

Economically Effective Potential of Algae for Biofuel Production

¹Khalid Hussain, ¹Khalid Nawaz, ¹Abdul Majeed and ²Feng Lin

¹Department of Botany, University of Gujrat, Gujrat, Pakistan

²Shenyang Agricultural University, China

Abstract: With petrol and gas prices soaring, people are looking for cheaper, renewable sources of fuel for their vehicles. Biodiesel fuel is used in diesel engines and is made from domestically available, renewable organic resources, such as vegetable oils and animal fats. Biodiesel burns cleaner (i.e. produces fewer emissions) than traditional petroleum diesel fuel and is biodegradable. Algae mainly microalgae have recently gained a lot of attention as a new biomass source for the production of renewable energy. Microalgae can provide several different types of renewable biofuel including methane produced by anaerobic digestion of the algal biomass, biodiesel derived from microalgal oil and photobiologically produced biohydrogen. The idea of using microalgae as a source of fuel is not new but is now being taken seriously because of the escalating prices of petroleum and more significantly, the emerging concern about global warming that is associated with burning fossil fuels. Biodiesel is produced currently from plant and animal oils but not from microalgae. Biodiesel is a proven fuel and it is technically feasible. It is the only renewable biodiesel that can potentially displace liquid fuels derived from petroleum. The conversion of the extracted algae lipids to biodiesel is relatively easy and the product price can easily be compared with fossil fuel prices. This review summarizes the advantages, fatty acid composition, transesterification, economically effective and efficient potential of algae for biofuel production.

Key words: Algae • Biodiesel • Biofuel • Lipids • Biomass

INTRODUCTION

Recent soaring oil prices, diminishing world oil reserves and the environmental deterioration associated with fossil fuel consumption have generated renewed interest in using algae as an alternative and renewable feedstock for biofuel production [1]. Oxygenic photosynthetic microalgae and cyanobacteria represent an extremely diverse, yet highly specialized group of micro-organisms that live in diverse ecological habitats such as fresh, brackish, marine and hyper-saline water, with a range of temperatures, pH and unique nutrient availability [2]. With over 40,000 species of algae has been identified and classified in major groups as: cyanobacteria (Cyanophyceae), green algae (Chlorophyceae), diatoms (Bacillariophyceae), yellow-green algae (Xanthophyceae), golden algae (Chrysophyceae), red algae (Rhodophyceae), brown algae (Phaeophyceae), dinoflagellates (Dinophyceae) and 'picoplankton' (Prasinophyceae and Eustigmatophyceae). Several additional divisions and classes of unicellular algae have been described and details of their structure and biology are available in literature [3].

Microalgae are sunlight-driven cell factories that convert carbon dioxide to potential biofuel, foods, feeds and high value bioactives [4-14]. In addition, these photosynthetic microorganisms are useful in bioremediation applications and as nitrogen fixing biofertilizers [15-18]. Microalgae can provide several different types of renewable biofuel including methane produced by anaerobic digestion of the algal biomass, biodiesel derived from microalgal oil [5] and photobiologically produced biohydrogen is also obtained from algae [4, 7, 10, 19, 20].

The idea of using microalgae as a source of fuel is not new [21, 22] but it is now being taken seriously because of the escalating price of petroleum and more significantly, the emerging concern about global warming that is associated with burning fossil fuels [23]. Currently biodiesel is produced from plant and animal oils but not from microalgae. This is likely to change as several companies are attempting to commercialize microalgal biodiesel as a proven fuel. Technology for producing and using biodiesel has been known for more than 50 years [24-30]. Other sources of commercial biodiesel include canola oil, soybeans animal fat, palm oil, corn oil,

waste cooking oil [26, 29] and Jatropha oil [24]. Production of methyl esters, or biodiesel from microalgal oil has been demonstrated [31].

Advantages of Algae for Biofuel Production: As many algal species have been found to grow rapidly and produce substantial amounts of triacylglycerol (TAG) or oil referred to as oleaginous algae. It has long been postulated that algae could be employed as a cell factories to produce oils and other lipids for biofuel and other biomaterials [32-34]. The potential advantages of algae as feedstock for biofuel and biomaterials include their ability to [35-37]:

- Synthesize and accumulate large quantities of neutral lipids/oil (20-50% DCW).
- Grow at high rates (e.g. 1-3 doublings per day).
- Thrive in saline, brackish and sea water for which there are few competing demands.
- Tolerate marginal lands (e.g. desert, arid and semi-arid lands) that are not suitable for conventional agriculture.
- Utilize growth nutrients such as nitrogen and phosphorus.
- Sequester carbon dioxide from flue gases emitted from fossil fuel-fired power plants and other sources, thereby reducing emissions of a major greenhouse gas.
- Produce value added products or byproducts (e.g. biopolymers, proteins, polysaccharides, pigments, animal feed, fertilizer and Hydrogen).
- Biomass productivity, on area basis exceeding than that of terrestrial plants by approximately tenfold.

Based upon the photosynthetic efficiency and growth potential of algae, theoretical calculations indicated that annual oil production of >30,000 liters or about 200 barrels of algal oil per hectare is achievable in mass culture of oleaginous algae, which is 100 fold greater than that of any other feedstock such as soybeans [37-39].

