Korean J. Chem. Eng., **30**(12), 2119-2126 (2013)
DOI: 10.1007/s11814-013-0161-1
pISSN: 0256-1115
eISSN: 1975-7220

REVIEW PAPER

Advances in cultivation and processing techniques for microalgal biodiesel: A review

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(*Received 1 June 2013 • accepted 26 August 2013*)

Abstract−The key technologies for producing microalgal biodiesel include microalgae screening, economical cultivation, and efficient methods in lipid extraction and conversion. Recent advances in microalgae cultivation, lipid extraction, and biodiesel preparation are reviewed in this work, with emphasis on photosynthetic metabolisms, separation efficiency and catalytic kinetics. The mutual exclusion between lipid accumulation and fast growth limits total lipid productivity, while only triglycerides in neutral lipids are converted to biodiesel through transesterification. The hurdles in large scale culture and low neural lipids yield are discussed, as well as the relationship between high unsaturation and fuel properties. This review aims to provide technical information to guide strain screening and lipid conversion for microalgal biodiesel industry.

Key words: Microalgal Oil, Poly-unsaturated Fatty Acids (PUFAs), Biodiesel, Lipid Extraction, Purification

Many countries and regions established sustainability targets in CO_2 reduction under the Kyoto Protocol, facing new opportunities in biofuels production to diversify income and fuel supply sources with reduced green house decarbonisation process [1-4]. Better than the first generation biofuels (mainly from food and oil crops), second generation biofuel consumes waste residues with reduced land requirements due to their presumed higher energy yields per hectare [5-10]. Third generation technology (based upon microalgae or cyanobacteria) yields oil of many fold higher than plant systems [11-15], with advantages of a higher growth rate, a shorter maturity [13,15], a higher biomass production rate [12], and low environmental impacts. Together with the selection of the economically valuable microalgae, it is of great importance to establish optimal operation strategy in the separation, purification, and conversion. Researchers have studied cultivation [16], harvesting [17], and processing techniques, together with metabolic engineering and genetic methods [18,19], to improve productivity, specialty and energy value in utilizing water unsuitable for human consumption [8-10,20]. This review addresses the current status of biodiesel production from microalgae, in which microalgae screening and culture, lipid purification, and lipid conversion are the main technical issues, covering the advances not only in the feedstock (cultivating and harvesting), but also in the conversion methods (purification, catalysis, and analysis).

THE SCREENING, METABOLIC REGULATION AND CULTURE OF MICROALGAE

For the goals of high lipid content, fast growth, good adaptability, and specific lipid composition, most researchers reported the screening and culturing of microalgae mainly by conventional methods instead of molecular ones, though the genetic, metabolic and enzymatic characteristics in photosynthetic production are being extensively studied [21,22]. Genetic engineering of key enzymes in specific fatty acid production pathways within lipid biosynthesis concerns the improvement of both quantity and quality of lipids, e.g., by up-regulation of fatty acid biosynthesis or by down regulation of beta-oxidation. By knocking out or modifying enzymes responsible for the synthesis of polyunsaturated lipids in the cell, it should be possible to dramatically increase the proportion of monounsaturated lipids, though the long-time and less liability make the genetic modification a difficult technology in microalgae screening. The rate at which triglyceride and free fatty acid are produced by microalgae may be a more important factor than simply total lipid content, predicting the necessity of checking the productivity of triglycerides and free fatty acids, other than the mere consideration of total lipid content during the microalgae screening.

Metabolic engineering was used to improve microalgae, concerning the fast growth, high oil content or fatty acid composition. By converting water, carbon dioxide into oxygen and biomass, several known species of microalgae accumulate significant amounts of lipids [23], though the change in protein content is associated with the change in total lipid content. The carbon source required for the cultivation represents 60% of the costs for the nutrients, showing the ability of fixing 183 tons of CO_2 by 100 tons of microalgae biomass [24]. Similar to the mutual exclusion between high lipid content and high microalgal productivity, the percentage of lipid, carbohydrate and protein in living microalgae cells changes unevenly. Keeping the protein content constant for some time to facilitate the adaption process, the carbohydrates content preferentially decreases as the lipid content increases, while the percentage of other components goes down as shown in the reported relative proportions of protein, lipid and carbohydrate [24-28]. Since protein molecules set the level of the cell's metabolism, a triangular plot can be made of the proportions of lipid, protein and carbohydrate content in microal-

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‡This paper is dedicated to commemorate Prof. Ji-Won Yang (KAIST) for his retirement.

