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Review

Constraints to commercialization of algal fuels

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ABSTRACT

Production of algal crude oil has been achieved in various pilot scale facilities, but whether algal fuels can be produced in sufficient quantity to meaningfully displace petroleum fuels, has been largely overlooked. Limitations to commercialization of algal fuels need to be understood and addressed for any future commercialization. This review identifies the major constraints to commercialization of transport fuels from microalgae. Algae derived fuels are expensive compared to petroleum derived fuels, but this could change. Unfortunately, improved economics of production are not sufficient for an environmentally sustainable production, or its large scale feasibility. A low-cost point supply of concentrated carbon dioxide colocated with the other essential resources is necessary for producing algal fuels. An insufficiency of concentrated carbon dioxide is actually a major impediment to any substantial production of algal fuels. Sustainability of production requires the development of an ability to almost fully recycle the phosphorous and nitrogen nutrients that are necessary for algae culture. Development of a nitrogen biofixation ability to support production of algal fuels ought to be an important long term objective. At sufficiently large scale, a limited supply of freshwater will pose a significant limitation to production even if marine algae are used. Processes for recovering energy from the algal biomass left after the extraction of oil, are required for achieving a net positive energy balance in the algal fuel oil. The near term outlook for widespread use of algal fuels appears bleak, but fuels for niche applications such as in aviation may be likely in the medium term. Genetic and metabolic engineering of microalgae to boost production of fuel oil and ease its recovery, are essential for commercialization of algal fuels. Algae will need to be genetically modified for improved photosynthetic efficiency in the long term.

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1. Introduction

Biofuels derived from crops and agroindustrial residues are a potential alternative to petroleum derived transport fuels (Durrett et al., 2008; Gressel, 2008), but have their limitations (Ulgiati, 2001; Patzek, 2004; Chisti, 2007; Pimentel et al., 2009; Borowitzka and Moheimani, 2013; Pate, 2013; Klein-Marcuschamer et al., 2013). Fuel oils derived from microalgae via photosynthesis (Chisti, 2007, 2008a; Hu et al., 2008; Brennan and Owende, 2010; Huang et al., 2010), have been advanced as potentially superior to crop derived fuels, but whether they can be produced inexpensively in a sufficient quantity to significantly replace petroleum derived transport fuels, is in doubt (Malcata, 2011; Pate et al., 2011; Klein-Marcuschamer et al., 2013; Pate, 2013). This review is focused on the prospects of algal crude oil as a replacement feedstock for petroleum oil, for use in production of transport fuels. Oil production via the photosynthetic route is of primary concern here.

Microalgae are more efficient than terrestrial plants in converting sunlight to biochemical energy (Stephenson et al., 2011). The green microalgae share the same basic photosynthetic machinery as the C3 land plants. The maximum efficiency of photosynthesis in C3 plants growing in the normal atmosphere has been estimated to be 4.6% (Zhu et al., 2008), that is 4.6% of the total solar energy received can be converted to biomass energy. In practice, the annual averaged efficiency does not usually exceed 2.4% (Zhu et al., 2008). In contrast, for algae growing in a carbon dioxide supplemented culture outdoors, the annual averaged photosynthetic efficiency of >5% has been observed and a possible maximum of 8.3% has been estimated for wildtype species (Chisti, 2012). Reasons for a higher photosynthetic efficiency of green algae relative to plants have been discussed elsewhere (Chisti, 2012).

Potentially, microalgae can provide fuels in several distinct forms (Kröger and Müller-Langer, 2012)—algal biomass for combustion; algal crude oil for direct combustion, or for use in production of other transportation fuels such as diesel, gasoline and jet fuel (kerosene) (Lestari et al., 2009); biogas via anaerobic digestion of the biomass (Zamalloa et al., 2011; Markou et al., 2013); biohydrogen; bioethanol via fermentation of carbohydrates derived from algae (Matsumoto et al., 2003; Ho et al., 2013); and bioethanol produced directly through algal photosynthesis (Williams, 2009; Lü et al., 2011). Of the different forms of fuels that algae can provide, algal oil for making liquid transport fuels is the focus of most attention, including commercialization effort by many companies. Of the several companies attempting to commercialize liquid fuels from algae (Table 1), most are focused on oils produced via the photosynthetic route. Others are attempting to photosynthetically convert carbon dioxide and water to directly useable fuels. For example, Algenol Biofuels is developing direct production of bioethanol from carbon dioxide in genetically modified algae and Joule Unlimited, Inc. (www.jouleunlimited.com) is taking a similar approach for producing other fuels. In contrast to the photosynthetic route, Solazyme, Inc., is using sugars sourced from plants to grow oil-rich algae.

Many algae accumulate oils (Banerjee et al., 2002; Chisti, 2007; Griffiths and Harrison, 2009; Griffiths et al., 2012). Oil content of algal biomass is usually greatly enhanced if a nutrient deficient culture stage, or other stress imposing stage, follows the nutrient sufficient growth stage (Illman et al., 2000; Rodolfi et al., 2009; Mazzuca Sobczuk and Chisti, 2010; Griffiths et al., 2012; Dillschneider et al., 2013). In some instances at least, oil is accumulated also in a nitrogen-sufficient medium if carbon is provided in excess (Fan et al., 2012). Algal oil includes diverse components: in addition to triglycerides, the oil may contain terpenoid hydrocarbons (Banerjee et al., 2002), polar lipids, oxygen-containing nontriglyceride carotenoid oils and chlorophylls. All these compounds are energy rich and carbon rich. Algal crude oil has an energy content of around 35,800 kJ kg^{-1}, or around 80% of the average energy contained in petroleum (Chisti, 2012). Algal crude oil is a potential substitute for petroleum for producing various kinds of transport fuels.

Algal crude oil tends to be rich in long chain polyunsaturated fatty acids (Belarbi et al., 2000; Ward and Singh, 2005; Guschina and Harwood, 2006; Harwood and Guschina, 2009; Nichols et al., 2010) and, consequently, may not be as suitable for producing biodiesel as some of the other vegetable oils (Knothe, 2011; Stansell et al., 2012). Nonetheless, algal oil has been successfully transformed into gasoline, diesel and kerosene fuels that are indistinguishable from the equivalent fuels derived from petroleum (Chisti, 2012). Liquid fuels derived from algae have performed outstandingly in tests (Johnson and Wen, 2009; Haik et al., 2010; Babich et al., 2011; Haik et al., 2011; Chen et al., 2012; Khan et al., 2012); therefore, algal transport fuels are a proven technology, although unaffordable at present. In the rush to commercialize, serious impediments to large scale production of algal fuels have been overlooked. These stumbling blocks are identified here and approaches to overcoming them are proposed.

2. Impediments to commercialization

2.1. Availability of carbon dioxide

Carbon dioxide is essential for growing algae for biofuels. Production of each ton of algal biomass requires at least 1.83 tons of carbon dioxide (Chisti, 2007). Nearly all pilot scale algae culture depends on purchased carbon dioxide that contributes substantially (∼50%) to the cost of producing the biomass. Algae culture for fuels is not feasible unless carbon dioxide is available free (Chisti, 2007). Potentially, carbon dioxide emitted by coal-fired power stations can be used for growing algae, but the amount of available carbon dioxide is a major limitation for large scale culturing of algae.

The dispersed tailpipe carbon dioxide emissions that are a consequence the use of petroleum as transport fuel cannot be directly used for growing algae. Concentrated sources of carbon dioxide are mainly the flue gases produced during power generation from combustion of coal. The cement industry is another source of concentrated carbon dioxide emissions. In 2008, coal burning power plants in the US produced 1945.9 million metric tons of carbon dioxide (U.S. Department of Energy, 2009). In the same year, the use of petroleum transportation fuels released 1889.4 million metric tons of carbon dioxide in the US (U.S. Department of Energy, 2009). In comparison, the cement manufacture in the US generated only 42.2 million metric tons of carbon dioxide (U.S. Department of Energy, 2009).

