9

Advantages and Challenges of Microalgae as a Source of Oil for Biodiesel

Melinda J Griffiths, Reay G Dicks,
Christine Richardson and Susan TL Harrison
Centre for Bioprocess Engineering Research (CeBER), University of Cape Town, South Africa

1. Introduction

Microalgal oil is currently being considered as a promising alternative feedstock for biodiesel. The present demand for oil for biofuel production greatly exceeds the supply, hence alternative sources of biomass are required. Microalgae have several advantages over land-based crops in terms of oil production. Their simple unicellular structure and high photosynthetic efficiency allow for a potentially higher oil yield per area than that of the best oilseed crops. Algae can be grown on marginal land using brackish or salt water and hence do not compete for resources with conventional agriculture. They do not require herbicides or pesticides and their cultivation could be coupled with the uptake of CO_2 from industrial waste streams, and the removal of excess nutrients from wastewater (Hodaifa et al., 2008; An et al., 2003). In addition to oil production, potentially valuable co-products such as pigments, antioxidants, nutraceuticals, fertilizer or feeds could be produced (Mata et al., 2010; Rodolfi et al., 2009).

Despite these advantages, algal fuel is not currently in widespread use, largely due to its high cost of production (Chisti, 2007; Miao & Wu, 2006). Despite strong interest from the commercial and scientific sectors, there are currently no industrial facilities producing biodiesel from algae (Lardon et al., 2009). One of the major economic and technological bottlenecks in the process is biomass and lipid production by the algae (Borowitzka, 1992; Sheehan et al. 1998; Tsukahara & Sawayama, 2005). Productive strains and optimized culture conditions able to produce cells with a simultaneously high growth rate and lipid content are required. The high cost and energy demand of harvesting unicellular algae also remains a major challenge. The small cell size (often < 10 μm in diameter) and dilute biomass produced requires innovative solutions to minimize the consumption of water and energy as well as processing costs (Rodolfi et al., 2009).

This chapter provides an overview of microalgae as a source of oil for biodiesel, focusing on:

- A description of algae and their properties with regards to oil production
- Requirements and key factors in microalgal cultivation
- Methods and challenges in harvesting and processing of algal biomass
- Economic and environmental feasibility of microalgal biodiesel
- Mechanisms to enhance lipid productivity of microalgae and future research directions.

2. Microalgae

The term 'algae' is used to describe a huge variety of prokaryotic (strictly termed Cyanobacteria) and eukaryotic organisms with a range of morphologies and phylogenies. They represent a wide array of species, inhabiting environments from deserts to the Arctic Ocean, including both salt and fresh water. They vary in colour, shape and size, from picoplankton (0.2 to 2 μm) to giant kelp fronds up to 60 m in length (Barsanti & Gualtieri, 2006). Macroalgae (e.g. seaweeds) are generally large (can be seen without the aid of a microscope), multicellular and often show some form of cellular specialisation. Microalgae are usually less than 2 mm in diameter and unicellular or colonial. Microalgae have been investigated for a variety of commercial applications. Annual global microalgal production is currently estimated at about 10 000 metric tons, with the main algae cultivated being *Spirulina* (accounting for roughly half of the worldwide algal production), *Chlorella*, *Dunaliella* and *Haematococcus*.

Algae have been investigated as a source of energy in many different contexts, from direct combustion to the production of hydrogen gas. Anaerobic digestion can be applied for the generation of methane or biogas (Golueke et al., 1957). Algal species with high oil content are particularly attractive as a feedstock for biodiesel production. Research into algae for the mass-production of oil has focused on the microalgae due to their high lipid content compared to macroalgae. Most algal species considered for biodiesel production are either green algae (Chlorophyta) or diatoms (Bacillariophyta) (Sheehan et al., 1998). They are generally photosynthetic, but several species are able to grow heterotrophically or mixotrophically (Barsanti & Gualtieri, 2006).

Microalgae have higher growth rates than land-based plants. Due to their simple cellular structure and existence in an aqueous environment, the entire cell surface is available for light capture and mass transfer, leading to high rates of substrate uptake and photosynthetic efficiency (Miao & Wu, 2006; Sheehan et al., 1998). In contrast to land-based oil crops, where only the seeds are harvested, each algal cell contains lipid and hence the yield of product from biomass is much higher (Becker, 1994). Due to these differences, the oil yield per area of microalgal cultures potentially exceeds that of the best oilseed crops (Table 1).

Oil source	Yield ($L.m^{-2}.yr^{-1}$)	Reference
Algae	4.7 to 14	Sheehan et al., 1998
Palm	0.54	Mata et al., 2010
Jatropha	0.19	Sazdanoff, 2006
Rapeseed	0.12	Sazdanoff, 2006
Sunflower	0.09	Sazdanoff, 2006
Soya	0.04	Sazdanoff, 2006

Table 1. Average productivities of some common oil seed crops compared to algae

3. Biodiesel from microalgae

Microalgal lipids can be extracted to yield oil similar to that from land-based oilseed crops. The amount and composition of the oil varies between algal species. Algal oil can be converted to biodiesel through the same methods applied to vegetable oil. The idea of using microalgae as a source of transportation fuel is not new. Research in this field has been

conducted since the 1950s (Oswald & Golueke, 1960). In the 1970s, several large, publicly funded research programs were set up in the USA, Australia and Japan (Regan & Gartside, 1983; Sheehan et al., 1998). The US Department of Energy invested more than US$ 25 million between 1978 and 1996 in the Aquatic Species Program to develop biodiesel production from algae (Sheehan et al., 1998). The main focus of the program was the production of biodiesel from high lipid-content algae grown in open ponds, utilizing waste CO_2 from coal fired power plants. Over 3000 species were collected and many of them screened for lipid content.

Early in the program, it was observed that environmental stress, particularly nutrient limitation (nitrogen for green algae and silicon for diatoms) led to an increase in accumulation of lipids. Promising species were investigated to determine the mechanism of this 'lipid trigger'. Researchers in the program were the first to isolate the enzyme Acetyl CoA Carboxylase from a diatom. This enzyme catalyzes the first committed step in the lipid synthesis pathway. Acetyl CoA Carboxylase was over-expressed successfully in algae; however, the anticipated increase in oil production was not demonstrated. The program close out report (Sheehan et al., 1998) concluded that, although algae used significantly less land and water than traditional crops, and sufficient resources did exist for algal fuel to completely replace conventional diesel, the high cost of microalgae production remained an obstacle. Even with the most optimistic lipid yields, production would only have become cost effective if petro-diesel had risen to twice its 1998 price.

The last decade has seen a renewal of interest in biofuels and microalgae as a feedstock source. An increase in oil prices, additional pressure to find alternatives to dwindling oil supplies and an urgent need to cut carbon emissions contributing to global warming has led to a renewed interest in algae as a source of energy, particularly lipid producing algae as a source of biodiesel.

4. Microalgal lipids

The main components of algae cells are proteins, carbohydrates and lipids (Becker, 1994). Microalgae naturally produce lipids as part of the structure of the cell (e.g. in cell membranes and as signalling molecules), and as a storage compound, similar to fat stores in animals and humans (Tsukahara & Sawayama, 2005). The term lipid encompasses a variety of compounds with different chemical structures (e.g. esters, waxes, cholesterol). The most common lipids are composed of a glycerol molecule bound to three fatty acids, known as triacylglycerol or TAG, or to two fatty acids with the third position taken up by a phosphate (phospholipids) or carbohydrate (glycolipids) group. Fatty acids consist of a long unbranched carbon chain. They are classified according to the number of carbon atoms in the chain and the number of double bonds, for example saturated (no double bonds), monounsaturated (one double bond) or polyunsaturated (more than one double bond). Microalgae commonly contain fatty acids ranging from C12 to C24, often with C16 and C18 unsaturates. Certain species contain significant amounts of polyunsaturated fatty acids.

Storage lipids, generally in the form of TAG, accumulate in lipid vesicles called oil bodies in the cytoplasm. Most fast-growing species have relatively low lipid content during normal growth, with these lipids mainly consisting of phospho- or glycolipids associated with cell membranes. Under certain conditions, generally triggered by stress or the cessation of growth, lipid content can increase to over 60% of cell dry weight (DW), mostly composed of

TAG (Shifrin & Chisholm, 1981; Piorreck et al., 1984; Spoehr & Milner, 1949; De la Pena, 2007; Becker, 1994).

TAGs are the most suitable class of lipids for biodiesel production. Phospholipids are particularly undesirable as they increase consumption of catalyst and act as emulsifiers, impeding phase separation during transesterification (Mittelbach & Remschmidt, 2004; Van Gerpen, 2005). Phospholipids, and some sulphur-containing glycolipids, also increase the phosphorous and sulphur content of the fuel respectively, which must both be below 10 $mg.L^{-1}$ to meet the European biodiesel standard EN 14214. The type of fatty acids found in the oil can have a profound effect on the biodiesel quality. The fatty acid chain length and degree of saturation (determined by the number of double bonds) affects properties such as the viscosity, cold flow plug point, iodine number and cetane number of the fuel (Ramos et al., 2009). For biodiesel production, it is therefore important to maximize not only total lipid production, but also TAG content and appropriate fatty acid profile.

Lipid synthesis relies on carbon compounds generated from CO_2 by photosynthesis, as well as energy and reducing power (in the form of ATP and NAD(P)H respectively). The latter are produced during the light reactions of photosynthesis, while CO_2 uptake is mediated by the Calvin cycle during the dark reactions of photosynthesis. The output of the Calvin cycle is a three-carbon compound (glyceraldehyde 3-phosphate), which is converted through glycolysis into acetyl CoA. The conversion of acetyl CoA to malonyl CoA is the first committed step in lipid biosynthesis (Livne & Sukenik, 1992). Throughout metabolism there are a number of branch points at which metabolic intermediates are partitioned between the synthesis of lipids and other products such as carbohydrates and proteins (Lv et al., 2010). For example, acetyl CoA is a substrate for lipid synthesis as well as entry into the TCA cycle, which generates energy and biosynthetic precursors for proteins and nucleic acids. Both external and internal constraints, such as the availability of nutrients and the enzymatic reaction rates, limit the supply of metabolic intermediates. The production of storage lipids is particularly energy and resource intensive (Dennis et al., 1998; Roessler, 1990) and therefore usually occurs at conditions of reduced growth.