Use of Microalgae for Biodiesel: Microalgae are the only source of biodiesel that has the potential to completely displace fossil diesel. Unlike other oil crops, microalgae grow extremely rapidly and many are exceedingly rich in oil. Microalgae commonly double their biomass within 24 hours [40]. Biomass doubling times during exponential growth are commonly as short as 3.5 hours. Oil content in microalgae can exceed 80% by weight of dry biomass as described in Table 1 and 2. Oil levels of 20-50% are quite

Table 1: Oil content of microalgae

Microalga	Oil content (% dry weight)
<i>Botryococcus braunii</i>	25-75
<i>Chlorella sp.</i>	28-32
<i>Cryptocodinium cohnii</i>	20
<i>Cylindrotheca sp.</i>	16-37
<i>Nitzschia sp.</i>	45-47
<i>Phaeodactylum tricoratum</i>	20-30
<i>Schizochytrium sp.</i>	50-77
<i>Tetraselmis suecia</i>	15-23

Source: Adapted from Chisti [42]

Table 2: Oil yields based on crop type

Crop	Oil yield (gallons/acre)
Corn	18
Soybeans	48
Canola	127
Jatropha	202
Coconut	287
Oil Palm	636
Microalgae	6283-14641

Source: Adapted from Chisti 42

Oil content ranges from 30 percent to 70 percent of dry biomass

common. Oil productivity, that is 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 [14]. Microalgae with high oil productivities are desired for producing biodiesel. Depending on the species, microalgae produce many different kinds of lipids, hydrocarbons and other complex oils [5, 12, 41].

The ability of algae to survive or proliferate over a wide range of environmental conditions, to a large extent, reflects its tremendous diversity and sometimes unusual pattern of cellular lipids as well as the ability to modify lipid metabolism efficiently in response to changes in environmental conditions have been observed [41, 43, 44]. The lipids may include polar lipids, wax esters, sterols and hydrocarbons, as well as prenyl derivatives such as tocopherols, carotenoids, teepees, quinines and phytylated pyrrole derivatives such as the chlorophylls. Under optimal conditions of growth, algae synthesize fatty acids principally for esterification into glycerol-based membrane lipids, which constitute about 5-20% of their dry cell weight (DCW). Fatty acids include medium-chain (C10- C14), long-chain (C16-18) and very-long-chain (C20) species and fatty acid derivatives. The major membrane lipids are the glycosylglycerides (e.g. monogalactosyldiacylglycerol, digalactosyldiacylglycerol and sulfoquinovosyldiacylglycerol), which are enriched in the chloroplast, together with significant amounts of

phosphoglycerides (e.g. phosphatidylethanolamine, PE and phosphatidylglycerol, PG), which mainly reside in the plasma membrane and many endoplasmic membrane systems [45-48]. The major constituents of the membrane glycerolipids are various kinds of fatty acids that are polyunsaturated and derived through aerobic desaturation and chain elongation from the 'precursor' fatty acids palmitic (16:0) and oleic (18:1x9) acids [49].

Lipids and Biodiesel: Over the past few decades, several thousand algae and cyanobacteria species have been screened for high lipid contents. Several hundred oleaginous species have been isolated and characterized under laboratory or outdoor culture conditions [50]. Oleaginous algae can be found among diverse taxonomic groups and the total lipid content may vary noticeably among individual species or strains between or within taxonomic groups [38]. Lipids are one of the main components of microalgae, depending on the species and growth conditions, it is about 2-60 percent of total cell dry matter. These lipids can be used as a liquid fuel in adapted engines as Straight Vegetable Oil (SVO). Tri-glycerides and free fatty acids, a fraction of the total lipid contents can be converted into biodiesel [39]. In order to efficiently produce biodiesel from algae, strains have to be selected with a high growth rate and oil content. If an open culture system is used, the selected strain must have the ability to remain dominant under varying environmental conditions such as temperature. In practice the use of locally occurring strain is useful in most cases [37]. Ratledge [51] showed an almost consistent productivity and almost doubling of the lipid content (up to 60 percent) after switching to nutrient-deficient conditions in an outdoor pilot reactor under natural light. From all energy carriers produced from algae, biodiesel has received the most attention and is the only initiative which is on the border of pilot-scale and full-scale deployment.

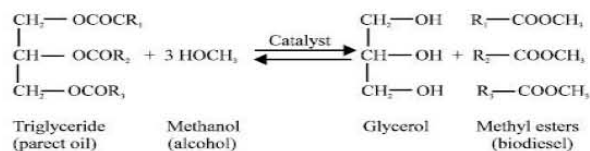
Fatty Acid Composition in Algae Groups: Algae synthesize fatty acids as building blocks for the formation of various types of lipids [39]. The most commonly synthesized fatty acids have chain lengths that range from C16 to C18 similar to those of higher plants⁵². Fatty acids are either saturated or unsaturated and unsaturated fatty acids may vary in the number and position of double bonds on the carbon chain backbone. In general, saturated and mono-unsaturated fatty acids are predominant in most algae examined [53]. Specifically, the major fatty acids are C16:0 and

C16:1 in the Bacillariophyceae, C16:0 and C18:1 in the Chlorophyceae, C16:0 and C18:1 in the Euglenophyceae, C16:0, C16:1 and C18:1 in the Chrysophyceae, C16:0 and C20:1 in the Cryptophyceae, C16:0 and C18:1 in the Eustigmatophyceae, C16:0 and C18:1 in the Prasinophyceae, C16:0 in the Dinophyceae, C16:0, C16:1 and C18:1 in the Prymnesiophyceae, C16:0 in the Rhodophyceae, C14:0, C16:0 and C16:1 in the Xanthophyceae and C16:0, C16:1 and C18:1 in cyanobacteria [54].

