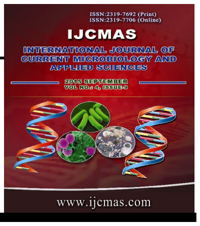

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Review Article

Perspectives and Advances of Microalgae as Feedstock for Biodiesel Production

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ABSTRACT

Keywords

Algal oil,
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Growth conditions,
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Microalgae

At present, the demand for fossil fuels is the global threatening picture. The fossil fuel is now widely accepted as unsustainable due to depleting resources and the accumulation of toxic greenhouse gases in the atmosphere, which exceeded the threshold level. To meet the inevitabilities, an alternative renewable source would be breathtaking for the rising demand. Renewable energy plays a critical role in addressing issues of energy security and climate change at global and national scales. To reduce the uses and to replace fossil fuel, the suitable alternate is biofuel. Microalgae are an attractive option for biodiesel production due to its rapid growth rate, rich source of lipids, and cheaper cultivation methods. Microalgal systems have the advantage as potential oil feedstock for the production of next generation fuels such as biodiesel, bioethanol, and biohydrogen. This review highlights the advantages of microalgae as suitable feedstock for biodiesel production; improved biomass production, their growth strategies, improvement of oil production, and genetic improvement are discussed.

Introduction

The extensive use of oil is leading to energy depletion, and it increases the greenhouse gas (GHG) emission. The greenhouse gases like nitrous oxide (N_2O) and methane (CH_4) shows a great impact on global warming. It is expected that with the development of new growing economies, such as India and China, the global consumption of energy will raise and lead to more environmental damage (IEA, 2006). GHG backs not only to global warming but also poses impacts on the environment, human life and biodiversity in earth. One side of the problem fears the reduction of crude oil reserves and difficulties in their extraction and processing, priming to an increase of its cost (Chisti, 2007). The threat is observed to be acute in transportation sector, as they have no alternate for fossil fuels.

The plea for clean and renewable energy sources is one global peril and most challenging treat today. In addition, the associated hazards like economic prosperity, environmental health, and global stability have long-term problems (Mata *et al.*, 2010). There is a need for researching a process in obtaining environmental and economic sustainability in fuel production. The process should be renewable and should be capable of sequestering atmospheric CO_2. The renewable energy sources, i.e., hydroelectric, solar, wind, tidal and geothermal is now targeting the electricity markets, but the fuel market has a global energy demand for approximately 66% to be addressed. Conventional fuels include solar energy, photovoltaic, hydroelectric, geothermal, wind biofuels (Benemann *et al.*, 1978), and fossil fuels (petroleum fuel, coal, propane, and natural gas).

Alternate fuels are fuels, which can be, used other than conservative or conventional fuels. The term “alternative fuels” refers to a source of energy, which is renewable. Alternative fuels include biodiesel, bioalcohol (methanol, butanol, and ethanol), chemically stored electricity (batteries and fuel cells), hydrogen, non-fossil methane, and other biomass sources. Biofuels can be solid, liquid or gas produced from organic sources. Recently biofuel are derived from living organisms and from their metabolic byproducts (e.g. biogas production from cow dung). Biodiesel is a renewable alternative to petroleum-based diesel fuel. Biodiesel contains no petroleum, but it can be mixed at any level with petrodiesel. Biodiesel can be made from any plant oil, animal oil, or even used cooking oil. Animal oils (tallow and lard) and used cooking oil are generally the least expensive feedstocks to purchase; however, there may be considerable additional expenses for the logistics to transport and handle these materials.

Biodiesel is quantified as diesel prepared from biological origin like plant or animal oils. It can be prepared from all plant oils such as canola, mustard, sunflower, safflower, corn oil, palm oil, pulses’ oil, animal fat and cooking oils. Biodiesel is gaining reputation due to its renewable properties and environmental benefits. However, the major hindrance in commercialization of the biodiesel is its cost. Biofuel is not cost effective when it is looked using biological material and used cooking oil or adapting continuous transesterification process and easy recovery of the fuel (Fangrui and Hanna, 1999). Biodiesel has lower level of greenhouse gas emission than mineral diesel and hence considered superior because of the following properties;

1. It has greater lubricity than low sculpture mineral oil.
2. It is more biodegradable than mineral diesel.
3. It is possible to manufacture on farm from oil seeds like canola.
4. It is considered as a renewable fuel.

Continuous and increasing consumption of petroleum would increase pollution and magnify global warming problems caused by CO_2 (Shay, 1993).

