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ORIGINAL ARTICLE

Production of biofuels from microalgae

Sophie Fon Sing · Andreas Isdepsky · Michael A. Borowitzka · Navid Reza Moheimani

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Abstract The production of biofuels from microalgae, especially biodiesel, has become a topic of great interest in recent years. However, many of the published papers do not consider the question of scale up and the feasibility of the various processes to be operated at the very large scale required if algal biofuels are to make a meaningful contribution to renewable fuels. All the steps in the process must also be very low cost. This paper discusses the unit processes required for algal biofuels production (i.e., growing the algae, harvesting, dewatering, extraction and conversion to biofuel) and their scalability. In many cases, especially in the lipid extraction step, little is known as yet as to the scalability and economic feasibility of the various processes proposed. We also highlight the key engineering and biological issues which must be resolved for the production of biofuels from microalgae to become an economic reality.

Keywords Biofuel · Algae · Scalability · Culture · Harvesting · Extraction · Transesterification

1 Introduction

The recognition that global oil supplies are finite and that the world is close to reaching 'peak oil', combined with the threat of global warming due to anthropogenic CO_2 emissions has created enormous interest in the last few years in the development of renewable energy sources, including biofuels (Martinot et al. 2007; Verbruggen and Al Marchohi 2010). Several different biofuels can be produced from plant biomass. These include biodiesel from oil seed plants such as soy bean, rape seed, canola, Jatropha, and oil palm, and ethanol from starch plants such as corn, wheat, sugar cane and cassava or from cellulosic biomass such as eucalypts, *Miscanthus* and sugar cane. The question of using potential food crops to make biofuels has led to discussions about 'food vs. fuel' and the

S. Fon Sing · A. Isdepsky · M. A. Borowitzka · N. R. Moheimani (✉)
Algae R & D Center, School of Biological Sciences and Biotechnology, Murdoch University, Murdoch, WA 6150, Australia
e-mail: n.moheimani@murdoch.edu.au

limitations of available agricultural land and fresh water (Hill et al. 2006). Algae have been proposed as an alternative source of plant lipids for the manufacture of biodiesel and possibly also of sugars for fermentation to ethanol. Algae are perceived to have the advantage of requiring less land area to produce an equivalent amount of fuel, and there are also many species which can be grown using saline water. The proposal to use algae for biofuels production is not new and was first suggested in the 1940s, and in the 1990s extensive research was carried out, especially at the Solar Energy Research Institute in Golden, Colorado, USA (Sheehan et al. 1998). Aside from photoautotrophic growth for algal lipid production the mixotrophic and/or heterotrophic culture of algae on sugars from higher plant sources has also been proposed (Lu et al. 2010) although it is hard to see what the advantage is of using these sugars which could be converted directly to ethanol by fermentation.

Currently significant research effort is being focused on developing a viable algae-to-biofuels process around the world, but the major challenge remains to be able to produce such algal biofuels at the scale required and to do this economically (Greenwell et al. 2010; Stephens et al. 2010). The scale of production for significant quantities of algae-derived fuel is extremely large. For example, Borowitzka and Moheimani (2011) have calculated that in order to produce 100,000 bbl of algae oil per year (equivalent to about 10% of Australia's daily requirement), assuming an areal annual average biomass productivity of 20 g dry weight m^{-2} d^{-1} and an 30% total lipid content, some 650 ha of pond and almost 4 GL of water $year^{-1}$ are required (this calculation assumes a 100% conversion efficiency of the lipid to biodiesel, a figure which is very unlikely to be achieved). The productivity figure used in this calculation is the best achieved in any long-term cultures so far outdoors using natural sunlight; the upper theoretical maximum productivity which could be achieved under ideal conditions is about 30–40 g m^{-2} d^{-1} (Grobbelaar 2009b; Walker 2009). High annual lipid productivity is the first essential step in producing biofuels from algae (Griffiths and Harrison 2009), but to achieve an economic process all steps in the production process from algal culture to actual fuel production must be efficient at scale and optimized. Therefore, as previously pointed out by Richmond (1999), the mass culture of microalgae for biofuel production is not an engineering or biological challenge, but is a combination of both.

Here we consider the various steps in the process and their alternatives, as well as aspects of scale-up to the extremely large scale required. Our aim is to highlight some of the key biological and engineering challenges presented by the very large scale of an algae biofuels plant which need to be resolved.

The process of production of biofuels from microalgae requires several steps:

- production of lipid-rich algal biomass;
- harvesting of the biomass from the growth medium generally combined with recycling of the medium;
- dewatering of the harvested biomass (possibly with drying of the biomass);
- extraction of the lipids (and sugars);
- conversion of the lipids and sugars (and possibly the remaining biomass) to biofuels;
- further processing of the remaining biomass to other products of some value.

These process steps are shown diagrammatically in Fig. 1. A variety of culture systems are possible (see section below), but all require an input of water [1], CO_2 preferably from a source such as a power station or a cement plant, with the addition controlled by a pH-stat system [2], nutrients (mainly an N and P source, but may also include trace elements and, if

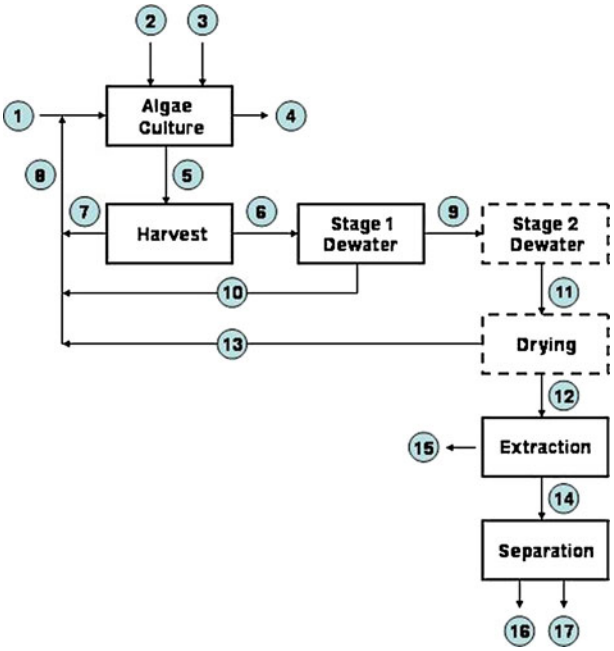

Fig. 1 Process flow diagram of algae to bio-oil. (*1*) Feed water; (*2*) CO_2; (*3*) Nutrients; (*4*) Evaporation water loss; (*5*) Harvested culture; (*6*) Concentrated culture; (*7*) Water removed during harvesting; (*8*) Recycled water; (*9*) Stage 1 dewatered culture; (*10*) Water removed from Stage 1; (*11*) Stage 2 dewatered culture; (*12*) Dried culture [<1% water]; (*13*) Water removed from dryer; (*14*) Extracted lipids; (*15*) Remaining biomass; (*16*) Polar lipids; (*17*) Nonpolar lipids. Note: *Boxes with dashed borders* show unit processes which possibly may be excluded depending on the requirements for extraction

diatoms are being cultured, silicon). In a fast growing culture approximately 30–50% of the culture volume would be removed each day [5] for harvesting and dewatering. The harvesting and dewatering steps are depicted as 3 stages depending on the processes used and the degree of dewatering required for the extraction step. If dry biomass is required for extraction then a drying step must also be included.