Polyunsaturated fatty acids (PUFAs) contain two or more double bonds [39]. Based on the number of double bonds, individual fatty acids are named dienoic, trienoic, tetraenoic, pentaenoic and hexaenoic fatty acids. Also, depending on the position of the first double bond from the terminal methyl end (x) of the carbon chain, a fatty acid may be either an x3 PUFA (i.e. the third carbon from the end of the fatty acid) or an x6 PUFAs (i.e. the sixth carbon from the end of the fatty acid). The major PUFAs are C20:5x3 and C22:6x3 in Bacillariophyceae, C18:2 and C18:3x3 in green algae, C18:2 and C18:3 x3 in Euglenophyceae, C20:5, C22:5 and C22:6 in Chrysophyceae, C18:3x3, 18:4 and C20:5 in Cryptophyceae, C20:3 and C20:4 x3 in Eustigmatophyceae, C18: 3x3 and C20:5 in Prasinophyceae, C18:5x3 and C22:6x3 in Dinophyceae, C18:2, C18:3x3 and C22:6x3 in Prymnesiophyceae, C18:2 and C20:5 in Rhodophyceae, C16:3 and C20:5 in Xanthophyceae and C16:0, C18:2 and C18:3x3 in cyanobacteria [54, 55].

Biodiesel Production: Parent oil used in making biodiesel consists of triglycerides in which three fatty acid molecules are esterified with a molecule of glycerol. In making biodiesel, triglycerides are reacted with methanol in a reaction known as Transesterification or alcoholysis. Transesterification produces methyl esters of fatty acids that are biodiesel and glycerol⁵⁶. The reaction occurs stepwise: triglycerides are first converted to diglycerides, then to monoglycerides and finally to glycerol.

Transesterification of Oil to Biodiesel: Transesterification requires 3-mol of alcohol for each mole of triglyceride to produce 1 mol of glycerol and 3-mol of methyl esters. During industrial processes 6-mol of methanol are used for production of each mole of triglyceride. This large excess of methanol ensures that the reaction is driven in the direction of methyl esters, i.e. towards biodiesel [27]. Transesterification is catalyzed by acids, alkalis [30] and lipase enzymes [57]. Alkali-catalyzed Transesterification is about 4000 times faster than the acid catalyzed reaction [27].



Consequently, alkalis such as sodium and potassium hydroxide are commonly used as commercial catalysts at a concentration of about 1% by weight of oil. Alkoxides such as sodium methoxide are even better catalysts than sodium hydroxide. Use of lipases offers important advantages, but is not currently feasible because of the relatively high cost of the catalyst [27, 57]. Alkalicatalyzed Transesterification is carried out at approximately 60°C under atmospheric pressure, as methanol boils off at 65 °C at atmospheric pressure. Under these conditions, reaction takes about 90min to complete. A higher temperature can be used in combination with higher pressure, but this is expensive. Methanol and oil do not mix, hence the reaction mixture contains two liquid phases. Other alcohols can be used, but methanol is the least expensive [30].

Algal Cultivations: Algal growth requires light, carbon dioxide, water and inorganic salts. Temperature must remain generally within 20 to 30°C. To minimize expense, biodiesel production must rely on freely available sunlight, despite daily and seasonal variations in light levels. Growth medium must provide the inorganic elements that constitute the algal cell. Essential elements include nitrogen (N), phosphorus (P), iron and in some cases silicon. Minimal nutritional requirements can be estimated using the approximate molecular formula of the microalgal biomass that is CO_{0.48}H_{1.83}N_{0.11}P_{0.01}. This formula is based on data presented by Grobbelaar [58]. Nutrients such as phosphorus must be supplied in significant excess because the phosphates added complex with metal ions is not available to plants. Sea water supplemented with commercial nitrate and phosphate fertilizers and a few other micronutrients is commonly used for growing marine microalgae [59]. Microalgal biomass contains approximately 50% carbon by dry weight [60]. All of this carbon is typically derived from carbon dioxide. Producing 100 tons of algal biomass fixes roughly 183 tons of carbon dioxide. Biodiesel production can potentially use some of the carbon dioxide that is released in power plants by burning fossil fuels [22]. This carbon dioxide is often available at little or no cost. Ideally, microalgal biodiesel would be carbon neutral, as all the power needed for producing and processing

the algae would come from biodiesel itself and from methane produced by anaerobic digestion of biomass residue left behind after the oils has been extracted. Although microalgal biodiesel can be carbon neutral, it will not result in any net reduction in carbon dioxide that is accumulating as a consequence of burning of fossil fuels [37].

As much as 25% of the biomass produced during daylight, may be lost during the night because of respiration. The extent of this loss depends on the light level under which the biomass was grown, the growth temperature and the temperature at night. The only practicable methods of large-scale production of microalgae are raceway ponds [59, 61] and tubular photobioreactor [60, 62].

Open Pond System: These are the oldest and simplest systems for large scale cultivation of microalgae. In open pond system, one foot deep shallow ponds are made and algae are cultured under conditions matching to their natural environment [59, 63]. The pond is designed in a raceway configuration, in which a paddlewheel circulates and mixes the algal cells and nutrients as explained in Figure 1 and 2. The raceways are typically made from poured concrete, or they are simply dug into the earth and lined with a plastic liner to prevent the ground from soaking up the liquid. Baffles in the channel guide the flow around the bends in order to minimize space [59].

