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Microalgal-based biorefineries: the nextgen renewable biofuels

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Abstract— The sustained and prolonged usage of fossil fuels is unsustainable - this is now widely recognized and accepted. The reasons for this are two-fold. These include the associated environmental pollution and their depleting supplies. Going forward, it becomes an imperative that we identify new renewable and carbon neutral fuels. This is the need of the hour, from both an economic as well as a sustainability standpoint. It is in this context that biodiesel derived from oil crops can serve as the saviour. Biodiesel has high potential to be a renewable, while being carbon neutral, alternative to fossil fuels. However, there are some challenges. The foremost challenge is that biodiesel from oil crops currently cannot support the scale that the transportation industry requires. In this context, biodiesel that is generated from microalgae can hold significant potential as an alternate source of transport fuel. This way, it does not impact the supply of crop products too. Microalgae use sunlight highly efficiently to produce oil, unlike crop plants. In this review, we discuss how such an environmentally friendly and economically competitive microalgal biodiesel can be produced.

Key words— Biofuel, Lipids, Microalgae, Metabolic engineering, Processing, Technology, Sustainability.

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1 INTRODUCTION

All nations have been challenged with the energy crisis due to exhaustion of finite fossil fuel reserves. Their continued consumption as sustainable energy source is at alarming stage due to depletion of resources and emissions of greenhouse gases GHGs in the environment (Demirbas 2010). A constant rising worldwide requirement of fossil fuels to satisfy demand of motor and power generation fuels, cause increasing anthropogenic GHG emissions and depletion of fossil reserves. Therefore, it is highly important to think about various environmental friendly alternate sources of energy to meet the global demand. Finding sufficient supplies of clean energy for the future is one of the world's most frightening challenges and is intimately linked with economic prosperity, global stability and quality of life. In the total global energy requirements, 70% of fuel is used particularly in manufacturing, transportation, and domestic heating. Electricity accounts only 30% of global energy consumption. In recent years, a lot of thrust has been put for the search for the potential biomass feedstock from different sources, which can be converted to liquid as well as gas fuels for energy generation. Various type of biomasses from different alternate sources including agricultural, forestry and aquatic sources have been identified for the production of different biofuels, including biodiesel, bio–ethanol, bio–oil, bio–hydrogen, and bio–gas.

These years, most of industrial biodiesels are made from oil (triglycerides) of raw materials (rapeseed, sunflower, soybean, etc.). To use these triglycerides as an alternative to petroleum-based biodiesel, their physicochemical properties are changed by trans-esterification into fatty acid alkyl esters, and can be used in a conventional engine without modifications (Knothe 2010). On the ecological side, in addition to the ability of oleaginous plants to reduce pollutant emissions of greenhouse gases (GHG) by their capacity to trap and use the carbon dioxide (CO_2), using biodiesel also reduces net emissions of pollutants. It is reported that, the emissions of carbon monoxide (CO), CO_2, particulate matter (PM) and hydrocarbons (HC) by 11%, 15.5%, 10% and 21%, respectively can be reduced by the addition of 20% (v/v) of soybean-based biodiesel in petro-diesel (Sheehan et al. 1998a; United States Environmental Protection Agency, 2002). Moreover biodiesel production through oil containing crops which represents a route for renewable and carbon – neutral fuel production, accounts for only 0.3% of the current demand for transport fuels. Hence, increasing biofuel production on arable land could have severe consequences for global food supply. On the contrary, producing biodiesel from algae is regarded and widely accepted as one of the most efficient ways of generating biofuels and also appears to represent the only current renewable source of oil that could meet the global demand for transport fuels. Out of the three generations of biofuels that have emerged till now; the 1st generation biofuel which is based on edible plant parts (oilseeds, grains, *etc.*); the 2nd refers to energy production from non-edible plants or non-edible parts of plants; and the 3rd is based on energy production from photosynthetic microorganisms such as microalgae. As photosynthetic microalgae, can use and remove nitrogen, phosphorus in wastewater, sequester CO_2 in the air, and synthesize lipids which can be converted into biodiesel. The decrease in supply of conventional fossil fuel and concern about global warming make microalgae-based biodiesel a very promising alternative.

The use of algae as a renewable feedstock for biofuel production has been known for many years. The advantages of using lipid based fuels from algae are listed below.

- Algae can be easily grown in large outdoor cultures and harvested.
- The algal biomass will contain a large amount percentage of lipids, though not necessarily in the form of triacylglycerides (TAGs).
- Algal oil can be obtained from harvested biomass by known means, with sub optimal yield, cost and thermodynamic efficiencies.
- At non-commercial scales biodiesel (fatty acid methyl ester, FAME), hydrogenation-derived renewable diesel (HDRD) and synthetic jet fuel production from algal oil have been demonstrated.