5. Cultivation of microalgae

The use of microalgae for energy generation requires large-scale, low-cost production. This demands cheap, scalable reactor design with efficient provision of the requirements for high algal productivity. Design considerations include optimum surface area to volume ratio for light provision, optimal mixing to keep cells in suspension and for distribution of nutrients, control over water balance and sterility, as well as maintenance of favorable temperature. A wide variety of reactor designs have been proposed, each with advantages and drawbacks.

5.1 Reactor systems

Microalgal production is a technology halfway between agriculture, which requires large areas for sunlight capture, and fermentation, which involves liquid culture of microorganisms (Becker, 1994). As light does not penetrate more than a few centimetres through a dense algal culture, scale-up is based on surface area rather than volume (Scott et al., 2010). Many different types of algal cultivation systems have been developed, but they can be divided into two main categories: open and closed.

Open systems consist of natural waters such as lakes, ponds and lagoons, or artificial ponds and containers that are open to the atmosphere. Most commercial production to date has taken place in open ponds as these systems are easy and cheap to construct (Pulz, 2001). The most common technical design is the raceway pond: an oblong, looped pond mixed by a paddlewheel, with water depths of 15 to 20 cm (Becker, 1994). Biomass concentrations of between 0.1 and 1 $g.L^{-1}$ and biomass productivities of between 50 and 100 $mg.L^{-1}.day^{-1}$ are possible (Chisti, 2007; Pulz, 2001). The main advantages of open systems are their low cost and ease of construction and operation. They also offer the potential for integration with wastewater treatment processes or aquaculture systems (Chen, 1996).

Disadvantages of open systems include contamination with unwanted species such as foreign algae, yeast, bacteria and predators, evaporation of water, diffusion of CO_2 to the atmosphere and low control over environmental conditions, particularly temperature and solar irradiation (Becker, 1994; Pulz, 2001). In addition, the relatively low cell densities achieved can lead to higher cost of cell recovery (Chen, 1996). Only a few microalgal species have been successfully mass cultivated in open ponds. These tend to be either fast-growers that naturally outcompete contaminating algae (e.g. *Chlorella* and *Scenedesmus*), or species that grow in a specialised environment such as high salt (e.g. *Dunaliella salina*) or high pH (*Spirulina platensis*), which limits growth of competitors and predators (Chen, 1996). Due to the lack of control over cultivation conditions resulting in low productivity, and the fact that many desirable species cannot be effectively maintained in open systems, attempts have been made to overcome some of these limitations through the use of enclosed reactor systems.

Closed systems, or photobioreactors, consist of containers, tubes or clear plastic bags of various sizes, lengths and orientations (Pulz, 2001). Commonly used designs include vertical flat-plate reactors and tubular reactors, either pumped mechanically or by airlift (Scott et al., 2010). Closed reactors offer a much higher degree of control over process parameters, leading to improved heat and mass transfer, and thus higher biomass yields. They can also offer a much higher surface area to volume ratio for light provision, better control of gas transfer, reduction of evaporation and easier installation in any open space (Chen, 1996). Additionally, the risk of contamination is reduced, CO_2 can be contained, production conditions can be reproduced and temperature can be controlled.

Productivity in closed systems can be much higher than open systems, with biomass concentrations of up to 8 $g.L^{-1}$ and productivities of between 800 and 1300 $mg.L^{-1}.day^{-1}$ (Pulz, 2001). However, they are generally much more costly to build and more energy demanding to operate than open systems (Table 2). Closed systems can also have problems with fouling and oxygen build-up. Large systems can be difficult to clean and sterilize and long sections of enclosed tubing may require oxygen purging. High oxygen concentrations cause the key enzyme Rubisco to bind oxygen instead of carbon dioxide, leading to photorespiration instead of photosynthesis (Dennis et al., 1998). Although closed bioreactors offer a much higher degree of control over process parameters and can have higher yields, it is uncertain whether the increased productivity can offset the higher cost and energy requirements. For a commodity product such as vegetable oil for biodiesel, low cost, high volume production is demanded, while quality is less critical (Pulz, 2001). In this case, the more favourable economics and energy requirements of open ponds may well outweigh the advantages of closed reactors.

A hybrid system combining the cost effectiveness of open ponds with the controlled environment of closed systems is appealing and has been tested in a few cases. Generally

production is divided into an initial growth or inoculum production stage in closed reactors, followed by a stress or scaling up stage in open ponds (Huntley & Redalje, 2006).

Parameter	Open	Closed
Control over process parameters	Low	High
Contamination risk	High	Low
Water loss due to evaporation	High	Low
CO_2 loss	High	Low
O_2 build-up	Low	High
Area required	High	Low
Productivity	Low	High
Consistency and reproducibility	Low	High
Weather dependence	High	Low
Cost	Low	High
Energy required	Low	High

Table 2. Comparison of open ponds and closed photobioreactors. Adapted from Pulz (2001).

5.2 Cultivation parameters

Several factors need to be considered in the cultivation of algal biomass. These include the provision of light, carbon and nutrients such as nitrate, phosphate and trace metals, the mixing regime, maintenance of optimal temperature, removal of O_2 and control of pH and salinity (Becker, 1994; Grobbelaar, 2000; Mata et al., 2010). The optimal and tolerated ranges tend to be species specific, and may vary according to the desired product.

5.2.1 Temperature

Light and temperature are among the most difficult parameters to optimise in large-scale outdoor culture systems. Daily and annual fluctuations in temperature can lead to significant decreases in productivity. Optimal growth temperatures are generally between 20 and 30°C (Chisti, 2008). Many algal species can tolerate temperatures of up to 15°C lower than their optimum, with reduced growth rates, but a temperature of only a few degrees higher than optimal can lead to cell death (Mata et al., 2010). Closed systems in particular often suffer from overheating during hot days, when temperatures inside the reactor can reach in excess of 50°C. Heat exchangers or evaporative water-cooling systems may be employed to counteract this (Mata et al., 2010). Low seasonal and evening temperatures can also lead to significant losses in productivity.

5.2.2 Light and mixing

The efficient production of algal biomass relies on the optimal provision of light energy to all cells within the culture. Most algal growth systems become light limited at high cell densities. Due to absorption and shading by the cells, light only penetrates a few centimetres into a dense algal culture (Richmond, 2004). The average provision of light is linked to reactor depth or diameter, cell concentration and mixing. A larger surface area to volume ratio, usually achieved through areas of thin panelling or narrow tubing, results in higher light provision.

Photosynthetic efficiency is highest at low light intensities. At high light levels, although photosynthetic rate may be faster, there is less efficient use of absorbed light energy.

Above the saturation point, damage to photosynthetic machinery can occur in a process known as photoinhibition (Scott et al., 2010). In a dense culture exposed to direct sunlight, cells at the surface are likely to be photoinhibited, while those at the centre of the reactor are in the dark. Mixing is therefore important not only in preventing cell settling and improving mass transfer, but also exposing cells from within a dense culture to light at the surface.

The frequency of light-dark cycling has been reported to affect algal productivity (Grobbelaar, 1994; Grobbelaar, 2000). Algae are less likely to become photoinhibited when the light is supplied in short bursts because the photosystems have time to recover during the dark period (Nedbal et al., 1996). While high rates of mixing facilitate rapid circulation of cells between light and dark zones in the reactor, high liquid velocities can damage algal cells due to increased shear stress (Mata et al., 2010). High rates of mechanical mixing or gas sparging also have large energy requirements, jeopardizing the process energy balance and increasing costs (Richardson, 2011).

5.2.3 Gas exchange

In order to maintain a high photosynthetic rate, the influx of carbon and energy must be non-limiting. In photoautotrophic growth, energy is provided by light and carbon in the form of CO_2. In order to be taken up by cells, the CO_2 must dissolve in the water. The rate of dissolution is determined by the CO_2 concentration gradient as well as by the temperature, rate of gas sparging and surface area of contact between the liquid and gas (a function of agitation and bubble size). Reactor geometry, methods of gas introduction and reactor mixing can all influence the rate of CO_2 delivery (Bailey & Ollis, 1977). Certain strains of microalgae can tolerate up to 12% CO_2 (Pulz, 2001). The 0.03% CO_2 content of ambient air is suboptimal for photosynthesis (Pulz, 2001), hence for optimal microalgal growth, additional CO_2 must be provided. This is usually done by direct injection of a CO_2 enriched air stream. As the addition of CO_2 acidifies the medium, care must be taken not to adversely decrease the pH (Anderson, 2005). It is debatable whether direct gas injection is the optimal method of CO_2 delivery. Efficiencies of carbon uptake are very low at high CO_2 concentrations, as most CO_2 exits the top of the reactor. Novel strategies of CO_2 provision include microporous hollow fibre membranes and separate gas exchanger systems (Carvalho et al., 2006).

5.2.4 Salinity, nutrients and pH

The major nutrient requirements for microalgal growth are nitrogen and phosphorous, with certain diatoms, silicoflagellates and chrysophytes also requiring silicon (Anderson, 2005). Requirements of nutrients, pH and osmolarity are species dependent. Deviation from optimal levels may cause a decrease in biomass productivity, but can have other advantages, for example, high salinity may limit contamination. Sufficient supply of all essential nutrients is a prerequisite for efficient photosynthesis and growth, but limitation of key nutrients (e.g. nitrate, phosphate or silica) may cause accumulation of desired products such as lipid.

5.2.5 Nutritional mode

Most microalgae are photoautotrophs (utilizing sunlight as their source of energy and CO_2 as a carbon source). This is the most common growth mode employed in algal cultivation

(Chen, 1996). However, several species (e.g. *Chlorella*, *Chlamydomonas, Phaeodactylum, Nitzschia, Tetraselmis* and *Crypthecodinium*) are also capable of heterotrophic growth (utilizing organic carbon such as glucose, acetate or glycerol as the sole source or carbon and energy) or mixotrophic growth (photoautotrophic growth supplemented by an organic carbon source).

The advantages of using an organic carbon substrate are that it decreases dependence on light provision, allowing growth in conventional fermenters in the dark. Optimal growth conditions can be maintained, allowing higher cell concentrations and hence increased volumetric productivities to be reached (Chen, 1996). Higher productivities of both biomass and lipid have been reported under heterotrophic growth compared to autotrophic (Ceron Garcia, 2000; Miao & Wu, 2006). Disadvantages of feeding an organic carbon source include the fact that there are a limited number of algal species that can utilize organic carbon sources, the risk of bacterial contamination is greatly increased and the carbon substrate adds an additional cost, along with the environmental burden of its production. The use of a substrate such as glucose, commonly sourced from crop plants, adds a trophic level to the process, thereby removing the simplicity of the concept of microalgae as cellular factories producing liquid fuel from pure sunlight and CO_2.

