Potential of Microalgae as a Source of Biodiesel

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Abstract

Petro-diesel fuel has been used as a source of energy since its discovery in early 6th century. Reserves of petroleum have been extracted since then and these reserves are still not renewable. Increasing fuel demand worldwide, rapid rise of crude oil price, limited reserve and its effect on environment, push scientists to look for a clean and renewable fuel in replacement of petroleum based fuel. Bio diesel has been loomed as a potential replacement for petro-fuel. There are various sources for extracting biofuel-jatropha, vegetable oils, soybeans, corn etc. But they all are food sources which could result in food crisis. These days' algae have emerged as a suitable source for biodiesel due to its abundance and high lipid content. Microalgae have the potential to produce large amount of biodiesel as they require very small cultivation area, having faster growth rate, shorter life cycle and high oil content. The production of biofuels from algae does not reduce atmospheric carbon dioxide (CO_2), because any CO_2 taken out of the atmosphere by the algae is returned when the biofuels are burned. They do however potentially reduce the introduction of new CO_2 by displacing fossil hydrocarbon fuels. Several companies and government agencies are funding efforts to reduce capital and operating costs and make algae fuel production commercially viable. However, there are challenges remain to produce large amount of algae biomass with high lipid content and the effective technique to harvest the grown algae, extract the algal oil and transesterify the oil to biodiesel.

In this review all the methods, techniques, research and advancements made in biodiesel production using microalgae are discussed.

Keywords - Microalgae, Biodiesel, Biofuel, Transesterification, Photobioreactor, Raceway Pond, Biofuel Industries.

Introduction

Continued use of petroleum sourced fuels 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. Renewable, carbon neutral, transport fuels are necessary for environmental and economic sustainability. Bio diesel derived from oil crops is a potential renewable and carbon neutral alternative to petroleum fuels. Unfortunately, bio diesel from oil crops, waste cooking oil and animal fat cannot

realistically satisfy even a small fraction of the existing demand for transport fuels. As demonstrated here, micro algae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels. Like plants, microalgae use sunlight to produce oils but they do so more efficiently than crop plants. Oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops. Approaches for making microalgal biodiesel economically competitive with petrodiesel are needed to be discussed.

Why microalgae?

Micro algae have much faster growth rates than terrestrial crops. The per unit area yield of oil from algae is estimated to be from between 5,000 to 20,000 US gallons per acre per year (4,700 to 18,000 m3/km2•a) (Schimizu, 1996; 2003). This is 7 to 30 times greater than the next best crop, Chinese tallow (700 US gal/acre •or 650 m3/km2•a) (Walter *et al.*, 2005). Studies show that some species of algae can produce up to 60% of their dry weight in the form of oil. Because the cells grow in aqueous suspension, where they have more efficient access to water, CO_2 and dissolved nutrients, micro algae are capable of producing large amounts of biomass and usable oil. This oil can then be turned into bio diesel which could be sold for use in automobiles. Regional production of micro algae and processing into bio fuels will provide economic benefits to rural communities (www.wikipedia.org//benefit of using microalgae for biodiesel production). Microalgae are more favored than macroalgae for biodiesel production as they grow very quickly and also because they have much higher lipid content. For microalgae, the theoritical yield is 158 tons per hectare and for macroalgae an yield is between 60 - 100 tons per hectare (Lorenz and Cysewski, 2000).

Microalgae use sunlight that convert carbon dioxide to potential biofuel, food, feeds and high-value bioactives (Lorenz and Cysewski, 2000). In addition, these photosynthetic microorganisms are useful in bioremediation applications (Munoz and Guieysse, 2006). Microalgae can provide several different types of renewable biofuels such as methane from anaerobic digestion of the algal biomass (Spolaore *et al.*, 2006), biodiesel derived from microalgal oil (Dunahay *et al.*, 1996), and biohydrogen that is produced photobiologically (Kapdan and Kargi 2006). Technology for producing and using biodiesel has been known for more than 50 years (Meher *et al.*, 2006). Now several companies are more emphasizing on commercialization of microalgal biodiesel.