The system is often operated in a continuous mode, i.e., the fresh feed (containing nutrients including nitrogen phosphorus and inorganic salts) is added in front of the paddlewheel and algal broth is harvested behind the paddlewheel after it has circulated through the loop. Depending on the nutrients required by algal species, several sources of wastewater such as dairy/swine lagoon effluent and municipal wastewater can be used for algal culture. For some marine-type microalgae, seawater or water with high salinity can be used [63].

Photobioreactors: Photobioreactors have been successfully used for producing large quantities of microalgal biomass [59, 64, 65]. A tubular photobioreactor consists of an array of straight transparent tubes that are usually made of plastic or glass for sunlight capturing. 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 (Figure 3 and 4).



Fig. 1: Open pond systems
Source: Adapted from Wen and Johnson [63]

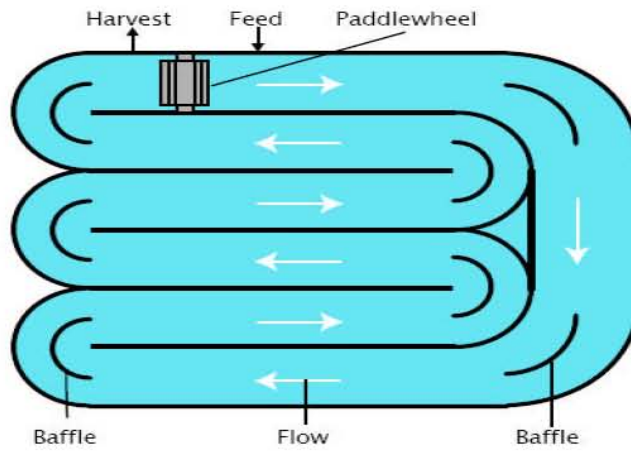


Fig. 2: Schematic for open pond system
Source: Adapted from Wen and Johnson [63]



Fig. 3: Photobioreactors
Source: Adapted from Wen and Johnson [63]

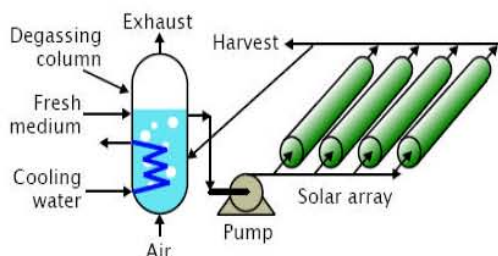


Fig. 4: Schematic tubular photobioreactor
Source: Adapted from Wen and Johnson [63]

Microalgal broth is circulated from a reservoir to the solar collector and back to the reservoir. The solar collector is oriented to maximize sunlight capture [59, 60]. In a typical arrangement, the solar tubes are placed parallel to each other and flat above the ground. Horizontal and 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 [64, 65].

Other variants of tubular photobioreactor exist but are not widely used [59, 64]. Artificial illumination of tubular photobioreactor is technically feasible but expensive compared with natural illumination. Nonetheless, artificial illumination has been used in large-scale biomass production particularly for high-value products [65]. Biomass sedimentation in tubes is prevented by maintaining highly turbulent flow. Flow is produced using either a mechanical pump, or a gentler airlift pump. Mechanical pumps can damage the biomass but are easy to design, install and operate [66-69]. Airlift pumps have been used quite successfully [70]. Airlift pumps for use in tubular photobioreactor are designed using the same methods that were originally developed for designing conventional airlift reactors. Airlift pumps are less flexible than mechanical pumps and require a supply of air to operate [71].

Photobioreactors require cooling during daylight hours. Furthermore, temperature control at night is also useful. For example, the nightly loss of biomass due to respiration can be reduced by lowering the temperature at night [59]. Outdoor tubular photobioreactor are effectively and inexpensively cooled using heat exchangers. A heat exchange coil may be located in the degassing column. Alternatively, heat exchangers may be placed in the tubular loop. Evaporative cooling by

water sprayed on tubes can also be used and has proven successful in dry climates. Large tubular photobioreactor have been placed within temperature controlled greenhouses [65]. Selecting a suitable microalgal biomass production method for making biodiesel requires a comparison of capabilities of raceways and tubular photobioreactor [69].

Acceptability of Microalgal Biodiesel: For user acceptance, microalgal biodiesel will need to comply with existing standards. In the United States the relevant standard is the ASTM Biodiesel Standard D 6751. In European Union, separate standards exist for biodiesel intended for vehicle use (Standard EN 14214) and for use as heating oil Standard EN 14213 describe by Knothe [72]. Microalgal oils differ from most vegetable oils in being quite rich in polyunsaturated fatty acids with four or more double bonds [31]. For example, eicosapentaenoic acid (EPA, C20:5n-3; five double bonds) and docosahexaenoic acid (DHA, C22:6n-3; six double bonds) occur commonly in algal oils. Fatty acids and fatty acid methyl esters (FAME) with 4 and more double bonds are susceptible to oxidation during storage and this reduces their acceptability for use in biodiesel. Some vegetable oils also face this problem. For example, vegetable oils such as high oleic canola oil 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 DHA and EPA, the European Standard EN 14214 limits linolenic acid methyl ester content in biodiesel for vehicle use to 12% (mol). No such limitation exists for biodiesel intended for use as heating oil, but acceptable biodiesel must meet other criteria relating to the extent of total unsaturation of the oil. Total unsaturation of oil is indicated by its iodine value. Standards EN 14214 and EN 14213 require the iodine value of biodiesel to not exceed 120 and 130 g iodine/ 100 g biodiesel, respectively [73]. Furthermore, both the European biodiesel standards limit the contents of FAME with four and more double bonds, to a maximum of 1% mol. In view of the composition of many microalgal oils, most of them are unlikely to comply with the European biodiesel standards, but this need not be a significant limitation. The extent of unsaturation of microalgal oil and its content of fatty acids with more than 4 double bonds can be reduced easily by partial catalytic hydrogenation of the oil as the same technology that is commonly used in making margarine from vegetable oils [74].

