Rangsit Journal of Arts and Sciences, January-June 2013 RJAS Vol. 3 No. 1, pp. 87-98

Review on green energy from microalgae

Sasirin Labua* and Vanida Osiripun

Faculty of Biotechnology, Rangsit University, Patumthani 12000, Thailand
E-mail: sasirin.l@rsu.ac.th

*Corresponding author

Submitted 8 November 2012; accepted in final form 15 May 2013

Abstract

Liquid fuels derived from petroleum have played a prominent role in our daily lives for centuries. Day after day, oil demand has been increasing because of increases in industrialization and population. Despite the many advantages of oil based fuels, there are many major disadvantages as well such as atmospheric pollution due to green house gases. Bioenergy is a clean energy and is one of the most important proposals to mitigate greenhouse gas emissions and substitute fossil fuels. Bioenergy can be an alternative energy source with the added benefit of fixing CO_2 in the atmosphere through photosynthesis. Given the reliance on petroleum based fuels sources for most industrial machinery, finding an alternative is critical. Biodiesel fuel production from microalgae is technically feasible due to the highest yielding feedstock, short harvesting time and being exceedingly rich in oil.

Keywords*: green energy, microalgae, algae oil, biodiesel*

บทคัดย่อ

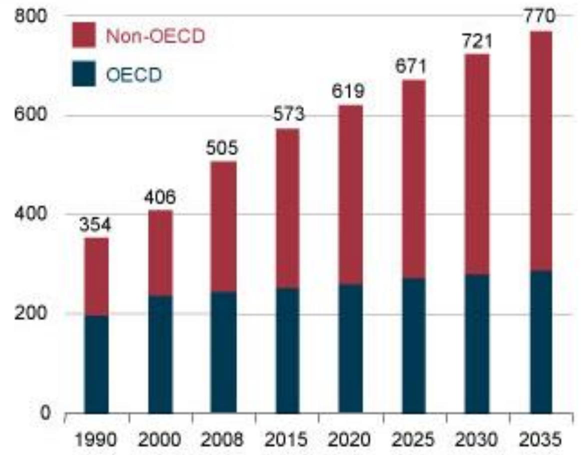
นานนับศตวรรษที่เชื้อเพลิงปิโตรเลียมเหลวเข้ามามีบทบาทสำคัญต่อการดำเนินชีวิตของมนุษย์และนับวันยิ่งมีความต้องการใช้เพิ่มขึ้นอย่างต่อเนื่องตามจำนวนประชากรและอุตสาหกรรมที่เพิ่มมากขึ้น ซึ่งข้อเสียของการใช้เชื้อเพลิงดังกล่าวคือการปล่อยก๊าซเรือนกระจกซึ่งเป็นมลพิษต่อสิ่งแวดล้อม พลังงานชีวภาพหรือพลังงานสะอาดถือเป็นแหล่งพลังงานที่สามารถทดแทนเชื้อเพลิงจากซากดึกดำบรรพ์ที่สำคัญที่สุดเนื่องจากสามารถลดปริมาณก๊าซคาร์บอนไดออกไซด์ในชั้นบรรยากาศได้โดยผ่านกระบวนการสังเคราะห์แสง การผลิตไบโอดีเซลจากสาหร่ายขนาดเล็กจึงเป็นอีกวิธีหนึ่งที่มีความเป็นไปได้สูงเนื่องจากมีปริมาณมาก เจริญเติบโตเร็วและสาหร่ายขนาดเล็กหลายสายพันธุ์ยังมีปริมาณน้ำมันที่สูงมากอีกด้วย

คำสำคัญ: *พลังงานสะอาด, สาหร่ายขนาดเล็ก, น้ำมันจากสาหร่าย, ไบโอดีเซล*

1. Introduction and trends in world primary energy demand

Population growth and income levels are the key drivers behind increasing demand for energy. By 2030 the world's population is projected to reach 8.3 billion, meaning an additional 1.3 billion people will need energy (BP's Energy Outlook 2013-annual report, 17 January 2013). Moreover, the world energy consumption and the energy use have been increasing continuously as shown in Figure 1. The U.S. Energy Information Administration (EIA, 2013) reported in 2013 that the world will need almost 51% more energy in 2035 than today. Energy consumption in developing countries is projected to grow at an average annual rate of 3 percent from 2013 to 2020. In industrialized countries, where national economies are mature and population growth is expected to be relatively low, the demand for energy is projected to grow at a lower rate of 0.9 percent per year, albeit from a much higher starting point. Energy consumption in developing regions is projected to surpass that in industrialized regions by 2010. If this trend continues, the world will be confronted with an energy crisis because the worldwide fossil oil reserves will be exhausted in fewer than 45 years (IEA, 2010). About half of the increase in global energy demand by 2030 will be for power generation and one-fifth for transport needs mostly in the form of petroleum-based fuels. Therefore, fossil fuels as a source of energy should be replaced with renewable, clean energy sources to reduce carbon dioxide and green house gas emissions (Amin, 2009). Renewable, carbon neutral, transport fuels are necessary for environmental and economic sustainability (Chisti, 2007).

DOI : 10.14456/rjas.2012.25



OECD = Organization for Economic Co-operation and Development

Figure 1 World energy consumption, 1990-2035. (EIA, 2013)

The vast majority of the world's energy is generated from non-renewable sources, specifically oil, coal and gas (Figure 2). The remainder of renewable energy comes from hydro-, geothermal, solar, wind, and tidal and wave sources. Projections of total global energy consumption show that between 2000 and 2030, fossil fuels will provide the bulk of the increase, with nuclear and other sources providing relatively minor contributions in absolute terms. Gas and coal are likely to show the greatest change. The ultimate contributions from different sources will be highly dependent on policy directions (IEA, 2002; IPIECA, 2004).