1. Potential of Microalgae for Biodiesel Production

Algae are the important food sources for many animals and belong to the bottom of the food chain. Moreover, they are the principal producers of oxygen on earth (Khan et al. 2009) and are one of the best sources of biodiesel production (Shay et al. 1993). They are the highest yielding feedstock for biodiesel. It has the ability to produce up to 250 times the amount of oil per acre as soybeans and 7-31 times greater oil than palm oil. Extraction of oil from algae is quite simple and convenient. Microalgae are ideal for the production of biodiesel. Recent research has clearly reported that oil production from microalgae is superior to that of terrestrial plants such as soyabean, rapeseed, Jatropha or palm.

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms having simple structure, which can grow rapidly and live under adverse conditions because of their unicellular or simple multicellular structure (Mata et al. 2010). They have the ability to convert carbon dioxide to potential biofuels, food, feeds and high-value bio actives and can function as sun light driven cell factories (Metting and Pyne 1986; Schwartz 1990; Kay 1991; Shimizu 1996, 2003; Borowitzka

1999; Ghirardi et al. 2000; Akkerman et al. 2002; Banerjee et al. 2002; Melis 2002; Spolaore et al. 2006; Walter et al. 2005).They are capable of producing several different types of renewable biofuels and by-products. These include renewable biofuel (Subramanium et al. 2010; Demirbas et al. 2007), biohydrogen (Chisti et al. 2007; Fedorov et al. 2005; Illman et al, 2000; Schenk et al, 2008), hydrocarbon (Bajhaiya et al. 2010; Barupal et al. 2010; Kojima et al. 1999), methane (Mata et al. 2010), ethanol (Bush et al. 2009), phycocolloids and carotenoids (Azocar et al. 2011), minerals, vitamins, polysaturated fatty acids (PUFAs), α-linolenic, eicosapentanoic and docosuccinic acids, belong to w-3 group (Khan et al. 2009; Shimizu et al. 2003), propylene glycol, acetol, butanol (Verma et al. 2010), biogas (Costa et al. 2011; Gunaseelan et al. 1997; Ras et al. 2011), neutral lipid (Chen et al. 2011), polar lipid, carbohydrates (Moreno et al. 2008), sterols, carotenoids, tocopherols, quinines, terpenes and phytylated pyrrole derivatives such as the chlorophylls (Bisen et al. 2010), β-carotene (Singh et al. 2011) antioxidants, antibiotics, astaxanthin and pigments (Ratledge et al. 2008).

Algal biomass is one of the emerging sources of sustainable energy. Therefore introducing large-scale biomass could contribute to sustainable development environmentally, socially and economically (Turkenburg et al. 2000). Moreover microalgae commonly double their biomass within 24h (Bajhaiya et al. 2010). During exponential growth the biomass doubling time is commonly as short as 3.5h (Chisti et al. 2008; Patil et al. 2008) which can double their biomass in less than 2-5 days, achieving large yields, without the need for the applying any pesticides, fungicides or herbicides (Costa et al. 2011). They are capable of synthesizing more oil per acre than the terrestrial plants which are currently used for the fabrication of biofuels. Moreover using microalgae for biodiesel production of will not compromise in the production of food, fodder and other products derived from crops (Kozlovska et al. 2012) (Table 1). More than 50,000 microalgae species exist in the world, but only 30,000 species have been studied and analyzed (Richmond et al. 2004). They can also be used as a source of waste water remediation by removal of NH_4^+, NO_3^-, PO_4^{3-} from a variety of waste water run-off for example industrial and municipal wastewaters, concentrated animal feed operations. It produces value added co-products or by products like biopolymers, proteins, polysaccharides, pigments, animal feed, fertilizer and H_2, that can be used in different industrial sectors. Algae can be grown in suitable photo-bioreactor all throughout the year with an annual biomass production (Naik 2010).

The idea of using microalgae as a source of fuel is not new (Chisti 1980; Nagle and Lemke 1990; Sawayama et al. 1995), because of the increasing petroleum price and, more significantly, the emerging concern about global warming that is associated with burning fossil fuels (Gavrilescu and Chisti 2005) it is now being taken seriously. The biodiesel generated from biomass from various plants and animal oils is a mixture of monoalkyl ester, which currently obtained from transesterification of triglycerides and monohydric alcohols. But this trend is changing as several companies are attempting to generate large scale algal biomass as there are several advantages associated with it, which can be used for commercial production of algal biodiesel.

