

Review

Microalgae: A promising feedstock for biodiesel

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Biodiesel is a renewable and environmentally friendly energy source fuel produced through transesterification of plant oils or animal fats with short chain alcohols. The global market for biodiesel has been growing rapidly during the past few years, and it is poised for explosive growth in the next years. However, the lack of oil feedstocks limits the large-scale development of biodiesel to a large extent. Recently, microalgae have attracted increasing attention due to their many advantages for biodiesel production. Compared to traditional feedstocks such as rapeseed and soybean, microalgae can rapidly grow on nonagricultural land or in brackish water with high oil content and rapid growth rate. Moreover, they can absorb carbon dioxide as the carbon source for growth. Although many challenges remain in microalgal biodiesel production, more and more inventors committed to believe that the rewards would eventually outweigh the risks. To date, microalgae investments have reached over \$900 million world wide. In this review paper, the related researches about microalgae as promising sources for biodiesel production were discussed.

Key words: Biodiesel, microalgae, investment, review.

INTRODUCTION

As dwindling fossil fuel reserves and growing concerns about global warming, biodiesel has gained immense popularity due to its being renewable and carbon dioxide-neutral properties in recent years (Basha and Jebaraj, 2009; Demirbas, 2009; Durrett et al., 2008; Koonin, 2006). From 2004 to 2008, the production of biodiesel increased almost four-fold in European Union, the world's biggest biodiesel producer, rising from 1.9 million metric tons in 2004 to 7.7 million metric tons in 2008 (Figure 1) (Bozbas, 2008). Biodiesel production in the United States also has expanded dramatically in the past five years, which was over 2 million metric tons for 2008 compared to 0.08 million metric tons produced in 2004 (Figure 1). Apart from developed countries, developing countries such as Brazil and Argentina are accelerating their biodiesel production as well. In Brazil, the production was estimated at 0.96 million metric tones in 2008, and it is expected to surpass those in the USA and European by the year 2015. Thus, the global market for biodiesel is expected for an exponential growth in the coming decade. It is predicted that global biodiesel production would in-

crease significantly from 11.1 million metric tons in 2008 to approximately 121million metric tons in 2016.

Biodiesel consists of fatty acid methyl esters, which typically are derived from triacylglycerols (TAGs) by transesterification with short chain alcohols such as methanol, with glycerol as a byproduct (Figure 2). The current feedstocks for commercial biodiesel include waste cooking oil, animal fat and various oleaginous species such as soybean, rapeseed, corn, sunflower, peanut, jatropha and oil palm (Barnwal and Sharma, 2005; Felizardo et al., 2006; Vasudevan and Birggs, 2008). Rapeseed oil is the predominant feedstock in European Union and soybean oil is the main contributor in the USA. However, these food-based raw materials have resulted in the debate "food vs. fuels". Since biodiesel production grew rapidly in the world, there was a dramatic increase in food prices for vegetable oils (Chakravorty et al., 2009). According to Martin (2008), one-quarter to one-third of the price increase can be explained by the increased production of energy from land. For biodiesel production, feedstock costs account for a large portion percent of the total costs. For instance, Haas et al. (2006) reported that soybean oil input contributed to 88% of the total production costs of biodiesel in the USA. Therefore, higher vegetable oil prices ultimately made biodiesel more expensive and

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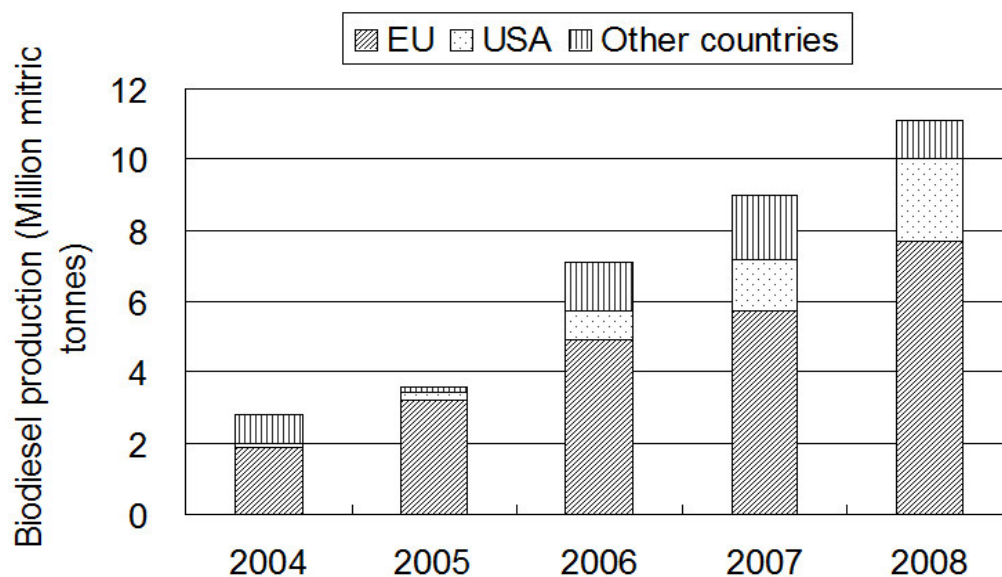