Economics for Algae Biodiesel: The production cost of algal oil depends on many factors, such as yield of biomass from the culture system, oil content, scale of production systems and cost of recovering oil from algal biomass [63]. Currently, algal-oil production is still far more expensive than petroleum diesel fuels. For example, Chisti [42] estimated the production cost of algal oil from a photobioreactor with an annual production capacity of 10,000 tons per year. Assuming the oil content of the algae to be approximately 30 percent, the author determined a production cost of \$2.80 per liter (\$10.50 per gallon) of algal oil. This estimation did not include costs of converting algal oil to biodiesel, distribution and marketing costs for biodiesel and taxes. At the same time, the petroleum-diesel price in Virginia was \$3.80 to \$4.50 per gallon.

Whether algal oil can be an economic source for biofuel in the future is still highly dependent on the petroleum oil price [63]. Chisti [42] used the following equation to estimate the cost of algal oil where it can be a competitive substitute for petroleum diesel:

$$C_{(\text{algal oil})} = 25.9 \times 10^{-3} C_{(\text{petroleum})}$$

Where: $C_{(\text{algal oil})}$ is the price of microalgal oil in dollars per gallon and $C_{(\text{petroleum})}$ is the price of crude oil in dollars per barrel.

This equation assumes that algal oil has roughly 80 percent of the caloric energy value of crude petroleum. For example, with petroleum priced at \$100 per barrel, algal oil should cost no more than \$2.59 per gallon in order to be competitive with petroleum diesel [63].

Microalgal oils can potentially completely replace petroleum as a source of hydrocarbon feedstock for the petrochemical industry. For this to happen, microalgal oil will need to be sourced at a price that is roughly related to the price of crude oil, as follows: $C_{\text{algal oil}} \approx 0.8 C_{\text{petroleum}}$ where $C_{\text{algal oil}}$ (\$ per liter) is the price of microalgal oil and $C_{\text{petroleum}}$ is the price of crude oil in \$ per barrel [63]. For example, if the prevailing price of crude oil is \$60/barrel, then microalgal oil should not cost more than about \$0.41/L, if it is to substitute for crude oil. If the price of crude oil rises to \$80/barrel as sometimes predicted, then microalgal oil costing \$0.55/L is likely to economically substitute for crude petroleum. It is assumed that algal oil has roughly 80% of the energy content of crude petroleum. Microalgal biodiesel refinery: producing multiple products from algal biomass [42].

Improving Economics of Microalgal Biodiesel: Cost of producing microalgal biodiesel can be reduced substantially by using a biorefinery based production strategy, improving capabilities of microalgae through genetic engineering and advances in engineering of photobioreactor.

Biorefinery Based Production Strategy: Like a petroleum refinery, a biorefinery uses every component of the biomass raw material to produce useable products. Because all components of the biomass are used, the overall cost of producing any given product is lowered. Integrated biorefinery are already being operated in Canada, the United States and Germany for producing biofuel and other products from crops such as corn and soybean. This approach can be used to reduce the cost of making microalgal biodiesel. In addition to oils, microalgal biomass contains significant quantities of proteins, carbohydrates and other nutrients [60]. Therefore, the residual biomass from biodiesel production processes can be used potentially as animal feed. Some of the residual biomass may be used to produce methane by anaerobic digestion, for generating the electrical power necessary for running the microalgal biomass production facility. Excess power could be sold to defray the cost of producing biodiesel [42]. Although the use of microalgal biomass directly to produce methane by anaerobic digestion that is technically feasible, it cannot compete with the many other low-cost organic substrates that are available for anaerobic digestion. Nevertheless, algal biomass residue remaining after the extraction of oil can be used potentially to make methane. A microalgal biorefinery can simultaneously produce biodiesel, animal feed, biogas and electrical power. Extraction of other high-value products may be feasible, depending on the specific microalgae used [75, 76].

Enhancing Algal Biology: Genetic and metabolic engineering are likely to have the greatest impact on improving the economics of production of microalgal diesel^{42,77}. Genetic modification of microalgae has received little attention. Molecular level engineering can be used to potentially:

- Increase photosynthetic efficiency to enable increased biomass yield on light.
- Enhance biomass growth rate.
- Increase oil content in biomass.
- Improve temperature tolerance to reduce the expense of cooling.

- Eliminate the light saturation phenomenon so that growth continues to increase in response to increasing light level.
- Reduce photo inhibition that actually reduces growth rate at midday light intensities that occur in temperate and tropical zones.
- Reduce susceptibility to photo oxidation that damages cells.
- In addition, there is a need to identify possible biochemical triggers and environmental factors that might favor accumulation of oil. Stability of engineered strains and methods for achieving stable production in industrial microbial processes are known to be important but have been barely examined for microalgae [78-80].

