REVIEW PAPER

Microalgae as second generation biofuel. A review

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Abstract Microalgae are autotrophic microorganisms having extremely high photosynthetic efficiency and are valued as rich source of lipids, hydrocarbons, and other complex oils for biodiesel besides being an invaluable source of bioethanol, biomethane, and biohydrogen. Biodiesel produced from oilseed crops such as jatropha and soy have lower yields per unit land area and threaten food security. Indeed, microalgae have higher oil yields amounting to about 40 times more oil per unit area of land in comparison to terrestrial oilseed crops such as soy and canola. Further, microalgae production does not require arable land for cultivation. Biofuel is regarded as a proven clean energy source and several entrepreneurs are attempting to commercialize this renewable source. Technology for producing and using biofuel has been known for several years and is frequently modified and upgraded. In view of this, a review is presented on microalgae as second generation biofuel. Microalgal farming for biomass production is the biggest challenge and opportunity for the biofuel industry. These are considered to be more efficient in converting solar energy into chemical energy and are amongst the most efficient photosynthetic plants on earth. Microalgae have simple cellular structure, a lipid-rich composition, and a rapid rate of reproduction. Many microalgal strains can be grown in saltwater and other harsh conditions. Some autotrophic microalgae can also be converted to heterotrophic ones to accumulate high quality oils using organic carbon. However, there are several technical challenges that need to be addressed to make microalgal biofuel profitable. The efficiency of microalgal biomass production is highly influenced by environmental conditions, e.g., light of proper intensity and wavelength, temperature, CO_2 concentration, nutrient composition, salinities and mixing conditions, and by the choice of cultivation systems: open versus closed pond systems, photobioreactors. Currently, microalgae for commercial purpose are grown mostly in open circular/elongated "raceway" ponds which generally have low yields and high production costs. However, a hybrid system combining closed photobioreactor and open pond is a better option. The biggest hurdle in commercialization of microalgal biofuel is the high cost and energy requirement for the microalgal biomass production, particularly agitation, harvesting, and drying of biomass. In order to conserve energy and reduce costs, algae are often harvested in a two-step process involving flocculation followed by centrifugation, filtration, or micro-straining to get a solid concentration. However, the major bottlenecks in algal biodiesel production within the cell can be identified and handled by adopting a system approach involving transcriptomics, proteomics, and metabolomics. Research and developments in the field of new materials and advanced designs for cultivation in closed bioreactors, use of waste water for biomass production, screening of efficient strains, high-value coproduct strategy, and cutting-edge metabolic engineering are thought to provide the biggest opportunities to substantially improve the cost effectiveness of such production systems.

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Contents

1 Introduction

Microalgae are photosynthetic cell factories, microscopic in nature, have high surface area–to–volume ratio, and are primary synthesizers of organic matter in aquatic habitats. It comprises a vast group of simple single-celled organisms that occur as discrete individuals alone, in pairs, in clusters, or in sheets of individuals all looking alike; but cannot form roots, stems, or leaves. The most abundant microalgae are single-cell drifters in plankton, generally called phytoplankton. They are capable of rapid uptake of nutrients and CO_2, possess faster cell growth and have much higher photosynthetic efficiency than most of the land–based plants. Since ancient time, microalgae have been used in human health food products (Kim 1990; Khan et al. 2005), feeds for fish and livestock (Duerr et al. 1998; Becker 2007), and have been cultured for their high–value oils (Belarbi et al. 2000; Molina Grima et al. 2003; Wen and Chen 2003; Spolaore et al. 2006), chemicals, pharmaceutical products (Guerin et al. 2003; Pulz and Gross 2004; Spolaore et al. 2006), and pigments (Pulz and Gross 2004; Spolaore et al. 2006). Microalgae can provide diverse forms of renewable biofuels including biomethane (by anaerobic digestion of the algal biomass), biodiesel (from microalgal oil), bioethanol (by fermentation of the microalgal carbohydrates), and photobiologically produced biohydrogen (Akkerman et al. 2002; Banerjee et al. 2002; Gavrilescu and Chisti 2005; Kapdan and Kargi 2006; Ragauskas et al. 2006; Spolaore et al. 2006; Huntley and Redalje 2007; Dismukes et al. 2008).

The term "renewable" in this article refers to bioenergy, i.e., the energy coming from biomass, the supply of which is limited and their availability depends on the primary natural resources (e.g., lands, water, ecosystems, etc.). The word "biofuel" refers to the origin of the energy converted through the metabolism of living organisms with, at the basis of the food chain, autotrophic organisms converting solar energy into chemical energy contained in the molecules they produce via photosynthesis. Biofuels are a wide range of fuels which are in some way derived from biomass. However, biomass, in the energy sector, refers to biological material which can be used as fuel for transport, or an energy source to produce industrial or domestic heat and electricity (feedstock) (Bessou et al. 2010). The concept of using microalgal biomass as a potential source of biofuel is not new, but it is now being taken seriously because of the escalating petroleum prices and more significantly, the emerging concern about global warming and climate change which is more significantly associated with burning fossil fuels (Nagle and Lemke 1990; Sawayama et al. 1995; Gavrilescu and Chisti 2005). A conceptual model for integrated microalgal biomass and biofuel (biomethane, biodiesel, biohydrogen, and biogas) production is shown in Fig. 1.
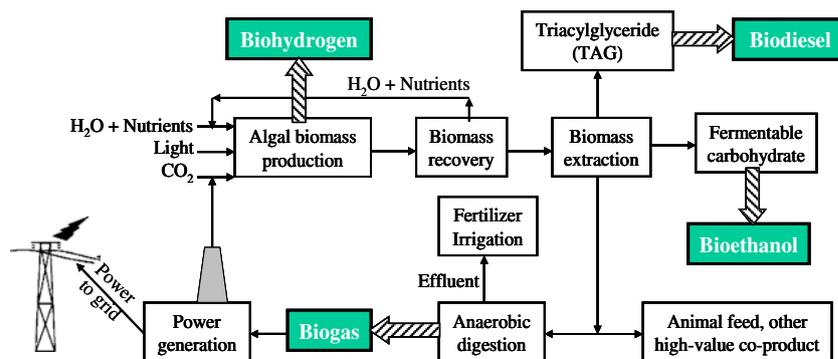
Currently, biodiesel is produced commercially from soybeans, canola oil, palm oil, corn oil, waste cooking oil, and jatropha oil, but not from microalgae (Barnwal and Sharma 2005; Felizardo et al. 2006; Kulkarni and Dalai 2006). The matter of concern is that these sources of biofuel are not sustainable in long term (Patzek and Pimentel 2005) as the sources currently used are either food/feed crops or tree crops. The food crops are not suitable for biofuel because of rising feeding requirements of the booming world population. The crop plants have a comparatively low potential and are usually harvested once or twice a year. The tree crops like Jatropha and Pongamia, presently used in several parts of the world, require 4–5 years to come to fruiting. Moreover, these require much more area for production of the same amount of biofuel as compared to microalgae.

This tendency is likely to change because biodiesel is a proven fuel and several entrepreneurs are attempting to commercialize microalgal biodiesel. Technology for producing and using biodiesel has been known for many years and it is noteworthy to mention that the high quality oil produced by these microalgae can be converted to biofuel using the existing technology (Barnwal and Sharma 2005; Demirbas 2005; Van Gerpen 2005; Felizardo et al. 2006; Kulkarni and Dalai 2006; Meher et al. 2006; Chisti 2007). Biofuels produced from microalgae have the potential to replace a portion of fossil fuel consumption with a renewable alternative. Previous research in the early 1990s by the National Renewable Energy Laboratory showed that under controlled conditions algae are capable of producing 40 times the amount of oil for biodiesel per unit area of land, compared to terrestrial oilseed crops such as soy and canola (Sheehan et al. 1998).

2 Microalgal second generation biofuel systems

A model second-generation biofuel system should have much higher net energy balance, should be more water efficient and should require much less arable land. A range of second-generation microalgae-based biofuel production systems are now under development (Kruse et al. 2005; Hankamer et al. 2007). Microalgae are reported to produce 15–300 times more oil for biodiesel production than traditional crops on area basis (Chisti 2007). Microalgae have a very short harvesting cycle ($\approx$1–10 days depending on the process) allowing multiple or continuous harvests with significantly increased yields (Table 1; Schenk et al. 2008). Higher photosynthetic efficiency ultimately leads to reduced fertilizer and nutrient inputs, thereby, resulting in less waste and pollution.

Microalgal cultivation for biofuel production can potentially be carried out on marginal land and thus, can reduce the competition for land and may open up new economic opportunities for these arid, drought or salinity-affected regions (Schenk et al. 2008). Moreover, good amount of fresh water can be saved if closed bioreactor systems are used for algal cultivation especially using marine and halophilic strains of microalgae. The microalgal biomass generated in bioreactors can also be gasified or pyrolysed to produce a range of biofuels and can act as a complement in CO_2 sequestration strategy (Weissman and Tillett 1992; Zeiler et al. 1995). In this way, transgenic organism's wastes can also be effectively disposed off in an environmentally sensitive way. The carbon-rich biomass pellets can also be stored as part of a carbon sequestration strategy which could utilize CO_2 from power plants as an input for biomass production (Bridgwater and Maniatis 2004).