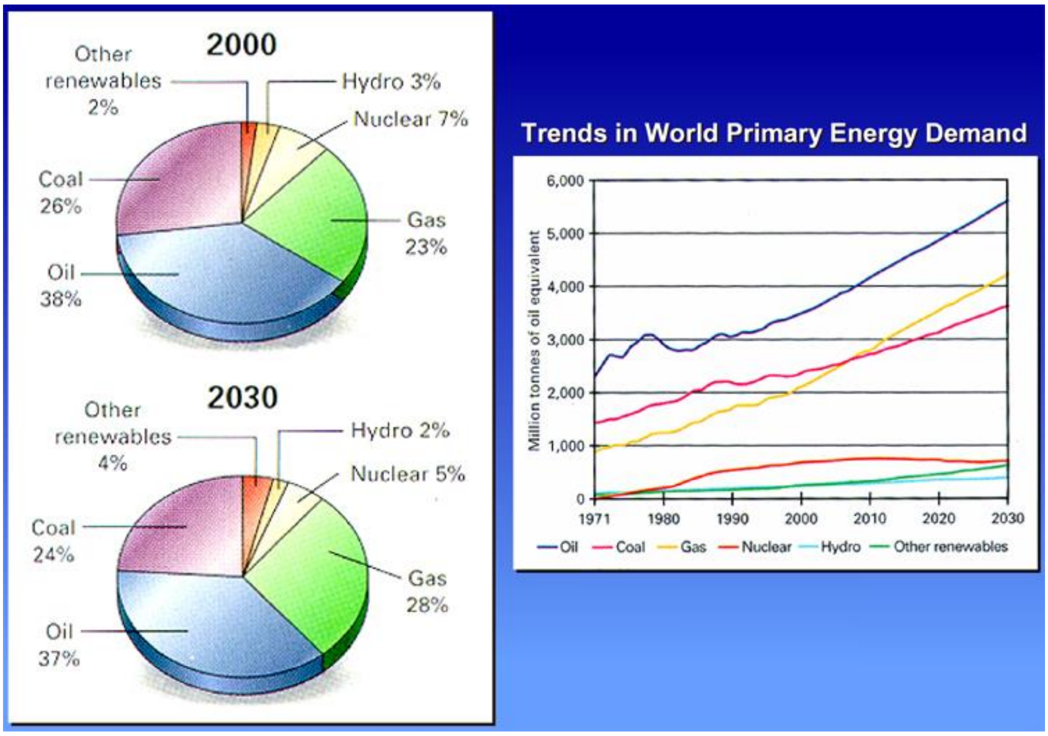


Figure 2 Trends in world primary energy demand (From: -IEA, 2002 and IPIECA, 2004)

Delivered energy consumption in the transportation sector will remain relatively constant at about 27 quadrillion Btu from 2011 to 2040 in the *Annual Energy Outlook 2013 (AEO2013)* Reference case (Figure 3). Energy consumption by Light-Duty Vehicles (LDVs) (including commercial light trucks) is projected to decline, from 16.1 quadrillion Btu in 2011 to 14.0 quadrillion Btu in 2025, due to incorporation of higher Green House Gas (GHG) and Corporate Average Fuel Economy (CAFÉ) standards for LDVs for model years 2017 to 2025. Higher industrial output in *AEO2013* could lead to greater growth in vehicle-miles traveled by freight trucks, which may lead to higher energy demand by heavy vehicles in *AEO2013* as compared with *AEO2012*. Factors used to calculate the economic effectiveness of heavy-duty alternative-fuel vehicles have been updated to represent the travel behavior of first-time buyers and economic breakeven hurdles that, when coupled with very competitive natural gas prices, significantly increases demand for natural gas fuel in heavy trucks. As a result, natural gas use in heavy-duty vehicles will increase to 1.7 trillion cubic feet in 2040, displacing 0.7 million barrels of liquid fuels per day. The *AEO2013* Reference case includes the GHG Emissions and Fuel Efficiency Standards for Medium- and Heavy-Duty Engines and Vehicles published by the Environmental Protection Agency (EPA) and the National Highway Traffic Safety Administration in September 2011 (BP's Energy Outlook Booklet 2013).

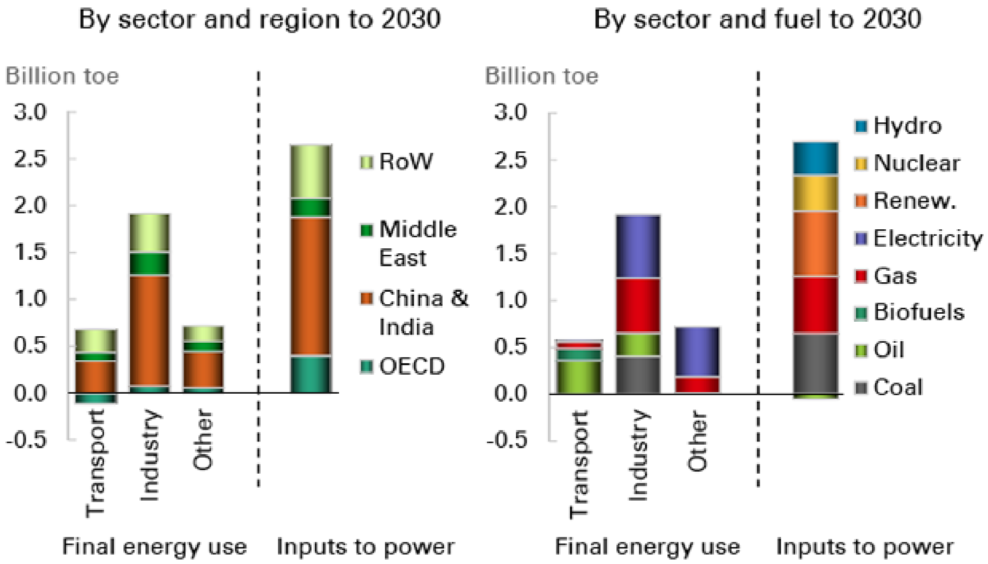


Figure 3 Energy use by sector and region to 2030 (From: BP Energy Outlook 2030)

Petroleum is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment. It is widely accepted that the continued burning of fossil fuels has caused global warming. Other detrimental effects of global warming include a potential increase in sea levels and subsequent submerging of lowlands, deltas and island, as well as changing weather patterns (Hassan, Yacob, & Ghani, 2005). Biomass has been focused on as an alternative energy source, since it is a renewable resource and it fixes CO_2 in the atmosphere. The rate of depletion of fossil fuels and the effect of greenhouse gas emissions on global climate change are creating much interest in biodiesel (Lang, Dalai, Bakhshi, Reaney, & Hertz, 2001; Lee, Yoo, Jun, Ahn, & Oh, 2010).