5.2.6 Cultivation strategy

The optimal cultivation strategy (e.g. batch, fed-batch or continuous cultivation mode) is determined by the kinetics of growth, product accumulation and substrate uptake (Shuler & Kargi, 2005). For production of a primary product such as protein or biomass for food or feed, optimisation of biomass productivity is the main objective. In this case, batch or continuous systems are generally used. For production of a secondary product such as carotenoids or storage lipids, the use of two or more production stages to enhance yield has been proposed (Ben-Amotz, 1995; Huntley & Redalje, 2006; Richmond, 2004). The first stage is designed to optimize growth, while the second stage provides conditions that retard growth and encourage product synthesis, usually by applying some form of stress, e.g. nutrient deprivation in the case of lipid accumulation. Another potential two-stage strategy that could enhance lipid productivity is an initial photosynthetic stage, followed by a second heterotrophic phase, where feeding with an organic carbon source such as glucose may boost lipid content.

6. Harvesting and processing

The economic recovery of microalgal biomass remains a major challenge. Microalgae for biofuel are a low value product suspended in large volumes of water. Harvesting contributes 20 to 40% of the total cost of biomass production (Gudin & Therpenier, 1986; Molina Grima et al., 2003). The difficulty in separation can be attributed to the small size of the cells (3 to 300 μm, Henderson et al., 2008), their neutral buoyancy and the fact that photoautotrophic microalgal cultures are relatively dilute, achieving concentrations in the order of 1 to 8 $g.L^{-1}$ (Pulz, 2001). Each algal species presents unique challenges due to the array of sizes, shapes, densities and cell surface properties encountered. A low-cost, energy efficient method with a high recovery efficiency and concentration is required, minimizing cell damage and allowing for water and nutrient recycle (Fig. 1).

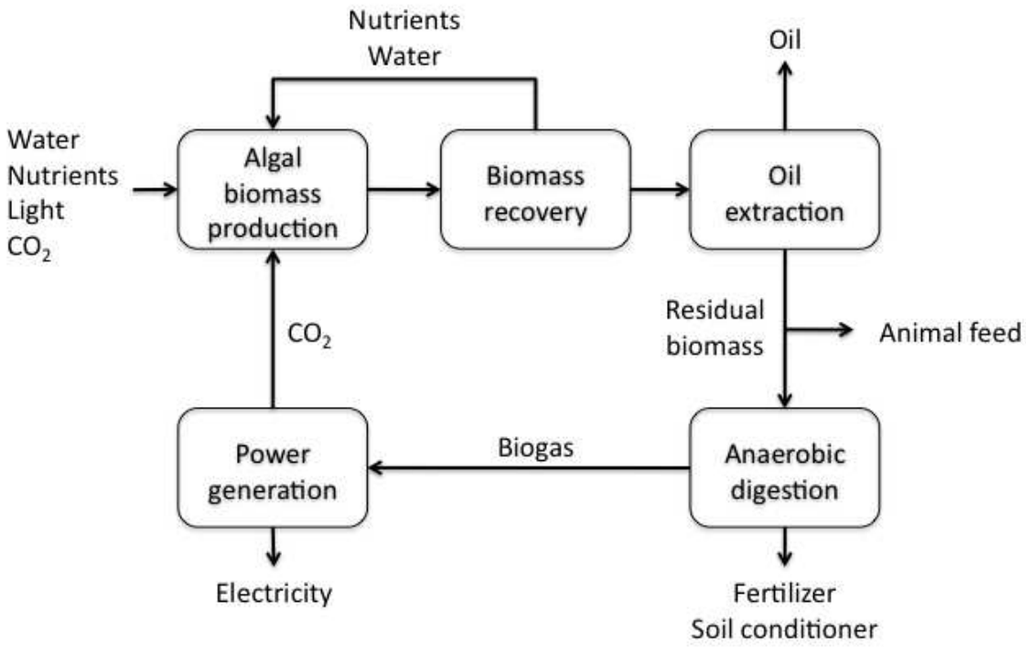


Fig. 1. Conceptual overview of microalgae process options (adapted from Chisti, 2008)

6.1 Factors affecting separation

Several natural properties of microalgal cells affect the choice and efficiency of harvesting methods. Factors relevant to separation include density, surface charge, size, shape, hydrophobicity, salinity of the medium, adhesion and cohesion properties and settling or floating velocities. Table 3 highlights the variability in some of these parameters between species, indicating that species-specific solutions may be required. Algal cell characteristics can vary with culture age and growth conditions. For example, changes in biochemical composition such as lipid content could affect the buoyancy of algal cells. Surface charge, the chemical structure of the cell wall and the amount and composition of the extracellular organic matrix (EOM) can vary with growth phase and greatly influence the degree to which cells repel or stick to one another (Bernhardt & Clasen, 1994; Henderson et al., 2008). Danquah et al. (2009) found a strong correlation between growth phase and settling efficiency, with improved filtration, flocculation and sedimentation rates during the stationary phase.

Species	Density ($kg.m^{-3}$)	Zeta potential (mV)	Culturing pH	Morphology	Diameter, length (μm)
Microcystis	1200	-7.5 to -26	5.6 - 9.5	Globular sphere	3-7
Chlorella vulgaris	1070	-17.4	7	Single cell spherical	3.5
Cyclotella sp.	1140	-19.8 to -22.3	4 - 10	Chains of spheres	6.1
Syendra acus	1100	-30 to -40	7.6	Needles	4.5-6, 100-300

Table 3. Characteristics relevant to harvesting of some microalgae (Henderson et al., 2008). Zeta potential is a measure of the degree of repulsion between adjacent particles due to surface charge.

Morphological characteristics that influence harvesting include cell motility, size, shape, cell wall elongations such as spines and flagella, colony formation, and the presence of extracellular mucilage layers or capsules (Petrusevski et al., 1995; Jarvis et al., 2009). Larger particles allow for easier separation due to increased surface area and mass. Filamentous morphology or appendages also allow for easier filtration as cells cannot pass through filter pores. Density affects sedimentation and flotation. Most algae have a specific gravity close to that of water, rendering them with a neutral buoyancy. Some cyanobacteria can adjust their density through gas vacuoles (Anderson, 2005), rendering sedimentation difficult but enabling potential surface collection.

Cell surface charges influence the electrostatic interactions between cells and between cells and surfaces or bubbles. This directly affects the adhesion, adsorption, flotation and flocculation properties of algal cells. Most algae have a negatively charged cell surface, leading to electrostatic repulsion between cell walls. Addition of positively charged ions to the solution can help to neutralize the negative surface charge and aid cell flocculation. Changing the pH of the solution can also cause flocculation (Chen et al., 1998). Hydrophobicity is another, non-electric property affecting interaction of algal cells with each other and external surfaces. Most algae are naturally hydrophilic (Fattom & Shilo, 1984), but this can be altered by surfactants and pH (Jameson, 1999). Increasing the hydrophobicity of cells could cause them to adhere to bubbles, filters or other separation catalysts.

6.2 Harvesting methods

Harvesting requires one or more solid-liquid separation techniques (Molina Grima et al., 2003). In order to achieve the levels of concentration required, various chemical, biological and physical separation steps may be necessary. Common methods of cell harvesting include flocculation, filtration, sedimentation, centrifugation and flotation (Mata et al., 2010). The small cell size of microalgae makes them difficult to dewater. Flocculation is used to 'clump' the cells, grouping them together to form larger particle sizes. This is often suggested as a pretreatment step prior to filtration, sedimentation or flotation. Flocculation occurs when the repulsion between cells is reduced, allowing them to either aggregate directly onto each other or through an intermediate bridging surface. The extent of flocculation is dependent on pH, temperature, density, hydrophobicity, surface charge and culture age (Lee et al., 1998). Flocculation can be induced by addition of positively charged ions or polymers, e.g. minerals such as lime, calcium and salts, metal salts such as aluminium sulphate and ferric chloride, and naturally occurring flocculants such as starch derivatives and tannins. Drawbacks to the use of chemical flocculants are the high dosages required, the need for pH correction (Pushparaj et al., 1993) and the contamination of the biomass and media with the flocculant, meaning that media cannot be recycled without removal of the chemical. Autoflocculation can be induced through pH change (Csordas & Wang, 2004), nutrient limitation (Schenk et al., 2008), excretion of macromolecules (Benemann et al., 1980) or aggregation between microalgae and bacteria (Lee et al., 2009).

Conventional filtration is only effective for larger (> 70 μm) or filamentous species such as *Coelastrum* and *Spirulina* (Brennan & Owende, 2010, Lee et al., 2009). For smaller cells, micro-filtration, ultra-filtration and membrane-filtration can be used, though usually only for small volumes (Brennan & Owende, 2010, Petrusevski et al., 1995, Borowitzka, 1997). Fouling (accumulation of material on the surface of the membrane, slowing filtration) is a major problem. If filtration were to be considered for mass production, a high driving force for separation (high pressure or suction) would be required, which necessitates a high energy

input. Microstraining (filtration by natural gravity using low speed rotating drum filters) is a promising method due to ease of operation and low energy consumption (Mohn, 1980). Another option is cross-flow membrane filtration (Zhang et al., 2010). Using a tangential, turbulent flow of liquid across the membrane prevents clogging of the filter with cells. The efficiency of the process is very dependent on cell morphology and the transmembrane pressure (Petrusevski et al., 1995). A more unconventional approach is magnetic filtration. Here addition of magnetic metals, either taken up by algal cells, or used to flocculate them, could allow capture using a magnetic field (Bitton et al., 1975).

Sedimentation is the process whereby solid particles suspended in a fluid are settled under the influence of gravity or some other force. In microalgae, it depends on coagulation or flocculation of cells to produce flocs with a large enough size (> 70 μm) or high enough density to induce settling (Vlaski et al., 1997). Sedimentation is typically used in wastewater treatment. It is suitable for large throughput volumes and has low operational costs. Flocculation, using a dense substance such as calcium carbonate, can greatly reduce settling time. Ultrasound (acoustic energy) can be used to induce aggregation and facilitate sedimentation (Bosma et al., 2003), however the energy requirement may be too high for large-scale use.