Other significant aspects of second generation microalgal systems are that they are amenable to highly innovative biotechnology approaches that have the potential for rapid improvements of algal strains and promise increase in yield by metabolic engineering (Sheehan et al. 1998; Ratledge 2004; Mussgnug et al. 2007). Although salt stress has been shown to play a role in oil production, freshwater species can undergo a stress response at osmolarities well below those of seawater, which can be strongly inhibitory or lethal (Vazquez-Duhalt and Arredondo-Vega 1991a, b; Takagi and Karseno 2006; Ranga et al. 2007). Several interesting findings have been obtained regarding osmo-adaptation in microalgae and the genes that exhibit anti-salt activity in microalgae (Kirst

Table 1 Biodiesel production efficiency of plant oils and microalgae

Plant source	Biodiesel (L/ha/year)	Area to produce global oil demand (hectares $\times 10^6$)	Area required as percent global land mass	Area as percent global arable land
Cotton	325	15,002	100.7	756.9
Soybean	446	10,932	73.4	551.6
Mustard seed	572	8,524	57.2	430.1
Sunflower	952	5,121	34.4	258.4
Rapeseed/canola	1,190	4,097	27.5	206.7
Jatropha	1,892	2,577	17.3	130
Oil palm	5,950	819	5.5	41.3
Algae (10 gm^{-2} day^{-1} at 30% triacylglyceride)	12,000	406	2.7	20.5
Algae (50 gm^{-2} day^{-1} at 50% triacylglyceride)	98,500	49	0.3	2.5

1990; Galinski 1995; Tanaka et al. 2007). Often, these genes are generic anti-stress genes that likely act through alleviation of oxidative stress. Moreover, these microalgae exhibit increased photosynthetic efficiency and tolerance to grow in saline or wastewater streams (Aslan and Kapdan 2006; Singh and Dhar 2006, 2007; Shi et al. 2007).

3 Microalgal biofuel history

Renewable energy production from microalgae is not a new concept and has been carried out since last century. Early work on the anaerobic digestion of microalgae for producing methane fuel was carried out by Golueke et al. (1957). Microalgae are known to make far more efficient use of solar energy than conventional agriculture and therefore, there has a larger potential for biomass production with comparatively less land requirement (Benemann 2007). Oswald (1962) and Oswald et al. (1957) conducted experiments and concluded that the average algal biomass productivity was also much higher as compared to wheat. The most extensive research into the development of biofuels from algae was performed by the National Renewable Energy Laboratory (NREL) from 1978 to 1996 (Sheehan et al. 1998). It concluded that a more practical approach for near-term production of algal biodiesel is to utilize wastewater treatment for algae propagation, an already well-developed technology (Sheehan et al. 1998; Oswald 2003). Other analyses of full-scale algae to biodiesel production have been conducted by several workers (Benemann et al. 1982; Weissman and Goebel 1987; Chisti 2007). It is assumed that the economics of algae biodiesel will be achievable with improvements to algal biology through genetic and metabolic engineering, and the use of photobioreactors which can provide a controlled environment (Chisti 2007). On the other hand, some workers

conclude that algae to biodiesel will have a large impact with combination of wastewater treatment in agreement with the NREL study (Benemann et al. 1982).

4 Microalgal biodiesel potential

Microalgae appear to be the only source of biodiesel that has the potential to completely displace fossil diesel, grow extremely rapidly and many are exceedingly rich in oil (Table 1). These commonly double their biomass within 24 h and during exponential growth, biomass doubling time may be as short as 3.5 h. Microalgae with high oil productivities are desired for producing biodiesel and oil levels of 20–50% are quite common (Table 2). Oil content in microalgae can exceed 80% by weight of dry biomass (Metting 1996; Spolaore et al. 2006). However, oil productivity (the mass of oil produced per unit volume of the microalgal broth per day) depends to a large extent on the algal growth rate and the oil content of the biomass.

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). Not all algal oils are satisfactory for making biodiesel, but suitable oils do occur commonly. Moreover, production of algal oils requires an ability to inexpensively produce large quantities of oil-rich microalgal biomass. Potentially, instead of microalgae, oil producing heterotrophic microorganisms grown on a natural organic carbon source such as sugar, can be used to make biodiesel; however, heterotrophic production is not as efficient as using photosynthetic microalgae (Ratledge 1993; Ratledge and Wynn 2002). This is because the renewable organic carbon sources required for growing heterotrophic microorganisms are produced ultimately by photosynthesis, usually in crop plants.

Table 2 Oil content of certain microalgae suitable for biodiesel production

Microalga	Oil content (% dry wt)	Reference
B. braunii	25–75	Chisti 2007
Chlorella emersonii	63	Illman et al. 2000
Chlorella minutissima	57	Illman et al. 2000
C. vulgaris	56.6	Liu et al. 2007
C. vulgaris	40	Illman et al. 2000
C. protothecoides	23	Illman et al. 2000
Chlorella sorokiniana	22	Illman et al. 2000
C. cohnii	20	Chisti 2007
Cylindrotheca sp.	16–37	Chisti 2007
D. primolecta	23	Chisti 2007
Isochrysis sp.	25–33	Chisti 2007
M. salina	>20	Chisti 2007
Monodus subterraneus	39.3	Khozin-Goldberg et al. 2006
Nannochloris sp.	20–35	Chisti 2007
Nannochloropsis sp.	31–68	Chisti 2007
N. oleoabundans	54	Metting 1996
N. oleoabundans	35–54	Chisti 2007
Nitzschia laevis	69.1	Chen et al. 2008b
Nitzschia sp.	45–47	Chisti 2007
Parietochloris incisa	62	Solovchenko et al. 2009
P. tricornutum	20–30	Chisti 2007
Schizochytrium sp.	50–77	Chisti 2007
T. sueica	15–23	Chisti 2007
Chlorella sp.	28–32	Chisti 2007

4.1 Autotrophic microalgae

Autotrophic microalgae can utilize carbon dioxide as the carbon sources and sunlight as the energy source for oil accumulation under special conditions. It has been found that many autotrophic microalgae, such as *Chlorella vulgaris*, *Botryococcus braunii*, *Navicula pelliculosa*, *Scenedesmus acutus*, *Crypthecodinium cohnii*, *Dunaliella primolecta*, *Monallanthus salina*, *Neochloris oleoabundans*, *Phaeodactylum tricornutum*, and *Tetraselmis sueica* can accumulate oils (Liang et al. 2006; Chisti 2007). The microalgal biomass production and oil accumulation is influenced by a large number of factors and therefore, different species of microalgae vary widely in their ability of oil production. Apart from inherent potential of algal species, cultivation parameters, such as temperature, light intensity, pH, salinity, mineral, and nitrogen sources, also influence oil production.

4.2 Heterotrophic microalgae

Some autotrophic microalgae can be converted to heterotrophic ones through changing cultivating conditions or using genetic engineering. Such microalgae also can accumulate high quality oils using organic carbon as the carbon source instead of using CO_2 and sunlight (autotrophic mode of nutrition). For example, *Chlorella protothecoides* normally growing as autotrophic microalgae could use organic carbon sources for oil production and oil content in biomass was about four times than that in the corresponding autotrophic cells (Miao and Wu 2004). Some heterotrophic microalgae like *Prototheca moriformis* could even use cheap organic carbon sources for oil accumulation (Han et al. 2006). Generally, heterotrophic microalgae can easily be cultivated and controlled in normal fermenters. But, these require organic carbon sources for oil accumulation, which may limit the application of such microalgae for oil/biodiesel production to some extent.

5 Environmental conditions for microalgae biomass production

Successful microalgae cultivation requires specific environmental conditions, which vary from species to species. The major parameters influencing biomass production include light of proper intensity and wavelength, temperature, CO_2 concentration, nutrient composition, salinities, and mixing conditions.

5.1 Light

Being the basic energy source for phototrophic algae, the availability and intensity of light is one of the key parameters affecting the success or failure of algae cultures. The net growth of the algae culture at very low light intensities is zero (compensation point) (Lee 1997). With increase in light intensities, photosynthesis increases until a point is reached where the growth rate is the maximum attainable (saturation point) (Goldman 1979; Lee 1999; Richmond 2000). Increasing the light intensity beyond this point does not increase the growth rate and can lead to photo-oxidation, damaging the light receptors and thereby, decreasing the photosynthetic rate and productivity (photo inhibition).

The light-harvesting antennae of algal cells are extremely efficient and absorb all the light striking them even though it cannot all be used for photosynthesis. Thus, at high algal cell concentrations, almost all the available light is absorbed by a thin top layer of cells, leaving the rest in the dark (mutual shading). However, most algae get light saturated at about 20% of solar light intensities (Goldman 1979; Toreillo et al. 2003), hence, while the cells below the surface of the culture may be light limited, the cells in the top layer may face the opposite problems of light saturation and inhibition. The problem can be overcome by reducing the light path length of algae culture system to increase light penetration and increasing cell density to a point at which mutual shading minimizes the photo-inhibition of algal cells. This is also facilitated by proper mixing which ensures that individual cells are not stationed exclusively in the dark or light zones of the culture as well as increasing mass transfer (Weissman and Goebel 1985; Grobbelaar 1994).

5.2 Temperature

Generally, rise in temperature leads to exponential increase in algal growth until an optimum level is reached, after which growth declines. For outdoor cultures and open systems, the ability to control temperatures is often limited and is determined by atmospheric temperature, solar irradiance, and humidity. Ambient temperature fluctuations can result in diurnal temperatures differences of as much as 20°C which can affect the productivity (Olaizola 2000; Borowitzka 2005).

Large water bodies have long response times to air temperatures and may suffer lack of synchronization between air temperature and optimal culture temperatures (Richmond 1987; Borowitzka 2005). Thus, this affects photosynthesis and creates a situation under which photo inhibition may occur during low levels of light intensity and sub–optimal temperatures (Lu and Vonshak 1999;

Vonshak et al. 2001). Generally sustained temperatures above the optimal range may kill algal cells and temperatures below the optimal range may not, except for freezing conditions. Furthermore, biomass loss has been reported to increase at higher temperatures during dark periods (Weissman and Goebel 1985). Therefore, it is necessary to achieve optimal temperatures quickly in the morning and rapidly decreased temperatures after darkness, thereby, maintaining high productivity during the day and minimizing biomass loss at night.