Biodiesel is the main alternative to fossil fuel and is an attractive energy resource for several reasons. It is a renewable fuel that could be sustainably supplied, is highly biodegradable and has minimal toxicity. Also, it appears to cause significant improvement of rural economic potential (Cadenas & Cabezudo, 1998). It is environmentally friendly, resulting in very low sculpture release and no net increase release of carbon dioxide, aromatic compounds or other chemical substances that are harmful to the environment (Khan, Rashmi, Hussain, Prasad, & Banerjee, 2009; Vicente, Martinez, & Aracil, 2004; Antolin, Tinaut, & Briceno, 2002). Biodiesel is better than petroleum-based diesel in term of its lower combustion emission profile, and it does not contribute to global warming because of it closed carbon cycle. An important goal for every country is to strive for energy independence. The production and use of biodiesel allows this and decreases dependence on foreign crude oil. Importantly, it can be used in existing diesel engines with little or no engine modification and with continuing efficient performance (Demirbas, 2002). Should there be an issue with combustive power output, it can be blended in any ratio with traditional petroleum-based diesel fuel in a diesel engine (Peterson, Feldman, Korus, & Auld, 1991). When added to regular diesel fuel in an amount of 1-2%, it can convert fuel with poor lubricating properties into an acceptable fuel (Gerpen, 2005). And finally, it can provide improved combustion petroleum-based diesel because of its high oxygen content.

Thailand's primary energy consumption is mostly from fossil fuels, accounting for over 80% of the country's total energy consumption. Oil accounted for 39% of total energy consumption in 2010. The country imports over 60% of its total petroleum needs and almost 85% of its crude oil consumption. Thailand is a net importer of oil and natural gas, although it is a growing producer of natural gas. In the next 20 years (2011-2030), if there is no energy conservation or energy efficiency improvement measures or no significant reform of the industrial structure and transportation system, energy demand under the business-as-usual (BAU) scenario will increase from 71,000 kilo tonnes of oil equivalent (ktoe) per year at present, to 151,000 ktoe, or about 2.1 times the present amount. This will equate to an annual average growth rate of 3.9%, under the assumption that the GDP will grow at an annual average rate of 4.2%. Hence, greenhouse gas emission from the energy sector will increase accordingly (EEDP, 2011-2030). Thailand's new alternative energy development plan will increase the biodiesel consumption target to 37,550 barrel per day (bbl/d) by 2021(EIA, 2013). Microalgae biodiesel in Thailand is one of the most attractive fossil fuels subsidies and can also decrease GHG that are causing global warming.

2. Potential of microalgal biodiesel

Biodiesel can be made from oil crops, waste cooking oil and animal fat. The major components of these sources are triglycerol molecules (TAGs) (Figure 4). The average oil yield per hectare from various crops and the farming area needed to meet 50% of the U.S. transport fuel are shown in Table 1.

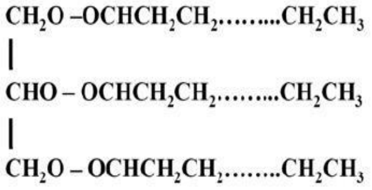


Figure 4 Molecular structure of triacylglycerol (TAG) (Adapted from Chisti, 2007)

Table 1 Comparison of some sources of biodiesel

Crop	Oil yield (L/ha)	Land area needed (M ha) [a]
Corn	172	1540
Soybean	442	594
Canola	1190	223
Jatropha	1892	140
Coconut	2689	99
Oil palm	5950	45
Microalgae[b]	136,900	2
Microalgae[c]	58,700	4.5

[a] For meeting 50% of all transport fuel needs of the United States
[b] 70% oil (by wt) in biomass
[c] 30% oil (by wt) in biomass

Table 1 shows that oil palm, a high-yielding oil crop can be grown 24% of the total cropland will need to be devoted to its cultivation to meet only 50% of the transport fuel needs. Algae can produce up to 300 times more oil per unit area than conventional crops such as rapeseed, palms, soybeans, or jatropha. As algae have a harvesting cycle of 1–10 days, their cultivation permits several harvests in a very short time-frame, a strategy differing from annual crops. This prompted the investigation into algae versus other biofuels, such as corn-based or sugar-cane-based ethanol. One reason is that algae can be grown using land or water that is unsuitable for plant or food production. In other words, unlike many other biofuels, algae biofuels do not compete with the food supply. Algae can yield more biofuel per acre than plant-based biofuels, currently 2,000 gallons of fuel per acre, per year which is almost five times more fuel per acre than sugar cane and almost 10 times more fuel per acre than corn. Algae consume CO_2 as they grow, so algae biofuels could help mitigate greenhouse gas emissions (Shirvani, Yan, Inderwildi, Edwards, & King, 2011). Shay (1993) reported that algae can produce up to 250 times the amount of oil per acre as compared to soybeans or 7 to 31 times greater oil than palm oil. Clearly, oil from crops cannot significantly contribute to replacing petroleum derived liquid fuels in the foreseeable future. This scenario changes dramatically if microalgae are used to produce biodiesel. Between 1% and 3% of the total U.S. farming area would be sufficient for producing algal biomass that satisfies 50% of the transport fuel needs. Microalgae appear to be the only source of biodiesel that has the potential to completely displace fossil fuel derived diesel. Unlike other oil producing crops, microalgae grow extremely rapidly and are exceedingly rich in oil. Microalgaes are one of the most promising sustainable energy sources for biodiesel production. Tilman, Socolow, Foley, Hill, Larson and Lynd (2009) concisely summarized the food, energy and environmental implications of biofuel development. In their policy forum, they argued cogently that "biofuels done right" must be derived from feedstocks with low greenhouse gas emissions and little or no competition with food production. With a little water, a few nutrients and carbon dioxide, microalgae use energy from the sun to grow, easily doubling their population in a day. However, when microalgae divert energy into accumulating oil, they do not grow very fast, if at all, and do not make much oil, a trade-off that can result in little increase in the overall production of oil (Waltz, 2009). This trade-off has been studied by researchers who are trying to improve the cultivation of microalgae.