Mass Cultivation of Microalgae

The commercial culture of microalgae is now over 30 years old with the main microalgal species grown being *Chlorella* and *Spirulina* for health food, *Dunaliella salina* for β-carotene, *Haematococcus pluvialis* for astaxanthin and several species for aquaculture (Borowitzka,1999). The culture systems currently used to grow these algae are generally fairly unsophisticated. For example, *Dunaliella salina* is cultured in large (up to approx. 250 ha) shallow open-air ponds with no artificial mixing. Similarly, *Chlorella* and *Spirulina* also are grown outdoors in either paddle-wheel mixed ponds or circular ponds with a rotating mixing arm of up to about 1 ha in area per pond (Borowitzka, 1999). The production of microalgae for aquaculture is generally on a much smaller scale, and in many cases is carried out indoors in 20–40 l carboys or in large plastic bags of up to approximately 1000 l in volume. More recently, a helical tubular

photobioreactor system, the BIOCOIL™, has been developed which allows these algae to be grown reliably outdoors at high cell densities in semi-continuous culture (Borowitza, 2005). Other closed photobioreactors such as flat panels are also being developed. The main problem facing the commercialisation of new microalgae and microalgal products is the need for closed culture systems and the fact that these are very capital intensive. The high cost of microalgal culture systems relates to the need for light and the relatively slow growth rate of the algae. Although this problem has been avoided in some instances by growing the algae heterotrophically, not all algae or algal products can be produced this way (Borowitzka, 1999).

Pond and Photobioreactor Cultivation Methods

Algae can be cultured in open-ponds (such as raceway-type ponds and lakes) and photobioreactors. Raceway ponds may be less expensive.

Open-ponds

Raceway-type ponds and lakes are open to the elements. Most commercial microalgae production such as Chlorella sp., or extremophile species, such as Arthrospira sp., D. salina and H. pluvialis; these are grown in shallow fertilized ponds or raceways (Sheehan *et al.*, 1998). Open ponds are highly vulnerable to contamination by other microorganisms, such as other algal species or bacteria. Thus cultivators usually choose closed systems for monocultures. Open systems also do not offer control over temperature and lighting. The growing season is largely dependent on location and, aside from tropical areas, is limited to the warmer months.

Open pond systems are cheaper to construct in comparison to other system of mass culturing. Large ponds have the largest production capacities relative to other systems of comparable cost. Also, open pond cultivation can exploit unusual conditions that suit only specific algae. For instance, *Spirulina sp.* thrives in water with a high concentration of sodium bicarbonate and *Dunaliella salina* grow in extremely salty water (Bilanovic and Shelef, 1988). Open culture can also work if there is a system of culling the desired algae and inoculating new ponds with a high starting concentration of the desired algae.

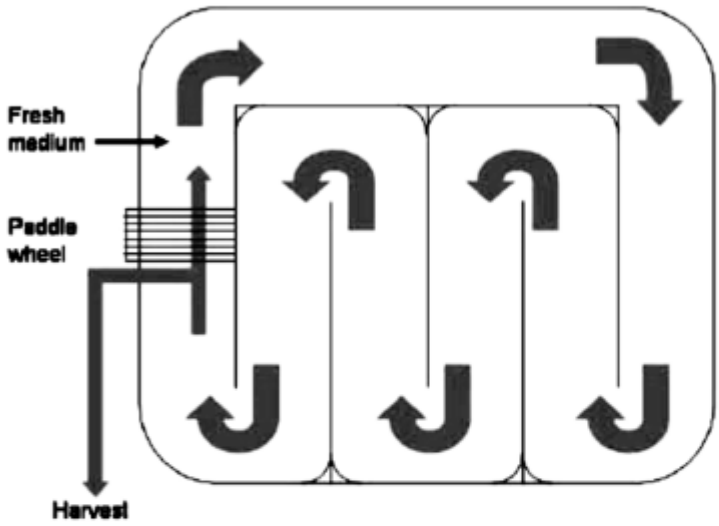

Figure 1: Schematic of a high rate pond (HRP), or paddlewheel raceway design (Chisti, 2007)

Enclosing a pond with a transparent or translucent barrier effectively turns it into a greenhouse. This solves many of the problems associated with an open system. It allows more species to be grown; it allows the species that are being grown to stay dominant; and it extends the growing season – and if heated the pond can produce year round.

