Status of Algae as Vehicles for Commercial Production of Fuels and Chemicals

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Abstract This chapter provides a brief overview of role of algae for the production of fuels and chemicals. Characteristics of algae and its production in open raceway ponds have been covered to identify the critical areas that require further exploration and development.

Keywords Microalgae • Photobioreactors • Raceway ponds • Fracking • Harvesting • Extraction • Technoeconomics • Life-cycle analysis • Algae cake

1 Introduction

Microalgae have come into prominence in the past several decades due to their ability to utilize solar energy to fix atmospheric carbon dioxide, and produce biomass and lipids at productivities much higher than those possible with terrestrial plants (Dragone et al. 2010). Indeed, their ability to grow on non-arable lands and thus potential for producing fuels and chemicals without competing with food production has been commented extensively (Zhou et al. 2013). Growing concerns about the limited reserves of crude petroleum, energy security, and adverse impacts of increasing greenhouse gases have also prompted many to explore the cultivation of algae, both autotrophically as well as heterotrophically. Even though the advances in fracking technology have resulted in recent years into increased production of natural gas and crude oil (USEIA 2011), and thus some respite from the immediate worries of energy supply and energy security, sources of liquid hydrocarbons for the increasing global needs of transportation sector remain a major cause for concern. Still, few examples exist of commercial use of algal system for production of biomass and lipids (Day et al. 2012; Borowitzka and Moheimani 2013) for reasons adequately pointed out by Richmond (2004).

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A seminal paper on mass cultivation technologies was published by Tamiya (1957) describing the state of the art of cultivation of algae. This report focused on cultivation of algae for the purposes of production of value-added compounds, for the purposes of nitrogen fixation, for treatment of wastewater, and outlined the characteristics of algae for different tasks and their production. Another USDOE report (Sheehan et al. 1998) summarized the results of a two-decade long experience of algae program. This program focused on production of biofuels using algae and concluded that algal technologies were way too costly for making a bulk chemical such as fuel. Both the reports concluded that outdoor open systems would be most economical for cultivation purposes, and that remains true even today (Greenwell et al. 2010), even though considerable progress has been made in terms of understanding the metabolic processes in algae and in cultivating pure algal cultures in closed photobioreactors. Unfortunately, most of the economic analyses still conclude that biofuels produced using photoautotrophic algae cannot compete with fuels derived from crude oil in spite of the spike in oil prices over the past decade (ANL, NREL, PNNL 2012; Lundquist et al. 2010; Sun et al. 2011). In all the cases, a running theme is need for improved strains (those with characteristics of fast growth while still sporting high neutral lipid content, auto-flocculation thereby facilitating easy recovery of cells from broth, capability to withstand the onslaught of viruses, contaminants, and predators), recycle and reuse of spent broth, and economic recovery of lipids from cells. Further research and developments in the areas of cell harvesting, product recovery, product fractionation into high value products, and research and market development for algal cake (residue) are also deemed necessary in order to make biofuels from algae economical, since cell harvesting and lipid recovery are themselves reported to account for 50 % of the cost of lipid produced (Greenwell et al. 2010).

Considerable efforts have recently been devoted to developing algal strains, systems for cultivation and harvesting, and processing of algae produced for fuels and chemicals (Adarme-Vega et al. 2012; Afify et al. 2010; Agwa et al. 2012; Beer et al. 2009; Chen et al. 2011; Faria et al. 2012, Khola and Ghazala 2012; Miranda et al. 2012; Rajvanshi and Sharma 2012; Rosenberg et al. 2011; Rulong et al. 2012). Most of these stem from the results of Aquatic Species Program (ASP) of US Department of Energy (USDOE) that created a significant database of potential algal species and reactor designs for their cultivation (Sheehan et al. 1998). The results of ASP efforts established that ‘open-pond’ cultivation systems represent the most promising pathways for large-scale bulk production of algal biomass. Recent analysis by Davis et al. (2011) reaffirmed this observation. A renewed effort starting in 2008 under the auspices of National Algal Biofuels Technology Roadmap Work (USDOE-EERE 2012) organized by USDOE identified the algal cultivation, cell harvesting and processing to produce lipids and other desirable valuable byproducts, and conversion technologies as major challenges to economic production of biofuels from algae. This chapter will focus on providing a summary of recent developments relating to economics of autotrophic cultivation of algae, especially as it relates to raceway-type outdoor open cultivation, economic models, and identify areas that need radical improvements and demonstrations for commercialization

of algal biofuel production. A similar report involving high density incline surface thin-film outdoor open cultivators is presented separately by Doucha and Livansky in a later chapter in this book.

2 Advances in Algal Strains

Algae have commercially been produced in various parts of the world and their annual sales in 1997 already exceeded US $ 30 billion (Spolaore et al. 2006). However, most of this production was for nutraceuticals and aquaculture. Typical algal strains used for these purpose are *Chlorella*, *Arthrospira*, *Dunaliella*, and *Haematococcus*. Other species such as *Tetraselmis*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema*, *Thalassiosira*, *Porphyridium*, *Phaeodactylum*, *Isochrysis*, and *Nitzschia*. These are utilized for their protein content and polyunsaturated fatty acids which are typical high value products. From the perspective of commodity items such as biofuels, the strains selected for production must show the following attributes: (a) high growth rate and sustainable production in an open culture (Dragone et al. 2010; USDOE-EERE 2012), (b) high photosynthetic efficiency (Brennan and Owende 2010), (c) reusability of filtrate from harvesting operations, (d) capability of withstanding toxic components in gases used to deliver carbon dioxide, and (e) ease of harvesting and extraction of intracellular components. Sustainable outdoor production in open systems involves cells that can withstand onslaught of contaminants/predators as well as weather conditions (to keep cultivation systems from crashing), and those that have low internal metabolism during conditions of low photon irradiation, including night time (to keep cells from reutilizing the chemicals being targeted for production).

