can be obtained using Eq. (6) [83,91].

$$C_aH_bN_d + \left(\frac{4a - b - 2c + 3d}{4}\right)H_2O \rightarrow \left(\frac{4a + b - 2c - 3d}{8}\right)CH_4 + \left(\frac{4a - b + 2c + 3d}{4}\right)CO_2 + d\,NH_3 \quad (6)$$

Microalgae having low intracellular nitrogen or sulfur content can enhance the suitability of algal biomass in biogas production process. For example, microalgae harvested at stationary phase, microalgae grown in nitrogen/sulfur limiting or depleted conditions can improve the quality of biomass for biogas production. Anaerobic digestion of sulfur deprived *C. reinhardtii* biomass after H_2 production was found to enhance the biogas formation up to 123%. This was due to significantly increased storage compounds such as starch and lipids at the end of H_2 production. *C. reinhardtii* was found a promising microalga for biohydrogen production by biophotolysis as well as biogas production (587 mL $\pm$ 8.8 g VS^{-1}) in anaerobic digestion. In addition, H_2S generation during the anaerobic digestion was found to reduce when sulfur deprived biomass was used as a feedstock [18].

2.5.2. Biogas from macroalgae

Biomass of macroalgae species such as *Macrocystis pyrifera* (Bird et al., 1982), *Ascophyllum nodosum* [92], *Saccharina latissima* (former *Laminaria saccharina*) [92,93], *Laminaria hyperborean* [92], *M. pyrifera* [23], *Ulva lactuca* [94] were investigated with respect to their biomethane potential. Some of these studies have also focused on determining the optimal pretreatment conditions for improving the solubilization of macroalgal biomass thereby enhancing its biodegradability.

Macroalgae do not have lignin and low level of cellulose, which make them superior feedstocks for anaerobic digestion compared to other terrestrial feedstocks. For example, the biochemical methane potential of *M. pyrifera* was comparable to or exceeded that from all terrestrial biomass sources by over three-fold [23]. Macroalgae have higher content of total solids (TS) (8.3–22%) as compared to microalgae (0.1–1%) [95]. Macroalgae cultivation does not involve energy intensive harvesting processes. Brown seaweeds have low content of protein and high content of carbohydrates (high C/N ratio) in their biomass and therefore more suitable for anaerobic digestion compared to green algae [95].

Initially, the research was focused on the effect of several variables on the anaerobic digestion process such as biomass composition, separation of juice and non-juice fractions, temperature, inoculum, nutrients, freshwater versus seawater dilution, and non-dilution. Later the focus moved towards advanced digester designs, process optimization and kinetics [23]. In general, these studies concluded that this particular macroalgae is the suitable feedstock for the anaerobic digestion process as demonstrated by high conversion efficiencies, rapid conversion rates and good process stability [23].

Depending on macroalgal composition and type of pretreatment, reported methane yields were typically between 94-340 $\pm$ 48 mL CH_4 g VS^{-1} (Table 4). One specific pretreatment can effectively work for a specific macroalgae, but it might not have the same results for another macroalgal species. For instance, mechanical and thermal pretreatments have been shown to solubilize the macroalgal biomass by breaking down the available carbohydrates into simple sugars, and this resulted in a significant increase (approximately 20%) in methane yield [93]. Nevertheless, when thermal pretreatment was applied to *U. lactuca*, this seemed to have the opposite effect, which decreased the methane yields [94] as shown in Table 4. *Saccharina latissimi* was found one of the best promising macroalgae for methane production based on the

Table 4
The methane production potential of some of the microalgae in anaerobic digestion.

Algal feedstock	Co-digested feedstock	OLR (g VS L^{-1} d^{-1})	Specific methane yield (L CH_4 g^{-1} VS d^{-1})	Volumetric reactor productivity (L CH_4 L^{-1} d^{-1})	Methane content (%)	Mode of operation	Solid removal rate (%)	References
Spirulina maxima	–	–	0.25–0.34 g^{-1} VS	–	46–76	Fed-batch	–	[19,85]
Tretraselmis sp.	–	–	0.31 g^{-1} VS	–	72–74	Continuous	–	[19,85]
Dunaliella sp.	–	–	0.44–0.45 g^{-1} VS	–	–	Batch	–	[19,85]
Chlorella vulgaris	–	–	0.31–0.35 g^{-1} VS	–	68–75	Batch	–	[19,85]
LEA of *Nannochloropsis salina*	–	–	0.14 g^{-1} VS	–	–	Batch (19 d)	–	[35]
Nannochloropsis salina	–	–	0.43 g^{-1} VS	–	–	Batch (19 d)	–	[35]
Chroococcus sp.	–(9.26)[a]	–	0.32 g^{-1} VS	–	–	Batch (45 d)	70.0 –89.3	[80]
LEA of *Nannochloropsis salina* (50%)	Fat, oil, and grease waste	3	0.54	1.62	69	Semi-continuous	> 60 (lipid)	[83]
Algal biomass	–(6:1)[a]	6	0.155	0.96	68.76	Continuous	42.24	[84]
Algal biomass (65%)	corn straw (20:1)[a]	6	0.234	1.4	62.35	Continuous	63.17	[84]

[a] (C:N) ratio of final feedstock.

specific methane yield [97]. However, *U. lactuca* cultivation can be more profitable as it forms natural blooms near the seashore. The cleaning of macroalgae can generate additional revenue [97]. Macroalgae cultivation for methane production can have energy productivity up to 365 GJ ha^{-1} yr^{-1} [97].