5.3 Gas exchange

The low percentage of CO_2 in the air (0.033%) will quickly limit the growth if, supplementary carbon is not supplied as roughly 45–50% of algal biomass is made of carbon (Doucha et al. 2005; Chae et al. 2006). This CO_2 is generally blended with air in aerated cultures or injected into the algal cultures via gas exchange vessels in photo-bioreactors or sumps in open raceways. Various methods adopted to reduce the losses of expensive CO_2 in open algae cultures include bubbling through air stones, plastic dome exchangers with perforated pipes, injection into deep sumps, trapping the CO_2 under floating gas exchangers, and maintaining high alkalinities in the culture water (Berzin 2005).

If oxygen concentrations exceed saturation level in algae cultures, photo–oxidative damage occurs to the chlorophyll reaction centers inhibiting photosynthesis and reducing productivity (Molina et al. 2001; Pulz 2001; Ugwu et al. 2007). Presence of an interface between the culture and the atmosphere in agitated cultures is not usually a problem as the O_2 concentrations will remain similar to that of ambient air. However, in closed photobioreactors, additional facilities such as gas exchange chambers are required.

5.4 Nutrients

Nutrients supplied to algal cultures must include the inorganic elements that make up the algal cell besides macronutrients, vitamins, and trace elements. While there is limited reports on optimal levels of nutrients required for mass algal cultures, normally the macronutrients required are nitrogen and phosphorus (ratio 16 N:1P) and silicon (Brzezinski 1985; Harrison and Berges 2005). However, to avoid nutrient limitation, nutrients are usually added in excess and widely different ratios are used even when the same alga is cultured (Richmond 1999; Sanchez et al. 1999; Acien Fernandez et al. 2001).

Trace elements such as cobalt, copper, molybdenum, zinc, and nickel are considered effective in hydrogen production (Ramchandran and Mitsui 1984). Many of these metals have shown pronounced enhancement of hydrogen

production which is thought to be due to their involvement in the nitrogenase enzyme activity. For example, *Anabaena variabilis* SPU003 is highly sensitive to copper, cobalt, manganese, zinc, nickel, and iron ions and shows no hydrogen production at concentrations below 10 mM for these ions (Moezelaar et al. 1996). A culture of *Anabaena cylindrica* grown with 5.0 mg of ferric ions/liter produce hydrogen at a rate about twice that of culture with 0.5 mg of ferric ions/liter (Jeffries et al. 1978).

5.5 Contamination

The open pond system has a serious disadvantage of being susceptible to contamination by unwanted species if utmost care is not taken. A new open pond is typically inoculated with the desired strain of microalgae with the aim of initiating growth and dominating the pond flora. However, over time it is likely that some undesired species may inevitably be introduced and can severely reduce yields and even outcompete the inoculated species. If a significant competitor has taken residence in a pond, it is extremely difficult to eradicate (Schenk et al. 2008). However, cultures of microalgae are kept relatively pure by using culture medium that is specific to the organisms (Spolaore et al. 2006). Sustained and reliable cultivation of the desired species in open pond systems can be encouraged by cultivating extremophiles that tolerate and outcompete other species in a particular environment (e.g., high/low pH or salinity). A high bicarbonate concentration allows *Spirulina* to be grown in open ponds with few invasive algae and a high saline environment allows *Dunaliella salina* to be grown in "relative pure cultures" (Anderson 2005). Producers keep *Chlorella* cultures in pure conditions by use of large inoculum doses and by operating in short batches with harvesting done before major contamination can take place (Benemann 2008).

5.6 Mixing

At high algae concentrations, almost all the available light is absorbed only by a thin top layer of cells which can be avoided by proper mixing. Mixing must be sufficient enough to keep the algae cells in suspension and to provide uniform exposure of light to all the cells. Mixing also decreases the boundary layer around cells facilitating the increased uptake and exudation of metabolic products. In photobioreactors, mixing prevents sedimentation of the cells and supports distribution of CO_2 and O_2 (Molina Grima et al. 1999). For CO_2, a partial pressure of at least 0.15 kPa has to be maintained to prevent kinetic CO_2 uptake limitation and a stoichiometric demand of 1.7 g CO_2 g^{-1} biomass has to be provided. This makes supply of CO_2 purified from external flue gas (e.g., from a power plant)

useful (Doucha et al. 2005). The rate of CO_2 fixation in the crude extract of 50 g of thalli of a marine alga (*Codium vermilara*) was estimated to be 39 μmoles/mg chlorophyll^{-1} h and was constant for at least 15 min (Schonfeld et al. 1973). The light intensity required for saturation of photosynthesis in intact thalli of *Codium* when measured in the presence of 10 mm $NaHCO_3$ was found to be 5×10^4 erg cm^{-2} s^{-1}, a value also markedly lower than that required for saturating photosynthesis in intact higher plants (Rabinowitch 1951). While light attenuation inside the reactor is not influenced by mixing, there is a complex interplay between culture mixing and light attenuation as each single algal cell passes through dark and light zones of the reactor in a more or less statistical manner (Barbosa et al. 2003).

6 Culture techniques

The choice of cultivation systems is an important aspect that significantly affects the efficiency and cost effectiveness of a microalgal biofuel production process (Lee 2001; Pulz 2001; Carvalho et al. 2006). A wide variation exists among the microalgal cultivation systems for the production of biomass. The three most widely used culture systems of raceways, photobioreactors, and fermenters have been discussed briefly.

6.1 Open pond culture

Large-scale cultivation of algae and cyanobacteria in outdoor open pond systems is well established (Benemann and Oswald 1996; Borowitzka 2005). Ponds can be excavated and used unlined or lined with impermeable materials or they can be built up with walls. Open ponds are suitable for a small number of algal species that can tolerate extreme environmental conditions to the exclusion of most other species. Such algal species include fast growers such as *Chlorella*, *Spirulina*, and *Dunaliella* which thrive in highly alkaline or saline environments (Chisti 2007).

Current technology envisages four main types of open ponds: (a) unmixed open ponds, (b) raceway ponds, (c) circular ponds, and (d) thin layer, inclined ponds. Unmixed open ponds are generally used for the mass culture of *D. salina*, have low productivities (less than 1 gm^{-2} d^{-1}) and are comparatively unsuitable for the culture of most algal species (Benemann and Oswald 1996; Borowitzka 2005). Whereas, raceway ponds are the most widely used open pond culture systems (Fig. 2; Chisti 2007) and are used frequently for the commercial cultivation of *Spirulina*, *Haematococcus*, and *Dunaliella* (Benemann and Oswald 1996). Circular ponds are mainly used in Asia for the production of *Chlorella*. It usually has provision of a centrally located rotating arm (similar to those used in wastewater treatment) for mixing

and may have productivities ranging between 8.5 gm^{-2} d^{-1} and 21 gm^{-2} d^{-1} (Benemann and Oswald 1996). However, thin layer, inclined ponds consist of slightly inclined shallow trays and may achieve productivities up to 31 gm^{-2} d^{-1} (Doucha and Livansky 2006).

6.2 Photobioreactors

The problems associated with open systems including contamination, uncontrolled environments, evaporation, limited species suitability, low volumetric productivities, and the need for large land area have encouraged the development of closed (or mostly closed) photobioreactors in order to overcome inefficiency of the earlier systems. Several reports exist regarding the design, efficiency and cost-effectiveness of a microalgal biofuel production process (Lee 2001; Chisti 2007). Many closed systems in detail have also been explained by several workers (Carvalho et al. 2006; Schenk et al. 2008). Few open systems have been systematically compared with closed systems over different geographical regions (Lee 2001).

Even though the open pond systems seem to be favored for commercial cultivation of microalgae at present due to their low capital costs, closed systems offer better control over contamination, mass transfer, and other cultivation conditions. The combination of the closed photobioreactor and open pond combines the benefits of the two and has been demonstrated to be effective at a 2-ha scale (Huntley and Redalje 2007). Photobioreactors can be located indoors or outdoors depending upon the light collection and distribution systems and their commercial feasibility. Photobioreactors of diverse designs have been developed and patents have been awarded, e.g., tubular photobioreactors, vertical bubble columns and airlift reactors, combined bubble column and inclined tubular reactors, helical photobioreactors, and flat plate photobior-

eactors (Tredici and Zittelli 1998; Sanchez et al. 1999; Berzin 2005; Ugwu et al. 2005).

6.3 Fermenters

While most algae grow phototrophically, some are capable of heterotrophic growth using organic substrates as the sole carbon and energy sources. This mode of algal cultivation is well established and has several advantages over phototrophic modes of growth (Barclay et al. 1994; Behrens and Kyle 1996). These include, the large existing fermentation technology knowledge base, high degree of process control for consistent reproducible production, elimination of light requirements, independence from weather and climatic conditions, and lower harvesting costs (Barclay et al. 1994; Ceron Garcia et al. 2000; Chen and Chen 2006). However, the heterotrophic cultivation of algae requires sufficient oxygen supply for catabolism of organic substrates (Moo-Young and Blanch 1987; Clark et al. 1995).