Table 2 Oil content of some microalgae (Chisti, 2007)

Microalgae	Oil content (% dry wt)
Botryococcus braunii	25-75
Chlorella sp.	28-32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella primolecta	23
Isochrysis sp.	25-33
Monallanthus salina	>20
Naanochloris sp.	20-35
Naanochloropsis sp.	31-68
Neochloris oleoabundans	35-54
Nitzschia sp.	45-47
Phaeodactylum tricornutum	20-30
Schizochytrium sp.	50-77
Tetraselmis sueica	15-23

Table 2 shows that oil content in microalgae can exceed 80% by weight of dry biomass (Metting, 1996; Spolaore, Joannis-Cassan, Duran, & Isambert, 2006). Average oil levels of 20-50% are quite common. The mass of oil produced per unit volume of the microalgal broth per day, depends on the algal growth rate and the oil content of the biomass. Microalgae with high oil productivities are desired for producing biodiesel. Depending on species, microalgae produce many different kinds of lipids, hydrocarbons and other complex oils (Banerjee, Sharma, Chisti, & Banerjee, 2002; Metzger & Largeau, 2005; Guschina & Harwood, 2006). Not all algal oils are satisfactory for making biodiesel, but suitable oils occur commonly. Using microalgae to produce biodiesel will not compromise production of food, fodder and other products derived from crops. Instead of microalgae, oil producing heterotrophic microorganisms grown on a natural organic carbon source such as sugar, can be used to make biodiesel (Ratledge, 1993; Ratledge & Wynn, 2002); however, heterotrophic production is not as efficient as photosynthetic microalgae. This is because the renewable organic carbon sources required for growing heterotrophic microorganisms are produced ultimately by photosynthesis, usually in crop plants. Production of algal oils requires an ability to inexpensively produce large quantities of oil-rich microalgal biomass. While algae could offer great potential as a transportation fuel, there are a number of challenges. First, there are more than 20,000 algae strains. The challenge is to find a certain types of algae that can produce bio-oils, and production systems that can produce bio-oils at scale with an attractive economic return. We need to learn which of these strains can achieve the greatest production of bio-oils at the lower cost.

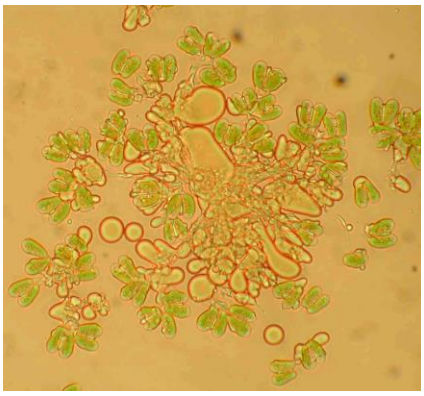


Figure 5 Oil droplets of *Botryococcus braunii* (400x magnified) [From: www.algae.wur.nl/UK/applications/energy/Biofuels/ (9/9/2012)]

3. Microalgal production

Microalgae have high water content (80-90%) (Patil, Tran, & Giselrod, 2008). The approximate molecular formula of the microalgal biomass is $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Grobbelaar, 2004). Producing microalgal biomass is generally more expensive than growing crops. Photosynthetic growth requires light, carbon dioxide, water and inorganic salts. Temperature must remain stable, generally between 20 to 30 °C. To minimize expense, biodiesel production must rely on freely available sunlight, despite daily and seasonal variation in light levels.

Growth medium must provide the inorganic elements that constitute the algal cell. Essential elements include nitrogen phosphorus, iron and silicon. Nutrients such as phosphorus must be supplied in significant excess the phosphates added complex with metal ions, therefore, not all the added phosphorus is bioavailable. Sea water supplemented with commercial nitrate and phosphate fertilizers and other micronutrient is commonly used for growing marine microalgae (Molina Grima, Fernandez, Acien Fernandez, & Chisti, 1999). Microalgae contain approximately 50% carbon by dry weight (S´anchez Mir´on, Molina Grima, Ceron Garcia, Contreras Gomez, Garcia Camacho, & Chisti, 2003), typically derived from carbon dioxide. Producing 100 tons of algal biomass fixes roughly 183 tons of carbon dioxide. Carbon dioxide must be fed continually during daylight hours.

Microalgae can be cultivated in scales ranging from small to large. Large-scale production uses continuous culture during daylight. Fresh culture medium is fed at a constant rate and the same quantity of microalgal broth is withdrawn continuously (Molina Grima et al., 1999). Large scale productions of microalgae are raceway ponds (Figure 6) and tubular photobioreactors (Figure 7).

Figure 6 A raceway pond of microalgae (From: Chisti, 2007)

Figure 7 Tubular photobioreactor (From:www.brae.calpoly.edu/CEAE/images/biofuels3.gif)

In raceways ponds, temperature fluctuates within a diurnal and seasonal cycle. Evaporative water loss can be significant. Because of significant losses to atmosphere, raceway ponds use carbon dioxide much less efficiently than photobioreactors. The biomass concentrations in raceway ponds are quite low because raceways are poorly mixed and cannot sustain an optically dark zone. A tubular photobioreactor consists of an array of straight transparent tubes that are usually made of plastic or glass. The solar collector tubes are generally 0.1 m or less in diameter. Tube diameter is limited because light does not penetrate deeply in the dense culture broth that is necessary for ensuring a high biomass productivity of the photobioreactor (Carvalho, Meireles, & Malcata, 2006).

After selecting the right strains of microalgae, they also need testing in several production systems. If the right strains are identified in conjunction with the corresponding production system, the final challenge of upscaling production still remains to be addressed. It will take large, integrated system to combine all these steps into a full scale, economic operation to produce, upgrade and commercialize biofuels from algae.

4. Oil extraction from microalgae

Microalgae are cultivated in an aqueous environment and removing water content beyond a paste consistency (typically 10-30 wt% dry biomass) is energy intensive. An ideal lipid extraction process for microalgal biodiesel production needs to be not only lipid specific in order to minimize the co-extraction of non-lipid contaminants but also selective toward desirable lipid fractions (neutral lipids containing mono-, di-, and trienoic fatty acid chains) (Fajardo, Cerdan, Medina, Fernandez, Moreno, & Grima, 2007). The lipid extraction based on Bligh and Dyer methods are shown in Figure 8 (Bligh & Dyer, 1959).