It has been suggested that native algal strains isolated from local environments stand the best chance for meeting the above mentioned criteria of sustainability (Sheehan et al. 1998; Chen et al. 2009). Since there are around ten million algal species on our planet and only a few thousand have been identified (Norton et al. 1996), there is considerable potential for finding natural algal strains capable of producing biofuels. Using this approach, Ravikumar (2013) was able to maintain open raceway cultivation systems predominantly monoculture by using locally isolated *Scenedesmus* strains. Stephens et al. (2010) have reported that several outdoor open pond systems have been operated without significant contamination for as long as six months. Still, cultivating desirable algal strain in open ponds remains very challenging (NRC 2012). While selecting algal strains for production of lipids, it is obvious that the focus be on cells that can generate a high fraction of their dry weight as lipids (Hussain et al. 2010). However, the conditions that result in high lipid content in the cells (environmental stresses or nutritional limitations) are generally also the conditions that cause the lipid productivity to drop, and vice versa (Chen et al. 2009). As a result, strain selection strategies such as those based on lipid productivity (the amount of neutral lipids produced per unit time per unit lighted area or volume), ease of harvesting of cells and recovery of lipids from cells,

ability of cells to overcome the environmental and nutritional shocks, and adaptability of cells to new environments, need to be emphasized (Griffiths and Harrison 2009; Mata et al. 2010; Day et al. 2012). Mutanda et al. (2011) have provided a summary of techniques for isolation of algae from natural samples and concluded that micromanipulation methods coupled with fast screening methods such as those based on dye fluorescence (either lipophilic dye BODIPY 505/525 or lipid-soluble dye Nile Red) can be used to rapidly identify desirable algal strains. In doing so, it is necessary to note that the goal is to cultivate cells with high neutral-lipid productivity, rather than cells that can accumulate just high concentration of lipids in them (Zhou et al. 2013; Pareira et al. 2011). Such fluorescence-based methods are especially attractive since lipid accumulation in cells normally takes place during the stationary phase of cell growth caused by the nutrient limitations in cell broth; in other words, cell growth and lipid production is generally decoupled. It would be interesting to identify / develop cell lines in which lipid production is coupled with cell growth.

Courchesne et al. (2009) have summarized the status of genetic and transcriptional factor approaches being explored to enhance lipid production in algae. These authors reported that multiple bottlenecks as well as competing pathways to those for lipid synthesis exist in cells; although successful overexpression of some of rate-limiting enzymes has been reported, no successful enhancement of lipid production in cells has been demonstrated as yet. On the other hand, genetic engineering approaches have demonstrated enhanced production of hydrogen by algal cells (Beer et al. 2012). Since multi-enzyme systems are generally involved in production of lipids, it may be more appropriate to utilize a transcription factor approach to enhance the production of lipids in algae (Courchesne et al. 2009). In such a case, efforts need to focus on characterizing the transcription factors that participate in lipid synthesis in algae (Nguyen et al. 2008).

Considering that heterotrophic cultivation of selected algal cells result in higher lipid production (Chen et al. 2009), several researchers have explored isolating strains capable of growing on organics in waste water. Agwa et al. (2012) optimized biomass production using a *Chlorella* sp. growing on different animal wastes. The strategy involving heterotrophic growth of algal cells on lignocellulosic hydrolyzates is being pursued by a San Francisco based algal-lipid producer, the Solazyme Inc., at semi-commercial scale. At the same time, other groups are focusing on naturally selected proprietary strains in raceway reactors for commercial cultivation of algae for biofuels. The most prominent of these is Saffire Energy based in San Diego, CA. As suggested by Luque (2010), the basic productivity of lipids by many of these companies may be placed too high resulting in unrealistic expectations. It is, therefore, not surprising that many companies fold their doors within a few years after generating considerable investor interest.

Selection of algae need not be based solely on lipid production by the cells (Rodolfi et al. 2009). Since production of lipids in microorganisms is influenced by environmental stress factors (Brennen and Owende 2010), accumulation of large quantities of lipids in cells is often accompanied by reduced growth rates of cells which can be a major disadvantage for cells growing in open culture systems.

Hence, strategies for selection of algal strains must account for utilization of high-value byproducts such as PUFA, as well as residual cake that may serve as source of carbon skeletons for making additional biofuels or of nutrition. Another consideration may also be the ease of recovery of lipids and the quality of biofuels that can be made from the extracted lipids. Towards this end, Afify et al. (2010) evaluated growth conditions and extraction solvents for eight algal species for lipid recovery and biodiesel production. Recently Liu and Curtiss (2012) have explored genetically modified algal cells that release free fatty acids when subjected to CO_2 limitation followed by exposure to increased temperatures. Although this strategy was time consuming and cumbersome, further developments in this direction can result in significantly reduced cost of lipid recovery.

Other major considerations in making algal systems economical include the sources of nutrients and their reutilization from spent media after harvesting of cells. Although algae are characterized by simple nutrient requirements (Brennan and Owende 2010; Chisti 2007), residual nutrients in spent media represent a major cost of production both in terms of their procurement as well as need to meet the regulatory requirements in any discharge waters. Nitrogen can be efficiently taken-up by the cells in the form of ammonium ions, urea, or even as nitrates, and its limitations have been reported to influence the production of neutral lipids in the cells (Rodolfi et al. 2009). As a result, almost all the nitrogen in broth is available to cells for uptake. On the other hand, phosphorous is required in the form of phosphates which complex with several cations present in broth and thus are not available to the cells completely. Proper optimization of N and P needs in the culture broth is an on-going research area that needs to be vigorously pursued. In this respect, the statistical techniques of medium optimization based on response-surface methodology may come very handy (Ponnusamy and Subramaniam 2013). In order to address these nutritional needs, several authors have considered use of wastewaters for cultivation of algae (Batten et al. 2013; Olguin 2012; Park et al. 2011; Pittman et al. 2011). In such cases, selection of algal strains capable of photoheterotrophic growth would result not only in reducing N and P content of waste water, but also much higher efficiencies of cell growth and lipid production. Doucha and Livansky (2006) have reported that algal cells may be grown to cell densities up to 100 times more in thin-film photobioreactors than in raceway ponds. This can be particularly useful since harvesting of cells is a major cost item in production of biofuels using algal systems.