2 Algae culture systems

The development of large-scale microalgae culture began approximately 60 years ago (Burlew 1953; Tamiya 1957). Two basic types of culture systems were recognized and developed at that time: 'open' pond systems and 'closed' photobioreactors. There now is extensive experience in commercial microalgae culture which began with *Chlorella* production as a health food in the 1950s in Japan and Taiwan (Soong 1980), followed by *Spirulina* (*Arthrospira*) in the 1970s in Mexico and the USA (Vonshak 1997), *Dunaliella salina* for the production of β-carotene in the 1970s in Australia, Israel and the USA (Borowitzka and Borowitzka 1989; Borowitzka 2010b), *Haematococcus pluvialis* for astaxanthin production in the 1980s in the USA (Lorenz and Cysewski 2000), and *Crypthecodinium cohnii* for eicosapentaenoic acid production in the USA (Mendes et al. 2009). Commercial-production of these algae is now taking place in many countries, especially in Australia, China, India, Israel, Taiwan and the USA. Almost all commercial producers use open ponds where the algae culture is mixed either by paddle wheels (raceway ponds) or by a centrally pivoted rotating arm. The exceptions are the culture of *Haematococcus pluvialis* in Israel and *Chlorella* in Germany, both of which are grown in tubular photobioreactors, the culture of *H. pluvialis* in Hawaii using dome-shaped photobioreactors, and the heretotrophic production of the thraustrochytrid *Crypthecodinium*

which is grown heterotrophically in fermenters. *Dunaliella salina* is also grown in very large (>200 ha in area), shallow unmixed ponds in Australia (Borowitzka and Hallegraeff 2007), and *Chlorella* is also grown on a small scale in a very shallow (as low as 10 mm) cascading system in Trebon, Czech Republic (Doucha et al. 2005). A number of other species of microalgae (e.g., *Isochrysis, Pavlova, Nannochloropsis, Tetraselmis, Chaetoceros* etc.) are also produced for use a feed in aquaculture at a wide range of scales uses both 'open' and 'closed' culture systems, mainly large 'bags' or tanks (Borowitzka 1997; Fulks and Main 1991; Zmora and Richmond 2004). Aside from the production of microalgae for valuable compounds there is also significant large-scale culture of algae in wastewater treatment, especially in the so-called high rate oxidation ponds (Craggs et al. 2003; Green et al. 1996; Oswald 1988).

The production of algal biomass for biofuels, however, requires significantly larger scale algal culture than is currently practiced commercially. Production costs must also be substantially lower. For example, the lowest production cost in commercial algae production today is about US$4–5 kg^{-1} algal biomass (Borowitzka 1999b), for algal biomass for biofuel production this cost must be less than US$1 kg^{-1}.

2.1 Open culture systems

The main reason why most commercial producers of microalgae use open pond systems is that these systems are much cheaper to construct and operate at a large scale compared to closed photobioreactors. A variety of open systems with different design exist and they vary in size, shape, material for construction, type of agitation and inclination (Borowitzka 1999a, 2005). These systems can be classified as (a) extensive shallow unmixed ponds; (b) circular ponds mixed with a rotating arm, (c) 'raceway' ponds, usually mixed with a paddle wheel, and (d) sloping thin-layer cascade systems. Some of the key characteristics of these systems are summarized in Table 1. The possibility of contamination is often cited as a serious limitation of open systems and it is true that most of the species cultured in such systems currently do grow in selective environments; i.e. *Arthrospira* (*Spirulina*) [high alkalinity]; *Dunaliella salina* [high salinity], *Chlorella* [high nutrients] (Belay 1997; Borowitzka 2010b; Soong 1980). However, other species with 'normal' growth requirements have also been grown successfully in open ponds, either in batch mode [e.g. *Haematococcus pluvialis* (Cysewski and Lorenz 2004)], or continuously for very long periods [e.g., *Phaeodactylum tricornutum; Nannochloropsis* and *Pleurochrysis carterae* (Ansell et al. 1963; Matsumoto et al. 1996; Moheimani and Borowitzka 2006)] without significant contamination problems.

A key limitation to productivity in open pond culture is light. As most open ponds have to be operated a depths of 20–30 cm due to hydraulic limitations this means that at the surface of the pond the algae are exposed to very high light, whereas at the bottom of the ponds the algae are in near darkness (Grobbelaar 1981). The culture needs to be mixed to keep the algae in suspension and move them from the lower (darker) layers to the upper (high light) layers of the ponds to maximize the amount of light the cells receive (see Section 3 below). An alternative approach to overcome this light limitation in raceway ponds was developed by Setlík and co-workers (1970) in the Czech Republic and further improved by Doucha and Livansky (1999, 1995) . In this system the algae culture flows over a sloping surface in a thin (0.5–1 cm) layer before being collected at the bottom and being pumped again to the top of the slope. This 'cascade' system can achieve high biomass densities of up to 10 g L^{-1} for *Chlorella* and achieves higher productivities compared to a raceway pond (Doucha et al. 2005).

Table 1 Characteristics of open algae culture systems

System	Maximum size of single unit	Relative capital cost	Relative operating cost	Operating depth (length of light path)	Main limitations	Typical species cultured	Long-term areal and volumetric productivity	Selected references
Circular Central Pivot Pond	~0.5 ha	High	Low	30–70 cm	Uneven mixing, becoming more so with increasing diameter of pond; light limited	*Chlorella, Spirulina*	10–20 $g.m^{-2}.d^{-1}$ 0.2–0.16 g L^{-1} d^{-1}	(Kanazawa et al. 1958)
Raceway Pond with paddle wheel	0.5–1 ha (at flow rate of about 30 cm s^{-1} and 25–30 cm depth)	Medium	Low	20–30 cm	Uneven flow in most large ponds, especially at corners; light limited	*Chlorella, Spirulina, Dunaliella salina, Haematococcus pluvialis, Phaeodactylum tricornutum, Pleurochrysis*	15–25 $g.m^{-2}.d^{-1}$ (annual average) [Up to ~40 g. $m^{-2}.d^{-1}$ for short periods under optimum conditions] 0.05–0.3 g L^{-1} d^{-1}	(Borowitzka 2005; Jiménez et al. 2003; Moheimani and Borowitzka 2006; Oswald 1988; Pushparaj et al. 1997)
Thin layer cascade system	1 ha ?	Medium	Low	>5 mm	Circulation system (pumping) may damage fragile cells	*Chlorella, Scenedesmus*	25 $g.m^{-2}$ day^{-1} 10 g L^{-1} d^{-1} (summer only)	(Doucha and Livansky 1995; Lee 2001; Setlík et al. 1970)
Covered raceway pond	0.5–1 ha	High	Low	20–30 cm	Uneven flow in most large ponds, especially at corners; Light limited; High temperatures due to limited cooling by evaporation may require cooling of the pond		?	(Walmsley and Shillinglaw 1984)