2.6. Thermochemical conversions

2.6.1. Pyrolysis and gasification

Thermochemical conversion methods such as pyrolysis, gasification, hydrothermal liquefaction and direct combustion can be used in all the algal feedstocks including LEA for the production of liquid and gaseous biofuels [21]. Pyrolysis and gasification require dried algal biomass and operates under normal atmospheric pressure and high temperature. Pyrolysis takes place at reducing environment producing primarily CH_4 and H_2 [102]. Algal biomass can be gasified into a gaseous mixture consisting of primarily of hydrogen and carbon monoxide in the presence of steam and a controlled amount of oxygen [103]. Syngas is a mixture consisting of primarily H_2 (30–40%), CO (20–30%), CH_4 (10–15%) and a little amount of C_2H_4 (1%), N_2, CO and water vapor. Syngas has a calorific value of 4–6 MJ L^{-1} [28].

2.6.2. Hydrothermal liquefaction (HTL)

Hydrothermal liquefaction (HTL) takes place at medium temperature (200–400 °C), and medium pressure (5–40 MPa) in aqueous environment with or without catalysts [4,28]. HTL process can be considered as a pressurized aqueous pyrolysis, but having a lower oxygen and moisture content in biocrude [28]. Most of the organic materials such as (proteins, lipids and carbohydrates) can be used for the HTL process except lignin and cellulose. HTL process produces biocrude looking similar to crude oil and comprises of solid, asphaltene, non-asphaltene. The direct handling of wet biomass into the reactor reduces the harvesting cost and makes the process attractive. A biocrude is composed of 71–73% carbon, 7–8% hydrogen, 10–11% oxygen, 6–7% nitrogen and 0–1% sulfur [104]. Compared to heating values of raw algal biomass (22–24 MJ kg^{-1}), a biocrude has significantly higher values, which generally varies between 33–38 MJ kg^{-1}. The study of Sapphire Energy indicated a lower amount of GHG emissions in the HTL process than petroleum fuels and corn ethanol [21]. Biocrude is upgraded into refined fuel products CH_4 using catalytic hydrothermal gasification. Contrary to HTL of cellulosic substrate, use of catalysts is not required. Higher energy content of the products than the reactants is due to polymerization into a non aqueous phase liquid energized by temperature and pressure [4]. Biocrude can be catalytically upgraded to produce fuels such as gasoline, jet fuel by removing oxygen, nitrogen, and double bonds. However, HTL process has several disadvantages. HTL process has a significantly lower energy return on investment than petroleum fuels [21]. The requirement of high temperature and pressure make the process less energetically and economically feasible. Nitrogen content in the biocrude generally varies between 5–8%, which has to be removed by refining because nitrogen causes emission of toxic NO_x [104].

A high carbon content in algal feedstock produces a higher biocrude yield [104]. Being rich in the lipid, the performance of microalgae in HTL process is better as compared to macroalgae [104]. A detail literature survey of the bio-oil yield by the HTL treatment of microalgae and macroalgae has been shown in Table 5. Hydrothermal liquefaction of *Dunaliella tertiolecta* biomass at 300 °C and 10 MPa having a moisture content of 78.4% resulted into 37% oil yield having a calorific value comparable to that of fuel oil [4,106]. The fast rate of heating and cooling the reactor was observed to have a better yield due to better decomposition of proteins and carbohydrates along with a favored formation of liquid hydrocarbons rather than solid or gaseous compounds [104]. Several researchers investigated the effect of different catalysts such as Na_2CO_3 [106,108,112] KOH [111], Pd/C, Pt/C, Ru/C, Ni/SiO_2-Al_2O_3, $CoMo/\gamma$-Al_2O_3(sulfided), and zeolite [108] to improve the biocrude yield. However, the yield was not significant towards the presence or identity of catalyst, especially under high pressure of hydrogen [106,108]. A catalyst was found to suit more to macroalgae as compared to microalgae due to enhanced conversion of carbohydrates into biocrude [104,112].