Generally, heterotrophic cultivation has been found to increase the total lipid content in algae compared to phototrophically grown cells (Miao and Wu 2006; Li et al. 2007). The heterotrophically grown *Chlorella* cells accumulated lipids to about 55.2% of the cellular dry weight as opposed to 14.6% in phototrophically grown cells (Miao and Wu 2006). Furthermore, heterotrophic cultivation of algae usually (but not always) results in higher yields (Wen and Chen 2000; Yu et al. 2009). For species that can utilize both light energy and chemical substrates, mixotrophic mode of cultivation offers a superior alternative to phototrophic and heterotrophic growth as both biomass and productivity increases have been reported (Fang et al. 2004; Yu et al. 2009). Productivities as high as 127 gm^{-2} d^{-1} (daytime) and 79 gm^{-2} d^{-1} (night time) have been reported for mixotrophic cultures of *Chlorella* in comparison to 35.8 gm^{-1} d^{-1}41.4 g m^{-1} d^{-1} obtained in photosynthetic cultures (Borowitzka 1992; Lee and Low 1992).

7 Harvesting of microalgal biomass

Biomass harvesting cost can be a significant proportion of the total algal production cost and range between 3.3 and 30% (Gudin and Therpenier 1986; Nakamura et al. 2005). This is because obtaining the algae biomass from the relatively dilute culture broths requires processing large volumes of water. In order to conserve energy and reduce costs, algae are often harvested in a two-step process. In the first step, the algae are concentrated, often by flocculation, which concentrates the dilute cultures to about 1–5% solids. In the second step, the cells are further concentrated by centrifugation, filtration, or micro-straining to get a solid concentration up to 25% (Golueke and Oswald 1965; Shelef et al. 1984).

Fig. 2 Arial view of a raceway pond

7.1 Flocculation

Microalgae carry negative cell surface charges which when neutralized lead to the agglomeration of the biomass into large clumps or "flocs". These flocs can then be more readily separated from the culture medium (Tilton et al. 1972; Benemann and Oswald 1996). Flocculation can be induced in various ways like, chemical flocculation (inorganic chemicals), chemical flocculation (polyelectrolytes), bioflocculation, electroflocculation, and dissolved air floatation. Inorganic flocculants like $Al_2(SO4)_3$ (alum), $Fe_2(SO4)_3$ (ferric sulfate), $FeCl_3$ (ferric chloride), or $Ca(OH)_2$ (lime) neutralize or reduce the negative surface charge of the cells causing the formation of flocs (Golueke and Oswald 1965; Sheehan et al. 1998; Heasman et al. 2000). However, it was concluded that chemical flocculation was too expensive for biofuels production and incorporation of large amount of metal salts in the harvested biomass limits its use and creates disposal problems (Sheehan et al. 1998; Molina Grima et al. 2003). Polymeric organic flocculants (polyelectrolytes) on the other hand are highly charged organic aggregates, non-toxic, required in small amounts, produce more stable flocs and thus, are more attractive flocculation option (Barclay et al. 1985; Sheehan et al. 1998).

Some algal species are reported to naturally flocculate after transfer to settling ponds when left quiescent for some time (Nakamura et al. 2005). This occurrence has been attributed to environmental stimuli, some of which have been identified, including nitrogen limitation, pH, and dissolved oxygen level (Lee et al. 1998; Blanchemain and Grizeau 1999). Electroflocculation is a coagulation/flocculation process which is based on the movement of electrically charged particles in an electric field in which active coagulant species are produced by oxidation of a metal anode (Poelman et al. 1997). Dissolved air floatation involves pressurizing some of the liquid to dissolve additional air (Levin et al. 1961; Borodyanski and Konstantinov 2003).

7.2 Centrifugation

This is a well–established industrial process that uses gravitational force to achieve separation. The morphology and sizes of the cells being harvested affect the recovery (and costs) as filamentous cells and large colonial cells will settle more readily than single smaller cells (Nakamura et al. 2005). Centrifugation is energy intensive and the estimates of the energy consumption required for various types of centrifuges are estimated to range from 0.3 to 8 $kWh.m^3$ (Molina Grima et al. 2003). The high capital and running costs associated with centrifuges limit their use to second–stage filtration in the processing of microalgae for biofuels.

7.3 Filtration

Filtration involves introducing the particles onto a screen of given aperture size. The particles either pass through or are retained on the screen according to their size. Filtration can be performed under pressure or vacuum with energy requirement estimates ranging from 0.2 to 0.88 $kWh\ m^3$ and 0.1–5.9 $kWh\ m^3$, respectively (Shelef et al. 1984; Molina Grima et al. 2003). Filtration can also be carried out by microstrainers consisting of a rotating drum covered by a straining fabric where a backwash spray collects the particles into an axial trough and requiring lower power consumption between 0.02 and 0.2 $kWh\ m^3$ (Benemann et al. 1977; Shelef et al. 1984). Although, the costs associated with filtration are low, screen clogging and membrane fouling limits its suitability to larger species of microalgae.

8 Microalgal biomass to biofuels

For conversion of microalgal biomass to biofuel, numerous options exist. Approaches like open ponds, raceways, or photobioreactors have been adopted by a large number of companies and research organizations for large-scale production of microalgal biomass. Although production of biodiesel and the use of flue gas is the main approach chosen so far; bioethanol, biomethane, and biohydrogen are also the important bioenergies obtained from microalgae.

8.1 Biodiesel

All microalgal oils are not satisfactory for biodiesel production but suitable oils occur commonly and are known to produce many different kinds of lipids, hydrocarbons, and other complex oils (Banerjee et al. 2002; Guschina and Harwood 2006). On the basis of chemical characteristics, lipids are subdivided in two main classes, polar and nonpolar/neutral/simple lipids (Christie 2003). Neutral lipids include the tri-, di-, and monoglycerides; waxes; and isoprenoid-type lipids. However, polar lipids include phospholipids (e.g., phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine) and glycolipids (combinations of oligosaccharides and lipids; e.g., monogalactosyl diglyceride), esters of fatty acids, and glycerol. This distinction in the main lipid classes is important for the subsequent conversion of microalgal oils to biofuels, as the composition of the lipid feedstock affects the efficiency and yield of fuel conversion by catalytic routes.

To obtain microalgal oil, the algae are first concentrated and the oil is extracted, using an organic solvent (90% oil

recovery) or a filter press (75% oil recovery) (Nagle and Lemke 1989). The residue contains starch and proteins and can be further processed to make ethanol, animal feed, or used as a feedstock in an anaerobic fermenter. Biodiesel production is a transesterification process involving transformation of highly viscous oils into alkyl esters with lower viscosity, similar to normal diesel fuel and glycerine (Fig. 3). Transesterification requires 3 mol of alcohol for each mole of triglyceride to produce 1 mol of glycerol and 3 mol of methyl esters. This reaction is reversible in nature and arrives at equilibrium at a certain stage (Fukuda et al. 2001).

The relative proportion of polar lipids to neutral lipids (triglycerides) and the high amount of long-chain polyunsaturated fatty acids (greater than C18) are common in microalgae but are not produced in significant quantities in higher plants. Both of these aspects affect the efficiency of biodiesel synthesis, as well as influence the fuel properties (e.g., very low oxidative stability of highly unsaturated fatty acids). In general, it appears that algal cells synthesize triglycerides at times when the energy input, through carbon assimilation, exceeds the immediate metabolic needs of the cell. The relative composition of microalgal lipids depends greatly on the species used and the nutrient, environmental, and developmental conditions in which the cells are cultured and harvested. It has been reported that the composition of microalgal lipids varies considerably with the growth cycle, under nutrient limitation and during a diurnal light dark cycle (Sukenik and Carmeli 1990; Ekman et al. 2007). For example, *Nannochloropsis* sp. accumulate triglycerides during the day and subsequently rapidly mobilize it in the dark to supply the energy needed for cell division (Sukenik et al. 1989). Triglycerides may serve as a sink for free fatty acids, to remove these from the cytoplasm and thereby avoiding lipotoxicity; they may act as energy and electron sinks during stress conditions (Roessler 1990; Kurat et al. 2006; Hu et al. 2008).

The physico-chemical properties of biodiesel (alkyl esters of fatty acid constituents of lipids) are largely determined by the structure of the constituent acyl chains (Knothe 2005). The most important fuel characteristics, according to the American Society for Testing and Materials (D6751-09 biodiesel standard), are ignition quality, cold-flow properties, and oxidative stability. Polyunsaturated fatty acids are more prone to oxidation and biodiesel made from these oils are not likely to meet the
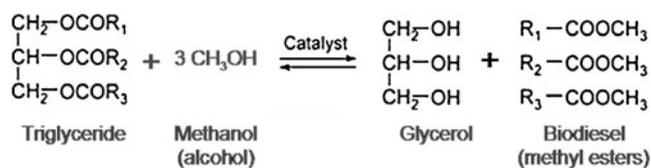
international standards (Chisti 2007). Despite this, biodiesel produced from these oils has a lower melting point and thus, possess excellent cold flow properties compared to biodiesel from saturated fats (which tends to gel at ambient temperatures) (Hu et al. 2008; Schenk et al. 2008). To meet the biodiesel fuel quality standards, the extent of unsaturation in algal oils can be reduced by partial catalytic hydrogenation (Dijkstra 2006; Chisti 2007).

8.2 Bioethanol

The starch content of algae is reported to reach much higher values which are comparable to corn, wheat and other conventional ethanol feed stocks (Feinberg 1984; S and T 2003). Microalgae have high potentiality for bioethanol production; which may be because of their high rate of productivity, high fermentable carbohydrates content of the biomass and lack of lignin in comparison to the terrestrial feedstocks (Table 3; Moore 2009). The potential of microalgae can be judged by the fact that 75% of algal complex carbohydrates can be hydrolyzed into a fermentable hexose monomer and fermentation yield of ethanol is 80% of the theoretical value (Huntley and Redalje 2007). However, lignin, a recalcitrant substance (not easily degraded) present in the biomass, cannot be converted to bioethanol and its processing is a major impediment for bioethanol production (Ragauskas et al. 2006).