If 10% of the carbon dioxide emitted annually from the US coal-fired power stations (∼1.5 billion tons

Table 1
Some startup companies attempting to commercialize algal fuels (Chisti and Yan, 2011).

Company	Location	Reference
Algenol Biofuels	Bonita Springs, FL, USA	www.algenolbiofuels.com
Aquaflow	Nelson, New Zealand	www.aquaflowgroup.com
Aurora Algae, Inc.	Hayward, CA, USA	www.aurorainc.com
Bioalgene	Seattle, WA, USA	www.bioalgene.com
Bionavitas, Inc.	Redmond, WA, USA	www.bionavitas.com
Bodega Algae, LLC	Boston, MA, USA	www.bodegaalgae.com
Joule Unlimited, Inc.	Bedford, MA, USA	www.jouleunlimited.com
LiveFuels, Inc.	San Carlos, CA, USA	www.livefuels.com
OriginOil, Inc.	Los Angeles, CA, USA	www.originoil.com
Parabel, Inc.	Melbourne, FL, USA	www.parabel.com
Phyco Biosciences	Chandler, AZ, USA	www.phyco.net
Sapphire Energy, Inc.	San Diego, CA, USA	www.sapphireenergy.com
Seambiotic Ltd.	Tel Aviv, Israel	www.seambiotic.com
Solazyme, Inc.	South San Francisco, CA, USA	www.solazyme.com
Solix Biofuels, Inc.	Fort Collins, CO, USA	www.solixbiofuels.com
Synthetic Genomics Inc.	La Jolla, CA, USA	www.syntheticgenomics.com

in 2012; U.S. Energy Information Administration: www.eia.gov/todayinenergy/detail.cfm?id=7350) can be converted to algal biomass, nearly 82 million tons of algal biomass could be produced. At 40% by weight oil in the biomass and 95% oil recovery, this is equivalent to 31 million tons of algal crude oil annually, or nearly 27,982 million liters of petroleum. (In energy terms, on average, 1 L of petroleum is equivalent to 1.25 L of algal crude oil (Chisti, 2012).) This is the equivalent of about 9 days of the total 2010 consumption of petroleum in the US. Clearly, therefore, the availability of point sources of carbon dioxide is a major impediment to production of algal fuel oils at a meaningful scale. Although algal biomass with a total lipid content of at least 40% by dry weight can be readily produced in outdoor processes (Quinn et al., 2012), the logic of relying on carbon dioxide from burning coal or other fossil fuel, to produce algal oil is flawed: no oil can be produced unless fossil fuels are burned.

The normal atmosphere contains around 0.039% of carbon dioxide by volume (Kumar et al., 2010). If somehow this source can be efficiently tapped at a low cost, the need for fossil-derived carbon dioxide will disappear altogether. Nor would there be a need for point sources of carbon dioxide. In addition, using carbon dioxide from the atmosphere is likely to greatly reduce the carbon footprint of algal fuels. Unfortunately, no method exists for growing algae at a high productivity using only the carbon dioxide available at the concentration in the normal atmosphere.

Many algae and cyanobacteria are known to possess mechanisms for concentrating carbon dioxide from the culture medium into the cell (Badger and Price, 1992; Kaplan and Reinhold, 1999; Badger et al., 2002; Giordano et al., 2005; Meyer and Griffiths, 2013), but carbon dioxide absorption from the standard atmosphere into the culture medium is never sufficiently fast to rapidly grow a large concentration of algae. Inadequacies in natural carbon concentrating mechanisms are such that carbon dioxide supplementation of an algal culture nearly always enhances biomass growth rate compared to what is possible under a normal atmosphere. Therefore, low-energy physical–chemical strategies are needed to cheaply capture and concentrate the carbon dioxide that is already in the atmosphere, for use in algal culture. The available technologies for nonbiological capture of carbon dioxide (Metz et al., 2005; Folger, 2010) are currently expensive.

Accelerating carbon capture via genetic modifications, for example, appears to be possible (Savile and Lalonde, 2011), but carbon dioxide will still need to be somehow transferred from the dilute level of the atmosphere to the algal broth. Transferring carbon dioxide from the atmospheric air to an algal culture is not easy because of the low concentration in the atmosphere. Also, in seawater the carbon dioxide solubility is low compared to in freshwater and this impedes carbon dioxide absorption. Low energy methods of achieving carbon dioxide absorption are necessary.

For algae that can be grown in highly alkaline conditions, carbon dioxide may be supplied in the form of bicarbonate (Chi et al., 2011). This may help in reducing the cost of supplying carbon dioxide (Chi et al., 2011), but may not be applicable for culturing marine algae as sea salts tend to precipitate once the pH exceeds about 8. Unfortunately, freshwater microalgae are not an option for large scale production of fuels as discussed elsewhere in this review (see Section 2.3).

Insufficiency of concentrated sources of carbon dioxide may suggest perhaps the use of organic carbon for algae culture. Growing certain microalgae on fixed organic carbon sources derived from plants is of course possible (Huang et al., 2010; Bumbak et al., 2011; Tabernero et al., 2012). Such heterotrophic growth achieves extremely high biomass productivities compared with growth on inorganic carbon and sunlight. Oil productivity is also high and this indeed is the fuel production model used by Solazyme, Inc. Unfortunately, heterotrophic production relies on fixed carbon that must be produced ultimately via photosynthesis. Heterotrophic growth can make use of suitably processed agroindustrial waste, but the amount of fuel available will be limited. Also, heterotrophic production of biomass in agitated and aerated fermenters consumes fossil energy. Anaerobic digestion of the residual biomass, discussed later (see Section 2.2.1), may be necessary to reduce reliance on fossil energy. An economic analysis of heterotrophic production of algal biodiesel found it to be expensive in view of the high cost of the bioreactors (Tabernero et al., 2012). This analysis was based on the use of supercritical carbon dioxide for extraction of the oil from the biomass that had been dried under vacuum (Tabernero et al., 2012). The choice of the drying and extraction methods was poor as neither are likely to be economically viable for the production of low value products such as fuel oils.

A growth mode combining photosynthesis and heterotrophy, or mixotrophic production, has a limited potential as a large-scale photosynthetic culture cannot be operated as a pure culture and contamination with bacteria growing on organic carbon will inevitably reduce productivity.

2.2. Supply of N and P nutrients

In addition to carbon dioxide, algal growth requires nitrogen (N) and phosphorous (P) as principal nutrients. The supply of P is of course finite (Cordell et al., 2009; Gilbert, 2009). Concerning N, almost as much N fertilizer can be produced as desired, but doing so will require fossil energy. Existing supply of N and P fertilizers for agriculture is insufficient to provide for any significant scale production of algal biomass for extraction of oils.

Fixing atmospheric nitrogen by the Haber–Bosch process (Travis, 1993), as currently used, requires a tremendous amount of energy. Nearly 1.2% of global energy consumption goes to producing N fertilizers for agriculture (Metz et al., 2007).

Total US consumption of N in 2010 was 12,285,000 tons (United States Department of Agriculture: www.ers.usda.gov/data-products/fertilizer-use-and-price.aspx#26720). Producing 82 million tons of algal biomass, as constrained by the supply of carbon dioxide (see Section 2.1), will consume 5.4 million tons of N, or nearly 44% of the existing usage. Similarly, the P requirements for producing 82 million tons of algal biomass are 1.1 million tons. If these vast quantities of nutrients are used to grow algae, how would we provide for crop agriculture?

Production of fertilizers requires energy (Metz et al., 2007). Ammonium nitrate used in algae culture media has an embodied energy value of 67 MJ kg^{-1} N. Embodied energy in P fertilizers is about 7.6 MJ kg^{-1} P_2O_5, or 3.3 MJ kg^{-1} P. Thus, the N and P nutrients contribute about 4.47 MJ per ton of algal biomass. This is low compared with the energy content of the biomass, but does need to be accounted for in addition to the other forms of fossil energy used in algal oil production processes.