2.2 'Closed' systems

A very wide variety of closed photobioreactors has been developed (Tredici 2004), but fundamentally they can be classified as (a) bags or tanks, (b) towers, (c) plate reactors, or (d) tubular reactors (Borowitzka 1999a). Large bag systems consist of clear plastic bags usually supported by a metal mesh frame or hung from supports and are widely used in the aquaculture industry (Fulks and Main 1991). Similarly the 'tower' systems, which are vertical cylinders usually made of fiberglass or acrylic, are also widely used in aquaculture. Plate reactors come in many forms ranging from alveolar panels with internal baffles, to vertical thin plate-like tanks made of glass, Perspex or of metal mesh frames containing a plastic bag (Hu et al. 1998; Tredici and Materassi 1992). The tubular photobioreactors are generally constructed of glass, clear Teflon tubing, or clear PVC tubing arranged either in parallel straight lines (Torzillo et al. 1993) or helically wound around a central support tower (the 'Biocoil'; Oxley and Startari 1999; Robinson et al. 1988). In the parallel tube photobioreactors the tubes may be arranged vertically (the 'bio-fence' types) or the tubes may lay on the ground in one or more layers (Acién Fernández et al. 2001; Torzillo et al. 1993). Table 2 compares some of the properties of these systems. Circulation of the algae culture is achieved by a variety of pumps including airlifts, centrifugal, diaphragm or lobe pumps. Temperature control, which generally means cooling during the day, is by installed heat exchangers or by evaporative cooling by spraying water over the reactor surface. One of the earliest large tubular photobioreactors had the tubes floating in a large pool for temperature control (Chaumont et al. 1988), and new variations of this are being developed. Photobioreactors also have been placed in glasshouses for temperature control, especially in temperate locations, but this increases further the capital costs of the culture system. Furthermore, several lifecycle assessments of closed photobioreactors systems (e.g., Jorquera et al. 2010; Stephenson et al. 2010) have indicated that the energy requirement of operating these is significantly higher than the energy requirement of open raceway-type ponds and can potentially exceed the energy content of the algae produced.

An often cited advantage of 'closed' photobioreactors is the ability to eliminate contamination. However, it is also impossible to keep a large 'closed' photobioreactor sterile; i.e. to maintain an axenic culture for the relatively long periods required to grow the algae, even in batch mode. The largest steam sterilizable photobioreactors built are a 250 L internally lit photobioreactor (Pohl et al. 1988) and the 100 L tubular glass 'Medusa' photobioreactor (Walter et al. 2003). Other reactors need to be chemically sterilized. However, as all photobioreactors need to be supplied with air and CO_2 and as the O_2 generated by algal photosynthesis must be removed sterility cannot be maintained for longer than about 10–12 days. Our experience with long-term algal culture in 1,000 L 'closed' helical tubular photobioreactors (Biocoils) has shown that contamination (including contamination by protozoa) can be managed, but not eliminated.

2.3 Hybrid systems

Hybrid culture systems using a combination of a closed photobioreactor and an open raceway pond have also been proposed. The closed photobioreactor produces a high density inoculum for the open ponds, thus optimising biomass production in the shortest time possible (Grobbelaar 2000). This 2-step concept has been employed, for example, for the production of astaxanthin in *Haematococcus pluvialis* in Hawaii (Olaizola 2000), but it is generally more expensive in both capital and operating costs as it is a batch rather than a continuous culture process and thus is not suitable for a low cost product such as biofuels.

Table 2 Comparison of some characteristics of 'closed' algal culture systems. Productivity figures are examples only as comparison between published figures is difficult due to the differences in the duration of culture. Long-term productivity data are almost non-existent

System	Maximum size of single unit	Relative capital cost	Relative operating cost	Main limitations	Volumetric productivity	Selected references
Vertical Cylinder	?	Low?	Low	Poor mixing in larger systems		
Bags	>10,000 L	Low	Low	Poor mixing		(Zmora and Richmond 2004)
Flat Panel	~1,000 L	High—very high	High	Outdoor systems usually require cooling; Sticking of algae to reactor surface	0.14–0.27 g L^{-1} d^{-1}	(Cheng-Wu et al. 2001; Rodolfi et al. 2009; Tredici et al. 1991)
Tubular Photobioreactor (helical—Biocoil)	~2,000–4,000 L	Very high	Very high	Outdoor systems usually require cooling; Sticking of algae to reactor surface; O_2 build-up in the tubes; Shear damage to cells by pumping system	~1 g L^{-1} d^{-1} (annual average)[a]—up to 1.4 g L^{-1} d^{-1} (short term)	(Borowitzka 1998b; Hall et al. 2003; Scragg et al. 2002)
Tubular Photobioreactor (straight—Biofence-type)	~350,000 L	Very high	Very high		0.58 g L^{-1} d^{-1} (annual average)[b]	(Torzillo et al. 1991)
Tubular Photobioreactor (on ground)		High—very high	Very high		Up to 0.76 g L^{-1} d^{-1} (short term in summer); Long-term average 0.075 g L^{-1} d^{-1} (*Isochrysis*)	(Garcia-Camacho et al. 1999; Torzillo et al. 1993; van Bergeijk et al. 2010; Zittelli et al. 1999)
Tubular Photobioreactor (α-type)		Very high	Very high			(Lee et al. 1995)

[a] Borowitzka unpublished results, 1,000 L Biocoil in Perth, Western Australia, growing *Tetraselmis chuii*

[b] Recalculated from published production figures for the Klötze reactor in Germany growing *Chlorella*

3 Scaling up of microalgal cultures- constraints and requirements

Production of microalgae for biofuels requires extremely large-scale systems and a major challenge in the development of such systems is the scaling up from the laboratory or small scale to the commercial scale while maintaining high productivity and culture reliability. As illustrated in Table 3, even a relatively small algae biofuel production plant will have a very large number of ponds or photobioreactors and very large volumes of water will be used and processed.

