Chapter 13
Liquid Fuels Production from Algal Biomass

Shantonu Roy and Debabrata Das

13.1 Introduction

Energy crisis is looming the global economy and environment. The rate at which fossil fuels are depleting, a necessity of alternate fuel has been gaining importance. The use of fossil fuels for energy is unsustainable and causes build up of greenhouse gases in the atmosphere leading to global warming. Biofuels store energy chemically that can be harnessed easily. It can also be used in existing combustion engines after blending with petroleum diesel to various degrees. No separate transportation infrastructures would be required for such fuels (Amaro et al., 2011). In biorefinery concept, every component of the biomass material would be used to produce commercially important products. At present, first generation biofuels are produced using sucrose and starch crops. Second generation biofuels are produced using lignocellulosic biomass. Lignocellulosic biomass gained importance because of their abundant availability but need of pretreatment and saccharification processes has hindered their usage as feedstock. Moreover, bioenergy production using agricultural crops or agricultural wastes as feedstock is disadvantageous as resources for water and agriculture lands are limited (Li et al., 2008). Algal biomass has been considered as third generation feedstock for biofuel production (Metzger and Largeau, 2005). Many algal species having high lipid content thus could be explored for oleo-fuel generation. Similarly, algal species having high carbohydrate content can be exploited for bioethanol or biogas production.

Algae have superior annual productivity and oil content as compared to seed crops. Oil productivity of soybean, canola and palm are 450, 1200 and 3000 litres per hectare, respectively. Algae can yield approximately 90,000 litres per hectare (Chisti, 2007). Algae do not require arable land for cultivation and thus it does not

S. Roy • D. Das (✉)
Department of Biotechnology, Indian Institute of Technology Kharagpur, Kharagpur 721302, India
e-mail: ddas.iitkgp@gmail.com

D. Das (ed.), *Algal Biorefinery: An Integrated Approach*,
DOI 10.1007/978-3-319-22813-6_13

necessitate agricultural space. In fact, algae cultivation facilities can be built on marginal land that has few other uses. The water resources available for algal cultivation are fresh water, saline and industrial wastewaters (Brown and Zeiler, 1993). Moreover, algae fix atmospheric CO_2 with greater competence than land plants.

13.2 Biodiesel

Biodiesel consists of monoalkyl esters that are derived from different oil-rich sources e.g. organic oils extracted from plants, animal, algae, etc. via transesterification process. The chemical reaction for biodiesel transesterification involves hydrolysis of ester bond between glycerol and fatty acid chain and then further esterification with methanol as shown in Eqn. (13.1).

$$\text{Triglyceride} + 3\text{Methanol} \underset{\longleftarrow}{\overset{\text{catalyst}}{\longrightarrow}} \text{Glycerine} + 3\text{Methylesters}\ \left(\textit{Biodiesel}\right) \quad (13.1)$$

The presence of a catalyst and alkali such as potassium hydroxide enhances transesterification. Since it's a reversible reaction, an excess of methanol could be used to force the reaction in forward direction. Solvent recovery could be done by soxhlet technique and can be reused. Elevating temperature to 60 °C could increase the kinetics of the reaction and the process could be completed in 90 minutes. The biodiesel production process involves following steps (Xu et al., 2006):

- Reactants such as triglycerides, methanol and catalyst were to be placed in a controlled reactor so as to initiate transesterification reaction.
- The initial products formed after transesterification are placed in a separator to remove the glycerine and other unreacted components
- Recovery of excess methanol from the methyl esters is done by evaporation.
- The final biodiesel needs to be rinsed with water, neutralized and dried.

Many reports are available suggesting that under stressed or unfavourable conditions, algae produce more oil as compared to optimal growth conditions. Under favourable growth conditions, fatty acids are synthesized principally for esterification into glycerol-based membrane lipids. It constitutes about 5–20 % w/w of their dry cell weight (DCW). Composition of fatty acids present under such conditions can be categorized into following species: medium-chain (C10–C14), long-chain (C16–C18) and very long chain (C20) and fatty acid derivatives. Under unfavourable or stress conditions algae redirect their lipid biosynthetic pathways towards the formation and accumulation of neutral lipids (20–50 % DCW). Major constituents of such lipids are in the form of triacylglycerol (TAG). The role of TAGs is not of forming cell membrane, rather it serves as storage energy (Hu et al., 2008). The accumulated TAGs are stored in the cytoplasm of the algal cell as densely packed lipid bodies. Lipid accumulation also takes place in the inter-thylakoid space of the chloroplast in certain green algae. However, the challenges in production cost of

high grade algae oils is the constraint in operational conditions (low temperature, low light intensity and nitrogen deficiency) that leads to accumulation of high grade oil in microalgae. In current scenario, it is quite difficult to obtain cheap algae biomass with 20 % w/w lipid content. With the advent of recent advances in photobioreactors configurations (closed and open), production of algal biomass is still a costly affair.

13.2.1 Microbiology

Algae have a very simple cellular structure as compared to higher autotrophic photosynthetic. They have higher nutrient assimilation ability because of their large surface to volume body ratio. The mechanism of CO_2 fixation via photosynthetic pathway is similar to that of higher plants. Because of their simple cellular structure, algae are generally more efficient in harnessing solar energy (Ghirardi, 2000; Singh et al., 2008). Therefore, microalgae produce 30 times the amount of oil per unit area of land as compared to terrestrial oilseed. This makes microalgae as a potential source for biodiesel production, thus completely displacing dependence on fossil oils such as diesel (Table 13.1). Many microalgae have oil content up to 80 % w/w of dry cell biomass. Generally oil content are in the range of 20–50 % w/w of dry cell biomass (Mata et al., 2010; Khan et al., 2009). Under stressed conditions accumulation of lipids is induced in microalgal species. The average lipid contents in algal cells vary between 1 % and 70 % w/w of dry cell weight (Table 13.1). Even within the same genus, different lipid content has been reported. *Botryococcus braunii* is a well known microalgae reported to have highest oil content (75 % w/w of dry cell weight), but is associated with a low productivity and much of it is secreted into the cell wall (Banerjee et al., 2002). In general, lipid content of microalgae like *Chlorella, Dunaliella, Isochrysis, Nannochloris, Nannochloropsis, Neochloris*, *Nitzschia*, *Phaeodactylum* and *Porphyridium* sp. lies in between 20 % and 50 % w/w DCW. Each of the algae has distinct productivity and growth characteristics. Many reports were available on *Chlorella* sp. as promising option for biodiesel production. On other hand, marine microalgae have greater lipid productivities. Thus marine algae could be used for mass production. There are certain advantages in marine algae cultivation e.g. a high salinity environment prevents extensive exogenous and endogenous contamination and moreover, sea water can be directly used for cultivation instead of putting burden on fresh water resources.