Production of fertilizers releases carbon dioxide. The whole point of attempting to switch to biofuels is to reduce emissions of carbon. Therefore, producing more fertilizer through conventional technology, for use in algae culture may be counterproductive. All this suggests that N and P must be recovered from the algal biomass once the oil has been extracted and, somehow, recycled. As the oil contains relatively little N and P, most of the nutrients are in the spent biomass. Reclamation of nutrients is essential for sustainable production of algal fuels (Chisti, 2010a).

The only available technology for nutrient recovery appears to be anaerobic digestion of the spent biomass (Chisti, 2008a). Although, the algal biomass residue left after extraction of the oil could be used as protein rich animal feed, this would be feasible only if a compensatory reduction is made in crop production for forage so that the net demand for N and P fertilizers does not increase compared to the present situation. Recycling nutrients from an anaerobic digester to grow algae is difficult because of the dark color of the digester effluent which impedes light penetration in an algal culture. Reuse of the recovered nutrients for crop agriculture will potentially allow a certain amount of algal oil to be produced without diverting nutrients from agriculture. Unfortunately, once the annual production of algal biomass exceeds ∼186 million tons, equivalent to 20 days of the US petroleum consumption, nutrient recycling will be insufficient to meet demand and the production of N and P must expand beyond the current levels. Potentially, the Haber–Bosch process and its improved variants (IFA, 2009) can be used to make the N needed for algae culture, as the fossil energy input to algal biomass via the typically used ammonium nitrate is relatively small.

An alternative to recycling of N may be the following: nitrogen can be biologically fixed using cyanobacteria and other bacteria (Bergman et al., 1997; Brill, 1980; Zehr Jonathan, 2011) and this has the potential to be developed into a source of N for algae culture. The algae may be engineered for N fixation, or co-cultured with N-fixing cyanobacteria, or preferably with nonphotosynthesizing pale colored diazotrophs (Dobbelaere et al., 2003) that do not impede light penetration in a culture as much as would cyanobacteria. Nitrogen biofixation processes that are totally independent of algae culture, may be developed to provide fixed nitrogen. All these require a significant developmental effort, but have the potential to obviate the need for nitrogen fertilizers. Attempts are now being made to engineer a nitrogen fixing capability in some nonlegume crops (Saikia and Jain, 2007) to make them independent of soil nitrogen. Similar approaches are worth investigating in microalgae. If a nitrogen fixing capability can be engineered in combination with an enhanced photosynthetic efficiency, the loss in biomass productivity that would occur as a consequence of diversion of energy to nitrogen fixation may be minimized, or eliminated. An enhanced N-fixing capability attained via genetic engineering has already been demonstrated in the naturally diazotrophic cyanobacterium *Anabaena* (Chaurasia and Apte, 2011).

Nitrogen-fixation is of course extremely sensitive to inactivation by oxygen (Fay, 1992; Gallon, 1992) and therefore may appear to be incompatible with oxygen evolving photosynthetic processes. Nevertheless, nature has evolved many strategies for achieving nitrogen fixation in the presence of atmospheric oxygen (Fay, 1992; Gallon, 1992; Tuli et al., 1996; Bergman et al., 1997; Bohme, 1998; Adams, 2000; Gallon, 2001) and some of these may be enhanced via metabolic engineering to achieve simultaneous nitrogen fixation with photosynthesis. In principle, nitrogenases, the enzymes involved in nitrogen fixation, may be made more resistant to inactivation by oxygen.

In crop farming, application rate of the N fertilizers is the main contributor to the carbon foot print of agriculture (Hillier et al., 2009). In algae production, the carbon footprint per unit of N applied may be lower than in agriculture as N can be supplied to algae in such a way that most of it is taken up by the cells. In agriculture, only 30–50% of the total N applied is taken up by the plants (Hillier et al., 2009). Notwithstanding a more complete use of N fertilizers by algae, making algae independent of an external supply of nitrogen is essential for sustainable production of algal fuels.

The P supply can be enhanced through recovery from animal fecal matter (Schoumans et al., 2010), at least for crop use, so that more P is available to be channeled to algae culture. Some opportunity exists for using the nitrogen and the other compounds present in the flue gas of a coal fired power station, as nutrients for algal culture (Van Den Hende et al., 2012).

2.2.1. *Anaerobic digestion*

In addition to enabling reclamation and reuse of nutrients, the biogas produced by anaerobic digestion can be burnt to supply all the electrical power that is needed for production of algal biomass and its separation from the water (Chisti, 2008b; Harun et al., 2011). Drying of the biomass for extraction is not feasible, or the net energy recovery in the oil would be low (Chisti, 2012), or even negative. A biomass paste (86–90% moisture) will need to be used for extraction of the oil, but this is already possible (Goodall et al., 2011).

As previously mentioned, algae culture in seawater, or other saline water, is the only possible option for biomass production for making fuels at any significant scale. The saline algal biomass from such a process may have to be washed with freshwater to reduce the salt content so that the N and P fertilizer material recovered from the anaerobic digester is not too saline for use in crop agriculture.

Anaerobic digestion of saline biomass is of course possible in principle using halophilic methanogens (Paterek and Smith, 1985; Mathrani et al., 1988; Zhilina and Zavarzin, 1990; Nakatsugawa and Horikoshi, 1994; Riffat and Krongthamchat, 2007), but the commonly used technology of anaerobic digestion is suited only to biomass with relatively little salt in it. Therefore, research on anaerobic digestion for biogas production and nutrient recovery, is essential for commercializing algal fuels.

Significant information already exists on anaerobic digestion of algae biomass (Nyns and Naveau, 1981; Samson and Le Duy, 1982; Sánchez Hernández and Travieso Córdoba, 1993; Vergara-Fernández et al., 2008; Ras et al., 2011; Markou et al., 2013), but not much on the digestion of the residual biomass after the oil has been extracted. Economic assessments suggest that the biomass productivity of the culture system strongly influences the cost of producing methane by anaerobic digestion of the whole algal biomass (Zamalloa et al., 2011). Therefore, the algal biomass needs to be produced inexpensively.

Up to 90% conversion of the biomass chemical oxygen demand (COD) to biogas has been reported (Sánchez Hernández and Travieso Córdoba, 1993) as have lesser values (Ras et al., 2011). Nearly 0.18 $m^3\ kg^{-1}\ d^{-1}$ methane production has been reported on dry biomass basis with a methane content in the biogas of 65% by volume (Vergara-Fernández et al., 2008). This is equivalent to a biogas energy yield of ~4.3 $MJ\ kg^{-1}$ of dry algal biomass. All this is for whole biomass, not residual biomass. The relatively low energy recovery in the biogas is certainly due to incomplete digestion of the biomass. The biomass left after extraction of the oil, is likely to be more easily digested as the cells are likely to be broken by the extraction process and therefore should be more accessible to the digesting microorganisms.

Algal biomass produced under nutrient starved conditions typically has an energy content of 23,000 $kJ\ kg^{-1}$. The crude algal oil has an energy content of 35,800 $kJ\ kg^{-1}$ (Chisti, 2012). If the biomass contains 40% oil by weight, the residual biomass should have a quite substantial energy content of about 14,466 $kJ\ kg^{-1}$. Oil content in nutrient starved biomass produced under relatively controlled conditions have often exceeded 40% (Mazzuca Sobczuk and Chisti, 2010; Griffiths et al., 2012; Dillschneider et al., 2013), but high oil levels (≥40% by dry weight) have been achieved also in long-term outdoor biomass production operations (Quinn et al., 2012).