Figure 1. The global production of biodiesel from 2004 to 2008. Data were obtained from the National biodiesel board (<http://www.biodiesel.org>), the European Biodiesel Board (<http://www.ebb-eu.org>) and Emerging Markets Online (<http://www.emerging-markets.com/biodiesel/>) and converted to metric tonnes.

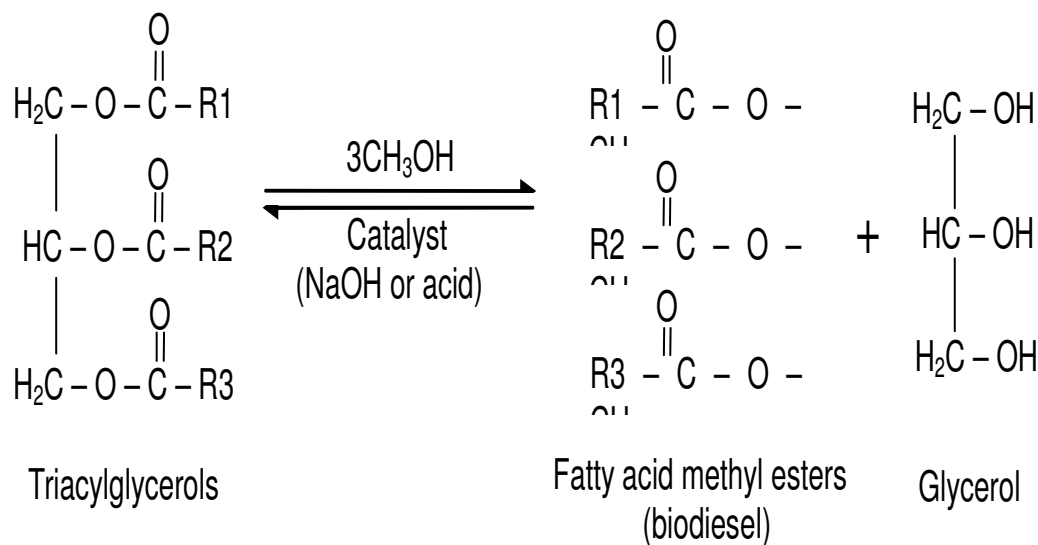


Figure 2. Biodiesel production via transesterification of triacylglycerols.

less competitive with fossil fuel at today's energy prices. On the other hand, the limited supply of these feedstocks would severely impede the further expansion of biodiesel production to a large extent. Durrett et al. (2008) suggested that even converting total 2005 USA soybean crop to biodiesel would replace only 10% of conventional diesel consumed. In reality, according to American Soybean Association, more than 80% of the soybean production entered the market for human consumption or

animal feed and less than 20% of those were used in biodiesel production (<http://www.soystats.com/>). In attempt to address these problems, dedicated energy crops must be explored, separately and distinctly from food.

Microalgae are a large and diverse group of photosynthetic eukaryotes with a simple cellular structure, ranging from unicellular to multicellular forms; they can be found anywhere water and sunlight co-occur, including

Table 1. Oil yield of some biodiesel feedstocks.