The fatty acid composition of the microalgal cell is also important. The heating power of biodiesel depends on its composition. In general, it consists of saturated and unsaturated fatty acids containing 12–22 carbon atoms, often belonging to omega 3 and omega 6 types. The fatty acid compositions of many fresh water microalgae species consist of C14:0, C16:0, C18:1, C18:2 and C18:3 fatty acids. Many fatty acid residues were species-specific, e.g. *Ankistrodesmus* sp. contains C16:4 and C18:4, *Isochrysis* sp. contains C18:4 and C22:6, *Nannochloris* sp. contains C16:3 and C20:5, and *Nitzschia* sp. contains C16:3 and C20:5. The fatty acid composition

Table 13.1 Potential of different algal species for biodiesel production

Habitat	Microalgal species	Lipid content (%w/w DCW)	Lipid productivity (mg L^{-1} d^{-1})
Fresh water	*Botryococcus* sp.	25.0–75.0	–
	Chaetoceros muelleri	33.6	21.8
	Chaetoceros calcitrans	14.6–16.4/39.8	17.6
	Chlorella emersonii	25.0–63.0	10.3–50.0
	Chlorella protothecoides	14.6–57.8	1214
	Chlorella sorokiniana	19.0–22.0	44.7
	Chlorella vulgaris	5.0–58.0	11.2–40.0
	Chlorella pyrenoidosa	2	–
	Chlorella sp.	18.0–57.0	18.7
	Chlorococcum sp.	19.3	53.7
	Cylindrotheca sp.	16–37	
	Ellipsoidion sp.	27.4	47.3
	Haematococcus pluvialis	25	–
	Scenedesmus obliquus	11.0–55.0	–
	Scenedesmus quadricauda	1.9–18.4	35.1
	Scenedesmus sp.	19.6–21.1	40.8–53.9
	Schizochytrium sp.	50–77	–
Marine water	*Dunaliella salina*	6.0–25.0	116
	Dunaliella tertiolecta	16.7–71.0	-
	Dunaliella sp.	17.5–67.0	33.5
	Isochrysis galbana	7.0–40.0	-
	Isochrysis sp.	7.1–33	37.8
	Nannochloris sp.	20.0–56.0	60.9–76.5
	Nannochloropsis oculata	22.7–29.7	84.0–142.0
	Nannochloropsis sp.	12.0–53.0	60.9–76.5
	Neochloris oleoabundans	29.0–65.0	90.0–134.0
	Nitzschia sp.	30.9	30.9
	Pavlova salina	30.9	49.4
	Pavlova lutheri	35.5	40.2
	Phaeodactylum tricornutum	18.0–57.0	44.8
	Porphyridium cruentum	9.5	34
	Spirulina platensis	4.0–16.6	–
	Tetraselmis sp. F&M-M34	14–18	43

of microalgae is greatly influenced by different nutritional and processing factors, cultivation conditions and growth phases. Under nitrogen deficiency and salt stress, accumulation of C18:1 is induced in all species. In *B. braunii*, under above conditions, accumulation of C20:5 takes place. Even CO_2 assimilation also influences the fatty acid profile in algal cells. The biomass productivity and carbon dioxide fixation ability of *Scenedesmus* sp. is high at 10 % (v/v) CO_2. Similarly, *B. braunii* grown under 10 % (v/v) CO_2 gave higher biomass productivity and it is also suitable for biodiesel production due to its high proportion of oleic acid (Yoo et al., 2010).

Following factors are associated with different microalgae cultivation:

(i) Growth rate – it is measured as total amount of biomass accumulated per unit time and unit volume.
(ii) Lipid quantity and quality – in the harvested biomass, the actual distribution of fatty acid residues within acylglycerols.
(iii) Robustness of the process – biomass productivity influenced by variation in temperature, nutrient input and light. Competition with other microalgae and/or bacterial species also important.
(iv) Nutrient predilection and rate of substrate utilization – during growth, CO_2, nitrogen and phosphorus utilization varies species to species.
(v) Ease of biomass harvesting – e.g. efficiency of cell lysis, extraction and purification of lipids depends upon the ease of harvesting of biomass.

Photo-biological formulae needed to be optimized for individual species followed with cost-effective cultivation techniques so as to make this process commercially viable. Using existing technologies, the commercial viability of biodiesel production from native microalgae is trivial. A significant degree of development would be required in volumetric productivity of biomass so as to make the process lucrative for commercialization. This goal could be achieved by exploring genetic engineering of the microalgae cells. Redirection of metabolic pathway towards appropriate lipids by usage of molecular biology tools like synthetic biology, gene knockout etc. along with improvement of bioprocess parameters could propel the biodiesel production process to better yields.

13.2.2 Genetic Engineering of Microalgae

When the wild species available in formal collections and described in the literature are not feasible for commercial production of biodiesel, one may resort to genetic engineering so as to improve and tune the features of native microalgae, with the aim of enhancing productivity and yield (Hu et al., 2008). However, the pending concerns of biological contamination—materialized in restrictive legislation at large—have hindered a broader utilization of genetically engineered microalgae. The several shortcomings found at present include: very few strains of microalgae that underwent genetic modification (with the notable exception of *Chlamydomonas reinhardtii*—the genome of which has been fully elucidated, but which is a fresh water species); poor elucidation of the mechanisms underlying regulation of gene expression; and lack of specific molecular biology tools, e.g., efficient nuclear transformation, availability of promoter and selectable marker genes, and stable expression of transgenes. Although lengthy in time and cost-intensive, transformation of microorganisms to better respond to non-expensive operating conditions brings about a few advantages. Metabolic engineering may increase the yields of acylglycerols, or even lead to different molecules with better performance as biodiesel. In higher plants, several studies have explored the effects of over expressing

enzymes associated with lipid synthesis, yet little change in oil content was achieved in species containing higher levels of acetyl-CoA carboxylase – the rate limiting step in fatty acid biosynthesis, possibly because of the complex regulation of this enzyme.

At present, one is indeed still far from globally understanding the detailed molecular pathways and forms of regulation of lipid metabolism in microalgae. Bioinformatics applied to already sequenced microalgal genomes has unfolded essentially similar biochemical routes. Therefore, little experimental validation of putative enzyme activities has so far been done. Lipid accumulation is easily induced in microalgae by nitrogen deprivation. This provides a useful experimental basis for observing changes in gene transcription, protein synthesis and metabolic activities that prevail during lipid accumulation in microalgae. Nitrogen depletion coupled with RNAi suppression on the changes in the lipid and protein qualitative and quantitative profiles in *C. reinhardtii* during lipid droplet formation has been investigated. In cultures transferred to N-depleted media, the total fatty acid content per cell basis, increased by 2.4-fold within 72 h, of which 65 % of the total fatty acids were esterified to triacylglycerols (Moellering and Benning, 2010). Proteomic analysis provided evidence of a 'major lipid droplet protein' (MLDP) which was rather abundant in said lipid bodies. The mRNA transcript abundance of this protein followed the observed increase in lipid droplets after N-depletion, and RNAi lines of *C. reinhardtii*, with a 55–60 % reduction of MLDP transcription, produced lipid droplets characterized by 40 % larger diameters than the control line – thus implying that this protein is involved in regulation of lipid droplet size. Another efficient way of increasing lipid yield is to delete 'redundant' pathways in the selected microorganism, thus leading to precursor metabolites more suitable for biofuel production. An increase in triacylglycerol contents of lipid droplets was observed in a *C. reinhardtii* starchless mutant which was deficient in ADP-glucose pyrophosphorylase (an essential enzyme in starch production) following 48 h of N-depletion. Wild-type cells had increased their lipid droplet content by 15-fold.