Algal biomass can contain substantial quantities of proteins (González López et al., 2010) and other useful products (Belarbi et al., 2000; Ward and Singh, 2005; Spolaore et al., 2006; Nichols et al., 2010). Therefore, the residual biomass is often suggested as a potential source of fodder and other products (Chisti, 2008a,b; Wijffels and Barbosa, 2010) that may help defray the cost of producing the algal oil. Unfortunately, in view of the limited energy content of the residual biomass, its diversion to uses other than anaerobic digestion does not seem to be realistically possible: attaining an energy ratio (see Section 2.4) of well above unity for the algal oil is essential for algal fuels to make any sense. An algal oil that is economically competitive with petroleum on an equal energy basis, but fails to achieve an energy ratio of unity, does not make sense from a sustainability perspective. Microalgae have been successfully engineered to produce certain high-value products (Rasala et al., 2010) that are needed in relatively small amounts. Producing such products in combination with fuel oils can certainly improve the economics of oil production so long as most of the residual biomass is used for energy recovery.

An alternative to oil extraction and subsequent anaerobic digestion of the spent biomass may be the use of hydrothermal liquefaction of the entire moist biomass to simultaneously produce crude fuel oil and combustible gases (Minowa et al., 1995; Sawayama et al., 1999; Brown et al., 2010; Biller and Ross, 2011; Biller et al., 2011; Duan and Savage, 2011; Valdez et al., 2011; Yu et al., 2011a,b; Alba et al., 2012; Vardon et al., 2012; Barreiro et al., 2013; Faeth et al., 2013; Frank et al., 2013). The inorganic nutrients may be recovered in the aqueous stream of hydrothermal liquefaction. Hydrothermal liquefaction of whole biomass (Minowa et al., 1995; Sawayama et al., 1999; Brown et al., 2010; Vardon et al., 2012; Barreiro et al., 2013; Faeth et al., 2013), or the biomass remaining after the extraction of oil (Yu et al., 2011a,b; Vardon et al., 2012), can actually enhance the overall oil production from a given quantity of biomass in comparison with solvent extraction of oil (Frank et al., 2013). Under certain conditions, the combustible gases produced through hydrothermal liquefaction may be used to supply sufficient energy to run the biomass production processes (Frank et al., 2013). Hydrothermal liquefaction offers opportunities for recycling of the inorganic nutrients, but some nutrient loss may occur through incorporation in the oil (Frank et al., 2013). Hydrothermal liquefaction requires further development and assessment.

Oil rich algal biomass could be produced using the N and P nutrients in domestic wastewater, but this option has a limited potential as discussed next.

2.2.2. *Wastewater as a source of nutrients*

Every large metropolis generates a significant quantity of domestic wastewater. Wastewater is once again attracting attention as a source of the N and P nutrients for producing algae (Kosaric et al., 1974; Woertz et al., 2009; Kumar et al., 2010; Christenson and Sims, 2011; Craggs et al., 2011). Unfortunately, algal fuels from wastewater can make only a minuscule contribution to the fuel supply.

In a best case scenario, algal oil from wastewater can contribute at most 1% to the petroleum demand of a large US city. The wastewater produced by a city of 10 million could at best provide about 425,000 metric tons of algal oil annually. This estimate is based on the following: a per capita wastewater generation rate of 378 $L\ d^{-1}$; a relatively high nitrogen content of 85 $mg\ L^{-1}$ in the wastewater; a relatively high phosphorous content in the wastewater of 10 $mg\ L^{-1}$ (8 $mg\ L^{-1}$ would have been more realistic); 6.6% nitrogen by weight in typical algal biomass; 1.3% phosphorous by weight in typical algal biomass; a full bioavailability of P; a 40% by weight oil content in the biomass; an algal oil density of 887 $kg\ m^{-3}$ (assumed to be the same as for palm oil); 1.25 L of algal oil being energetically equivalent to a liter of petroleum (Chisti, 2012); and an annual per capita petroleum consumption of nearly 3577 L in the United States.

Only about 73% of the total US petroleum consumption goes to making the main transport fuels of gasoline, diesel and kerosene. Therefore, if algal oil is used to displace only the petroleum consumed for making transport fuels, up to 3% of the annual fuel requirements of a large city of 10 million could be provided using algae grown on wastewater produced by the city. Clearly, the nutrients in wastewater cannot supply any substantial amount of algal biofuels.

2.3. *Water constraints*

The supply of freshwater is insufficient to support any substantial scale production of algal fuels anywhere. Supply of brackish water is also relatively limited. Therefore, use of seawater and marine algae are the only realistic options for making algal fuels. Using seawater for algae culture, unfortunately, does not totally eliminate the need for freshwater. Freshwater is needed to compensate for evaporative losses and a consequent increase in culture salinity. Evaporative loss depends on the local climatic conditions, particularly on the irradiance level, the air temperature, the wind velocity and the absolute humidity (Chisti, 2012). For example, in a dry region such as Goodlands, Western Australia (25°14′S, 123°55′E), the average monthly evaporation rate is 241.4 mm (Chisti, 2012). A freshwater evaporation rate of 10 $L\ m^{-2}\ d^{-1}$ has been mentioned for some tropical areas (Becker, 1994). This is equivalent to 0.01 $m^3\ m^{-2}\ d^{-1}$, or 10 $mm\ d^{-1}$.

Freshwater is necessary also for washing the biomass of salt prior to extraction of the oil (see Section 2.2.1). Therefore, production efforts focused on using freshwater algae species are shortsighted. To minimize consumption of freshwater, evaporative loss must be minimized. This may require a combination of closed production facilities and water recycling strategies (Pate et al., 2011). In addition, the location should be selected to minimize evaporation. Cultivation perhaps in shallow seas, saline lakes, constructed marine wetlands (Liu et al., 2012a,b) located adjacent to coastal areas may be some options. Use of halophilic marine algae that can withstand elevated salt levels, may be the way to go, but too high a salt concentration will incur a metabolic cost because the cell must either pump out the excess salt, or produce compounds that allow it to maintain an osmotic balance (Chen and Jiang, 2009).

An evaluation of the freshwater footprint of algal fuels is of course essential (Dominguez-Faus et al., 2009). For marine algae, the water footprint is smaller than the water footprint of oils derived from terrestrial crops (Rodolfi et al., 2009; Harto et al., 2010; Wijffels and Barbosa, 2010; Yang et al., 2011a). For algae grown in open ponds in freshwater, a total water footprint of nearly 3700 kg kg^{-1} of biodiesel has been estimated in the absence of water recycle (Yang et al., 2011a). Recycling reduces the water footprint to around 600 kg kg^{-1} of biodiesel (Yang et al., 2011a). Further reductions in water footprint may be feasible if the biomass can be grown in closed photobioreactors. If algae are grown in seawater in open ponds, freshwater is needed to compensate for evaporation, but the life-cycle freshwater use may be reduced to as low as 370 kg kg^{-1} of biodiesel (Yang et al., 2011a). Assuming a calorific value of 37.27 MJ kg^{-1} for biodiesel, the water footprint of algal biodiesel from seawater ponds is about 10 L MJ^{-1}. A different estimate suggests an average water consumption of 216 L per liter of algal biodiesel from open culture systems (Harto et al., 2010). This is equivalent to 6.59 L MJ^{-1}, assuming a biodiesel density of 880 kg m^{-3}.

The water footprint of biodiesel production from soybean has been estimated at 13,676 kg kg^{-1} of biodiesel (Yang et al., 2011a), or about 367 L MJ^{-1}. For ethanol from corn, the average water footprint is 138 L per liter of fuel (Harto et al., 2010), or about 6.54 L MJ^{-1}. For lignocellulosic ethanol from nonirrigated crops, the average water use is 6.5 L L^{-1} (Harto et al., 2010), or 0.31 L MJ^{-1}. In comparison, the estimated water footprint of petroleum extraction and refining is at most 0.05 L MJ^{-1}, based on published data (U.S. Department of Energy, 2006). Thus, the water footprint of algal biodiesel is much higher than for petroleum fuels, much lower than for biodiesel from soybean, and at least 32-fold that of lignocellulosic bioethanol.