Table.1. Amount of biodiesel produced by some of the plants and algae (Mata et al. 2010)

Crop	**Oil Yeild (L/ha)**	**Land Area needed (Mha)[a]**	**Percent of existing US cropping area [a]**
Corn	172	1540	846
Soyabean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil palm	5950	45	24
Microalgae [b]	1,36,900	2	1.1
Microlagae [C]	58,700	4.5	2.5

In view of table 1, it is clearly visible that microalgal strains with high oil content are of great interest in search for sustainable feedstock for biodiesel production (Spolaore et al. 2006; Chisti et al. 2007) and have the capacity to replace fossil fuels. Algae have 20-80% of oil by weight of dry mass (Table.1). Moreover, microalgae grow extremely rapidly and many are exceedingly rich in oil unlike other oil crops. Oil content in some microalgae can exceed 80% by weight of dry biomass (Metting 1996; Spolaore et al. 2006). 20–50% of oil levels are quite common (Table 1). The amount of oil produced per unit volume of the microalgal broth per day, depends on the growth rate of algae and the oil content of the biomass. Moreover lipid accumulation in algae typically occurs during environmental stress, including nutrient inadequate conditions. Biochemical studies have proposed that acetyl-CoA carboxylase (ACCase), a biotin containing enzyme which catalyzes an early step in biosynthesis of fatty acids, may be involved in the control of this lipid accumulation process. Therefore, for enhanced lipid production this enzyme activity can be increased by genetic engineering.

Microalgae can represent a great potential in economic development as they do not compete with food and wood production, as they can be cultivated even in marginal lands. Moreover, using microalgae for biofuel production would reduce deforestation and preserving forest heritage. Hence development of valorisation of microalgae could indulge the energetic autonomy of all countries. In comparison with any other terrestrial biomass, microalgae have the potential to produce greater amounts of biomass and lipids per hectare. They are basically cultivated using sunlight and carbon dioxide as carbon source. They may be grown in Shallow lagoons or raceway ponds on marginal land or closed ponds. Plastic tubes in ponds offer up to seven times the productivity of open ponds. A number of closed photobioreactors are being investigated, for cost effective production of the algae. These include horizontal tubes, vertical tubes, thin film and open/closed systems. Productivity is higher in the controlled, contained environment of a photobioreactor, but capitals as well as operating expenses are also substantially higher than for open systems. Thus, the industrial production of microalgae could be considered as a sustainable solution to energetic, environmental and food problems. Although some disadvantages are associated with microalgae for fuel production such as the low biomass concentration in the microalgal culture due to the limit of light penetration, because of the small size of algal cells, which makes the harvest of algal biomasses relatively costly and also rigorous care of microalgal farming area must be taken in comparison to conventional agricultural crops. Even then, microalgae have vast potential as the most efficient primary producers of biomass if proper technology is developed, that solves the problem between the production of food and that of biofuels in the near future which can meet the global demand for transport fuels.

2. Present Scenario of microalgal biodesel production:

Microalgae are simple photosynthetic organisms that can fix CO_2 and synthesize organic compounds, such as lipids, carbohydrates and proteins in large amounts in short periods of time. But worldwide, there is no significant amount of microalgal biofuels are produced commercially. As of today, approximately 9000 tonnes of algal biomass is produced commercially, mainly for the production of high-value, low-volume food supplements and neutraceuticals. Traditional methods of microalgae cultivation based on photoautotrophic mode have many shortcomings, among which low cell density is a major issue giving rise to low productivity, harvesting difficulty, associated high costs, and hence poor techno-economic performance. Therefore, a significant effort towards commercializing microalgae biomass production is to develop high density cultivation processes. Two approaches are being actively researched and developed: (1) metabolic pathways control; (2) cultivation system design.

2.1. Metabolic Pathways: There are three major metabolic pathways that can be utilized by microalgae depending on light and carbon conditions: photoautotrophy, heterotrophy, and mixotrophy (Chojnacka and Noworyta 2004). Most microalgae are competent of photoautotrophic growth. For large scale biomass production, open ponds photoautotrophic cultivation is a simple and low-cost way; however the biomass density is low because of less light transmission, contamination by other species or bacteria, and low organic carbon concentration (Greenwell et al. 2010). Some microalgae can propagate rapidly by heterotrophic pathway by making use of organic carbons and O_2. Heterotrophic cultivation has drawn increasing attention and it is regarded as the most practical and promising way to increase the productivity (Chen 1996; Li et al, 2007; Douch and Lívansky 2012). Presently, heterotrophic cultivation of microalgae is mainly focused on *Chlorella*. Cell densities as high as 104.9 g·L−1 (dry cell weight, *Chlorella pyrenoidosa*) have been reported (Wu and Shi, 2006). Microalgae can adapt to different organic matters such as glycerol, sucrose, xylan, organic acids in slurry after acclimatization (Heredia-Arroya et al. 2011). The potential of heterotrophic microalgae to utilize a wide variety of organic matter provide an opportunity to reduce the cost of microalgae biodiesel production as these organic substrates can be found in the waste streams such as effluents from anaerobic digestion, animal and municipal wastewaters, food processing wastes, *etc.* Based on research of heterotrophic cultivation, researchers have carried out studies on mixotrophic cultivation which can greatly enhance the growth rate, as it realizes the combined effects of photosynthesis and heterotrophy. Park et al (2012) after examining the biomass and lipid productivities characteristics of 14 microalgae found that biomass and lipid productivities were boosted by mixotrophic cultivation. It was reported by Andrade *et al.* (2007) the biomass production on mixotrophic growth of *Spirulina platensis* was stimulated by molasses, which suggested that this industrial by-product could be used as a low-cost supplement for the growth of this species. It was also found the mixotrophic growth of *Chlamydomonas globosa*, *Chlorella minutissima* and *Scenedesmus bijuga* resulted in 3–10 times more biomass production compared to that obtained under phototrophic growth conditions (Bhatnagar *et al.*2011). The maximum lipid productivities of *Phaeodactylum tricornutum* in mixotrophic cultures with glucose, starch and acetate in medium were 0.053, 0.023 and 0.020 g·L−1·day−1, which were respectively 4.6-, 2.0-, and 1.7-fold of those obtained in the corresponding photoautotrophic control cultures (Wang et al. 2012).