Crop	Oil yield (L/ha /year)	Biodiesel Productivity (Kg/ha/year)	Crop	Oil yield (L/ha/year)	Biodiesel Productivity (Kg/ha/year)
Rapeseed	1190	862	Sunflower	952	946
Oil palm	5950	4747	Jatropha	1892	656
Corn	172	152	Microalgae ^a	58700	51927
SOYBEAN	446	562	Microalgae ^b	136900	121104

^aAlgae contain 30% oil (/wt) in biomass. ^b Algae contain 70% oil (/wt) in biomass. Source: http://journeytoforever.org/biodiesel_yield.html; Chisti (2007); Mata et al., 2009.

soils, ice, lakes, rivers, hotspots and ocean (Parker et al., 2008); and they have the ability to capture carbon dioxide and convert energy of sunlight to chemical energy. Algal oils, which can be used to produce biodiesel, are usually accumulated as membrane components, storage products, metabolites and sources of energy under some special conditions. Recently, microalgae have attracted considerable attention from researchers and entrepreneurs as an alternative non-food biodiesel feedstock owing to their high oil content and rapid biomass production. In this review paper, the related researches about microalgae as promising sources for biodiesel production were discussed.

Advantages of microalgae for biodiesel

Microalgae yield more oils per hectare than some traditional biodiesel feed stocks. As shown in Table 1, microalgae containing 30% oil by weight of dry biomass could yield almost 587000 liters per hectare, far in excess of what can be generated from rapeseed or soybean grown in the same area. Furthermore, the 30% oil level is quite common in microalgae and the total oil content can be up to 70% in some microalgae species such as *Botryococcus braunii* and *Schizochytrium spp* (Chisti, 2007). Biodiesel productivity could reach 121 104 Kg/ha per year using 70% oil content algae as feedstock (Table 1). Thus it was feasible to use algae oil production to completely replace fossil diesel. Aside from higher oil yields, microalgae are able to grow extremely rapidly, generally doubling their biomass within a day. Moreover, they can grow on non-arable land or in brackish water, avoiding competition for land with crops and tapping into freshwater resources for irrigation. Oil from algae on 20-30 million acres of marginal land would replace the entire US supply of imported oil, leaving 450 million acres of fertile soil in the country entirely for food production (Um and Kim, 2009). Another reason why microalgae are attractive is that they can assimilate carbon dioxide as the carbon source for growth (Pokoo-Aikins et al., 2009). This contributes to both atmospheric CO₂ emission mitigation and high microalgal biomass production. In addition, microalgal oils are similar to those produced by crops such as soybean, and can be

used directly to run existing diesel engines or as a mixture with crude oil diesel.

Investments in microalgae biodiesel

From the mid 1970's to mid 1990's, The National Renewable Energy Laboratory (NREL) had devoted to the seminal work on developing microalgae as a source of biodiesel through Aquatic Species Program (ASP). Unfortunately, the program was shut down by U.S. Department of Energy (DOE) because of the low gasoline prices (about one dollar per gallon) of the day in the mid-90's (Sheehan et al., 1998). Fortunately, DOE renewed its investment in microalgae projects in 2008. Several companies also are attempting to commercialize microalgal biodiesel. For example, ExxonMobil, the world's second largest company, well partner with California-based Synthetic Genomics Inc. (SGI) and spend up to \$600 million to produce biofuels from microalgae in next 5 to 6 years. SGI plans to build a large facility in San Diego to test thousands of algal species in hopes of finding or reengineering high oil content strains and ExxonMobil attempts to scale up biofuels production from microalgae and refine the resulting oils into finished fuels (Service, 2009). Two algae fuel makers, Sapphire Energy in San Diego and Solazyme in South San Francisco have invested \$100 million and \$76 million in advancing the use of microalgae for biofuels, respectively (Service, 2009). Other companies such as Green Fuels, Partisan, Aurora Biofuels and UK Carbon Trust also have announced that they would take millions of dollars in biodiesel production from microalgae. Up to now, microalgae investments have reached over \$900 million world wide. Recently, algae aviation fuels were developed rapidly by governments, universities, research labs and private companies. The US defense industry, Sapphire Energy, Solazyme and the Netherlands based Algaelink all have been contracts to produce algal fuels that meet aviation petroleum-based jet fuel standards (Thurmond, 2009). In January 2009, The world's first commercial algae-based biofuels test flight, using a mixture of algae, jatropha and petroleum-based jet fuel, were successfully performed by Sapphire Energy, GE, Continental Airlines, UOP and Boeing, demonstrating the significant commercial potential of algae in aviation.

Table 2. Lipid content of many microalgae species (Chisti, 2007; Li et al., 2008; Mata et al., 2009; Sialve et al., 2009; Um and Kim, 2009).