Centrifugation is essentially sedimentation under a rotational force rather than gravity. The efficiency of centrifugation depends on the size and density of the particles, the speed of the rotor, the time of centrifugation and the volume and density of the liquid. Almost all microalgae can be harvested by centrifugation. It is a highly efficient and reliable method, can separate a mixture of cells of different densities and does not require the addition of chemicals, but has a high energy consumption (Chisti, 2007). It is routinely used for recovery of high value products, or for small scale research operations, although large, flow-though centrifuges can be used to process large volumes. Many algae require speeds of up to 13 000 g which results in high shear forces (Harun et al., 2010; Knuckey et al., 2006) and can damage sensitive cells.

Flotation operates by passing bubbles through a solid-liquid mixture. The particles become attached to the bubble surface and are carried to the top of the liquid where they accumulate. The concentrated biomass can be skimmed off (Uduman et al., 2010). Flotation is considered to be faster and more efficient than sedimentation (Henderson et al., 2008). It is associated with low space requirements and moderate cost. Addition of chemical coagulants or flotation agents is often required to overcome the natural repulsion between the negatively charged algal particles and air bubbles. The pH and ionic strength of the medium are important factors to optimize this recovery technique.

6.3 Processing

After harvesting, the major challenge is in releasing the lipids from their intracellular location in the most energy efficient and economical way possible. Algal lipids must be separated from the rest of the biomass (carbohydrates, proteins, nucleic acids, pigments) and water. Common harvesting methods generally produce a slurry or paste containing between 5 and 25% solids (Shelef et al., 1984). Removing the rest of the water is thought to be one of the most expensive steps with literature values ranging from 20 to 75% of the total processing cost (Uduman et al., 2010, Molina Grima et al., 2003). Shelef et al. (1984) highlight a number of possible techniques for drying biomass: flash drying, rotary driers, toroidal driers, spray drying, freeze-drying and sun drying. Because of the high water content, sun-drying is not an effective method and spray-drying is not economically

feasible for low value products (Mata et al., 2010). The selection of drying technique is dependent on the scale of operation, the speed required and the downstream extraction process (Mohn, 1980).

Lipid extraction can be done in a number of ways. Solvent extraction techniques are popular, but the cost and toxicity of the solvent (e.g. hexane) is of concern and solvent recovery requires significant energy input. Other methods involve disruption of the cell wall, usually by enzymatic, chemical or physical means (e.g. homogenization, bead milling, sonication (Mata et al., 2010)), allowing the released oil to float to the top of the solution. Ultrasound and microwave assisted extraction methods have been investigated (Cravotto et al., 2008). Supercritical CO_2 extraction is an efficient process, but is too expensive and energy intensive for anything but lab-scale production. Direct transesterification (production of biodiesel directly from algal biomass) is also possible. Some of these techniques do not require dry biomass, but the larger the water content of the algal slurry, the greater the energy and solvent input required.

Once the algal oil is extracted, it can be treated as conventional vegetable oil in biodiesel production. Direct pyrolysis, liquefaction or gasification of algal biomass have also been suggested as means of producing fuel molecules. One of the concerns for biodiesel production through transesterification, shared with any biodiesel feedstock, is the quality of the biodiesel produced. Biodiesel must meet certain international regulations, for example, the ASTM international standards or the EN14214 in Europe. It has been calculated that the fatty acid profile of certain microalgal species will produce biodiesel that does not meet these specification, therefore blending or additives may be required (Stansell, 2011).

7. Economic and environmental feasibility

In order to be economically feasible, microalgal biodiesel must be cost competitive with petroleum-based fuels. We have investigated the relationship between algal lipid productivity and cost in order to determine the range of productivities that need to be achieved for economic viability. Based on values from Chisti (2007), a model was set up to estimate cost per litre of algal oil as a function of algal biomass productivity and lipid content. Where the cost of producing a litre of algal biodiesel was below the price of a litre of fossil-fuel derived diesel, it was considered economically viable (i.e. no profit margin was introduced). The price of fossil-fuel derived diesel is partly dependent on the price of crude oil, which has varied widely in the last few years, hence several scenarios were evaluated.

Assumptions made in the execution of the model were:

1. Cost per kg algal biomass: US$ 0.6 for raceway ponds, and US$ 0.47 for photobioreactors (Chisti, 2007)
2. In order to be economically viable, the cost of algal oil per litre must be less than 6.9×10^{-3} times the cost of crude oil in US$ per barrel (Chisti, 2007)
3. Density of algal oil: 0.86 $g.cm^{-3}$ (Barsanti & Gualtieri, 2007)

The economic model was run for three prices of crude oil, based on fluctuations over the last few years. These scenarios of 'high' ($ 130), 'medium' ($ 90) and 'low' ($ 50) cost of crude oil per barrel gave the price limits for algal oil of 0.90, 0.62 and 0.35 US$ per L respectively. The results of the model are shown in Fig. 2a (raceway ponds) and 2b (closed photobioreactors).

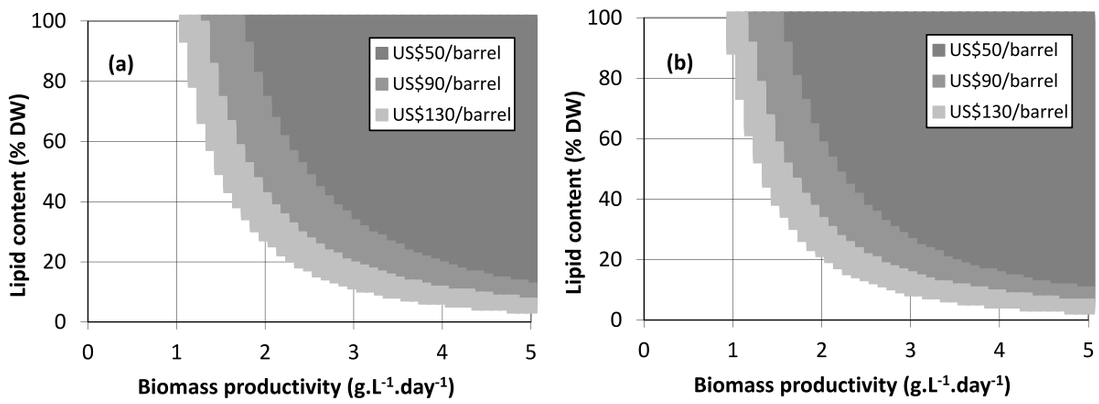


Fig. 2. Lipid contents and biomass productivities required for economic feasibility in (a) large-scale, outdoor raceway ponds and (b) large-scale, outdoor photobioreactors. Dark grey region: productivities economically feasible at US$ 50 per barrel crude oil (cost of algal oil per L lower than cost of regular diesel per L). Additional region for crude oil price US$ 90 per barrel = mid-grey and US$ 130 = light grey

Based on this model, the results for raceway ponds show that algal biodiesel will not be economically feasible, either in ponds or photobioreactors, at current costs below a biomass productivity of 1 $g.L^{-1}.day^{-1}$. Assuming a maximum realistically achievable lipid content of 50% DW, algal biodiesel becomes economically feasible at biomass productivities of 1.5 $g.L^{-1}.day^{-1}$ (US$ 130 per barrel crude oil), close to 2 $g.L^{-1}.day^{-1}$ (US$ 90), and 2.5 $g.L^{-1}.day^{-1}$ (US$ 50) in raceway ponds. At lower lipid contents, higher biomass productivity is required, e.g. at a lipid content of 25% DW, algal biodiesel only becomes cost effective at 2 $g.L^{-1}.day^{-1}$ for US$ 130 per barrel. The model for photobioreactors is based on a lower cost per kg algal biomass than raceway ponds, hence economic feasibility is reached at slightly lower biomass productivities and lipid contents, e.g. at a biomass productivity of 2 $g.L^{-1}.day^{-1}$, a lipid content of only 20% DW is required to be viable at US$ 130 per barrel crude oil.

Currently reported biomass productivities in outdoor raceway ponds average around 0.17 $g.L^{-1}.day^{-1}$, with a lipid content of 26% DW (Griffiths and Harrison, 2009), which is far from being economically feasible. Biomass productivities for closed photobioreactors (1.33 $g.L^{-1}.day^{-1}$) are closer to being within the economically viable range, if they can be maintained in the long term, concurrent with sufficiently high lipid content. As a reflection of this, there are currently no industrial facilities producing biodiesel from microalgae (Lardon, 2009). For cultivation to be economically viable, productivities must be increased, costs lowered, or additional income streams developed. The economics of algal biofuel production could be greatly improved through the production of co-products. For example, high value compounds such as pigments could be produced along with lipid. The residual biomass after lipid extraction could be sold as animal feed, fertilizer or soil conditioner, anaerobically digested to produce biogas, gasified or merely burned to provide some of the heat or electricity required in the process.

In addition to economic feasibility, algal biodiesel must be environmentally desirable. It is critical that the energy embodied in the fuel produced is greater than the energy input required to produce it. Net energy analysis and life cycle analysis (LCA) are tools used to quantify the environmental burdens at every stage of production, from growth of the

biomass to combustion of the fuel. Lardon et al. (2009) conducted a life-cycle analysis of a hypothetical algal biodiesel production facility. Two different culture conditions: fertilizer feeding and nitrogen starvation, as well as two different extraction options: dry or wet, were investigated. The study confirmed the potential of microalgae as an energy source, but highlighted the necessity of decreasing energy and fertilizer consumption. Energy inputs, such as the energy required for mixing and pumping, the embodied energy in the materials used and the energy cost of harvesting and processing must be minimized. Recycling of material and energy from waste streams is also important wherever feasible (Scott et al., 2010). The use of nitrogen stress, as well as the optimization of wet extraction were indicated as desirable options. The anaerobic digestion of residual biomass was also suggested as a way of reducing external energy usage and recycling of nutrients.

We conducted a LCA on a hypothetical algal biodiesel process. Biomass production in three different reactor types (open ponds and two types of closed reactor: horizontal tubular and vertical tubular) was evaluated. In all cases, harvesting was modeled as an initial settling step followed by centrifugation. Hexane extraction was used to recover the oil, with the residual biomass sent for anaerobic digestion and the resulting energy from biogas production recycled to the process. The hexane was recovered and the oil converted to biodiesel using an enzymatic process. The basis chosen was production of 1000 kg of biodiesel from *Phaeodactylum tricornutum*. The net energy return (the energy embodied in the biodiesel produced divided by the energy input required) was positive (1.5) for the open pond, neutral (0.97) for the horizontal tubular reactor and negative (0.12) for the vertical tubular reactor. In this model, open ponds were the most energetically favorable reactor type, yielding 50% more energy than was put in. Horizontal tubular reactors required an energy input equivalent to the output, and vertical tubular reactors were the most unfavorable, requiring several times the energy input as that in the product, where system optimization was not conducted.