After oil extraction from the microalgal biomass, a fermentation process utilizing enzymes alpha–amylase and gluco–amylase and yeast for fermenting the sugars to ethanol and carbon dioxide has been devised (Dismukes et al. 2008). The fermented mash so resulting contains about 11–15% ethanol by volume as well as the non–fermentable solids from the algae and the yeast cells. Ethanol is distilled off the mash at 96% strength, while the residual stillage can be recovered from the base of the column and dried to obtain dried distiller's grain. Another interesting method of microalgal bioethanol production is demonstrated under dark and anaerobic conditions in which the starch within the cells of microalgae was fermented (Hirano et al. 1997). This essentially means that ethanol can be made within the microalgal cells. The potential for simple and low cost methods of bioethanol production are apparent. The next phase of this research should try to develop methods of improving the efficiency of this intracellular ethanol production.

8.3 Biomethane

Biogas production from microalgal biomass is a focus of interest as the efficiency of biomass production/ha is estimated to reach five to 30 times than that of crop plants and a major part of the methane demand can be satisfied

Fig. 3 Transesterification of triglyceride (oil) to biodiesel (methyl esters)

Table 3 Differences in productivity, fermentable carbohydrate and lignin content in some bioethanol feed stocks (Moore 2009)

Feedstock	Productivity (dry Mg/ha/year)	% Fermentable carbohydrate	% Lignin	Fermentable carbohydrate productivity (dry Mg/ha/year)	Lignin productivity (dry Mg/ha/year)
Woody biomass	10–22[a]	70–85[f]	25–35[f]	7–18.7	4–7.7
Switch grass	3.6–15[b]	76.4[f]	12[f]	2.8–11.5	0.4–1.8
Corn	6–14	80–92[f,g]	15[f]	5.6	1.05
Chlorella sp	127.8–262.8[e]	33.4[c]	0[c]	42.7–87.8	0
T. sueica	38–139.4[d]	11–47[b]	0[b]	4.2–65.5	0
Arthrospira sp	27–70[b]	15–50[b]	0[2]	4.1–35	0

[a] Ragauskas et al. 2006

[b] Dismukes et al. 2008

[c] Kristensen 1990

[d] Zittelli et al. 2006

[e] Chisti 2007

[f] Sanchez 2009

[g] Zhu et al. 2007

with biogas (Sheehan et al. 1998). One of the first feasibility study using microalgae for biomethane production was published almost 50 years ago which concluded that the process could be feasible and could further be optimized (Golueke and Oswald 1959). Successful cultivation of microalgae requires huge quantities of nitrogen and phosphate for which environmental and economic impact may not be sustainable. It is therefore desirable to recycle the nitrogen and phosphorus contained in the microalgal waste after lipid extraction in order to reduce the use of fertilizers (Sialve et al. 2009). Such problem can effectively be handled by anaerobic digestion involving biotechnological approaches, which can mineralise algal waste containing organic nitrogen and phosphorus, resulting in a flux of ammonium and phosphate that can then be used again as a substrate for the microalgae (Olguin 2000; Phang et al. 2000). Chisti (2007) has also proposed that in order to reach an economical balance, the biomass obtained after lipid extraction needs to be transformed into methane. Therefore, algal waste after being processed by anaerobic digestion will not only recycle nitrogen and phosphorus but also produce methane.

The energetic value of the methane thus produced can potentially lead to an energetic balance of the microalgae to biofuel process (Sialve et al. 2009). Anaerobic degradation of phytoplanktonic cells is a process which takes place naturally in aquatic environments after algal cells sink in the anoxic and aphotic zones where they eventually die and break up. Nutrient remineralisation in these anoxic layers of aquatic environments is responsible for ammonium and phosphate release and hence recycling such important

nutritive elements, which can eventually sustain growth of phytoplanktonic communities (Vandenbroucke and Largeau 2007).

For biomethane production, either a multispecific biomass is harvested from a wastewater treatment pond, or a monospecific biomass is grown in the laboratory (Asinari Di San Marzano et al. 1982; Chen 1987; Yen and Brune 2007). A significant enhancement of the methane production has been reported with addition of waste paper to algal sludge feedstock (Yen and Brune 2007). They reported a maximum methane production rate of 1.61 $LL^{-1} d^{-1}$ under mesophilic condition when the loading rate was 5 g VS $L^{-1} d^{-1}$ when algal sludge was mixed with 60% of waste paper. Indeed, it was an indication of the increased cellulase activity stimulated by the specific nature of the waste paper. It appeared that the addition of waste paper had a positive effect on the digestion of algal cell walls and therefore on the anaerobic digestion itself. Moreover, codigestion led to the dilution of certain toxic compounds maintaining them under their toxic threshold. Theoretically, the higher the lipid content of the cell, the higher the potential methane yield. The high energetic content of lipids makes microalgae attractive substrates for anaerobic digestion due to their higher gas production potential compared with carbohydrates and proteins (Li et al. 2002; Cirne et al. 2007).

The hydraulic and solid retention time are key parameters in anaerobic processes. The hydraulic and solid retention time should be high enough to allow the active populations to remain in the reactor, especially methanogens, and not to limit hydrolysis which is generally

regarded as the rate-limiting step in the overall conversion of complex substrates to methane. In the case of slowly degradable complex organic pollutants, hydraulic retention time is a deciding factor (Speece 1996). The methane yield remains maximal and constant when loading rate is low and hydraulic retention time is high. Moreover, optimal loading rates and hydraulic retention times must be chosen carefully for efficient conversion of organic matter, depending on the type/composition of the algal substrate. It is because, accessibility of the microalgal intracellular content to the anaerobic microflora is limited by the resistance of the microalgal cell wall to hydrolysis when the cells are directly injected into the anaerobic process (Asinari Di San Marzano et al. 1982; Chen 1987).

Regardless of the operating conditions and species, the proportion of methane in the biogas produced for the majority of the studies falls in the range 69–75%. The quality of conversion of the microalgal organic matter into methane is also satisfactory. However, pH has been reported to be the most important factor impacting CH_4 proportion in the biogas from algal biomass as it controls the speciation of the carbonate system and the release of CO_2. At high pH, due to high alkalinity from NH_3 release, the gas content will shift more to CH_4. The biogas quality is also affected by the oxidation state of the biomass, which drives the proportion of released methane (Sialve et al. 2009). However, due to less content of sulphurated amino acids, the microalgal biomass digestion releases a lower amount of hydrogen sulfide than other types of organic substrates (Becker 1988).

Anaerobic digestion of wastewater is a well-known technology and is widely used in agro-industrial processes such as dairy lagoons. The traditional wastewater treatment ponds can be modified by creating a controlled anaerobic environment such as a covered pond or tank which allows the methane to be captured and combusted for energy production. In the biochemical process of anaerobic digestion, the waste goes through three steps namely hydrolysis, fermentation and methanogenesis as it is converted to methane (Metcalf and Eddy 2003). Anaerobic digestion is a very effective process for biological oxygen demand removal but it is not an effective way to remove nutrients. Thus, there is a need for further treatment of the effluent from anaerobic digesters before it can be discharged into the environment. The relatively high lipid, starch, and protein contents and the absence of lignin make microalgae an ideal candidate for efficient biomethane production by fermentation in biogas plants. Similar to biodiesel, lipids play an important role since their conversion capacity into biomethane is higher (1,390 L biogas kg^{-1} organic dry substance) than that of proteins and carbohydrates (VDI 2004). Using highly efficient closed algal bioreactors for biomass feedstock and bio-

methane production, microalgae can now be grown in large amounts (150–300 t/ha/year; Pulz and Gross 2004; Chisti 2007). This quantity of biomass can theoretically yield 0.2–0.4 million m^3 of methane/ha/year.

High protein and sodium (marine species) content are other significant factors that have the potentiality to affect the methane yield and productivity. High nitrogen concentrations in the microalgal biomass lead to the release of a significant amount of ammonia during anaerobic digestion, which accumulates in the liquid phase. At high biomass concentration in the influent; NH_3 concentrations and the resultant alkalinity will increase and, as a consequence, inhibition of fermentation by free ammonia may occur (Mc Carty 1964). Samson and LeDuy (1986) reported that anaerobic digestion of Spirulina maxima (containing about 60% proteins on dry weight basis) releases an extremely high concentration of ammonia (up to 7,000 mg/L). The methanogenic bacteria are probably among the most sensitive to high NH_3 concentration (Angelidaki and Ahring 1993). The unionized hydrophobic form of nitrogen diffuses passively across the cell membranes where, it shows its toxicity and therefore inhibits the anaerobic microflora. This inhibition is affected by factors such as acclimation period, nature of substrate and inoculum, operating conditions, high ammonia concentration (1.7–14 gL^{-1}), and thermophilic condition. However, high concentrations of Na^+, Ca^{2+}, and Mg^{2+} increase the alkalinity and decrease the fraction of unionized NH_3 and thus can lower the inhibition effects. Methanogenic bacteria also have the tendency to acclimatize to high ammonium concentration and therefore may increase the level of toxicity threshold (Chen et al. 2008a).

