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Microalgae as Feedstocks for Biodiesel Production

Jin Liu[1], Junchao Huang[2,3] and Feng Chen[3,4]
[1]*Department of Applied Sciences and Mathematics, Arizona State University, Polytechnic Campus, Mesa,*
[2]*Kunming institute of Botany, Chinese Academy of Sciences,*
[3]*School of Biological Science, The University of Hong Kong, Hong Kong,*
[4]*Institute for Food & Bioresource Engineering, College of Engineering, Peking University, Beijing,*
[1]*USA*
[2,3,4]*China*

1. Introduction

Fossil-based fuels including oil, coal and gas play a pivotal role in modern world energy market. These fossil fuels, according to world energy outlook 2007, will remain the major sources of energy and are expected to meet about 84% of energy demand in 2030. However, fossil fuels are non-renewable and will be finally diminished. It has been recently estimated that the global oil, coal and gas last only approximately for 35, 100 and 37 years respectively, based on a modified Klass model (Shafiee & Topal, 2009). In order to sustain a stable energy supply in the future, it is necessary to develop other sources of energy, e.g., renewable energy. Renewable energy is derived from natural processes that are replenished constantly, including hydropower, wind power, solar energy, geothermal energy, biodiesel, etc. An estimated $150 billion was invested in renewable energy worldwide in 2009, around 2.5 times of the 2006 investment (Figure 1).

It is well known that transport is almost totally dependent on petroleum-based fuels, which will be depleted within 40 years. An alternative fuel to petrodiesel must be technically feasible, easily available, economically competitive, and environmentally acceptable (Demirbas, 2008). Biodiesel is such a candidate fuel for powering the transport vehicles. Biodiesel refers to a biomass-based diesel fuel consisting of long-chain alkyl (methyl, propyl or ethyl) esters. In addition to being comparable to petrodiesel in most technical aspects, biodiesel has the following distinct advantages over petrodiesel (Knothe, 2005a):

1. derived from renewable domestic resources, thus reducing dependence on and preserving petroleum;
2. biodegradable and reduced exhaust emissions, being environment-friendly;
3. higher flash point, being safer for handling and storage; and
4. excellent lubricity.

Like petrodiesel, biodiesel operates in compression ignition engines. Biodiesel is miscible with petrodiesel in all ratios. Currently, the blends of biodiesel and petrodiesel instead of net biodiesel have been widely used in many countries and no engine modification is

required (Singhania et al., 2008). These blends of biodiesel with petrodiesel are usually denoted by acronyms, for example B20 which indicates a blend of 20% biodiesel with petrodiesel (Knothe, 2005a).

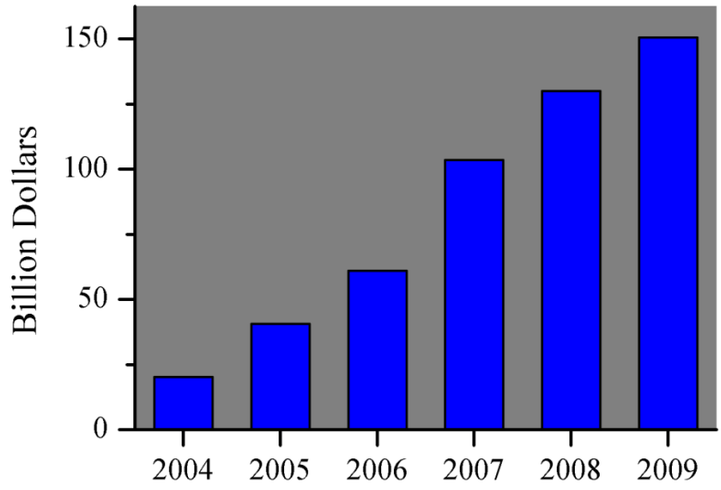

Fig. 1. Global investment in renewable energy, 2004-2009. Adapted from REN21 (2010)

The global markets for biodiesel are entering a period of rapid and transitional growth. In the year 2007, there were only 20 nations producing biodiesel for the needs of over 200 nations; by the year 2010, more than 200 nations become biodiesel producing nations and suppliers (Thurmond, 2008). Global biodiesel production has massively increased to 16.6 billion liters per year over the last nine years (Figure 2). Much of the growth is happening in just three countries: the United States, Brazil and Germany, which together account for over half of biodiesel (Checkbiotech, 2009). The International Energy Agency's report suggests that world production of biodiesel could top 25 million tons per year by 2012 if the recent trends continue.

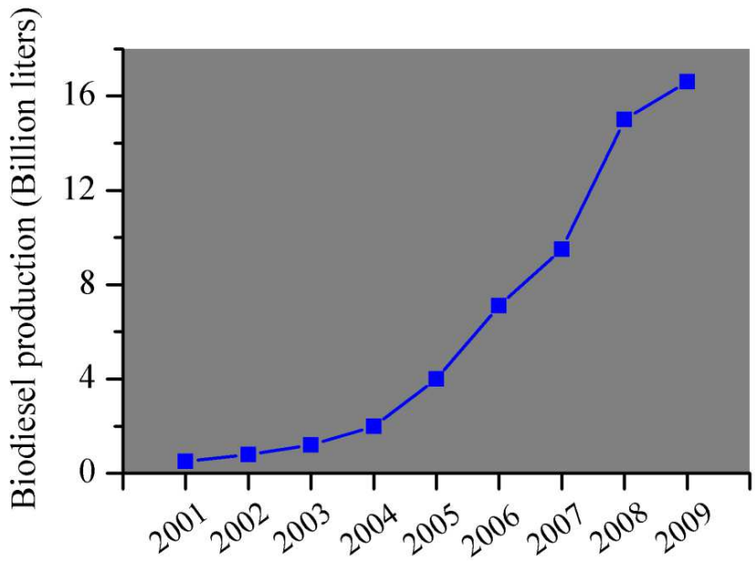

Fig. 2. Global biodiesel production, 2001-2009. Adapted from REN21 (2010)

Biodiesel can be produced from a variety of feedstocks, including plant oils, animal fats and waste oils as well as microalgae (Demiras, 2008). Each feedstock has its advantages and disadvantages in terms of oil content, fatty acid composition, biomass yield and geographic distribution. Depending on the origin and quality of feedstocks, changes may be required for the production process of biodiesel.

The use of plant oils as biodiesel feedstocks has been long recognized and well documented in numerous studies (Abdullah et al., 2009; de Oliveira et al., 2005; Graef et al., 2009; Hawash et al., 2009; Hill et al., 2006; Jain & Sharma, 2010; Nakpong & Wootthikanokkhan, 2010; Patil & Deng, 2009; Rashid & Anwar, 2008; Sahoo & Das, 2009; Saka & Kusdiana, 2001). These feedstocks include the oils from soybean, rapeseed, palm, canola, peanut, cottonseed, sunflower and safflower. Based on the geographic distribution, soybean is the primary source for biodiesel in USA, palm oil is used as a significant biodiesel feedstock in Malaysia and Indonesia, and rapeseed is the most common base oil used in Europe for biodiesel production (Demiras, 2008). The vast majority of these plants are also used for food and feed production, which means that possible food versus fuel conflicts are present. Thus, the use of these plant oils as feedstocks for biodiesel seems insignificant for the developing countries which are importers of edible oils (Meher et al., 2008). In addition to these edible oils, various non-edible, tree-borne oils from jatropha, karanja, jojoba and neem are the potential biodiesel feedstocks (Jain & Sharma, 2009; Meher et al., 2008; Sahoo & Das, 2009). Jatropha and karanja are two oilseed plants that are not widely exploited due to the presence of toxic components in the oils. In India, they are popularly used as biodiesel feedstocks.

In addition to the plant oils, animal fats and waste oils are the potential sources for commercial biodiesel production (Thompson et al., 2010). Among these feedstocks, tallow, lard, yellow grease and waste cooking oils have received most interest (Banerjee et al., 2009; Canakci, 2007; da Cunha et al., 2009; Dias et al., 2009; Diaz-Felix et al., 2009; Oner & Altun, 2009; Phan & Phan, 2008). However, animal fats and waste oils usually contain large amounts of free fatty acids, which can be as high as 41.8% (Canakci, 2007). Free fatty acids cannot be directly converted to biodiesel in alkali-catalyzed transesterificatoin but react with alkali to form soaps that inhibit the separation of biodiesel from glycerin and wash water fraction (Huang et al., 2010). A two-step process was developed for these high fatty acid feedstocks: acid-catalyzed pretreatment and alkali-catalyzed transesterificaton. Because animal fats and waste oils have relatively high level of saturation (Canakci, 2007), the biodiesel from these sources exhibits poor cold flow properties.