gae [24-28]. The average values for active growth are lipid 24.2%, protein 48.3%, carbohydrate 27.5%, with a shift in composition from unlimited logarithmic growth to N-limited growth, concerning the trajectory on increasing lipid composition at the cost of the consumption of protein and carbohydrate. Proteins preferentially run down as the carbohydrates decrease in relative proportions. Among microalgal species, lipid content for the nitrogen limiting growth can reach theoretically up to 80% by dry weight, while levels of 20-50% are quite common [26]. Assuming that microalgae that had the typical composition ($C_{106}H_{181}O_{45}N_{16}P$), 16 : 1 (N : P, molar ratio) was suitable for the nutrients, though high productivity with efficient nutrient availability in culture media could be achieved even at relatively low N:P ratios (from 4 : 1 to 40 :1) for some microalgal species [28]. The growth media must provide nitrogen, phosphorus, iron and in some cases silicon [28]. Estimated maximum lipid production and growth rates are valuable for process design and management to allow culture dilution rates to be optimized without deleterious effect on overall lipid yield. Generally, microalgal cultures in the exponential growth phase contain more protein, while cultures in the stationary phase have more carbohydrates and glycogen. The growth factors include not only organic carbon (such as sugars, proteins and fats), vitamins, salts and other nutrients (nitrogen and phosphorous), but also equilibrium between operational parameters (oxygen, carbon dioxide, pH, temperature, light intensity, and product and byproduct removal). Water pH is a function of microalgal productivity, respiration, the alkalinity and ionic composition of the culture media, and the efficiency of CO_2 addition. By enhancing ammoniacal nitrogen removal from the media via ammonia volatilization and phosphorous removal through phosphate precipitation with unchelated ferric iron, calcium and magnesium, water pH affects many of the biochemical processes associated with microalgal growth and metabolism, as the equilibrium shift to higher free ammonia concentrations. A pH above or below 8 (the optimal pH) decreases not only microalgal growth but also nitro-

Table 1. Lipid content and productivities of different microalgal species under different cultivation conditions

	Lipid content (% dry cell weight)	Carbohydrate/ protein (wt%)	Lipid productivity (mg/l/day)[a]	Volumetric productivity of biomass (g/L/day)	Cultivation condition[b]	References
Ankistrodesmus sp.	24.0-31.0	25/43	12.4	0.29	P	[37]
Botryococcus braunii	33.6	18.9/17.8	5.5	0.03	P	[38]
Chaetoceros muelleri	25.0-63.0	19.3/46.9	21.8	0.07	P	[37]
Chaetoceros calcitrans	19.0-22.0	-	17.6	0.04	P	[37]
Chlorella emersonii	5.0-58.0;	26/44	10.3-50.0	0.036-0.041	P	[39]
Chlorlla protothecoides	10.0-48.0	8.70/41.60	0.2-5.4	2.00-7.70	P	[36]
Chlorlla protothecoides	43.0-46.0	-	1881.3-1840.0	0.01	H	[30]
Chlorella sorokinian	2.0	-	44.7	0.23-1.47	P	[36]
Chlorella vulgaris	18.0-57.0	18/22	11.2-66.3	0.02-0.20	P	[36, 39, 40]
Chlorella vulgaris	23.0-36.0	-	27.0-35.0	0.08-0.15	H	[30]
Chlorella vulgaris	21.0-34.0	-	22.0-54.0	0.09-0.25	M	[30]
Chlorela sp.	19.3	19/48	42.1	21.6-34.0	P	[37]
Chlorell pyrenoidosa	11.76	-	34.8	2.90-3.64	P	[37]
Chlorococuum sp.	20.0-51.1	22/41	53.7	0.28	P	[37]
Crythecodinium cohnii	6.0-45.0	-	28.0	0.20	P	[41]
Dunaliella salina	23.1	32/57	116.0	0.20-0.34	P	[41]
Dunaliella primolecta	10-71	19/48	36.4	0.09	P	[37]
Dunaliella tertiolecta	17.5-67.0	-	60.6-69.8	0.12	P	[40]
Dunaliella sp.	27.4	5/47	33.5	0.20	P	[37]
Elliposodium sp.	14.0-20.0	20/46	47.3	0.17	P	[37]
Euglena gracilis	25.0	18/39	32.4	7.70	P	[37]
Haematcocuus pluvialis	13.58	18/34	-	0.05-0.06	P	[17]
Isochrysis galbana	7.0-40.0	26.8/47.9	-	0.32-1.60	P	[19, 37]
Isochrysis sp.	7.1-33	12.9/50.8	37.8	0.08-0.17	P	[37, 41]
Monodus subterraneus	16.0	22/33	30.4	0.19	P	[37]
Monalantus salina	20.0-22.0	17/49	-	0.08	P	[37]
Nannochloropsis sp.	20.0-56.0	19.81/32.82	84.0-142.0	0.37-0.48	P	[35]
Nannochloropsis oculata	22.7-29.7	23/44	37.6-90.0	0.17-1.43	P	[40]
Nannochloropsis sp.	12.0-53.0	28/48	90.0-134.0	0.18	P	[37]
Neochloris oleoabundans	29.0-65.0	9.2/16.8	-	0.20	P	[37]
Nitzschia sp.	16.0-47.0	26/48	-	0.24	P	[37]
Ocystis pusilla	10.5	19/44	49.4	0.16	P	[37]