2.2. **Cultivation system design:** For large scale biodiesel production from microalgae, an economical cultivation system is of great importance. This system includes both open and closed forms. The open style stimulates the growth environment in lakes, which is characterized by simple and low cost operation. However there will be less biomass concentration and poor system stability. On the other hand closed culture systems like photobioreactors which are quite stable, easier to control the process conditions, maintain monoculture and thus attain high cellular density. But the operational costs of PBR's are quite expensive. The detailed commercial technology is discussed further.

3. Current Technology used for biodiesel production:

Currently for various nutritional products, microalgae have been cultivated commercially in several dozen small to medium scale production system, which producing few tons to several hundreds of tons of biomass annually. The main algae genera currently cultivated photosynthetically (e.g. with light energy) for various nutritional products are *Spirulina, Chlorella, Dunaliella and Haematococcus*. Total world production of dry algal biomass for these algae is estimated at about 10,000 tons per year. About half of this produced takes place in mainland China, with most of the rest in Japan, Taiwan, U.S.A., Australia and India, and a few small producers in some other countries. Proposed commercial algal biofuels production will require the development of strains and conditions for culture that allow rapid production of algal biomass with high lipid content and minimal growth of competing strains. And can grow both in both open (ponds) and closed (tubes, also known as photobioreactors) cultivation systems. The details are discussed below.

3.1.Strain selection: Microalgae can be found in a large range of places where light and water are present including ocean, soils, lake, rivers, ice, etc. (Deng et al. 2009). Approximately 22,000-26,000 species of microalgae exist of which only a few have been identified for successful commercial application (Norton et al. 1996). The best performing microalgae strain can be obtained by screening of a large range of naturally available isolates and their efficiency can be improved by selection, adaptation and genetic engineering (Singh et al.2011). Isolation of appropriate microalgae from the natural environment is the first critical step in developing oil-rich strains, and can then further exploited in engineered systems for the production of biodiesel feedstock (Doan et al. 2011). Depending on species, microalgae produce many different kinds of lipids, hydrocarbons and other complex oils (Banerjee et al. 2002; Metzger and Largeau 2005; Guschina and Harwood 2006). Among the algal strains the amount of lipid produced varies within a vast range (4-80% dry weight basis) and the variation is as result of the environmental conditions. For example some microalgae species like *Botryococcus braunii* or *Schizochytrium* sp. can contain up to 80% of their dry weight of lipids (Deng et al. 2009). These species can produce lipid yields of by acre up to 770 times higher than oleaginous plants (colza, sunflower, etc.). A list of protein, carbohydrate and lipid, composition of some microalgae is shown in Table 2.

Table.2. Some of the microalagal chemical composition based on their % dry matter (Kim et al. 2009, Sydney et al. 2010)

Algal species	**Proteins**	**Carbohydrates**	**Lipids**
Anabaena cylindrica	43-56	25-30	4-7
Botryococcus braunii	8-17	8-20	25-75
Chlamydomonas rheinhardii	48	17	21
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	51-58	12-17	14-22
Dunaliella biocula	49	4	8
Dunaliella salina	57	32	6
Euglena gracilis	39-61	14-18	14-20
Isochrysis sp.	31-51	11-14	20-22
Neochloris oleoabundans	20-60	20-60	35-54
Porphyridium cruentum	28-39	40-57	9-14
Prymnesium parvum	28-45	25-33	22-38
Scenedesmus dimorphus	8-18	21-52	16-40
Scenedesmus obliquus	50-56	10-17	12-14

Scenedesmus quadricauda	48	17	21
Spirogyra sp.	6-20	33-64	11-21
Spirulina maxima	60-71	13-16	6-7
Spirulina platensis	46-63	8-14	4-9
Synechoccus sp.	63	15	11
Tetraselmis maculata	52	15	3

Microalgae which can grow heterotrophically, uses exogenous carbon source as chemical energy that is stored as lipid droplets (Konur et al. 2012). For example *Chlorella protothecoides* which was cultivated heterotrophically has accumulated higher lipids (about 55% dry weight) when compared to the one grown photoautotrophically (14% of dry weight). Nitrogen starvation is another mechanism to alter the lipid content in microalgae. In the green alga *Haematococcus pluvialis,* because of nitrogen deficiency there is inhibition cell cycle and production of cellular components. However the rate of lipid accumulation is higher, which leads to the accumulation of oil in starved cells and also enhances the accumulation of antioxidant pigment astaxanthin (Boussiba et al. 2000). Both these responses help the alga to survive under stress conditions.