Microalgae represent a wide variety of aquatic photosynthetic organisms with the potential of producing high biomass and accumulating high level of oil. The production of biodiesel from microalgal oil has long been recognized and been evaluated in response to the United States Department of Energy for research in alternative renewable energy (Sheehan et al., 1998). Currently, the commercialization of algae-derived biodiesel is still in its infancy stage. Using microalgae as biodiesel feedstocks has received unprecedentedly increasing interest, including but not restricted to microalgal strain selection and genetic engineering, mass cultivation for biomass production, lipid extraction and analysis, transesterification technologies, fuel properties and engine tests (Abou-Shanab et al., 2011; Brennan & Owende, 2010; Demirbas, 2009; Greenwell et al., 2010; Miao & Wu, 2006; Pruvost et al., 2011; Radakovits et al., 2010; Rodolfi et al., 2009; Ross et al., 2008; Sydney et al., 2011). Considering their unique characteristics, microalgae have been considered as the most promising feedstock of biodiesel that has the potential to displace fossil diesel (Chisti, 2007). This review mainly focuses on the potential of using microalgae as biodiesel feedstocks, biodiesel production pipeline, and possibility of employing genetic engineering for improving microalgal productivity.

2. Potential of using microalgae as biodiesel feedstocks

Microalgae represent a large and diverse group of prokaryotic or eukaryotic photosynthetic microorganisms that are in unicellular or multicellular form. Examples of prokaryotic microorganisms are cyanobacteria (commonly referred to as blue-green algae) that are closely related to Gram-negative bacteria and eukaryotic ones are for example green microalgae and diatoms (Graham et al., 2009). Microalgae can be found in a wide range of environmental conditions, including water, land, and even unusual environments such as snow and desert soils (Lee, 2008). It is estimated that there are more than 50,000 species around the world, among which only about 30,000 have been studied and analyzed (Mata et al., 2010). Extensive collections of microalgae have been established by researchers in different countries, including the Freshwater Microalgae Collection of University of Coimbra (Portugal), the Collection of the Goettingen University (Germany), the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP, USA), the University of Texas Algal Culture Collection (USA), the CSIRO collection of Living Microalgae (CCLM, Australia), the National Institute for Environmental Studies Collection (NIES, Japan), the American Type Culture Collection (ATCC, USA), and the Freshwater Algae Culture Collection of Institute of Hydrobiology (China). Together more than 10,000 microalgal strains are available to be selected for use in a broad range of applications, for example, as biodiesel feedstocks.

The use of microalgae for biodiesel production has long been recognized and its potential has been widely reported by many research studies recently (Abou-Shanab et al., 2011; Afify et al., 2010; Ahmad et al., 2011; Cheng et al., 2009; Damiani et al., 2010; Gouveia et al., 2009; Liu et al., 2010; Rodolfi et al., 2009; Yoo et al., 2010). Microalgae reproduce themselves autotrophically using CO_2 from air and light through photosynthesis. Compared with higher plants, microalgae exhibit higher photosynthetic efficiency and grow much faster, finishing an entire growth cycle within a few days (Christi, 2007). Typical growth rates are presented in Figure 3 as the doubling time for each microalgal species. A low doubling time corresponds to a high specific growth rate. Microalgae double themselves with an average time of 26 h, and some can even reproduce within 8 h. Moreover, they can be adapted to grow in a broad range of environmental conditions, suggesting the possibility of finding species best suited to local environments which is not suitable for cultivating oil plants (e.g. palm, soybean and rapeseed).

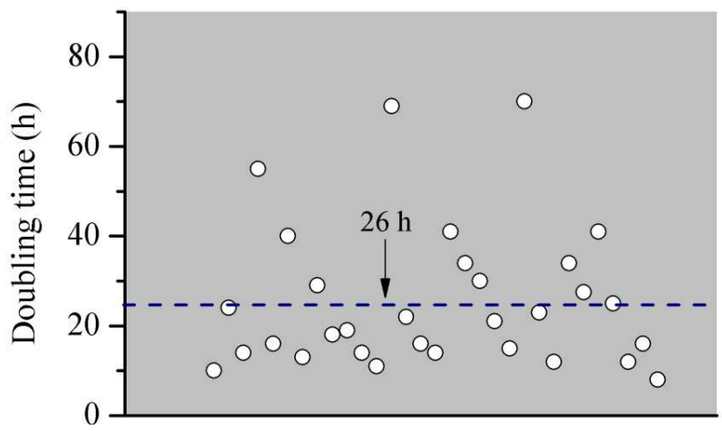

Fig. 3. Doubling time for some microalgal species. The dash line indicates the average value. T_d=ln(2)/μ, T_d, doubling time, μ, specific growth rate.

In addition to growth rate, lipid content is another important factor to assess the potential of microalgae for biodiesel production. Over the past few decades, thousands of algae and cyanobacterial species have been screened for high lipid production, and numerous oleaginous species have been isolated and characterized. The lipid contents of these oleaginous algae are species- and/or strains-dependent, vary greatly, and may reach as high as 68% of dry weight, as shown in Table 1. Generally, microalgae synthesize a low content of lipids under nutrient replete conditions (Figure 4), with membrane lipids (e.g., phospholipids and glycolidips) being the main components; whereas under stress conditions such as nitrogen deficiency, a great increase in total lipids was observed (Figure 4) with neutral lipids in particular triacylglycerols (TAGs) being the dominant components (Hu, 2004). TAGs are considered to be superior to phospholipids or glycolipids for biodiesel feedstocks because of their higher percentage of fatty acids and lack of phosphate (Pruvost et al., 2009). Unlike higher plants in which individual classes of lipids may be synthesized and localized in a specific cell, tissue or organ, algae produce these different lipids in a single cell (Hu et al., 2008b). The synthesized TAGs are deposited in lipid bodies located in cytoplasm of algal cells (Damiani et al., 2010; Rabbani et al., 1998).

Algal species	Culture conditions	Lipid content (%)	biomass productivity (g/L/day)	Lipid productivity (mg/L/day)	References
Chlorophyta					
Botryococcus braunii	Phototrophic	9.5-13.5	0.02-0.04	2.6-4.5	Chinnasamy et al., 2010
Botryococcus braunii	Phototrophic	17.85	0.346		Órpez et al., 2009
Botryococcus braunii	Phototrophic	24	0.077	21	Yoo et al., 2010
Botryococcus sp.	Phototrophic	15.8-35.9	0.14-0.22	21.3-46.9	Yeesang and Cheirsilp, 2011
Chlamydomonas reinhardtii	Mixotrophic	12.2-46	0.21-0.36	29-95	Li et al., 2010a
Chlorella ellipsoidea	Phototrophic	32	0.07	22.4	Abou-Shanab et al., 2011
Chlorella ellipsoidea	Phototrophic	15-43		11.4	Yang et al., 2011
Chlorella protothecoides	Heterotrophic	48.1-63.8	1.02-1.73	3432-6293	De la Hoz Siegler et al., 2011
Chlorella protothecoides	Heterotrophic	49	1.2	586.8	Gao et al., 2010
Chlorella saccharophila	Phototrophic	12.9-18.1	0.02	2.7-4.2	Chinnasamy et al., 2010
Chlorella sorokiniana	Phototrophic	19.3	0.23	44.7	Rodolfi et al., 2009
Chlorella sp.	Phototrophic	33.9	0.528	178.8	Chiu et al., 2008
Chlorella sp.	Phototrophic	22.4-66.1	0.08-0.34	51-124	Hsieh and Wu., 2009
Chlorella sp.	Phototrophic	34.1 [a]	0.053	22	Matsumoto et al., 2010
Chlorella sp.	Phototrophic	18.7	0.23	42.1	Rodolfi et al., 2009
Chlorella vulgaris	Phototrophic	20-42	0.21-0.35	44-147	Feng et al., 2011
Chlorella vulgaris	Phototrophic, Mixotrophic, heterotrophic	21-38	0.01-0.26	4-54	Liang et al., 2009
Chlorella vulgaris	Phototrophic	19.2	0.17	32.6	Rodolfi et al., 2009
Chlorella vulgaris	Phototrophic	26-52		11.6-13.2	Widjaja et al., 2009
Chlorella vulgaris	Phototrophic	35	0.117	41	Yeh et al., 2010
Chlorella zofingiensis	Heterotrophic	52	0.72	374.4	Liu et al., 2010