Table 1. Continued

	Lipid content (% dry cell weight)	Carbohydrate/ protein (wt%)	Lipid productivity (mg/l/day)[a]	Volumetric productivity of biomass (g/L/day)	Cultivation condition[b]	References
Pavlova salina	30.9	28/42	31.2	0.14	P	[37]
Pavalova lutheri	35.5	28/42	40.2	0.18	P	[37]
Phaeodactylum tricornutum	18.0-57.0	28/42	29.2	0.14	P	[37]
Porphyridium cruentum	10.37	28/42	40.2	0.13	P	[37]
Scenedesmus obliquus	11.0-55.0	15/50	7.14	0.04-0.74	P	[40, 42]
Scenedesmus obliquus	6.6-11.8	-	11.6-58.6	0.10-0.51	M	[42]
Scenedesmus quadricauda	1.9-18.4	14/47	35.1	0.19	P	[37]
Scenedesmus sp.	19.6-21.1	21/18	40.8-53.9	0.03-0.26	P	[37-38]
Skeletonema sp.	13.3-31.8	22/38	27.3	0.09	P	[37]
Skeletonema costatum	13.5-51.3	22/38	17.4	0.08	P	[37]
Spirulina platensis	4.0-16.6	14/26	14.2	0.06-4.3	P	[37]
Spirulina maxima	4.0-9.0	13/46	21.0	0.21-0.25	P	[40]
Tetraselmis suecica	12.78	33/57	27.0-36.4	0.12-0.32	P	[37]
Tetraselmis sp.	8.5-23.0	28/50	43.4	0.30	P	[37]
Thalassiosira pseudonana	12.6-14.7	12/44	17.4	0.08	P	[37]

[a]The neutral lipid content in microalgae shift to lipid storage when under environmental stress, being not predominantly straight chain saturated hydrocarbons but multi-branched and/or polyunsaturated components. Overall lipid production will be more complex than this simple analysis, together with many other considerations, suggesting high lipid content alone cannot be regarded as the sole, perhaps not even the major, factor in searching for suitable microalgal strains or optimum growth conditions

[b]The abbreviation: P is phototrophic, M is mixotrophic, and H is heterotrophic, respectively. Most of the species listed in Table 1, were cultivated under phototrophic condition

gen removal [28-30]. The carbohydrate content diminishes as the lipid content increases, concerning the ratio of protein/carbohydrate, which is taken as a growth factor in Table 1, showing much variance between species. Note that the data is only for reference, concerning the experimental errors by different methods and different researchers, especially when lipid yield is treated as a key index in overall economic evaluation [29]. The cultivation factors on microalgal growth have been reported by various authors [30], including abiotic factors such as light (quality, quantity), temperature, nutrient concentration, O_2, CO_2, pH, salinity, and toxic chemicals; biotic factors such as pathogens (bacteria, fungi, viruses) and microalgae competition; operational factors such as shear produced by mixing, dilution rate, depth, harvest frequency, and addition of bicarbonate [18]. Temperature is the most important limiting factor, after light, for culturing microalgae in both closed and open outdoor systems, since exceeding the optimum temperature may result in the total culture loss [31]. To avoid osmotic stress, ion stress, and changes of the cellular ionic ratios due to the membrane selective ion permeability, the easiest way for salinity control is adding fresh water or salt [32]. Mixing homogenizes the cells distribution, heat, metabolites, while high liquid velocities and degrees of turbulence can damage microalgae due to shear stress [33]. A better growth was reported in airlift bioreactor than in an unaerated column, showing an increase in cell density and specific growth rate with an increase in the light intensity up to a certain limit, above which the growth was inhibited [34]. Cultivation under nitrogen deficient conditions increased the neutral lipid contents, while the greatest neutral lipid production occurred in the resting stage and the greatest amount showed in the conversion from the green to the brown growth phase. In general, nitrate deficiency reduces the protein content and the chlorophyll level, showing a species-specific change in the content of carbohydrates and lipids [34,35]. Commercial microalgae cultivation is commonly carried out in open system using solar energy as the light source. Although many research works in the laboratory scale cultivation have been made to develop a cost-effective photobioreactor, artificial illumination remains a major problem, let alone the high cost of installing and facility repairing, high power consumption, and high operating cost [11]. Furthermore, the light conversion efficiency has been limited, due to the generation of a considerable amount of heat in close contact with conventional light sources. Some microalgal species showed higher lipid content during heterotrophic growth, and a 40 wt% increase in lipid content was obtained in *Chlorella protothecoides* than that in phototrophic cultivation [21]. Microalgae can assimilate a variety of organic carbon sources (such as glucose, acetate, glycerol, fructose, sucrose, lactose, galactose, and mannose) for growth, and give a lipid productivity as high as 179 mg/L/d, which is nearly 20 times higher than the average value, by *Chlorella* sp. under phototrophic cultivation [23]. The main difference between mixotrophic and photoheterotrophic cultivation is that the latter requires light as the energy source. Phototrophic cultivation is most frequently used, due to the advantage of uptake CO_2 from the flue gas of factories in an open pond system, though the lipid productivity of this approach is usually lower than that of heterotrophic cultivation [26].