First generation biofuels primarily produced from food crops and mostly oil seeds, compete for arable land, freshwater or biodiverse natural landscapes and are limited in their ability to achieve targets for biofuel production.

Second generation and third generation biofuels such as lignocellulosic and microalgae respectively, do not compete for arable land and precious freshwater (Dragone *et al.*, 2010).

The three main advantages of biodiesel than petroleum diesel,

1. First, it is not a petroleum-based fuel, which means that using biodiesel would reduce our dependency on foreign oil.
2. Second, it can be produced domestically. It can create jobs and contribute to local economy.
3. Third, it is cleaner and produces significantly less harmful emission when burned in a combustion engine.

The idea of using microalgae is implemented seriously only now, due to the hike in petroleum costs and growing awareness of global warming, associated with burning fossil fuels (Gavrilescu and Chisti, 2005). According to 2009 (look for a recent update.. incase u don't find then retain this) report from Science Progress, "the entire world petroleum demand which is now provided for by 31 × 10^9 barrels of crude oil might instead be met from algae grown on an area equivalent to 40% of the of the United States" (Rhodes, 2009).

Vegetable oil methyl esters (Biodiesel)	**Kinetic viscocity at 38°c (mm^2/sec)**	**Cetane number**	**Heating value (MJ/Kg)**	**Flash point**
Peanut	4.9	54	33.6	176
Soyabean	4.5	45	33.5	178
Babassu	3.6	63	31.8	127
Palm	5.7	62	33.5	164
Sunflower	4.6	49	33.5	183
Tallow	-	-	-	96
Microalgal	4.35	58.5	41	166
Diesel	3.06	50	43.8	76
20% Biodiesel	3.2	51	43.2	128

What are microalgae?

Algae can be classified into two groups, i.e., microalgae (phytoplankton) and macroalgae (macrophytes). In particular, microalgae are unicellular plant rich in chlorophyll that lack lignin or cellulose and contains proteins, carbohydrates and lipids in strain specific proportions (Schenk *et al.*, 2008). In particular, microalgae are photosynthetic microorganisms that convert sunlight, water, and carbon dioxide to algal biomass. Many microalgae are reported to be rich in oil, which is converted into biodiesel using the existing technologies (Chisti, 2007). Thus, oil extracted from plants and photosynthetic microorganisms like microalgae, have the potential to produce much more significant quantities of biodiesel (Gilman, 2007). Scientists are interested in oil production from microalgae, as they have high lipid content, especially nonpolar triglycerides (TAGs). The total lipid content varies from 1% to 8% of the dry weight for different species. A microalga produces lipids, which can be converted into transportation fuel under favorable growth conditions (Spoehr and Milner, 1989).

Many microalgae that produce biodiesel are *Botryococcus* sp., *Chlorella* sp., *Chlamydomonas* sp., *Crythecodium* sp., *Spirulina* sp., *Cylindrotheca* sp., *Euglena* sp., *Isochrysis* sp., *Dunaliella* sp., *Monochioropsis* sp., *Neochloris* sp., *Nitzschia* sp., *Phacodactylum* sp., *Porophyridium* sp., *Prymnesium* sp., *Scenedesmus* sp., *Schizochytrium* sp., *Spirulina* sp., and *Tetraselmis sueica* (Shimada *et al.*, 1999).

Oil content of microalgae (Mata *et al.*, 2010)

Microalgae	**Oil content (% dwt)**
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
Nannochloropsis sp	31 – 68
Nanochloris oleoabundans	35 – 54
Nitzschia sp	45 – 47
Phaeodactylum tricornutum	20 – 30
Schizochytrium sp	50 – 77
Tetraselmis suecica	15 – 23

Application of microalgae for biodiesel production

US Department of Energy states that due to algae's simple cellular structure, it can yield 30 times more energy per acre than land crops like soyabeans. Algae can feed on CO_2 and release oxygen; hence, the existing coal power emissions would act as a thriving breeding ground to produce algal biofuel thereby reducing greenhouse gas emissions. Second generation biofuel, uses a variety of non-food material mainly agricultural crops. They can be used as other than animals' feed or human food such as soybean, switch grass, and Jatropha (Van Beilen, 2010).

The main advantage in using biodiesel is it reduces greenhouse gas emission and helps in removing carbon dioxide from industrial flu gas by algae biofixation. In addition, waste water treatment involves removal of ammonium nitrite and phosphate, it makes algae to grow using these water contaminants as nutrients. After oil extraction the used algal biomass can be used as live feed stock as organic fertilizer due to its high N:P ratio (Li *et al.*, 2008).