2.6.3. Direct combustion of algal biomass

The burning of biomass in the presence of air is called combustion. The hot gases produced after combustion can be used to produce energy in the form of heat, electricity or mechanical power [113]. A suitable feedstock should have lower contents of inorganic compounds as well as compounds containing sulfur and nitrogen [4]. A high concentration of these components releases toxic gases such as SO_x, NO_x, H_2S etc. Due to the fact that algal biomass have several economical routes for energy generation, direct combustion of algal biomass is never attempted for power generation on a large scale [102]. Microalgae have a higher ash content, which is comparatively lower compared to terrestrial energy crops [28]. The moisture content of biomass reduces the heat availability and the direct combustion of biomass is possible only for biomass with a moisture not greater than 50% [28]. Dry macroalgae can be easily combust, but have a low thermal value

Table 5
The effect of hydrothermal liquefaction on microalgae and macroalgae for bio-oil production.

Algae	Species	Bio-oil yield (%, w/w)	Dry biomass to water ratio (w/v)	HHV (MJ/kg)	Temperature (°C)	Catalysts	References
Microalgae	*Spirulina* (freshwater)	31	moisture 78.4% (w/w)	35–37	300	–	[105]
	Scenedesmus (freshwater)	45.4	moisture 78.4% (w/w)	35.5	300	–	[105]
	LEA of *Scenedesmus* (freshwater)	36	moisture 78.4% (w/w)	35.3	300	–	[105]
	Dunaliella tertiolecta (marine)	37	moisture 78.4% (w/w)	36	340	with & without	[106]
	Desmodesmus (freshwater)	49	~1:13	22–36	375	with & without	[107]
	Nannochloropsis sp. (marine)	57	1:18	38	350	Pd/C, Pt/C, Ru/C, Ni/SiO2-Al2O3, CoMo/γ-Al2O3 (sulfided), and zeolite	[108]
	Botryococcus braunii (freshwater)	64	3:2	~50	300	Na_2CO_3	[109]
Macroalgae	*Laminaria saccharina* (marine)	79	1:10	35.97	350	–	[110]
	Laminaria saccharina (marine)	19.3	1:10	36.5	350	KOH	[111]
	Enteromorpha prolifera (marine)	23	2:15	28–30	220–320	Na_2CO_3	[112]
	Oedogonium (freshwater)	26.2	1:14	33.7	330–340	–	[104]
	Cladophora (freshwater)	19.7	1:14	33.5	330–340	–	[104]
	Cladophora (marine)	13.5	1:14	33.3	330–340	–	[104]
	Derbesia (marine)	19.7	1:14	33.2	330–340	–	[104]
	Ulva (marine)	18.7	1:14	33.8	330–340	–	[104]

typical of carbohydrate-rich biomass (14–16 MJ kg^{-1}) [28]. Therefore, combustion of algal biomass is discouraged to produce heat and electricity. Alternatively, the blending of algal with other feedstocks has been reported. The blending of microalgal biomass with coal powder in the ratio of 1:1 had the comparable calorific value to the pure coal powder [114].

3. Techno-economic analysis

Microalgae is a highly productive but a cost intensive source of biofuels. For example, besides an additional dewatering stage, the energy required to support microalgae cultivation is nearly 2.1 times higher as compared to soy cultivation [115]. However, oil extraction from microalgae requires less energy as compared to soy. Based on the life cycle assessment and techno-economic analysis, different researchers have recommended different routes of biofuel generation from algae, and allocation of LEA in order to reduce the minimum fuel selling price (MFSP). This is because of different assumptions used in the simulation study and a large variation in the data available in the literature. Chisti estimates the MFSP of the biodiesel per liter to be $0.78 and $1.0 for PBR and open ponds respectively [5]. The biodiesel costs in 2012 were estimated in the range of $0.42–0.97 L^{-1} (2012 USD values), which was marginally improved as compared to a decade-old cost analysis by U. S. National Renewable Energy Laboratory [116]. Batan [114] conducted a detailed techno economic analysis to calculate the MFSP of the biodiesel and concluded that MFSP of biodiesel is dependent upon the application of the LEA. The production costs of the microalgal raw crude oil and refined diesel were $3.46 and $3.72 per liter [115]. The selling of the LEA as a fish feed replacement was calculated to be more beneficial as compared to energy extraction from it in the form of co-firing. This is because Monte Carlo simulations demonstrated that MFSP is reduced to negative, when the LEA is sold as an aqua feed indicating that the revenue generated by only selling co-products is sufficient to recover capital and operating costs to produce algal biodiesel [115]. Contrary to this, Davis demonstrated the higher reduction in the MFSP, when the carbohydrates and lipid extracted algal biomass was utilized in anaerobic digestion as compared to other routes of algal residue utilization such as animal feed or butanol production. The MFSP was found majorly sensitive to feedstock cost, daily feed rate supply, lipid extraction yield, and total capital investment [116]. The economic feasible of biodiesel production was claimed to enhance by increasing lipid content in microalgae as compared to biomass productivity. However, this effect was more pronounced in case of low lipid content of 20%(w/w), thereafter the effect became less significant [116].