Irrespective of which culture system, either open ponds or closed photobioreactors, is the most suited for large-scale microalgal cultivation (Grobbelaar 2009a; see for example discussion in Pulz 2001), it is very important for any culture system to maintain a balance between turbulence, culture depth (length of light path) and biomass concentration for maximum light capture and optimum biomass productivities with increasing reactor size. High productivities require high cells densities as *productivity = cell density × doubling time*, and this means a high degree of self-shading by the algal cells (Lee 2001). Therefore mixing is required to continually transfer the algae cells between the surface high light environment and the darker environment deeper within the culture to achieve the optimum average irradiance required for efficient utilization of the available irradiance (Richmond 1996). The cycle of high light-low light to which the cells are exposed to due to the mixing (turbulence) appears also to enhance productivity is some cases due to what is known as the 'flashing-light effect' (Kok 1953; Laws et al. 1983). For example, light/dark (L/D) cycles of the order of 0.1 to 10 Hz have been shown to result in a 2-fold increase in photosynthetic rates (Grobbelaar et al. 1996). The theoretical photosynthetic

Table 3 Calculated culture volume and total pond area or number of photobioreactor units required to produce 100,000 bbl (= 15,898,700 L) of lipid per annum at different productivities and lipid contents of the cells. *Note*: for raceway pond cultures annual average productivities of about 20 g dry wt m^{-2} d^{-1} at lipid contents of 20–40% have been reported in the literature and maximum productivities of up to about 35 g dry wt m^{-2} d^{-1} have been reported for short periods of 1–2 days. Long-term productivities of up to 1 g L^{-1} d^{-1} have been recorded in closed photobioreactors in continuous culture of over 9 months (1,000 L Biocoil tubular photobioreactor in Perth, Western Australia; Borowitzka unpubl. results), but long term productivities of 0.2–0.4 g L^{-1} d^{-1} are found for most species. The annual average productivity of the Klötze photobioreactor system in Germany is approx. 0.58 g L^{-1} d^{-1} (recalculated from annual maximum production figures reported by the company)

RACEWAY PONDS						
Productivity (g m^{-2} d^{-1})	20	30	40	20	30	40
(g L^{-1} d^{-1})	0.07	0.10	0.13	0.07	0.10	0.13
Lipid Content (% AFDW)[a]	30	30	30	40	40	40
Total Culture Volume Required (m^3)	1,960,114	1,306,742	980,057	1,470,085	980,057	735,043
Total Pond Area Required (ha)	653	436	327	490	327	245
PHOTOBIOREACTORS						
Productivity (g L^{-1} d^{-1})	0.2	0.5	1	0.2	0.5	1
Lipid Content (% AFDW)	30	30	30	40	40	40
Total Culture Volume Required (m^3)	806,631	322,652	161,326	604,973	241,989	120,995
No of Production Units required (@ 35 m^3 per unit)[b]	23,047	9,219	4,609	17,285	6,914	3,457

[a] AFDW Ash free dry weight

[b] based on the Klötze plant

efficiency of algae, like all plants, is about 11% of solar irradiation (Huntley and Redalje 2007; Weyer et al. 2010) but actual efficiencies observed lie between about 2–4%, with cultures grown in tubular photobioreactors generally at the upper end of this range (Del Campo et al. 2001; data from: Laws et al. 1983; Moheimani and Borowitzka 2006; Molina-Grima et al. 1997; Moreno et al. 2003; Pushparaj et al. 1997; Tredici and Zittelli 1998). For example, Hase et al. (2000) observed long-term photosynthetic efficiencies of 6.56% PAR (or 2.8% solar) in *Chlorella* cultures grown in small raceway ponds in a glasshouse in Sendai, Japan.

Turbulence (mixing) also is an essential prerequisite in the maintenance of high productivity microalgal cultures for other reasons (Bosca et al. 1991; Richmond et al. 1990). Turbulence minimises the development of nutritional, gaseous and thermal gradients by reducing the boundary layer around the cells, which results in better mass transfer of nutrients and cellular metabolites between the cells and the growth medium, and the elimination of unwanted metabolites such as oxygen (Grobbelaar 1994). Heterogeneous mixing throughout the culture also reduces cell settling and the formation of biofilms on the reactor surfaces. However, many algae species, especially the flagellates, are very shear-sensitive and the mixing system used must not damage the cells (Barbosa et al. 2003; Sánchez Mirón et al. 2003).

Algae biofuel plants will also require the transfer of very large amounts of water (see Table 3) and therefore the location of the plant with relation to the water source (i.e. height and distance needed to pump the water) will affect the energy requirements for pumping and the overall economics. Similarly, the distance from the CO_2 source will impact on the cost of supplying the CO_2 to the algal cultures (Doctor et al. 2005).

3.1 Raceway pond scale-up

The long and extensive use of raceway ponds for commercial algae culture and wastewater treatment means that their characteristics are well understood. In raceway ponds, turbulence is dependent on the culture velocity which in turn is dependent on the dimensions of the ponds, the materials of construction (concrete, plastic lined, clay lined etc.) and the water depth. While culture velocities as low as 5 $cm.s^{-1}$ are sufficient to maintain many microalgae cells in suspension, velocities between about 20 and 30 $cm.s^{-1}$ are optimal and are essential for many algae species to prevent the cells from settling (Borowitzka 2005; Oswald 1988). Higher velocities require significantly more energy. As the depth of raceway ponds should not exceed 30 cm for optimum light exposure of the algal cells, the size limit for a raceway pond at these mixing velocities is about 1 ha, with ponds of about 0.5 ha area being the most common. A major limitation to achieving very high productivities in open raceway ponds is that they need to be operated at depths of 20–30 cm and that mixing and turbulence which affect both nutrient availability and the average irradiance received by the cells is relatively low (Borowitzka 1998a; Grobbelaar 2009a; Richmond et al. 1990). Greater mixing and higher productivities can be achieved by faster flow rates, but the energy cost to achieve this is very high (Oswald 1988). The main options to maximize productivity available to the algaeculturalist are to manipulate pond depth and cell density to maximize the effective use of the available irradiance (e.g., Vonshak et al. 1982). However, operating at lower biomass densities will affect the costs of harvesting as larger volumes have to be processed. The use of aeroplane-type ‘wings’ placed in the pond to enhance the generation of turbulence has also been proposed by Laws et al. (1983) to stimulate the ‘flashing light effect’ and thus enhance productivity, but these have never been applied on a large scale.

As the size of raceway ponds increases, the hydrodynamics of the system change and the pattern of water flow, especially at the ends of the ponds, changes significantly and will affect productivity and culture stability. At the ends of the raceway ponds where the direction of flow changes the flow pattern is very uneven. This leads to regions of very high flow, regions of low flow and eddies in different parts of the pond leading to pontential localized settling out of the algal cells. Several methods to create a more even flow pattern to minimize settling of the algal cells have been developed. One early innovation to achieve a more even flow pattern in the ponds was the installation of flow-rectifiers in the pond corners (Shimamatsu 1987). More even flow can also be achieved by having an eccentrically placed curved wall and baffles at the end of the pond furthest away from the paddle wheel (Dodd 1986). This creates a curved zone of accelerating flow followed by a flow expansion zone after the directional change has been made. The rate of constriction of the curved zone needs to be sufficient to avoid eddies and leads to more even water flow.