The anaerobic microorganisms require sodium only in trace quantity (0.002–0.004 M) for their metabolism and are strongly inhibited at sodium concentration above 0.14 M (Mc Carty 1964; Kugelman and McCarty 1965; Rinzema et al. 1988). Marine microalgae have been reported to require a culture medium with high sodium chloride (0.5–1 M). However, it is also reported that NaCl has no particular effect up to 0.3 M, at 0.4 M the methane production becomes affected and it causes toxicity only above 0.5 M (Chen 1987). Moreover, it is feasible to use salt-adapted microorganisms capable of withstanding high salinities through adaptation of the sludge to high salt concentrations (Chen et al. 2008a). There are sufficient evidences suggesting no inhibition of saline waste anaerobic digestion at concentrations close to marine water (Asinari Di San Marzano et al. 1982; Omil et al. 1995). Additionally, sodium turns out to be less inhibitory in mesophilic as compared to the thermophilic conditions. The potential toxicity of sodium is also affected by the presence and concentration of various cations (Ca^{2+}, Mg^{2+}, and K^+) (Chen et al. 2008a). However, the knowledge of the

biological processes taking place in a biogas production facility is limited and therefore, more research in this field is needed to improve the biomethane production process.

8.4 Biohydrogen

A large number of unicellular, filamentous, fresh water, and marine cyanobacterial species and strains have been reported to produce large quantity of hydrogen (Fig. 4; Table 4). *Gloeocapsa alpicola*, *A. variabilis*, *Anabaena azollae*, *Arthrospira* (*Spirulina*) *platensis*, *A. cylindrica*, *Cyanothece*, *Nostoc muscorum*, etc. has been reported to produce a high quantity of hydrogen gas (Jeffries et al. 1978; Aoyama et al. 1997; Antal and Lindblad 2005). *Anabaena* sp. is reported to produce extraordinarily significant amount of hydrogen. Among them nitrogen-starved cells of *A. cylindrica* produces highest amount of hydrogen (30 ml H_2/lit/h; Margheri et al. 1990).

These cyanobacterial strains use two sets of enzymes to generate hydrogen gas. The first one is nitrogenase and it is found in the heterocysts of filamentous cyanobacteria when they grow under nitrogen-limiting conditions. Hydrogen is produced as a by-product of fixation of nitrogen into ammonia. The reaction consumes 16ATP for fixation of 1 mol of N_2 and results into formation of 1 mol of hydrogen gas.

The other hydrogen-metabolizing/producing enzymes in cyanobacteria are hydrogenases, which occur as two distinct types in different cyanobacterial species. The first one is uptake hydrogenase (encoded by *hupSL*) which has the ability to oxidize hydrogen in a reaction known as oxyhydrogenation or Knallgas reaction whereas, the other type of hydrogenase is reversible or bidirectional hydrogenase (encoded by *hoxFUYH*) having capacity to either take up or produce hydrogen (Schmitz et al. 1995; Tamagnini et al. 2002). Hydrogen is an important fuel and is widely applied in fuel cells, liquefaction of coal, upgrading of heavy oils, and several other operation. Hydrogen can be produced biologically by a variety of means, including the steam reformation of bio oils, dark and photo fermentation of organic materials, and photolysis of water catalyzed by special microalgal species (Kapdan and Kargi 2006; Ran et al. 2006; Wang et al. 2007).

9 Enhancing cost-effectiveness of microalgal biofuels

The biggest impediment in commercialization of microalgal biofuel is the high cost and energy requirement for the mass production of microalgae. Each and every part of the technology used during agitation, harvesting, and drying of biomass should be made more perfect so as to prevent excessive power expenditure. Moreover, efficient strain selection, metabolic engineering, utilization of waste water

for biomass production, and high value coproduct strategy should be taken into consideration.

9.1 Efficient strain selection and microalgal response to starvation

Out of several different renewable fuels produced from microalgae, the desired end product is occasionally the primary factor influencing the choice of species and selection of strain. Other factors that should be considered during species selection include growth rate, optimal temperature range, lipid accumulation, and response to nutrient deprivation; as these factors affect the performance and productivity of the algae in the proposed culture system. Normally, lipid production of most oleaginous algae from various taxa is about 25% of the dry weight (Ben-Amote et al. 1985; Hu et al. 2008). However, under stress of nutrient deprivation, the cell division stops while the cells continue to accumulate storage products at about the same rate. This result in the accumulation of neutral lipids (including hydrocarbons) and increase in the percentage of lipids to around 35–45% (Benemann and Oswald 1996; Sheehan et al. 1998).

The responses of different algae (in terms of their storage product) to nutrient deprivation are variable; therefore, this method can be adapted for the generation of desired storage product (Opute 1974). For instance, while *B. braunii* increased its lipid content from 46% to 54% under nitrogen starvation, *D. salina* decreased its lipid content from 25% to 9% and instead increased carbohydrates from 16% to 56% (Ben-Amote et al. 1985). It was also reported that the limitation of nitrogen could increase the oil content in all *Chlorella* strains (Illman et al. 2000). After extensive study, Fe_3^+ concentration and its addition time was concluded to affect oil accumulation to some extent (Li et al. 2007; Liu et al. 2007). However, high light intensity, nutrient stress, and nitrogen deficiency are considered to be the key factors for satisfactory oil accumulation by microalgae (Solovchenko et al. 2009).

This of course, requires rigorous screening for identification and selection of strains producing and accumulating the desired end product. A phased approach was suggested, whereby, the algae are grown under non–limiting conditions in the first phase, followed by culture under nitrogen–limiting (not starvation) conditions in the second phase (Benemann and Oswald 1996). This imposes a growth rate limit on the algae and enables the algae to accumulate nutrients without the resultant decrease in biomass that nitrogen–deficient cultures face.

9.2 Metabolic engineering

Metabolomics aims to determine metabolic profiles to define the metabolome of a given lipid-rich algae. Metabo-
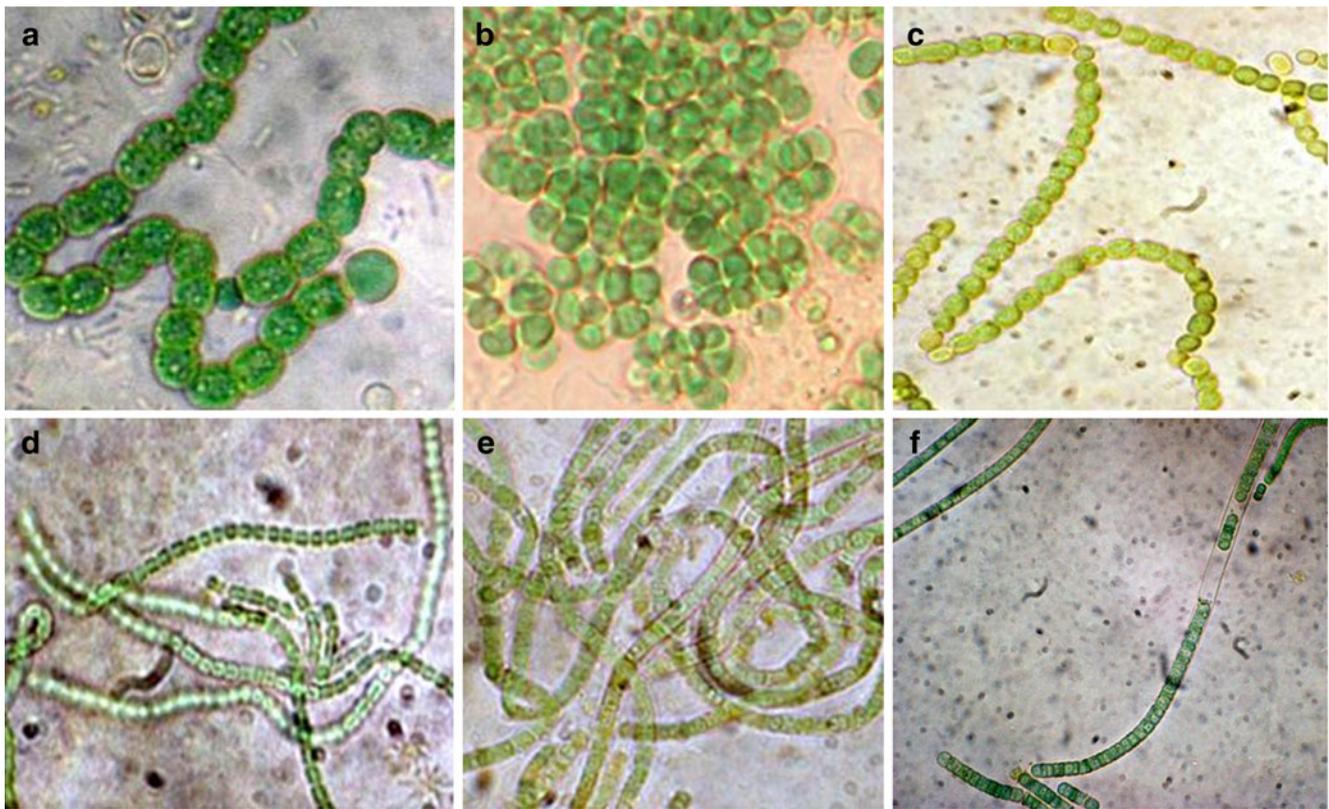
Fig. 4 Potential biohydrogen producing strains. **a** *Anabaena*, **b** *Gloeocapsa*, **c** *Nostoc commune*, **d** *Microcystis*, **e** *Phormidium*, **f**. *Oscillatoria*

nomics on the other hand helps in the statistical identification of differences in metabolite levels due to genetic or environmental changes and allows determination of accumulated metabolic intermediates and end products (Fiehn 2002; Nicholson et al. 2002; Lindon et al. 2004). To resolve the metabolic dynamics of microalgae, the metabolic flux can be studied by various techniques such as the monitoring of consumption and production of key compounds or the isotopic labeling of key metabolite precursors or intermediates and the monitoring of these isotopes in a time-dependent manner (Yang et al. 2000; Fernie et al. 2005; Dong et al. 2006). Moreover, transcriptomics and proteomics offer possibility of identifying differentially expressed genes and proteins that are either directly involved in lipid biosynthesis and degradation or that are co-ordinately regulated (Anderson et al. 2004; McGrath et al. 2005; Dombrecht et al. 2007). The interpretation of these results alone is not straightforward as the accumulation can be due to an upregulated enzyme downstream or a down-regulated enzyme upstream in the metabolic pathway. The identification of differentially expressed genes, proteins or metabolites gives clues to rate-limiting processes in the cell which can be backed up by the determination of metabolic flux. The system biology approach will allow fine-tuning of algal properties by genetic or metabolic engineering (Mus et al. 2007).