Microalgae has the ability to grow quickly when compared with other high oil containing crops, which takes a season to grow and only contain a maximum of about 5% dry weight of oil, when their nutrients are varied; during the peak growth phase, microalgae can double every once in three hour (Chisti, 2007).

Microalgae produce many kinds of oils or lipids, not all of which are suitable for conversion to biodiesel. Conventionally, only the neutral triglyceride lipids are used to make biodiesel; however, using existing chemistry, all algal lipids can be used to make diesel, gasoline, and jet fuel (Hu *et al.*, 2008).

Microalgae lipid content and productivities

The biochemical fractions like lipid, carbohydrate and protein can be converted into fuels. Among the three, lipids have the highest energy content. The lipid content of some species resembles the hydrocarbons found in petroleum, which can be converted to a synthetic diesel fuel (ester fuel) by the process known as transesterification (Benemann *et al.*, 1978). Microalgae contains 60% of oil in weight and in which 90% can be extracted; the oil production will be around 54 tons per ha/yr (Van Harmelen and Oonk, 2006). The major constituents of the membrane glycolipids are various kinds of fatty acids that are polyunsaturated and derived through aerobic desaturation from the precursor fatty acids palmitic acid and oleic acids (Tsuzuki *et al.*, 1990). *Botryococcus* sp has the ability to fix atmospheric carbon into energy storage components like protein, carbohydrate, and lipids in the cell. Currently the algae are reported to convert 15% of the photosynthetically available solar radiation into new cell mass. The conversion of solar energy into renewable liquid fuels and other products could become economically competitive with petroleum (Dayananda *et al.,* 2007).

Botryococcus sp plays a prominent role among the high lipid producing species. It can produce 40% of total lipids, which are hydrocarbons that can be extracted (Benemann *et al.,* 1978). Some of the acids which are found in *Botryococcus braunii* are palmitic acid (C16:0), oleic acid (C18:1), linolenic acid (C18:2) and linolenic acid (C 18:3) (Fang *et al.,* 2004). *B.braunii* is a microalgae belonging to the order Chlorococcales (Class Chlorophyceae), which are green, pyramid shaped. The colonies are held by a lipid biofilm matrix. They are found in temperate, tropical, oligotrophic lakes and estuaries. It can bloom well in elevated inorganic phosphorous. They are noted to produce triterpenes, i.e, 30-40% of the dry weight. The algae have thick cell wall, which makes the extraction of the cellular components difficult. However, most of the useful hydrocarbons are present outside the cell as the lipid biofilm matrix (Metzger and Largeau, 2005).

Most of the triacylglycerols (TAGs) obtained from microalgae are C_{14} to C_{18}, that are saturated or monosaturated. The methyl ethyl esters functions very well as a diesel fuel. Microalgae have membrane lipids, which are rich, elongated and polysaturated fatty acids. The less desirable fatty acids comprise of 20-80% of the total cell mass. It is also advantageous that they can manipulate the chain length and unsaturation level of the membrane lipid fatty acids (Christie, 1982).

Microalgae cultivation

A growth medium provides all the sufficient nutrients for the growth of microalgae. Algal cells constitutes of carbon, nitrogen, phosphorus and sulfur. Other essential elements include iron, magnesium, trace elements, and in some cases silicon (Chisti, 2007). Under nutrient deprivation, many algae alter their biosynthetic pathways to mainly produce triglyceride (Hu *et al.,* 2008).

A growth medium deficient of nitrogen greatly affects the synthesis of lipids and fatty acids (Saha *et al.,* 2003). The lipid content of microalgae appears to be highly variable and related to environmental factors, culture conditions, and compositions of nitrogen starvation or other stress factors (Pernet *et al.,* 2003). *B. braunii* accumulated 54.2% of lipids in dry weight when it was grown in a nitrogen deficient growth condition in fresh water (Feinberg, 1984).

Open and closed culture system

The main key aspect to be considered for efficient production and cost effectiveness is choice of cultivation system for microalgal biofuel production process. Raceway pond and photo bioreactors (PBRs) are the main production systems for microalgae (Van Beilen, 2010). Raceway ponds are open systems which are shallow with a typical operating depth of 20–30 cm and the channels have a width to length ratio from 5 to over 10, covering an area of up to about 3000 cm^2. PBR is a closed system. Water, nutrients and CO_2 are provided and oxygen is removed internally or by using a separate degassing chamber. All the microbes, other algae, rotifers and other grazers (Tredici, 2004), can easily contaminate raceway ponds.