Photobioreactors

Algae can also be grown in a photobioreactor (PBR). A PBR is a bioreactor which incorporates a light source. Virtually any translucent container could be called a PBR, however the term is more commonly used to define a closed system, as opposed to an open tank or pond. Because PBR systems are closed, the cultivator must provide all nutrients, including CO_2. A PBR can operate in "batch mode", which involves restocking the reactor after each harvest, but it is also possible to grow and harvest continuously. Continuous operation requires precise control of all elements to prevent immediate collapse. The grower provides sterilized water, nutrients, air, and carbon dioxide at the correct rates (Carvalho and Malcata, 2006). This allows the reactor to operate for long periods. An advantage is that algae that grows in the "log phase" is generally of higher nutrient content than old "senescent" algae. Maximum productivity occurs when the "exchange rate" (time to exchange one volume of liquid) is equal to the "doubling time" (in mass or volume) of the algae (Carvalho *et al.*, 2006). Closed microalgae bioreactors offer theoretical advantages in terms of avoiding contamination, yielding higher culture densities and providing closer control over physico-chemical conditions. A PBR system typically incorporate the following integrated components: (i) a transperant culture vessel containing the microalgal culture, (ii) the light delivery system either artificially illuminated fluorescent or metal halide lamps that provide photosynthetically active radiation (λ 400– 700 nm) to the culture, while outdoor reactors use natural incident light or solar collection devices of varying complexity, (iii) the gas exchange system that delivers carbon dioxide and removes photosynthetically generated oxygen that may inhibit metabolism or otherwise damage the microalgae if allowed to accumulate, and (iv) the harvesting system that is involved in concentrating the microalgae for downstream processing and product recovery.

Different types of PBRs are known such as tanks, Polyethylene sleeves or bags, tubular (Glass or plastic tubes). Unlike open raceways, photobioreactors permit essentially single-species culture of microalgae for prolonged durations. Photobioreactors have been successfully used for producing large quantities of microalgal biomass (Carvalho, 2006). A tubular photobioreactor consists of an array of straight transparent tubes that are usually made of plastic or glass. This tubular array, or the solar collector, is where the sunlight is captured. The solar collector tubes are generally 0.1 m or less in diameter. Tube diameter is limited because light does not penetrate too deeply in the dense culture broth that is necessary for ensuring a high biomass productivity of the photobioreactor. Microalgal broth is circulated from a reservoir to the solar collectorand back to the reservoir. Continuous culture operation is used, as explained above. The solar collector is oriented to maximize sunlight capture (Sánchez *et al.*, 1999). In a typical arrangement, the solar tubes are placed parallel to each other and flat above the ground. Horizontal, parallel straight tubes are sometimes arranged like a fence, in attempts to increase the number of tubes that can be accommodated in a given area. The tubes are always oriented North–South. The ground beneath the solar collector is often painted white, or covered with white sheets of plastic to

increase reflectance, or albedo. A high albedo increases the total light received by the tubes (Rodolfi *et al.*, 2008).

Mulumba and Farag (2009), were designed a tubular photobioreactor that is mounted for algal biomass production. It consisted of clear PVC tubing mounted in two spiral, main tank containing algal solution, fluorescence lamps as source of light, carbon dioxide and air sources, and pump which keeps the algal broth in motion in order to avoid microalgae biomass sticking on tubing hall. Carbon dioxide, nutrients with water placed in main tank and source of photons are needed to grow microalgae. In this design, fluid culture , which is composed of nutrients and water, is transferred into the tubing using a pump. The pump keeps the fluid culture in motion during the full cycle till the end of the batch when the culture reaches a stationary phase. An inoculum of microalgal strain is transferred to the batch in a ration of 1:10 (inoculums:medium by volume) in order to minimize the lag phase. Thus the exponential phase is reached within three days and lasts about 10 days. The yield in microalgae biomass in this TPBR is four times higher than yield of microalgae biomass in a cylindrical batch reactor or an open pond. Besides, this TPBR is a closed system, it lessens risks of contamination.