Table 1 presents significant differences between the various species, indicating that heterotrophic growth is beneficial for better oil productivity than other cultivation conditions. To show the general trend of the complex interaction, the contents of carbohydrate and protein are also given in Table 1. Actual lipid content depends on

growing conditions and growth phase. Lipid production rates are the greatest during the period of high growth rate of microalgae biomass, since the lipid production rate dramatically decreases as the microalgal culture reaches the late stationary phase at the death phase. For microbial oil production, both phototrophic and heterotrophic cultivation are restricted by contamination risk and light requirement for a special photo-bioreactor in large scale operation [23].

Though many factors are listed in Table 1, only lipid content is attracting much attention during microalgae screening, due to the direct relationship with biofuel output. The lipid index in Table 1 is manipulated by microalgae growth with profound metabolism mechanics. Lipid productivity and volumetric productivity of biomass could be improved, to some extent, during the culture process. The ratio of carbohydrate to protein is actually an important growth factor, concerning its key roles in lipid reserve metabolism and growth regulation [35]. As shown in Table 1, oil content in microalgae can reach 75% by weight of dry biomass but is associated with low productivities.

Among the above-mentioned species, some species such as *Chlorella* sp., *Nannochloropsis* sp., and *Dunaliella salina* seem to be good options of microalgae resources for biodiesel production.

LIPID EXTRACTION

Lipid content of some species of microalgae is between 30 and 100 times higher than that of conventional biodiesel sources, while the abundance (% of total lipids) of dominant fatty acids in total lipid, neutral lipid, glycolipid, phospholipid are different [15]. Lipid