Algal species	Culture conditions	Lipid content (%)	biomass productivity (g/L/day)	Lipid productivity (mg/L/day)	References
Chlorella zofingiensis	Phototrophic	25.8	0.136	35.1	Liu et al., 2011
Chlorococcum sp.	Phototrophic	19.3	0.28	53.7	Rodolfi et al., 2009
Choricystis minor	Phototrophic	21-59.3	0.35	82	Sobczuk and Chisti, 2010
Dunaliella tertiolecta	Phototrophic	12.2-15.2	0.03-0.04	4.0-4.6	Chinnasamy et al., 2010
Dunaliella tertiolecta	Phototrophic	16.7	0.12	20	Gouveia and Oliveira, 2009
Haematococcus pluvialis	Phototrophic	15.6-34.9			Damiani et al., 2010
Micractinium pusillum	Phototrophic	24	0.108	25.7	Abou-Shanab et al., 2011
Neochloris oleabundans	Phototrophic	19-56	0.03-0.15	10.7-38.8	Gouveia et al., 2009
Neochloris oleabundans	Phototrophic	7-40.3	0.31-0.63	38-133	Li et al., 2008
Ourococcus multisporus	Phototrophic	52	0.045	23.3	Abou-Shanab et al., 2011
Parietochloris incisa	Phototrophic	18-34 [a]	0.23-0.47	46-160	Solovchenko et al., 2008
Pseudochlorococcum sp.	Phototrophic	24.6-52.1	0.234-0.76	53-350	Li et al., 2011a
Scenedesmus obliquus	Phototrophic	21-58	0.08-0.09	19-43.3	Abou-Shanab et al., 2011
Scenedesmus obliquus	Phototrophic	17.7	0.09	15.9	Gouveia and Oliveira, 2009
Scenedesmus obliquus	Phototrophic	12-38.9	0.20-0.29	35.1-78.7	Ho et al., 2010
Scenedesmus obliquus	Phototrophic, Mixotrophic	12.6-58.3	0.51	270	Mandal and Mallick, 2009
Scenedesmus rubescens like	Phototrophic	11.3-27 [a]	0.44-0.54	108-133	Lin and Lin, 2011
Scenedesmus quadricauda	Phototrophic	18.4	0.19	35.1	Rodolfi et al., 2009
Scenedesmus sp.	Phototrophic	22-53	0.08	20.3	Xin et al., 2010
Scenedesmus sp.	Phototrophic	18	0.203	39	Yoo et al., 2010
Scenedesmus sp.	Phototrophic	21.1	0.26	53.9	Rodolfi et al., 2009
Tetraselmis chui	Phototrophic	17.3-23.5	1-2.6	235-450	Araujo et al., 2011
Tetraselmis sp.	Phototrophic	8.7-33	0.21	22.86	Huerlimann et al., 2010
Tetraselmis suecica	Phototrophic	8.5-12.9	0.28-0.32	27-36.4	Rodolfi et al., 2009
Tetraselmis tetrathele	Phototrophic	29.2-30.3	3.1-4.4	905-1333	Araujo et al., 2011
Bacillariophyceae					
Chaetoceros calcitrans	Phototrophic	39.8	0.04	17.6	Rodolfi et al., 2009
Chaetoceros gracilis	Phototrophic	15.5-60.3	3.4-3.7	530-2210	Araujo et al., 2011
Chaetoceros muelleri	Phototrophic	11.7-25.3	1.2-2.7	1404-6831	Araujo et al., 2011
Chaetoceros muelleri	Phototrophic	33.6	0.07	21.8	Rodolfi et al., 2009
Cylindrotheca closterium	Phototrophic	17-30			Pruvost et al., 2011
Navicula sp.	Phototrophic	47.6 [a]	0.055	26.4	Matsumoto et al., 2010
Nitzschia cf. pusilla	Phototrophic	48	0.065	31.4	Abou-Shanab et al., 2011
Nitzschia laevis	Heterotrophic	12.8	2.02	258.6	Chen et al., 2008

Algal species	Culture conditions	Lipid content (%)	biomass productivity (g/L/day)	Lipid productivity (mg/L/day)	References
Nitzschia sp.	Phototrophic	32	0.013		Moazami et al., 2011
Phaeodactylum tricornutum	Phototrophic	18.7	0.24	44.8	Rodolfi et al., 2009
Skeletonema costatum	Phototrophic	21.1	0.08	17.4	Rodolfi et al., 2009
Skeletonema sp.	Phototrophic	31.8	0.09	27.3	Rodolfi et al., 2009
Thalassiosira pseudonana	Phototrophic	20.6	0.08	17.4	Rodolfi et al., 2009
Eustigmatophyceae					
Ellipsoidion sp.	Phototrophic	27.4	0.17	47.3	Rodolfi et al., 2009
Monodus subterraneus	Phototrophic	12.9-15 [a]	0.34-0.49	47.5-67.5	Khozin-Goldberg and Cohen, 2006
Monodus subterraneus	Phototrophic	16.1	0.19	30.4	Rodolfi et al., 2009
Nannochloropsis oculata	Phototrophic	22.8-23	2.4-3.4	547.2-782	Araujo et al., 2011
Nannochloropsis oculata	Phototrophic	26.2-30.7	0.37-0.50	84-151	Chiu et al., 2009
Nannochloropsis oculata	Phototrophic	7.9-15.9	0.06-0.13	9.1-16.4	Converti et al., 2009
Nannochloropsis sp.	Phototrophic	52	0.0465		Moazami et al., 2011
Nannochloropsis sp.	Phototrophic	23.1-37.8	0.06	20	Huerlimann et al., 2010
Nannochloropsis sp.	Phototrophic	28.7	0.09	25.8	Gouveia and Oliveira, 2009
Nannochloropsis sp.	Phototrophic	21.6-35.7	0.17-0.21	37.6-61	Rodolfi et al., 2009
Others					
Aphanothece microscopica	Heterotrophic	7.1-15.3	0.26-0.44	30-50	Queiroz et al., 2011
Crypthecodinium Cohnii	Heterotrophic	19.9	2.24	444.9	Couto et al., 2010
Isochrysis galbana	Phototrophic	24.6	0.057	14.02	Lin et al., 2007
Isochrysis sp.	Phototrophic	23.5-34.1	0.09	20.95	Huerlimann et al., 2010
Isochrysis sp.	Phototrophic	22.4-27.4	0.14-0.17	37.8	Rodolfi et al., 2009
Pavlova lutheri	Phototrophic	35.5	0.14	50.2	Rodolfi et al., 2009
Pavlova salina	Phototrophic	30.9	0.16	49.4	Rodolfi et al., 2009
Pavlova viridis	Phototrophic	24.8-32			Li et al., 2005
Pleurochrysis carterae	Phototrophic	9.7-12	0.03-0.04	2.7-4.4	Chinnasamy et al., 2010
Porphyridium cruentum	Phototrophic	9.5	0.37	34.8	Rodolfi et al., 2009
Rhodomonas sp.	Phototrophic	9.5-20.5	0.06	6.19	Huerlimann et al., 2010
Schizochytrium limacinum	Heterotrophic	50.3 [a]	3.48	1750	Ethier et al., 2011
Schizochytrium mangrovei	Heterotrophic	68 [a]	2.44	1659	Fan et al., 2007
Spirulina maxima	Phototrophic	4.1	0.21	8.6	Gouveia and Oliveira, 2009
Thalassiosira weissflogii	Phototrophic	6.3-13.2	0.5-1.5	31.5-198	Araujo et al., 2011

[a] Total fatty acid content

Table 1. Lipid content and productivity of various microalgal species.

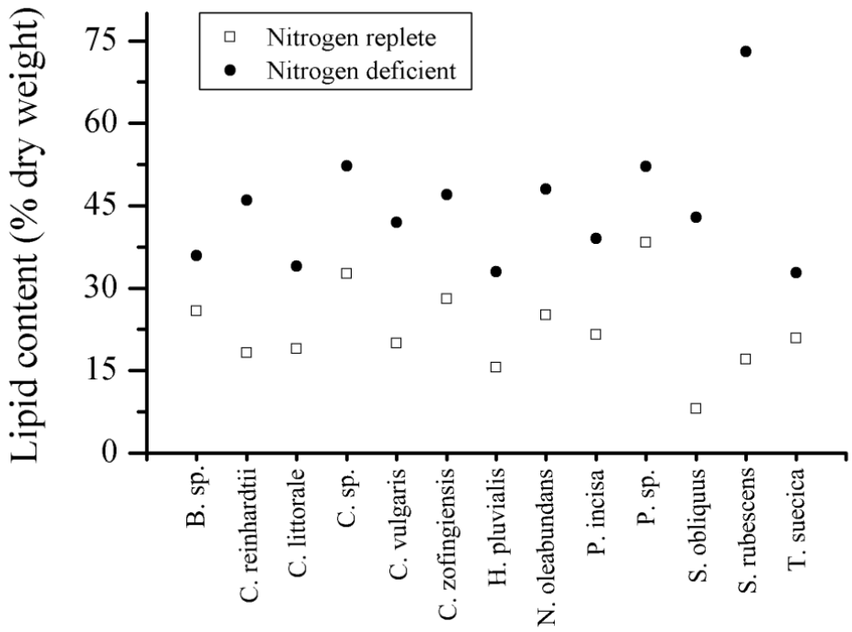

Fig. 4. Lipid content under nitrogen replete (open squares) and nitrogen deficient (filled circles) conditions for *Chlorophyta*. *B*. sp., *Botryococcus* sp. (Yeesang and Cheirsilp, 2011); *C. reinhardtii*, *Chlamydomonas reinhardtii* (Li et al., 2010); *C. littorale, Chlorocuccum littorale* (Ota et al., 2009); *C.* sp., *Chlorella* sp. (Hsieh and Wu, 2009); *C. vulgaris*, *Chlorella vulgaris* (Feng et al., 2011); *C. zofingiensis*, *Chlorella zofingiensis* (Liu et al., 2010); *H. pluvialis*, *Haematococcus pluvialis* (Damiani et al 2010); *N. oleabundans*, *Neochloris oleabundans* (Gouveia et al., 2009); *P. incisa, Parietochloris incisa* (Solovchenko et al., 2010); *P*. sp., *Pseudochlorococcum* sp. (Li et al., 2011); *S. obliquus*, *Scenedesmus obliquus* (Mandal and Mallick, 2009); *S. rubescens*, *Scenedesmus rubescens* (Mandal and Mallick, 2009); *T. suecica*, *Tetraselmis suecica* (Rodolfi et al., 2009).