4. Conclusion

Microalgae and macroalgal have potential for the production of various types of biofuels (solid, liquid, gaseous) through different routes. Biodiesel attracts more attention to the researchers and policy makers as this can be directly replace the fossil diesel without any modification of the engine, whereas ethanol needs to be blended with the diesel in limited ratio. However, currently the energy requirement and cost of biofuels production from algae is not competitive with fossil fuel based sources of energy. Technological challenges have not been overcome to make the biofuel production process energetically and commercially viable. The genetic engineering approaches should be applied to target the limiting factor controlling the lipid synthesis pathway. Hydrogen production by microalgae through biophotolysis seems interesting as it directly converts the solar energy into hydrogen. However, the production rate is too low to be scaled up and the process has not been scaled-up even in the laboratory. Being a relatively simple process in terms of engineering and infrastructure requirement, most of researchers agree AD process as a more promising specially for macroalgae. The use of macroalgae is restricted as a substrate for fermentative ethanol, butanol production and as a feedstock in biogas generation and thermochemical conversion. The focus should be given to cost effective way of biomass hydrolysis to release fermentable sugars. Among the thermochemical conversion methods of energy generation, HTL seems more promising due to handling of wet biomass at moderate temperature and pressure, and conversion of the whole

biomass into high quality oil.

An integration of different routes of bioenergy production and judicial use of entire algal biomass can make the process more promising. It can be recommended to use the algal biomass after hydrogen production by biophotolysis for the production of biodiesel, and bioethanol from lipid and starch respectively as these energy storage compounds were found to enhance after sulfur deprivation. The spent lipid and sugar extracted algal biomass can be further utilized in anaerobic digestion because of better biodegradability of algal biomass and having a low sulfur content. Alternatively, the LEA can be allocated for non-energy purpose, for example, the aqua feed supplement was found to decrease the MFSP of oil to negative.

Acknowledgments

The authors gratefully acknowledge the Department of Biotechnology (DBT), Government of India, New Delhi, India and the Danish Council for Strategic Research (DSF) for Indo-Danish project on Algal Biorefinery (project no.09–067601) for financial support and the Council of Scientific and Industrial Research (CSIR), Govt. of India and University Grants Commission (UGC) for the fellowship. The valuable help by Mrs. Anshu for shaping this manuscript is highly appreciated.