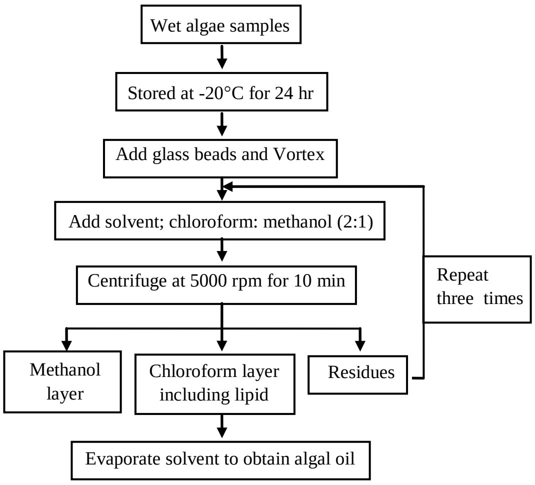


Figure 8 Schematic diagram of algal oil extraction procedure (From: Tang, Abunasser, Garcia, Chen, Simon, & Salley, 2011)

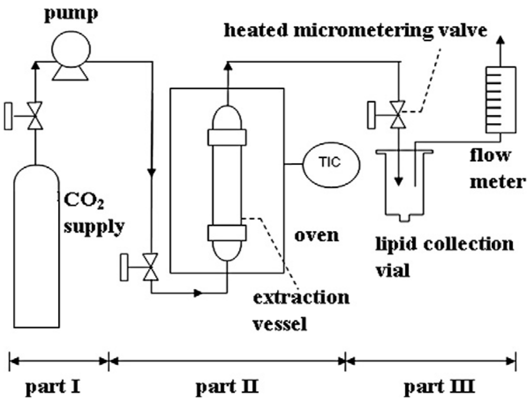


Figure 9 Schematic of $SCCO_2$ extraction unit (From: Halim, Gladman, Danquah, & Webley, 2011)

Supercritical carbon dioxide ($SCCO_2$) extraction is a promising green technology that can potentially displace the use of traditional organic solvents for lipid extraction. All $SCCO_2$ extractions were carried out in a dynamic mode with a constant decompressed CO_2 flow rate of 400 ml/min (residence time between 4.9 and 14.1 min depending on fluid density). The pressure was varied from 10 to 50 MPa and the temperature was set to either 60 or 80°C. Extractions lasted between 80 and 120 min. For each extraction, a new pre-weighed glass vial for lipid collection was used every 20 min. The crude lipid in each vial was gravimetrically quantified. Associated with $SCCO_2$ extraction include tunable solvating power, low toxicity of the supercritical fluid, favorable mass transfer equilibrium due to intermediate diffusion/viscosity properties of the fluid, and the production of solvent-free extract (Macias-Sanchez, Mantell, Rodriguez, De la Ossa, Lubian, & Montero, 2007; Thana, Machmudah, Goto, Sasaki, Pavasant, & Shotipruk, 2008). The main disadvantage of the process is high cost associated with its infrastructure and operation. Even though the classic Folch chloroform-based lipid extraction protocol is effective for the majority of microalgal lipid analyses, an alternative organic solvent method that is more user-friendly is needed for scale-up. Hexane, despite being reported to be less efficient than chloroform when extracting from microalgae, is less toxic, has minimal affinity towards non-lipid contaminants, and apparent higher selectivity towards neutral lipid fractions (Lee, Yoo, Jun, Ahn, & Oh, 2010).

Microalgal oils differ from most vegetable oils in being rich in polyunsaturated fatty acids with four or more double bonds (Belarbi, Molina, & Chisti, 2000). Fatty acids and fatty acid methyl esters (FAME) with 4 and more double bonds are susceptible to oxidation during storage. Some vegetable oils *e.g.* canola contain large quantities of linoleic acid (C18:2n-6; 2-double bonds) and linolenic acid (C18:3n-3; 3-double bonds). Although these fatty acids have much higher oxidative stability compared with docosahexaenoic acid (DHA, C22:6n-3; six double bonds) which commonly occur in algal oils, the European Standard EN 14214 limits linolenic acid methyl ester content in biodiesel for vehicle use to 12% (mol). Total unsaturation of an oil is indicated by its iodine value. Furthermore, both the European biodiesel standards limit the contents of FAME with four and more double bonds, to a maximum of 1% (mol).

From an economic and energy point-of-view, oil extraction directly from a wet algal slurry is thought to be preferable (Xu, Brilman, Withag, Brem, & Kersten, 2011), but issues regarding stability of the oils in harvested wet algae still have to be addressed. Cellular lipids in wet algae biomass may be enzymatically degraded by internal enzymes (Singh, Olsen, & Nigam, 2011). During long-term storage, cellular lipids can be degraded to volatile organic acids or free fatty acid (Alencar, Faroni, Peternelli, Silva, & Costa, 2010). Krohn, McNeff, Yan, and Nowlan (2011) reported that free fatty acid in oil extraction from algae biomass can reach as high as 84% (oil weight). Such high levels FFAs are unlikely to have been present in the algae during growth since they would have had a cytotoxic effect on the cells (Wu, Chiang, Huang, & Jane, 2006). In the current study, changes in FFA and TAG in wet algae biomass stored under various conditions were investigated. Generally, the longer the biomass was stored, the more FFA were detected. Algae oil from the fresh water species *Scendesmus* sp., the marine species *Nanochloropsis* sp. and a heterotrophic *Dinoflagellate*, containing different fatty acid levels are shown in Table 3.

Table 3 Fatty acids composition (%) of three microalgae oil

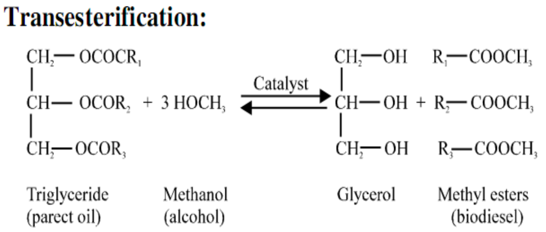
	Scendesmus sp.	*Nanochloropsis* sp.	*Dinoflagellate*
C14:0	ND	5.37	6.01
C16:0	18.42	28.83	16.65
C16:1	2.31	32.93	3.35
C16:2	3.26	ND	ND
C18:0	3.43	0.98	ND
C18:1	49.64	21.16	2.10
C18:2	11.30	2.24	ND
C18:3	8.26	ND	ND
C20:5	ND	6.33	2.89
C22:5	ND	ND	18.27
C22:6	ND	ND	44.98
C24:0	ND	ND	2.65
Saturated	21.85	35.18	25.31
Mono-unsaturated	51.95	54.09	5.45
Poly-unsaturated	22.82	8.57	66.14

ND: not detected
(From: Chen, Liu, Zhang, Chen, & Wang, 2012)

Chen, Liu, Zhang, Chen, and Wang (2012) described the extraction of oil from wet algae. The algae paste was thawed at room temperature and mixed with ethanol before it was loaded into the chamber of a high pressure extractor. Nitrogen gas was driven into the chamber to maintain a pressure of 1.5 MPa. The temperature of the extractor was maintained at 120°C for 50 min. Samples were cooled to room temperature before the pressure was decreased. The extraction mixture was centrifuged at 2632 g for 5 min to separate the oil solution and residual algae. Finally, the solvent was evaporated using a rotary evaporator to recover algae oil.