The important properties of biodiesel such as cetane number, viscosity, cold flow, oxidative stability, are largely determined by the composition and structure of fatty acid esters which in turn are determined by the characteristics of fatty acids of biodiesel feedstocks, for exmaple carbon chain length and unsaturation degree (Knothe, 2005b). Fatty acids are either in saturated or unsaturated form, and the unsaturated fatty acids may vary in the number and position of double bones on the acyl chain. Based on the number of double bones, unsaturated fatty acids are clarified into monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The fatty acid profile of a great many algal species has been investigated and is shown in Table 2. The synthesized fatty acids in algae are commonly in medium length, ranging from 16 to 18 carbons, despite the great variation in fatty acid composition. Specifically, the major fatty acids are C16:0, C18:1 and C18:2 or C18:3 in green algae, C16:0 and C16:1 in diatoms and C16:0, C16:1, C18:1 and C18:2 in cyanobacteria. It is worthy to note that these data are obtained from algal species under specific conditions and vary greatly when algal cells are exposed to different environmental or nutritional conditions such as temperature, pH, light intensity, or nitrogen concentration (Guedes et al 2010; James et al., 2011; Sobczuk & Chisti, 2010; Tatsuzawa et al., 1996). Generally, saturated fatty esters possess high cetane number and superior oxidative stability; whereas unsaturated, especially

Fatty acids / Algal species	C12:0	C14:0	C15:0	C16:0	C16:1	C16:2	C16:3	C16:4	C17:0	C18:0	C18:1	C18:2	C18:3	C18:4	C20:0	C20:4	C20:5	C22:5	C22:6	Refs
Chlorophyta																				
Botryococcus braunii				29.5	3.4					1	44.9	21.2								Yoo et al., 2010
Botryococcus sp.		3.95	1.56	30.04	0.94				1.54	12.02	37.68	5.01	7.35		0.63					Yeesang and Cheirsilp, 2011
Chlamydomonas reinhardtii				30.7	3	1.8	1.6	2.7		3.2	27.2	18.3	11		0.5					James et al., 2011
Chlorella ellipsoidea		2		26							4	40	23			5				Abou-Shanab et al 2011
Chlorella protothecoides				14.3	1				0.32	2.7	71.6	9.7								Cheng et al 2009
Chlorella pyrenoidosa		0.7		17.3	0.8	7	9.3			1.2	3.3	18.5	41.8							D'Oca et al 2011
Chlorella sorokiniana				25.4	3.1	10.7	4.1			1.4	12.4	34.4	7.1							Chen and Johns, 1991
Chlorella sp.	3.78		5.24	16.1	10.88	9.79			4.74	4.35	8.45	14.36	18.79							Li et al., 2011b
Chlorella vulgaris				24	2.1					1.3	24.8	47.8								Yoo et al., 2010
Chlorella zofingiensis				22.62	1.97	7.38	1.94	0.22		2.09	35.68	18.46	7.75	0.49						Liu et al., 2010
Chlorocuccum littorale				20.9	5.6			14.4			29.7	7.2	22.2							Ota et al., 2009
Choricystis minor				36					0.4	12.3	31.2	9.9	3.8		1.9					Sobczuk and Chisti, 2010
Dictyochloropsis splendida		13.88		69.59					1.21	0.38	1.11	12.14	0.42							Afify et al 2010
Dunaliella tertiolecta				26.4		2.3	1.27			0.6	16.8	13.1	39.6							Chen et al 2011
Haematococcus pluvialis	0.21	1.25		22.5	0.64				0.19	3.15	19.36	26.9	17.04		0.2	0.89	0.57			Damiani et al 2010
Micractinium pusillum				33	1						31	17	18							Abou-Shanab et al 2011
Neochloris oleabundans				23.3	0.6	1.6	2.4		0.2	4.5	43	17.8	5.8							Levine et al., 2011
Neochloris sp.		5.22		29.4					5.2	6.6	17.5	23.6	12.6							Moazami et al., 2011
Ourococcus multisporus		2		19	1					5	26	11	36							Abou-Shanab et al 2011
Parietochloris incise				9.1	0.7	0.6				2.1	15.1	9.3	1.6	1.2		58.9				Khozin-Goldberg et al., 2002
Scenedesmus obliquus		1.48		21.8	5.95	3.96	0.68	0.43		0.45	17.93	21.74	3.76	0.21						Gouveia and Oliveira, 2009
Scenedesmus sp.				36.3	4					2.7	25.9	31.1								Yoo et al., 2010
Tetraselmis sp.		0.6		27.8						0.9	28.2	9.3	23.9	3.7		0.9	3.4			Huerlimann et al., 2010
Bacillariophyceae																				
Chaetoceros sp.		23.6		9.2	36.5	6.9	2.6		2		3		1.4	0.6		4.1	8		1	Renaud et al., 2002
Cyclotella cryptica		1.4		15.2	10.7						3.9	1.2	3.5				9.7		1.7	Pahl et al., 2010
Navicula sp.				45	52.7					0.6	1.1	0.6								Matsumoto et al., 2010
Nitzschia cf. pusilla		6		31	57	0.27							6							Abou-Shanab et al 2011
Nitzschia laevis		16.9		28.5	23.9					0.7	5.1	3.4	4.1			5	11.7			Chen et al 2008
Nitzschia sp.		9	3.5	37.4					4.6	5.3	16.9	11.6								Moazami et al., 2011
Cyanobacteria																				
Nostoc commune				23.5	22.5						5.6	21.1	14.1							Pushparaj et al., 2008
Nostoc flagelliforme		0.65		21.27	14.91					6.2	22.59	15.03	19.35							Liu et al., 2005
Spirulina				49.2	5.9					1.7	2.9	22.7	17.5							Chaiklahan et al 2008
Spirulina maxima		0.34		40.16	9.19		0.42	0.16		1.18	5.43	17.89	18.32	0.08	0.06					Gouveia and Oliveira, 2009
Synechocystis PCC6803				52	3				1		3	9	29	3						Wada and Murata, 1990
Eustigmatophyceae																				
Monodus subterraneus		3.3		19.8	34.3					9.7	9	0.8	0.7			2.8	15.5			Khozin-Goldberg and Cohen, 2006
Nannochloropsis oculata				62						11	5	8	15							Converti et al 2009
Nannochloropsis sp.				23.4						7.14	45.4	11.7	12.2							Moazami et al., 2011

Fatty acids / Algal species	C12:0	C14:0	C15:0	C16:0	C16:1	C16:2	C16:3	C16:4	C17:0	C18:0	C18:1	C18:2	C18:3	C18:4	C20:0	C20:4	C20:5	C22:5	C22:6	Refs
Prymnesiophyceae																				
Isochrysis galbanan		19.3		18.1							29.5	2.6	3.6	13.8				4.1	7.5	Lin et al., 2007
Isochrysis sp.		8.9	0.4	13.7	5.1					0.2	22.8	2.3	4.8	22.5		0.1	0.6	1.7	12.7	Huerlimann et al., 2010
Pavlova lutheri		5.54		19	31.46					1.11	2.55	4.46	5.37	6.63			16.07		7.8	Guedes et al 2010
Pavlova viridis		19.9		13.9	16.1												21.2		8.7	Hu et al 2008a
Pavlova viridis		10.34		17.3	17.87					3.16	1.33	2.48	2.23				10.46		14.78	Li et al., 2005
Rhodophyta																				
Porphyridium cruentum				14.5	8.5					10.5	14				10.8	6.1			10.5	Oh et al., 2009
Others																				
Crypthecodinium cohnii	2.9	13.4		22.9	0.4					2.6	7.6							0.5	49.5	Couto et al., 2010
Glossomastrix chrysoplasta		22		4.4	4						6.6	3.9				5.5	39.2	13.3		Kawachi et al., 2002
Rhodomonas sp.		7.8	0.4	19.7	1.5					3	8.4	3	29.8	11.7		0.6	8.6	1.7	3	Huerlimann et al., 2010
Schizochytrium limacinum		3.96		54.61						3.86								6.47	31.09	Ethier et al 2011

Table 2. Fatty acid composition of various algal species (% of total fatty acids)

polyunsaturated, fatty esters have improved low-temperature properties (Knothe, 2008). In this regard, it is suggested that the modification of fatty esters, for example the enhanced proportion of oleic acid (C18:1) ester, can provide a compromise solution between oxidative stability and low-temperature properties and therefore promote the quality of biodiesel (Knothe, 2009). Thus, microalgae with high oleic acid are suitable for biodiesel production.