References

[1] Alabi AO, Tampier M, Bibeau E. Microalgae technologies and bioprocesses for bioenergy production in British Columbia: current technology, suitability & barriers to implementation. Report to the British Columbia Innovation Council. 2009. p. 1–88.
[2] Demirbas A, Demirbas MF. Importance of algae oil as a source of biodiesel. Energy Convers Manag 2011;52(1):163–70.
[3] Kumar K, Dasgupta CN, Nayak BK, Lindblad P, Das D. Development of suitable photobioreactors for CO_2 sequestration addressing global warming using green algae and cyanobacteria. Bioresour Technol 2011;102(8):4945–53.
[4] Wegeberg S. Algae biomass for bioenergy in Denmark. Biological/technical challenges and opportunities. 2010.
[5] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25(3):294–306.
[6] Schenk PM, Thomas-Hall SR, Stephens E, Markx UC, Mussgnug JH, Posten C, Kruse O, Hankamer B. Second Generation Biofuels: High-Efficiency Microalgae for biodiesel Production. Bioenergy Res 2008;1:20–43.
[7] Brennan L, Owende P. Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev 2010;14:557–77.
[8] Cagliari A, Margis R, Maraschin FS, Turchetto Zolet AC, Loss G, Margis-Pinheiro M. Biosynthesis of Triacylglycerols (TAGs) in plants and algae. Int J Plant Biol 2011;2:40–52.
[9] Gaffron H, Rubin J. Fermentative and photochemical production of hydrogen by algae. J Gen Physiol 1942;26:219–40.
[10] Ghirardi ML, Togasaki RK, Seibert M. Oxygen sensitivity of algal H_2 production. Appl Biochem Biotech 1997;63:141–51.
[11] Kumar K, Roy S, Das D. Continuous mode of carbon dioxide sequestration by *C. sorokiniana* and subsequent use of its biomass for hydrogen production by *E. cloacae* IIT-BT 08. Bioresour Technol 2013;145:116–22.
[12] Roy S, Kumar K, Ghosh S, Das D. Thermophilic biohydrogen production using pre-treated algal biomass as substrate. Biomass Bioenergy 2014;61:157–66.
[13] Rojan PJ, Anisha GS, Nampoothiri KM, Pandey A. Micro and macroalgal biomass: a renewable source for bioethanol. Bioresour Technol 2011;102:186–93.
[14] Yen H-W, Brune DE. Anaerobic co-digestion of algal sludge and waste paper to produce methane. Bioresour Technol 2007;98(1):130–4.
[15] Ras M, Lardon L, Bruno S, Bernet N, Steyer J-P. Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*. Bioresour Technol 2012;102:200–6.
[16] Golueke CG, Oswald WJ, Gotaas HB. Anaerobic digestion of Algae. Appl Microbiol 1957;5(1):47–55.
[17] Vertès AA, Qureshi N, Blaschek HP, Yukawa H. Biomass to biofuels. Strategies for global industries. First edition. West Sussex, United Kingdom: John Wiley & Sons, Ltd.; 2010.
[18] Mussgnug JH, Klassen V, Schlüter A, Kruse O. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. J Biotechnol 2010;150:51–6.
[19] Sialve B, Bernet N, Bernard O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol Adv 2009;27(4):409–16.
[20] Angelidakil MG, Georgakakis D. Carbohydrate-enriched cyanobacterial biomass as feedstock for bio-methane production through anaerobic digestion. Fuel 2013;111:872–9.
[21] Driver T, Bajhaiya A, Pittman JK. Potential of Bioenergy Production from Microalgae. Curr Sust Renew Energ Rep 2014;1:94–103.
[22] Bird KT, Habig C, Debusk T. Nitrogen allocation and storage patterns in *Gracilaria tikvahie* (Rhodophyta). J Phycol 1982;18:344–8.
[23] Chynoweth DP. Review of biomethane from marine biomass. Department of Agricultural and Biological Engineering.Gainesville, Florida: University Of Florida; 2002.
[24] Gong Y, Jiang M. Biodiesel production with microalgae as feedstock: from strains to biodiesel. Biotechnol Lett 2011;33:1269–84.
[25] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. Renew Sustain Energy Rev 2010;14(1):217–32.
[26] Li X, Xu H, Wu Q. Large-scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors. Biotechnol Bioeng 2007;2007(98):764–71.
[27] Williams P, Laurens L. Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics. Energy Environ Sci 2010;3(5):554–90.