Since algae cultures generally are also CO_2-limited, a supply of CO_2 is required to achieve maximum productivity. The source of the CO_2 can be flue gas from power stations (coal, gas or oil fired), CO_2 from cement plants, or almost any other CO_2 source. The CO_2 can be supplied by using a diffuser/carbonator and several different designs have been developed. Becker (1994): summarised these as:

(1) active gas transfer by sparging small gas bubbles into the medium or spraying the liquid through the gas phase; and

(2) passive transfer by creation of large contact areas between a CO_2-rich atmosphere and the surface of the culture medium.

The simplest, but most effective, type basically consists of a plastic sheet supported by a floating frame made from PVC pipe (Vasquez and Heussler 1985). Diffusers on the pond bottom release CO_2 into the water and the gas inflates the plastic dome which prolongs its contact with the water. Spoilers across the injector produce a high turbulence in the running algal suspension for more efficient gas transfer into the liquid. As CO_2 addition causes acidification of the medium the CO_2 supply is regulated by a pH-stat system thus maintaining a constant pH in the medium.

3.2 Photobioreactor scale-up

The scale-up of closed photobioreactors is much harder to discuss because of the plethora of closed photobioreactor designs. Some of the key design criteria are: surface-to-volume ratio, reactor orientation and inclination, mixing and degassing, cleaning, temperature regulation, transparency and durability of the construction material, and ease of operation (Borowitzka 1996; Richmond 2000; Tredici 2004). Early attempts in scaling-up photobioreactors (see for example Tredici 2004) have met with problems such as reactor instability and premature break-down, inadequate mixing, leading to low productivities, as well as high oxygen build-up and rapid biofouling of internal reactor walls, inadequate temperature control and contamination.

The size (volume) of column/tower reactors is limited by two main factors: the column diameter is limited by the penetration of light into the culture, and the height is limited to a maximum height above which axial mixing rate would decrease and above which the elongated structure will not be able to withstand strong wind loads (Janssen et al. 2002).

Flat panel reactors are limited to a capacity of about 1 m^3 (Richmond and Cheng-Wu 2001). This is due to materials constraints to support the culture volume as the thickness of the reactor should not exceed about 5 cm for optimum light for the algae and to achieve adequate mixing of the culture.

The largest closed photobioreactors are the tubular photobioreactors. As with the plate reactors the maximum tube diameter for optimum light supply to the cells is about 4–5 cm. In the period the algae are in the tube the CO_2 concentration declines (and pH rises) and the O_2 concentration increases due to photosynthesis. Thus the maximum length of the tube depends on the flow rate (usually 30–50 $cm.s^{-1}$ to prevent cell settling) and is determined by the need to provide CO_2 to the cells and to remove the photosynthetically-produced O_2 which inhibits photosynthesis due to the oxygenase activity of Rubisco (Raven 1997), and there must be an efficient degasser at the end of the tube (Molina Grima et al. 1999) to remove the excess O_2. Even so, if airlifts are used to circulate the medium, the oxygen content of the medium will always be greater than air saturation. Foaming at the top of the degasser can also lead to biomass loss and build-up of a biofilm which can lead to culture instability. Longer tube lengths also lead to loss in turbulence due to frictional loss (Weissmann and Goebel 1988), which translates into a bigger laminar sub-layer on the internal tube surface and longer light/dark cycles (Janssen et al. 2002) as well as a higher energy requirement. Various studies have found that the maximum allowable tube lengths are between 60 and 100 m. Larger reactors are constructed by connecting the tubes to manifolds where gas exchange occurs (Borowitzka 1999a). The largest operational closed photobioreactor to date is the 700 m^3 tubular photobioreactor plant in Klötze, Germany, which consists of 20 reactor modules with a total glass tube length of about 500 km and produces about 150 t of *Chlorella* per year (Moore 2001).

Generation of higher velocities and shorter light/dark cycles close to 10 Hz would be desirable. However, an upper limit is set by the limited pressure tolerance of the typically employed transparent materials of construction and by the sensitivity of many algal species, especially the flagellated species, to hydrodynamic shear, especially shear generated by the pumping system. A possible alternative to improving photosynthetic efficiency is the inclusion of light through optical fibers within the reactor, as suggested by Csögör et al. (2001) and Janssen et al. (2002), or static helical mixers to generate more turbulence (Rosello Sastre et al. 2007). Hydrodynamic shear also significantly limits the species which can be cultivated and is an important factor in the choice of circulation system (Barbosa et al. 2003; Chisti and Moo-Young 1996). The scale-up of the pumping system is especially difficult as the micro-scale of shear within the pumps and its effect on the algae (which is very species dependent) is usually unknown. The types of pumps used to circulate the culture in large photo-bioreactors include airlifts, centrifugal pumps, lobe pumps and diaphragm pumps. Closed photobioreactors also have a potential problem caused by the formation of biofilms on the inner surface of the reactor which affect light penetration and flow.

As shown above, individual closed photobioreactors are limited in capacity and therefore a production plant will have very many reactors. The arrangement of these reactors is important as shading from adjacent reactors will affect overall areal productivity and the optimum spacing between the reactors needs to be determined (Chini Zittelli et al. 2006).

4 Culture management

Quite often, the success of outdoor cultivation attempts has been dampened and cut short due to an inadequate understanding of the biology and ecology of the algae. Microalgae

cultivated outdoors are exposed to daily and annual climatic changes and to non-sterile growth conditions (Note: even the 'closed' photobioreactors cannot be maintained sterile for periods longer than about 1–2 weeks at best). As a result, there is always a dynamic shift in the environmental and biological parameters within the culture which, if left uncontrolled, can quickly lead to culture collapse, making reliable outdoor cultivation, let alone on a large scale, quite challenging. Common impediments encountered in outdoor cultures are:

- reduced photosynthetic activity in the middle of the day due to high light and high O_2 (Grobbelaar 2009a) and, on cool mornings, due to sub-optimal culture temperatures (Vonshak et al. 2001), thereby reducing photosynthetic efficiency,
- photoinhibition and photooxidation due to excessive dissolved oxygen (Richmond and Grobbelaar 1986; Vonshak et al. 1996) [This is especially a problem in closed photobioreactors],
- Significant biomass loss through respiration at night (Grobbelaar and Soeder 1985) which is higher at suboptimal temperatures (Torzillo et al. 1991), hence leading to additional losses in biomass productivity,
- Gradual change in phenotypic and genotypic traits of the algae through adaptation (Lakeman et al. 2009),
- Contamination, competition and/or predation by other organisms (other algae, bacteria, viruses, fungi, zooplankton).

Culture reliability and stability are essential for commercial microalgal cultivation on a large scale and therefore careful selection of species and strains best suited to the culture system and the prevailing environmental conditions plays a crucial role. Some of the desirable characteristics are: (a) broad tolerance to temperature (approx. 0–35°C—i.e., the minimum night temperature and the maximum likely day temperature encountered in dry desert regions), (b) smaller photosynthetic antenna size for more efficient use of light (Melis et al. 1999; Polle et al. 2002), (c) a high light saturation coefficient (E_s) so that the algae are less likely to be photoinhibited at high irradiances, (d) a high photosynthetic efficiency (α) (Meyers 1953), (e) higher affinity for carbon dioxide and/or HCO_3^- and less sensitivity to high dissolved oxygen levels (Vonshak et al. 1996), (f) algae which display 'weedy' features such as more rapid and efficient uptake of nutrients, and (g) fast growth in non-fastidious (cheap) media. Species control/retention is best managed by keeping culture conditions (temperature range, pH, salinity, nutrient type and quantity) optimum for the desired algal species and/or increasing the frequency of dilution to wash out unwanted species and by prophylactic treatment of the water, although the last two methods are generally impractical and too costly on a large scale.

4.1 Culture system control and production facility operation

Given that labour is an important cost factor in an algal production plant (Borowitzka 1992, 1999b), and given the required scale of operation with very many ponds or photobioreactors, the integration of semi-automated and fully automated control systems in the daily monitoring and maintenance of the cultures is essential (Olaizola 2000). A wide array of sophisticated methods is now available combined with process control software and a large commercial algae production plant will need to employ these. For example, cell counting of dead and live cells and bacterial detection can be conveniently determined by flow cytometry (Sosik et al. 2010) and the physiological state of the algae can now be readily and easily determined using fluorescence techniques such as the pulse amplitude

modulated fluorometry (PAM) (Kromkamp et al. 2009; Masojidek et al. 2010). The integration of such quick measurement techniques into process control algorithms is highly desirable so that culture 'health' can be assessed rapidly and preventive measures can be implemented earlier to maintain optimum growth conditions and avoid culture crashes. Already, the application of intelligent modeling systems (see references in Greenwell et al. 2010) for real time control of system parameters such as pH (including CO_2 addition), nutrients, (and light and temperature in closed photobioreactors) and in the harvesting and post-harvesting processes are being used in several commercial algae plants.

5 Harvesting, thickening and dewatering

A key step in the production of algal biofuels is harvesting and dewatering of the algal biomass before extraction or other processing. The high cost of harvesting and dewatering presents major challenges to the development of commercially viable microalgae-based biofuels. For a process to be considered suitable for the recovery of microalgal biomass for biofuel production, it must be able to process very large volumes (see Table 4), be highly reliable, be flexible and it must be cost effective. As microalgae vary greatly in those properties which affect harvesting processes (e.g., size, surface charge, resistance of cell to breakage, compressibility etc.) between species and with growth phase (Danquah et al. 2009; Lee et al. 1998), the biomass recovery processes must be tailored to the species of microalgae and the growth system. As very large amounts of water are used in microalgae culture the medium, which still contains nutrients, must also be able to be recycled after cell harvesting. At the large volumes of an algae biofuels production plant these remaining nutrients represent a valuable resource, and if not recycled this nutrient-containing medium would also represent considerable disposal issues.

The harvesting, thickening and dewatering of microalgae cultures has been extensively reviewed by Shelef (1974); Moraine et al. (1980), Mohn (1988) and Molina Grima et al. (2004). Here we will only outline some of the key aspects.

Key properties of microalgae which influence their separation are: (a) shape: [rods, spheres or chains or filaments], (b) size: [generally between 2 and 30 μm], (c) specific weight: [1.05–1.1], (d) surface charge: [usually negative]. Microalgal cultures to be harvested usually contain between 0.2 to 2 $g.L^{-1}$ solids and for lipid extraction a concentration of at least 20 $g.L^{-1}$ solids is required. Filamentous algae such as *Spirulina* can be harvested by filtration (Belay 1997), but almost all of the algae under consideration as a source of biofuels (e.g., *Nannochloropsis* or *Chlorella*) are unicellular and too small for effective filtration. Centrifugation as the only step is too energy intensive (Mohn 1988) and not practical for the extremely high volumes required to be processed for algal biofuels production. Sedimentation is also a possibility, but is generally too slow to be effective (Shelef et al. 1984).

The most commonly considered processes are flocculation followed by flotation or by settling as the first step. Flocculation can be achieved by the use of inorganic flocculants such as alum (Knuckey et al. 2006; Papazi et al. 2009) or organic flocculants such as chitosan (Morales et al. 1985) or starch (Vandamme et al. 2010), although the cost of these flocculants is substantial. The flocculant used must be compatible with the need to recycle the water back to the growth system without complex pretreatment of this recycled water. Other flocculation methods which are being explored include autoflocculation, where the algae are induced to self-flocculate usually by raising the pH due to photosynthetic CO_2 uptake (Sukenik and Shelef 1984; Yahi et al. 1994), or bioflocculation by co-cultured

Table 4 Example of volumes of water that need to be processed daily through the stages of harvesting and dewatering outlined in Fig. 1 and based on the raceway pond scenario in Table 3. It is assumed that 50% of the culture volume is harvested every day. The numbers in [] refer to the steps shown in Fig. 1

Productivity (g m^{-2} d^{-1})	20	30	40	20	30	40
Lipid Content (% AFDW)	30	30	30	40	40	40
Cell density (g L^{-1})	0.07	0.10	0.13	0.07	0.10	0.13
Total Pond Volume (L)	1,960,113,699	1,306,742,466	980,056,849	1,470,085,274	980,056,849	735,042,637
Volume harvested daily (L.day^{-1}) [*5*]	980,056,849	653,371,233	490,028,425	735,042,637	490,028,425	367,521,318
Harvesting (concentration by 10×)						
Volume removed (L) [*7*]	882,051,164	588,034,109	441,025,582	661,538,373	441,025,582	330,769,186
Final Solids (g L^{-1})	0.67	1.00	1.33	0.67	1.00	1.33
Final% water	99.93	99.90	99.87	99.93	99.90	99.87
Final volume remaining (L) [*6*]	98,005,685	65,337,123	49,002,842	73,504,264	49,002,842	36,752,132
Stage 1 dewatering (concentration by 10×)						
Volume removed (L) [*10*]	88,205,116	58,803,411	44,102,558	66,153,837	44,102,558	33,076,919
Final Solids (g L^{-1})	6.67	10.00	13.33	6.67	10.00	13.33
Final% water	99.33	99.00	98.67	99.33	99.00	98.67
Final volume remaining (L) [*9*]	9,800,568	6,533,712	4,900,284	7,350,426	4,900,284	3,675,213
Stage 2 dewatering (centrifugation)						
Volume removed (L)	9,473,883	6,207,027	4,573,599	7,105,412	4,655,270	3,430,199
Final Solids (g L^{-1})	200.00	200.00	200.00	200.00	200.00	200.00
Final% water	80.00	80.00	80.00	80.00	80.00	80.00
Concentration factor	30	20	15	30	20	15
Final volume remaining (L)[*11*][a]	326,686	326,686	326,686	245,014	245,014	245,014

[a] If a drying step is necessary than this is the volume which needs to be dried daily

bacteria or other algae species (Eisenberg et al. 1981; Lee et al. 2009, 2010a), and alternate methods such as electroflocculation (Poelman et al. 1997; Sandbank et al. 1974) and ultrasound (Bosma et al. 2003). Flocculation of marine and halophilic algae species is generally easier than flocculation of freshwater species because of the high ionic strength of the medium (Ayoub et al. 1986; Sukenik et al. 1988). An alternative process to flocculation may be magnetic separation using magnetic particles such as Fe_3O_4. The coagulated particles can then be passed through a magnetic field for separation (Bitton et al. 1975; Snook 1983).