5. Methods for biodiesel production from algae oil

Biodiesel production from algae oil is generally done by one of three methods. The first is a two-step protocol in which algae oil is extracted with organic solvent and then converted to biodiesel using a catalyst, such as an acid (Krohn, McNeff, Yan, & Nowlan, 2011; Nagle & Lemke, 1990), a base (Umdu, Tuncer, & Seker, 2009; Vijayaraghavan & Hemanathan, 2009), or an enzyme (Li, Xu, & Wu, 2007). The second method directly produces biodiesel from algae biomass using an acid catalyst at atmospheric pressure and ambient temperature (Ehimen, Sun, & Carrington, 2010; Johnson & Wen, 2009; Wahlen, Willis, & Seefeldt, 2011). The third method is one-step conversion to biodiesel at high pressure and high temperature in the absence of a catalyst (Huang, Yuan, Zeng, Wang, Li, Zhou, Pei, You, & Chen, 2011; Patil, Gude, Mannarswamy, Deng, Cooke, Munson-McGee, Rhodes, Lammers, & Nirmalakhandan, 2011). Each method has advantages and disadvantages. The second method requires high concentrations of sulfuric acid since moisture in the biomass is a limiting factor for conversion efficiency. In contrast, moisture can be ignored under the subcritical or supercritical conditions of the third method. However, side reactions happen at subcritical or supercritical conditions that produce organic acids and heterocyclic nitrogen compounds from the degradation of proteins and carbohydrates. These contaminants lower the quality of biodiesel or interfere with the purification process. The transesterification of oil to biodiesel are shown in Figure 10.



$$\begin{matrix} CH_2-OCOCR_1 & & & CH_2-OH & R_1-COOCH_3 \\ | & & & | & \\ CH-OCOR_2 & + 3\,HOCH_3 & \underset{}{\overset{Catalyst}{\rightleftarrows}} & CH-OH & + R_2-COOCH_3 \\ | & & & | & \\ CH_2-OCOR_3 & & & CH_2-OH & R_3-COOCH_3 \end{matrix}$$

Figure 10 Transesterification of oil to biodiesel. $R_{1\text{-}3}$ are hydrocarbon group (From: Chisti, 2007)

Transesterification requires 3 mol of alcohol for each mole of triglyceride to produce 1 mol of glycerol and 3 mol of methyl ester. Industrial processes use 6 mol of methanol for each mole of triglyceride (Fukuda, Kondo, & Noda, 2001).

The algae oil was degummed by stirring with 1% phosphoric acid and 10% water at 85°C for 1 h to remove most of phosphoric acids and non-lipid impurities. The degummed oil or mixtures of degummed oil and FFA were first pretreated with acid catalyst to lower FFA level. A 5g sample was mixed with 2 ml methanol containing 3.3% sulfuric acid. The mixtures were stirred at 65°C for 120-180 min. The sample with highest FFA level, 2 ml methanol was added to the sample mixture and the esterification was repeated. The acid value of each oil sample was determined every 15 min. The treated oils (5g) were mixed with 2 ml of methanol containing catalyst (10% KOH or $KOCH_3$) at 65°C for 60 min under continuous stirring at 100 rpm for 1 h. Samples were washed with water to remove un-reacted methanol and catalysts.

6. Purification of crude algae biodiesel

The crude biodiesel contained impurities, such as chlorophyll, soap and phospholipids which diminish the qualities of biodiesel (Balasubramanian & Obbard, 2011). Using bleaching earth, these impurities were removed. The crude biodiesel had strong absorbance peaks at 667 and 470 nm, due to chlorophyll and carotene, respectively (Kulkarni, Dalai, & Bakhshi, 2006). At 80°C under vacuum condition, 10 g of bleaching earth was added to 50 g crude algae biodiesel for 1 h in rotary evaporator to remove pigments and other impurities. The bleaching earth was separated using centrifugation at 3790 g for 10 min. Algae cost more per unit mass (as of 2010, food grade algae costs ~$5000/ton), due to high capital and operating costs, yet are claimed to yield between 10 and 100 times more fuel per unit area than other second-generation biofuel crops (Greenwell, Laurens, Shields, Lovitt, & Flynn, 2010). The United States Department of Energy estimates that if algae fuel replaced all the petroleum fuel in the United States, it would require 15,000 square miles (39,000 km^2) which is only 0.42% of the U.S. map (Hartman, 2008), or about half of the land area of Maine. This is less than $^1/_7$ the area of corn harvested in the United States in 2000 (Dyer, 2008).

7. Conclusion

Green energy from microalgae is technologically feasible and can potentially completely displace liquid fuels derived from petroleum. The microalgal production needs to improve substantially to make it competitive with petrodiesel. The primary improvements of microalgae, through genetic and metabolic engineering, demanded high productivity to attain a consistently good annual yield of oil. However, these claims remain unrealized commercially. According to Mary Rosenthal, the head of 170-member of the Algal Biomass Organization (ABO), algae fuel can reach price parity with oil in the year 2017 or 2018 if granted production tax credits (Feldman, 2010). "We're hoping to be at parity with fossil fuel-based petroleum in the year 2017 or 2018, with the idea that we will be at several billions of gallons", Rosenthal told SolveClimate News in a phone interview (Feldman, 2010). However in 2013, Exxon Mobil Chairman and CEO Rex Tillerson, in a joint venture with J. Craig Venter's Synthetic Genomics, said that after spending $600 million on development since 2009, algae fuel is “probably further” than 25 years away from commercial viability (Carroll, 2013). These challenges are significant, and overcoming them will take a considerable investment of time, money and scientific expertise. But we believe it is an effort worth making, particularly given the potential of algae to help enhance the world's transportation fuel supply and assist in reducing greenhouse gas emissions. Since 2009, there have been many important milestones achieved. Even though the researchers have isolated and engineered a large number of candidate algal strains and developed growth conditions under which these strains could be made more productive, more work is needed. The life cycle and sustainability studies to assess the impact of each step in the process on greenhouse gas emission, and land and water use should be studied in parallel.

In writing up this review article, the authors aimed to motivate researchers in the developing countries, especially in Thailand, to put efforts in to growing more microalgae for green energy production as well as enhancing the production of green energy from microalgae. A realistic goal should be to up-scale production to a level that can dramatically replace at least 50% or more of the amount of energy consumption from fossil fuels needed in 2030.