[28] Milledge JJ, Smith B, Dyer PW, Harvey P. Macroalgae-derived biofuel: a review of methods of energy extraction from seaweed biomass. Energies 2014;7:7194–222.
[29] Holdt SL, Kraan S. Bioactive compounds in seaweed: functional food applications and legislation. J Appl Phycol 2011;23:543–97.
[30] Greenwell HC, Laurens LML, Shields RJ, Lovitt RW, Flynn KJ. Placing microalgae on the biofuels priority list: a review of the technological challenges. J R Soc Interface 2010;7:703–26.
[31] Knothe G. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Process Technol 2005;86:1059–70.
[32] Leonardi PI, Popovich CA, Damiani MC. Feedstocks for second-generation biodiesel: microalgae's biology and oil composition. Econ Eff Biofuel Prod 2011:317–46.
[33] Jones CS, Mayfield SP. Algae biofuels: versatility for the future of bioenergy. Curr Opin Biotechnol 2012;23:346–51.
[34] Kumar K, Das D. Growth characteristics of Chlorella sorokiniana in airlift and bubble column photobioreactors. Bioresour Technol 2012;116:307–13.
[35] Quinn J, de Winter L, Bradley T. Microalgae bulk growth model with application to industrial scale systems. Bioresour Technol 2011;102(8):5083–92.
[36] Huerlimann R, de Nys R, Heimann K. Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. Biotechnol Bioeng 2010;107:245–57.
[37] Schlagermann P, Göttlicher G, Dillschneider R, Rosello-Sastre R, Posten C. Composition of algal oil and its potential as biofuel. J Combust 2012:1–14.
[38] Jiang H, Gao K. Effects of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae). J Phycol 2004;40:651–4.
[39] Zhila NO, Kalacheva GS, Volova TG. Effect of salinity on the biochemical composition of the alga *Botryococcus braunii* Kutz IPPAS H-252. J Appl Phycol 2010;23:47–52.
[40] Abou-Shanab RAI, Matter IA, Kim S, Oh Y, Choi J, Jeon B. Characterization and identification of lipid-producing microalgae species isolated from a freshwater lake. Biomass Bioenergy 2011;35:3079–85.
[41] Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 2008;54:621–39.
[42] Yu WL, Ansari W, Schoepp NG, Hannon MJ, Mayfield SP, Burkart MD. Modifications of the metabolic pathways of lipid and triacylglycerol production in microalgae. Microb Cell Fact 2011;10:91.
[43] Skjånes K, Rebours C, Lindblad P. Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. Crit Rev Biotechnol 2012;33:172–215.
[44] Shifrin NS, Chisholm SW. Phytoplankton lipids: Interspecific differences and effects of nitrate, silicate and light-dark cycles. J Phycol 1981;17:374–84.
[45] Guarnieri MT, Nag A, Smolinski SL, Darzins A, Seibert M, Pienkos PT. Examination of triacylglycerol biosynthetic pathways via de novo transcriptomic and proteomic analyses in an unsequenced microalga. PLoS One 2011;6:1–13.
[46] Siaut M, Cuine S, Cagnon C, Fessler B, Nguyen M, Carrier P, Beyly A, Beisson F, Triantaphylidès C, Li-Beisson Y, Peltier G. Oil accumulation in the model green alga *Chlamydomonas reinhardtii*: characterization, variability between common laboratory strains and relationship with starch reserves. BMC Biotechnol 2011;11:7.
[47] Ohlrogge J, Browse J. Lipid biosynthesis. The Plant Cell 1995;7:957–70.
[48] Durrett TP, Benning C, Ohlrogge J. Plant triacylglycerols as feedstocks for the production of biofuels. Plant J 2008;54:593–607.
[49] Sheehan J, Dunahay T, Benemann J, Roessler P. A look back at the US department of energy's aquatic species program: biodiesel from algae.Golden, CO: National Renewable Energy Laboratory; 1998.
[50] Chen G, Peng Z-y, Shan L, Xuan N, Tang G-y, Zhang Y, Li L, He Q-f, Bi Y-p. Cloning of Acyl-ACP Thioesterase FatA from *Arachis hypogaea* L. and Its Expression in *Escherichia coli*. J Biomed Biotech 2012;652579.
[51] Zhou N, Zhang Y, Wu X, Gong X, Wang Q. Hydrolysis of *Chlorella* biomass for fermentable sugars in the presence of HCl and $MgCl_2$. Bioresour Technol 2011;102:10158–61.