Table 2. Some extraction methods and their effectiveness at recovering lipids

Method	Species	Lipid yield (%)	Fatty acids (%)	NL/GL/PL (% of total lipids)	References
Bligh and Dyer	*Alexandrium minutum*	22.4	C16:1-52.0	2/12/4	[43]
Solvent/saponification	*Ankistrodesmus* sp.	24.6	C16:1-55; C18:1-38	28/18/2	[43]
Super -critical CO_2	*Arthropira maxma*	2.1	GLA-31.3	30/14/2	[43]
Super -critical CO_2	*Arthropira maxma*	40	GLA-13.0	32/12/8	[43]
Bligh and Dyer	*Chaetoceros muelleri*	33.6	C16:1-46.5	42/18/6.2	[43]
Bligh and Dyer	*Chaetoceros calcitrans*	39.8	C16:1-45.9	37/36/25	[44]
Soxhlet	*Chaetoceros* sp.	27.2	C16:0, 1-45.6	42/18/6.8	[43]
Chloroform/methanol	*Chlorella emersonii*	63	C18:3-30.2	44.2/6.4/2.2	[36]
Chloroform/methanol	*Chlamydomonas*	40.2	C18:1-28.7	48/18/10.2	[45]
Bead beater	*Chlorlla protothecoides*	18.8	C18:1, 2, 3-45.4	44/12/6.2	[45]
Super -critical CO_2	*Chlorlla protothecoides*	14.9	C16:0-17.7	45/18/10	[43]
Wet milling	*Chlorlla protothecoides*	14.4	C18:3-28.9	38/18/6	[43]
Sonnication	*Chlorlla protothecoides*	10.7	C18:1, 2, 3-46.8	26/16/8	[43]
Soxhlet	*Chlorlla protothecoides*	5.6	C18:1, 2, 3-47.4	28/18/4.6	[45]
Bligh and Dyer	*Chlorella sorokinian*	46.2	C18:1, 2, 3-49.4	32/18/4.4	[43]
Bligh and Dyer (dry)	*Chlorella vulgaris*	52.5	C16:0-20.4	38/12/4.3	[43]
Bligh and Dyer	*Chlorell pyrenoidosa*	28.4	C18:1, 2, 3- 44.8	38/14/8	[43]
Bligh and Dyer	*Chlorella* sp.	26.4	C18: 3-29.6; EPA-10.6	32/4.6/2.2	[43]
Supercritical CO_2	*Crypthecodinium cohnii*	8.6	DHA-42.7	34/14/6	[47]
Bligh and Dyer	*Crypthecodinium cohnii*	19.9	DHA-49.5	36/12/8	[46]
Bligh and Dyer	*Dunaliella salina*	28.2	C18:1, 2, 3-48.3	46/14/4.3	[46]
Bligh and Dyer	*Dunaliella tertiolecta*	19.0	C18:1, 2, 3- 47.9	44/10.3/8.4	[43]
Bligh and Dyer	*Dunaliella* sp.	22.6	C16:0-41.7; C18:3-30.5	44/12/8	[48]
Bligh and Dyer	*Dunaliella viridis*	28.2	C18:1, 2, 3-34.6	44/8.6/4.2	[43]
Bligh and dyer	*Ellipsodion* sp.	27.4	C18:1, 2, 3-46.6	46/12/6	[37]
Bligh and dyer	*Euglena gracilis*	24.4	C18:1, 2, 3-26.6	72.5/12.5/13.0	[43]
Supercritical CO_2	*Haematcocuus phuvialis*	26.4	C18:1, 2, 3-42.4	32/12/8	[43]
Bligh and Dyer	*Nannochloropsis salina*	34.8	C16:1-40.8	65/14/18	[43]
Bligh and Dyer	*Nannochloropsis oculata*	18.0	C16:0-90.8	9.0/2.1/2.0	[37]
Bligh and Dyer	*Nannochloropsis* sp.	24.4-29.2	AA-9.6; EPA-32.1	38/16/8	[49-51]

1, Neutral lipids (NL); Glycolipids (GL); Phospholipids (PL); γ-linolenic acid (GLA); Arachidonic acid (AA), Eicosapentaenoic acid (EPA); Docosahexaenoic acid (DHA)

2, Concerning one of the main challenges in processing lipid-producing microalgae is to efficiently extract lipids from the cells, This table summarizes extraction methods, which include mainly traditional solvent extraction and supercritical fluid extraction. There are a few well-documented procedures for extracting oil from microalgae by mechanical pressing, homogenization, milling, solvent extraction, supercritical fluid extraction, enzymatic extractions, ultrasonic-assisted extraction and osmotic shock. All of these methods have their individual benefits and drawbacks [46]

can be separated into three fractions, neutral lipids, glycolipids, and phospholipids [36-41]. Fatty acid chains cannot easily be separated, due to the similarities in molecular structure and chemical properties. In the unit operation of lipid extraction, protein, carbohydrate and salt were removed from the microalgae cells, producing a mixture of neutral lipids, glycolipids and phospholipids. The overall fatty acid composition in microalgal lipids is possibly different from the fatty acid composition in microalgal biodiesel, and only triglycerides in neutral lipids are converted to biodiesel by methanol transesterification [36-39]. Fatty acid in glycolipids and phospholipids could be utilized in methanol esterification, at the high cost of mass loss in purification. Although many methods for microalgal lipid extraction have been recommended for higher efficiency, less extraction time, low or moderate costs, and minimal toxicity, the most popular is similar to the Bligh and Dyer method [42,43]. Solvent extraction is still the main extraction method due to its simplicity and being relatively inexpensive requiring almost no investment for equipment. Non-solvent extraction technologies include the use of pulse electric field, enzymes, microwaves, ultrasonic energy and mechanical disruption. Supercritical fluids extract high-value products from microalgae, producing highly purified extracts that are free of potentially harmful solvent residues, with the advantage of quick extraction and separation, as well as the protection of thermally sensitive products [44-51].