2.4. *Energy ratio considerations*

Production of algal biofuels requires input of energy derived from fossil fuels. Fossil energy is used to mix and pump the culture broth, recover the biomass from the water via filtration and other processes, and extract the oil from the biomass. The algal crude oil produced has a certain energy content. Energy ratio, the ratio of the energy contained in the oil to the fossil energy required for making it, is an important measure that determines whether production of oil is worthwhile (Chisti, 2008b). An energy ratio of unity implies a nil net recovery of energy in the oil. Ideally, an energy ratio of well above unity is wanted, preferably a value of at least 7. Achieving a high energy ratio requires the spent biomass to be used in production of biogas for generating power to run the biomass production processes. Recovery of N and P fertilizers in the effluent of anaerobic digester can actually improve the energy ratio of algal crude oil. In addition, the processes of biomass production, recovery from water and oil extraction must be engineered to minimize fossil energy input (Chisti, 2008b; Sompech et al., 2012; Wongluang et al., 2013). Not much attention has been given to this issue so far. An energy ratio of 1.4 has been estimated for algal diesel (Liu et al., 2012a), but this may be low and has the potential to be greatly improved (Chisti, 2008b). Ideally, an energy ratio of at least 7 is wanted (Chisti, 2008b).

Energy ratio estimates for algal biodiesel have generally been less than unity (Khoo et al., 2011), but many such estimates are based on unrealistic assumptions. For example, an energy ratio of ~0.5 has been estimated for biodiesel production from algae in a process involving drying of biomass prior to solvent extraction and no recovery of energy from the spent biomass (Khoo et al., 2011). Other similar theoretical assessments have produced low estimates of energy returns (Cooney et al., 2011; Beal et al., 2012).

Any oil production process involving high-g centrifugal recovery of the biomass, energy-intensive physical breakage of the algal cells, drying of the biomass prior to extraction, use of the supercritical extraction methods, and membrane-based oil recovery techniques, is unlikely to be energy efficient. As discussed elsewhere in this review, production methods involving flocculation–sedimentation in combination with low-g centrifugation or continuous belt filtration should be considered for biomass recovery. The oil must be extracted from a moist biomass paste, unless the cells have been engineered for autolysis or secretion of the oils. The energy efficiency as well as biomass productivity of raceway ponds can be improved to some extent by changes to design (Sompech et al., 2012). Similar improvements in energy efficiency have been envisaged for photobioreactors (Wongluang et al., 2013). Hydrothermal liquefaction (Barreiro et al., 2013) of whole biomass, or spent biomass, may offer other opportunities for enhancing the energy ratio of algal fuels.

2.5. *Carbon footprint*

Carbon footprint (Zamboni et al., 2011) is a measure of the amount of carbon released in production and use of a given quantity of a material. The carbon footprint of an algal fuel relative to the carbon footprint of the petroleum fuel it displaces, is a key factor in determining whether its production is worthwhile. The carbon footprint of an acceptable biofuel must be smaller than the footprint of petroleum on an equal energy basis. This aspect has been so far largely ignored for algal fuels. Algal oil production technologies need to be designed to minimize the carbon footprint of the oil.

The published life-cycle analyses of algal fuels (Lardon et al., 2009; Batan et al., 2010; Clarens et al., 2010; Jorquera et al., 2010; Sander and Murthy, 2010; Stephenson et al., 2010; Brentner et al., 2011; Campbell et al., 2011; Clarens et al., 2011; Khoo et al., 2011; Kucukvar and Tatari, 2011; Pfromm et al., 2011; Shirvani et al., 2011; Singh and Olsen, 2011; Yang et al., 2011a,b; Frank et al., 2012) have been generally inconclusive with respect to their sustainability. In view of the developing technology and the often poor assumptions on which analyses are based, they are by no means definitive. A meta-analysis of several life-cycle assessments suggests that the energy consumption and greenhouse gas emissions associated with algae-derived biodiesel would be about the same as for soy biodiesel and for bioethanol from corn (Liu et al., 2012a).

Greenhouse gas emissions of 49 g MJ^{-1} of recovered energy have been claimed for soybean biodiesel (Hill et al., 2006). In comparison, the greenhouse gas emissions for petroleum diesel are 82.3 g MJ^{-1} of energy content (Hill et al., 2006). Estimates for greenhouse gas emissions for algal biodiesel have ranged from 78 to 351 g MJ^{-1}, depending on how the electricity used in the production of the algal fuel is generated (Shirvani et al., 2011). For example, in regions where electricity generation uses relatively decarbonized technologies such as hydropower and nuclear reactors, the greenhouse gas emissions of algal oil production would be low compared with regions producing electricity by burning fossil fuels.

In contrast to other data (Shirvani et al., 2011), greenhouse gas emissions of production and use of algal biodiesel have been estimated to be at most about 50% of the same emissions for biodiesel from canola (Campbell et al., 2011). For biodiesel from canola the emissions were 35.9 g CO_2 equivalent per ton-kilometer travelled (Campbell et al., 2011). The emissions for algal biodiesel were estimated for a hypothetical production scenario involving clay-lined raceways with a somewhat low biomass productivity of 0.015 kg m^{-2} d^{-1} and delivery of pure liquefied carbon dioxide to the production site by truck from a distance of 100 km. Flocculation was used for harvesting the biomass and energy was recovered from the residual biomass by anaerobic digestion (Campbell et al., 2011).

A life-cycle analysis of marine algae production concluded that in terms of the vehicle kilometers travelled per unit of cultivation area, algae were much better than corn, canola and switchgrass when compared on a raw biomass energy basis (Clarens et al., 2011). In terms of water use per vehicle kilometer travelled, algal biodiesel was better than canola biodiesel and bioelectricity from switchgrass (Clarens et al., 2011). In terms of greenhouse gas emissions per vehicle kilometer travelled, algal biodiesel was much better than canola biodiesel but comparable to electricity from switchgrass (Clarens et al., 2011). The energy demand for production of algal biodiesel on a per vehicle kilometer travelled basis was higher than for biodiesel from canola and bioelectricity from switchgrass (Clarens et al., 2011).

Nitrous oxide (N_2O), a greenhouse gas that is much more potent than carbon dioxide, has been sometimes detected in microalgae culture systems. Apparently, nitrous oxide is produced from nitrate by the denitrifying bacteria contaminating the culture (Batan et al., 2010; Fagerstone et al., 2011). Nitrous oxide is produced mainly under anoxic conditions (Fagerstone et al., 2011) that do not generally occur during daylight. In continuously mixed open ponds and aerated photobioreactors, there is generally sufficient oxygen also during the dark to prevent significant emissions of nitrous oxide. A nitrous oxide emission level of 0.002% of the nitrogen fertilizer applied has been estimated for oxic algal cultures (Fagerstone et al., 2011). During dark periods in anoxic conditions, the emission level may rise to 0.390% of the nitrogen fertilizer applied (Fagerstone et al., 2011). Compared with this, about 1% of the nitrogen fertilizer applied to terrestrial crops has been estimated to be lost as nitrous oxide. In terms of the carbon dioxide equivalents of global warming potential, the release of methane and nitrous oxide from large-scale open ponds during culture of the marine diatom *Staurosira* sp. has been found to be two orders of magnitude smaller than the overall carbon dioxide uptake by the microalga (Ferrón et al., 2012). These data were measured in a 2.4 ha pilot facility located in Hawaii, United States (Ferrón et al., 2012).

2.6. Limitations of the algae biomass production technology

Production of algal oil to replace just 1% of the US annual consumption of petroleum will require the production of a vast quantity of algal biomass, approximately 31 million tons at 40% by weight oil in the biomass.