Photobioreactor incorporates a light source. A translucent container, which is a closed system, is usually termed as photobioreactor. As it is a closed system, the essential nutrients should be introduced for efficient cultivation of the algae. The essential nutrients are carbon dioxide, water, minerals and light. A continuous stream of sterilized water is introduced with the required essential nutrients. The algae are harvested after excess growth is observed. The maximum production in a photobioreactor occurs when exchange rate of one volume of liquid is equal to the doubling time of the algae. Different types of photobioreactors include

- tanks provided with a light source;
- polyethylene sleeves or bags;
- glass or plastic tubes.

Synthesis of TAGs

TAG synthesis in microalgae is through three sequential acylation process of glyceraldehyde – 3 – phosphate which is termed as Kennedy Pathway. Understanding of Kennedy pathway is quite complex as it involves many intermediates i.e. phosphatidic acid (PA) and diacylglycerol (DAG). These are also used in synthesis of membrane polar lipids that affect the reproduction and growth of microalgae.

Processing and biodiesel production

Microalgal cells are cultivated and the first step is it has to be harvested. Harvesting is considered an expensive and problematic part of industrial production of microalgal biomass due to the low cell density of microalgal cultures, which is typically in the range of 0.3–0.5g dry cell weight per liter and with exceptional cases reaching 5g dry weight per liter (Wang *et al.*, 2008). For biomass harvesting many methods are employed like chemical flocculation, biological flocculation, filtration, centrifugation and ultrasonic aggregation (Hu *et al.*, 2008). Microscreens, centrifugation or flocculation is also used to harvest algae. Algae and water are aerated to froth and the algae can be harvested, which is termed as froth flocculation. For harvesting alum and ferric chloride are used as chemical flocculants. There are many methods of extracting algal oil, and the simplest is mechanical crushing. Algae have a wide physical attribute and hence, various methods like screw press, expeller or piston are used for better results. Mechanical crushing with chemicals is used widely. It is a typical method for extraction of oil from oil seeds, which is then used as raw material for transesterification to produce biodiesel (McGill, 2008).

Octane number, exhaust emission, heat of combustion, cold flow, oxidative stability, viscosity, and turbidity are used to determine the quality of biodiesel (Knothe, 2005). The triglycerides or fatty acid methyl ester obtained have to be transesterified to obtain diesel. Acids, alkalis, and lipase enzymes catalyze transesterification. Alkali catalyzed transesterification is about 4000 times faster than the acid catalyzed reaction. The use of lipase offers important advantages but is not currently feasible because of the relatively high cost of the catalyst. The acid catalyst reaction takes about 90 min to complete. A higher temperature can be used in combination with higher pressure but this is expensive (Fukuda *et al.*, 2001).

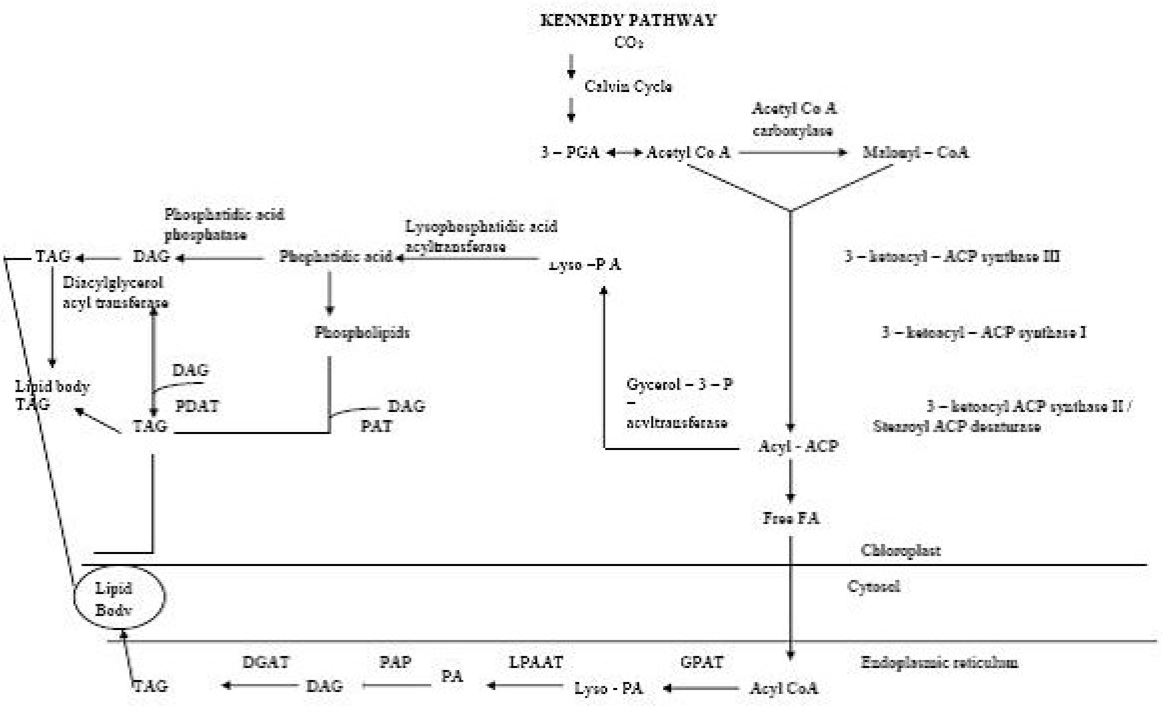
Genetic improvements of microalgal lipid production