Another important approach while selecting the microalgal strains is to combine their growth with CO_2 bio-mitigation. This is due to the fact that microalgae have much higher CO_2 fixing abilities as compared to agricultural and aquatic plants, conventional forestry (Borowitzka et al. 1999; Li et al. 2008). This approach of microalgal biofuel production becomes more attractive when combined with fixing industrial exhaust gases (flue gas) and integrating the cultivation of algae with wastewater treatment.

For profitable commercialization of microalgae, apart from species selection, genetic and metabolic engineering tools can be adapted. By manipulating the metabolic pathways, the cellular function can be redirected for the production of desired products and even expand the processing capabilities of microalgae. The metabolic engineering allows direct control over the organism's cellular machinery through mutagenesis or the introduction of transgenes. The development of a number of transgenic algal strains boasting recombinant protein expression, engineered photosynthesis, and enhanced metabolism encourage the prospects of designer microalgae (Rosenberg et al. 2008). Production of algal oils requires an ability to inexpensively produce large quantities of oil-rich microalgal biomass.

3.2.Production of microalgal biomass: Microalgal cultivation can be done in an open culture systems such as lakes or ponds and in closed-culture systems, which are highly controlled such as photobioreactors (PBRs). The photosynthetic growth of microalgae requires light, CO_2, water, organic salts and temperature of 20º-30ºC. As large quantities of algal biomass are required for the production of microalgal biodiesel, it is better to produce the biomass using free sunlight in order to minimize the expense.

Large scale production of microalgal biomass generally uses continuous culture during day light. In this method, fresh culture medium is fed continuously at a constant rate, while the same quantity of microalgal broth is withdrawn continuously (Grima et al. 1999). As feeding ceases during night because of low light, but the mixing of broth should continue to prevent the settling of the biomass (Grima et al. 1999). Around 25% of the biomass produced during the day, may be lost during the night because of respiration. This loss of biomass mainly depends on the light level, the growth temperature, and the temperature during night. The only feasible methods for large scale production of microalgal biomass can be achieved by growing them in raceway ponds (Terry and Raymond 1985) or photobioreactors (Grima et al. 1999; Tredici 1999; Sánchez Mirón et al. 1999),

3.2.1. Raceway Ponds:

A raceway pond is a shallow artificial pond used in the cultivation of algae. The pond is divided into a rectangular grid, with each rectangle containing one channel in the shape of an oval, like an automotive raceway circuit. In this, the algae, water and nutrients circulate around a race track. A raceway pond is made of a closed loop recirculation channel, is about 0.3m deep. In order to prevent sedimentation there is a paddle wheel which is used for mixing and circulating the algal biomass. Flow is guided around bends by baffles placed in the flow channel. Raceway channels are built in compacted earth or concrete, and may be lined with white plastic. During day time, the culture is fed continuously in front of the paddlewheel where the flow begins. Broth is harvested behind the paddlewheel, on completion of the circulation loop. The paddlewheel operates continuously to prevent sedimentation. Raceway ponds have been used since the 1950s for mass culture of microalgae. Open race way pond is a cost effective method of growing microalgae and in commercial raceway ponds, the biomass production is in excess of 0.5g/l (Rawat et al. 2010).The advantage for this method is that municipal waste water can be used as a medium for cultivation with added benefit of bioremediation. If the race pond is located near a power plant, cheaply available flue gas can be used speed up the photosynthetic rates in the pond or pure CO_2 can be bubbled into the pond (Rawat et al. 2010; Tsukahara et al. 2005). Some drawbacks of this method of cultivation are in raceways, temperature fluctuates within a diurnal cycle and seasonally and cooling is achieved only by evaporation, the water loss by evaporation is significant. As there is significant losses to atmosphere, raceways use carbon dioxide less efficiently than photobioreactors. Contamination with unwanted algae and microorganisms that feed on algae will affect the productivity. In raceways the biomass concentration remains low because there are poorly mixed and cannot sustain dark zone. Terry and Raymond (1985) further discussed about raceway ponds and other open culture systems for producing microalgae. Although raceways are low-cost, they have a low biomass productivity compared with photobioreactors.