3 Advances in Cultivation Technologies

Phototrophic cultivation of algal cells is carried out in laboratory in lighted environment in clear shake flasks, culture vessels, bottles, tanks, or specially-designed photobioreactors. In order to take advantage of solar energy, outdoor cultivation is carried in tubular or cylindrical photobioreactors or in open ponds/raceways. Due

to the attenuation of light in liquid media (Daultani 2010; Benson 2003), the depth of fluid in these reactors is limited to no more than 30 cm (Murphy et al. 2010). Based on the production capacities needed for biofuels, outdoor production facilities, mainly the raceways, are the only viable options although some very large capacity closed tubular photobioreactors are in use in Germany (Spolaore et al. 2006). Among open configurations, raceway design is the reactor of choice due to ease of creating circulation patterns and potential for addition of carbon dioxide and nutrients. These can be constructed on non-arable land also.

Recent advances in open outdoor photobioreactor designs have taken place in delivery of carbon dioxide to the cells, mixing of cells, and in control of contaminants/grazers. Since, the depth of culture medium is limited (approximately 30 cm or so, and no more than 50 cm), considerable amount of sparged carbon dioxide would escape into atmosphere unless an appropriate delivery system is designed and utilized. One such system is a floating CO_2 injector which consists of a floating compartment with a hollow enclosure. Gas under the cover filled with CO_2 permits the device to float. As the gas gets consumed, the floating device gets submerged in the medium causing a float valve to supply more gas under the cover. As the float emerges from the medium, the valve closes the supply of gas. Gas is introduced in the medium through a gas sparger due to pressure in the cover. Such systems result in very low (as little as 4%) losses of CO_2 (NRC 2012). Based on carbon content of the cells, a minimum of 1.8 kg of carbon dioxide would be required to produce 1 kg of dry cell mass. In practice, up to 5 kg carbon dioxide may be needed per kg of dry algae due to low efficiency of transfer of CO_2 from the gas phase (Seambiotic Ltd. 2010; Murphy et al. 2010; Doucha et al. 2005). In order to deliver CO_2 to cells, sparging of CO_2-laden gases may be conducted using either perforated tubes or diffusers. Since diffusers result in formation of much smaller bubbles compared to perforations in tubes, these have a significantly higher efficiency of mass transfer of CO_2 from gas to broth (Weiss and Lezion 2008). The efficiency of gas-liquid mass transfer can be enhanced further by increasing the height of medium at the sparging locations through creation of sparging (sump) wells (Murphy et al. 2010). Air, with its 395 ppm CO_2levels (NOAA 2013), cannot deliver enough carbon dioxide to achieve reasonable production rates (20 g DW algae/m^2 day) and a concentrated source of carbon dioxide is required. Such sources are available in the form of power plant stack gases (carbon dioxide concentrations between 9–14% v/v), monoethanol amine (MEA) scrubbers for CO_2 in natural gas, or even exhaust gases from alcoholic fermentations. Of these, the power plant stack gases represent the most obvious sources of plentiful carbon dioxide available in disperse locations where the energy from the hot gases may be utilized also for controlling temperatures in algal ponds. Power plant stack gases have been successfully used as CO_2 sources by Seambiotic (2010) in outdoor open ponds as well as by Olaizola (2000) in a large outdoor photobioreactor. The power plant stack gases contain, in addition to carbon dioxide, other components as well, such as particulates, oxygen, nitrogen, carbon monoxide, nitrogen and sulfur oxides, and trace metals depending on the source of carbon and operation of combustor. Of these, sulfur oxides are most harmful to the algal cells and needs to be reduced to concentrations below 60 ppm. On the other

Table 1 Effect of fluid velocity and open channel length on liquid circulation time and the fluid head needed to overcome flow resistance

Velocity, m/s	Channel length, m	Liquid head, mm	Circulation time, min
0.3	500	10.0	27.8
	1000	20.0	55.6
	2000	39.9	111.1
0.4	500	18.2	20.8
	1000	36.4	41.7
	2000	72.5	83.3

hand, many of the trace metals in stack gases may even promote algal cell growth, as was found by Seambiotic (2010).

Another area in which significant progress has been made is in the designs of fluid mixing and propulsion systems. The central part of these systems in raceway systems consists of paddles mounted on an axial shaft connected to a motor either directly or through a pulley. The purpose of mixing is to keep the algal cells suspended in the growth medium (decaying cells in the sedimented material are good food for contaminating bacteria) and to ensure that the cells rotate routinely between the darker interior of the broth and the lighted air-liquid interface (light in these systems rarely penetrates beyond 15 cm liquid depth; Daultani 2010). Another important function of the paddle system is to circulate the suspended cells through the CO_2-rich zone near the spargers. In the pilot cultivation system of Seambiotic (2010), the paddles propel fluid forward with an average velocity of 20 cm/s and a single sparger zone is used in raceways such that a full circulation of fluid through the raceway takes place every 3–3.5 min. Such circulation times between sparged zones are similar to the circulation times experienced by cells in traditional agitated bioreactors. Murphy et al. (2010) suggest 30 cm/s linear fluid velocity as minimum to keep the cells in suspension.