The overriding energy input in the process was found to be that required to run the reactor. Reactor energy was by far the most dominant determinant of the overall process energy requirement. This was largest in the vertical tubular reactor as these were continually mixed by gas sparging. Energy required for pumping between unit processes was also significant, particularly at lower biomass concentrations due to the larger volume of culture to be processed. The major energy inputs in downstream processing were that embodied in the lime used as a flocculation agent, and the energy required for solvent recovery. Lipid productivity and species choice had a significant impact on the energy balance.

8. Optimizing lipid productivity

Increasing microalgal lipid productivity improves both the economics and energy balance of the process. The land area and size of culture vessels required, as well as the energy and water requirements for large-scale algal culture are strongly dependent on algal productivity. With a higher productivity, lower cultivation, mixing, pumping and harvesting volumes would be required to yield the same amount of product, resulting in lower cost and energy requirements. More concentrated cell suspensions could also make downstream processing more efficient. The genetic characteristics of an algal species determine the range of its productivity. The levels reached in practice within this range are determined by the culture conditions. The two main approaches to enhancing productivity are: 1. selection of highly productive algal species and 2. designing and maintaining optimal conditions for productivity.

The choice of algal strain is a key consideration. The diversity of algal species is much greater than that of land plants (Scott et al., 2010) allowing selection of species best suited to the local environment and goals of the project. Although there have been several screening programs, building on the work of the Aquatic Species Program (Sheehan et al., 1998), the majority of strains remain untested, few species have been studied in depth and the data reported in the literature is often not comparable due to the different experimental procedures used. We conducted a broad literature review of the growth rates and lipid contents of 55 promising microalgal species under both nutrient replete and limited conditions. The original study (Griffiths & Harrison, 2009) has been extended here through the use of two key assumptions to convert data into common units of biomass and lipid productivity.

Lipid productivity is determined by both growth rate and lipid content. Lipid content (P) was typically reported as percentage dry weight (% DW). Data presented in pg lipid.cell^{-1} was discarded if no cell weight was available for conversion. Growth rates were reported as doubling time (T_d) or specific growth rate (μ). These were inter-converted according to Equation 1.

$$T_d = \frac{\ln 2}{\mu} \tag{1}$$

Standard units of g.L^{-1}.day^{-1} were chosen for biomass productivity. Specific growth rate (μ, in units of day^{-1}) can be converted to volumetric biomass productivity (Q_V, in g.L^{-1}.day^{-1}) where the biomass concentration (X, in g.L^{-1}) is known (Equation 2). Biomass productivity is often reported on the basis of surface area (Q_A), in units of g.m^{-2}.day^{-1}. This can be converted to Q_V using Equation 3 where the depth (D, in m) of the culture vessel can be calculated from the reactor geometry.

$$Q_V = \mu \times X \tag{2}$$

$$Q_V = \frac{Q_A}{D \times 1000} \tag{3}$$

Lipid productivity (Q_P) was infrequently reported in the literature, and was generally reported in g.L^{-1}.day^{-1} or mg.L^{-1}.day^{-1}. This parameter could be calculated from volumetric biomass productivity (Q_V, in g.L^{-1}.day^{-1}) and lipid content (P in % DW) where appropriate data were available (Equation 4).

$$Q_P = Q_V \times P \tag{4}$$

The calculation of lipid productivity for the majority of species necessitated two assumptions:

1. Conversion of areal productivities (in g.m^{-2}.day^{-1}) to volumetric productivities (g.L^{-1}.day^{-1}), using an average depth of 0.1 m, based on best fit of the data
2. Conversion of specific growth rate to biomass productivity using an average biomass concentration of 0.15 g.L^{-1}, based on typical experimental results.

The average literature values for the 55 species are shown in Table 4. Among the species with the highest reported lipid productivity were *Neochloris oleoabundans*, *Navicula pelliculosa*, *Amphora*, *Cylindrotheca* and *Chlorella sorokiniana* (Fig. 3). Other findings were that green algae (Chlorophyta) generally showed an increase in lipid content when nitrogen deficient, whereas

			Lipid content			Biomass productivity			Lipid productivity	
			N replete	N defficient	T_d	Q_A	Q_V	Ave Q_V	Calculated	Literature
Species	**Taxa[a]**	**Media[b]**	**% dw**	**% dw**	**days**	**g.m^{-2}.day^{-1}**	**g.L^{-1}.day^{-1}**	**g.L^{-1}.day^{-1}**	**mg.L^{-1}.day^{-1}**	**mg.L^{-1}.day^{-1}**
Amphiprora hyalina	B	M	22	28	0.41			0.30	67	
Amphora	B	M	51		0.83	40.0		0.23	117	160
Anabaena cylindrica	Cy	F	5	5	1.00			0.10	5	
Ankistrodesmus falcatus	C	F	24	32	0.33	31.6	0.46	0.36	85	
Chaetoceros calcitrans	O	M	40				0.04	0.04	16	18
Chaetoceros muelleri	O	M	19	27	0.46		0.07	0.26	50	22
Chlamydomonas applanata	C	F	18	33						
Chlamydomonas reinhardtii	C	F	21		0.26			0.40	83	
Chlorella emersonii	C	F	29	63	0.80		0.03	0.08	23	
Chlorella minutissima	C	M	31	57	1.60		0.03	0.05	15	
Chlorella protothecoides	C	F	13	23	1.68			0.07	8	
Chlorella pyrenoidosa	C	F	16	64	0.28			0.47	76	
Chlorella sorokiniana	C	F	18	18	0.35		0.55	0.62	110	45
Chlorella vulgaris	C	F	24	42	0.70	10.7	0.11	0.16	40	30
Crypthecodinium cohnii	D	M	25		0.38			0.28	70	
Cyclotella cryptica	O	M	18	34	0.56			0.20	36	
Cylindrotheca	B	M	27	27	0.30			0.43	114	
Dunaliella primolecta	Pr	S	23	14		9.1		0.09	21	
Dunaliella salina	Pr	S	19	10	0.44			0.27	53	
Dunaliella tertiolecta	Pr	S	15	18	0.48			0.22	35	
Euglena gracilis	Eg	F	20	35	0.60			0.18	37	
Hymenomonas carterae	H	M	20	14	1.71			0.06	12	
Isochrysis galbana	H	M	25	29	0.89	11.5	0.16	0.15	37	38
Monodopsis subterranea	E	F	25	13			0.19	0.19	48	30
Monoraphidium minutum	C	F	22	52	0.35			0.30	65	
Nannochloris	C	M/F	28	30	0.49	31.9	0.23	0.27	74	77
Nannochloropsis	E	M	31	41	1.20		0.27	0.24	72	52
Nannochloropsis salina	E	M	27	46		13.9		0.14	38	
Navicula acceptata	B	F	33	35	0.42			0.29	96	
Navicula pelliculosa	B	F	27	45	0.23			0.46	124	
Navicula saprophila	B	F	24	51	0.38			0.28	68	
Neochloris oleoabundans	C	F	36	42			0.46	0.46	164	136
Nitzschia communis	B	M			0.96			0.18		
Nitzschia dissipata	B	M	28	46	0.39			0.27	73	
Nitzschia frustulum	B	M	26							
Nitzschia palea	B	M	47	40						48
Oscillatoria	Cy	F	7	13	0.28			0.37	27	
Ourococcus	C	F	27	50	3.01			0.03	9	
Pavlova lutheri	H	M	36				0.21	0.21	75	50
Pavlova salina	H	M	31				0.16	0.16	49	49
Phaeodactylum tricornutum	B	M	21	26	1.02	20.0	0.34	0.18	38	45
Porphyridium purpureum	R	M	11		0.45		0.23	0.23	24	35
Prymnesium parvum	H	M	30		0.74			0.14	42	
Scenedesmus dimorphus	C	F	26		0.46			0.23	57	
Scenedesmus obliquus	C	F	21	42	2.74		0.12	0.10	22	
Scenedesmus quadricauda	C	F	18				0.19	0.19	35	35
Selenastrum gracile	C	F	21	28						
Skeletonema costatum	O	M	16	25	0.66		0.08	0.15	24	17
Spirulina maxima	Cy	S	7		1.34			0.16	11	
Spirulina platensis	Cy	S	13	10	0.60	25.0		0.23	29	
Synechococcus	Cy	M	11		0.36			0.29	32	75
Tetraselmis suecica	P	M	17	26	1.51	28.1	0.59	0.39	65	32
Thalassiosira pseudonana	O	M	16	26	0.49		0.08	0.26	43	17
Thalassiosira weissflogii	O	M	22	24	0.58			0.18	41	
Tribonema	O	M	12	16	1.82		0.51	0.28	33	
			Average	**Average**	**Average**	**Average**	**Average**	**Average**	**Average**	**Average**
Total			23	32	0.80	22.2	0.23	0.23	52	51
Freshwater			21	36	0.82	21.2	0.24	0.26	54	35
Marine			25	31	0.82	22.7	0.21	0.21	49	47
Chlorophyta			23	41	1.01	24.7	0.24	0.25	58	65
Other taxa			25	30	0.72	20.4	0.24	0.23	57	50
Cyanobacteria			8	9	0.72	25.0		0.23	21	75

[a] Key to taxa: C = Chlorophyta, Cy = Cyanobacteria, D = Dinophyta, E = Eustigmatophyta, Eg = Euglenozoa, H = Haptophyta, O = Ochrophyta, Pr = Prasinophyta, [b] Key to media: F = Freshwater, M = Marine, S = Saline

Table 4. Growth and lipid parameters of 55 species of microalgae, along with their taxonomy and media type (adapted from Griffiths and Harrison, 2009). The average of literature values for lipid content under nitrogen (N) replete and deficient growth conditions, doubling time (T_d), and areal (Q_A) and volumetric (Q_V) biomass productivities are shown in columns 4 to 8. Average biomass productivity calculated from T_d, μ, Q_A and Q_V is shown in column 9, and calculated and literature lipid productivity in columns 10 and 11 respectively. Blanks represent no data available

diatoms and other taxa were more variable in their response, although all those subjected to silicon deprivation showed an increase in lipid content. This increase in lipid content, however, does not necessarily translate into increased lipid productivity due to decreased growth rates under nutrient stress conditions. Response of biomass productivity to nutrient deprivation is variable between species and further investigation is necessary.