Large scale culture technologies for producing algal biomass have been around for decades. Of these, the most commonly used raceway ponds (Terry and Raymond, 1985; Spolaore et al., 2006; Chisti, 2007, 2012) have an exceedingly low productivity compared to what the algal biology allows. A practical upper limit to algal oil productivity of conventional raceways is about 37,000 L ha^{-1} $year^{-1}$ (Chisti, 2012). Whether raceways can be used to produce sufficient algal biomass inexpensively for making biofuels is debatable (Chisti, 2012). Closed culture systems such as photobioreactors (Sánchez Mirón et al., 1999; Molina Grima et al., 2000; Acién Fernández et al., 2001; Carvalho et al., 2006; Wang et al., 2012) are more productive and can achieve a much higher concentration of algal cells in the water (e.g. 5 kg m^{-3}) than is possible in raceways (e.g. 0.5–1.5 kg m^{-3}), but are expensive and require a lot of energy to operate. A high energy expenditure for producing algal biomass is of course unacceptable (Chisti, 2008b; Sompech et al., 2012; Wongluang et al., 2013), or the net energy recovered in the algal oil will be low (see Section 2.4).

Novel biomass production methods that rely on sunlight, achieve high productivity and biomass concentration in the broth, and are inexpensive and energy efficient, are needed. Perhaps shallow raceways, or other low cost systems with a high surface-to-volume ratio to maximize sunlight capture and transmission into the algal broth, may be worth considering. Given a sufficiency of all nutrients, a high density algae culture is attainable only at high light intensities and only in a growth system with a shallow depth (Zijffers et al., 2010). Production processes involving wastewater-filled plastic bags floating in coastal waters, mixed by wave action and dewatered by forward osmosis, have been proposed by Jonathan Trent of the US National Aeronautics and Space Administration (National Aeronautics and Space Administration: www.nasa.gov/centers/ames/research/OMEGA/index.html; Trent et al., 2010, 2012).

What can be done to consistently maintain a high productivity during the entire cultivation period at least in regions where sunlight and temperature are essentially invariant with time, is a question yet to be answered for large scale outdoor culture of algae. Productivity can be substantially enhanced not only through engineering of a growth system, but also by addressing issues of algal biology as discussed later in this review.

A theoretical maximum productivity of algal crude oil has been estimated to be 354,000 L ha^{-1} yr^{-1} (Weyer et al., 2010) and the best case estimates have ranged from 40,700 to 53,200 L ha^{-1} $year^{-1}$, depending on geographic location (Weyer et al., 2010). The above estimate of the theoretical upper limit on oil productivity is substantially greater than some of the other estimates (Chisti, 2007).

In terms of the biomass and oil productivity, the existing culture systems do not come close to the biological limits of productivity. This is mainly because an algal culture is inevitably light limited due to mutual shading by the cells and is susceptible to photoinhibition at a light level that is only about 10% of the peak midday sunlight level in a tropical region. For an alga with an oil content of 40% by weight, a theoretical oil productivity of $\sim$159,000 L ha^{-1} yr^{-1} has been estimated (Cooney et al., 2011). Theoretical estimates of biomass productivity (40% by weight oil in biomass) have been as high as $\sim$0.095 kg m^{-2} d^{-1} (Cooney et al., 2011), but the limitations of the culture systems such as raceways reduce the average annual biomass productivity to only around 0.025 kg m^{-2} d^{-1} (Chisti, 2012). Biomass productivity would be lower if the oil content is higher. A theoretical maximum biomass productivity exceeding 0.080 kg m^{-2} d^{-1} (Cooney et al., 2011) has been estimated for biomass with an oil content of 60% by dry weight.

In terms of areal productivity of fixed carbon, the theoretical maximum has been claimed to be about 0.012 kg C m^{-2} d^{-1}, or 29.8×10^{-3} kg m^{-2} d^{-1} in terms of the dry algal biomass (Grobbelaar, 2009). Turbulence-induced light-dark cycling of the algal cells is claimed to increase the photosynthetic efficiency (Camacho Rubio et al., 2003; Mussgnug et al., 2007; Grobbelaar, 2009) so much so that the theoretical dry biomass productivity may approach 0.2 kg m^{-2} d^{-1} (Grobbelaar, 2009). Achieving this level of productivity would require a light-dark cycle frequency of about 100 s^{-1}, or a cycle time of 10 ms (Grobbelaar, 2009), but attaining this intensity of turbulence is impractical in a commercial culture device in view of the energy expenditure that will be necessary for it. Also, many algae are susceptible to damage by intense turbulence (García Camacho et al., 2001, 2011; Sánchez Mirón et al., 2003; Mazzuca Sobczuk et al., 2006; Gallardo Rodríguez et al., 2009, 2011; Chisti, 2010b) and, therefore, are unlikely to attain the predicted productivity.

2.7. Cost of production

If the problems of resource supply (water, carbon dioxide, fertilizers) can be resolved, algal crude oil will have to be produced competitively relative to petroleum. The price of petroleum fluctuates considerably and resembles little the actual cost of producing it. Price fluctuations mean an uncertain investment scenario for biofuels, including algal fuels. A potentially substantially reduced carbon footprint of algal crude oil compared to petroleum fuels, ought to be a primary driver for a move to biofuels. Nonetheless, the price at which algal fuels can be produced will be an important consideration, as it determines stakeholders' commitment to commercialization and the user affordability.

Inexpensive and low-energy processes for recovering the algal biomass from a fairly dilute broth and for extracting the oil from the moist biomass paste, are certainly required, but biomass recovery and oil extraction are not the major impediments to commercialization of algal oil as is sometimes claimed (Cooney et al., 2009; Uduman et al., 2010; Williams and Laurens, 2010; Mercer and Armenta, 2011; Halim et al., 2012; Lam and Lee, 2012; Sharma et al., 2013). The major difficulty is actually in producing a large quantity of the biomass sustainably and at a sufficiently low cost.

Estimates suggest that the algal biomass with an oil content of 40% by weight will have to be produced at a cost of no more than \$0.25 per kg (Chisti, 2012), if algal oil is to compete with petroleum at around the current price of \$629 per cubic meter (\$100/barrel). The actual cost of producing the biomass at present appears to be at least 10-fold greater.

Data from an algal biomass production facility operating multiple 3 m^3 tubular photobioreactors suggest a cost of €69 kg^{-1} for producing dry algal biomass (Acién et al., 2012). A simplification of the production technology and operation at a more economic scale of 200 tons/year are estimated to reduce the cost of producing the dry biomass to €12.6 kg^{-1} (Acién et al., 2012). If a supposedly lower cost raceway-based production facility can produce biomass at 10% of this price, the biomass would still be nearly 5-fold more expensive compared to the maximum acceptable production price of \$0.25 kg^{-1} (Chisti, 2012) required to achieve cost competiveness of the algal fuels with petroleum derived fuels. A biomass production cost of €4.95 kg^{-1} has been estimated for raceways (Norsker et al., 2011). Under optimized production conditions, this may be reduced to \$0.68 kg^{-1} according to one estimate (Norsker et al., 2011), but this nearly is nearly 3-fold greater than required. All existing commercial algal biomass production technologies are quite expensive for producing algal oils (Williams and Laurens, 2010; Richardson et al., 2012). Therefore, making algal fuels economically competitive with petroleum fuels will take quite some effort.

Depending on the production scenario, the estimates of cost of production of algal biodiesel have ranged from \$1.68 to >\$75 per liter (Nagarajan et al., 2013). A recent estimate suggests a final biodiesel production cost in the range of \$0.42–0.97 L^{-1} (Nagarajan et al., 2013). This is for production in raceway ponds with an assumed biomass productivity of $\geq$30 g m^{-2} d^{-1} and an oil content of 50% by weight in the biomass (Nagarajan et al., 2013). Both these number are rather high. Some estimates suggest that in terms of cost per ton-kilometer travelled, algal biodiesel may actually be less expensive compared with biodiesel from canola (Campbell et al., 2011).