Murphy et al. (2010) projected using raceways of 245 m length and 18 m width (aspect ratio of around 12). This ratio is in the range of 10:1–20:1 for aspect ratio suggested by Benemann and Oswald (1996) whereas the aspect ratio (length to width of each arm or raceway) used in the pilot plant raceways of Seambiotic (2010) appears to be around 4 (based on the pilot plant photographs). The proposed length of raceways by Benemann and Oswald (1996) is as large as 1000 m. With increasing length of the raceway, liquid head needed for flow of fluid will also increase. Using correlations and governing equations for subcritical flow in open channels (Tilton 1997), the liquid heads and circulation times between different channel lengths can be calculated for specified fluid velocities and these are listed in Table 1. Here channel length is the total distance between paddle wheels; the calculations are based on horizontal channels of zero slope and identical to natural streams with clean straight bank full stage.

Thus in a raceway of 1000 m equivalent length (2000 m channel length), the liquid head to be generated by a single paddle for 30 cm/s liquid velocity is modest (around 1/7th to 1/8th of the liquid depth), but the circulation times are of the order

of 110 min. This implies that several carbon dioxide spargers will need to be placed every 80–100 ms in the raceway channel in order to ensure a mean residence time around 4 min for cells between sparged zones. Doucha et al. (2005) recommend using carbon dioxide spargers every 50 m in order to prevent CO_2 limitation for the cells which in their case corresponds to around 30 s residence time between spargers.

For most of the algal cells, the fluid velocity of 40 cm/s would keep the cells in suspension (Murphy et al. 2010). Fast settling cells such as *scenedesmus* may require higher velocities to keep them suspended. In such a case, higher fluid velocities up to 1 m/s may be employed by reducing the length of raceways to 100–125 ms. This would keep the liquid head modest around 7–8 cm and require only two sparging stations in the raceway.

In deciding the flow rate of fluid in the raceways, shear sensitivity of algal cells being cultivated must be considered. For shear sensitive cells, however, such high velocities may be detrimental (Michaels et al. 2010). The shear sensitivity of algal cells follows the order below of sensitivity to shear stresses: *green algae* < *bluegreen algae* < *diatoms* < *dinoflagellates* (Thomas and Gibson 1990). In a study of shear stress on algal cells, Contreras et al. (1998) reported that cells of *Phaeodactylum tricornutum* grew fastest at a shear rate of 7000 s^{-1} whereas the dinoflagellate *Protoceratium reticulatum* cells showed damage already at a shear rate of 0.12 s^{-1} (García Camacho et al. 2007). *Chlorella* and *Scenedesmus* are relatively shear tolerant (Dragone et al. 2010; Setlik et al. 1970). Michaels et al. (2010) recently showed that cells of microalgae *Chaetoceros muelleri* experience an abrupt but definite loss of cell viability between shear rate of 750 and 975 s^{-1} without showing any external sign of cell damage, but increasing shear rate further to 14500 s^{-1} did not show any further loss of cell viability. Several algal varieties such as *Dunaliella* show sensitivity to turbulence created by sparging of gases (Barbosa et al. 2004).

Although dissolved oxygen and temperature control are not major issues in raceways, evaporation of water in dry season and flooding of raceway during rains are major issues. pH in the broth also must be controlled within the parameters of the strains being cultivated. When sparging CO_2-enriched gases, broth pH may drop and appropriate measures should be taken to control it back to the desired range. Another major issue in these reactors is the potential for contamination by viruses, bacteria, and grazers. The issues of contamination have been addressed either by use of extremophiles (high pH, high salinity) or small quantities (1–3 ppm) of antibiotics in broth (Brennan and Owende 2010). Protozoa population in the medium can be controlled by deliberately lowering broth pH for a short period and then raising it again. Chemical agents such as chlorine or UV-treatment may also be used to control contaminant population, especially in the reclaimed broth after it is made-up for losses due to evaporation and during cell harvesting and before it is recycled. In any case, regular cleaning of channels for any sediment is strongly advised to control bacterial contamination. Seambiotic (2010) reports restarting the raceway frequently with fresh culture inoculated with as much as 30% v/v inoculums of good algal cell suspension. In some cases, judicious use of local strains of algae

along with carefully designed operational techniques has also been used to keep the raceway systems running for several weeks in a row (Ravikumar 2013, Stephens et al. 2010). Still, lack of appreciable authenticated data for long-term algae production (of the order of a year or more) in outdoor raceways is hindering scale-up of production facilities (Murphy et al. 2010).

4 Advances in Harvesting and Extraction Technologies:

Recovering cells from broth and extraction of lipids from algal cells is critical for economic production of biofuels using open algal ponds. Recovering cells from broth involves several operations (Sing et al. 2013) including bulk harvesting (increasing cell concentration to ~0.5–1 % w/v, dry weight basis), thickening (final cell concentration ~5–10 %), dewatering (final concentration to 15–25 %), and drying (if required). Cell recovery itself costs as much as 20–30 % and more towards the cost of algal lipids (Brennan and Owende 2010). The costs occur due to difficulties in separation of cells from broth (due to small cell size as well as low cell concentrations), need for processing of large volumes of liquids, tendency of cells to reutilize the lipids as energy source during stationary phase, and intracellular nature of product(s). One of the biggest difficulties faced in harvesting is the low concentration of cells which in open raceway bioreactors ranges between 300 to 500 g m^{-3}. This means that almost a million m^3 (or 264.2 million US gallons) water must be separated in order to obtain 300–500 ton dry algae. Considering an average 20 % extractable neutral lipids in dry algae, it amounts to 34–57 m^3 (or 9000 to 15000 gal) broth that must be processed to make a gallon of lipids from algae. In this sense, some cell lines such as cyanobacterium *Spirulina* that are filamentous, may permit considerable cost savings in harvesting (Benemann and Oswald 1996). Most others such as *Chlorella*, *Nannochloropsis*, and *Scenedesmus* are unicellular. High density algal cell cultivations in thin-film open outdoor systems, as proposed by Doucha and coworkers (Doucha et al. 2005; Doucha and Livansky 2006), are highly desirable as these reduce not only the liquid volumes but also the frequency of liquid handling resulting in considerable savings in operating costs. Unfortunately, the inclined bed systems suggested by these authors increase capital costs several fold.