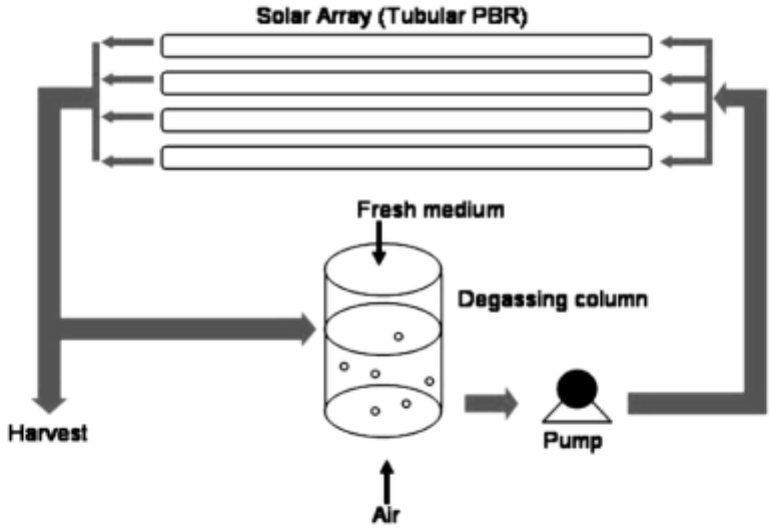

Figure 2: Schematic representation of an enclosed photobioreactor (tubular variety).

Rodolfi *et al.*, (2008) were screened thirty microalgal strains for their biomass productivity and lipid content. Four strains (two marine and two freshwater) selected as they were highly productive and with a relatively high lipid content. The algal cultures were cultivated under nitrogen deprivation in 0.6-L bubbled tubes. One of them, the *eustigmatophyte Nannochloropsis* sp. F&M-M24, which attained 60% lipid content after nitrogen starvation. The same culture was grown in a 20-L Flat Alveolar Panel photobioreactor to study the influence of irradiance and nutrient (nitrogen or phosphorus) deprivation on fatty acid accumulation (Rodolfi, 2008). Fatty acid content increased with high irradiances (up to 32.5% of dry biomass) and following both nitrogen and phosphorus deprivation (up to about 50%). Lipid production potential of *Nannocloropsis sp* was studied under natural sunlight, the strain was grown outdoors in 110-L Green Wall Panel photobioreactors under nutrient sufficient and deficient conditions (Rodolfi *et al.*, 2008). Lipid productivity increased from 117 mg/L/day in nutrient sufficient media (with

an average biomass productivity of 0.36 g/L/day and 32% lipid content) to 204 mg/L/day (with an average biomass productivity of 0.30 g/L/day and more than 60% final lipid content) in nitrogen deprived media. In a two-phase cultivation process (a nutrient sufficient phase to produce the inoculum followed by a nitrogen deprived phase to boost lipid synthesis) the oil production potential could be projected to be more than 90 kg per hectare per day (Rodolfi, 2008) This is the first report of an increase of both lipid content and a real lipid productivity attained through nutrient deprivation in an outdoor algal culture. The experiments showed that this marine eustigmatophyte has the potential for an annual production of 20 tons of lipid per hectare in the Mediterranean climate and of more than 30 tons of lipid per hectare in sunny tropical areas (Dustan, 2009)

Harvesting

Algae can be harvested using microscreens, centrifugation, flocculation and by froth flotation (Molina *et al*, 2003). Interrupting the carbon dioxide supply can cause algae to flocculate on its own, which is called "autoflocculation". "Chitosan", a commercial flocculant, more commonly used for water purification, is far more expensive. The powdered shells of crustaceans are processed to acquire chitin, a polysaccharide found in the shells, from which chitosan is derived via de-acetylation. Water that is more brackish, or saline requires larger amounts of flocculant. Flocculation is often too expensive for large operations. Alum and ferric chloride are other chemical flocculants (Molina *et al.*, 2003).

In froth flotation, the cultivator aerates the water into a froth, and then skims the algae from the top. Ultrasound and other harvesting methods are currently under development (Bosma *et al.*, 2003).

Lipid Content in Microalgae

Microalgae are tiny (in the size of micrometers) unicellular algae that normally grow in suspension within a body of water. Microalgae are often responsible for the appearance of cloudiness within a pond or even an aquarium. Microalgae grow very quickly compared to terrestrial crops. They commonly double every 24 h. During the peak growth phase, some microalgae can double every 3.5 h (Chisti, 2007). Oil contents of microalgae are usually between 20- 50% (dry weight) (Table 1), while some strains can reach as high as 80% (Metting 1996; Spolaore *et al.*, 2006). Microalgae are known to contain large amount of lipids within their cell structure, and so they are increasingly becoming an interest as a biofuel feedstock.