Currently the commercial production of biodiesel is mainly from plant oils and animal fats. However, the plant oil derived biodiesel cannot realistically meet the demand of transport fuels because large arable lands are required for cultivation of oil plants, as demonstrated in Table 3. Based on the oil yield of different plants, the cropping area needed is calculated and expressed as a percentage of the total U.S. cropping area. If soybean, the popular oil crop in United States is used for biodiesel production to meet the existing transport fuel need, 5.2 times of U.S. cropland will need to be employed. Even the high-yielding oil plant palm is planted as the biodiesel feedstock, more than 50% of current U.S. arable lands have to be occupied. The requirement of huge arable lands and the resulted conflicts between food and oil make the biodiesel from plant oils unrealistic to completely replace the petroleum derived diesel in the foreseeable future. It is another case, however, if microalgae are used to produce biodiesel. As compared with the conventional oil plants, microalgae possess significant advantages in biomass production and oil yield and therefore the biodiesel productivity. In terms of land use, microalgae need much less than oil plants, thus eliminating the competition with food for arable lands (Table 3).

In addition to biodiesel, microalgae can serve as sources of other renewable fuels such as biogas, bioethanol, bio-oil and syngas (Chisti, 2008; Demirbas, 2010; Mussgnug et al., 2010). Moreover, microalgal biomass contains significant amounts of proteins, carbohydrates and other high-value compounds that can be potentially used as feeds, foods and pharmaceuticals (Chisti, 2007). Thus, integrating the production of such co-products with biofuels will provide new insight into improving the production economics of microalgal biodiesel. Microalgae can

also be used for sequestration of carbon dioxide from industrial flue gases and wastewater treatment by removal of nutrients (Chinnasamy et al 2010; Fulke et al., 2010; Levine et al., 2011; Yang et al., 2011). Coupled with these environment-beneficial approaches, the production potential of microalgae derived biodiesel is desirable.

Feedstocks	Oil content (% dry weight)	Oil yeild (L/ha year)	Land area needed (M ha)[a]	Percentage of existing US cropping area[a]
Corn	44	172	3480	1912
Hemp	33	363	1650	906
Soybean	18	636	940	516
Jatropha	28	741	807	443
Camelina	42	915	650	357
Canola	41	974	610	335
Sunflower	40	1070	560	307
Castor	48	1307	450	247
Palm oil	36	5366	110	60.4
Microalgae (low oil content)	30	58,700	10.2	5.6
Microalgae (medium oil content)	50	97,800	6.1	3.4
Microalgae (high oil content)	70	136,900	4.4	2.4

[a] For meeting all transport fuel needs of the United States. Adapted from Chisti, 2007 and Mata et al., 2010.

Table 3. Comparison of microalgae with other biodiesel feedstocks.

3. Biodiesel production from microalgae

The biodiesel production from microalgal oil shares the same processes and technologies as those used for other feedstocks derived oils. However, microalgae are microorganisms living essentially in liquid environments and thus have particular cultivation, harvesting, and downstream processing techniques for efficient biodiesel production. The microalgal biodiesel production pipeline is schematically presented in Figure 5, including strain selection, mass culture, biomass harvesting and processing, oil extraction and transesterification.

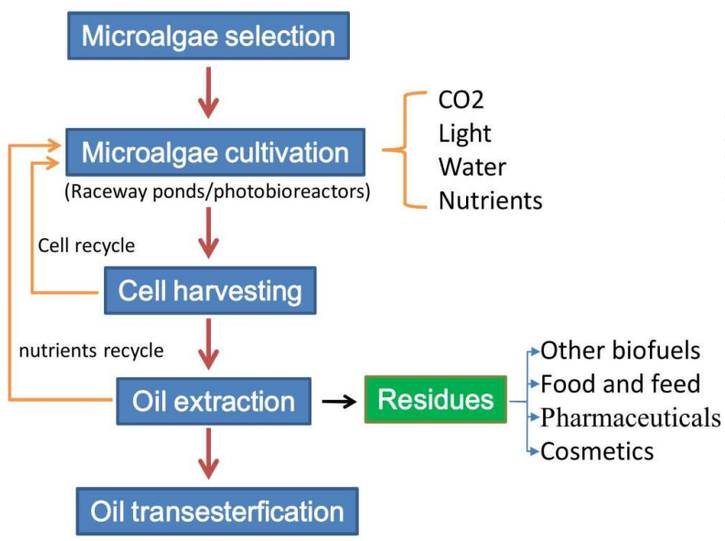

Fig. 5. Microalgal biodiesel production pipeline

3.1 Microalgae selection

There are more than 50,000 microalgal species around the world. Selection of an ideal species is of fundamental importance to the success of algal biodiesel production. Theoretically, an ideal species should own the following desirable characteristics: rapid growth rate, high oil content, wide tolerance of environmental conditions, CO2 tolerance and uptake, large cell size, easy of disruption, etc. However, it is unlikely for a single species to excel in all above mentioned characteristics. Thus, prioritization is required. Commonly, fast-growing strains with high oil content are placed on the priority list for biodiesel production. Fast growth makes sure the high biomass productivity and reduces the contamination risk owing to out-competition of slower growers. High oil content helps increase the process yield coefficient and reduce the cost of downstream extraction and purification. The selected species should be suitable for mass cultivation under local geographic and climatic conditions, for example, the inland prefers freshwater algae while the coastal place desires marine algal species. Ease of harvesting is an often-overlooked criterion and should be taken into account. Algal biomass harvest requires significant capital and accounts for up to 30% of total biomass production cost (Molina Grima et al., 2003). Therefore, it is desirable to choose algal species with properties that simplify harvesting, including large cell size, high specific gravity and autofloculation potential (Griffiths & Harrison, 2009). These properties can greatly influence the process economics for biodiesel production from algae. An additional algal characteristic is the suitability of lipids for biodiesel production; for example, neutral lipids in particular TAG are superior to polar lipids (phospholipids and glycolipids) for biodiesel and C18:1 has advantages over other fatty acids for improving biodiesel quality (Knothe, 2009).

3.2 Microalgae cultivation

3.2.1 Factors affecting algal lipids and fatty acids

Microalgae require several things to grow, including a light source, carbon dioxide, water, and inorganic salts. The lipid content and fatty acid composition are species/strain-specific and can be greatly affected by a variety of medium nutrients and environmental factors. Carbon is the main component of algal biomass and accounts for *ca* 50% of dry weight. CO_2 is the common carbon source for algal growth. But some algal species are also able to utilize organic carbon sources, for example sugars and glycerol (Easterling et al., 2009; Liu et al., 2010). Sugars particularly glucose are preferred and can be used to boost production of both algal biomass and lipids (Liu et al., 2010). Nitrogen is an important nutrient affecting lipid metabolism in algae. The influence of nitrogen concentration on lipid and fatty acid production has been investigated in numerous algal species. Nitrate was suggested to be superior to other nitrogen sources such as urea and ammonium for algal lipid production (Li et al., 2008). Generally, low concentration of nitrogen in the medium favors the accumulation of lipids particularly TAGs and total fatty acids. But in some cases, nitrogen starvation caused decreased synthesis of lipids and fatty acids (Saha et al., 2003). Nitrogen concentration also affects algal fatty acid composition. For example, in cyanobacteria, increased levels of C16:0 and C18:1 and decreased C18:2 levels were observed in response to nitrogen deprivation (Piorreck & Pohl, 1984). In the marine alga *Pavlova viridis*, nitrogen depletion resulted in an increase in saturated, monounsaturated fatty acids and C22:6 (n-3) contents (Li et al., 2005). Nitrogen starvation brought about a strong increase in the proportion of C20:4 (n-6) in the green algal *Parietochloris incisa* (Solovchenko et al., 2008). Similar to nitrogen, silicon is a key

nutrient that affects lipid metabolism of diatoms, and can promote the accumulation of neutral lipids as well as of saturated and monounsaturated fatty acids when depleted from culture medium (Roessler, 1988). Other types of nutrient deficiency include phosphorus and sulfur limitations are also able to enhance lipid accumulation in algae (Khozin-Goldberg & Cohen, 2006; Li et al., 2010b; Sato et al., 2000). These types of nutrient deficiency, however, do not always lead to elevated overall lipid production, because they at the same time exert negative effect on algal growth and contribute to the reduced algal biomass production that compromises the enhanced lipid yield resulting from increased lipid content. Therefore, the manipulation of these nutrients needs to be optimized to induce lipid accumulation while maintaining algal growth for maximal production of lipids. Iron is a micro-nutrient required in a tiny amount for ensuring algal growth. Within a certain range of concentrations, high concentrations of iron benefit algal growth as well as cellular lipid accumulation and thus the overall lipid yield in the green alga *Chlorella vulgaris* (Liu et al., 2008).