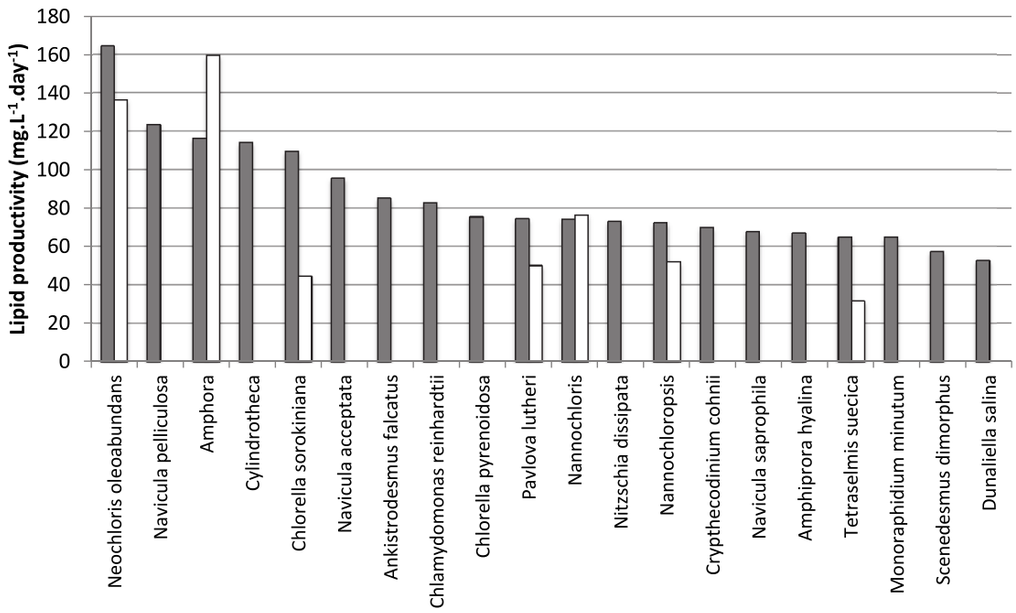


Fig. 3. Average calculated (grey bars) and literature (empty bars) biomass productivity for the 20 most productive species investigated (adapted from Griffiths & Harrison, 2009)

In Fig. 4, the impact of biomass productivity and lipid content on calculated lipid productivity is analyzed through correlation. A relationship is demonstrated between lipid productivity and biomass productivity. All species with a high biomass productivity (above 0.4 $g.L^{-1}.day^{-1}$), and all but one above 0.3 $g.L^{-1}.day^{-1}$, have a high lipid productivity, greater than 60 $mg.L^{-1}.day^{-1}$. However, there are a few species with high lipid productivity despite an average biomass productivity, indicating that lipid content is also a factor. Lipid content correlates poorly with lipid productivity, indicating that lipid content alone is not a good indicator of suitability for biodiesel production. There are several species with low lipid productivity despite an above-average lipid content (> 22%). The species with high lipid productivities (> 60 $mg.L^{-1}.day^{-1}$) range in lipid content from 16% DW to 51%. Further, species with high lipid content (> 30%) vary in lipid productivity between 15 and 164 $mg.L^{-1}.day^{-1}$.

Once the species has been chosen, the next critical factor is the optimisation of culture conditions. In addition to optimal temperature and pH, conditions that maximize autotrophic growth rate are optimal light, carbon and nutrient supply. Microalgal lipid accumulation is affected by a number of environmental factors (Guschina & Harwood 2006; Roessler 1990), and often enhanced by conditions that apply a 'stress' to the cells. Lipids appear to be synthesised in response to conditions when energy input (rate of photosynthesis) exceeds the capacity for energy use (cell growth and division) (Roessler 1990). Enhanced cell lipid content has been found under conditions of nutrient deprivation (Hsieh & Wu, 2009; Illman et al., 2000; Li et al., 2008; Shifrin & Chisholm, 1981; Takagi et al.,

2000), high light intensity (Rodolfi et al., 2009), high temperature (Converti et al., 2009); high salt concentration (Takagi et al., 2000) and high iron concentration (Liu et al., 2008).

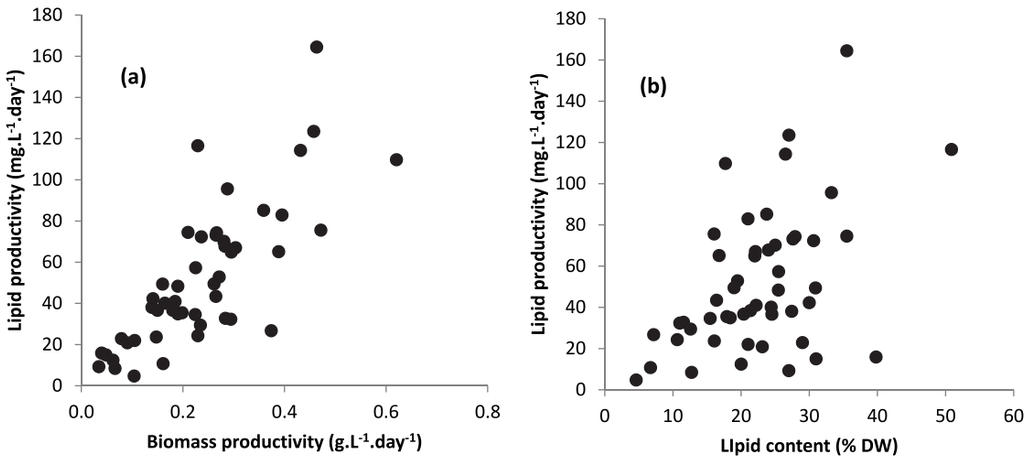


Fig. 4. Correlation of calculated lipid productivity with (a) biomass productivity and (b) lipid content under nutrient replete conditions

Nitrogen (N) deprivation is the most frequently reported method of enhancing lipid content, as it is cheap and easy to manipulate. N deficiency has a reliable and strong influence on lipid content in many species (Chelf, 1990; Rodolfi et al., 2009; Shifrin & Chisholm, 1981). Unfortunately, stress conditions that enhance lipid content, such as nitrogen deprivation, typically also decrease the growth rate, and thus the net effect on lipid productivity must be ascertained (Lardon et al., 2009). Maximum biomass productivity and lipid content in *Chlorella vulgaris* occur under different conditions of nitrogen availability, suggesting that a two-stage cultivation strategy may be advantageous. From studies we have conducted on *C. vulgaris*, it appears that an intermediate level of nitrogen limitation creates the optimum balance between biomass and lipid production. The optimum cultivation strategy tested was batch culture, using a low starting nitrate concentration (between 250 and 300 $mg.L^{-1}$ nitrate), ensuring that nitrogen in the medium was depleted towards the end of exponential growth. Other cultivation strategies (e.g. two-stage batch, fed-batch or continuous) were found not to improve upon the productivity achieved in N limited batch culture.

Although high lipid productivity is a key factor in species selection, other characteristics such as ease of cultivation, tolerance of a range of environmental conditions (particularly temperature and salinity), flue-gas contaminants and high O_2 concentrations, as well as resistance to contaminants and predators are likely to be equally as important.

9. Conclusion and future research directions

Algal biodiesel continues to hold promise as a sustainable, carbon neutral source of transportation fuel. The technical feasibility of algal biodiesel has been demonstrated (Miao & Wu, 2006; Xiong et al., 2008), but the economics and energy demands of production require substantial improvement. The necessary changes appear attainable through the enhancement of productivity, the reduction of cost and energy demand for key processes and the application of the biorefinery concept (co-production of valuable products or

processes). Current research is focussed on achieving this through a combination of biological and engineering approaches. The major challenges currently being addressed are:

- Increasing productivity in large-scale outdoor microalgal culture
- Minimizing contamination by predators and other algal species
- Mitigating temperature changes and water loss due to evaporation
- Optimizing supply of light and CO_2
- Developing cheap and efficient reactor designs
- Developing cost and energy-efficient methods of harvesting dilute suspensions of small microalgal cells
- Decreasing the overall energy and cost requirements, particularly for pumping, gas transfer, mixing, harvesting and dewatering
- Improving resource utilization and productivity through a biorefinery approach
- Producing valuable co-products
- Decreasing environmental footprint through recycling of water, energy and nutrients.

These topics have captured the imagination of several researchers and some innovative solutions are being investigated. The overall goal of biofuel production is to optimise the conversion of sunlight energy to liquid fuel. In algal cultivation, techniques to improve light delivery include manipulating the reactor design, the use of optics to deliver light to the centre of the reactor, optimising fluid dynamics to expose all cells to frequent light flashes, increasing the efficiency of photosynthesis and carbon capture (e.g. enhancing the carbon concentrating mechanism), and using mixed-species cultures to utilise different intensities or wavelengths of light (Scott et al., 2010).

One of the major problems with light delivery is poor penetration of light into dense cultures due to mutual shading by the cells. Under high light conditions, microalgal cells absorb more light than they can use, shading those below them and dissipating the excess energy as fluorescence or heat. In nature, this confers individual cells an evolutionary advantage, however, in mass production systems it is undesirable as it decreases overall productivity. It would be advantageous to minimize the size of the chlorophyll antennae in cells at the surface, so as to permit greater light penetration to cells beneath (Melis, 2009). Reducing the size of the light harvesting complexes through genetic modification has been shown to improve productivity (Nakajima et al., 2001). The goal now is to engineer cells that change antennae size according to light intensity.

Although the TAG content of cells can be enhanced by manipulation of the nutrient supply, there is a tradeoff between growth and lipid production. For optimum productivity, cells that can maintain a simultaneously high growth rate and lipid content are required. Strategies to achieve this include screening for novel species, and genetic engineering of well characterised strains. The genes and proteins involved in regulation of lipid production pathways are currently being investigated through synthetic biology and the modelling of carbon flux through metabolism. Key enzymes and branch-points can then be manipulated to improve productivity. For example, carbohydrate and lipid production compete directly for carbon precursors. Shunting carbon away from starch synthesis by downregulation of the enzyme ADP-glucose pyrophosphorylase in *Chlamydomonas* has been shown to enhance TAG content 10-fold (Li et al., 2010).