[52] Rabelo SC, Filho RM, Costa AC. Lime pretreatment of sugarcane bagasse for ethanol production. Appl Biochem Biotechnol 2009;153:139–50.
[53] Zhou N, Zhang Y, Wu X, Gong X, Wang Q. Hydrolysis of *Chlorella* biomass for fermentable sugars in the presence of HCl and $MgCl_2$. Bioresour Technol 2011;102:10158–61.
[54] Laurens LML, Davis NNR, Sweeney N, Wychen SV, Lowell A, Pienkos PT. Acid-catalyzed algal biomass pretreatment for integrated lipid and carbohydrate-based biofuels production. Green Chem 2015;17:1145–58.
[55] Harun R, Danquah MK. Influence of acid pre-treatment on microalgal biomass for bioethanol production. Process Biochem 2011;46(1):304–9.
[56] Rojan PJ, Anisha GS, Nampoothiri KM, Pandey A. Micro and macroalgal biomass: a renewable source for bioethanol. Bioresour Technol 2011;102:186–93.
[57] Bush RA, Hall KM. Process for the production of ethanol from algae. US Patent 2006; 7:135,308.
[58] Ueda R, Hirayama, S, Sugata K, Nakayama H. Process for the production of ethanol from microalgae. US Patent 1996;5:578,472.
[59] Harun R, Danquah MK, Forde GM. Microalgal biomass as a fermentation feedstock for bioethanol production. J Chem Technol Biotechnol 2010;85:199–203.
[60] Deng MD, Coleman JR. Ethanol synthesis by genetic engineering in Cyanobacteria. Appl Environ Microbiol 1999;65:523–8.
[61] Wargacki AJ, Leonard E, Nyan Win M, Regitsky DD, Santos CNS, Kim PB, Cooper SR, Raisner RM, Herman A, Sivitz AB, Lakshmanaswamy A, Kashiyama Y, Baker D, Yoshikuni Y. An engineered microbial platform for direct biofuel production from brown macroalgae. Science 2012;335:308–13.
[62] Algenol Biofuels, 2015, ⟨http://www.algenolbiofuels.com⟩ and ⟨http://www.algenol.com/sites/default/files/news_articles/Going%20Commercial-Biofuels%20Journal%20-%20Second%20Quarter%20Issue%202014.pdf⟩ (accessed 21.06.15).
[63] Jones CS, Mayfield SP. Algae biofuels: versatility for the future of bioenergy. Curr Opin Biotechnol 2012;23(3):346–51.
[64] Schiener P, Black KD, Stanley MS, Green DH. The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea, Saccharina latissima* and *Alaria esculenta*. J Appl Phycol 2015;27:363–73.
[65] Talebnia F, Karakashev D, Angelidaki I. Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation. Bioresour Technol 2010;101(13):4744–53.
[66] Horn SJ, Aasen IM, Østgaard K. Ethanol production from seaweed extract. J Ind Microbiol Biotechnol 2000;25:249–54.
[67] Adams JM, Gallagher JA, Donnison IS. Fermentation study on Saccharina latissima for bioethanol production considering variable pretreatments. J Appl Phycol 2009;21:569–74.
[68] Alvarado Morales M, Gunnarsson IB, Fotidis I, Vasilakou E, Lyberatos G, Angelidaki I. *Laminaria digitata* as a potential carbón source for succinic acid and bioenergy production in a biorefinery perspective. Algal Res 2015;9:126–32.
[69] Horn SJ. Bioenergy from brown seaweeds (PhD thesis). Trondheim, Norway: Norwegian University of Science and Technology (NTNU); 2000. p. 22–6.
[70] Aizawa M, Asaoka K, Atsumi M, Sakou T. Seaweed bioethanol production in Japan-The Ocean Sunrise Project. Oceans 2007. Vancouver, Canada; p. 1–5.
[71] Alvarado-Morales M, Terra J, Gernaey KV, Woodley JM, Gani R. Biorefining Computer aided tools for sustainable design and analysis of bioethanol production. Chem Eng Res Des 2009;87:1171–83.
[72] Koppram R, Olsson L. Combined substrate, enzyme and yeast feed in simultaneous saccharification and fermentation allow bioethanol production from pretreated spruce biomass at high solids loadings. Biotechnol Biofuels 2014;7:54.
[73] Melis A. Green alga hydrogen production: progress, challenges and prospects. Int J Hydrog Energy 2002;27:1217–28.
[74] Kumar K, Das D. CO_2 sequestration and hydrogen production using cyanobacteria and green algae. In: Razeghifard R, editor. Natural and artificial photosynthesis: solar power as an energy source. Hoboken, NJ, USA: John Wiley & Sons Inc.; 2013. p. 173–215.
[75] Dasgupta CN, Suseela MR, Mandotra SK, Kumar P, Pandey MK, Toppo K, Lone JA. Dual uses of microalgal biomass: an integrative approach for biohydrogen and biodiesel production. Appl Energy 2015;146:202–8.
[76] Niehaus TD, Okada S, Devarenne TP, Watt DS, Sviripa V, Chappell J. Identification of unique mechanisms for triterpene biosynthesis in *Botryococcus braunii*. PNAS 2011;108(30):12260–5.
[77] Dayananda C, Sarada R, Bhattacharya S, Ravishankar GA. Effect of media and culture conditions on growth and hydrocarbon production by *Botryococcus braunii*. Process Biochem 2005;40:3125–31.
[78] Khatri W, Hendrix R, Niehaus T, Chappell J, Curtis WR. Hydrocarbon production in high density *Botryococcus braunii* race B continuous culture. Biotechnol Bioeng 2014;111:493–503.
[79] Khan NE, Nybo SE, Chappell J, Curtis WR. Triterpene Hydrocarbon Production Engineered Into a Metabolically Versatile Host *Rhodobacter capsulatus*. Biotechnol Bioeng 2015;112:1523–32.
[80] Prajapati SK, Kumar P, Malik A, Vijay VK. Bioconversion of algae to methane and subsequent utilization of digestate for algae cultivation: a closed loop bioenergy generation process. Bioresour Technol 2014;158:174–80.
[81] Tommaso G, Chen W-T, Li P, Schideman L, Zhang Y. Chemical characterization and anaerobic biodegradability of hydrothermal liquefaction aqueous products from mixed-culture wastewater algae. Bioresour Technol 2015;178:139–46.
[82] Torres A, Fermoso FG, Rincón B, Bartacek J, Borja R, Jeison D. Challenges for cost-effective microalgae anaerobic digestion. In: Chamy R, Rosenkranz F, editors. Biodegradation - Engin Technol. . p. 139–59.
[83] Park S, Li Y. Evaluation of methane production and macronutrient degradation in the anaerobic co-digestion of algae biomass residue and lipid waste. Bioresour Technol 2012;111:42–8.