Once flocculated the algae then must be concentrated further by settling or by flotation. Flocculation and dissolved air flotation (DAF) are commonly used in wastewater treatment plants to remove the algal/bacterial biomass (Féris and Rubio 1999) and are easily scaled and fairly well understood processes. However, for the production of a relatively low-value product such as biodiesel the cost of the flocculants and the energy cost of a DAF system are significant. Furthermore, an additional thickening step, or steps, such as belt filtration or centrifugation, will probably be required to achieve the solids concentration needed for the next processing step. These steps are illustrated in Fig. 1 and Table 4 gives an indication of the scale-up required to process volumes in each of these steps.

In conclusion, reducing the cost harvesting and dewatering of microalgae cultures for biofuels production with a high volume throughput presents significant challenges. It is most likely that multi-staged separation processes need to be used to amplify the solid content. Due to algae interspecies differences, it is likely that for each microalga species a unique harvesting, thickening and dewatering process will need to be designed (Borowitzka 2010a). The recovery system must (1) be able to handle extremely large volumetric throughputs, (2) be highly reliable, (3) flexible, (4) operate at extremely low cost and have a low energy requirement, and (5) be compatible with recycling of the growth medium and other downstream processes. If microalgae are to be grown in saline water, which is essential for sustainability in most regions (Borowitzka and Moheimani 2011), then the materials of the recovery systems must also be resistant to corrosion. For the recovery of microalgae biomass from the culture medium and before extraction several unit processes are likely to be required (cf. Fig. 1 and Table 4): (1) harvesting (primary concentration) which increases biomass concentration by about 10 fold, (2) thickening (secondary concentration) which thickens the primary concentrate by an additional 10 fold, (3) dewatering (tertiary concentration) which increase the solid content to 15–25% and, if required, (4) drying (quaternary concentration) which removes unbound water (for algae biofuels production drying of the biomass is probably impossible because of the high energy requirement—see below). It is apparent that there is no one unique, ideal, or universal operation, or even sequence of operations, which can be recommended; individual unit operations must be combined in the most suitable, economical and sustainable way for the recovering microalgae and the selection of methods will be strongly influenced by the species of algae.

6 Extraction and further downstream processing

Low cost scalable extraction methods for lipids and sugars for subsequent conversion to biodiesel and ethanol at an industrial scale still remain to be developed, optimized and trialed (see Cooney et al. 2009 for a recent review). Many methods are available, but few, if any, have been trialed as yet at scale. Because of the prohibitively high energy requirement that would be needed to dry the algal cells because of the high latent heat of water (Cooney

et al. 2009; Yang et al. 2004), the extraction process must be able to work with wet biomass. Solar drying has been proposed, but the large volumes which need to be processed mean that a very large land area is required and the capital and labor costs are likely to be prohibitive. Alternatively, it may be possible to integrate waste heat (i.e., from a power station) into a drying process (Katchanov 2010) in some cases.

The extraction process will determine the degree of dewatering required in the preceding harvesting and dewatering steps. The extraction process also will be species-dependent, mainly because of the variety of cell coverings of different microalgae species; in many algae the cell walls need to be broken to disrupt the cells in order to achieve efficient extraction.

6.1 Cell disruption

Breaking of the algal cell covering is essential for efficient extraction and negates the need to use elevated temperature and pressure extraction processes. Microalgae have a variety of cell coverings, ranging from the 'naked' cells of *Dunaliella* which only have a thin outer glycocalyx, to the highly-resistant algenin containing cell walls of *Nannochloropsis* and *Chlorella*, and the silica frustules of diatoms (Atkinson et al. 1972; Corre et al. 1996; Hatanaka et al. 1998; Schmid et al. 1981). Methods which have been used to break the cells include mechanical processes such as bead mills, sonication, cavitation and autoclaving. Non-mechanical methods include freezing, osmotic shock, enzymatic digestion, use of organic solvents, and acid or base reactions. (Cooney et al. 2009; Cravotto et al. 2008; Kanel and Guelcher 1998; Lee et al. 2010b; Pernet and Tremblay 2003; Ranjan et al. 2010). Because of the wide variety of cell wall types and chemistry between microalgae, different methods will be required for different species, but so far none have been applied on a large scale, nor has the energy requirement and cost impact on the whole process been evaluated as yet.

6.2 Extraction of lipids

Lipids are mostly hydrophobic molecules (e.g., neutral lipids) which interact with relatively non-polar solvents such as ethyl ether, chloroform and benzene, while membrane-associated polar lipids require polar solvents such as ethanol and methanol to disrupt the hydrogen bonding and electrostatic forces between the lipids and proteins. The most common laboratory methods for lipid extraction are the Soxhlet extraction (usually with n-hexane as solvent), and the Folch (Folch et al. 1951) and Bligh and Dyer (Bligh and Dyer 1959; Kates and Volcani 1966) methods which use chloroform and methanol in varying ratios as solvents. Because of the toxicity of methanol and chloroform, and the fact that chloroform extracts more than just saponifiable lipids (e.g., pigments and other lipids and non-lipid contaminants), methods using other alcohols such as ethanol, 1-butanol and isopropanol are being developed to replace the methanol (Nagle and Lemke 1990). Other combinations of co-solvents also have been proposed for the extraction of lipids from microalgae are hexane/ethanol (Cartens et al. 1996), and hexane/isopropanol (Nagle and Lemke 1990). In the hexane system the hexane and alcohol will readily separate into two separate phases when water is added, thereby improving downstream separations (Schaefer 1998). Furthermore, the combination of ethanol and hexane at a 5:1 *v/v* ratio in a 2-step extraction procedure reduces the solvent requirement for the same amount of biomass by approximately 10-times compared to the chloroform/methanol extraction procedure (Fajardo et al. 2007). It also eliminates the use of the toxic chloroform. This is

advantageous in terms of organic solvent utilization in a very large scale production of biodiesel from microalgae as it reduces the solvent requirement and where the ethanol could be produced as a byproduct by fermentation of the microalgae sugars. Solvent extraction can be accelerated by the use of microwave or ultrasound energy (Cravotto et al. 2008; Lee et al. 2010b) but so far the energy costs at scale have not been evaluated.