The main targets in Table 1 are fast microalgae growth and high lipid content, of which the latter restricts the maximum yield of microalgal lipids in Table 2. Microalgae membranes are mainly constructed from phospholipids and glycolipids, where the hydrophilic polar phosphate or sugar moieties and the level of saturation of the fatty acyl chains determine the fluidity of the membranes. Long-chain polyunsaturated fatty acids (PUFAs), being pharmacologically important for dietetics and therapeutics, are susceptible to oxidation during storage, reducing their acceptability for vehicle use (European standard EN 14214 limits to 12%). Iodine value, representing total unsaturation, must not exceed 120 g iodine/100 g biodiesel, according to the European standard.

Solvent extraction may not always be the best solution for recovering oil from microalgae biomass when the safest and most environmentally sustainable manner is concerned. The raw microalgal oil, containing free fatty acid in the range of 20-50% (wt%, total lipid bases), tends to form soap in alkaline catalyst based transesterification process and prevents separation of biodiesel and glycerol [23,28,37,38]. Cell disruption causes no clear differences in total extracted lipids [37]. Bead beating gives a higher amount of total lipids during the first extraction, which recovers almost all neutral lipids, with small differences present in fatty acid composition of glycolipid and phospholipid [37]. An efficient extraction requires the solvent to fully penetrate the biomass and to match the polarity of the targeted compounds. One way is to mechanically disrupt cells prior to exposing them to the solvent [52]. Differences were caused by cell shape, chemical composition, cell structure, and the interrelationship between the components. Solvent extraction can be enhanced by using organic solvents at temperatures and pressures above their boiling point [53]. Electric pulses enhance mass transfers across cell membranes [54], while enzymatic treatment of microalgae disrupts cells with minimal damage to the inside product [55]. Several methods involve simultaneous extraction and transesterification of microalgae biomass to recover fatty acids [56,57].

LIPID CONVERSION TO BIODIESEL

Conventional biodiesel production occurs via the transesterification of neutral lipids in the presence of an alcohol and a catalyst, which can be a strong acid or base. Factors such as alcohol volume, moisture content, temperature, time of incubation and catalyst concentration determine the yield and conversion efficiency, which are the key issues in utilizing microalgal lipids with different composition [28,37,38]. Neutral lipids mainly contain triglycerides and free

Table 3. Conversion of microalgal lipid to biodiesel

Species	MeOH /lipid, molar	Catalyst	Condition	Time	Yield (%)	References
Botryococcus braunii	30 : 1	HCl or KOH	350 °C	-	32-40	[15]
Chaetoceros calcitrans	6 : 1	KOH	600 RPM, 1 atm, 50 °C	50 min	85	[53]
Chlamydomonas sp.	32 : 1	Titania catalyst	360 °C, 2295 psi	30 s	80-85	[55]
Chlorella protothecoides	56 : 1	100% H_2SO_4	30 °C	4 hr	63	[60]
Chlorella protothecoides	3 : 1	*Candidia* sp. lipase (30 wt%)	38 °C	12 hr	98.15	[53]
Chlorella protothecoides	10 : 1	KOH	65 °C	5-20 min	97.34	[54]
Chlorella sp.	32 : 1	Titania catalyst	360 °C, 2295 psi	30 s	80-85	[60]
Chlorella vulgaris	6 : 1	*Penicillium expansum* lipase	Ionic liquid[a]	24 hr	90.7	[1]
Chlorella pyrenoidosa	6 : 1	*Candidia* sp. Lipase	Ionic liquid[a]	24 hr	86.2	[1]
Dunaliella salina	32 : 1	2% sulphuric acid	30 °C	2-4 hr	40	[61]
Dunaliella tertiolecta	32 : 1	Titania catalyst	360 °C, 2295 psi	30 s	80-85	[62]
Monoraphidium minutum	6 : 1	HCl or NaOH	38 °C	30 min	68	[37]
Nannochloropsis sp.	6 : 1	KOH	Stirring, 60 °C	0.5-2 hr	89.7	[40, 62]
Nannochloropsis sp.	4 : 1	Acetyl chloride	260 °C, 1200 psi	10-30 min	85	[15]
Nannochloropsis oculata		Al_2O_3 supported CaO & MgO	Supercritical, 360 °C	10 min	97.5	[37]
Spirulina platensis	3 : 1	*Scenedesmus dimorphus* lipase	40 °C	24 hr	44.8	[61]

[a]Ionic liquid here is referred to 1-butyl-3-methyl-imidazolium hexa-fluoro-phosphate

fatty acids, of which the latter affects the catalysis in transesterification process by neutralizing the basic sites in the catalysts, though it could be esterified with methanol for biodiesel production [42]. The low FAME content (from 3 to 31 wt%) in reaction mixture indicated the presence of unsaponifiable materials [50]. In the conversion of microalgal oils with high content of free fatty acid, acid catalysts resulted in side reactions and consistently higher yields [58], while enzymes were expensive and unable to provide the degree of reaction completion (ASTM, fuel specification), and supercritical transesterification process was also restricted due to economic and safety concerns [55].