Microalgae species	Lipid content (% dry weight biomass)	Microalgae species	Lipid content (% dry weight biomass)
<i>Ankistrodesmus sp.</i>	24–31	<i>Monodus subterraneus</i>	16
<i>Botryococcus braunii</i>	25–75	<i>Monallanthus salina</i>	20–22
<i>Chaetoceros muelleri</i>	33	<i>Nannochloris sp.</i>	20–56
<i>Chlamydomonas reinhardtii</i>	21	<i>Nannochloropsis oculata.</i>	22–29
<i>Chlorella emersonii</i>	25–63	<i>Nannochloropsis sp.</i>	12–53
<i>Chlorella minutissima</i>	57	<i>Neochloris oleoabundans</i>	29–65
<i>Chlorella protothecoides</i>	14–57	<i>Pyrrosia laevis</i>	69.1
<i>Chlorella sorokiniana</i>	19–22	<i>Pavlova salina</i>	30
<i>Chlorella sp.</i>	10–48	<i>Prostanthera incisa</i>	62
<i>Chlorella vulgaris</i>	5–58	<i>Prymnesium parvum</i>	22-39
<i>Cryptocodinium cohnii</i>	20–51	<i>Pavlova lutheri</i>	35
<i>Dunaliella salina</i>	6–25	<i>Phaeodactylum tricornutum</i>	18–57
<i>Dunaliella primolecta</i>	23	<i>Scenedesmus obliquus</i>	11–55
<i>Dunaliella tertiolecta</i>	16–71	<i>Skeletonema costatum</i>	13–51
<i>Dunaliella sp.</i>	17–67	<i>Scenedesmus dimorphus</i>	16-40
<i>Euglena gracilis</i>	14-20	<i>Schizochytrium sp.</i>	50-77
<i>Ellipsoidion sp.</i>	27	<i>Thalassiosira pseudonana</i>	20
<i>Haematococcus pluvialis</i>	25.0	<i>Isochrysis galbana</i>	7–40
<i>Isochrysis sp.</i>	7–33	<i>Zitzschia sp.</i>	45-47

Collection and screening of microalgae strains for biodiesel production

Looking for the microalgal strains with the combination of high oil content and a rapid growth rate is the start of biodiesel production. In the world, over 50000 microalgae species are present in not only aquatic but also terrestrial environments, implying their widespread availability (Richmond, 2004). A limited number, about 4000 species have been identified, which can be divided into several groups including cyanobacteria (Cyanophyceae), green algae (Chlorophyceae), diatoms (Bacillariophyceae), yellow-green algae (Xanthophyceae), golden algae (Chrysophyceae), red algae (Rhodophyceae), brown algae (Phaeophyceae), dinoflagellates (Dinophyceae) and 'pico-plankton' (Prasinophyceae and Eustigmatophyceae) (Hu et al., 2008). Among these, Diatoms and green algae are relatively abundant (Khan et al., 2009). Most common microalgae (*Botryococcus*, *Chlamydomonas*, *Chlorella*, *Dunaliella*, *Neochloris*, etc.) have oil levels between 20 and 75% by weight of dry biomass (Table 2). They are all potential sources for biodiesel production. NREL remains around 300 strains, mostly green algae and diatoms, for oil-production via screening over 3000 microalgal strains. Nowadays the collection has been transferred to the University of Hawaii and is still available to interested researchers.

Besides the oil content of microalgae, biomass productivity should be considered simultaneously in the

selection of the most adequate species for biodiesel production. In general, lower oil strains grow faster than high oil strains (Vasudevan and Briggs, 2008). Microalgae containing 30% oil grow 30 times faster than those containing 80% oil (Becker, 1994). Another challenge is that microalgae usually accumulate oil under stress conditions with slow growth rate. Hu et al. (2008) reported that neutral lipids content, mainly in the form of triacylglycerol (ATG), double or triple increased when the cells were subjected to unfavorable culture conditions. The nitrogen limitation also could increase the oil content in *Neochloris oleoabundans* (Li et al., 2008) and five *Chlorella* strains including *Chlorella vulgaris*, *Chlorella emersonii*, *Chlorella protothecoides*, *Chlorella sorokiniana* and *Chlorella minutissima* (Illman et al., 2000). So, the ability of microalgae to thrive in extreme conditions should be taken into account for seeking efficient strains to biodiesel production.