The challenge of harvesting small algae cells from dilute suspensions has yet to be solved in a cheap, energy efficient manner. Ideally the addition of chemical agents that impede the recycling of the culture medium and nutrients should be avoided. A series of methods is likely to be used e.g. flocculation followed by sedimentation, or settling followed by

centrifugation. Promising ideas for harvesting techniques include concentration using sound waves and triggering of autoflocculation on command. Another attractive idea is direct product excretion, where algae secrete fuel molecules into the medium as they are produced, allowing continuous production and harvesting without cell disruption. The Cyanobacterium *Synechocystis* has recently been successfully modified to excrete fatty acids (Liu, 2011).

The use of nutrients from waste sources (e.g. CO_2 from flue-gas and nitrate and phosphate from wastewater) could help to reduce costs and energy input, as well as contributing to environmental remediation. Potential co-products include fine chemicals such as astaxanthin, B-carotene, omega-3 fatty acids, polyunsaturated fatty acids, neutraceuticals, therapeutic proteins, cosmetics, aquafeed and animal feed (Mata et al., 2010). Algae could also potentially be modified to synthesize other types of fuel e.g. ethanol, butanol, isopropanol and hydrocarbons (Radakovits et al., 2010) or downstream processing of algae could be modified to process the entire biomass to energy containing fuels through thermal processes.

10. Acknowledgements

This work is based upon research supported by the South African National Energy Research Institute (SANERI), the South African Research Chairs Initiative (SARChI) of the Department of Science and Technology, the Technology Innovation Agency (TIA) and the National Research Foundation (NRF). The financial assistance of these organizations is hereby acknowledged. Opinions expressed and conclusions arrived at are those of the authors and are not necessarily to be attributed to SANERI, SARChI, TIA or the NRF.

11. References

An, J-Y, Sim, S-J, Lee JS & Kim BW. (2003). Hydrocarbon production from secondarily treated piggery wastewater by the green alga *Botryococcus braunii*. *Journal of Applied Phycology*, 15(2-3): 185-191

Anderson, RA. (2005). *Algal Culturing Techniques*. Elsevier, London

Bailey, J & Ollis, D. (1977). *Biochemical Engineering Fundamentals*. McGraw-Hill, New York

Barsanti, L & Gualtieri, P. (2006). *Algae: Anatomy, Biochemisty and Biotechnology*. CRC Press, Taylor and Francis Group, Florida

Becker, EW. (1994). *Microalgae: Biotechnology and Microbiology*. Cambridge University Press, Cambridge

Ben-Amotz, A. (1995). New Mode of *Dunaliella* Biotechnology: Two-Phase Growth for B-Carotene Productio. *Journal of Applied Phycology,* 7(1):65-68

Benemann, J, Koopman, B, Weissman, J, Eisenberg, D & Gobell R. (1980). Development of Microalgae Wastewater Treatment and Harvesting Technologies in California. In *Algae Biomass: Production and Use*. Edited by Shelef, G & Soeder, C. pp. 457-495 . Elsevier, Amsterdam

Bernhardt, H & Clasen, J. (1994). Investigations into the Flocculation Mechanisms of Small Algal Cells. *Journal of Water Supply: Research and Technology – AQUA,* 43(5): 222-232

Bitton, G, Fox, JL & Strickland, HG. (1975). Removal of Algae from Florida Lakes by Magnetic Filtration. *Applied and Environmental Microbiology*, 30(6):905-908

Borowitzka, MA. (1992). Algal Biotechnology Products and Processes - Matching Science and Economics. *Journal of Applied Phycology*, 4:267-279

Borowitzka, MA. (1997). Microalgae for Aquaculture - Opportunities and Constraints. *Journal of Applied Phycology,* 9(5):393-401

Bosma, R, van Spronsen, WA, Tramper, J & Wijffels, RH. (2003). Ultrasound, a New Separation Technique to Harvest Microalgae. *Journal of Applied Phycology*, 15(2-3):143-153

Brennan, L & Owende, P. (2010). Biofuels from microalgae: A Review of Technologies for Production, Processing, and Extractions of Biofuels and Co-Products. *Renewable and Sustainable Energy Reviews,* 14(2):557-577

Carvalho, AP, Meireles, LA & Malcata, FX. (2006). Microalgal Reactors: A Review of Enclosed System Designs and Performances. *Biotechnology Prog*ress, 22(6):1490-1506

Ceron Garcia, MC, Fernandez Sevilla, JM, Acien Fernandez, FG, Molina Grima, E & Garcia Camacho, F. (2000). Mixotrophic Growth of *Phaeodactylum tricornutum* on Glycerol: Growth Rate and Fatty Acid Profile. *Journal of Applied Phycology*, 12:239-248

Chelf, P. (1990). Environmental Control of Lipid and Biomass Production in Two Diatom Species. *Journal of Applied Phycology,* 2(2):121-129

Chen, F. (1996). High Cell Density Culture of Microalgae in Heterotrophic Growth. *Trends in Biotechnology,* 14(11):421-426

Chen, YM, Liu, JC & Ju, Y. (1998). Flotation Removal of Algae from Water. *Colloids and Surfaces B: Biointerfaces,* 12(1):49-55

Chisti, Y. (2007). Biodiesel from Microalgae. *Biotechnology Advances*, 26(3):294-306

Chisti, Y. (2008). Biodiesel from Microalgae Beats Bioethanol. *Trends in Biotechnology*, 25(3):126-31

Converti, A, Casazza, A, Ortiz, EY, Perego, P & Del Borghi, M. (2009). Effect of Temperature and Nitrogen Concentration on the Growth and Lipid Content of Nannochloropsis Oculata and *Chlorella vulgaris* for Biodiesel Production. *Chemical Engineering and Processing: Process Intensification,* 48(6):1146-1151

Csordas, A. (2004). An Integrated Photobioreactor and Foam Fractionation Unit for the Growth and Harvest of *Chaetoceros* spp. in Open Systems. *Aquaculture Engineering,* 30(1-2):15-30

Danquah, MK, Gladman, B, Moheimani, N & Forde, GM. (2009). Microalgal Growth Characteristics and Subsequent Influence on Dewatering Efficiency. *Chemical Engineering Journal,* 151(1-3):73-78

De la Pena, M. (2007). Cell Growth and Nutritive Value of the Tropical Benthic Diatom, *Amphora* sp., at Varying Levels of Nutrients and Light Intensity, and Different Culture Locations. *Journal of Applied Phycology,* 19(6):647-655

Dennis, DT, Turpin, DH, Lefebvre, DD & Layzell, DB. (1998). *Plant metabolism*. Longman, Singapore

Fattom, A & Shilo, M. (1984). Hydrophobicity as an Adhesion Mechanism of Benthic Cyanobacteria. *Applied and Environmental Microbiology,* 47(1):135-143

Golueke, CG, Oswald, WJ & Gotaas, HB. (1957). Anaerobic Digestion of Algae. *Applied Microbiology*, 5(1):47-55

Griffiths, MJ & Harrison, STL. (2009). Lipid Productivity as a Key Characteristic for Choosing Algal Species for Biodiesel Production. *Journal of Applied Phycology*, 21(5):493-507

Grobbelaar, JU. (1994). Turbulence in Mass Algal Cultures and the Role of light/dark Fluctuations. *Journal of Applied Phycology,* 6(3):331-335

Grobbelaar, JU. (2000). Physiological and Technological Considerations for Optimising Mass Algal Cultures. *Journal of Applied Phycology*, 12:201-206

Gudin, C & Therpenier, C. (1986). Bioconversion of Solar Energy into Organic Chemicals by Microalgae. *Advanced Biotechnology Processes,* 6:73-110

Harun, R, Singh, M, Forde, GM & Danquah, MK. (2010). Bioprocess Engineering of Microalgae to Produce a Variety of Consumer Products. *Renewable and Sustainable Energy Reviews* 14(3):1037-1047

Henderson, R, Parsons, SA & Jefferson, B. (2008). The Impact of Algal Properties and Pre-Oxidation on Solid/Liquid Separation of Algae. *Water Research*, 42:1827-1845

Hodaifa, G, Martínez, M & Sánchez, S. (2008). Use of industrial wastewater from olive-oil extraction for biomass production of *Scenedesmus obliquus*. *Bioresource Technology*, 99(5):1111-1117

Hsieh, C & Wu, W. (2009). Cultivation of Microalgae for Oil Production with a Cultivation Strategy of Urea Limitation. *Bioresource Technology*, 100(17):3921-3926

Huntley, ME & Redalje, DG. (2006). CO_2 Mitigation and Renewable Oil from Photosynthetic Microbes: A New Appraisal. *Mitigation and Adaptation Strategies for Global Change,* 12(4):573-608

Illman, A, Scragg, AH & Shales, SW. (2000). Increase in *Chlorella* Strains Calorific Values when Grown in Low Nitrogen Medium. *Enzyme MicrobialTechnology*, 27(8):631-635

Lee, SJ, Kim, S, Kim, J, Kwon, G, Yoon, B & Oh, H. (1998). Effects of Harvesting Method and Growth Stage on the Flocculation of the Green Alga *Botryococcus braunii*. *Letters in Applied Microbiology*, 27(1):14-18

Jameson, GJ. (1999). Hydrophobicity and Floc Density in Induced-Air Flotation for Water Treatment. *Colloids and Surfaces A: Physicochemical and engineering aspects,* 151:269-281

Jarvis, P, Buckingham, P, Holden, B & Jefferson, B. (2009). Low Energy Ballasted Flotation. *Water Research*, 43:3427-3434

Knuckey, R, Brown, M, Robert, R & Frampton, D. (2006). Production of Microalgal Concentrates by Flocculation and their Assessment as Aquaculture Feeds. *Aquaculture Engineering*, 35(3):300-313

Lardon, L, HeÌ lias, A, Sialve, B, Steyer, JP & Bernard, O. (2009). Life-Cycle Assessment of Biodiesel Production from Microalgae. *Environmental science & technology*, 43(17):6475-6481

Lee, AK, Lewis, DM & Ashman, PJ. (2008). Microbial Flocculation, a Potentially Low-Cost Harvesting Technique for Marine Microalgae for the Production of Biodiesel. *Journal of Applied Phycology*, 21(5):559-567

Li, Y, Han, D, Hu, G, Dauvillee, D, Sommerfeld, M, Ball, S & Hu, Q. (2010). Chlamydomonas Starchless Mutant Defective in ADP-Glucose Pyrophosphorylase Hyper-Accumulates Triacylglycerol. *Metabolic Engineering*, 12(4):387-91

Li, Y, Horsman, M, Wang, B, Wu, N & Lan, CQ. (2008). Effects of Nitrogen Sources on Cell Growth and Lipid Accumulation of Green Alga Neochloris Oleoabundans. *Applied microbiology and biotechnology,* 81(4):629-36

Liu, X, Sheng, J & Curtiss, R. (2011). Fatty Acid Production in Genetically Modified Cyanobacteria. *Proceedings of the National Academy of Sciences of the United States of America,* 108(17):6899-904

Liu, Z, Wang, G & Zhou, B. (2008). Effect of Iron on Growth and Lipid Accumulation in *Chlorella vulgaris. Bioresource Technology,* 99(11):4717-22

Livne, A & Sukenik, A. (1992). Lipid Synthesis and Abundance of Acetyl CoA Carboxylase in *Isochrysis galbana* (Prymnesiophyceae) Following Nitrogen Starvation. *Plant and Cell Physiology*, 33(8):1175-1181

Lv, J, Cheng, L, Xu, X, Zhang, L & Chen, H. (2010). Enhanced Lipid Production of *Chlorella vulgaris* by Adjustment of Cultivation Conditions. *Bioresource Technology*, 101(17):6797-804.