13.2.3 Chemistry Behind Biodiesel Production

Alcohols are the most frequently used acyl-acceptors, particularly methanol and, to a lesser extent, ethanol. Other alcohols can be used, e.g. propanol, butanol, isopropanol, tert-butanol, branched alcohols and octanol but the cost is much higher. Regarding the choice between methanol and ethanol, the former is cheaper, more reactive and the fatty acid methyl esters (FAME) are more volatile than those of the fatty acid ethyl esters (FAEE). However, ethanol is less toxic and it can be considered more renewable because it can be easily produced from renewable sources by fermentation. In contrast, methanol is mainly produced from non-renewable fossil sources, such as natural gas. Regarding their characteristics as fuels, FAME and FAEE show slight differences e.g. FAEE have slightly higher viscosities and slightly lower cloud and pour points than the corresponding FAME. The transesterification

of triacylglycerols can be carried out by different catalytic processes, or in supercritical conditions (Marchetti et al., 2007). The catalyst used may be classified as: (1) alkaline-catalyst (sodium hydroxide, NaOH; Potassium hydroxide, KOH; sodium metoxide, NaOMe); (2) acid-catalyst (sulphuric acid, phosphoric acid, hydrochloric acid, sulphonic acid); (3) enzymatic-catalyst (lipases); and (4) inorganic heterogeneous catalyst (solid phase catalyst) (Fig. 13.1).

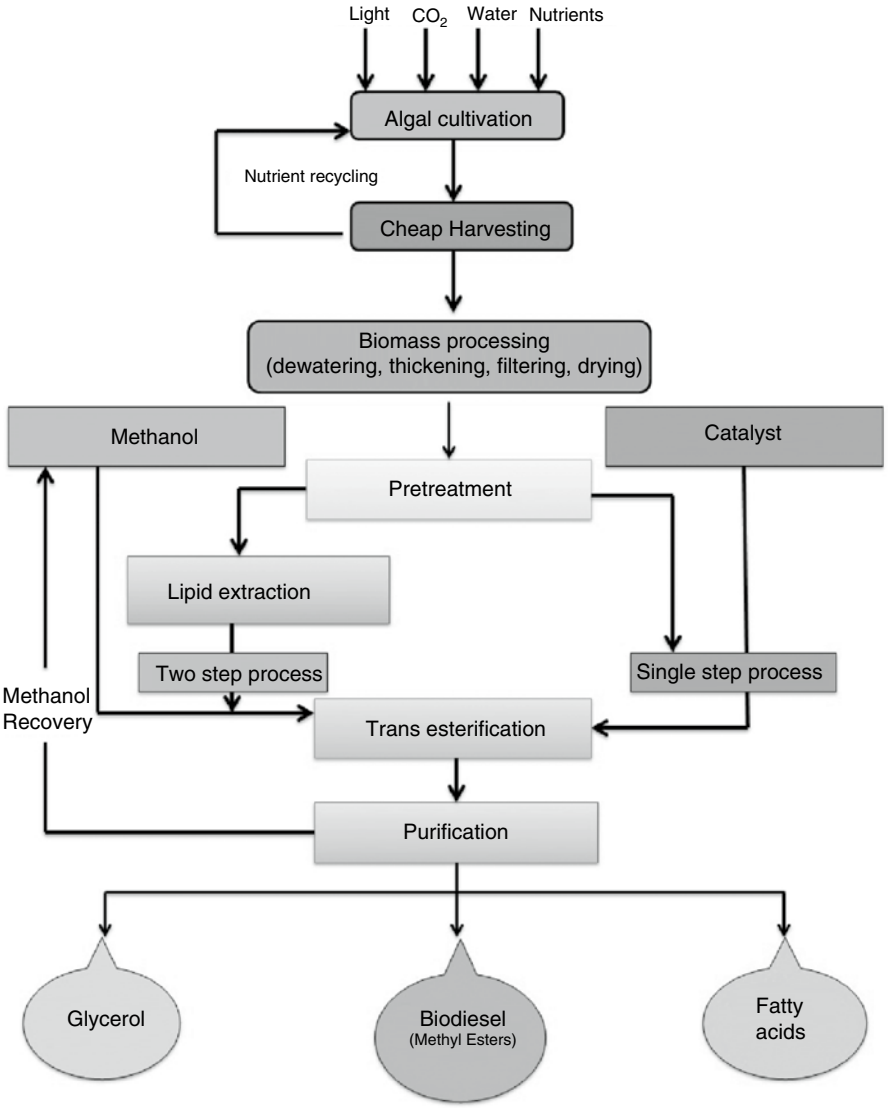

Fig. 13.1 Algal biodiesel production processes.

The transesterification using enzymes has been reported to be very expensive (the enzyme costs are very high), shows deactivation problems and requires a much longer reaction time (Vyas et al., 2010). On the other hand, acid and basic transesterification are widely used for biodiesel production. It is well known that basic catalyzed transesterification is faster than the acid catalyzed reaction (about 4000 times). However, acid catalysts can simultaneously promote esterification of free fatty acids (FFAs) and transesterification of triglycerides (Demirbas, 2007). Traditionally, homogeneous catalysts have been used for both acid and basic catalyzed reaction. Sulphuric acid is the main acid catalyst used for biodiesel production whereas NaOH, KOH and Na_2CO_3 mixed with alcohol, are commonly used for homogeneous basic catalysis (Helwani et al., 2009). However, one of the major disadvantages of homogeneous catalysts is that they cannot be reused or regenerated, because they are consumed in the reaction and separation of catalysts from products is difficult and requires more equipment, which could result in higher production costs. Besides, the process is not environmentally friendly because a large amount of wastewater is produced in the separation step. Based on the above premises, the use of solid catalysts seems to be an appropriate solution to overcome problems associated with homogeneous catalysts. Nevertheless, one of the major problems associated with heterogeneous catalysts is the formation of three phases with alcohol and oil, which leads to diffusion limitations thus lowering the rate of the reaction.

One way of overcoming mass transfer problem in heterogeneous catalysts is using certain amount of co-solvent to promote miscibility of oil and methanol and accordingly accelerate the reaction rate. Tetrahydrofuran (THF), dimethyl sulphoxide (DMSO), n-hexane and ethanol were used more frequently as co-solvent in transesterification of vegetable oils with methanol and solid catalysts. CaO as a solid base catalyst for transesterification of rapeseed oil with methanol and after 170 min of reaction time methyl ester gave improved yield of 93 % (Zabeti et al., 2010). However, by adding certain amount of THF into rapeseed oil/methanol mixture the same yields of 93 % were observed after 120 min of reaction time. Another way to promote mass transfer problems associated with heterogeneous catalysts is using structure promoters or catalyst supports which can provide more specific surface area and pores for active species where they can anchor and react with large triglyceride molecules.