Genetic engineering is also applied to improve fatty acids or biodiesel synthesis in *Escherichia coli* by improving the acetyl Co A biosynthetic pathway (Kalscheuer *et al.*, 2006).

Genetic and metabolic engineering in microalgae has mostly focused on

(i) to enhance the photosynthetic efficiency and increase biomass yield on light;
(ii) to increase temperature tolerance;
(iii) to increase the oil content in biomass;
(iv) to eliminate the light saturation phenomenon and reduce the susceptibility to photo-oxidation that damage cells (Leon Banares *et al.*, 2004).

Fig.1 Kennedy Pathway – synthesis of TAG in microalgae



Abbreviations

GHG – Greenhouse gas
TAGs – Triacylglycerols
PBRs - Photobioreactors

Environmental applications

Biofuel offers several interesting and attractive properties, e.g., biodegradability and nontoxicity compared to petroleum based diesel. The most important advantage of biodiesel maintaining a balanced carbon dioxide cycle. Additionally, biodiesel combustion results in reduced emission of carbon monoxide, sulfur, aromatic hydrocarbon, and soot particles (Nielson *et al.*, 2008).

Microalgae can produce up to 300 times the amount of oil per acre as soybeans, 24 times greater than palm oil. On average, algae produce about 15,000 gallons per acre per year, while the best energy crop palm oil yields 635 gallons/acre/year. It is reported that one company could produce 180,000 gallons of biodiesel every year from just one acre of algae, which comes to about 4,000 barrels; at a cost of $25 per barrel or $0.59 per gallon. It takes 3,750 acres of soybean to make the same amount of biodiesel at a cost of $2.50 per gallon for 4000 barrels. It is estimated that over eight times of total cropland areas in the United States are needed for corn-based biofuel production in order to meet 50% of US transport fuel needs. Only 1% to 3% of total US cropland would be sufficient for producing biofuel from algae biomass to meet the same need (Chisti, 2007).

The three roles of microalgae are CO_2 fixation, wastewater treatment, and biofuel production. It has the potential to maximize the impact of microalgal biofuel production systems but it has to be investigated accordingly (Kumar, 2009). Further research in the field of biodiesel production from microalgae should be focused on the reduction of the costs of small and large-scale systems. Moreover, the reduction of costs through maintenance of purity of waters and nutrients, the utilization of sewage, and the nutrients contained in it and utilization of CO_2 emitted by the industry are all related with the protection of the natural environment in its broad sense. Algae can also be utilized in other branches of human activity such as agriculture, medical sciences, and in chemical, cosmetics, and pharmaceutical industries. Despite the above mentioned challenges, microalgae are promising feedstock for biodiesel production. Research on microalgae based biodiesel production should be continued and commercial-scale use of microalgae for biodiesel production would require massive investments in production facilities. The ExxonMobil Report, 2009 states that, Baltimore's AltCar program shows, putting advanced technology behind the wheel is a good start to solving our energy challenges. The biological challenge is to identify the most appropriate strain of algae that is most prolific at producing the desired bio-oil. Algae-based fuels also offer major benefits. Unlike other biofuels, hydrocarbons from algae can be produced using land and water unsuitable for crop plant or food production. Corn and sugar cane, for example, are increasingly employed to make fuel, but their use has an impact on their availability as food. Algae production does not require the use of farmable land. Since growing algae feed on carbon dioxide, algae production can help reduce greenhouse gas emissions.

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