3.2.2. Photobioreactors (PBRs):

Fully closed photobioreactors provide opportunities for production of monoseptic culture of a greater variety of algae than is possible in open systems. Photobioreactors have been successfully used for producing large scale microalgal biomass (Grima et al. 1999; Tredici 1999; Pulz 2001; Carvalho et al. 2006). Photobioreactors with tubular solar collectors are the most promising (Grima. 1999; Tredici. 1999) for producing algal biomass on a large scale needed for biofuel production.

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 for photosynthesis. 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. Microalage, required nutrients and water are circulated from a reservoir such as feeding vessel to the solar collector and back to the reservoir. It is usually operated as a continuous culture during day light. In continuous culture fresh medium is fed continuously at a constant rate and the same quantity of microalgal broth is removed. PBRs require cooling during the day time. The loss of biomass because of respiration can be reduced by decreasing the temperature at night. To utilize the sunlight to the maximum extent, the tubes of the solar collector are generally placed horizontally flat on the surface. The ground underneath the soar collector are pointed with white sheets of plastic to increase the reflectance (Miron et al. 1999; Chisti et al. 1999; Banerjee et al. 2002), which in turn increase the total light received by the tubes. To prevent sedimentation of biomass, highly turbulent flow is maintained. This flow is produced either using a gentle airlift pump (Garcia et al. 2001, 2007; Mazzuca et al. 2006; Sanchez et al. 2003) or a mechanical pump. The typical biomass produced by a photo bioreactor is nearly 30 times the concentration of biomass produced by raceponds.

3.2.3. Comparison of raceways and tubular photobioreactors:

The microalgal biomass produced from photobioreactor and raceway methods as shown in Table.3. This comparison is for an annual production level of 100 t of biomass in both cases. The amount of carbon dioxide consumed by both methods are almost identical (Table.3). The production methods in Table 3 are compared for optimal combinations of biomass productivity and concentration that have been actually achieved in large-scale photo bioreactors and raceways. The amount of oil produced per hectare was significantly higher in photo bioreactors as compared to race way ponds (Table.3). This is because the volumetric biomass productivity of photo bioreactors is more than 13-fold greater in comparison with raceway ponds (Table.3). Both raceway and photo bioreactor production methods are technically feasible. Production facilities using photo bioreactors and raceway units of dimensions similar to those in Table 3 have indeed been used extensively in commercial operations (Terry and Raymond 1985; Grima 1999; Tredici 1999; Pulz 2001; Lorenz and Cysewski 2003; Spolaore et al. 2006).

Table.3. Comparison of photobioreactor and raceway production methods (Chisti, 2007)

Variable Photobioreactor	**Facility**	**Raceway ponds**
Annual biomass production (kg)	100	100 000
Volumetric productivity (kg m−3 d−1)	1.535	0.117
Areal productivity (kg m−2 d−1)	0.048 [a] 0.072 [c]	0.035 [b]
Biomass concentration in broth (kg m−3)	4.00	0.14
Dilution rate (d−1)	0.384	0.250
Area needed (m2)	5681	7828
Oil yield (m3 ha−1)	136.9 [d] 58.7 [e]	99.4 [d] 42.6 [e]
Annual CO2 consumption (kg)	183,333	183,333
System geometry	132 parallel tubes/unit; 80 m long tubes; 0.06 m tube diameter	978 m2/pond; 12 m wide, 82 m long, 0.30 m deep
Number of units	6	8

a Based on facility area.

b Based on actual pond area.

c Based on projected area of photobioreactor tubes.

d Based on 70% by wt oil in biomass.

e Based on 30% by wt oil in biomass.

Each of these has advantages and disadvantages, but photobioreactors are much more expensive to build than open ponds. Photobioreactors have not been engineered to the extent of other bioreactors in commercial practice, and so there is certainly room for cost reductions. Neither open ponds nor closed photobioreactors are mature technologies. Until large-scale systems have actually been built and can show demonstrated performance over many years of operation, many uncertainties will remain. Cultivation issues for both open and closed systems such as reactor construction materials, mixing, optimal cultivation scale, heating/cooling and CO2 administration have been considered and explored to some degree, but more definitive answers await detailed and expensive scale-up evaluations. Nevertheless, as an important part of the development process, there are algal demonstration projects that are in progress.

4. Processing of Microalgae Lipid

4.1. Microalgae Harvesting

Microalgae cells are very small (typically in the range of Φ2–70 μm) and the cell densities in culture broth are low (usually in the range of 0.3–5 g·L−1). For commercial scale production and processing of microalgae requires, harvesting microalgae from the culture broth and dewatering them, which are energy intensive process and therefore a major obstacle. Various harvesting technologies, such as centrifugation, filtration, flocculation, gravity sedimentation, electrophoresis and floatation techniques have been tested (Uduman et al. 2010). The choice of harvesting technique depends on, the characteristics of microalgae (such as their size and density) and the target products.