Table 1: Oil content of some microalgae (Chisti, 2007; Gouveia and Oliveira, 2009)

Microalgae	**Oil content (% dry weight)**
Botryococcus braunii	25-80
Chlorella protothecoides	23-30
Chlorella vulgaris	14-40
Crypthecodinium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella salina	14-20
Neochloris oleoabundans	35-65

Nitzschia sp	45-47
Phaeodactylum tricornutum	20-30
Schizochytrium sp.	50-77
Spirulina maxima	04-09
Tetraselmis suecia	15-23

Three species of microalgae were grown in mass culture to investigate the influence of culture technique and growth phase on the production of Polyunsaturated fatty acids (Dunstan *et al.*, 2007). The species of microalgae examined were *Nannochloropsis oculata, Pavlova lutheri* and *Isochrysis* sp. Production of PUFA could be maximised by harvesting at specific times and growth phases. Maximum cellular content (pg cell-1) of long-chain PUFA was found in logarithmic phase batch cultures of N. *oculata* and in stationary phase cultures of *P. lutheri*. The cellular content of PUFA in cultures of *Isochrysis* sp. did not change significantly with culture technique or growth phase. Alternatively, stationary phase cultures of all three species showed increased proportions (%) and cellular contents of triacylglycerols.

Conversion of Lipid to Biodiesel: Transesterification

Algal oil is highly viscous, with viscosities ranging 10–20 times those of no. 2 Diesel fuel. The high viscosity is due to the large molecular mass and chemical structure of oils which in turn leads to problems in pumping, combustion and atomization in the injector systems of a diesel engine. Therefore, a reduction in viscosity is important to make high-viscous oil a suitable alternative fuel for diesel engines. There are a number of ways to reduce vegetable oil's viscosity. These methods include; transestrification, pyrolysis, micro emulsion, blending and thermal depolymerization. One of the most common methods used to reduce oil viscosity in the Biodiesel industry is called transesterification. It involves chemical conversion of the oil into its corresponding fatty ester.

Transesterification consists of a number of consecutive, reversible reactions. The triglycerides are converted stepwise to diglycerides, monoglycerides and finally glycerol. A mole of ester is liberated in each step. The reactions are reversible, although the equilibrium lies towards the production of fatty acid ester and glycerol (Ma and Hanna, 1999). Transesterification reaction can be base or acid catalyzed.

$$\text{Triglyceride (TG)} + \text{R'OH} \longrightarrow \text{Diglyceride (DG)} + \text{R'COOR}_1$$

$$\text{Diglyceride (DG)} + \text{R'OH} \longrightarrow \text{Monoglyceride (MG)} + \text{R'COOR}_2$$

$$\text{Monoglyceride (MG)} + \text{R'OH} \longrightarrow \text{Glycerol (GL)} + \text{R'COOR}_3$$

Chemically, transesterification means taking a triglyceride molecule or a complex fatty acid, neutralizing the free fatty acids, removing the glycerin and creating an alcohol ester. This is accomplished by mixing methanol with sodium hydroxide to make sodium methoxide. This liquid is then mixed into algal oil. The entire mixture then settles. Glycerin is left on the bottom and methyl esters, or biodiesel, is left on top. The glycerin can be used to make soap (or any one of 1600 other products) and the methyl esters is washed and filtered (http://www.oilgae.com/algae/cult/pbr/typ.html).

Companies Reported for Developing Genetically Modified Algae for Biofuels

In February 2010, the Environmental Protection Agency, in issuing final regulations under the Renewable Fuels Standard, announced its determination that diesel produced from algal oils complies with the 50% greenhouse gas reduction threshold and therefore qualifies as an "advanced biofuel" that could be used to satisfy biodiesel mandates; and in January of this year, the Energy Department announced nearly $80 million in funding for research and development on algae-based fuels, most of which will go to two research consortia. In addition, as mentioned, there has been a large uptick of interest in the use of biological systems, including algae, for the production of jet fuels, with several airlines conducing highly-publicized test flights using jet biofuel, and with the announcement of several high-profile research collaborations relating to microbial, algal or biomass-based production of jet fuel.