Basic solids like CaO and MgO supported on alumina and hydrotalcites (Suppes, 2004) have been used for biodiesel production from vegetable oils. To avoid diffusion limitations, catalysts with higher surface area (porous silica-metal oxide composites) were tested in the transerification of vegetable and animal oils providing high conversion to (C10–C30) alkyl methyl esters and glycerin. On the other hand, zeolites, ion-exchange resins, mixed metal oxides or mesostructured solids have shown promising results in the acid esterification and transesterification of vegetable oil with high content of free fatty acids (FFAs) to obtain FAMEs. Recently, the transesterification of triglycerides contained in waste oilseed fruits with methanol has been studied using zeolites as strong acid catalysts (USY, BEA, FAU-X), together with weak acid catalysts (siliceous MCM-41 and ITQ-6) and base cata-

lysts such as K-MCM-41 and K-ITQ-6 (Macario et al., 2010). Zeolites are microporous crystalline metallosilicates featured by exhibiting molecular sieve and shape selective properties, which have found widespread applications in catalytic, adsorption and ion exchange processes. Zeolites have usually been synthesized with crystal sizes in the micrometre range and, therefore, with negligible external surface area. These properties impose severe limitations for their use in the conversion of bulky compounds. A huge interest has emerged recently for the synthesis of new zeolitic materials with enhanced accessibility. In this sense, nanocrystalline hierarchical zeolites contain a bimodal porosity (micro- and mesopores) and high external surface area where active sites can catalyze reactions involving large molecules like triglycerides. The synthesis of hierarchical nanozeolites is based on the incorporation of organosilanes in the synthesis gel to prevent zeolite crystal growth and thereby to stabilize zeolitic particles with ultra small sizes.

In any case, acid catalyst is the recommended process when the starting materials are low grade or have a high concentration of free fatty acids. The fatty acids would deactivate the alkaline catalyst. Acid catalysts could be used in conjunction with base catalysts (two-stage process). This two-stage process allows the use of low-cost feedstock like waste oil with high content of free fatty acids. The acid catalysts are used in the primary stage to convert free fatty acid to methyl esters, followed by a base catalyst process to convert the remaining triglycerides to methyl esters. Acid catalysts should be the method when using oils extracted from microalgal biomass. For example, a maximum yield of 10 mg of FAME from 250 mg of lipids was observed under following conditions: 0.6 N hydrochloric acid–methanol catalysts for 0.1 h at 70 °C, using the lipids extracted from *Chaeotoceros mulleri* (Nagle and Lemke, 1990). In comparison, only 3.3 mg of FAME were produced when sodium hydroxide was used as the catalyst, at the same conditions that gave maximum FAME yield. A simultaneous extraction and transesterification method for microalgal fatty acids using an acid catalyst was also studied (Belarbi et al., 2000). Fatty acids were extracted either from freeze-dried microalgal biomass or from centrifugally harvested biomass paste that has been freeze stored. The biomass paste had a moisture content of 82 % by weight. The biomass belonged to either the diatom *Phaeodactylum tricornutum* or the green alga *Monodus subterraneus* and a maximum yield of 8.37 g of FAME was obtained from 10.8 g of lipids in the following conditions: biomass paste (500 g, 82 % moisture, 12 % of lipids by wt.) of *P. tricornutum*, methanol, 1 L and 50 mL of acetyl chloride. The resulting slurry was heated in a boiling water bath for 120 min at 2.5 atm. The most usual method to transform oil into biodiesel is transesterification. This consists of the reaction between triacylglycerols (contained in the oils) and an acyl-acceptor. The acyl group acceptors may be carboxylic acids (acidolysis), alcohols (alcoholysis) or another ester (inter esterification). Only alcoholysis and inter esterification are of interest to produce biodiesel. The starting esters in both are triacylglycerols (oils), and if the transformation is quantitative they yield a mixture of monoalkyl esters (biodiesel) and glycerol (alcoholysis) or another triacylglycerol (inter esterification).

13.2.4 Quality of Biodiesel

The physical and chemical properties of biodiesel such as density, viscosity, flash point, cold filter plugging point, solidifying point and heating value must be determined for assessment of the potential of biodiesel as a substitute of diesel fuel. One of the important interfering factor in biodiesel production process is bound glycerol. It is associated with the residual amount of triglycerides and partial glycerides in the biodiesel. Biodiesel fuel, in the form of FAME, is now manufactured in many countries. The relevant standard for biodiesel is the ASTM Biodiesel Standard D 6751 which is followed in the United States (Knothe, 2006). European Union follows a separate standard for biodiesel when it is used for internal combustion engines (Standard EN 14214) and for use as heating oil (Standard EN 14213). The acid number of biodiesel limit according to ASTM D 6751 standard (US) was synchronized with the European biodiesel value of 0.5. Algal oils are quite rich in polyunsaturated fatty acids unlike most of the vegetable oils. Biodiesel intended to be used as heating oil is not affected by PUFA content. For getting acceptance as heating oil, it must meet criteria relating to the extent of total unsaturation of the oil, which is indicated by its iodine value. According to European standard EN 14214 and EN 14213, the iodine value of biodiesel should exceed 120 and 130 g iodine/100 g biodiesel, respectively.

13.3 Bioethanol

In the global scenario, ethanol is produced from sugar plants (55 %), grain (37 %), synthetically (8 %) and other raw materials (2 %) (İçöz et al., 2009). Bioethanol for fuel purpose has certain characteristics viz. they are derived from carbohydrate-rich biomass. They are biodegradable and environment friendly. Different feedstock such as cellulosic biomass, agricultural waste, and wood waste were commonly used for bioethanol production. Complex sugars present in the feedstock are first converted to simple sugars (mainly hexose). Then these simple sugars are utilized by solventogenic organisms to produce ethanol by fermentation (Eq. 13.2):

$$C_nH_{2n}O_n \rightarrow \frac{n}{3}CH_3CH_2OH + \frac{n}{3}CO_2 + 227.0\ kJ/mol \tag{13.2}$$

The ethanol thus produced can be recovered by fractional distillation. The ethanol on combustion gives 1370.7 kJ mol^{-1} (Eq. 13.3) of energy that can be harnessed for cooking, automobile combustion engine etc.

$$CH_3CH_2OH + 3O_2 \xrightarrow{Combustion} 2CO_2 + 2H_2O + 1370.7\ kJ/mol \tag{13.3}$$

The exemplary characteristics of bioethanol are as follows (Yoon and Lee, 2011):

- Easy for storage and no need of separate infrastructure for distribution.
- Highly suitable automobile fuel when blended with naturally occurring fossil fuels.
- Emission of harmful unburned hydrocarbon and carbon monoxide are extremely low as compared to fossil fuel combustion.

The technologies and skills of ethanol production were once confined to handful countries around the globe. Its production and usage as fuel have started to show its presence globally. At present, the viability of bioethanol production from starch or sugar in a wide variety of crops is debatable as a replacement for fossil fuels. It has led to a debate of "food vs. fuel". There is the potential for rising food prices as food and fuel markets compete for scarce arable land. The requirement of large amount of arable land, the amount of energy to be spent and environmental pollution etc. are the major public concerns. Production of ethanol from lignocellulosic biomass promises to assuage the above concerns. Lignocellulosic biomass such as sugarcane bagasse, wheat straw, rice husk, rice straw, corn straw and other lignocellulosic biomass are explored as feedstock for ethanol production (Binod et al., 2010; Talebnia et al., 2010). To exploit the entrapped sugars from their complex polymeric forms, various pretreatment techniques have been developed viz. physical, chemical, physiochemical and biological pretreatment methods. The plant cell wall is rigid and complex structure composed of lignin, cellulose, hemicelluloses, etc. which makes them resistant to pretreatment techniques and thus leads to poor sugar yield.