CONCLUSION

Microalgal biodiesel is technically feasible and it is the only renewable biodiesel that can potentially displace liquid fuels derived from petroleum. Algae for biodiesel are generally the favored algae-for-energy option and have been researched the most. Both open and closed land based cultivation systems appear suitable for this option. The conversion of the extracted lipids to biodiesel is relatively easy and the product price is relatively low as compared with fossil fuel prices.

REFERENCES

1. Shimizu, Y., 2003. Microalgal metabolites. *Curr Opin Microbiol.*, 6: 236-43.
2. Falkowski, P.G. and J.A. Raven, 1997. *Aquatic Photosynthesis*. Malden, MA: Blackwell Sci., pp: 45-60.
3. Liang, Y., J. Beardall and P. Heraud, 2006. Changes in growth, chlorophyll fluorescence and fatty acid composition with culture age in batch cultures of *Phaeodactylum tricornutum* and *Chaetoceros muelleri* (Bacillariophyceae). *Bot Mar.*, 49: 165-173.
4. Akkerman, I., M. Janssen, J. Rocha and R. Wijffels, 2002. Photo biological hydrogen production: photochemical efficiency and bioreactor design. *Int. J. Hydrogen Energy*, 27: 1195-208
5. Banerjee, A., R. Sharma, Y. Chisti and U.C. Banerjee, 2002. *Botryococcus braunii*: a renewable source of hydrocarbons and other chemicals. *Crit Rev. Biotechnol.*, 22: 245-79.
6. Borowitzka, M.A., 1999. Pharmaceuticals and agrochemicals from microalgae, In Z. Cohen, editor. *Chemicals from microalgae*, Taylor and Francis Pulishesr, pp: 313-352.
7. Ghirardi, M.L., J.P. Zhang, J.W. Lee, T. Flynn, M. Seibert and E. Greenbrae, 2000. Microalgae: a green source of renewable H₂. *Trends Biotechnol.*, 18: 506-11.
8. Kay, R.A., 1991. Microalgae as food and supplement. *Crit Rev Food Sci. Nutr.*, 30: 555-73.
9. Lorenz, R.T. and G.R. Cysewski, 2003. Commercial potential for *Haematococcus microalga* as a natural source of astaxanthin. *Trends Biotechnol.*, 18: 160-7.
10. Melis, A., 2002. Green alga hydrogen production: progress, challenges and prospects. *Int. J. Hydrogen Energy*, 27: 1217-28.
11. Metting, B. and J.W. Pyne, 1986. Biologically-active compounds from microalgae. *Enzyme Microb Technol.*, 8: 386-94.
12. Metzger, P. and C. Largeau, 2005. *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. *Appl. Microbial. Biotechnol.*, 66: 486-96.
13. Singh, S., B.N. Kate and U.C. Banerjee, 2005. Bioactive compounds from cyanobacteria and microalgae: an overview. *Crit Rev. Biotechnol.*, 25: 73-95.
14. Spolaore, P., C.C. Joannis, E. Duran and A. Isambert, 2006. Commercial applications of microalgae. *J. Biosci Bioeng*, 101: 87-96.
15. Kalin, M., W.N. Wheeler and G. Meinrath, 2005. The removal of uranium from mining waste water using algal/microbial biomass. *J. Environ. Radioact*, 78: 151-77.
16. Mallick, N., 2002. Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. *Bimetals*, 15: 377-90.
17. Munoz, R. and B. Guieysse, 2006. Algal-bacterial processes for the treatment of hazardous contaminants: a review. *Water Res.*, 40: 2799-815.
18. Suresh, B. and G.A. Ravishankar, 2004. Phytoremediation, a novel and promising approach for environmental clean-up. *Crit Rev. Biotechnol.*, 24: 97-124.
19. Fedorov, A.S., S. Kosourov, M.L. Ghirardi and M. Seibert, 2005. Continuous H₂ photoproduction by *Chlamydomonas reinhardtii* using a novel two-stage, sulfate-limited chemostat system. *Appl. Biochem. Biotechnol.*, 121: 403-12.