4.2. Microalgae Lipid Extraction and Refining

Microalgal intracellular lipids can be extracted by a variety of methods, such as mechanical

crushing extraction, enzymatic extraction, chemical extraction, supercritical carbon dioxide (SCCO2) extraction (Halim et al. 2011), microwave extraction (Koberg et al. 2011), *etc.* Microalgae lipids which are in the form of triglycerides or fatty acids can be converted to biodiesel through transesterification/(esterification for fatty acids) reactions after the extraction (Johnson and Wen 2009). In order to achieve efficient reaction, the choice of catalyst plays a major role. The traditional liquid acid and alkali catalyst are called homogeneous catalysts as they act in the same liquid phase as the reaction mixture. In biodiesel industry, homogenous catalysts are most commonly used because of their simple usage and they take less time for lipids conversion. However, the transesterification catalyzed by homogeneous catalysts needs high purity feedstock and complicated downstream processing (Borges and Diaz 2012), so high efficiency and low pollution catalysts such as solid acid catalysts, enzyme catalyst, solid alkali catalysts, supercritical catalyst systems and ionic liquid catalysts are receiving increasing attention. To catalyze the simultaneous transesterification and esterification of triglycerides and free fatty acids to biodiesel, Krohn *et al.* (2011) studied the catalytic process using supercritical methanol and *porous titania* microspheres in a fixed bed reactor .The process was able to reach conversion efficiencies of up to 85%. Patil *et al.* In 2011 also reported a process involving simultaneous extraction and transesterification of wet algal biomass containing about 90% of water under supercritical methanol conditions.

4.3. Pyrolysis of Microalgae Lipid

Thermochemical conversion includes various processes such as direct combustion, gasification, thermochemical liquefaction, and pyrolysis. In pyrolysis, the reaction rate gets affected by the heating rate which in turn affects the composition of the product. To achieve rapid temperature rise with poor process control, traditional heating methods require expensive heating mechanisms. Microwave assisted pyrolysis (MAP) has various advantages like: fine grinding of biomass is not necessary; microwave heating is mature and scalable technology which is suitable for distributed biomass conversion. Due to insufficient understanding of the mechanism of pyrolysis and the lack of effective control of the pyrolysis process, pyrolytic bio-oils are complex mixture with low calorific value, high acidity, high oxygen volume, and poor stability. However, when compared to cellulosics, bio-oil from pyrolysis of microalgae appears to have higher quality (Du et al. 2011). In recent years, the role of catalyst and minerals in biomass pyrolysis was investigated. Lu *et al.* (2011) reported that corn cob could be catalysed by $ZnCl_2$ by fast pyrolysis method, there by producing the main products furfural and acetic acid. Du *et al.* (2010) used 1-butyl-3-methylimidazolium chloride and 1-butyl-3-methylimidazolium boron tetrafluoride as the catalysts in MAP of straw and sawdust.

5. Major Limiting Factors in Commercialization of Microalgal Biodiesel

Oil crops are potential renewable and carbon neutral source of biodiesel that can replace petroleum fuels. But, it did not find its way to become a top competitor for petroleum fuels because of very limited supply in comparison to global demand as well as high cost. Therefore, biodiesel is bright and attractive hope to both investors and consumers. Because biodiesel from oil crops, waste cooking oil and animal fat cannot fulfill a small fraction of the existing demand for transport fuels, microalgae appears to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels (Hossain et al. 2008). But, at the same time it has to be thought whether algal fuels can be produced in sufficient quantity to genuinely replace petroleum fuels. Restraint to commercialization of algal fuels needs to be understood and addressed for any future commercialization (Chisti 2013). First of all, carbon dioxide availably is major impediment to any significant production of biodiesel (Chisti 2013). Production of each ton of algal biomass requires at least 1.83 tons of carbon dioxide (Chisti 2007). Almost all commercial algae culture set ups buy CO_2 that contributes substantially (~ 50%) to the cost of producing the biomass. Hence, amount of CO_2 available is a major cause which is hampering biodiesel sizeable amount of production and commercialization and algae culture for fuels is not feasible unless carbon dioxide is available free (Chisti 2007). Moreover nutrient supply in the form of nitrogen (N) and phosphorous (P) to grow algal biomass is another hindrance in algal biodiesel commercialization. Although phosphorous is present in ample amount to supply (Cordell et al. 2009; Gilbert 2009). Whatever, existing nitrogen is there, not even sufficient for agricultural food crops and cannot be provided for any significant scale production of algal biomass for fuel production and fixation of environmental nitrogen by the Haber–Bosch process (Travis 1993), requires extensive amount of energy.

In addition to enabling reclamation and reuse of nutrients the biogas produced by anaerobic digestion can be burnt to supply all the electrical power that is needed for production of algal biomass and its separation from the water (Chisti 2008b; Harun et al. 2011). Algae has to be dried for efficient extraction of oil but process of drying is not feasible, as net energy recovery in the oil would be very less or can be even negative. Presently techniques used for anaerobic digestion suits to less salt containing algal biomass only and not to marine microalgal biomass which contains high salt in it. Therefore, researchers have to concentrate to invent some new technology of anaerobic digestion for biogas productions and nutrient recovery for growth of algal biofuel or biodiesel market.