So need of the hour is a source of carbohydrate-rich biomass that has simple cellular structure and doesn't compete for arable land. Algal biomass as feedstock holds the perfect future for bioethanol production because of their high carbohydrate content, cellulosic cell walls and starch based cytoplasm. Less harsh pretreatment techniques are required for saccharification for algal biomass. Moreover, lignin removal is a rate-limiting step for lignocellulosic biomass. Since there is an absence of lignin in algal biomass, it reduces the costs, time and difficulty of the saccharification process (Sarkar et al., 2012). The efficiency of fermentative bioethanol production strongly depends on the pretreatment and saccharification conditions. Under optimized conditions of pretreatment and saccharification it would lead to solubilization of carbohydrates and conversion of them to simple fermentable sugars. However, improper pretreatment and saccharification might lead to further degradation of fermentable sugars to undesirable products such as formic acid, acetic acid, and some furanic compounds (Lee et al., 2013).

13.3.1 Microbial Insight on Algae as a Source of Energy Crop

The algae are generally categorized into two groups viz. microalgae and macroalgae on the basis of their size and morphology. Algae are photosynthetic, eukaryotic organisms devoid of multicellular sex organs. It contains green chlorophyll along

Table 13.2 Ethanol yield from different feedstock (Mussato et al. 2010)

Feedstock	Ethanol yield (L ha^{-1})
Corn stover	1,050–1,400
Wheat	2,590
Cassava	3,310
Sweet sorghum	3,050–4,070
Corn	3,460–4,020
Sugar beet	5,010–6,680
Switch grass	10,760
Microalgae	46,760–140,290

with other photosynthetic pigments that give them characteristic colour which further helps in identification of key divisions. They fix atmospheric CO_2 to complex carbohydrates such as starch and cellulose via photosynthesis (Singh et al., 2011). Macroalgae also known as seaweeds are inhabitants of both intertidal and sub-tidal zone of coastal region where there is sufficient light penetration. They are composed of multiple cells organized into structures having analogy with roots, stems and leaves of higher plants. On the other hand, microalgae belongs to a large group of microscopic unicellular photosynthetic organisms. The yield of ethanol that could be achieved from microalgae is approximately 5000–15,000 gal of ethanol ac^{-1} yr^{-1} (46,760–140,290 L ha^{-1}). This yield is of higher magnitude as compared to other feedstock (Mussato et al., 2010) (Table 13.2).

Fermentative methods have been developed to utilize carbohydrate-rich microalgae for the production of bioethanol.

Major commercial advantages that are attracting researchers and entrepreneurs in the field of bioethanol production from algal biomass are:

(i) Countering the perception of "Food vs. Fuel", bioethanol production using algae wouldn't compete for either land or water.
(ii) As they are rich in carbohydrates, both marine and freshwater algae may be used for ethanol production (Singh et al., 2011) (Table 13.3).
(iii) Algae don't have lignin in their cellular ultra structure. Moreover, it has very low hemicelluloses content. This endorses for improved hydrolysis efficiency when subjected to pretreatment thus enhancing fermentation yields (Gouveia and Oliveira, 2009).
(iv) Rapid growth of algae and its versatility to grow in a variety of aquatic environments such as fresh water, saline water, or municipal wastewater are two most contrasting features that make them an ideal feedstock for bioethanol production.
(v) They have a high photosynthetic efficiency which is much higher than that of terrestrial biomass (Harun et al., 2009; Ross et al., 2008).

Table 13.3 List of algae rich in carbohydrates

Habitat	Algal source	% starch or biomass (g/dry weight)
Marine water	*Saccharina latissima*	50.0 (reserve food material)
	Green alga NKG 121701	>50.0 (starch)
	Laminaria hyperborean	55.0 (reserve food material)
	N. maculiforme TISTR 8406	30.1 (starch)
	Synechococcus sp.	15.0 (starch)
	Kappaphycus alvarezii	64 (starch)
Fresh water	*Spirogyra* sp.	43.3 (biomass after oil extraction)
	Oedigonium sp.	33.6 (biomass after oil extraction)
	Chlamydomonas reinhardtii UTEX 90	53.0 (starch)
	C. reinhardtii (UTEX2247)	45.0 (starch)
	C. reinhardtii	17.0 (starch)
	C. vulgaris	37.0 (starch)
	Chlorella sp. TISTR 8485	27.0 (starch)
	Chlorella sp. TISTR8593	22.0 (starch)
	Chlorococcum sp. TISTR8583	26.0 (starch)
	Scenedesmus sp. TISTR 8579	20.4 (starch)
	S. acutiformis TISTR 8495	16.4 (starch)
	S. acutus TISTR 8447	18.6 (starch)
	S. arcuatus TISTR 8587	12.9 (starch)
	S. armatus TISTR 8591	15.4 (starch)
	S. obliquus TISTR 8522	23.7 (starch)
	S. obliquus TISTR 8546	23.4 (starch)
	Nostoc sp. TISTR 8872	30.7 (starch)
	Nostoc sp. TISTR 8873	32.9 (starch)
	N. muscorum TISTR 8871	33.5 (starch)
	N. paludosum TISTR 8978	32.1 (starch)
	N. piscinale TISTR 8874	17.4 (starch)
	Oscillatoria sp. TISTR 8869	19.3 (starch)
	O. jasorvensis TISTR 8980	9.7 (starch)
	O. obscura TISTR 8245	12.6 (starch)
	Phormidium angustissimum	28.5 (starch)
	Spirulina fusiformis	37.3–56.1 (starch)

Steps involved in bioethanol production from algae are similar to that of cellulosic bioethanol production which requires four major unit operations viz. pretreatment, hydrolysis for saccharification, fermentation and distillation. Pretreatment process destroys the physical barriers in the cell wall. This enhances the accessibility of complex carbohydrate towards enzymatic hydrolysis. As compared to lignocellulosic biomass, algal biomass has a soft cellular organization and high moisture content rendering ease towards pretreatment. Different types of pretreatment used for algal biomass are physical, physico-chemical, chemical and biological.

13.3.2 Pretreatment and Saccharification of Algal Biomass

There are two ways of bioethanol production from algal biomass i.e. by either the sugar fermentation pathway or syngas pathway. Algal biomass on pretreatment and saccharification gives simple sugars that could be directly fermented to produce ethanol. In syngas pathway, hydrocarbons present in algal biomass are converted to syngas through gasification. The syngas thus generated could be subjected to fermentation to produce bioethanol. Such fermentation are carried out by strict autotrophic bacteria like *Clostridium ljungdahlii* (Younesi et al., 2005).

There are few important points to assess efficacy of pretreatment techniques which are as follows (Agbor et al., 2011):

(a) Quantitative and qualitative estimation of sugar and carbohydrate content in the liquid- and solid-fractions, respectively.
(b) Screening fermentation inhibitors like furfurals and neutralizing them prior to fermentation.
(c) Selecting the source from which bioethanol will be produced. It is judged on the basis of sugar and carbohydrate analyses whether liquid hydrolysates or WIS (water insoluble solids) to be taken for fermentation.
(d) Exploring pretreated samples for producing other value added products.