The following are the companies that are believed to be using or developing algae genetically enhanced in some manner, in biofuel production.

- Algenol Biofuel
- Aurora Biofuels
- AXI LLC
- Global Green Solutions
- Kuehnle AgroSystems
- Planktonix Corporation
- Sapphire Energy
- Solazyme
- Synthetic Genomics
- Exxon Mobil

Source: (www.google.co.in//companies reported to be developing genetically modified algae for biofuels<<advance biotechnology for biofuels.html)

Recent Advances in Microalgae Biodiesel Production

The main aim is to develop a highly-efficient system for lowcost algal oil production and to optimize conversion to efficient biodisel. Utilization of algal byproducts and their marketing can be helpful to reduce the total capital cost of microalgal biodiesel production. Cost-effective harvesting has been and still is a major limiting factor. A very cost-effective way for bioflocculation is the use of Skeletonema to co-bioflocculate high lipid Nannochloropsis (Mallick, 2002). Cheap bioreactors designs are now reported using disposable plastic materials (Schenk *et al.*, 2008). Open pond yields averaging 20 g m−2 day−1 and that overall production costs is viable if the lipid content is high enough. Molecular improvements of microalgae can increase the biodiesel yields. Biofuel production can be enhanced by (1) screening a wide range of natural isolates, (2) improving them by metabolic (genetic) engineering or (3)

by selection and adaptation. By changing temperature of culture medium of microalgae also affects on lipid content as by providing low temperatures like 10-20°C enhance the lipid content in few microalgal sp. Significantly (Renald *et al.*, 2010). The US Aquatic Species Program had collected 3000 algal strains and assessed these for potentials of biofuel production. Global algae collections and species maintained from the Aquatic Species Program combined with recent advances in genetic engineering and material sciences, provide a good starting point for further development of microalgal biodiesel production systems (Spolare *et al.*, 2006). Future algal strain improvement will utilize methodologies such as lipidomics, genomics, proteomics, and metabolomics to screen for and develop new strains that exhibit high growth and lipid biosynthesis rates, broad environmental tolerances, and that produce high value-add by-products.

Conclusion

Global atmospheric CO_2 increases and depletion of mineral oil reserves require the rapid development of carbon-neutral renewable alternatives. Biodiesel production from microalgae provides technical and economic feasibility that also has the potential for CO_2 sequestration and is therefore likely to find wide acceptance. Algal biofuels appear to be the only current renewable source that could meet the global demand for transport fuels. Microalgal biofuels are also likely to have much lower impacts on the environment and the world's food supply than conventional biofuel-producing crops. The main reasons for this are high yields, a near-continuous harvest stream, and the potential to site the algal bioreactors on non-arable land. The biggest challenge over the next few years in the biodiesel field will be to reduce costs for cultivation and to further improve the biology of oil production. New materials and designs for cultivation in closed bioreactors and the use of cutting-edge metabolic engineering and screening/selection techniques are thought to provide the biggest promises.

References

Bilanovic, D., Sukenik, A., Shelef, G. 1988. Flocculation of microalgae with cationic polymers-Effects of medium salinity. *Biomass*, 17(1), 65-76.

Borowitzka, M.A. 1999. Pharmaceuticals and agrochemicals from microalgae. In: Cohen Z, editor. Chemicals from microalgae. Taylor & Francis; pp. 313–352.

Borowitzka, M.A. 2005. Culturing microalgae in outdoor ponds. In Algal culturing techniques (ed. R. A. Andersen), London, UK: Academic Press, pp. 205–218.

Bosma R., Tramper J., Wijffels, R.H. 2003. ULTRASOUND A new technique to harvest microalgae. *Journal of Applied Phycology,* 15(2-3) 143-153.

Carvalho, A.P., Meireles, L.A., Malcata, F.X. 2006. Microalgal reactors: a review of enclosed system designs and performances. *Biotechnology Progress*, 22:1490–1506.

Chisti, Y. 2007. Biodiesel from microalgae. *Biotechnology Advances*, 25, 294-306.