Table 3 gives a summary of lipid conversion methods used in microalgal biodiesel production. Although the theoretical molar ratio is 3 : 1 (alcohol : oil), the molar ratio of 6 : 1 is generally used to complete the reaction, as the relationship between the feedstock mass input and biodiesel mass output is about 1 : 1 [36]. The conversion efficiency of homogeneous acid of base-catalyzed transesterification of triglycerides is higher dependent on the fatty acid and water content in the lipid feedstock. The base catalyzed method, used in the majority of current commercial operations, cannot tolerate free fatty acid content higher than 0.6% [49]. More than 90% of the triglycerides in the highly refined neutral lipids from microalgae could be converted to fatty acid methyl esters (FAME). This final product makes up less than 10% of dry biomass, while the content of total lipids (20-50 wt%, dry microalgae bases) is much higher. This is caused by both the mass loss during removal of polar lipids and the presence of other chemicals (hydrocarbons, wax and sterol esters, free alcohols, and sterols) in neutral lipids at a ratio of 10-30 wt% [37]. About 30% of the original lipid mass must be deducted after removal of the polar phase. A yield of <70% from microalgal neutral lipids could be expected even if triglycerides have a biodiesel yield >99% [59,60]. Low FAME yield (less than 20 wt% of dry cells) after the whole process illustrates the importance of high contents of triglycerides and free fatty acids in microalgae for the commercial viability of biodiesel industry [56]. The lipid class composition will also greatly affect the potential fuel yield, as discussed in the next section.

FUEL PROPERTIES OF MICROALGAL BIODIESEL

Concerning the chemical composition, the main differences are the atom numbers of the carbon chain, together with the numbers, positions of the unsaturated bonds. For pure ASTM grade biodiesel, only the content of free fatty acids and triglycerides in neutral lipids rather than the total lipid content must be considered for biodiesel production [49]. Attention is commonly given to both total lipids and neutral lipids, though only neutral lipids are suitable for biodiesel preparation [57]. Neutral lipids only comprised 30-35 wt% of the total lipids for a wide variety of microalgal species [37]. Being composed of saturated and unsaturated fatty acids with 12-22 carbon atoms, the lipid composition of microalgal species has a significant effect on the characteristics of biodiesel produced, concerning the relative intensity of individual fatty acids chains, as shown in Table 2.

A summary of the fuel properties of biodiesel from microalgae and soybean oil is given in Table 4. The predominant components (C_{16} and C_{18}), shown in traditional oil crops (soybean, corn, rapeseed, and palm), make up 85-90% of the fatty acids in the microalgal lipids. The level of unsaturation, the chain length of the acyl

Table 4. Comparison between biodiesels from microalgal oil and vegetable oil

	Microalgal biodiesel	Soybean biodiesel	ASTM limits	Diesel fuel	ASTM method	References
Flash point (°C)	149	122	93	38-52	D93	[7]
Distillation, 90% recovery (°C)	371	339	360	282-338	D1160	[9]
Carbon residue (wt%)	0.018	0.019	0.050	0.15-0.35	D4530	[37]
Total glycerin (wt%)	0.169	0.161	0.240	-	D6584	[42]
Free glycerin (wt%)	0.006	0.05	0.02	-	D6584	[36]
Water and sediment (wt%)	0.005 (max)	3.07	0.05	0.05 (max)	D2709	[37]
Sulfur, total (ppm)	8.43	3.07	15	38	D5453	[38]
Cetane number (%)	71.67	50	47	40-45	D613	[39]
Cloud point (°C)	−16	2	-	-	D2500	[40]
Solidifying point (°C)	−12	-	-	−50 to 10	-	[41]
Sulfated ash (ppm)	0.008	0.011	0.020 (max)	0.05	D874	[42]
Copper strip corrosion (scale 1-4)	1	1	3 (max)	3	D130	[36]
Acid number (mg KOH/g)	0.01	0.07	0.50	0.5 (max)	D664	[37]
Kinematic viscosity at 40 °C (mm^2/s)	11-35.4	4.5	1.9-6.0	1.9-4.1	D445	[38]
Cold filter plugging point (°C)	126	185	360 (max)	−6.7 (max)	D6217b	[39]
Phosphorus (ppm)	1 (max)	2	10 (max)	0.1	D4951	[61]
H/C ratio	1.81	1.81	-	1.81	-	[62]
Density (kg/L)	0.864	0.838	-	0.83	-	[63]
Heating value (mJ/ kg)	37-41	-	-	40-45	-	[64]