8. References

Alencar, E. R. D., Faroni, L. R. D., Peternelli, L. A., Silva, M. T. C., & Costa, A. R. (2010). Influence of soybean storage conditions on crude oil quality. *Rev. Bras. Eng. Agric. Ambient*, *14*, 303-308.

Amin, S. (2009). Review on biofuel oil and gas production processes from microalgae. *Energy Convers Manage.*, *50*, 1834-1840.

Annual Energy Outlook 2013 (AEO2013). (2013). See: BP's Energy Outlook. (2013). Projections of long-term energy trends (through 2030). BP's Energy Outlook. (2013) – annual report, January 17, 2013. URL: http://petronomist.com

Antolin, G., Tinaut, F. V., & Briceno, Y. (2002). Optimization of biodiesel production by sunflower oil transesterification. *Bioresource Technol*., *83*, 111-114.

Balasubramanian, R. K., & Obbard, J. P. (2011). Heterogeneous catalytic transesterification of phosphatidylcholine. *Bioresour Technol.*, *102*, 1942-1946.

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

Belarbi, E. H., Molina G. E, & Chisti, Y. (2000). A process for high yield and scaleable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil. *Enzyme Microb Technol.*, *26*, 516-529.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol., 37*, 911-917

BP's Energy Outlook. (2013). Projections of long-term energy trends (through 2030). BP's Energy Outlook. Booklet 2013 – annual report, January 17, 2013.

Cadenas, A., & Cabezudo, S. (1998). Biofuels as sustainable technologies: perspectives for less developed countries. *Technol Forecast Social Change*, *58*, 83-103.

Carroll, J. (2013). Exxon at least 25 years away from making fuel from algae. Insight Exchange. Mar 9, 4-11

Carvalho, A. P., Meireles, L. A., & Malcata, F. X. (2006). Microalgal reactors: a review of enclosed system designs and performances. *Biotechnol Prog.*, *22*, 1490-1506.

Chen, L., Liu, T., Zhang, W., Chen, X. & Wang, J. (2012). Biodiesel production from algae oil high in fatty acids by two-step catalytic conversion. *Bioresource Technology*, *111*, 208-214.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advance*, *25*, 294-306.

Demirbas A. (2002). Diesel fuel from vegetable oil via transesterification and soap pyrolysis. *Energy Convers Mgnmt*, *43*, 2349-235

Dyer G. (2008). "A replacement for oil". *The Chatham Daily News*. http:www.chathamdailynews.ca/ArticleDisplay.aspx?e=1075914. Retrieved 18 June 2008.

Ehimen, E. A., Sun, Z. F., & Carrington, C. G. (2010). Variables affecting the in situ transesterification of microalgae lipids. *Fuel*., *89*, 677-684.

Fajardo, A. R., Cerdan, L. E., Medina, A. R., Fernandez, F. G. A., Moreno, P. G. A., & Grima, E. M. (2007). Lipid extraction from the microalga *Phaedactylum tricornutum. European Journal of Lipid Science and Technology*, *109*, 120-126.

Feldman, S. (2010). "Algae Fuel Inches Toward Price Parity with Oil". Reuters. http:www.reuters.com/article/idUS108599411820101122?pageNumber. Retrieved 14 February 2011.

Fukuda, H., Kondo, A., & Noda, H. (2001). Biodiesel fuel production by transesterification of oils. *J Biosci Bioeng.*, *92*, 405-416.

Gerpen, V. (2005). Biodiesel processing and production. *Fuel process Technol*., *86*, 1097-1107.

Greenwell, H. C., Laurens, L. M. L., Shields, R. J., Lovitt, R. W., & Flynn, K. J. (2010). Placing microalgae on the biofuels priority list: a review of the technological challenges. J. R. Soc. Interface 6 May 2010, *7*(46), 703–726.

Grobbelaar, J. U. (2004). Turbulence in algal mass cultures and the role of light/dark fluctuations. *J Appl Phycol.*, *6*, 331-335.

Guschina, I. A., & Harwood, J. L. (2006). Lipids and lipids metabolism in eukaryotic algae. *Prog Lipid Res*., *45*, 160-186.

Halim, R., Gladman, B., Danquah, M. K., & Webley, P. A. (2011). Oil extraction from microalgae for biodiesel production. *Bioresource Technology*, *102*, 178-185.

Hartman, E. (2008). "A Promising Oil Alternative: Algae Energy". *The Washington Post*. http://www.washingtonpost.com/wp-dyn/content/article/2008/01/03/AR2008010303907.html. Retrieved 10 June 2008.

Hassan, M. A., Yacob, S., & Ghani, B. A. (2005). Utilization of biomass in Malaysia-potential for CDM business. University Putra Malaysia, Faculty of Biotechnology.

Hussain, A. B. M., Salleh, A, Boyce, A. N., Chowdhury, P., & Naqiuddin, M. (2009). Biodiesel fuel production from algae as renewable energy. *American Journal of Biochemistry and Biotechnology*, *4*(3), 250-254.

Huang, H. J., Yuan, X. Z., Zeng, G. M., Wang, J. Y., Li, H., Zhou, C. F., Pei, X. K., You, Q. A., & Chen, L. A. (2011). Thermochemical liquefaction characteristics of microalgae in sub- and supercritical ethanol. *Fuel Process Technol*., *92*, 147-153.

International Energy Agency (IEA). (2002). World Energy Outlook. Paris, France

International Energy Agency (IEA). (2010). Energy Balances of OECD Countries. Paris, France.

International Energy Agency (IEA). (2012). World Energy Outlook. Paris, France

Johnson, M. B., & Wen, Z. Y. (2009). Production of biodiesel fuel from the microalga *Schizochytrium limacinum* by direct transesterification of algal biomass. *Energy Fuels*, *23*, 5179-5183.

Khan, S. A., Rashmi, Q. Q., Hussain, M. Z., Prasad, S., & Banerjee, U. C. (2009). Prospects of biodiesel production from microalgae in India. *Renew Sust Energy Rev*., *13*, 2361-2372.

Krohn, B. J., McNeff, C. V., Yan, B., & Nowlan, D. (2011). Production of algae-based biodiesel using the continuous catalytic Mcgyan process. *Bioresour. Technol.*, *102*, 94-100.

Kulkarni , M. G., Dalai, A. K., & Bakhshi, N. N. (2006). Utilization of green seed canola oil for biodiesel production. *J. Chem. Technol. Biotechnol*., *81*, 1886-1893.