Crystalline structure of cellulose present in algal biomass is derived from β-D-glucopyranose units condensed by β-1,4-glycosidic bonds (Mittal et al., 2011). The initial degree of crystallinity is a crucial element to assess the pretreatment process. The distortion of crystallinity can be studied with the help of X-ray crystallography (XRD) analysis. The wide angle X-ray diffraction counts at 2θ angle close to 22° and 18° according to the Segal empirical method. The CrI can be calculated by the following equation:

$$CrI = \frac{I_{22} - I_{18}}{I_{22}} \times 100\% \tag{13.4}$$

where I_{22} is the peak intensity of the crystalline material (2θ = 22°) and I_{18} is the peak intensity of the amorphous material (2θ = 18°).

Disordered crystalline or amorphous cellulose shows higher hydrolysis rates as compared to partially disordered structure. In recent years, many studies have been performed on various pretreatment techniques to improve bioethanol fermentation.

Since algal cells are less rigid than plant cells, an extremely low acid pretreatment process is widely used. It obliterates the algal cell wall and releases carbohydrates to the liquid hydrolysates (Fig. 13.2).

Sugar recovery in such process can be maximized by cumulative optimization of three parameters viz. pretreatment time, temperature and acid concentration. It was reported that the temperature (50 to 180 °C) influences efficiency of saccharification at extremely low acid pretreatment (Lee et al., 2013). At 170 °C, extremely low acid pretreatment gave maximum glucan content of 32 % w/w using brown *Laminaria*

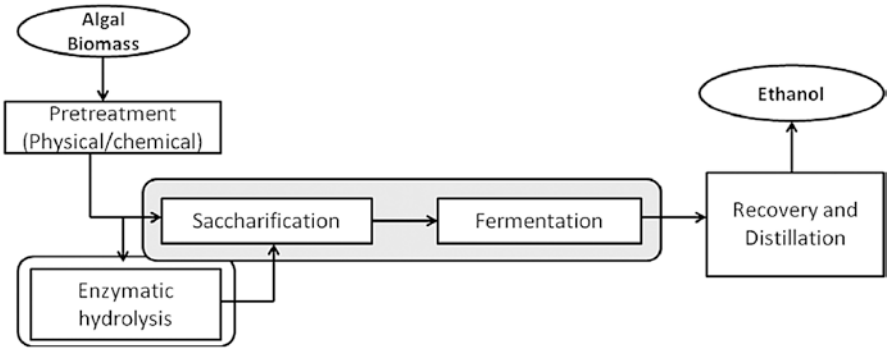

Fig. 13.2 Ethanol production process using algal biomass.

japonica biomass. In other reports, an increase in glucan content and enzyme digestibility was observed in ELA-treated *S. japonica* as compared to untreated algal biomass. Use of acids for pretreatment may lead to formation of furfural formation. Furfurals are growth inhibitors. Thus in some cases, the removal of furfurals was done prior to fermentation to avoid process inhibition. Dilute acid pretreatment of *Kappaphycus alvarezii* was treated by activated charcoal to remove hydroxyl methyl furfural (Hargreaves et al., 2013).

When acid treated algal biomass are neutralized using NaOH, it generates high concentrations of Na_2SO_4 that inhibit fermentation of *Ulvareticulate* sp. (Yoza and Masutani, 2013). Alkaline pretreatment was also explored for algal biomass pretreatment. Alkaline pretreatment of the green alga *Ulva lactuca* showed gelling property during alkaline pretreatment abolishing such pretreatment process for this algal species (Kim et al., 2011).

13.3.3 Enzymatic Hydrolysis of Pretreated Algal Biomass

Enzymatic hydrolysis has certain advantages as it does not generate or get affected by furfural content. The enzymatic liquefaction of starch in *Chlamydomonas reinhardtii* (UTEX90) could be used for dark fermentation. Hydrolytic enzymes such as α-amylase and amyloglucosidase are commercially available and are used for liquefaction and saccharification, respectively. The α-amylase disrupted the cell wall completely resulting in release of all carbohydrates. Ethanol yield was low as compared to acid pretreatment. Maximum enzymatic activity could be achieved by optimizing certain parameters like temperature, pH and time of exposure to enzyme. For *C. reinhardtii* and *U. pinnatifida,* the optimum enzymatic reaction conditions were 45 °C, pH 4.6 and 60 min for maximum extraction of glucose (Choi et al., 2010).

In biorefinery concept, the algal biomass left after extraction of lipid during biodiesel production could be a potential substrate for bioethanol production. The con-

version of defatted microalgae biomass to bioethanol could be more economically viable than direct conversion of microalgae to bioethanol. After biodiesel production, the defatted biomass of *Dunaliella tertiolecta* and *Gracilaria verrucosa* were used for bioethanol production (Lee et al., 2013). The major bottleneck in using defatted algal biomass is the difficulty of removing solvents used during biodiesel production.

13.3.4 Microbial Fermentation of Algal Biomass

After hydrolysis, glucose and mannose are yielded from cellulose whereas xylose and arabinose are yielded from hemicelluloses. From commercial aspect, microorganisms are needed to convert hemicellulosic sugars viz. xylose and arabinose into bioethanol. One of the major hindrance towards effective production of ethanol is the inability of many microorganisms to utilize pentose sugars. There are plethora of microorganisms (mainly bacteria, fungi and yeasts) that are available in nature that can utilize pentose and hexose sugars into bioethanol. Each sugar fermenting microorganisms is different with respect to very narrow substrate range, ethanol tolerance, etc. These limitations can be overcome by development of recombinant strains which are tolerant to high ethanol concentrations and are capable of metabolizing various sugars. Industrially important prominent microorganism involved in bioethanol production is *Saccharomyces cerevisiae*. It is also capable of fermenting galactose. *Brettanomyces custersii* (KCCM11490) is also an ethanol producing mold that is preferred over *S. cerevisiae* during fermentation of red algae *G. amansii*. The hydrolysates of *G. amansii* are rich in galactose. The *B. custersii* (KCCM11490) can produce high bioethanol yields from galactose as compared to other sugars. Biochemical pathway involved in bioethanol production from galactose involves three routes (Goh and Lee, 2010; Park et al., 2012): (a) The D-galactose-6-phosphate pathway; (b) The Leloir pathway; and (c) The Entner-Deudoroff pathway.

When there is abundance of mannitol in the fermentation media, *Enterobacter* sp. (JMP3) and *Escherichia coli* (KO11) are reported to use it effectively for ethanol production (Kim et al., 2011). Co-culture technique was developed where two different microorganisms were used to ferment various saccharified products. One such example is fermentation of *L. japonica* hydrolyzates using *S. cerevisiae* and *E. coli* (KO11), sequentially giving high ethanol yields. Solid state fermentation of *S. japonica* hydrolyzates was fermented with cocktail of four different yeasts (*Pichia angophorae* [KCTC 17574], *Pichia stipitis* [KCTC 7228], *S. cerevisiae* [KCCM 1129] and *Pachysolen tannophilus* [KCTC 7937]) with *Bacillus* sp. (JS1) for ethanol production (Jang et al., 2012). Saccharification was performed by *Bacillus licheniformis* and the sugars thus generated were used by the four different types of yeasts to produce ethanol. Highest yield of ethanol is reported by *P. angophorae*. The oxidation of glucose via glycolytic pathway forms pyruvate along with NADH and ATP. Pyruvate is converted to ethanol and CO_2 under anaerobic conditions. The

pyruvate is converted to acetaldehyde. The reaction is catalyzed by pyruvate decarboxylase enzyme. Acetaldehyde is then reduced to ethanol. This reaction is catalyzed by alcohol dehydrogenase. This conversion of pyruvate to ethanol can also be affected by intracellular electron balance i.e. $NADH/NAD^+$ ratio (Wang et al., 2013). *Zymomonas mobilis* is a well known bacterium capable of giving high ethanol yield. A strategy was developed to introduce pdc (pyruvate decarboxylase) and adhB (alcohol dehydrogenaseII) genes from *Zymomonas mobilis* into *E. coli*. This leads to development of an ethanologenic strain, *E. coli* (KO11).