In American Society for Testing and Materials (ASTM), the most important fuel properties considered to access the potential of biodiesel as substitute of diesel fuel are viscosity, cetane number (CN), density, cold filter plugging point, oxidative stability, lubricity, ignition quality and combustion heat. The physical characteristics of both fatty acids and triglycerides can be determined by chain length, number of double bonds and amount of each fatty ester components in both fatty acids and triglycerides. The saponification values (SV) and iodine value (IV) represent the ignition quality of fuel and presence of unsaturated fatty acid component in fatty acid methyl esters (FAME)

group, and the presence of polar lipids affect biodiesel properties such as viscosity, cetane number, density, cold filter plugging point, oxidative stability, lubricity, ignition quality and combustion heat. For example, fuels with a higher level of unsaturation of the acyl chains (with four or more double bonds) have a higher cloud point, which is desirable, but are also much more susceptible to oxidation [56]. The identification of lipid composition in selected microalgal strain is essential for determining the suitability to biodiesel and fuel quality. The high concentrations of unsaturated fatty acids in the extracted lipids, and ultimately in the resulting fuel, will be an important fuel quality determinant. The preponderance of the shorter chain fatty acids has significance for their potential as diesel fuels. Fatty acid composition in neutral lipids, commonly containing hydrocarbons, wax and sterol esters, free alcohols, and sterols, is not the same as that in total lipids [37,38]. It is unclear how large percentages of these compounds would affect the fuel properties of microalgal biodiesel [7,9]. Microalgal lipids differ from higher plant oils in their high phospholipid and glycolipid concentrations. These lipid classes contain nitrogen, phosphorous and sulfur that may be problematic with regards to engine performance, if present in fuels. The yields of polar fractions in total lipids, together with the high percentage of the unsaturated components in neutral lipids, limit the final production of biodiesel, which is nearly equal to the refined lipids in weight [56]. However, it is likely that the sulfur, phosphorus and nitrogen-containing compounds would end up in the water soluble fraction after transesterification, meeting the demand of low concentration to non-existence in microalgal biodiesel [61].

The overall heating value of crude microalgal lipid is somewhat depressed to a value of 36 kJ/g, due to the lower calorific value of the glycolipids and phospholipids. However, as these fractions would be almost certainly separated off after the transesterification process, their low calorific value would have no effect on the properties of the final biodiesel. Higher cetane number values indicate better ignition properties of the fuel. Higher unsaturated fatty acids (UFA) present in oil gave higher iodine values, causing the polymerization of glycerides and the formation of deposits or deterioration of the lubricating in heating [62]. Higher oleic acid content increases the oxidative stability for longer storage, and decreases the cold filter plugging point (CFPP) for use in cold regions. The pour and cloud points of feedstock decrease with increasing chain length and branching of the alcohol moiety [65]. Biodiesel feedstock with a high degree of saturation is more resistant to oxidation and more stable in presence of light, oxygen, high temperature, and metals. The chain length distribution of fatty acids in microalgal species is more diverse, compared with that in higher plants, pointing out the possibility of certain species to be cultured for selected fuel properties. The shorter carbon chains (e.g., C_{12}) result in a very high cetane number of 71.67, while an acid number less than 200 mg KOH indicates the presence of unsaponifiable compounds in the neutral lipids [65]. Compared to petroleum based fuels, microalgal biodiesel cultivated on nonarable area has less emissions and contaminations, utilizing industrial flue gas and non drinking water.

Microalgal biodiesel passed all ASTM standards, except for two tests (distillation 90% recovery and kinematic viscosity at 40 °C). The flash point and cetane number were also high compared to soybean based biodiesel, while the cloud point was notably lower at −16 °C [65].

CONCLUSION

High lipid content and fast microalgal growth could be achieved by metabolic regulation, to which optimal culture conditions are well documented. Total lipid productivity is limited due to growth depression at nitrogen stress. Large scale culture and low neutral lipids yields remain major problems for microalgal biodiesel industry. Neutral lipids, glycolipids and phospholipids could be extracted via various methods, in which the cell penetration and lipid diffusion restrict the processing. Triglycerides in neutral lipids could be converted to biodiesel via transesterification, while the unsaturation and the impurities greatly affect the fuel properties.

ACKNOWLEDGEMENT

This work was supported by the Advanced Biomass R&D Center (ABC) of Global Frontier Project funded by the Ministry of Science, ICT and Future Planning (ABC-2013M3A6A2072223).

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