Among the environmental factors, light is an important one that has a marked effect on the lipid production and fatty acid composition in algae (Brown et al., 1996; Damiani et al., 2010; Khotimchenko & Yakovleva, 2005; Napolitano, 1994; Sukenik et al., 1989; Zhekisheva et al., 2002, 2005). Generally, low light intensity favors the formation of polar lipids such as the membrane lipids associated with the chloroplast; whereas high light intensity benefits the accumulation of neutral storage lipids in particular TAGs. In *H. pluvialis,* for example, high light intensity resulted in a great increase of both neutral and polar lipids, but the increase extent of neutral lipids was much greater than that of polar lipids, leading to the dominant proportion of neutral lipids in the total lipids (Zhekisheva et al., 2002, 2005). Although the effect of light intensity on fatty acid composition differs among the algal species and/or strains, there is a general trend that the increase of light intensity contributes to the enhanced proportions of saturated and monounsaturated fatty acids and the concurrently the reduced proportion of polyunsaturated fatty acids (Damiani et al., 2010; Sukenik et al., 1989; Zhekisheva et al., 2002, 2005). Temperature is another important environmental factor that affects profiles of algal lipids and fatty acids. In response to temperature shift, algae commonly alter the physical properties and thermal responses of membrane lipids to maintain fluidity and function of membranes (Somerville, 1995). In general, increased temperature causes increased fatty acid saturation and at the same time decreased fatty acid unsaturation. For example, C14:0, C16:0, C18:0 and C18:2 increased and C18:3 (n-3), C18:4, C20:5 and C22:6 decreased in *Rhodomonas* sp., and C16:0 increased and C18:4 decreased in *Cryptomonas* sp. when temperature increased (Renaud et al., 2002). As for the effect of temperature on cellular lipid content, it differs in a species-dependent manner. In response to increased temperature, algae may show an increase (Boussiba et al., 1987), no significant change or even a decrease (Renaud et al., 2002) in lipid contents. Other environmental factors such as salinity, pH and dissolved O_2 are also important and able to affect algal lipid metabolism.

In addition to the nutritional and environmental factors, growth phase and aging of the culture affect algal lipids and fatty acids. Commonly, algae accumulate more lipids at stationary phase than at logarithmic phase (Bigogno et al., 2002; Mansour et al., 2003). Associated with the growth phase transition from logarithmic to stationary phase, increased proportions of C16:0 and C18:1 and decreased proportions of PUFAs are often observed. Besides, it is suggested that algal lipids and fatty acids can be greatly affected by cultivation modes. Algae growing under heterotrophic mode usually produce more

lipids in particular TAG and higher proportion of C18:1 than under photoautotrophic mode (Liu et al., 2011).

3.2.2 Raceway ponds and photobioreactors

Currently, the commonly used culture systems for large-scale production of algal biomass are open ponds and enclosed photobioreactors. An open pond culture system usually consists of a series of raceways-type of ponds placed outdoors. In this system, the shallow pond is usually about one foot deep; algae are cultured under conditions identical to their natural environment. The pond is designed in a raceway configuration, in which a paddle wheel provides circulation and mixing of the algal cells and nutrients (Chisti, 2007). The raceways are typically made from poured concrete, or they are simply dug into the earth and lined with a plastic liner to prevent the ground from soaking up the liquid. Compared with photobioreactors, open ponds cost less to build and operate, and are more durable with a large production capacity. However, the open pond system has its intrinsic disadvantages including rapid water loss due to evaporation, contamination with unwanted algal species as well as organisms that feed on algae, and low biomass productivity. In addition, optimal culture conditions are difficult to maintain in open ponds and recovering the biomass from such a dilute culture is expensive.

Unlike open ponds, enclosed photobioreactors are flexible systems that can be employed to overcome the problems of evaporation, contamination and low biomass productivity encountered in open ponds (Mata et al., 2010). These systems are made of transparent materials with a large surface area-to-volume ratio, and generally placed outdoors using natural light for illumination. The tubular photobioreactor is the most widely used one, which consists of an array of straight transparent tubes aligned with the sun's rays (Chisti, 2007). The tubes are generally no more than 10 cm in diameter to maximize sunlight penetration. The medium broth is circulated through a pump to the tubes, where it is exposed to light for photosynthesis, and then back to a reservoir. In some photobioreactors, the tubes are coiled to form what is known as a helical tubular photobioreactor. Artificial illumination can be used for photobioreactor. But it adds to the production cost and thus is used for the production of high value products instead of biodiesel feedstock. The algal biomass is prevented from settling by maintaining a highly turbulent flow within the reactor using either a mechanical pump or an airlift pump (Chisti, 2007). The result of photosynthesis will generate oxygen. The oxygen levels will accumulate in the closed photobioreactor and inhibit the growth of algae. Therefore, the culture must periodically be returned to a degassing zone, an area where the algal broth is bubbled with air to remove the excess oxygen. In addition, carbon dioxide must be fed into the system to provide carbon source and maintain culture pH for algal growth. Photobioreactors require cooling during daylight hours and temperature regulation in night hours. This may be done through heat exchangers located either in the tubes themselves or in the degassing column.

Table 4 shows the comparison between open ponds and photobioreactors for microalgae cultivation.

Photobioreactors have obvious advantages over open ponds: offer better control, prevent contamination and evaporation, reduce carbon dioxide losses and allow to achieve higher biomass productivities. However, enclosed photobioreactors cost high to build and operate and the scale-up is difficult, limiting the number of large-scale commercial systems operating globally to high-value production runs (Greenwell et al., 2010). In this context, a hybrid photobioreactor-open pond system is proposed: using photobioreactors to produce contaminant-free inoculants for large open ponds.

Culture systems	Open ponds	Enclosed bioreactors
Contamination control	Difficult	Easy
Contamination risk	High	Reduced
Sterility	None	Achievable
Process control	Difficult	Easy
Species control	Difficult	Easy
Mixing	Very poor	Uniform
Operation regime	Batch or semi-continuous	Batch or semi-continuous
Area/volume ration	Low	High
Algal cell density	Low	High
Investment	Low	Hight
Operation cost	Low	High
Light utilization efficiency	Poor	High
Temperature control	difficult	More uniform temperature
Productivity	Low	High
Hydrodynamic stress on algae	Very low	Low-high
Evaporation of growth medium	High	Low
Gas transfer control	Low	High
O_2 inhibition	< bioreactors	Great problem
Scale-up	Difficult	Difficult

Table 4. Comparison of open ponds and photobioreactors for microalgae cultivation (Mata et al., 2010)

3.3 Biomass harvesting and concentration

Algal harvesting is the concentration of diluted algal suspension into a thick algal paste, with the aim of obtaining slurry with at least 2–7% algal suspension on dry matter basis. Biomass harvest is a very challenging process and may contribute to 20-30% of the total biomass production cost (Molina Grima et al., 2003). The most common harvesting methods include sedimentation, filtration, centrifugation, sometimes with a pre-step of flocculation or flocculation-flotation. Flocculation is employed to aggregate the microalgal cells into larger clumps to enhance the harvest efficiency by gravity sedimentation, filtration, or centrifugation (Molina Grima et al., 2003). The selection of a harvesting process for a particular strain depends on size and properties of the algal strain. The selected harvest method must be able to handle a large volume of algal culture broth.

Filtration is the most commonly used method for harvesting algal biomass. The process can range from micro-strainers to pressure filtration and ultra-filtration systems. Vacuum filtration is feasible for harvesting large microalgae such as *Coelastrum proboscideum* and *Spirulina platensis* but unsuitable for recovering small size algal cells such as *Scenedesmus, Dunaliella*, or *Chlorella* (Molina Grima et al., 2003). Membrane-based microfiltration and ultrafiltration have also been used for harvesting algal cells for some specific application purposes, but overall, they are more expensive. Centrifugation is an accelerated sedimentation process for algae harvesting. Generally, centrifugation has high capital and operation costs, but its efficiency is much higher than natural sedimentation. Because of its high cost, centrifugation as an algae harvesting method is usually considered only feasible for high value products rather than biofuels.

3.4 Biomass processing for oil extraction

After harvesting, chemicals in the biomass may be subject to degradation induced by the process itself and also by internal enzyme in the algal cells. For example, lipase contained in

the cells can rapidly hydrolyze cellular lipids into free fatty acids that are not suitable for biodiesel production. Therefore, the harvested biomass need be processed rapidly. Drying is a major step to keep the quality of the oil. In addition, the solvent-based oil extraction can be difficult when wet biomass is used. Various drying methods such as sun drying, spray drying, freeze drying, and drum drying can be used for drying algal biomass (Mata et al., 2010). Due to the high water content of algal biomass, sun-drying is not a very effective method for algal powder production. Spray drying and freeze drying are rapid and effective, but also expensive and not economically feasible for biofuel production. Because of the high energy required, drying is considered as one of the main economical bottlenecks in the entire process.