9.2.1 Genetic engineering to improve algal productivity

After identification of the pathways and key enzymes involved, genetic engineering has the potential to improve algal productivity. Routine transformation is currently carried out only for a few selected algal model species including *Chlamydomonas reinhardtii*, however, the growing field of transgenic microalgae has considerable potential (Leon-Banares et al. 2004; Walker et al. 2005). Different transformation methods are available for the delivery of DNA into the algal genome with the "biolistic" technique being the most common one (Walker et al. 2005). This technique involves bombardment with DNA-coated micro-projectiles and has been successfully used for a variety of algae including green algae and diatoms and is also the method of choice for chloroplast or mitochondrial genome transformation (Apt et al. 1996; Remacle et al. 2006; Kroth 2007). Other methods to create transgenic algae are the agitation of cells in the presence of glass beads and DNA, agitation with silicon-carbide whiskers and electroporation (Kindle 1990; Dunahay et al. 1997; Sun et al. 2005). More recent developments for the improved overexpression of transgenes involve the use of vectors containing nuclear matrix attachment regions to increase the expression level of foreign genes which has been carried out in the halotolerant alga *D. salina* (Wang et al. 2007).

Table 4 Biohydrogen produced by selected cyanobacteria and their characteristics

Organism description	Organisms name	Maximum H_2 production (μmol/mg chl a/h)	References
Heterocystous filamentous cyanobacteria	A. cylindrica	0.91–2.1	Masukawa et al. 2001
	A. variabilis IAMM-58	4.2	Masukawa et al. 2001
	A. variabilis	0.05–167.6	Fedorov et al. 2001; Happe et al. 2000; Markov et al. 1995; Moezelaar et al. 1996; Sveshnikov et al. 1997; Tsygankov et al. 1998
	Anabaena flos-aquae UTEX 1444	1.7–3.2	Masukawa et al. 2001
	A. azollae	38.5	Sveshnikov et al. 1997
	A. cylindrica B-629	0.103[a]	Lambert and Smith 1977
	Anabaenopsis circularis IAM M-13	0.31	Masukawa et al. 2001
	Anabaena sp. PCC 7120	2.6	Masukawa et al. 2001
	Calothrix scopulorum 1410/5	0.128[a]	Lambert and Smith 1977
	Calothrix membrnacea B-379	0.108[a]	Lambert and Smith 1977
	N. muscorum IAM M-14	0.60	Masukawa et al. 2001
	Nostoc linckia IAM M-30	0.17	Masukawa et al. 2001
	Nostoc commune IAM M-13	0.25	Masukawa et al. 2001
Non-heterocystous filamentous cyanobacteria	Oscillatoria sp. Miami BG7	0.250[a]	Phlips and Mitsui 1983
	Oscillatoria limosa	0.83	Heyer et al. 1989
	Oscillatoria brevis B-1567	0.168[a]	Lambert and Smith 1977
Non-heterocystous unicellular cyanobacteria	Aphanocapsa montana	0.40	Howarth and Codd 1985
	Chroococcidiopsis thermalis	0.7	Serebryakova et al. 2000
	Gloebacter PCC 7421	1.38	Moezelaar et al. 1996
	G. alpicola CALU 743	0.58[c]	Antal and Lindblad 2005
	Microcystis PCC 7820	0.16	Moezelaar et al. 1996
	Mycrocystis PCC 7806	11.3[b]	Moezelaar and Stal 1994
	Synechococcus sp.	0.02–0.66	Howarth and Codd 1985
	Synechocystis sp.	0.07–0.13	Howarth and Codd 1985
	Cyanothece 7822	0.92	Van der Oost et al. 1989

[a] Values expressed in μmol/mg dry wt/h

[b] Values expressed in nmol/mg prot/h

[c] Value expressed in μmol/mg protein

9.2.2 Biosynthesis of quality lipids

The algal lipids biosynthesis requires acetyl-CoA as the starting point. Acetyl CoA carboxylase and other enzymes of the lipid biosynthesis pathway have been used as targets for improving oil production (Ratledge 2004; Richmond 2004). Lipid metabolism and the biosynthesis of fatty acids, glycerolipids, sterols, hydrocarbons, and ether lipids in eukaryotic algae have been studied in the context of optimization for biodiesel production (Metzger and Largeau 2005; Guschina and Harwood 2006). While C. reinhardtii serves as a model organism to study lipid biosynthesis in green algae, some unusual hydrocarbons and ether lipids (n-alkadienes, trienes, triterpenoid botryococcenes, methylated squalenes, and tetraterpenoids lycopadiene) from B. braunii have also been described (Achitouv et al. 2004; Metzger and Largeau 2005; Riekhof et al. 2005). Genetic engineering of key enzymes in specific fatty acid production pathways within lipid biosynthesis is a promising target for the improvement of both quantity and quality (chain length and saturation grade) of lipids.

9.2.3 Increasing the proportion of monounsaturated lipids

Microalgal lipids are predominantly polyunsaturated and therefore, are more prone to oxidation which is a serious issue with biodiesel while in storage. This drawback, however, can be corrected through partial catalytic hydrogenation of the oil (Chisti 2007). It is therefore, preferable that the level of polyunsaturated fatty acids in biodiesel is

Table 5 Some high-value bio-products extracted from microalgae

Product group	Applications	Examples/producer	References
Phycobiliproteins carotenoids	Pigments, cosmetics, pro vitamins, pigmentation	Phycocyanin (*Spirulina platensis*)	Furuki et al. 2003; Spolaore et al. 2006
		Phycocyanin, phycoerythrin (*Porphyridium cruentum*)	Borowitzka 1997; Roman et al. 2002; Servel et al. 1994
		C-phycocyanin-hepatoprotective effect (*Spirulina platensis*)	Bhat and Madyastha 2000; Vadiraja et al. 1998
		β-carotene (*D. salina*)	Borowitzka 1991; Ben-Amotz and Avron 1990; Masjuk 1973; Olaizola 2003
		Astaxanthin, leutin, zeaxantin and canthaxantin (*Haematococcus pluvialis*)	Borowitzka 1997; Hirata et al. 2000; Lorenz and Cysewski 2000; Olaizola 2003; Piccardi et al. 1999
Polyunsaturated fatty acids (PUFAs)	Food additive, nutraceutical	Eicosapentaenoic acid (EPA) (*Chlorella minutissima, Crypthecodinium*)	Apt and Behrens 1999; Belarbi et al. 2000; Cardozo et al. 2007; Cohen 1999; Radmer 1996
		Docosahexaenoic acid (DHA) (*Schizochytrium* sp, *Gymnodinium, Crypthecodinium, Ulkenia*)	Apt and Behrens 1999; Pulz and Gross 2004; Radmer 1996; Valencia et al. 2007
		Arachidonic acid (*Parietochlorisincise, Porphyridium cruentum*)	Borowitzka 1997; Bigogno et al. 2002
Vitamins	Nutrition	Biotin (*Euglena gracilis*)	Baker et al. 1981
		α-tocopherol (Vitamin E) (*Euglena gracilis*)	Survase et al. 2006
		Ascorbic acid (Vitamin C) (*P. moriformis, Chlorella* spp)	Bremus et al. 2006; Running et al. 2002
Proteins/Amino acids	Anti HIV-1	Cyanovirin-N (CV-N) (*Nostoc ellipsosporum*)	Boyd 2001, 2002, 2004; Burja et al. 2001; Yang et al. 1999
	Reversing activity against multi drug resistance	Hapalosin (*Hapalosiphon welwitschii*)	Burja et al. 2001
Alkaloids	Immuno-suppressive	Microcolin (*Lyngbya*)	Koehn et al. 1992
	Hormothamnin	*Harmathamnion enteromorphoides*	Gerwick et al. 1992
	Antillatoxin- ichthiotoxic	Cyclodepsipeptides	Orjala et al. 1995
	Acetogenin-cytotoxicity towards leukaemic cells	Curacin- inhibits tubulin polymerization	Burja et al. 2002; Lai et al. 1996
	Anti-tuberculosis Activity	(+)-8-hydroxymanzamine derivative, e.g., Ircinol A, Manzamine A, Litosterol (*Litophyton viridis*)	Donia and Hamann 2003
Protease Inhibitors	Elastase inhibiting activity	Cyclic desipeptides (*Scytonema hofmanni* pcc 7110)	Matern et al. 2001
	Inhibitor of platelet activating factor- induced platelet aggregation	Phosphoglyco-analog of acyl-acetylated sphingosine, glyco-analog of phosphatidyl-glycerol (*Scytonema julianum*)	Antonopoulou et al. 2005
	Inhibitors of serine proteases, including elastase	Micropeptins, aerugenosins, microginins, anabaenopeptins and microverdins	Grach-Pogrebinsky et al. 2003; Matern et al. 2001; Ploutno and Carmeli 2005
Microalgal Metabolites	Hypocholesterolemic effect	(*Aphanizomenon flos-aquae*)	Vlad et al. 1995
	Anticancer effect	Diacylglycerols (*Spirulina fusiformis, Dunaliella* and *Phormidium tenue*)	Mathew et al. 1995; Schwartz and Shklar 1987; Schwartz et al. 1988; Tokuda et al. 1996
	Cytotoxicity against epidermoid and adeno carcinoma cells	Borophycin (*Nostoc linckia* and *Nostoc spongiaeforme* var. tenue)	Burja et al. 2001
	Anti-inflammatory Activity	Carotenoids (β-carotene, lycopene, lutein; C-phycocyanin	Bhat and Madyastha 2000; Kerfeld 2004; Vadiraja et al. 1998
Polysaccharides	food coating, emulsifying and gelling agents, flocculants and hydrating agents; remove toxic metals	Acidic sugars (glucuronic and/or galacturonic acids) and other anionic organic (acetyl, pyruvil) and inorganic (phosphate and sulfate) substituent.	Otero and Vincenzini 2003
		Chrysolaminarin (a soluble β-1,3-polyglucan (*Phaeocystis pouchetii*)	Borowitzka 1997; Laing and Ayala 1990
	Inhibit tumor invasion and metastasis	Sulphated polysaccharide, calcium spirulans (*Aphanizomenon flos-aquae*)	Mishima et al. 1998