Lang, X., Dalai, A. K., Bakhshi, N. N., Reaney, M. J., & Hertz, P. B. (2001). Preparation and characterization of bio-diesels from various bio-oils. *Bioresource Technology, 80*, 53-62.

Lee, J. Y., Yoo C., Jun, S. Y., Ahn, C. Y., & Oh, H. M. (2010). Comparison of several methods for effective lipid extraction from microalgae. *Bioresource technology*, *101*, 575-577.

Li, X. F., Xu, H., & Wu, Q. Y. (2007). Large-scale biodiesel production from microalgal *Chlorella protothecoids* through heterotropic cultivation in bioreactors. *Biotechnol Bioeng.*, *98*, 764-771.

Macias-Sanchez, M. D., Mantell, C., Rodriguez, M., De la Ossa, E. M., Lubian, L. M., & Montero, O. (2007). Supercritical fluid extraction of caratenoids and chlorophyll A from *Synechococcus sp*. *Journal of Supercritical Fluids*, *39*, 323-329.

Metting, F. B. (1996). Biodiversity and application of microalgae. *J Ind microbial.*, *17*, 477-489.

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

Molina Grima, E., Fernandez, J., Acien Fernandez, F. G., & Chisti, Y. (1999). Photobioreactors: Light regime, mass transfer, and scale up. *J. Biotechnol*., *70*, 231-247.

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

Patil, V., Tran, K. G., & Giselrod, H. R. (2008). Toward sustainable production of biofuels from microalgae. *Int J Mol Sci., 9,* 1188-1195.

Patil, P. D., Gude, V. G., Mannarswamy, A., Deng, S. G., Cooke, P., Munson-McGee, S., Rhodes, I., Lammers, P., & Nirmalakhandan, N. (2011). Optimization of direct conversion of wet algae to biodiesel under supercritical methanol conditions. *Bioresour Technol.*, *102*, 118-122.

Peterson, C. L., Feldman, M, Korus, R., & Auld, D. L. (1991). Batch type transesterification process for winter rape oil. *Appl Eng Agric*., *7*(6), 711-716.

Pirt, S. J. (1975). Principles of Microbe and Cell Cultivation. Oxford, UK: Blackwell Scienfic Publication.

Ratledge, C, & Wynn J. P. (2002). The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. *Adv Appl Microbiol., 51*, 1-51

Ratledge, C. (1993). Single cell oils-have they a biotechnological future?. *Trends Biotechnol*., *11*, 278-293

S´anchez Mir´on, A., Molina Grima, E., Ceron Garcia, M-C., Contreras Gomez, A., Garcia Camacho, F., & Chisti, Y. (2003). Shear stress tolerance and biochemical characterization of *Phaeodactylum tricornutum* in quasi steady-state continuous culture in outdoor photobioreactors. *Biochem Eng J.*, *16*, 287-297.

Shay, E. G. (1993). Diesel fuel from vegetable oils: Status and opportunities. *Biomass Bioenergy*, *4*, 227-242.

Shirvani, T., Yan, X., Inderwildi, O. R., Edwards, P. P., & King, D. A. (2011). Life cycle energy and greenhouse gas analysis for algae-derived biodiesel. *Energy & Environmental Science*, *4*(10), 3773.

Singh, A., Olsen, S. I., & Nigam, P. S. (2011). A viable technology to generate third-generation biofuel. *J. Chem. Technol. Biotechnol*., *86*, 1349-1353.

Spolaore, P., Joannis-Cassan, C., Duran, E., & Isambert, A. (2006). Commercial applications of microalgae. *J Biosci Bioeng., 101*, 87-96.

Tang, H., Abunasser, N., Garcia, M. E. D., Chen, M., Simon, K. Y., & Salley, S. O. (2011). Potential of microalgae oil from *Dunaliella tertiolecta* as a feedstock for biodiesel. *Applied Energy*, *88*, 3324-3330.

Thana, P., Machmudah, S., Goto, M., Sasaki, M., Pavasant, P., & Shotipruk, A. (2008). Respond surface methodology to supercritical carbon dioxide extraction of astaxanthin from *Haematococcus pluvialis. Bioresource Technology*, *99*, 3110-3115.

Tilman, D., Socolow, R., Foley, J. A., Hill, J., Larson, E., & Lynd, L. (2009). Beneficial biofuels: the food, energy, and environmental trilemma. *Science*., *325*, 270-271.

The Energy Efficiency Develpoment Plan (EEPA). (2011-2030). Thailand 20 Year Energy Efficiency Develpoment Plan 2011-2030, Ministry of Energy, 2-3

The International Petroleum Industry Environmental Conservation Association (IPIECA). (2004). Statistical Review of World Energy 2012, Annual review.

Umdu, E. S., Tuncer, M., & Seker, E. (2009). Transesterification *of Nannochloropsis oculata* microalga's lipid to biodiesel on Al_2O_3 supported CaO and MgO catalysts. *Bioresour Technol.*, *100*, 2828-2831.

U.S. Energy Information Administration (EIA). (2010). World Energy Outlook (Paris, France, November), 128-134

U.S. Energy Information Administration (EIA). (2013). World Energy Outlook (Paris, France, October), 115

Vicente, G., Martinez, M., & Aracil, J. (2004). Integrated biodiesel production: a comparison of different homogeneous catalysts systems. *Bioresour Technol.*, *92*, 297-305.

Vijayaraghavan, K., & Hemanathan, K. (2009). Biodiesel production from freshwater algae. *Energy Fuels*, *23*, 5448-5453.

Wahlen, B. D., Willis, R. M., & Seefeldt, L. C. (2011). Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. *Bioresour Technol.*, *102*, 2724-2730.

Waltz, E. (2009). Biotech's green gold?. *Nature Biotechnology*, *27*, 15-18 www.algae.wur.nl/UK/applications/energy/Biofuels/ (9/9/2012)

Wu, J. T., Chiang, Y. R., Huang, W. Y., & Jane, W. N. (2006). Cytotoxic effects of free fatty acids on phytoplankton algae and cyanobacteria. *Aquat. Toxicol*., *80*, 338-345.

Xu, L., Brilman, D. W. F., Withag, J. A. M., Brem, G., & Kersten, S. (2011). Assestment of a dry and wet route for the production of biodiesel from microalgae: energy balance analysis. *Bioresource technology*, *102*, 5113-5122.