There are several approaches for extracting oil from the dry algal biomass, including solvent extraction, osmotic shock, ultrasonic extraction and supercritical CO_2 extraction. Oil extraction from dried biomass can be performed in two steps, mechanical crushing followed by solvent extraction in which hexane is the main solvent used. For example, after the oil extraction using an expeller, the leftover pulp can be mixed with cyclohexane to extract the remaining oil. The oil dissolves in the cyclohexane and the pulp is filtered out from the solution. These two stages are able to extract more than 95% of the total oil present in the algae. Oil extraction from algal cells can also be facilitated by osmotic shock or ultrasonic treatment to break the cells. Osmotic shock is a sudden reduction in osmotic pressure causing cells to rupture and release cellular components including oil. The algae lacking the cell wall are suitable for this process. In the ultrasonic treatment, the collapsing cavitation bubbles near to the cell walls cause cell walls to break and release the oil into the solvent. Supercritical CO_2 is another way for efficient extraction of algal oil, but the high energy demand is a limitation for commercialization of this technology (Herrero et al., 2010).

3.5 Oil transesterification

Algal oil contained in algal cells can be converted into biodiesel through transesterification. Transesterification is a chemical conversion process involving reacting triglycerides of vegetable oils or animal fats catalytically with a short-chain alcohol (typically methanol or ethanol) to form fatty acid esters and glycerol (Figure 6). This reaction occurs stepwise with the first conversion of triglycerides to diglycerides and then to monoglycerides and finally to glycerol. The complete transesterification of 1 mol of triglycerides requires 3 mol of alcohol, producing 1 mol of glycerol and 3 mol of fatty esters. Considering that the reaction is reversible, large excess of alcohol is used in industrial processes to ensure the direction of fatty acid esters. Methanol is the preferred alcohol for industrial use because of its low cost, although other alcohols like ethanol, propanol and butanol are also commonly used.

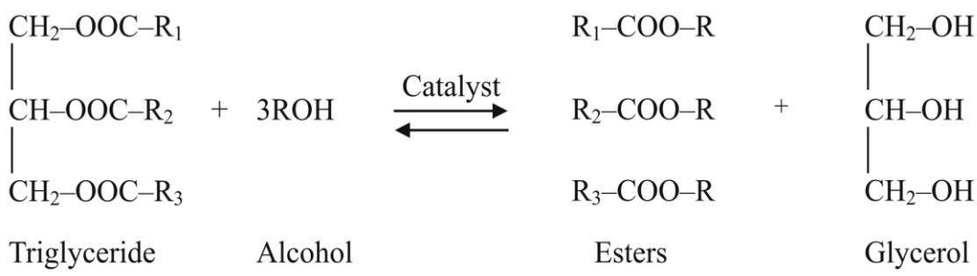

Fig. 6. Transesterification of oil to biodiesel. $R_{1\text{-}3}$ indicates hydrocarbon groups.

In addition to heat, a catalyst is needed to facilitate the transesterification. The transesterification of triglycerides can be catalyzed by acids, alkalis or enzymes. Acid transesterification is considered suitable for the conversion of feedstocks with high free fatty acids but its reaction rate is low (Gerpen, 2005). In contrast, alkali-catalyzed transesterification has a much higher reaction rate, approximately 4000 times faster than the acid-catalyzed one (Fukuda et al., 2001). In this context, alkalis (sodium hydroxide and potassium hydroxide) are preferred as catalysts for industrial production of biodiesel. The use of lipases as transesterification catalysts has also attracted much attention as it produces high purity product and enables easy separation from the byproduct glycerol (Ranganathan et al., 2008). However, the cost of enzyme is still relatively high and remains a barrier for its industrial implementation. In addition, it has been proposed that biodiesel can be prepared from oil via transesterification with supercritical methanol (Demirbas, 2002).

4. Genetic engineering of microalgae

4.1 Microalgal lipid biosynthesis

Although lipid metabolism, in particular the biosynthesis of fatty acids and TAG, is poorly understood in algae, it is generally recognized that the basic pathways for fatty acid and TAG biosynthesis are similar to those demonstrated in higher plants.

Algae synthesize the *de novo* fatty acids in the chloroplast using a single set of enzymes. A simplified schedule for saturated fatty acid biosynthesis is shown in Figure 7. Acetyl-CoA is the basic building block of the acyl chain and serves as a substrate for acetyl CoA

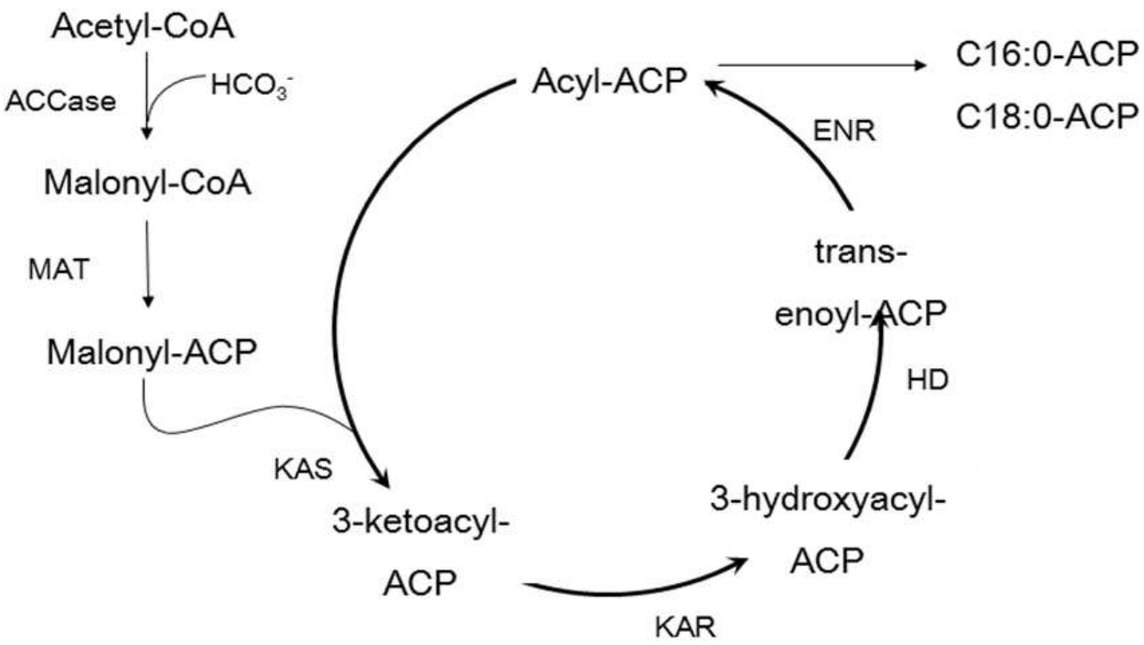

Fig. 7. Simplified overview of saturated fatty acid biosynthesis in algal chloroplast. ACCase, acetyl-CoA carboxylase; ACP, acyl carrier protein; CoA, coenzyme A; ENR, enoyl-ACP reductase; HD, 3-hydroxyacyl-ACP dehydratase; KAR, 3-ketoacyl-ACP reductase; KAS, 3-ketoacyl-ACP synthase; MAT, malonyl-CoA:ACP transacylase.

carboxylation and as well as a substrate for the initial condensation reaction. The formation of malonyl CoA from acetyl CoA is generally regarded as the first reaction of fatty acid biosynthesis, which is catalyzed by acetyl CoA carboxylase (ACCase). The malonyl group of malonyl CoA is transferred to a protein co-factor, acyl carrier protein (ACP), resulting in the formation of malonyl ACP that enters into a series of condensation reactions with acyl ACP (or acetyl CoA) acceptors. The first condensation reaction is catalyzed by 3-ketoayl ACP synthase III (KAS III), forming a four-carbon product. KAS I and KAS II catalyze the subsequent condensations. After each condensation, the 3-ketoacyl-ACP product is reduced, dehydrated, and reduced again, by 3-ketoacyl-ACP reductase, 3-hydroxyacyl-ACP dehydratase, and enoyl-ACP reductase, respectively, to form a saturated fatty acid. To produce an unsaturated fatty acid, a double bond is introduced onto the acyl chain by the soluble enzyme stearoyl ACP desaturase (SAD). Unlike plants, some algae produce long-chain acyl ACPs (C_{20}-C_{22}) that derive from the further elongation and/or desaturation of C_{18}. The fatty acid elongation is terminated when the acyl group is released from ACP by an acyl-ACP thioesterase that hydrolyzes the acyl ACP and produces free fatty acids or by acyltransferases that transfer the fatty acid from ACP to glycerol-3-phosphate or monoacylglycerol-3-phosphate. These released fatty acids serve as precursors for the synthesis of cellular membranes and neutral storage lipids like TAG.

It has been proposed that the biosynthesis of TAG occurs in cytosol via the direct glycerol pathway (Figure 8). Generally, acyl-CoAs sequentially react with the hydroxyl groups in glycerol-3-phosphate to form phosphatidic acid via lysophosphatidic acid. These two reactions are catalyzed by glycerol-3-phospate acyl transferase and lysophosphatidic acid acyl transferase respectively. Dephosphorylation of phosphatidic acid results in the release of DAG which accepts a third acyl from CoA to form TAG. This final step is catalyzed by diacylglycerol acyltransferase, an enzymatic reaction that is unique to TAG synthesis. In addition, an alternative pathway that is independent of acyl-CoA may also be present in algae for TAG biosynthesis (Dahlqvist et al., 2000). This pathway employs phospholipids as acyl donors and diacylglycerols as the acceptors and might be activated when algal cells are exposed to stress conditions, under which algae usually undergo rapid degradation of the photosynthetic membranes and concurrent accumulation of cytosolic TAG-enriched lipid bodies (Hu et al., 2008b).