20. Kapdan, I.K. and F. Kargi, 2006. Bio-hydrogen production from waste materials. *Enzyme Microb Technol.*, 38: 569-82.
21. Nagle, N. and P. Lemke, 1990. Production of methyl-ester fuel from microalgae. *Appl. Biochem Biotechnol.*, 5: 355-61.
22. Sawayama, S., S. Inoue, Y. Dote and S.Y. Yokoyama, 1995. CO₂ fixation and oil production through microalga. *Energy Convers Manag.*, 36: 729-31.
23. Gavrilescu, M. and Y. Chisti, 2005. Biotechnology-a sustainable alternative for chemical industry. *Biotechnol. Adv.*, 23: 471-99.
24. Barnwal, B.K. and M.P. Sharma, 2005. Prospects of biodiesel production from vegetables oils in India. *Renew Sustain Energy Rev.*, 9: 363-78.
25. Demirbas, A., 2005. Biodiesel production from vegetable oils via catalytic and non-catalytic supercritical methanol Transesterification methods. *Pror Energy Combust Sci.*, 31(5-6): 466-87.
26. Felizardo, P., M.J.N. Correia, I. Raposo, J.F. Mendes, R. Berkemeier, J.M. Bordado, 2006. Production of biodiesel from waste frying oil. *Waste Manag.*, 26(5): 487-94.
27. Fukuda, H., A. Kondo, H. Noda, 2001. Biodiesel fuel production by Transesterification of oils. *J. Biosci. Bioeng.*, 92: 405-16.
28. Knothe, G., R.O. Dunn and M.O. Bagby, 1997. Biodiesel: the use of vegetable oils and their derivatives as alternative diesel fuels. *Acs. Symp Ser.*, 666: 172-208.
29. Kulkarni, M.G. and A.K. Dalai, 2006. Waste cooking oil an economical source for biodiesel: A review. *Ind Eng. Chem. Res.*, 45: 2901-13.
30. Meher, L.C., S.D. Vidya and S.N. Naik, 2006. Technical aspects of biodiesel production by Transesterification - a review. *Renew Sustain Energy Rev.*, 10: 248-68.
31. Belarbi, E.H., G.E. Molina and Y. Chisti, 2000. A process for high yield and scaleable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil. *Enzyme Microb. Technol.*, 26: 516-29.
32. Benemann, J.R., R.P. Goebel, J.C. Weissman and D.C. Augenstein, 1982. Microalgae as a source of Liquid Fuels. Final Technical Report to US Department of Energy. Washington DC: US Department of Energy, pp: 70-75.
33. Borowitzka, M., 1988. Fats, oils and hydrocarbons. In *Microalgal Biotechnology* (M.A. Borowitzka and L.J. Borowitzka, eds). Cambridge, UK: Cambridge University Press, pp: 257-287.
34. Burlew, J.S., 1953. Algal Culture, in *Laboratory to Pilot Plant*, Washington DC: Carnegie Institution of Washington, pp: 60-75.
35. Hill, A., A. Feinberg, R. McIntosh, B. Neeman and K. Terry, 1984. Fuels from Microalgae: Technical Status, Potential and Research Issues, Report SERI/SP-231-255. Golden, CO: Solar Energy Research Institute.
36. Meier, R.L., 1955. Biological cycles in the transformation of solar Energy into useful fuels. In *Solar Energy Research* (F. Daniels and J.A. Duffie, eds). Madison, WI: University of Wisconsin Press, pp: 179-183.
37. Sheehan, J., T. Dunahay, J. Benemann and PA. Roessler, 1998. A look back at the U.S. Department of Energy's Aquatic Species Program-biodiesel from algae. National Renewable Energy Laboratory, Golden, Co, Report NREL/TP-580-24190.
38. Hu, Q., C.W. Zhang and M. Sommerfeld, 2006. Biodiesel from Algae: Lessons Learned Over the Past 60 Years and Future Perspectives. Juneau, Alaska: Annual Meeting of the Phycological Society of America, July 7-12, pp: 40-41.
39. Shahzad, I., K. Hussain, K. Nawaz and M.F. Nisar, 2010. Algae as an alternative renewable resource for biodiesel production. *The Biol.*, 1(1): 16-23.
40. Metting, B. and J.W. Pyne, 1986. Biologically-active compounds from microalgae. *Enzyme Microb Technol.*, 8: 386-94.
41. Guschina, I.A. and J.L. Harwood, 2006. Lipids and lipid metabolism in eukaryotic algae. *Prog Lipid Res.*, 45: 160-186.
42. Chisti, Y., 2007. Biodiesel from microalgae. *Biotechnology Advances*, 25: 294-306.
43. Thompson, G.A., 1996. Lipids and membrane function in green algae. *Biochim Biophys Acta*, 13(2): 17-45.
44. Wada, H. and N. Murata, 1998. Membrane lipids in cyanobacteria, in *Lipids in Photosynthesis: Structure, Function and Genetics* (P.A. Siegenthaler and N. Murata, eds). Dordrecht, The Netherlands: Kluwer Academic Publishers, pp: 65-81.
45. Guckert, J.B. and K.E. Cooksey, 1990. Triacylglyceride accumulation and fatty acid profile changes in *Chlorella* (Chlorophyta) during high-pH induced cell cycle inhibition. *J. Phycol.*, 26: 72-79.
46. Harwood, J.L., 1998. Membrane lipids in algae, in *Lipids in Photosynthesis: Structure, Function and Genetics* (P.A. Siegenthaler and N. Murata, eds). Dordrecht, the Netherlands: Kluwer Academic Publishers, pp: 53-64.