There is another constraint which has to be considered here is supply of freshwater. At present, algal fuel would cost about $8 a gallon. That same gallon would also require 350 gallons of water to be produced (Keune 2012). Therefore, use of marine and seawater algae are the only options for making biofuels, but even cultivation of these does not vanish the requirement of fresh water as it is needed to compensate some evaporative losses that depends on climatic conditions of the particular area. Production of algal biofuels requires inputs of energy obtained from fossil fuels (Chisti 2013). Energy ratio, the ratio of the energy contained in the oil to the fossil energy required for making it, is an important measure that determines whether production of oil is worthwhile (Chisti 2008b). An energy ratio of unity means a nil recovery of energy in the oil. Ideally, an energy ratio of at least 7 is wanted to make algae biodiesel commercial (Chisti 2008b) and that can be possible by the important in the recovery process of N and P fertilizer through anaerobic digestion, through minimizing fresh water and fossil energy input (Sompech et. al. 2012, Wongluang et. al. 2013). Algal biomass production process is the next important strategy; it has to be, economic and easier. On the basis of several available reports, the Algae 2020 study has reported the estimated costs of produce algae oils and algae biodiesel today between $ 9 and $ 25 per gallon in ponds and $ 15 - $ 40 in photo bioreactors (PBRs). Algal oil production has many processes in oil i.e. production, harvesting, extraction and drying systems, reducing the number of processes in algae biofuel production is essential to providing easier, better and lower cost systems. This can be addressed with the advent of cheaper photobioreactors (PBRs), in the next few years (Singh and Gu 2010).

Finally, but importantly the co-production of some more valuable fraction and their marketing is also important for the success. Even with algae species with up to 50% oil content, the additional

50% of the biomass remains. This biomass fractions contains valuable protein for livestock, poutry and fresh feed additives (Singh and Gu 2010). Remaining fractions of algal biomass still contains some important compounds that can be used to produce bio-byproducts (cleaners, detergents, plastic etc). These co-product marketing will be a way to get success of commercialization of algal biodiesel. Manipulation of metabolic pathways through genetic engineering can redirect cellular function towards the synthesis of products and even expand the processing capabilities of microalgae (Singh et al. 2011). The algal micro-refineries can avoid the harvesting, extraction and refining systems by excreting lipids directly from the cells using non-lethal extraction known as milking. Apart from these biofuels like bioethanol, biomethane and other valuable products can be co-generated to make commercialization process a profitable venture. Such methods have the capability to reduce the production cost significantly and future of algal biodiesel will be bright. Interest of consumers and investors in addition to present hour need in commercial production of biodiesel from microalgae is so strong that economically and simplified methods of viable production at large scale will be possible in a certain quantum of time. As Al Darzins, co-author of a Department of energy report on algae's viability, said, "The path of algal biofuels commercialization will not be totally dependent on any one unit operation" or technology but rather on the industry's ability to string together or "integrate robust and scalable technology solutions." It is no surprise that there are many limitations when it comes to algal biofuel production. This sector is still new when compared to the standard fossil fuel production line. However, many of these constraints can be overcome with careful planning and consideration of the needs of the algal cell. There is no doubt that the outdoor pond system is the most ecologically sound system of algae growth at the moment. With time, this system can become the leading method of biofuel production.

6. Conclusion

Microalgae comprise a vast group of photosynthetic, auto-/heterotrophic cell factories that convert CO_2 to potential biofuels, foods, feeds, and high-value additives that are also considered as energy crops. Microalgae offer great potential as a sustainable feedstock for the production of third-generation biofuels, such as biodiesel and bioethanol. Microalgal biodiesel has the potential to replace petroleum, and it is technically feasible and economically competitive. However, several important scientific and technical barriers remain to be overcome before the large-scale production of microalgal-derived biofuels can become commercial reality. Microalgal strain selection, biomass production, harvesting, drying, processing, water sources, nutrient and growth inputs, and advances in engineering of PBRs are important areas that must be optimized. The use of the biorefinery concept and genetically manipulated algal strain for more lipid production, less water requirement, etc., will further make algal biofuel sustainable. The cultivation of algal biomass also saves our environment from air and water pollution and minimizes the waste disposal problems by utilizing wastewater and flue gases for algal growth. The short and simple life cycle, energy balance, biofuel yield per unit area, carbon balance, N and P balance, water availability, land use, and nutrient sources are very important factors to decide the sustainability of algal biofuels including biodiesel. Considerations of these areas may lead enhanced cost-effectiveness and, therefore, successful commercial implementation of the biofuel from microalgal strategy.

7. REFERENCES

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