[84] Zhong W, Chi L, Luo Y, Zhang Z, Zhang Z, Wu W-M. Enhanced methane production from Taihu Lake blue algae by anaerobic co-digestion with corn straw in continuous feed digesters. Bioresour Technol 2013;134:264–70.
[85] Ehimen EA, Connaughton S, Sun Z, Carrington GC. Energy recovery from lipid extracted, transesterified and glycerol codigested microalgae biomass. GCB Bioenergy 2009;1(6):371–81.
[86] Collet P, Helias A, Lardon L, Ras M, Goy RA, Steyer JP. Life-cycle assessment of microalgae culture coupled to biogas production. Bioresour Technol 2011;102:207–14.
[87] Ehimen EA, Holm-Nielsen J-B, Poulsen M, Boelsmand JE. Influence of different pre-treatment routes on the anaerobic digestion of a filamentous algae. Renew Energy 2013;50:476–80.
[88] Angelidaki I, Ellegaard L, Ahring BK. A mathemathical model for dynamic simulation of anaerobic digestion of complex substrates, focusing on ammonia inhibition. Biotechnol Bioeng 1993;42:159–66.
[89] Chen Y, Cheng JJ, Creamer KS. Inhibition of anaerobic digestion process: a review. Bioresour Technol 2008;99(10):4044–64.
[90] Ehimen EA, Sun ZF, Carrington CG, Birch EJ, Eaton-Rye JJ. Anaerobic digestion of microalgae residues resulting from the biodiesel production process. Appl Energy 2011;88(10):3454–63.
[91] Angelidaki I, Sanders W. Assessment of the anaerobic biodegradability of macropollutants. Rev Environ Sci Biotechnol 2004;2004(3):117–29.
[92] Hanssen JF, Indergaard M, Østgaard K, Bævre OA, Pedersen TA, Jensen A. Anaerobic digestion of Laminaria spp. and Ascophyllum nodosum and application of end products. Biomass 1987;14:1–13.
[93] Vivekanand V, Eijsink VGH, Horn SJ. Biogas production from the brown seaweed *Saccharina latissima*: thermal pretreatment and codigestion with wheat straw. J Appl Phycol 2011;24:1295–301.
[94] Bruhn A, Dahl J, Nielsen HB, Nikolaisen L, Rasmussen MB, Markager S, Olesen B, Arias C, Jensen PD. Bioenergy potential of *Ulva lactuca*: Biomass yield, methane production and combustion. Bioresour Technol 2011;102:2595–604.
[95] Nielsen HB, Heiske S. Anaerobic digestion of macroalgae: methane potentials, pre-treatment, inhibitiom and co-digestion. Water Sci Technol 2011;54:1723–9.
[96] Allen E, Browne J, Hynes S, Murphy JD. The potential of algae blooms to produce renewable gaseous fuel. Waste Manag 2013;33:2425–33.
[97] Allen E, Wall DM, Hermann C, Xia A, Murphy JD. What is the gross energy yield of third generation gaseous biofuel sourced from seaweed. Energy 2015;81:352–60.
[98] Briand X, Morand P. Anaerobic digestion of Ulva sp. 1. Relationship between Ulva composition and methanisation. J Appl Phycol 1997;9:511–24.
[99] Morand P, Briand X, Charlier RH. Anaerobic digestion of *Ulva* sp. 3. Liquefaction juices extraction by pressing and a technico-economic budget. J Appl Phycol 2006;18:741–55.
[100] Adams JMM, Ross AB, Anastasakis K, Hodgson EM, Gallager JA, Jones JM, Donnison IS. Seasonal variation in the chemical composition of the bioenergy feedsctock Laminaria digitata for the thermochemical conversion. Bioresour Technol 2011;102:226–34.
[101] Orosz MS, Forney D. A comparison of algae to biofuel conversion pathways for energy storage off-grid. Report 2008:1–31.
[102] Ullah K, Ahmad M, Sharma VK, Lu P, Harvey A, Zafar M, Sultana S, Anyanwu CN. Algal biomass as a global source of transport fuels: overview and development perspectives. Prog Nat Sci: Mater Int 2014;24(4):329–39.
[103] Neveux N, Yuen AKL, Jazrawi C, Magnusson M, Haynes BS, Masters AF, Montoya A, Paul NA, Maschmeyer T, de Nys R. Biocrude yield and productivity from the hydrothermal liquefaction of marine and freshwater green macroalgae. Bioresour Technol 2014;155:334–41.
[104] Vardon DR, Sharma BK, Blazina GV, Rajagopalan K, Strathmann TJ. Thermochemical conversion of raw and defatted algal biomass via hydrothermal liquefaction and slow pyrolysis. Bioresour Technol 2012;109:178–87.
[105] Minowa T, Yokoyama S, Kishimoto M, Okakura T. Oil production from algal cells of *Dunaliella tertiolecta* by direct thermochemical liquefaction. Fuel 1995;74:1735–8.
[106] Alba LG, Torri C, Samori C, van der Spek J, Fabbri D, Kersten SRA, Brilman DWF. Hydrothermal treatment of microalgae: evaluation of the process as conversion method in an algae biorefinery concept. Energy Fuels 2012;26:642–57.
[107] Duan P, Savage PE. Hydrothermal liquefaction of a microalga with heterogeneous catalysts. Ind Eng Chem Res 2011;50(1):52–61.
[108] Dote Y, Sawayama S, Inoue S, Minowa T, Yokoyama S-y. Recovery of liquid fuel from hydrocarbon-rich microalgae by thermochemical liquefaction. Fuel 1994;73(12):1855–7.
[109] Bach Q-V, Sillero MV, Tran K-Q, Skjermo J. Fast hydrothermal liquefaction of a Norwegian macro-alga: screening tests. Algal Res 2014;6:271–6.
[110] 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.
[111] Zhou D, Zhang L, Zhang S, Fu H, Chen J. Hydrothermal liquefaction of

macroalgae *Enteromorpha prolifera* to bio-oil. Energy Fuels 2010;24:4054–61.
[112] Davis C. Heat of combustion of algae for use in a diesel engine. MS thesis; 2013.
[113] Ghayal MS, Pandya MT. Microalgae biomass: a renewable source of energy. Energy Procedia 2013;32:242–50.
[114] Batan LYD. Life cycle and techno-economic analysis of microalgae-based biofuel. PhD Dissertation; 2014.
[115] Nagarajan S, Chou SK, Cao S, Wu C, Zhou Z. An updated comprehensive techno-economic analysis of algae biodiesel. Bioresour Technol 2013;145:150–6.
[116] Davis R. DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review. Algal biofuels techno-economic analysis. 2015.
[117] Radakovits R, Jinkerson RE, Darzins A, Posewitz MC. Biofuel production genetic engineering of algae for enhanced biofuel production. Eukaryot Cell 2010;9:486–501.