Table 5 (continued)

Product group	Applications	Examples/producer	References	
Microbicides	Bactericide	*Spirogyra*	Muller-Feuga et al. 2003	
	Anti-viral	Anti-HIV	Cyanoviridin-N (*Lyngbya lagerheimeii* and *Phormidium tenue*)	Gustafson et al. 1989; Yang et al. 1997
		Inhibits Herpes simplex, human cytomegalovirus and measles virus	Calcium spirulan (Ca-SP) (*Lyngbya lagerheimeii* and *Phormidium tenue*)	Ayehunie et al. 1998; Hayashi and Hayashi 1996; Hayashi et al. 1996
	Anti-helminthic	Dihydroxy tetrahydrofuran, Jasplakinolide (brown algae-*Notheia anomala*)	Donia and Hamann 2003	
	Fungicide- apoptosis of tumor cells	Cryptophycin (*Nostoc* sp. ATCC 53789)	Burja et al. 2001; Panda et al. 1998	
	Anti-protozoan: Leishmaniasis treatment	Sodium stibogluconate and meglumine antimonite	Berman 1998	

kept to a minimum. In contrast, higher levels of polyunsaturated fats lower the cold filter plugging point; the temperature at which the fuel starts to form crystals/solidifies and blocks the fuel filters of an engine. It can be seen that the extent of unsaturation in oil lowers its melting point. Therefore, colder climates require a higher unsaturated lipid content to enable the fuel to perform at low temperatures (Knothe 2005). Microalgae have excellent potential for the genetic modification of their lipid pathways either by upregulation of fatty acid biosynthesis or by downregulation of β-oxidation. By knocking out or modifying enzymes responsible for the synthesis of polyunsaturated lipids in the cell, it should be possible to dramatically increase the proportion of monounsaturated lipids.

9.3 High-value coproduct strategy

Microalgae are regarded as biorefinery for their capacity to produce a wide range of chemicals and biofuels from biomass by the integration of bioprocessing and appropriate low environmental impact chemical technologies in a cost effective and environmentally sustainable manner (Chisti 2007). They have excellent capacity of producing a large number of high-value bioactive compounds that can be used as pharmaceutical compounds, health foods, and natural pigments (Jiang 2000). Few well-studied examples include acetylic acids, β-carotene, vitamin B, ketocarotenoid astaxanthin, polyunsaturated fatty acids, and lutein (Shi et al. 2002; Wen and Chen 2003; Jiang et al. 2004; He et al. 2005; Ip and Chen 2005; Huang et al. 2006; Del Campo et al. 2007; Table 5). The economical feasibility of microalgal biofuel production can be significantly enhanced by a high-value coproduct strategy, which would, concep-

tually, involve sequentially the cultivation of microalgae in a microalgal farming facility (CO_2 mitigation), extracting bioreactive compounds from harvested algal biomass, thermal processing (pyrolysis, liquefaction, or gasification), extracting high-value chemicals from the resulting liquid, vapor, and/or solid phases, and reforming/upgrading biofuels for different applications (Chisti 2007).

9.4 Cost-effective technologies for biomass harvesting and drying

Limited light penetration and small size of microalgal cells (typically in the range of 2–20 μm in diameter) leads to relatively low biomass concentration and thereby, enhances the costs and energy consumption for biomass harvesting. Diverse technologies including chemical flocculation, biological flocculation, filtration, centrifugation, and ultrasonic aggregation have been investigated for microalgal biomass harvesting (Divakaran and Pillai 2002; Bosma et al. 2003; Molina Grima et al. 2003; Olaizola 2003; Knuckey et al. 2006). In general, chemical and biological flocculation require low operating costs; however, they have the disadvantage of requiring long processing period and have the risk of bioreactive product decomposition. On the other hand, filtration, centrifuge and ultrasonic flocculation are more efficient but at the same time more costly. The selection of appropriate harvesting technology, therefore, depends on the value of the target products, biomass concentration, and the size of microalgal cells.

Biomass drying before lipid/bioproduct extraction and/or thermochemical processing is another step that needs to be taken into consideration. Sun drying is probably the cheapest drying method that has been employed for the processing of

microalgal biomass (Millamena et al. 1990; Prakash et al. 1997). However, this method requires long drying period, large drying surface, and risks the loss of some bioreactive products. Low-pressure shelf drying is another low-cost drying technology that has been investigated which, nevertheless, is considered to be a low efficiency method (Prakash et al. 1997). More efficient but costly drying technologies investigated for drying microalgae include drum drying, spray drying, fluidized bed drying, freeze drying, and refractance window dehydration technology (Millamena et al. 1990; Prakash et al. 1997; Leach et al. 1998; Desmorieux and Decaen 2006; Nindo and Tang 2007). It is therefore, important to find a balance between harvesting and drying efficiency with the cost effectiveness to maximize the net energy output of the fuels from microalgae.

9.5 Microalgal production with wastewater treatment

Municipal and industrial wastewater treatments are the key infrastructural facilities that keep our cities, communities, and ecosystems sanitary. A great deal of money and effort is being spent on the removal of pollutants such as biochemical oxygen demand, turbidity, nitrogen, and phosphorus. Large confined animal feeding operations such as dairies can also be major sources of water pollution (Centner 2001). Nutrients like nitrogen and phosphorus can be removed from wastewaters in several ways. The most common method for removing nitrogen is denitrification which leads to reduction of nitrate to nitrogen gas (Metcalf and Eddy 2003). Phosphorus, on the other hand, is often removed by chemical precipitation using ferric chloride. However, both phosphorus and nitrogen can also be removed satisfactorily by growing bacteria or algae in the wastewater and then the biomass can be removed. The technique of promoting microalgal growth for nutrient removal was first developed by Oswald et al. (1957). Later, Singh and Dhar (2006, 2007) also worked on nitrogen and phosphorous removal from secondary treated sewage effluent with microalgae.

Using microalgae for wastewater treatment offers some interesting advantages over conventional methods. It has been shown to be a more cost-effective way to remove biochemical oxygen demand, pathogens, phosphorus, and nitrogen than activated sludge (Green et al. 1996; Singh and Dhar 2006). Traditional wastewater treatment processes involve the high energy costs of mechanical aeration to provide oxygen to aerobic bacteria to consume the organic compounds in the wastewater, whereas, microalgae provide an efficient way to consume nutrients and provide the aerobic bacteria with the needed oxygen through photosynthesis (Oswald et al. 1953). It is estimated that roughly 1 kg of biological oxygen demand removed in an activated sludge process requires 1 kWh of electricity for aeration, which produces 1 kg of fossil CO_2 from power generation. By contrast, 1 kg of biological oxygen demand removed by photosynthetic oxygenation requires no energy inputs and produces enough microalgal biomass to generate methane that can produce 1 kWh of electric power (Oswald 2003).

10 Conclusions

Rapid industrial development, depletion of mineral oil reserves, and rise in atmospheric CO_2 require the development of carbon-neutral renewable alternatives. Biofuel production from microalgae is supposed to provide technical and economic feasibility that has the potential for CO_2 sequestration and is therefore, likely to get wide acceptance. Algal biofuels appear to be the only current renewable energy source that could meet the global demand for transport fuels (Schenk et al. 2008). For many centuries, various microalgal species have been exploited for the valuable protein source for animals. Currently, over 5,000 dry tons of microalgal biomass is produced annually in the world, mostly in "race way" or "high rate" type ponds for high-value nutritional supplements (Spolaore et al. 2006). The biggest challenge over the next few years in the microalgal biofuel field will be to reduce costs of cultivation and to further, improve the biology of biofuel production.

Economic feasibility of biofuels from microalgae can be enhanced by using efficient methods of biomass harvesting and drying, metabolic plus genetic engineering and system biology approaches, selection of efficient strains, high-value co-product strategy, and linking microalgal production with wastewater treatment. There is a need of more research on microalgal lipid structure and composition, quality of oil derived from waste-grown algae, fuel quality, suitability of algal oil for transportation, and its compatibility with newer technologies such as thermal depolymerization. Decision regarding location of biofuel production plant is crucial for land availability for large open ponds, requirement of appropriate climate (average temperature and solar insolation), communities and agro-industrial sites, need for wastewater treatment upgrades, proximities of algae production to biodiesel production sites and oil extraction plants, and ancillary industries for further processing of microalgal oil. Lastly, the bottlenecks in the complete cycle of microalgal biofuel production should be identified and overcome on pilot plants after optimization of growth conditions before the large-scale implementation potentials are realized.

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