The technology for recovery of the biomass and the oil is advancing (Chisti, 2012). Flocculation-sedimentation methods for inexpensively concentrating the biomass to a thick slurry already exist and are being further improved (Belarbi et al., 2000; Uduman et al., 2010; Spilling et al., 2011; Kim et al., 2012). A thickened slurry must of course be concentrated further to a paste for additional processing. This may be done using continuous belt filters and low-g centrifuges of the type used in concentrating the sludge produced in some wastewater treatment processes. In a pilot scale study of recovery of algal biomass from raceway ponds, dissolved air flotation and continuous belt filters were found to be more economical and energy efficient in comparison with centrifugation (Sim et al., 1988).

Existing and potential methods for extracting oils from algal biomass have been reviewed in the literature (Cooney et al., 2009; Mercer and Armenta, 2011; Halim et al., 2012). Conventional solvent extraction of the oil from moist biomass paste appears to be the most practicable method at present. Large-scale solvent extraction of algal oils from the biomass paste has been developed to some level (Goodall et al., 2011; Chisti, 2012), but further progress is needed. At a sufficiently large scale of operation, the oil can apparently be extracted for about \$21 per barrel of oil from a biomass paste that has about 40% oil by dry weight of biomass (Goodall et al., 2011). Nevertheless, there is a need to entirely eliminate the use of petroleum derived solvents from the recovery processes for algal oils. Ultimately, genetic and metabolic engineering of the algae will greatly reduce the cost and the energy demand of the recovery of the biomass and the oil. As discussed later in this review, some of these technologies have already been proven in principle. Hydrothermal liquefaction (Barreiro et al., 2013) as a solvent-free oil recovery technology deserves further study.

Notwithstanding their high cost and the other unresolved problems, the quest for sustainably produced algal fuels must continue. Fuels derived from terrestrial crops are not an option. This is because arable land cannot be diverted from existing use in producing food, forage and fiber; arable land area cannot be increased because of factors relating to deforestation and loss of biodiversity; and freshwater is already in short supply in many regions and must provide for other essential needs.

Petroleum is not likely to run out in the near future and there is a lot of coal available for producing liquid fuels for many decades. The need for alternative fuels is driven by global warming considerations associated with the consumption of fossil fuels and emission of carbon dioxide. An increasing demand for energy and the quest for energy security are other drivers for alternatives to fossil fuels. The real cost of fossil fuels is in their long-term environmental impact. This severe impact calls into question the continued existence of human civilization and indeed life, if allowed to go unchecked (Walther et al., 2002; Gosling et al., 2011; Sumaila et al., 2011; Matyssek et al., 2012). Fossil fuels may be cheap and readily available, yet we need to find alternatives to them, or develop technologies which negate the impact of burning fossil fuels. The latter technologies (Figueroa et al., 2008; Folger, 2010) could of course only take us so far, as at some point we will surely run out of inexpensive petroleum, coal and, eventually, natural gas. We need renewable biofuels.

3. Genetic and metabolic engineering for enhancing commercialization prospects

In developing fuels from algae, the algal biology has been the most neglected area but has the greatest potential for profoundly impacting the viability of oil production. Some of the questions that need attention are the following:

1. How to improve the efficiency of algal photosynthesis?
2. How to eliminate the need for external inputs of nitrogen?
3. How to improve penetration of light in a dense culture of algal cells?
4. How to channel the energy captured by the algal cell via photosynthesis, to production of oils?
5. How to enhance carbon capture by the cells?
6. How to coax the cells to excrete the oil?
7. How to achieve controlled autoflocculation of the biomass to facilitate recovery from the water?

All these questions can be addressed through genetic and metabolic engineering (Gust et al., 2008; Rosenberg et al., 2008;

Waltz, 2009; Radakovits et al., 2010, 2011; Lü et al., 2011; Stephenson et al., 2011; Zeng et al., 2011; Day et al., 2012; Larkum et al., 2012; Qin et al., 2012), therefore, genetic and metabolic engineering are essential for the development of a future viable algal oil industry. In addition, the use of synthetic biology methods may allow algae to be produced with new capabilities (Schmidt et al., 2010; Heidorn et al., 2011; Georgianna and Mayfield, 2012; Noor-Mohammadi et al., 2012; Maurino and Weber, 2013).

In terrestrial crops, for example, estimates suggest the potential for substantially enhancing the total biomass yield through improved efficiency of photosynthesis (Long et al., 2006). Similar improvements are certainly possible for microalgae. Some of the target genes for improving the photosynthetic efficiency of microalgae have been noted by Stephenson et al. (2011). The techniques of genetic engineering of algae have been reviewed by Qin et al. (2012). Other metabolic engineering aspects are discussed by Lü et al. (2011). Selection and breeding of algae for enhanced production of fuels have been discussed by Day et al. (2012) and Larkum et al. (2012).

Genetic and genomic methods are already being applied to improve production and recovery of algal fuels (Waltz, 2009), although much remains to be done in all the key areas. Genomes of only a few eukaryotic microalgae have been fully sequenced (Finazzi et al., 2010; Khozin-Goldberg and Cohen, 2011; Lü et al., 2011) and this greatly hinders their genetic and metabolic engineering. Furthermore, the molecular biology tools required for genetic transformation of algae are barely developed, but accelerating effort is being made to address this lack of knowledge (Finazzi et al., 2010; Radakovits et al., 2010; Lü et al., 2011; Tirichine and Bowler, 2011; Weiss et al., 2011). Some proof-of-concept progress has been made already in engineering algae as shown in the following sections.

3.1. Improving light supply to algae culture

Improved penetration of light in algae cultures has been shown to be possible by reducing the size of the light harvesting chlorophyll antenna (Nakajima and Ueda, 1997, 2000; Polle et al., 2003; Mussgnug et al., 2007; Melis, 2009). Antenna truncation, without reducing the total number of photosynthetic units in the cell, increases photosynthetic efficiency (Mussgnug et al., 2007; Oey et al., 2013). Efficiency improvements of up to 3-fold are predicted to be possible through this technology (Melis, 2009), but its usefulness is limited. This is because a suspension of algal cells that are entirely free of a pigment such as chlorophyll will still impede light penetration into the broth once the biomass concentration has exceeded a few grams per liter.

The strategy of reducing the antenna size is faced with other complicating factors: marine microalgae modify their pigment composition in response to light level (Finazzi et al., 2010). For example, under high light, production of photoprotective and antioxidative carotenoid pigments is enhanced (Finazzi et al., 2010). In low light, algae photoacclimatize by either increasing the amount of chlorophyll in the chloroplast, or increasing the size of the light-harvesting complexes (Finazzi et al., 2010).

The fraction of the sunlight spectrum available to a microalga for photosynthesis can be potentially broadened (Chen and Blankenship, 2011) through genetic engineering quite independently of any increase in the efficiency of photosynthesis. Most microalgae rely on chlorophylls *a* and *b* for light absorption, absorbing only the visible portion of the light, i.e. the light in the wavelength range of 400–700 nm, the photosynthetically active radiation (PAR). A modest increase in the absorption range to 750 nm can potentially increase the number of photons available for photosynthesis by about 19% (Chen and Blankenship, 2011). At least one marine cyanobacterium, *Acaryochloris marina*, is known to absorb light in the 700–750 nm range using chlorophyll *d* as the absorption pigment (Miyashita et al., 1996; Larkum and Kühl, 2005; Chen and Blankenship, 2011; Mielke et al., 2011). Potentially chlorophyll *d* could be coexpressed with chlorophylls *a* and *b* in microalgae to make more energy available for photosynthesis without actually affecting the efficiency of photosynthesis per se. Other totally different natural microbial light harvesting pigments (e.g. chlorophyll *f*; Chen et al., 2010) could provide opportunities for a greater expansion of the sunlight spectrum that is useable for photosynthesis. Some microorganisms use pigments with light absorption maxima of up to 760 nm to photosynthesize carbohydrates from carbon dioxide and water (Koehne et al., 1999; Schlodder et al., 2005; Wilhelm and Jakob, 2006). Overexpression of photosystems is another potential strategy for improving light capture (Stephenson et al., 2011).