Initial separation of algal cells from broth in raceway ponds will depend strongly on the size and shape of cells, density of wet cells and their agglomerates, and surface charge. Sizes of algal cells range from 2 to >200 μm. Shapes of cells can be spherical, rod-like, or filamentous (Henderson et al. 2008). Some cells are buoyant due to gas-vacoules while others are denser due to heavy presence of minerals (Greenwell et al. 2010). Presence of excessive lipids may also make some cells lighter than the broth, but these effects are generally not significant as the wet algal cells are over 80 % water. Generally algal cells carry negative charge under physiological conditions (zeta potential in the range of −5 to −40 mV). In light of these cell characteristics, different combinations of flocculation, floatation, fil-

tration, sedimentation, centrifugation, and electrically assisted cell separations are commonly employed for harvesting algal cells from broth.

4.1 Flocculation

Flocculation of cells is based on modifying cellular charge by changing culture conditions, culture pH, addition of chemical flocculants, or exposing the cells to electric field. Several algal species tend to autoflocculate as their metabolism slows down (Uduman et al. 2010). Sirin et al. (2012) found that the microalgae *Phaeodactylum tricornutum* efficiently self-coagulates at pH 9.75 which is just a tad over its cultivation pH of 9.12; at cultivation pH, the natural flocculation as well as sedimentation rate is very poor. In cases like this, autoflocculation may be the most efficient method for initial concentration of cell. The most common chemical flocculants are inorganic (aluminum, ferric, zinc sulfates or chlorides) or organic (cationic polymers, starches, chitosans, etc) in nature. Papazi et al. (2010) found aluminum salts to be most efficient among inorganic salts but caused some lysis of *Chlorella minutissima* cells. Chlorides caused an almost immediate aggregation of cells upon addition of requisite quantities, whereas with sulfates it took 2–5 h to cause aggregation. The concentrations of the salts ranged from 0.5 to 0.75 kg m^{-3} which though effective may make use of these salts cost prohibitive. Using these coagulants may also interfere with reuse of residual water in cultivation reactors. Chitosan is a natural cationic polysaccharide that is non-toxic, produces large flocs, and results in high sedimentation rate (Sirin et al. 2012). These authors found that addition of 20 mg/L chitosan to *Phaeodactylum tricornutum* broths at pH 9.75 enhanced flocculation as well as sedimentation rates, but not at pH 9.12. Polymeric coagulants such as nonionic polymer Magnafloc LT25 and cationic polymer Praestol are very effective in inducing coagulation in many algal systems at concentrations as low as 0.5 ppm and have no adverse effect on cell growth in recycled media (Milledge and Heaven 2012). Saline systems require higher doses of electrolytes and polymers in causing coagulation, even though a combination of polyelectrolytes and chemical coagulants has been reported to be highly effective (Knuckey et al. 2006). Zeng et al. (2012) reported concentration factor of greater than 20 and over 90 % cell recovery with the addition of around 20 mg/L poly (γ)-glutamic acid as organic flocculent for several marine and freshwater algae (*Chlorella vulgaris*, *Chlorella protothecoides*, *Nannochloropsis oculata* LICME 002, *Phaeodactylum tricornutum*, *Botryococcus braunii* LICME 003). At times, the efficiency of flocculation can be enhanced by pretreatment of the cells by ozonation (Greenwell et al. 2010).

Bioflocculation is the term associated with formation of flocs in an otherwise nonflocculating culture in presence of another organism that flocculates easily. In several waste treatment systems utilizing algae, presence of contaminating bacteria have been shown to cause considerable flocculation (Lee et al. 2009; Medina and Neis 2007). Recently, Salim et al. (2011) reported mixing of two microalgae as potential means of causing easy flocculation of algal mass from cultures. Nonfloc-

culating freshwater microalga *Chlorella vulgaris* was flocculated with the help of flocculating freshwater algae *Ankistrodesmus falcatus* and *Scenedesmus obliquus*, and nonflocculating marine alga *Neochloris oleoabundans* was harvested with the aid of flocculating marine alga *Tetraselmis suecica*. In all the cases, addition of flocculating coagulating cells to non-flocculating cells increased sedimentation rates of all cells in the mixture; bridging and entrapment were proposed as possible mechanism of enhanced recovery of the nonconforming cells.

Electrically-induced flocculation has also been proposed as a means of concentrating algal cells from broth (Vandamme et al. 2011). Electro-coagulation-flocculation utilizes a sacrificing anode (either iron or aluminum) in causing flocculation of algal cells; Vandamme et al. (2011) found that aluminum anodes were superior to iron anodes for *Chlorella* and *Phaedactylum* broths and power consumption was of the order of 0.3–2 kWh/kg cells harvested. Electroflocculation, on the other hand, does not require sacrificing anodes and flocculation takes place at the anode where cells give-up their charge. Power consumption in these systems is also of the order of 0.3 kWh/kg cells harvested, but significant fouling of anodes has been reported (Uduman et al. 2010). These methods have been shown to be effective at bench scale, but performance of any scaled-up unit has not been reported as yet.