An interesting new development is the use of switchable solvents. These solvents are capable of turning from a non-ionic form to an ionic liquid by the bubbling of CO_2, and can be reconverted to the non-ionic form by bubbling N_2. Switchable polarity solvents are lipophilic in the non-ionic form and hydrophilic in the ionic form (Handy 2003; Phan et al. 2009). The application of switchable solvents to algae has been limited so far. Samori et al. (2010) have used switchable solvents to extract hydrocarbons from *Botryococcus braunii*. Switchable solvents are part of the so-called 'green solvents' which have lower toxicity and flammability than more conventional solvents. Other 'green' solvent systems include subcritical water extraction and supercritical fluid extraction with CO_2 (Herrero et al. 2006), or co-solvent mixtures of ionic liquids and polar covalent molecules such as ethanol as has been used to extract lipids from *Chlorella* and *Dunaliella* by Young et al. (2010). A potential disadvantage of some of these 'green'-solvent extractions is the generally high energy requirement compared to conventional organic solvents.

Despite the wide range of lipid extraction methods available little is known about the scalability of the processes and the economics of their application on an industrial scale with microalgal biomass (Cooney et al. 2009).

7 Conversion into biofuel

The energy contained in the algal biomass can be recovered in a number of ways including direct thermochemical liquefaction (Demirbas 2010; Yang et al. 2004). However the current main interest in algae is the production of liquid fuels, especially biodiesel and jet fuel, from the algal lipids.

The oils or lipids from microalgae are very similar to plant oils which usually contain free fatty acids, phospholipids, sterols, water, and other impurities which cannot directly be used as fuel and which affect the efficiency of the transesterification reaction. Compared to higher plant lipids the lipids of many algal species are also relatively rich in polyunsaturated fatty acids which negatively affect the properties and quality of the biodiesel. These oils require chemical modification such as transesterification, pyrolysis or hydrogenation to produce fuel of acceptable quality (Murugesan et al. 2008). The current standards for biodiesel which must be met are discussed by Knothe (2006, 2010) Methods for production of biodiesel and the quality of the biodiesel produced from algal oils have been reviewed extensively recently (Amin 2009; Borowitzka 2010a; Brennan and Owende 2010; Demirbas 2009; Meher et al. 2006; Vasudevan and Briggs 2008), and will not be covered in detail in this paper. The three main approaches currently used for biodiesel production are base catalyzed transesterification, acid catalyzed transesterification (with simultaneous esterification of free fatty acids) and noncatalytic conversion (Demirbas 2003; McNeff et al. 2008).

Transesterification is currently the main process by which fuel is produced from different natural oil sources such as vegetable oil or cooking waste oil. Transesterification or alcoholysis is the reaction of a lipid with an alcohol to form esters and a by-product, glycerol. The type of catalyst is the most important variable in the conversion process. Nagle and Lemke (1990), using algal lipids, achieved a maximum yield of 68% with a

hydrochloric acid-methanol catalyst compared to a yield of 32% with sodium hydroxide as the catalyst. Although catalyst-free processes for biodiesel production exist, such as a supercritical methanol method with a reaction temperature of 350°C and a molar ratio of methanol of 42 (Kudsiana and Saka 2001), they have a large requirement of methanol and energy, as well as a requiring more costly equipment, probably making them uneconomical for large scale biodiesel production.

Methanol (methanolysis) is commonly used in the conventional biodiesel production process, and although ethanol (ethanolysis) has been shown to produce a more environmentally friendly fuel and is less toxic than methanol, however ethanolysis is more expensive. Both alcohols are not miscible with triglycerides under normal room temperature condition and so the mass transfer in the reaction mixture is usually increased by mechanical stirring and emulsions are formed. In methanolysis, the emulsion formed is unstable and will break down quickly and easily to form a lower glycerol-rich layer and upper methyl-rich layer, whereas the emulsion formed by ethanolysis is more stable and the separation and purification of ester is more complicated (Anastopoulos et al. 2009; Ehimen et al. 2010).

The lipids used for the transesterification reaction must be free of water as the presence of even small amounts of water reduces the efficiency of the reaction (Bikou et al. 1999; Canakci and Van Gerpen 1999). This means that the algal biomass must be dried before lipid extraction (a very energy intensive step), or the extracted lipids must be dried before transesterification. The presence of free fatty acids also reduces the efficiency of the reaction (Ma et al. 1998) and the extracted algal lipids may need pretreatment to reduce the free fatty acid content. Alternatively, acid catalyzed transesterification with simultaneous esterification of free fatty acids can be carried out using sulphuric, hydrochloric, phosphoric or sulphonic acid (Meher et al. 2006). However, acid catalyzed transesterification has a slower reaction rate than alkali catalyzed transesterification, and the acids are more corrosive, thus making the process more expensive.

Recently there have been several studies of biodiesel production from algae by direct (in situ) transesterification of the algal biomass (Ehimen et al. 2010; Johnson and Wen 2009; Koberg et al. 2010; Wahlen et al. 2011; Xu and Mi 2011) including one patent (Machacek and Smith 2009). In situ transesterification negates the need for the lipid extraction step which has a high energy requirement for the vacuum evaporation to recover the solvent (usually hexane), process heating and stirring during transesterification. Cooney et al. (2009) have compared several direct transesterification methods using various solvents (methanol or the ionic liquid 1-ethyl-3-methylimidazolium methyl sulphate) and catalysts (hydrochloric acid, sulphuric acid, acetyl chloride) on a number of species of microalgae. Koberg et al. (2010) used direct transesterification of *Nannochloropsis* biomass suspended in a mixture of methanol-chloroform (1:2 *v*/*v*) using microwaves and a SrO catalyst. Similarly, Wahlen et al. (2011) also used a microwave, but used methanol with sulphuric acid in various ratios as catalyst. Two of these studies (Koberg et al. 2010; Wahlen et al. 2011) present some evidence that direct transesterification produces significantly more biodiesel than would be expected from available tryglycerides alone, indicating conversion of fatty acids in other molecules such as phospholipids.

However, almost all of these in situ transesterification studies have used dried algal biomass thereby still requiring the energy intensive drying step of the harvested algal biomass which is probably impractical. In the few studies with wet algal biomass the presence of water has been found to significantly reduce the yield of the fatty acid methyl esters (FAME) (Wahlen et al. 2011) However, by increasing the amount of methanol, the effect of the water could be reduced but this would increase costs. So far all of these studies have only been carried out at the laboratory scale only and no comparative economic

studies between the various methods have been carried out. An important question to be answered is the economics of drying the algae and then using direct transesterification as compared to the more conventional extraction of the wet biomass, drying of the extract and then transesterifying the lipids.

8 Summary

In order to achieve the significant production of algal oils for biofuels required if these fuels are to partially replace fossil fuels requires the production of the algal biomass, harvesting and extraction of the biomass, and the efficient conversion of the lipids to fuels, all on a very large scale and at low cost. All the unit process steps shown in Fig. 1 need to be optimized and optimally integrated to minimize costs. As pointed out by Cooney et al. (2009), there is still a wide gap between existing technologies (especially in the extraction/fractionation process) and an industrial scale economic microalgae-based biofuel process.

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