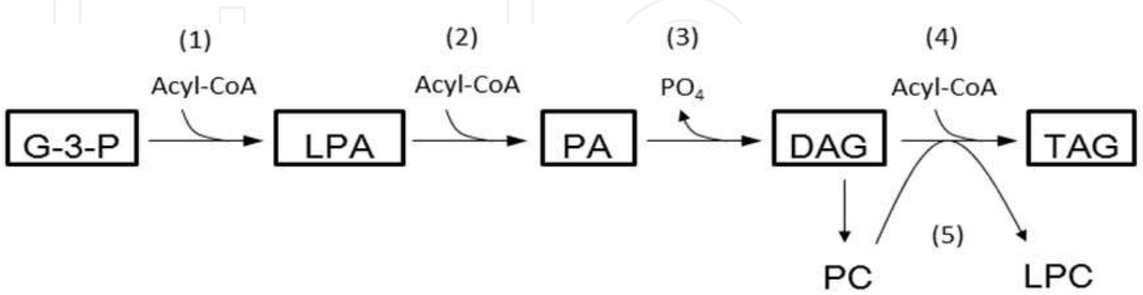

Fig. 8. Simplified illustration of the TAG biosynthesis in algae. DAG, diacylglycerol; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; G-3-P, glycerol-3-phosphate; PA, phosphatidic acid; PC, phosphatidylcholine; TAG, triacylglycerol. (1) glycerol-3-phosphate acyl transferase, (2) lysophosphatidic acid acyl transferase, (3) phosphatidic acid phosphatase, (4) diacylglycerol acyl transferase, and (5) phospholipid:diacylglycerol acyltransferase.

4.2 Genetic engineering of microalgal lipids

Genetic engineering is a feasible and complimentary approach to increase algal productivity and improve the economics of algal biodiesel production. This has long been recognized but it seems that so far little progress has been made. The lack of full or near-full genome sequences and robust transformation systems makes genetic engineering of algae lag much behind that of bacteria, fungi and higher eukaryotes. Although certain algal species have been reported for efficient transformation, it proves to be difficult to produce stable transformants of algae. Currently, sophisticated genetic engineering whereby several genes are concurrently down-regulated or overexpressed is only really applicable to the green alga *Chlamydomonas reinhardtii*. This situation, however, is likely to change because of the growing scientific and commercial interest in other algal species that are of great potential for industrial applications.

Understanding the algal lipid biosynthesis is of great help to engineer algal lipid production. Although lipid metabolism in algae is not as fully understood as that in higher plants, they have similar lipid biosynthetic pathway as mentioned above. Theoretically, overexpression of the genes involved in fatty acid synthesis is able to increase lipid accumulation, in that fatty acids required as precursors for lipid biosynthesis are produced in excess. However, overexpressoin of the native ACCase, the rate-limiting enzyme catalyzing the first committed step of fatty acid biosynthesis in many organisms, could not increase the lipid production in diatom (Dunahay et al., 1995). It is possible that under high flux conditions through ACCase, the condensing enzymes or other factors may begin to limit fatty acid synthesis rate. Therefore, more complete control may come from certain transcription factors that can increase expression of the entire pathway. Another feasible approach of increasing cellular lipid contents is to inhibit metabolic pathways that lead to other carbon storage compounds, such as starch. Starch synthesis shares common carbon precursors with lipid synthesis in algae. Blocking starch synthesis is able to redirect carbon flux to lipid biosynthetic pathway, resulting in overproduction of fatty acids and thus total lipids (Li et al., 2010a). Neutral lipids in particular TAG surpass other lipids for biodiesel production, attracting the interest of enhancing cellular TAG contents through genetic engineering. Overexpression of genes involved in TAG assembly, e.g., glycerol-3-phosphate acyltransferase, lysophosphatidic acid acyltransferase, or diacylglycerol acyltransferase, all significantly increase TAG production in plants. Such strategies may also be applicable to algae for enhancing TAG levels. Commonly, algae produce larger amounts of lipids under unfavorable conditions than logarithmic growing condition. Enhancing lipid biosynthesis through genetic engineering, therefore, is likely to reduce algal proliferation and biomass production. In this context, the use of inducible promoters could overcome the problem because the transgenic expression can only be activated when a high cell density is achieved.

The important properties of biodiesel such as cetane number, viscosity, cold flow, oxidative stability, are largely determined by the composition and structure of fatty acid esters which in turn are determined by the characteristics of fatty acids of biodiesel feedstocks, for example carbon chain length and unsaturation degree (Knothe, 2005b). Thus, the genetic modification of algal fatty acid composition is of also great interest. Generally, saturated fatty esters possess high cetane number and superior oxidative stability; whereas unsaturated, especially polyunsaturated fatty esters have improved low-temperature properties (Knothe, 2008). In this regard, it is suggested that the modification of fatty esters, for example the enhanced proportion of oleic acid (C18:1) ester, can provide a compromise solution between oxidative stability and low-temperature properties and therefore promote

the quality of biodiesel (Knothe, 2008, 2009). Oleic acid is converted to linoleic acid (C18:2) in a single desaturation step, catalyzed by a Δ12 desaturase enzyme encoded by the *FAD2* gene. Inactivation of this desaturation step can greatly increase the proportion of oleic acid in soybean and may represent a possible strategy for elevated accumulation of oleic acid in algae.

Genetic engineering can also be used potentially to improve tolerance of algae to stress factors such as temperature, salinity and pH. These improved attributes will allow for the cost reduction in algal biomass production and be beneficial for growing selected algae under extreme conditions that limit the proliferation of invasive species. Photoinhibition is another technical challenge to be addressed by genetic engineering. When the light intensities exceed the value for maximum photosynthetic efficiency, algae show photoinhibition, a common phenomenon for phototrophy under which the growth rate slows down. Engineered algae with a higher threshold of light inhibition will significantly improve the economics of biodiesel production.

Engineering algae for biodiesel production is currently still in its infancy. Significant advances have only been achieved in the genetic manipulation of some model algae. It is likely that many of these advances can be extended to industrially important algal species in the future, making it possible to use modified algae as cell factories for commercial biodiesel production. Nevertheless, many challenges yet remain open and should be addressed before profitable algal biodiesel become possible.

5. Conclusion and perspectives

Microalgae have the potential for the production of profitable biodiesel that can eventually replace petroleum based fuel. Algal-biodiesel production, however, is still too expensive to be commercialized as no algal strains are available possessing all the advantages for achieving high yields of oil via the economical open pond culturing system. Current studies are still limited to the selection of ideal microalgal species, optimization of mass cultivation, biomass harvest and oil extraction processes, which contribute to high costs of biodiesel production from microalgae. Future cost-saving efforts for algal-biofuel production should focus on the production technology of oil-rich algae via enhancing algal biology (in terms of biomass yield and oil content) and culture-system engineering coupled with advanced genetic engineering strategies and utilization of wastes. In addition to oils, microalgae also contain large amounts of proteins, carbohydrates, and other nutrients or bioactive compounds that can be potentially used as feeds, foods and pharmaceuticals. Integrating the production of such co-products with biodiesel is an appealing way to lowering the cost of algal-biofuel production.

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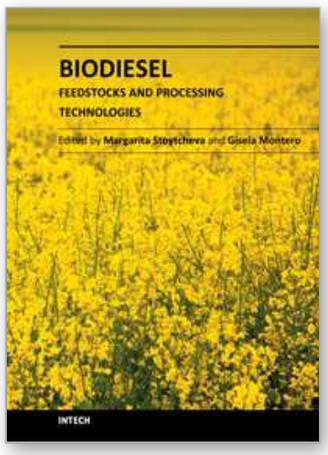

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The book "Biodiesel: Feedstocks and Processing Technologies" is intended to provide a professional look on the recent achievements and emerging trends in biodiesel production. It includes 22 chapters, organized in two sections. The first book section: "Feedstocks for Biodiesel Production" covers issues associated with the utilization of cost effective non-edible raw materials and wastes, and the development of biomass feedstock with physical and chemical properties that facilitate it processing to biodiesel. These include Brassicaceae spp., cooking oils, animal fat wastes, oleaginous fungi, and algae. The second book section: "Biodiesel Production Methods" is devoted to the advanced techniques for biodiesel synthesis: supercritical transesterification, microwaves, radio frequency and ultrasound techniques, reactive distillation, and optimized transesterification processes making use of solid catalysts and immobilized enzymes. The adequate and up-to-date information provided in this book should be of interest for research scientist, students, and technologists, involved in biodiesel production.

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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