47. Pohl, P. and F. Zurheide, 1979a. Fatty acids and lipids of marine algae and the control of their biosynthesis by environmental factors, in *Marine Algae in Pharmaceutical Science* (A. Hoppe, T. Levring and Y. Tanaka, eds). Berlin: Walter de Gruyter, pp: 473-523.
48. Pohl, P. and F. Zurheide, 1979b. Control of fatty acid and lipid formation in Baltic marine algae by environmental factors. In *Advances in the Biochemistry and Physiology of Plant Lipids* (L.A. Appelqvist and C. Liljenberg, eds). Amsterdam: Elsevier, pp: 427-432.
49. Shahzad, I., K. Hussain, K. Nawaz and M.F. Nisar, 2010. Algae as an alternative renewable resource for biodiesel production. *The Biol.*, 1(1): 16-23.
50. Grossman, A.R., M. Croft, V.N. Gladyshev, S.S. Merchant, M.C. Posewitz, S. Prochnik and M.H. Spalding, 2007. Novel Metabolism in *Chlamydomonas* through the lens of genomics. *Curr Opin Plant Biol.*, 10: 190-198.
51. Ratledge, C., 1993. Single cell oils, have they a biotechnological future? *Trends Biotechnol.*, 11: 278-84.
52. Ohlrogge, J. and J. Browse, 1995. Lipid biosynthesis. *Plant Cell*, 7: 957-970.
53. Borowitzka, M., 1988. Fats, oils and hydrocarbons, in *Microalgal Biotechnolog*, Cambridge, UK: Cambridge University Press, pp: 257-287.
54. Cobelas, M.A. and J.Z. Lechado, 1989. Lipids in microalgae. A review: *Biochemistry. Grasasy Aceites* 40: 118-145.
55. Basova, M.M., 2005. Fatty acid composition of lipids in microalgae. *Int. J. Algae*, 7: 33-57.
56. Durrett, T., C. Benning and J. Ohlrogge, 2008. Plant triacylglycerols as feedstocks for the production of biofuels. *Plant J.*, 54: 593-607.
57. Sharma, R., Y. Chisti and U.C. Banerjee, 2001. Production, purification, characterization and applications of lipases. *Biotechnol Adv.*, 19: 627-62.
58. Grobbelaar, J.U., 2004. Algal nutrition, in *Handbook of microalgal culture: biotechnology and applied phycology*. Blackwell, pp: 79-115.
59. Molina, G.E., 1999. Microalgae, mass culture methods, in *Encyclopedia of bioprocess technology: fermentation, biocatalysts and bioseparation* M.C. Flickinger and S.W. Drew, editors, Wiley, 3: 1753-69.
60. Sánchez, M.A., 2003. Cerón GMC, Contreras GA, García CF, Molina GE and Chisti Y, Shear stress tolerance and biochemical characterization of *Phaeodactylum tricornutum* in quasi steady-state continuous culture in outdoor photobioreactors. *Biochem Eng. J.*, 16: 287-97.
61. Terry, K.L. and L.P. Raymond, 1985. System design for the autotrophic production of microalgae. *Enzyme Microb. Technol.*, 7: 474-87.
62. Molina, G.E., A.F.G. Fernández, C.F. García and Y. Chisti, 1999. Photobioreactors: light regime, mass transfer and scale up. *J. Biotechnol.*, 70: 231-47.
63. Wen, Z. and M.B. Johnson, 2009. Microalgae as a feedstock for biofuel production. *Communications and Marketing*, College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University.
64. Carvalho, A.P., L.A. Meireles and F.X. Malcata, 2006. Microalgal reactors: a review of enclosed system designs and performances. *Biotechnol. Prog.*, 22: 1490-506.
65. Pulz, O., 2001. Photobioreactors: production systems for phototrophic microorganisms. *Appl. Microbial. Biotechnol.*, 57: 287-93.
66. Chisti, Y., 1999a. Shear sensitivity, In M.C. Flickinger and S.W. Drew, editors. *Encyclopedia of bioprocess technology, Fermentation, Biocatalysts and Bioseparation*, 5: 2379-2406.
67. Chisti, Y., 1999b. Modern systems of plant cleaning, in *Encyclopedia of food microbiology*. Academic Press, pp: 1806-1815.
68. García, C.F., R.J. Gallardo, M.A. Sánchez, G.M.C. Cerón, E.H. Belarbi and Y. Chisti, 2007. Biotechnological significance of toxic marine dinoflagellates. *Biotechnol, Adv.*, 25: 176-94.
69. Mazzuca, S.T., C.F. García, G.E. Molina and Y. Chisti, 2006. Effects of agitation on the microalgae *Phaeodactylum tricornutum* and *Porphyridium cruentum*. *Bioprocess Biosyst Eng.*, 28: 243-50.
70. Fernández, A.F.G., C.F. García, P.J.A. Sánchez, S.J. Fernández and G.E. Molina, 1998. Modelling of biomass productivity in tubular photobioreactor for microalgal cultures. Effects of dilution rate, tube diameter and solar irradiance. *Biotechnol Bioeng*, 58: 605-11.
71. Chisti, Y., 1998. Pneumatically agitated bioreactors in industrial and environmental bioprocessing: hydrodynamics, hydraulics and transport phenomena. *Appl. Mech Rev.*, 51: 33-112.

72. Knothe, G., 2006. Analyzing biodiesel: standards and other methods. *J. Am. Oil Chem. Soc.*, 83: 823-33.
73. Renaud, S.M., L.V. Thinh, G. Lambrinidis and D.L. Parry, 2002. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. *Aquaculture*, 211: 195-214.
74. Dijkstra, A.J., 2006. Revisiting the formation of Tran's isomers during partial hydrogenation of triacylglycerol oils. *Eur. J. Lipid Sci. Technol.*, 108(3): 249-64.
75. Raven, R.P. and K.H. Gregersen, 2007. Biogas plants in Denmark: successes and setbacks. *Renew Sustain Energy Rev.*, 11: 116-32.
76. Alvarez, M.J., S. Mace and P. Liabres, 2000. Anaerobic digestion of organic solid wastes: An overview of research achievements and perspectives. *Bioresour Technol.*, 74: 3-16.
77. Dunahay, T.G., E.E. Jarvis, S.S. Dais and P.G. Roessler, 1996. Manipulation of microalgal lipid production using genetic engineering. *Appl. Biochem. Biotechnol.*, 57: 223-3.
78. Bañares, L.R., D. González-Ballester, A. Galvan and E. Fernandez, 2004. Transgenic microalgae as green cell-factories. *Trends Biotechnol.*, 22: 45-52.
79. Chisti, Y., 2000. Animal-cell damage in sparged bioreactors. *Trends Biotechnol.*, 18: 420-32.
80. Chisti, Y., 2001. Hydrodynamic damage to animal cells. *Crit Rev. Biotechnol.*, 21: 67-110.