The composition of microalgal fatty acids has a significant effect on the fuel properties of biodiesel produced. Several researchers reported that reducing the saturated fatty acid content of plant oil can improve the cold temperature low properties of the biodiesel derived from it because long-chain saturated fatty esters significantly increase the cloud point and the pour point of biodiesel (Serdari et al., 1999; Stournas et al., 1995). Microalgal oils are mostly composed of four unsaturated fatty acids, namely palmitoleic (16:1), oleic (18:1), linoleic (18:2) and linolenic acid (18:3). Saturated fatty acids such

as palmitic (16:0) and stearic (18:0) also present with a small proportion (Meng et al., 2009). Some special microalgae could synthesize polyunsaturated fatty acids such as C16:4 and C18:4 in *Ankistrodesmus spp.*, C18:4 and C22:6 in *Isochrysis spp.*, C16:2, C16:3 and C20:5 in *Nannochloris spp.*, C16:2, C16:3 and C20:5 in *Nitzschia spp.* (Thomas et al., 1984). But biodiesel from highly unsaturated sources oxidizes more rapidly than conventional diesel, resulting in forming insoluble sediments to interfere with engine performance. Therefore, the proper percentage of saturated and unsaturated fatty acid is very important to microalgae as a biodiesel feedstock.

In brief, many parameters including lipid content, growth rate, fatty acid composition and cultivation conditions should be considered to identify the most promising microalgae species and to maximize oil yield per acre for biodiesel production.

Scaling up of microalgae for biodiesel production

Microalgae can be large-scale cultivated either by open or close culture systems or closed systems (Ugwu et al., 2008; Borowitzka, 1999). Raceway pond is the most commonly used design as an open culture system, and its structure has been well document by Chisti (2007). It comprise of a close loop recirculation channel operated at water depths of 15-20 cm. Flow is guided around bends by baffles located in the flow channel. Paddle wheel is used to mix and circulation the algae, driving water flow continuously around the circuit to prevent sedimentation of the biomass sedimentation. The raceway ponds are easy to operate, but they lose water rapidly by evaporation and are susceptible to contamination by unwanted species because of being open to atmosphere. Generally speaking, the low oil strains would rapidly take over the ponds due to their higher growth rate compared to high oil content strains (Vasudevan and Briggs, 2008). In addition, culture condition such as light and temperature are harder to control in the open raceway ponds.

Closed photobioreactors are recommended for scaling up of microalgae because they can be erected over any open space, can operate at high biomass concentration, can keep out atmosphere contaminants and can save water, energy, and chemicals compared to some other open culture systems (Amin, 2009; Schenk et al., 2008). Most closed photobioreactors are designed as tubular reactors, plate reactors, or bubble column reactors (Pulz, 2001; Weissman et al., 1988). Among these, tubular photobioreactor is the most common type. A tubular photobioreactor consists of an array of fence-like solar collectors, where sunlight is captured. The solar collector tubers are usually made of plastic or glass, which are either laid horizontally on the ground or coiled around a supporting frame to form a helical coil tubular photobioreactors. The diameter of tubers should be less than 0.1 m because light does not penetrate too deeply in the dense culture broth is that is necessary for ensuring a high

biomass productivity of the photobioreactor (Chisti, 2007). The microalgae broth is continuously pumped through the solar array tubes using either a mechanical pump or airlift pump. The broth must periodically return to a degassing zone that is bubbled with air to strip out the accumulation oxygen generated during photosynthesis. After the broth is harvested, fresh broth will be fed to the degassing column, where carbon dioxide is also fed to prevent carbon limitation and an excessive rise in PH (Molina Grima et al., 1999). Photobioreactors suffer from several drawbacks that need to be considered and solved. The main limitation is that the equipment is expensive, for example, several kilometers of tubes are necessary to produce commercial amounts of oil. If more efforts can be made to reduce its operating cost significantly, the prospect of photobioreactors used for large scale culture of microalgae is quit promising.

A combination of open ponds and closed photobioreactors is probably the most logical choice for cost-effective cultivation of high yielding strains for biodiesel (Schenk et al., 2008). In the first stage, the microalgal strain with high oil content is grown in photobioreactors to produce biomass. In the second stage, the microalgae enter an open raceway with nutrient limitations and other stressors to promote biosynthesis of oil. This process has been successfully demonstrated on a small commercial scale by Huntley and Redalje (2007).

Genetic engineering of microalgae for biodiesel production

To obtain the best microalgae strains that exhibit high oil content, high growth rate and broad environmental tolerances for biodiesel production, much of industry's previous work has focused on species selection and cultivation techniques. unexpectedly, genetic modification of microalgae has received little attention. With the development of microalgal molecular biology, it is possible to increase microalgal oil yield by genetic or metabolic engineering.