13.3.5 Future Prospects of Algal Ethanol

With the encouraging trends in the field of algal ethanol production, many entrepreneurs are focusing on commercialization of this process. The major bottleneck for bioethanol production in commercial scale is availability of algae at very large quantities and at very low cost. Through breakthrough technological innovations and advancement, algal bioethanol can be produced in commercial scale. There are several roadblocks in the algae-to-ethanol technology. In open pond cultivation, the chance of external contamination arises. This may lead to a situation where the desired algae has been consumed by predators like *Paramecium* sp. or other protozoan or other stronger algal species have dominated the cultivation pond. Transgenic algae have commercially important traits but they are less fit for open cultivation. It is hypothesized that usage of transgenic extremophilic algae may be more robust and have better chances of survival in open pond culture. In such extreme conditions, the contaminants/competitors would be limited to minimum. The high cost of enzymatic hydrolysis of starch/cellulose also makes the cost of algal bioethanol several folds higher. Genetically modifying microalgal strain in such a way that it accumulates higher amount of starch/cellulose which could make them more lucrative commercially.

Application of synthetic biology could open a lead to paraphernalia of enzymes in microalgae that might help in saccharification of stored starch and cellulose. This would minimize the need of external enzymatic hydrolysis. The up regulation or over expression of biosynthesis pathway for starch/cellulose accumulation would definitely increase algal biofuel production potential. Dependence on large amount of fresh water for production of algal biomass might compete for fresh water requirements of crops and human consumption. By the year 2050, commercial bioenergy production is expected to consume 18–46 % of fresh water resources. Globally, world is facing shortage of fresh water recourses. Since 70 % of our earth is covered with marine water, development of high salt and temperature tolerant microalgae could be the potential candidate for mass production (Sheridan, 2009; Waltz, 2009). Places with greater availability of coastlines are the ideal regions for trapping sunlight and thus wouldn't compete for terrestrial lands. Thus the path of sustainable growth and energy generation can be realized truly by algal biofuel technologies.

13.4 Conclusion

Biodiesel from algal biomass has promising potential towards commercialization. The techniques that are currently used for biodiesel production are still trivial in terms of net energy balance. A careful assessment of the life cycle energy balances is required to study the sustainability of the process. Information regarding large scale demonstration of biodiesel production are scares which lead to incorrect economic assessments. Thus large scale pilot studies should be conducted under realistic setups which would include typical weather conditions. This would be useful for estimating biodiesel productivities. Innovation and breakthroughs are still required for development of design and technologies that would lead to costs reduction. Selection of strains for high lipid concentration which could be amicabily adapted to regional conditions along with genetic improvement could eventually make this process economically viable. A biofinery concept encompasses the vision where spent biomass after lipid extraction could be used for production of alternative bulk or fine chemical production. At present, emphasis is given on the production of bioproducts such as bioethanol or biomethane from lipid-extracted algal biomass. Since this approach utilizes complete waste resources, the overall energy conversion efficiency increases.

Bioethanol production from biomass is generally focused on utilization of lignocellulosic biomass. The lignocellulosic biomass for bioethanol production are cheap, easily available and renewable. But the production of the second-generation bioethanol is commercially not viable because of their recalcitrant nature. The algal biomass as a raw material for bioethanol production could become a sustainable and eco-friendly resource for renewable biofuel production. Currently, commercial production of bioethanol from algae is not feasible because of low product yield when compared to other conventional substrates. Issues pertaining to high costs of algae cultivation such as algae cultivation, harvesting, and biomass pretreatment are the major bottlenecks towards its commercialization. It is high time for human civilization that needs to take decisive steps on issues related to climate and environment and maintain a sustainable growth. Marine algae cultivation hold a promising avenue towards this endeavour.

References

Agbor, V.B., Cicek, N., Sparling, R., Berlin, A. and Levin, D.B. (2011). Biomass pretreatment: Fundamentals toward application. *Biotechnol. Adv*., 29, 675–685.

Amaro, H.M., Guedes, A.C. and Malcata, F.X. (2011). Advances and perspectives in using microalgae to produce biodiesel. *Appl. Energy*, 88, 3402–3410.

Banerjee, A., Sharma, R., Chisti, Y. and Banerjee, U.C. (2002). *Botryococcus braunii*: A renewable source of hydrocarbons and other chemicals. *Crit. Rev. Biotechnol.*, 22, 245–279.

Belarbi, E.-H., Molina, E. and Chisti, Y. (2000). RETRACTED: A process for high yield and scaleable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil. *Process Biochem*., 35, 951–969.

Binod, P., Sindhu, R., Singhania, R.R., Vikram, S., Devi, L., Nagalakshmi, S., Kurien, N., Sukumaran, R.K. and Pandey, A. (2010). Bioethanol production from rice straw: An overview. *Bioresour. Technol.*, 101, 4767–4774.

Brown, L.M. and Zeiler, K.G. (1993). Aquatic biomass and carbon dioxide trapping. *Energy Convers. Manag.*, 34, 1005–1013.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnol. Adv.*, 25, 294–306.

Choi, S.P., Nguyen, M.T. and Sim, S.J. (2010). Enzymatic pretreatment of *Chlamydomonas reinhardtii* biomass for ethanol production. *Bioresour. Technol.,* 101, 5330–5336.

Demirbas, A. (2007). Importance of biodiesel as transportation fuel. *Energy Policy*, 35, 4661–4670.

Ghirardi, M. (2000). Microalgae: A green source of renewable H_2. *Trends Biotechnol*., 18, 506–511.

Goh, C.S. and Lee, K.T. (2010). A visionary and conceptual macroalgae-based third-generation bioethanol (TGB) biorefinery in Sabah, Malaysia as an underlay for renewable and sustainable development. *Renew. Sustain. Energy Rev*., 14, 842–848.

Gouveia, L. and Oliveira, A.C. (2009). Microalgae as a raw material for biofuels production. *J. Ind. Microbiol. Biotechnol*., 36, 269–274.

Hargreaves, P.I., Barcelos, C.A., da Costa, A.C.A. and Pereira, N. (2013). Production of ethanol 3G from *Kappaphycus alvarezii*: Evaluation of different process strategies. *Bioresour. Technol.*, 134, 257–263.

Harun, R., Danquah, M.K. and Forde, G.M. (2009). Microalgal biomass as a fermentation feedstock for bioethanol production. *J. Chem. Technol. Biotechnol*., 85(2), 199–203.