Chisti, Y. 2007. Biodiesel from microalgae beats bioethanol. *Biotechnology Advances,* 26, 126-137.

Dunahay, T.G., Jarvis, E.E., Dais, S.S., Roessler, P.G. 1996. Manipulation of microalgal lipid production using genetic engineering. *Applied Biochemistry Biotechnology*, 57-58, 223-231.

Dunstan, G A., Volkman J. K., Barrett S. M., Garland C. D. 2007. *Journal of Applied Phycology,* 5-1, 71-83.

Gavrilescu, M., Chisti, Y. 2005. Biotechnology—a sustainable alternative for chemical industry. *Biotechnology Advances,* 23,471–499.

Gouveia, L. Oliveira, A. C. 2009. Microalgae as a raw material for biofuels production. *Journal of Industrial Microbiology and Biotechnology,* 36, 269-274.

Kapdan, I.K., Kargi, F. 2006. Bio-hydrogen production from waste materials. *Enzyme Microbiology and Technology,* 38,569-582.

Lorenz, R.T., Cysewski, G.R. 2000. Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. *TIBTECH*, 18, 160–167.

Ma, F.R., Hanna, M.A. 1999. Biodiesel production: a review. *Bioresource Technology*, 70(1), 1-15.

Mallick, N. 2002. Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. *Biometals*, 15, 377–390.

Meher, L.C., Vidya Sagar, D., Naik, S.N. 2006. Technical aspects of biodiesel production by transesterification - a review. *Renewable and* Sustainable Energy *Reviews*, 10, 248–268.

Metting, F.B. 1996. Biodiversity and application of microalgae. *Journal of Industrial Microbiology*, 17, 477–489.

Michael A. Borowitzka 1998. Commercial production of microalgae: ponds, tanks and fermenter. *Progress in Industrial Microbiology*, 35, 313-321.

Molina, G.E., Belarbi, E.H., Fernandes, F.G.A., Robles, M., Christi, Y. 2003. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology Advances,* 20, 491–515.

Mulumba, N., Farag, I. Biodiesel production from microalgae, Chemical Engineering Department., University of New Hampshire 03824,USA.

Munoz, R. and Guieysse, B. 2006. Algal–bacterial processes for the treatment of hazardous contaminants. *Water Research,* 40, 2799–2815.

Renald, S., Zzhou, H., Parry, D., Woo, K. 2010. Effect of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae *Isochrysis* sp., *Nitzschia closterium, Nitzschia paleacea*, and commercial species *Isochrysis* sp. (clone T.ISO). *Journal of Applied Phycology,* 7(6), 595-602.

Rodolfi, L., Bonini, G., Tredici, R.M. 2008. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in low cost photobioreactors. *Biotechnology and Bioengineering,*102, 100-112.

Sánchez, M.A., Contreras, G.A., García, C.F, Molina, G.E., Chisti, Y. 1999. Comparative evaluation of compact photobioreactor for large-scale monoculture of microalgae. *Journal of Biotechnololgy,* 70, 249–270.

Sheehan, J., Dunahay, T., Benemann, J., Roessler, P. 1998. A look back at the US Department of Energy's aquatic species program—biodiesel from algae. Report NREL/ TP-580–24190, National Renewable Energy Laboratory,Golden, CO.

Schenk, P.M., Skye, R., Thomas, H., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O., Hankamer, B. 2008. Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production. *Bioengineering Research*, 1, 20-43.

Shimizu, Y. 1996. Microalgal metabolites: a new perspective. *Annual Review of Microbiology,* 50, 431–465.

Shimizu, Y. 2003. Micro-algal metabolites. *Current Opinion in Microbiology*, 6, 236-243.

Spolaore, P.C., Joannis-Cassan, E.D., Isambert, A. 2006. Commercial applications of microalgae. *Journal of Bioscience and Bioengineering,* 101, 87-96.

Tredici, M.R., 1999. Bioreactors, photo. In: Flickinger, M.C., Drew, S.W., editors. Encyclopedia of bioprocess technology: fermentation, biocatalysis and bioseparation Wiley, pp. 395–419.

Walter, T.L., Purton, S., Becker, D.K., Collet, C. 2005. Microalgae as bioreactor. *Plant Cell Report,* 24,629-641.