On one hand, genetic engineering can be used to increase photosynthetic efficiency and thus higher microalgal biomass yield. Since photosynthesis drives the first stage of biodiesel production, any increase in photosynthetic efficiency will benefit downstream biodiesel production (Schenk et al., 2008). However, excessive light can damage the photosystems and cells had to trigger photo-protective mechanisms that most of captured energy is radiated as heat or fluorescence. In the interest of engineering a microalgae strain to effectively capture light energy, researches focused on reducing the number of light-harvesting antenna complexes (LHCs) which capture sunlight and transfer the derived energy to PS and PS α to drive the photosynthetic reaction. For example, Mussnug et al. (2007) reported the successful down regulation of LHCs in *Chlamydomonas reinhardtii* for improved photosynthetic efficiency and light penetration

in liquid culture. The resulting LHC mutant offers more efficient conversion of solar energy to biomass.

On the other hand, genetic engineering can be used to increase microalgal biosynthesis of oil by regulating the activity of the enzyme that catalyzes the rate-limiting step in lipid production. It is generally believed that basic pathways of fatty acid and TAG biosynthesis in microalgae are directly analogous to those demonstrated in higher plants (Hu et al., 2008). The committed step in fatty acid synthesis is the conversion of acetyl CoA to malonyl CoA, catalyzed by acetyl CoA carboxylase (ACCCase). The ASP made efforts to over-express the gene encoding ACCCase in the diatom *Cyclotella cryptica*. However, the transformants did not show a detectable increase in lipid levels due to feedback inhibition. The ASP also failed in increasing lipid production by down-regulating *UPP1* gene encoding a fusion protein containing the activities for UDPglucose pyrophosphorylase and phosphoglucomutase in *C. cryptica*. It was postulated that decreasing expression of the *UPP1* gene could result in decrease in the proportion of newly assimilated carbon into the carbohydrate synthesis pathway, and consequently increase the flow of carbon to lipids. Even though these initial experiments were unsuccessful, the tools and methodologies acquired during this project have set the stage for future genetic engineering efforts in microalgae (Sheehan et al., 1998).

TAG biosynthesis in microalgae has been proposed to occur via the direct glycerol pathway. Fatty acids produced in the chloroplast are sequentially attached to glycerol backbone, which catalyzed by cytosolic glycerol-3 phosphate acyl transferase, lyso-phosphatidic acid acyl transferase (LPAT), phosphatidic acid phosphatase and diacylglycerol acyl transferase (DGAT), respectively (Roessler et al., 1994). Many researchers demonstrated that over-expression of *LPAT* and *DGAT* gene led to increased seed oil levels in higher plants (Bouvier-Nave et al., 2000; Hako et al., 2001; Zou et al., 1997). However, few result have reported in this field of microalgae. Such genetic modifications may be potential to utilize in microalgal oil production.

Concisely, the expression of genes involved in fatty acid synthesis and TAG biosynthesis is poorly understood in microalgae. A detailed knowledge of the lipid biosynthesis pathways should be developed before beginning to pursue genetic modification.

Conclusion

Biodiesel derived from microalgae appear to be the only current renewable source that can potentially completely substitute fossil fuels. Nonetheless, there are many challenges remained in biodiesel production. The biggest one is that microalgal biodiesel are not economically competitive with fossil fuels at today's energy prices. Many efforts have been done to reduce the production costs by governments, researchers and entrepreneurs.

The primary strategy is to identify algae species that have a high oil content that will also grow quickly to produce biodiesel. The second strategy is to develop photo-bioreactors that allow large-scale culture of microalgae. Photobioreactors have their advantages since they can provide environmental control and high biomass concentration, but the cost for oil production is relatively high, ranging from \$15 to \$40 per gallon. Designing lower-cost photobioreactors to bring down the costs should be taken into consideration. At present, available biochemical knowledge about fatty acid and TAG synthetic pathway in microalgae is fragmentary. Much strenuous research needs to do before we utilize lipido- mics, genomics, proteomics and metabolomics to improve microalgal oil production.

Despite these Challenges, microalgae are a promising feedstock for biodiesel production. A large number of companies are announcing that they will be producing microalgal biodiesel economically within next few years. One or more successful stories in this field will surely accelerate the world wide commercialization of this new type of energy source.

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