4.2 Floatation and Sedimentation

These gravity-based technologies have been proposed for separation of algal flocs from broth. A review of principles of these processes is provided by Milledge and Heaven (2011) and it clearly shows the role of particle size in the separation. As a result, floatation/sedimentation are generally used after flocculation of cells. Due to small density differences between cells and culture medium, settling of cells and flocs is easily disturbed by convection currents and sedimentation is normally not used as a unit-operation for separation of algal cells (Uduman et al. 2010). Floatation, on the other hand, is relatively fast as it can be assisted by addition of small air-bubbles in the mix. In dispersed or suspended-air-floatation (SAF), micro-bubbles are generated chemically in the broth using surfactant and depressurization from 2 atm (absolute) to atmospheric pressure. In dissolved air floatation (DSF), 10–100 micron size bubbles are generated by releasing high pressure fluid (~5 atm absolute) directly in the culture medium (Wiley et al. 2009). With the use of surfactants (~2.5 mL/L of floatation water equivalent to ~21 mL surfactant/m^3 medium) and relatively modest pressures to form microbubbles, the SAF process resulted in significant cost savings compared to DAF which is generally regarded as one of the most promising mode of separation of cells from medium. At the bench scale of 100 L, the energy requirements were calculated to be 7.6 kWh/m^3 sample treated using DAF compared to 3 Wh/m^3 sample treated using SAF for identical solid capture. Recently, Hanotu et al. (2012) have reported production of micron-sized bubbles from porous spargers using a fluidic oscillator based on Coanda effect with 2–3 orders of magnitude reduction in energy usage. Although a recent review by Brennan and Owende (2010) concluded that flocculation and floatation are not likely to be

cost effective in view of material (flocculant) and energy (microbubble generation) requirements, the advances in more effective polymeric flocculants coupled with algal surface modifications (via mild ozonation) and new techniques in formation of microbubbles make this a promising alternative to other methods of cell harvesting.

4.3 Filtration

Filtration is a commonly used unit operation for separation of solids from liquids. The conventional devices include pressure filters (plate and frame press, chamber filter press, belt press, pressure suction filter, cylindrical sieve filter, filter basket) and vacuum filters (vacuum leaf or Moore filter, vacuum Nutsche or batch bed filter, rotary drum filter). For the case of algae, two major issues need to be confronted in order to successfully use filtration for the desired separation. Firstly, algal cells are rather small (3 to 100 μm in size) and these are suspended in culture medium in an extremely dilute manner. Secondly, the algal cells often release extracellular organic materials (EOM or EPS—extracellular polymeric substances) depending on the cultivation conditions (Drews et al. 2006). EOMs have a high potential of fouling the filtration media. Generally cultivation under high light intensities and high temperatures results in stunted cell growth and production of larger amounts of EOMs (Round 1981).

Shelef et al. (1984) provided an excellent review of these devices for algal systems. Direct filtration, using conventional filtering devices, is a possibility for some algal cells (*Coelastrum* and *Spirulina*) that are large in size (>70 μm). As reported by Shelef et al. (1984), filtration of *Coelastrum* broths could produce algae cake containing as much as 27 % solids at energy cost ranging from 0.1–6 kWh/m^3 fluid processed; such cakes would be ready for extraction either as such or after drying. For algal cells of smaller size (such as those of *Dunaliealla*, *Scenedesmus*, *Chlorella*), these methods suffer from incomplete separation and rapid drops in filtration rates. In such cases, due to small cell size and low cell density, filtration alone is not a feasible operation for harvesting algal cells.

Microfiber membranes, esp. when used with cross-flow filtration to reduce the buildup of algae cake on filter have been proposed for use after process volumes have been reduced by a factor of 100 (Greenwell et al. 2010). Babel and Takizawa (2010) recently explored filtration of *Chlorella* cells using cellulose ester and polyvinylidine difluoride (PVDF) microfiber membranes and found that the extracellular organic material (EOM) released by the cells causes considerable fouling of both types of membranes; the *Chlorella* cells themselves did not foul the membranes. The cell cake was found to be compressible with a compressibility index of 0.44. Satone et al. (2011) demonstrated a novel tangential-flow filtration device in which shear rate around the filtration membranes (of 1.5 μm pore size) was increased by use of a concentric spiral guide within the ceramic membranes. In such a system it was possible to concentrated algal suspensions from 3 to 11 kg m^{-3} rapidly under a pressure of 6 bar with a relatively constant flux of 0.5 L m^{-2} min^{-1} and no

deposits were observed on the spiral guide rod; during batch filtration using this device, the filtrate flux dropped rapidly in the first few minutes from around >4 to 1 L m^{-2} min^{-1} and settled to around 0.5 L m^{-2} min^{-1} after several hours. Since it is the EOMs that interfere with filtration membranes, it is likely that surface treatments of membrane surface to reduce fouling would enable significant improvements in filtration rates. Milledge and Heaven (2012) have reported an ultrafiltration membrane (0.03 μm pore diameter) from Avanti Membrane Technology Inc. that resulted in filtration rate of ~1.1 L m^{-1} min^{-1} with 95% recovery of microalgae but only 20 fold concentration factor. No data were provided for the starting of final concentration of cells in the retentate. The energy consumption was between 1 and 3 kWh m^{-3} fluid processed.

4.4 Centrifugation

For any slowly settling system, such as the one with algal suspension, centrifugation is definitely desirable from the viewpoint of accelerating the separation process as it increases the driving forces for separation by several orders of magnitude. This is the reason several researchers (Rosch and Posten 2012; Seambiotic Ltd 2010; NRC 2012) have concluded that centrifugation is an essential part of final thickening of algal slurries, following at least flocculation and sedimentation/floatation. It is a highly energy intensive process, consuming around 1.4 kWh m^{-3} fluid processed. The centrifuges can be either disk stack type or decanter type. Disk stack centrifuges can be used for very dilute algal slurries (concentration 0.02%) and produce >20% solid slurry. Decanter centrifuges require higher feed cell concentrations (10% or more) but these can produce >40% solids in the cake; these centrifuges consume even more power than the disk stack centrifuges. Milledge and Heaven (2012) have reported on a new type of spiral-plate centrifuge in which the centrifugal force was used to force solids on the outer bottom edge of the bowl vanes and a solid paste (31.5% dry solids) was obtained from 0.025% suspension of *Nannochloropsis* for energy usage of 1.9 kWh kg^{-1} dry algae. This energy consumption is considerably lower than that for disk stack centrifuges. The operation was semi-batch and the capacity was only 4 m^3 h^{-1}. Based on the information available on Evodus Web site (http://www.evodos.eu/market-specific-solutions/algae.html), the energy picture improves even further as the feed stream cell concentration increases (1 kWh g^{-1} dry wt at 0.05% feed, 0.53 kWh kg^{-1} dry wt at 0.1% feed, and 0.45 kWh kg^{-1} dry wt at 0.15% feed).

4.5 Drying

The wet algae paste is highly perishable and it needs immediate processing to get the valuable products out of it. Alternatively, it can be frozen (for short term) or dried. Drying of cells may be necessary also depending on the process of extraction

of lipids from algal cells. This can be done by several means which include using energy directly from the sun (solar drying) or indirectly through hot gases (spray drying), hot surfaces (roller drying), or drying under vacuum at low temperature (freeze drying). In all of these cases, minimum energy to be removed per kg of water removed is established by thermodynamic considerations (i.e. 2.6 MJ kg^{-1}, Milledge and Heaven 2012). As a result, the cells must be dewatered as much as possible before drying. While using solar energy the only requirements are space, but it could be very slow depending on the global location. Freeze drying is also very slow and it is a very energy intensive process. Both roller and spray drying are quite fast, although they may incur loss of lipids as well as other valuable constituents in the cells. Drying temperature and duration together determine the lipid content in the cells (Brennan and Owende 2010). Drying at or below 80 °C retains most of the lipids (Graham 2011).

4.6 Extraction of Lipids

Lipids in algal cells are intracellular in the form of lipid bodies and require extraction utilizing solvents. The extractions can be conducted either from dry cells or from the wet cake. An excellent review of extraction principles and procedures has recently been published by Halim et al. (2012).

Common solvents used for extraction are hexane, chloroform, methanol, ethanol, isopropanol, butanol, ethyl acetate, petroleum ether (NRC 2012). The non-polar solvents such as hexane and chloroform are necessary to extract neutral lipids (triacylglycerols, TAGs) from the cells. Polar solvents such as methanol and isopropanol in solvent mixture generally serve the role of disruptors of hydrogen bonds and electrostatic forces between membrane-bound lipids and proteins, thus making the membrane porous and allowing the non-polar solvents better access to the non-polar lipids within the cell (Cooney et al. 2009). The use of polar solvents may even result in higher lipid extraction as these solvents are able to remove the polar lipids also from the cells. Extraction of polar lipids such as phosphoglycerides may even be problematic during subsequent processing of lipids into biofuels (Dufreche 2008; Halim et al. 2012).

Before lipid extraction, the cells need to be disrupted to ensure an intimate contact between the solvent and the intracellular lipid bodies. The cell disruption can be achieved using either mechanical devices (bead milling, sonication, French press, etc.) or using physical-chemical means (enzymatic digestion of cell walls, acid/alkali based hydrolysis of cell walls, or even osmotic shock if the cell structure permits, Lee et al. 2010). French presses disrupt cells by first pressurizing the suspension to 500–850 bar and then passing it through a small opening to lower pressure (Halim et al. 2012). The efficiency of single pass cell disruption is very high (~74 %) and it causes a quick solvent extraction of lipids from cells. This method is commonly utilized for cell disruptions at industrial scale. Bead mills and sonication are more laboratory scale operations.

The extraction methods commonly employed are variations of the classical Bligh and Dyer (1959) methods or its many variations. In this method, the cells are first disrupted in presence of solvent at room temperature. Water is then added forming a multi-phase system consisting of a heavy lipid-rich chloroform phase, a light aqueous phase containing methanol, and a solid phase that forms a layer between the light and heavy phases. Although absence of water in the beginning permits better access of the non-polar solvent to the cells, it is not necessary to completely dry the cells before using Bligh and Dyer extraction. This extraction utilizes chloroform and methanol in the ratio of 2:1 as solvent system. After removal of the solvent phase, the remaining phases are then extracted repeatedly with the solvent phase. The solvent phase is evaporated to recover the solvents for reuse and the lipids are retained for further processing. Unless already removed by special processing, the photosynthetic pigments also extract out with the lipids during extraction of algae and impart a greenish color to the algal oil. This method is mostly used at bench scale. Two other extraction methods used at bench scale are accelerated solvent extraction (ASE) and supercritical extraction (SCE). Both involve high pressures and temperatures, except subcritical conditions are maintained during ASE. These are highly efficient methods and do not generally involve any prior cell disruption. Both can utilize any of the solvent combinations that can be thought of for facilitating extractions.

Since both chloroform and methanol are toxic chemicals, alternative solvent system have been explored. In many situations, hexane/isopropanol solvent system does as good a job of extracting neutral lipids from the cells as the chloroform-methanol system. In a comparative study of four different solvent systems (chloroform-methanol, chloroform, hexane, hexane-isopropanol, methy tert-butyl ether MTBE), Subramaniam et al. (2011) compared the extent of extraction of lipids from freeze-dried as well as wet cells of oleaginous yeast *Lipomyces starkeyi* using Bligh and Dyer procedure; these authors found that the hexane-isopropanol (3:2) solvent system, the chloroform-methanol (2:1) solvent system, and MTBE performed almost identically in terms of extracting lipids from the yeast cells, hexane was the worst, and chloroform alone was in between. On the basis of cost of extraction, the hexane-isopropanol system was least costly. Use of wet cell cake in the extractions resulted in 50 % loss of extraction efficiency, although this may not be as bad economically considering the high costs of drying the cells and the fact that lipid-bearing residual algae cake will have higher energy content due to presence of lipids as well as better nutritional value.

Graham (2011) explored the effect of drying temperature on the extent of extraction and composition of lipids from a local strain of *Scenedesmus* cultivated in open raceway ponds. The algae cells were freshly obtained and dried at three different drying temperatures (80, 109, and 180 °C) followed by extractions in an accelerated solvent extractor. The data show that gravimetric yields of lipids extracted from samples dried at lower temperatures were significantly higher than those dried at 180 °C samples. Furthermore, the yields of overall esterifiable lipids from the samples dried at 109 and 80 °C were nearly two orders of magnitude higher than those from the sample dried at 180 °C. ASE procedure was evaluated also by

Rulong et al. (2012) who found the ASE to have 'wide applicability'. These authors found that ethanol and acetone were the best solvents for extraction of lipids from marine microalga *Nannochloropsis oculata*. Increasing temperature and pressure greatly facilitated solvent access to the lipids and increased extraction rate as well as efficiency of extraction.

Ionic liquids (IL) have also been suggested as solvents for fast extraction of lipids from algae. Kim et al. (2012) used (Bmin)-based hydrophilic as well as hydropobic ionic liquids in a mixture with methanol to extract lipids from *Chlorella vulgaris* cells and found that ionic lipids were able to extract more lipids from cells than the Chloroform-methanol solvent mixture. The extraction efficiency of ionic liquids was dependent on the anionic nature of ionic lipids and addition of water to the mixture increased the efficiency of separation of lipids.

Another unique solution to recovery of intracellular lipids from algal cells has been proposed by Liu and Curtiss (2012). This method involves utilizing genetically engineered cells in which lipases are induced by keeping cells limited in CO_2 for a day, followed by incubating the cells at increased temperature (46 °C) for two days. Such a treatment resulted in production of extracellular free fatty acids. This technique, however, is more suitable for algae cultivated in closed photobioreactors than in open raceway ponds.

5 Techno-economic and Life-cycle Analyses

Techno-economic analysis of photoautotrophic algal cultivation for production of lipids has been conducted by several researchers recently (Davis et al. 2011). These studies are based on experimental data that are at best only partially confirmed at the scale at which the analyses are conducted. The base-level production data are generally obtained from a considerably smaller scale and even those are from studies conducted over relatively short periods (several weeks to months at most). Moreover, the analyses of larger scale operations necessarily involves considering longer distances over which production occurs and making informed assumptions regarding when and how harvesting and processing of algal biomass is to be conducted. The difficulties posed in deciding the harvesting and processing sequences can be deemed from the algal biofuel life-cycle-analysis report of Murphy et al. (2010). Depending on the selection of unit operations in an algae cultivation- and processing-facility, and the assumptions made regarding their efficiencies and operational costs, a number of economic scenarios emerge for algal biofuels. Realistic guidelines for choosing one operation over the other do not exist since little experience with actual large scale systems exists. This is in spite of the fact that various unit operations under consideration have been in use in other industries for a long time, and considerable research activity has been undergoing to develop basic biological systems.

A number of researchers have attempted to put together process flow sheets for algal biofuel production and conducted techno-economic analyses for different hy-

pothetical production scenarios. In one such analysis, Darzins et al. (2010) considered three different production scenarios for a plant with 10 million gallon per year lipid production capacity. These were (a) algal raceway pond productivity of 10 g m^{-2} day^{-1} with 10% lipids in cells based on the experience of Roswell pond cultivation conducted under DOE's Aquatic Species Program, (b) a hypothetical case in which cellular productivity remains same, but cells accumulate 40% lipid intracellularly, and (c) a third case where the oleaginous algae of case (b) can be cultivated with cell productivity of 50 g m^{-2} day^{-1}. In each case, an efficient CO_2 transfer is hypothesized such that 2 kg CO_2 is needed per kg dry cell production. Only in case (c), a preliminary estimate of biodiesel cost get under $ 1 per liter. Such biomass productivities have been shown to take place under the most optimized conditions but not on a consistent basis under field conditions. Moreover, this case envisages a high lipid content as well which is also presently not feasible. All the techno-economic studies conducted so far confirm that the cost of production of algal biofuels under realistic present-day technology far exceed the cost of petroleum-derived fuels (Ribeiro and da Silva 2012; Davies et al. 2011; Andersson et al. 2011; Lundquist et al. 2010; Quinn et al. 2011; Williams and Laurens 2010). A very detailed life cycle analysis of large-scale algae cultivation in outdoor raceway ponds by Murphy et al. (2010) demonstrated the difficulties that will be faced in the area of materials handling and the real bottleneck that one can expect during harvesting of cells. The biggest problems occur because several of the steps in harvesting are likely to be too slow and energy intensive. The problem is exacerbated by the fact that the algal biomass is fragile in nature and it is rapidly spoiled once it is taken out of its natural environment in the cultivation system. Moreover, biofuels are a bulk commodity for which it is difficult to draw a significant premium. Other components in the residual algal cake are potentially high value items and it will be necessary to develop efficient fractionation processes for such compounds.

6 Conclusions

In summary, algae have potential to be carbon sources for future needs of fuels and chemicals. But there are major technical roadblocks in their utilization. Future of biofuels from algae depends on the following technical developments:

1. For each kg of neutral lipids produced by the cells, four or more kgs of residual cake is produced. Given the large amounts of biofuels that are targeted for production, it will be necessary to find useful avenues for utilization of the residual cake.
2. The cake residue has potentially high value components that need to be identified and for which efficient fractionation methods need to be developed.
3. There is a need for techniques (a) that can separate and concentrate algal cells from broths rapidly with ultralow energy consumption (of the order of 0.01 kWh per m^3 fluid processed), and (b) that can be easily automated and

scaled-up for local use so that transport of dilute cell suspensions even over short distances can be avoided.
4. Ultimately, sustained cultivation of dominant algal species in septic open systems with reuse of a substantial part of spent medium needs to be demonstrated and documented.

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