3.2. Reducing photoinhibition

During peak sunlight, photoinhibition substantially reduces the biomass productivity in open ponds (Grobbelaar, 2012) and photobioreactors. Elevated light levels induce photoinhibition of photosystem II (Finazzi et al., 2010) and this adversely impacts utilization of incident light. Photoinhibition may be potentially reduced, for example, by increasing the concentration of the photoprotective pigments, changing their compositional profile (Stephenson et al., 2011), or enabling the cell to produce entirely new and more effective protective pigments and reactive oxygen species scavenging molecules. Of course, there is always a metabolic cost associated with these strategies (Raven, 2011).

3.3. Engineering of Rubisco and other enzymes of the Calvin cycle

Molecular level engineering of the Rubisco enzyme (ribulose-1,5-bisphosphate carboxylase oxygenase) has the potential to enhance its affinity for carbon dioxide and reduce its affinity for oxygen (Stephenson et al., 2011). Rubisco engineering may be used to reduce photorespiration that results in the recently fixed carbon being converted back to carbon dioxide, thus effectively reducing the efficiency of photosynthesis. Engineering of Rubisco may have little associated metabolic cost compared with its overexpression, but has not been successful (Stephenson et al., 2011). The enzymes downstream of Rubisco in the Calvin cycle may be overexpressed, or engineered at the molecular level, to improve the efficiency of photosynthesis.

The Rubisco enzyme in some algae appears to be superior to Rubisco of most green plants in converting carbon dioxide to carbohydrates. A mathematical model suggests that the biomass productivity of many plants can be substantially enhanced by engineering them to express the Rubisco of the non-green alga *Griffithsia monilis* (Zhu et al., 2004). Could yet better Rubiscos be found in some algae?

3.4. Lipid synthesis, quality and recovery

Bioprocess engineering methods for enhancing lipid production rely on modifying the production environment to favor the production or accumulation of lipids. This generally involves imposing some kind of a physiological stress (e.g. nutrient starvation, temperature change, salinity increase) to redirect metabolic fluxes to accumulation of lipids (Illman et al., 2000; Courchesne et al., 2009; Rodolfi et al., 2009; Mazzuca Sobczuk and Chisti, 2010). There are other ways for improving the production of lipids (Huerlimann and Heimann, 2013). Biosynthetic pathways for lipid production can be genetically modified to enhance the synthesis of lipids, for example, by overexpressing the rate limiting enzymes (Courchesne et al., 2009; Huang et al., 2010; Khozin-Goldberg and Cohen, 2011). The enzymes involved in lipid synthesis may themselves be molecularly engineered for improved catalytic effectiveness. The transcriptional factors involved in regulating a metabolic pathway may be overexpressed to favor lipid synthesis (Courchesne et al., 2009). Entire metabolic pathways may be potentially reengineered to favor the production of desired lipids, or entirely novel molecules (Akhtar et al., 2013). Increasing information is available on the biosynthetic pathways for some algal lipids, but how the lipid production is regulated is less well known. Lipid metabolism of photosynthetic eukaryotic microalgae is reviewed elsewhere (Courchesne et al., 2009; Huang et al., 2010; Khozin-Goldberg and Cohen, 2011).

The main regulatory steps involved in fatty acid biosynthesis, import into the endoplasmic reticulum and further transformation into glycerolipids in microalgae appear to be similar to those of higher plants (Khozin-Goldberg and Cohen, 2011). The focus of research on lipid biosynthesis appears to be largely on enhancing the productivities of fatty acids and triacylglycerol oils (Courchesne et al., 2009; Khozin-Goldberg and Cohen, 2011; Yu et al., 2011c; Blatti et al., 2012) as these are of direct relevance to production of biodiesel from microalgae. As previously noted, all carbon-rich oils (Chisti, 2012) and not just triacylglycerols are potential feedstock for making various liquid transport fuels from algae. Therefore, maximizing the total lipid synthesis and not just the synthesis of triglycerides, ought to be considered.

Genetic modifications have altered the biochemical profiles of algal lipids (Radakovits et al., 2011; Yu et al., 2011c; Zhu et al., 2011) to provide more desirable types of oils. Various strategies of modifying the lipid production by an algal cell have been suggested (Courchesne et al., 2009). Engineering the cells to excrete lipids has been demonstrated (Liu and Curtiss III, 2009; Liu et al., 2011a,b). Microalgae such as *Botryococcus braunii* naturally secrete oils (Wolf et al., 1985; Banerjee et al., 2002).

3.5. Improving biomass recovery

Algae are known to be able to autoflocculate under certain conditions (Sukenik and Shelef, 1984). Engineering a cell age triggered autoflocculation mechanism may be possible to facilitate recovery of the cells from the water. Engineering of autoflocculation capability has been demonstrated in bacteria and yeasts (Ogden and Taylor, 1991; Hammond, 1995; Ostergaard et al., 2000; Bauer et al., 2010) and may be attainable in microalgae.

3.6. Use of cyanobacteria

In addition of microalgae, cyanobacteria have attracted much attention as a source of biofuels (Heidorn et al., 2011; Robertson et al., 2011; Liu et al., 2011a,b; Machado and Atsumi, 2012; Rosgaard et al., 2012). Genetic modification of cyanobacteria is easier in comparison with eukaryotic microalgae. Some cyanobacteria can naturally fix nitrogen and thrive in bicarbonate containing alkaline media. Photosynthetic conversion of carbon dioxide directly to secreted combustible hydrocarbon diesel using genetically modified cyanobacteria has been argued to be overall superior to the use of microalgae to produce triglyceride oils for conversion to biodiesel in a separate process (Robertson et al., 2011). Facets of such a technology have been demonstrated (Liu et al., 2011a,b). Genetic engineering of cyanobacteria for improved fixation of carbon dioxide has been discussed by Rosgaard et al. (2012). Production of alkanes, fatty acids and alcohols in cyanobacteria has been reviewed by Machado and Atsumi (2012).

Potentially, the metabolic productivities of nonphotosynthetic microorganisms may be enhanced by engineering them to incorporate light-driven processes (Johnson and Schmidt-Dannert, 2008).

3.7. Ecological issues

The use of genetically modified algae for biofuels may be inevitable, but does raise concerns relating to ecological impact (Menetrez, 2012; Snow and Smith, 2012; Borowitzka and Moheimani, 2013). Open culture systems with provisions for intercepting releases of culture broth may be used, but a total containment is unlikely to be feasible. Of course, genetically modified terrestrial crops have been widely cultivated in the open for decades (Gressel, 2008), but doing so with microalgae may pose new problems.

4. Concluding remarks

The impediments to commercialization of algal fuels are many: impossibly high demands on certain key resources; the high cost of production; and the need to achieve an energy ratio of well above unity. Potential solutions to all these issues are being investigated and production of certain niche fuels such as jet fuel may become feasible up to a certain scale. A widespread availability of algal fuels is certainly not likely in the near term. Consumption of petroleum continues to increase. Replacing a significant fraction of this consumption with algal oil cannot happen without development of new technologies. Focused research both on algal biology and the engineering of the production systems is necessary. In addition, peripheral but enabling technologies — carbon dioxide concentration from the atmosphere, for example — need to be developed to make algal fuels feasible. Interest in commercial production of algal fuels continues to be strong, suggesting that the possibility of an economically viable production at some scale and within a reasonable timeframe should not be totally discounted.

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