Helwani, Z., Othman, M.R., Aziz, N., Kim, J. and Fernando, W.J.N. (2009). Solid heterogeneous catalysts for transesterification of triglycerides with methanol: A review. *Appl. Catal. A Gen.*, 363, 1–10.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M. and Darzins, A. (2008). Microalgal triacylglycerols as feedstocks for biofuel production: Perspectives and advances. *Plant J.*, 54, 621–639.

İçöz, E., Mehmet Tuğrul, K., Saral, A. and İçöz, E. (2009). Research on ethanol production and use from sugar beet in Turkey. *Biomass and Bioenergy*, 33, 1–7.

Jang, J.-S., Cho, Y., Jeong, G.-T. and Kim, S.-K. (2012). Optimization of saccharification and ethanol production by simultaneous saccharification and fermentation (SSF) from seaweed, *Saccharina japonica. Bioprocess Biosyst. Eng.*, 35, 11–18.

Khan, S.A., Hussain, M.Z., Prasad, S. and Banerjee, U.C. (2009). Prospects of biodiesel production from microalgae in India. *Renew. Sustain. Energy Rev.*, 13, 2361–2372.

Kim, N.-J., Li, H., Jung, K., Chang, H.N. and Lee, P.C. (2011). Ethanol production from marine algal hydrolysates using *Escherichia coli* KO11. *Bioresour. Technol.*, 102, 7466–7469.

Knothe, G. (2006). Analyzing biodiesel: standards and other methods. *J. Am. Oil Chem. Soc*., 83, 823–833.

Lee, O.K., Kim, A.L., Seong, D.H., Lee, C.G., Jung, Y.T., Lee, J.W. and Lee, E.Y. (2013). Chemoenzymatic saccharification and bioethanol fermentation of lipid-extracted residual biomass of the microalga, *Dunaliella tertiolecta. Bioresour. Technol*., 132, 197–201.

Li, Y., Horsman, M., Wang, B., Wu, N. and Lan, C.Q. (2008). Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans. Appl. Microbiol. Biotechnol.*, 81, 629–636.

Macario, A., Giordano, G., Onida, B., Cocina, D., Tagarelli, A. and Giuffrè, A.M. (2010). Biodiesel production process by homogeneous/heterogeneous catalytic system using an acid–base catalyst. *Appl. Catal. A Gen*., 378, 160–168.

Marchetti, J.M., Miguel, V.U. and Errazu, A.F. (2007). Possible methods for biodiesel production. *Renew. Sustain. Energy Rev.*, 11, 1300–1311.

Mata, T.M., Martins, A.A. and Caetano, N.S. (2010). Microalgae for biodiesel production and other applications: A review. *Renew. Sustain. Energy Rev.*, 14, 217–232.

Metzger, P. and Largeau, C. (2005). *Botryococcus braunii*: A rich source for hydrocarbons and related ether lipids. *Appl. Microbiol. Biotechnol.*, 66, 486–496.

Mittal, A., Katahira, R., Himmel, M.E. and Johnson, D.K. (2011). Effects of alkaline or liquid-ammonia treatment on crystalline cellulose: Changes in crystalline structure and effects on enzymatic digestibility. *Biotechnol. Biofuels*, 4, 41.

Moellering, E.R. and Benning, C. (2010). RNA interference silencing of a major lipid droplet protein affects lipid droplet size in *Chlamydomonas reinhardtii. Eukaryot. Cell*, 9, 97–106.

Mussato, S.I., Dragone, G., Guimarães, P.M., Silva, J.P.A., Carneiro, L., Roberto, L.M., Vicente, A., Domingues, L. and Teixeira, J.A. et al. (2010). Technological trends, global market, and challenges of bio-ethanol production. *Biotechnol. Adv*., 28, 817–830.

Nagle, N. and Lemke, P. (1990). Production of methyl ester fuel from microalgae. *Appl. Biochem. Biotechnol.*, 24–25, 355–361.

Park, J.-H., Hong, J.-Y., Jang, H.C., Oh, S.G., Kim, S.-H., Yoon, J.-J. and Kim, Y.J. (2012). Use of *Gelidium amansii* as a promising resource for bioethanol: A practical approach for continuous dilute-acid hydrolysis and fermentation. *Bioresour. Technol*., 108, 83–88.

Ross, A.B., Jones, J.M., Kubacki, M.L. and Bridgeman, T. (2008). Classification of macroalgae as fuel and its thermochemical behaviour. *Bioresour. Technol*., 99, 6494–6504.

Sarkar, N., Ghosh, S.K., Bannerjee, S. and Aikat, K. (2012). Bioethanol production from agricultural wastes: An overview. *Renew. Energy*, 37, 19–27.

Sheridan, C. (2009). Making green. *Nat. Biotechnol*., 27, 1074–1076.

Singh, A., Nigam, P.S. and Murphy, J.D. (2011). Renewable fuels from algae: An answer to debatable land based fuels. *Bioresour. Technol.*, 102, 10–16.

Singh, S., Kate, B.N. and Banerjee, U.C. (2008). Bioactive Compounds from Cyanobacteria and Microalgae: An Overview. *Crit. Rev. Biotechnol.*, 25(3), 75–95.

Suppes, G. (2004). Transesterification of soybean oil with zeolite and metal catalysts. *Appl. Catal. A Gen*., 257, 213–223.

Talebnia, F., Karakashev, D. and Angelidaki, I. (2010). Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation. *Bioresour. Technol*., 101, 4744–4753.

Vyas, A.P., Verma, J.L. and Subrahmanyam, N. (2010). A review on FAME production processes. *Fuel*, 89, 1–9.

Waltz, E. (2009). Biotech's green gold? *Nat. Biotechnol*., 27, 15–18.

Wang, J., Kim, Y.M., Rhee, H.S., Lee, M.W. and Park, J.M. (2013). Bioethanol production from mannitol by a newly isolated bacterium, *Enterobacter* sp. JMP3. *Bioresour. Technol*., 135, 199–206.

Xu, H., Miao, X. and Wu, Q. (2006). High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. *J. Biotechnol*., 126, 499–507.

Yoo, C., Jun, S.-Y., Lee, J.-Y., Ahn, C.-Y. and Oh, H.-M. (2010). Selection of microalgae for lipid production under high levels carbon dioxide. *Bioresour. Technol*., 101, 71–74.

Yoon, S.H. and Lee, C.S. (2011). Lean Combustion and Emission Characteristics of Bioethanol and Its Blends in a Spark Ignition (SI) Engine. *Energy & Fuels*, 25, 3484–3492.

Younesi, H., Najafpour, G. and Mohamed, A.R. (2005). Ethanol and acetate production from synthesis gas via fermentation processes using anaerobic bacterium, *Clostridium ljungdahlii. Biochem. Eng. J*., 27, 110–119.

Yoza, B.A. and Masutani, E.M. (2013). The analysis of macroalgae biomass found around Hawaii for bioethanol production. *Environ. Technol*., 34, 1859–1867.

Zabeti, M., Daud, W.M.A.W. and Aroua, M.K. (2010). Biodiesel production using alumina-supported calcium oxide: An optimization study. *Fuel Process. Technol*., 91, 243–248.