Mata, TM, Martins, AA & Caetano, NS. (2010). Microalgae for Biodiesel Production and Other Applications: A Review. *Renewable and Sustainable Energy Reviews,* 14(1):217-232

Melis, A. (2009). Solar Energy Conversion Efficiencies in Photosynthesis: Minimizing the Chlorophyll Antennae to Maximize Efficiency. *Plant Science,* 177(4):272-280

Miao, X & Wu, Q. (2006). Biodiesel Production from Heterotrophic Microalgal Oil. *Bioresource Technology*, 97(6):841-846

Mittelbach, M & Remschmidt, C. (2004). *Biodiesel - The Comprehensive Handbook*. Martin Mittelbach

Mohn, FH. (1980). Experiences and Strategies in the Recovery of Biomass from Mass Cultures of Microalgae. In *Algae biomass: Production and Use*. Edited by Shelef, G & Soeder, C. pp. 547-571. Elsevier, Amsterdam

Molina Grima, E, Belarbi, E, Acien Fernandez, FG, Robles Medina, A & Chisti, Y. (2003). Recovery of Microalgal Biomass and Metabolites: Process Options and Economics. *Biotechnology Advances,* 20(7-8):491-515

Nakajima, Y, Tsuzuki, M &Ueda, R. (2001). Improved Productivity by Reduction of the Content of Light-Harvesting Pigment in *Chlamydomonas perigranulata*. *Journal of Applied Phycology,* 13(2):95-101

Nedbal, L, Tichy,V, Xiong, F & Grobbelaar, JU. (1996). Microscopic Green Algae and Cyanobacteria in High-Frequency Intermittent Light. *Journal of Applied Phycology,* 8(4-5):325-333

Oswald, WJ & Golueke, C. (1960). Biological Transformation of Solar Energy. *Advanced Applied Microbiology*, 2:223-262

Petrusevski, B, Bolier, G, van Breemen, A & Alaerts, GJ. (1995). Tangential Flow Filtration: A Method to Concentrate Freshwater Algae. *Water Research,* 29:1419-1424

Piorreck, M, Baasch, K & Pohl, P. (1984). Biomass Production, Total Protein, Chlorophylls, Lipids and Fatty Acids of Freshwater Green and Blue-Green Algae Under Different Nitrogen Regimes. *Phytochemistry,* 23(2):207-216

Pulz, O. (2001). Photobioreactors: Production Systems for Phototrophic Microorganisms. *Applied Microbiology and Biotechnology*, 57(3):287-293

Pushparaj, B, Pelosi, E, Torzillo, G & Materassi, R. (1993). Microbial Biomass Recovery using a Synthetic Cationic Polymer. *Bioresource Technology*, 43(1):59-62

Radakovits, R, Jinkerson, RE, Darzins, A & Posewitz, MC. (2010). Genetic Engineering of Algae for Enhanced Biofuel Production. *Eukaryotic Cell,* 9(4):486-501

Ramos, MJ, Fernandez, CM, Casas, A, Rodriguez, L & Perez, A. (2009) Influence of Fatty Acid Composition of Raw Materials on Biodiesel Properties. *Bioresource Technology*, 100(1):261-268

Regan, DL & Gartside, G. (1983). *Liquid Fuels from Microalgae in Australia*. CSIRO, Melbourne

Richardson, C. 2011. *Investigating the role of reactor design to maximise the environmental benefit of algal oil for biodiesel.* Master's thesis. University of Cape Town.

Richmond, A. (2004). *Microalgal culture: biotechnology and applied phycology*. Blackwell Science, Oxford

Rodolfi, L, Chini Zittelli, G, Bassi, N, Padovani, G, Biondi, N, Bonini, G & Tredici, MR. (2009). Microalgae for Oil: Strain Selection, Induction of Lipid Synthesis and Outdoor Mass Cultivation in a Low-Cost Photobioreactor. *Biotechnology and Bioengineering*, 102(1):100-112

Roessler, P. (1990). Environmental Control of Glycerolipid Metabolism in Microalgae: Commercial Implications and Future Research Directions. *Journal of Phycology*, 26:393-399

Sazdanoff, N. (2006). *Modeling and simulation of the algae to biodiesel fuel cycle*. Honour's thesis, Ohio State University

Schenk, PM, Thomas-Hall, S, Stephens, E, Marx, UC, Mussgnug, JH, Posten, C, Kruse, O & Hankamer, B. (2008). Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production. *BioEnergy Research* 1(1):20-43

Scott, SA, Davey, MP, Dennis, JS, Horst, I, Howe, CJ, Lea-Smith, D & Smith, AG. (2010). Biodiesel from Algae: Challenges and Prospects. *Current Opinions in Biotechnology*, 21:277-286

Sheehan, J, Dunahay, T, Benemann, J & Roessler, P. (1998). *A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae*. Close-Out report. National Renewable Energy Lab, Department of Energy, Golden, Colorado, U.S.A. Report number NREL/TP-580-24190, dated July 1998

Shelef G, Sukenik A, Green M (1984) *Microalgae Harvesting and Processing: a Literature Review*. SERI report.

Shifrin, NS & Chisholm, SW. (1981). Phytoplankton Lipids: Interspecific Differences and Effects of Nitrate, Silicate and Light-Dark Cycles. *Journal of Phycology*, 374-384

Shuler, ML & Kargi, F. (2005). *Bioprocess Engineering: Basic Concepts*. Pearson Education, Singapore

Spoehr, HA & Milner, HW. (1949). The Chemical Composition of *Chlorella*, Effect of Environmental Conditions. *Plant Physiology*, 24:120-149

Stansell, G, Gray, VM & Sym, S. (2011). Microalgal Fatty Acid Composition: Implications for Biodiesel Quality. *Journal of Applied Phycology,* In press. DOI: 10.1007/s10811-011-9696-x

Takagi, M, Watanabe, K, Yamaberi, K & Yoshida, T. (2000). Limited Feeding of Potassium Nitrate for Intracellular Lipid and Triglyceride Accumulation of *Nannochloris* sp. UTEX LB1999. *Applied Microbiology and Biotechnology*, 54(1):112-7

Tsukahara, K & Sawayama, S. (2005). Liquid Fuel Production using Microalgae. *Journal of the Japan Petroleum Institute,* 48(5):251-259

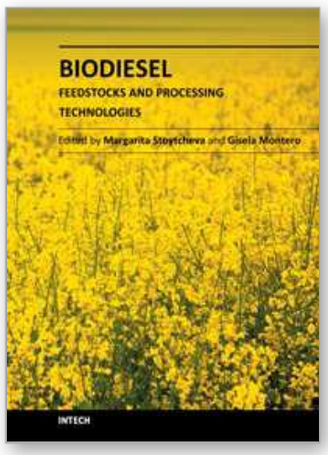
Uduman, N, Qi, Y, Danquah, MK, Forde, GM & Hoadley, A. (2010). Dewatering of Microalgal Cultures: A Major Bottleneck to Algae-Based Fuels. *Journal of Renewable and Sustainable Energy,* 2(1)

Van Gerpen, J. (2005). Biodiesel Processing and Production. *Fuel Processing Technology* 86:1097-1107

Vlaski, A, van Breemen, A & Alaers, G. (1997). The Role of Particle Size and Density in Dissolved Air Flotation and Sedimentation. *Water Science and Technology,* 36(4):177-189

Xiong, W, Li, X, Xiang, J & Wu, Q. (2008). High-Density Fermentation of Microalga *Chlorella protothecoides* in Bioreactor for Microbio-Diesel Production. *Applied Microbiology and Biotechnology*, 1:29-36

Zhang, X, Hu, Q, Sommerfeld, M, Puruhito, E & Chen, Y. (2010). Harvesting Algal Biomass for Biofuels using Ultrafiltration Membranes. *Bioresource Technology*, 101(14):5297-304



Biodiesel - Feedstocks and Processing Technologies

Edited by Dr. Margarita Stoytcheva

ISBN 978-953-307-713-0
Hard cover, 458 pages
Publisher InTech
Published online 09, November, 2011
Published in print edition November, 2011

The book "Biodiesel: Feedstocks and Processing Technologies" is intended to provide a professional look on the recent achievements and emerging trends in biodiesel production. It includes 22 chapters, organized in two sections. The first book section: "Feedstocks for Biodiesel Production" covers issues associated with the utilization of cost effective non-edible raw materials and wastes, and the development of biomass feedstock with physical and chemical properties that facilitate it processing to biodiesel. These include Brassicaceae spp., cooking oils, animal fat wastes, oleaginous fungi, and algae. The second book section: "Biodiesel Production Methods" is devoted to the advanced techniques for biodiesel synthesis: supercritical transesterification, microwaves, radio frequency and ultrasound techniques, reactive distillation, and optimized transesterification processes making use of solid catalysts and immobilized enzymes. The adequate and up-to-date information provided in this book should be of interest for research scientist, students, and technologists, involved in biodiesel production.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Melinda J. Griffiths, Reay G. Dicks, Christine Richardson and Susan T. L. Harrison (2011). Advantages and Challenges of Microalgae as a Source of Oil for Biodiesel, Biodiesel - Feedstocks and Processing Technologies, Dr. Margarita Stoytcheva (Ed.), ISBN: 978-953-307-713-0, InTech, Available from: http://www.intechopen.com/books/biodiesel-feedstocks-and-processing-technologies/advantages-and-challenges-of-microalgae-as-a-source-of-oil-for-biodiesel

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen