BioMara Project
Processing Technology Review

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1 Introduction

As global fossil fuel supplies dwindle and atmospheric carbon dioxide concentrations rise, there is increasing pressure to find viable biofuel alternatives to petroleum products. The European Parliament has set a target of 10% of road transport fuel to come from renewable sources by 2020. Usage in the U.K. is currently estimated at less than 3%, making this an urgent challenge.

In response to this challenge, amongst a climate of rising fuel prices and concerns over energy security, there has been an increased focus on the search for alternatives to petroleum derived products to act as transport fuel. The production of biofuels from biomass such as micro- and macro-algae has become very attractive to both academic researchers and energy companies; biodiesel (Ahmad et al., 2011), bioethanol (Harun et al., 2010), biohydrogen (Park et al., 2009), biogas (Salerno et al., 2009) and biobutanol (Maron, 2009) are all being investigated. Biodiesel and bioethanol are of special interest as these fuels are similar to existing petroleum diesel and gasoline and are therefore compatible with existing infrastructure (Chisti, 2008, Mata et al., 2010).

The production of biofuels and value-added chemicals from algal biomass involves several common unit operations, regardless of end product (Figure 1).

![Figure 1: Unit operations involved in algal fuel production](image)

Whilst this report focusses on the different options available for the downstream processing of algal biomass into transport fuel, it also included details of the cultivation step, as the choices during the cultivation stage have a significant impact for the other processes in the production line.

Previously, review of the production process have identified a number of challenges which must be overcome in order to make algal biofuels to viable (Brennan & Owende, 2010b):

1. Species selection must balance requirements for biofuel production and extraction of valuable co-products
2. Attaining higher photosynthetic efficiency
3. Development of single species cultivation, evaporation reduction and CO₂ diffusion losses
4. Potential for negative energy balance and accounting for requirements in water pumping, CO₂ transfer, harvesting and extraction.
5. Few plants in operation, therefore lack of information for scale-up (desire to be innovative)
6. Use of flue gases due to poisonous nature of NOₓ and SOₓ.

2 Cultivation

2.1 Requirements for algal growth
The potential of using ‘waste’ streams in algal cultivation is attractive; for example flue gas from power plants can be utilised as a carbon dioxide source (Stephenson et al., 2010), whilst nutrients can be obtained from industrial wastewater (Chinnasamy et al., 2010), or anaerobic digestate (Wang et al., 2010).

2.1.1 Light
The theoretical maximum efficiency of solar energy conversion (total solar energy to primary photosynthetic products) is calculated as being approximately 10% (Hulatt & Thomas, 2011), but metabolic activities within the cell, such as lipid and protein production, mean this figure is not obtained and measured values are typically in the range 1 – 3%.

2.1.2 Carbon dioxide
The growth of most photosynthetic algae is autotrophic, carbon is taken up in the inorganic form i.e. carbon dioxide, carbonate or hydrogencarbonate. Although this can be sourced from the atmosphere in open growth systems, the driving force for the replacement of CO₂ in the growth media is low and the CO₂ concentration can become the limiting factor in growth (Williams & Laurens, 2010). Therefore, to maintain high biomass production CO₂ needs to be continually replaced by addition of liquid or gaseous CO₂.

A low cost source is to use flue gases. CO₂ makes up between 3% - 15% of flue gases from fossil-fuel power plants, depending on the fuel source and plant design (Packer, 2009). This is also a form of carbon capture which adds to the attractiveness of this option.

CO₂ requirements can be calculated from growth rates and algal cell compositions (Stephenson et al., 2010), using an assumed value for efficiency of transfer to the growth media, and utilisation of, CO₂ (Carvalho et al., 2006).

The supply and uptake of CO₂ is a key processing parameter in the production of algal biomass for fuel production. Despite this, the technology for supply of CO₂ to algal
biomass is as yet poorly developed. This is in stark contrast to the large body of research on genetic improvement of algal species for specific applications (Carvalho et al., 2006).

2.2 Microalgae
A two-step process to maximise the production of fuel by microalgae is proposed by Stephenson et al. (2010). Microalgae would first be grown to a high concentration under nutrient sufficient conditions. The supply of nutrient is then discontinued, causing the microalgae to accumulate molecules such as triacylglycerides within their cells.

The mechanism by which microalgae stop producing new cells, and instead begin to accumulate fuel molecules in their cells, is triggered by stress conditions such as nutrient limitations (Chen et al., 2011).

2.2.1 Species selection
Evidence from the NREL Aquatic Species Programme (ASP) suggests that allowing the system to self-select the organism is the best approach for cultivation. The key parameters – temperature, light intensity, pH etc. – can then be optimized for the prevalent species following small scale modelling work (Sheehan et al., 1998).

Recently, commercial algae-fuel operations have taken one of two approaches: either choosing to first optimise the production methods (for example an oil-company), or to first optimise the algal species, through use of genetic modification (Kanellos, 2008).

The ease of downstreaming processing is also highly dependent on the algal species. For example, Park et al. (2011) found harvesting of algal cells with a settling process most effective when Pediastrum sp. dominated a mixed algal culture.

2.2.2 UV utilisation
It has been estimated that only about 10% of total solar energy can be utilised by microalgae (Sheehan et al., 1998). Above this level, light is simply wasted, and may in fact damage the photosynthetic apparatus (photoinhibition). This presents a major challenge in microalgae culture – how can high conversion of incident light be achieved? This is a major consideration in photobioreactors.

Chojnacka & Noworyta (2004) compared growth rates of algae in autotrophic and mixotrophic modes. The researchers found that, in both modes, no increase in the specific growth rate was observed above a light intensity of 30 W/m², with autotrophic growth being photoinhibited at light intensities above 50 W/m².

It has been shown experimentally that microalgae cultures exposed to short (milliseconds) of intense light followed by longer periods of darkness, achieved the same light conversion as cultures constantly exposed to the same average photon flux. Another
approach, using diffusion of light throughout the culture using optical fibres, was also attempted in Japan, but the high cost of optical fibres is likely to be prohibitive.

2.2.3 Oxygen poisoning
Algal cells take up carbon dioxide and use solar radiation to convert this to oxygen, which is then expelled from the algal cells. The level of dissolved oxygen present in the culture can become inhibitory in high concentrations, and become toxic to the algae at concentrations in excess of 35 mg/l (Carvalho et al., 2006). Chisti (2008) recommended that oxygen levels are maintained below 400% of air saturation levels to prevent inhibition occurring.

Although not a problem in open systems (e.g. raceway ponds), where oxygen levels will be in equilibrium with atmospheric conditions, in closed systems air bubbling zones may be required to strip excess oxygen from solution (Zebib, 2008).

2.2.4 Nutrient uptake
In a lab scale study, Wang et al. (2010) used diluted dairy slurry digestate as a nutrient source for cultivation of Chlorella sp. Using a 25 times dilution of the digestate, 100% ammoniacal nitrogen, 76-83% total nitrogen and 63-75% total phosphorous removal was achieved over a 21 day period. Up to 38% reductions in COD concentrations were also observed, with no significant difference in removals obtained at different dilutions.

A study utilising textile effluent for nutrient removal found 99%, 100% and 75% removals of nitrate-nitrogen, ammoniacal-nitrogen and phosphate-phosphorous respectively within 24 hours (Chinnasamy et al., 2010). Removal increased to 99.7%, 100% and 98.8% within 72 hours when bubbled with ambient air. Lower phosphate removal was obtained in cultures bubbled with CO₂ enriched (6%) air.

Use of wastewater to provide nutrient is beneficial as, in addition to the obvious savings in nutrient costs, 100 kg of nitrogen (as N) and 10 kg of phosphorous (as P) can potentially be removed from wastewater per tonne of algal biomass produced (Chinnasamy et al., 2010).

2.3 Growth methods
Brennan & Owende (2010a) summarised the advantages and disadvantages of the various cultivation options for microalgae production (Figure 2).
In general, open systems are characterised by low operational and capital costs and low productivities, whilst closed systems are characterised by high operational and capital costs and high productivities. Despite their higher productivities and other advantages, uptake of closed systems for existing commercial applications was not widespread, with the simple operation of open systems being considered preferred (Carvalho et al., 2006).

2.3.1 Raceway ponds

Raceway ponds are currently used for most commercial microalgae cultivation operations (Stephenson et al., 2010). Capable of producing algal suspensions with concentrations up to 600 mg/l (Shelef et al., 1984), the operation is simple, with a paddle wheel circulating the biomass and CO₂ added by sparging the gas into the bottom of a sump placed into the raceway (Stephenson et al., 2010).

Raceway ponds, however, have several disadvantages (Stephenson et al., 2010):
- Potential for high water losses by evaporation;
- Potential for contamination with alternative microalgae species.

The requirement for water for cultivation in raceway ponds has been estimated to be as high as 11-13 million L/ha.year. In a life cycle analysis study, Stephenson et al. (2010) report evaporative losses ranging from $10 \times 10^{-3}$ m$^3$/m$^2$/day for tropical climates to $3 \times 10^{-3}$ m$^3$/m$^2$/day for the climate experienced in the U.K.

The NREL review of the US Department of Energy’s ASP states that invasion by native species is inevitable, and that therefore the production system should self-select the organisms. The conditions and genetics of these species should then be optimised for fuel production (Sheehan et al., 1998).

<table>
<thead>
<tr>
<th>Production system</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raceway pond</td>
<td>Relatively cheap</td>
<td>Poor biomass productivity</td>
</tr>
<tr>
<td></td>
<td>Easy to clean</td>
<td>Large area of land required</td>
</tr>
<tr>
<td></td>
<td>Utilises non-agricultural land</td>
<td>Limited to a few strains of algae</td>
</tr>
<tr>
<td></td>
<td>Low energy inputs</td>
<td>Poor mixing, light and CO₂ utilisation</td>
</tr>
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<td></td>
<td>Easy maintenance</td>
<td>Cultures are easily contaminated</td>
</tr>
<tr>
<td>Tubular photobioreactor</td>
<td>Large illumination surface area</td>
<td>Some degree of wall growth</td>
</tr>
<tr>
<td></td>
<td>Suitable for outdoor cultures</td>
<td>Requiring large land space</td>
</tr>
<tr>
<td></td>
<td>Relatively cheap</td>
<td>Gradients of pH, dissolved oxygen and CO₂ along the tubes</td>
</tr>
<tr>
<td>Flat plate photobioreactor</td>
<td>High biomass productivities</td>
<td>Difficult scale-up</td>
</tr>
<tr>
<td></td>
<td>Easy to sterilise</td>
<td>Difficult temperature control</td>
</tr>
<tr>
<td></td>
<td>Low oxygen build-up</td>
<td>Small degree of hydrodynamic stress</td>
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<tr>
<td></td>
<td>Readily tempered</td>
<td>Some degree of wall growth</td>
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<tr>
<td></td>
<td>Good light path</td>
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<tr>
<td></td>
<td>Large illumination surface area</td>
<td></td>
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<tr>
<td></td>
<td>Suitable for outdoor cultures</td>
<td></td>
</tr>
<tr>
<td>Column photobioreactor</td>
<td>Compact</td>
<td>Small illumination area</td>
</tr>
<tr>
<td></td>
<td>High mass transfer</td>
<td>Expensive compared to open ponds</td>
</tr>
<tr>
<td></td>
<td>Low energy consumption</td>
<td>Shear stress</td>
</tr>
<tr>
<td></td>
<td>Good mixing with low shear stress</td>
<td>Sophisticated construction</td>
</tr>
</tbody>
</table>

Figure 2: Advantages and limitations of open ponds and photobioreactors (Brennan & Owende, 2010b)
Bennemann and Eisenberg (Sheehan et al., 1998) carried out investigations using a 0.1 ha raceway pond. Mixing velocities of 15 cm/s were achievable with a power input of 1 kWh/day, but this increased significantly to 10 kWh/day for a mixing velocity of 30 cm/s (as head loss is related to the square of velocity).

Power requirements decrease with increasing pond depth (Weissman et al., 1988), but obviously lead to reduced productivity due to reduced incident sunlight per unit volume.

### 2.3.2 Photobioreactors

Most photobioreactors are one of three types:
- (i) vertical airlift/bubble columns;
- (ii) horizontal tubular reactors;
- (iii) Continuous Stirred-Tank Reactor (CSTR).

The cost of biomass production in photobioreactors (PBRs) may be one order of magnitude higher than in ponds (Mata et al., 2010), but PBRs offer advantages in terms of better control of contaminants, temperature and substrate concentrations. Additionally, photobioreactors offer better volume to land area ratios and reduced harvesting costs (Carvalho et al., 2006). The main limitations include: overheating, bio-fouling, oxygen accumulation, difficulty in scale-up, high capital cost and damage of cells by shear stress (Mata et al., 2010).

In column reactors mixing is provided by the turbulence caused by the rising gas bubbles, whilst in horizontal reactors a circulation pump is required for mixing purposes. For this reason, horizontal reactors have higher energy consumption, although this is balanced by a higher biomass productivity due to the angle toward sunlight facilitating efficient light harvesting (Carvalho et al., 2006).

![Figure 3: Column reactors at Daithi O’Mhurchu Marine Research Station, Bantry, Cork, Ireland](image1)

![Figure 4: Horizontal bioreactor at SARDI, Adelaide, Australia](image2)
Large diameter tubes have a higher specific volume, but the energy required for mixing is larger and they have limited exposed surface area, meaning algal growth is limited by availability of UV radiation. The optimum size varies with solar conditions and algae species, but Zebib (2008), estimates the optimum diameter should not exceed 0.1 m.

The lengths of tubular bioreactors are limited by other factors including the need to remove excess oxygen and control CO₂ concentration with associated pH effects (Ugwu et al., 2008).

A CSTR consists of a single tank which is continuously agitated by a motor driven paddle. The mixing rate is such that concentrations of substrate and products are the same in all areas of the reactor; allowing easier control of CO₂ and O₂ concentration and pH.

CSTRs are commonly applied in the pharmaceutical industry for biosynthesis reactions and have been used by U.S. company OriginOil for the production of microalgae for the production of fuel and other valuable chemicals. The Helix BioReactor system uses an array of light emitting diodes (LEDs) as part of the agitator (Figure 5), which can be tuned to specific frequencies for optimisation of photosynthesis (Eckelberry, 2010).

Recently, the exterior of several buildings has been clad in algal photobioreactors, including a 15 unit apartment building in Hamburg, Germany (Figure 6). The bioreactors are provided with nutrients from the greywater produced by the building, and turn to face the sun to maximise production. The biomass is periodically harvested and converted to biogas which provides a portion of the energy required to heat the building (Yirka, 2013).
2.4 Macroalgae

The majority of macroalgae used for commercial operations is cultivated. Various approaches are used including the use of land-based tanks, coastal lagoons, inshore waters and more open waters.

Regardless of the location of cultivation, the first stage usually takes place on land, where seaweed is seeded onto string or nets prior to ongrowing in coastal waters (Figure 7). After a growing period of 6 – 8 months, the seaweed is ready for harvest for biofuel production or other uses (Figure 8).
Large near shore seaweed cultivation industries are well developed in countries such as China and Chile, but these facilities are very labour intensive and the economic viability of such operations relies on the availability of relatively cheap labour. For a seaweed production operation to succeed in the UK, especially for production of a relatively low value commodity such as energy, optimisation of all stages of the production and processing steps is required (Schmid et al., 2003b).

A combination of mechanical failures and issues with nutrient availability rendered early attempts to cultivate seaweed in offshore locations unsuccessful. Since these failures, however, there has been significant practical development, and prototypes for kelp production in the North Sea have been successfully tested (Schmid et al., 2003a).

A recent development in seaweed cultivation is Integrated Multi-Trophic Aquaculture (IMTA), an aquaculture technique in which the by-products of one process, including waste, are provided as inputs for another. This typically involves the combination of fed aquaculture (e.g. finfish, prawns) with organic extractive aquaculture (e.g. shellfish) and inorganic extractive aquaculture (i.e. seaweed) (Wanner et al., 1994).

In IMTA, finfish are grown in net cages and fed as in standard fish farming. In the same vicinity shell fish such as mussels are grown supported on ropes as shown in Figure 10, and seaweeds such as kelp are grown supported on strings similar to that illustrated in Figure 7 and Figure 8. All three cultivated organisms are valuable commodities, so that the overall productivity of the operation is greater than if the individual products were grown in monocultures.

IMTA is seen as more sustainable than single species aquaculture operations (Debus & Wanner, 1992). In fish farming, waste material such as uneaten food and faeces, fall to the seabed below the cages. Decomposition of this material leads to altered nutrient levels.
with associated changes to the ecosystem (Lynch, 1978). In IMTA, each element of the IMTA system is arranged in such a way so that the extractive shellfish and seaweed occupy the nutrient plume, where they uptake these nutrients and lessen the effect on the environment (Wanner et al., 1994). In this way, nutrient availability issues which hindered early commercial seaweed cultivation projects are avoided.

The majority of IMTA deployments to date have been for academic research purposes. Systems have been operated in various parts of Asia and Europe, South and North America. The greatest concentration of research has occurred in the Bay of Fundy, New Brunswick/Nova Scotia, Canada. In this area, industry, academia and government are collaborating to expand production to commercial scale, with a working industrial scale IMTA unit expected by 2014 (Modin et al., 2007/6).

In the U.K., the Scottish Marine Institute has carried out a number of IMTA projects. The REDWEED project (2003-2006) examined bioremediation by seaweed growing adjacent to sea based fish farms and the MERMAIDS project studied salmon/oysters/seaweed IMTA systems in the Western Isles (Schnobrich et al., 2007). The projects quantified the bioremediation potential of IMTA systems and made recommendations of best practice, but no assessment of the economic aspects of the use of such systems in the U.K. has yet been published.

3 Harvesting

3.1 Macroalgae

In Northern Ireland, The Environment and Heritage Service’s position is that mechanical harvesting will not be supported, unless it can be demonstrated that it will not significantly adversely affect the environment (EHS (N.I.), 2007).

Seaweed that washes up on beaches is regularly removed, especially in tourist areas where it, along with other deposits and litter, is considered an eyesore. Collection of beach cast material varies from manual cleaning with rakes to mechanical operations where the top layer of sand is removed, sieved to remove seaweed and litter, and returned to the beach (Zemke-White et al., 2005).

Beach cast seaweed begins to decompose within 2-3 days of being washed up on the high tide line. The decay of seaweed provides nutrients to an area which is likely to otherwise receive low levels of nutrient influx (McLaughlin et al., 2006). The removal of these deposits can have a very significant adverse impact on biodiversity (EHS (N.I.), 2007). However, in areas with high levels of eutrophication, removal of beach cast seaweed can have positive effects such as increasing water clarity (McLaughlin et al., 2006).

The Blue Flag criteria states that beach cast seaweed should only be removed where it is considered a nuisance, and that, when removed, it should be disposed in an “environmentally friendly manner” e.g. composting or use as fertiliser (EHS (N.I.), 2007).
Issues surrounding the use of beach cast seaweed concern sand content, which can give mechanical handling issues, and salt concentration, meaning the seaweed requires washing in order to prevent interference with fuel production processes.

3.2 Microalgae

The cultivation of microalgae typically results in suspensions of cells with concentrations of 200 – 600 mg/l. Harvesting refers to the concentration step into slurries or pastes containing 5000 – 25000 mg/l (Shelef et al., 1984). Due to the cost of concentrating the relatively dilute solutions produced by the cultivation step, up to 50% of the final product costs can be due to downstream processing (Greenwell et al., 2010), with 20 – 30% attributable to the harvesting step alone (Brennan & Owende, 2010b).

As such, the harvesting of algal cultures has been identified by researchers as a major bottleneck in the production of algal based biofuels (Uduman et al., 2010).

Harvesting can either take place a one-step process, where the chosen technology is capable of delivering a sufficiently concentration slurry for oil extraction/fuel production; or by a cascade approach utilising multiple technologies (Figure 11), similar to that suggested by Bilad et al. (2012) and discussed in Section 3.2.4.

![Figure 11: Schematic diagram of microalgal production and processing (Uduman et al., 2010)](image)

The efficient separation, dewatering and drying (if required) of microalgae is probably the most significant factor in determining the economic feasibility of any microalgal production system (Shelef et al., 1984).

Algae are difficult to concentrate, as they typically have negatively charged surfaces due to ionization of functional groups on cell walls. The intensity of these charges is a function of algal species, ionic strength of the medium, pH and temperature (Shelef et al., 1984).
3.2.1 Sedimentation
Sedimentation (or settling) is a technique by which a feed suspension is separated into a concentrated slurry and clarified liquid. Used extensively in the wastewater treatment industry, it is a low-cost separation technology. Suspensions are provided with quiescent conditions so that the denser solid particles sink to the bottom of the tank and are removed as an underflow, whilst the clarified liquid layer leaves as a top product (Figure 12).

![Figure 12: Settling tank at Antrim WwTW](image)

It is not widely practised with algal cultures, as settling rate is highly influenced by density difference, and algal cells have a density close to that of water. Without the addition of flocculants, the reliability of the process is poor (Uduman et al., 2010). With the addition of flocculants, slurries of up to 1.5% TSS can be obtained; without flocculation additional, reliability is poor (Uduman et al., 2010).

3.2.2 Flocculation
The addition of chemicals to induce algae flocculation is a common procedure where sedimentation, floatation, filtration and centrifugation processes are utilised (Shelef et al., 1984). The chemicals used fall into two broad groups:

a) Inorganic polyvalent metal ions
b) Polymeric organics

Harith et al. (2009) carried out a comparison study of the flocculation efficiency of two inorganic anionic flocculants (Magnafloc® LT 25 & LT 27) and organic chitosan. Over 90% flocculation efficiency was achieved with each of the flocculants at optimal conditions, but the authors noted the higher dosage requirement for chitosan (~20 mg/L) compared to the Magnafloc® (~1 mg/L) to achieve this efficiency.

However, there has been limited investigation on the effect the addition of these flocculation has to the fuel production, and the ability of the produced fuels to meet the relevant quality standards. Flocculants are often expensive and used in high concentrations, which can cause problems during further processing steps and in the reuse of waste materials.
3.2.3 Flotation

Flotation is a unit process by which bubbles of gas are used to drag suspended particles to the surface of a liquid, from where they can be removed from suspension. Two types of flotation are suitable for algal harvesting:

- Dissolved air flotation (DAF)
- Suspended air flotation (SAF)

DAF units use a compressor to supersaturate flotation water with air in a saturator. The flotation water is then released at atmospheric pressure, causing air to precipitate as small bubbles. SAF is similar to DAF, but bubbles are created by conventional sparging. Surfactants are used to reduce surface tension and prevent agglomeration of bubbles and therefore bubble size. This eliminates the need for a compressor and saturator and associated costs (Wiley et al., 2009).

The efficiency of flotation processes can be improved via the addition of flocculants. In jar tests involving alum, cationic, anionic & neutral polyacrylamide polymers and chitosan, Sim et al. (1988) found alum to be most effective for clarification of water. Solids concentrations of up to 5% were achieved. Chitosan was also effective at a dose of 50 mg/l, but none of the polyacrylamide polymers was effective at economically low doses.

3.2.4 Membrane filtration

Membrane filtration can be used to concentrate algal suspensions and remove protozoans and viruses allowing reuse of residual nutrients. Whilst the majority of studies investigating membrane filtration in relation to algal cells have explored methods of preventing fouling in water filtration applications, there have been some reports of use of membranes for algal cell harvesting.

Using hollow fibre PVC ultrafiltration membranes with a molecular weight cut-off (MWCO) of 50 kDa, Zhang et al. (2010) concentrated algal suspensions from 1 g/L by a factor of 155. Using a cross-flow configuration, backwashing and air scouring techniques were developed that allowed maintenance of consistently high flux levels in continuous operation.

Whilst the results from the Zhang et al. study are promising, cross-flow membrane filtration is associated with high energy usage. Using a submerged microfiltration membrane, Bilad et al. (2012) were able to achieve a 15 times concentration with a specific energy consumption of just 0.27 kWh/m³ for a culture of Chlorella vulgaris.

Although this is still a relatively low concentration, the authors suggest it would be economic to use centrifugation to bring the suspension up to the concentration required for fuel production.
3.2.5 Centrifugation

Centrifugation is a technique which utilises centrifugal force to accelerate sedimentation of suspensions. Suspensions are rotated around a fixed axis, with denser particles forced to the outside. Centrifuges are used in many applications including blood separation, separation of cream from milk and thickening of wastewater treatment sludge.

Centrifuges have been used for algal cell harvesting and dewatering in many investigations. Sim et al. (1988) carried out an investigation using a centrifuge for harvesting of sewage grown algae. Slurry with dry solids content in excess of 15% was obtained, with an energy consumption estimated at 1.3 kWh/m³ of pond water.

Despite the widespread use by academic studies, there has been no recent energy analysis of the use of centrifugation for harvesting of algal biomass.

4 Dewatering

The products of harvesting processes oftentimes have significant water content which must be reduced before further processing is possible. By dehydrating the harvested algal slurry to a moisture content of 12-15%, the product is stable and storable and suitable for all forms of lipid extraction processes (Shelef et al., 1984).

In lab scale experiments, lyophilisation or freeze drying is commonly employed (e.g. Wahlen et al., 2011), but due to the energy involved in maintaining a vacuum, this commands significant energy consumption and its industrial application is therefore limited (Huang et al., 2009). Whilst economic for high value pharmaceutical and food products, it is unlikely to be viable for algal fuel products.

For dewatering of low value products, such as wastewater sludges etc., thermal drying techniques such as rotary, spray and flash dryer are used (Shelef et al., 1984). Sun drying is also employed where climate permits, as this can realise an order of magnitude saving in energy use when compared to thermal techniques (Ciurzynska & Lenart, 2011).

For fuel production processes, unit operations which are capable of oil extraction from algal slurries, such as Origin Oil’s Single-Step Extraction™ (Eckelberry, 2010) are of great interest. This process uses an applied electromagnetic field to force algal cells to release their cells contents which are then easily separated by density difference (light lipids rise to the top, biomass sinks to bottom (Figure 13)). This, it is claimed, is capable of processing algal slurries of 1 g/L (dry basis) for as little of $0.20/kg – approximately one-sixth the cost of conventional techniques.
5 Lipids extraction
Most microalgae strains are relatively small and have a thick cell wall, meaning harsh conditions are required to rupture the cells for lipid extraction (Wijffels & Barbosa, 2010).

Lipids for fuel production can be extracted from algal biomass using two groups of methods: mechanical methods where pressure is used to rupture the cells and squeeze the oil from the cells and chemical methods where solvents are used to extract the lipids. A combination of both can also be used, which has been shown to most effective in some cases (Samori et al., 2010).

5.1 Mechanical methods
Mechanical methods involve placing dried algae cells under pressure, forcing the cells walls to rupture and release the contained lipids.

The most frequently used mechanical method is the screw press, which works in a similar way to an extruder for polymer processing (Figure 14). Dry algae are conveyed by a rotating screw along a horizontal annulus whose cross section decreases along the length. The ruptured cells and oil emerge through a nozzle and are easily separated. Approximately 75% oil recovery is possible (Farag, 2010).
The screw press is commonly used for dewatering operations in other industries such as paper production, sewage sludge and digestate separations, and for various food processes including extraction of lipids from olives and seeds.

Although the simplest technology, mechanical methods are very energy intensive, both in the requirement for dry input material and the energy involved in creating sufficient pressure for cell rupture.

5.2 Solvent extraction

Several solvents have been used successfully including hexane, ethanol and hexane-ethanol mixtures, obtaining up to 98% extraction of lipids. Although ethanol is a very good solvent, it does not selectively extract lipids, extracting also sugars, amino acids, salts and pigments. This is not desirable for biodiesel production, where the purpose of extraction is just the lipids and fatty acids (Mata et al., 2010), and the use of ethanol as a solvent reduces the amount of energy that can be recovered from residues via anaerobic digestion or other processes.

In a study aimed at optimising the analytical determination of lipid content in lyophilized microalgae, Ryckebosch et al. (2012) compared the effectiveness of a number of organic solvents and organic solvent blends. The study found a 1:1 blend of chloroform and methanol to be most effective for lipid extraction from Chlorella vulgaris, using a technique involving vortex mixing and separation of solvent and aqueous layers by centrifugation.
5.2.1 Switchable solvents
The potential of switchable solvents for extraction of lipids from algal biomass has also been investigated (Samori et al., 2010). Switchable solvents are capable of turning from a non-ionic form to an ionic liquid by bubbling CO$_2$ through the solution and recovered by bubbling N$_2$. Despite being described by the authors as having potential, the processing steps used appear very complex, with long extraction and centrifugation steps, and characterised by hydrocarbon recoveries of less than 20%.

5.3 Supercritical CO$_2$
Supercritical fluids are fluids which are held at or above a critical temperature and pressure. At these conditions, no distinct liquid and gas phases exist. Supercritical fluids typically are able to effuse through solids like a gas and dissolve materials like a liquid. Supercritical fluid extraction is more effective than traditional solvent separation methods, with recoveries of 100% being possible (Demirbas, 2009).

Capital costs involved in supercritical extraction methods are higher than traditional solvent extractions (Pellerin, 2003), however operating costs can be considerably lower as separation of solvent and product is simpler. Solvent can be easily recycled and residues are suitable for uses such as animal feed. Additionally, the process is relatively rapid, due to the inherent low viscosity and high diffusivities of supercritical fluids.

Supercritical carbon dioxide is often the choice for supercritical extractions due to its near ambient critical temperature (304 K) and similar solvation properties to hexane.

5.4 Ultrasonic extraction
Ultrasonic extraction utilises ultrasonic waves (20 kHz - 2 MHz) to create bubbles in a solvent material. When a bubble collapses near a cell wall, it creates a shock wave which causes the cell wall to rupture, releasing the cell contents into the solvent.

Cravotto et al. (2008) employed ultrasound and microwaves in order to improve solvent extraction of oil from biomass sources including cultivated microalgae. Ultrasound was found to be the most effective for microalgae, improving the extraction yield from 4.8% to 25.9% whilst reducing the extraction time from 4 hours to 20 minutes.

No details of large cell algal harvesting operations deploying this technology are currently published, but the technology is being tested in anaerobic digestion applications. Fitted onto a recycle loop, the Ultrawaves reactor (Figure 15) is designed to cause anaerobic sludge cells to collapse, allowing their cell contents to become available for biogas production and boost gas yields (Ultrawaves Reactors Ltd, 2011).
5.5 Flash depressurisation

Cellruptor, a technology developed by Eco-Solids International for boosting of biogas yields from wastewater sludge digestion, uses more moderate conditions than supercritical CO₂. Whereas supercritical CO₂ methods typically use temperatures and pressures in the ranges 313 – 333 K (40 – 60°C) and 20 – 30 MPa (Couto et al., 2010), the Eco-Solids method uses pressures up to 10 barg (1MPa).
Under these pressures, a gas (recycled biogas or exhaust gases) are forced to dissolve in the aqueous phase (including inside the cells) before it is rapidly depressurized. This sudden change in osmotic pressure causes the dissolved gases to form bubbles, the force of which causes cells to rupture (Gill, 2008). This method has been applied to rupture of wastewater sludge cells, microalgae cells and as a pre-treatment for ligno-cellulosic biomass for biofuels production (Figure 16).

The energy consumption of this process compares very favourably with other technologies such as supercritical CO₂ and ultrasonic methods. The energy consumption of the Cellruptor system is estimated to 3 orders of magnitude lower than that of ultrasonic methods (Gill, 2008). However, this technology is not yet mature and no data is available on the effectiveness with algal biomass.

6 Fuel Production

6.1 Biodiesel

The term Biodiesel refers to any fuel made from renewable feedstocks with characteristics similar to those of petroleum derived diesel (Ahmad et al., 2011). Biodiesel was first produced in the mid-19th century, approximately 40 years before Rudolf Diesel developed the first diesel engine.

Biodiesel is formed by the transesterification reaction of fats or oils with alcohols to form Fatty-Acid Methyl Esters (FAME) (Karmakar et al., 2010). As alcohols are barely soluble in fats or oils, the reaction proceeds very slowly without the use of catalysts to increase alcohol solubility, Catalysts can be either homogenous acidic or alkaline,
biocatalysts or heterogenous. The fastest reaction rates are achieved with alkali catalysts, and as such they are the most widely used for commercial applications, despite the formation of soaps as side products, with associated separation issues (Karmakar et al., 2010).

The basic process by which biodiesel is produced is the same regardless of feedstock or catalysis choice. The fats or oils are heated and blended with methanol containing catalysis. The reaction takes place at the interface between the two phases, with the reaction reaching completion after 1-2 hours (Lee & Saka, 2010). A simplified process flow diagram for the production of biodiesel from waste greases and vegetable oils is shown in Figure 18.

Wahlen et al. (2011) achieved single vessel lipid extraction and transesterification using methanol acidified with sulphuric acid. At a catalyst concentration of 2.0% (v/v), 32.9 mg of FAME was produced from 100 mg of lyophilised algal biomass. The reaction went to completion with 2.5 hours, with the majority of reaction taking place in the first 100 minutes.

A recently developed alternative to the use of catalysts to improve organic solubility is to employ supercritical conditions, at which distinct phases no longer exist (Madras et al., 2004). With the reaction taking place in one phase, esterification time is greatly reduced.

Saka & Kusdiana (2001) achieved catalyst free conversion of rapeseed oil and methanol to biodiesel at conditions of 45-65 MPa and 350°C with a reaction time of 240s. Approximately 95% conversion was obtained. Separation was significantly easier as no soap layer was formed in the absence of alkali catalyst.
Although the use of high temperatures favours a short reaction time and high conversion, it can have a negative effect on the biodiesel properties. Xin et al. (2008) found thermal decomposition took place when production took place at temperatures greater than 300°C, which adversely impacted the cold flow properties of the fuel.

Algal oils, unlike other plant oils, contain nitrogen, phosphorus and sulphur which are problematic with regard to engine performance. However, these impurities are likely to end up in the soluble fraction following transesterification, and be virtually absent from algal biodiesel (Williams & Laurens, 2010).

6.2 Bioethanol
Recent work by Lee et al. (2011), employed inverse metabolic engineering of Saccharomyces cerevisiae to enhance the fermentation of galactose to ethanol, without any decline in glucose fermentation. Although these studies were carried out in the laboratory with model sugar solutions, galactose is one the most abundant sugars contained in red seaweeds and therefore this work is of relevance in increasing the viability of bioethanol production from marine macroalgae.

Bioethanol has the potential to replace gasoline in today’s cars with little or no modification to engines (Mata et al., 2010), and has already been established as a fuel for transportation. In countries such as Brazil, where 15% of total energy is derived from sugarcane, bioethanol use is widespread (Sivakumar et al., 2010).

Unlike petroleum gasoline, however, ethanol is corrosive and miscible in water. As water infiltration is evitable in submerged pipeline systems, ethanol cannot be distributed using the existing pipeline infrastructure. Instead, it must be transported by rail or truck, considerably increasing the net energy cost (Sivakumar et al., 2010).

Estimates of fuel production efficiency indicate that potentially 2,000 gallons of ethanol per acre per year are possible using macroalgae (Bio Architecture Lab, 2010).

6.3 Biogas
Algal biomass is suitable for biogas production via anaerobic digestion of both whole cells and of the residue resulting from lipids extraction (Sialve et al., 2009). Anaerobic digestion is especially suitable for wastes with high moisture (80 – 90%) content (Brennan & Owende, 2010b).

Anaerobic digestion of algal biomass residue following lipid extraction can increase the viability of the process, as additional energy as biogas from algal biomass is recovered (Sialve et al., 2009).

Where oil content of the algal cells remains below 40%, there is little benefit in employing through an extraction step to recover oil followed by digestion of residues over anaerobic digestion of whole algal cells. Therefore, the better strategy is probably
the direct AD of harvested biomass (following a suitable concentration step) (Sialve et al., 2009).

The performance of an anaerobic digester may be compromised by the low C/N ratio of algal biomass (Brennan & Owende, 2010b). This problem can be resolved by co-digestion with a high C/N ratio waste product (e.g. waste paper). This also reduces the potential for inhibition by excess ammonium (Sialve et al., 2009).

The specific methane yield, expressed in litres of CH$_4$ per gram of volatile solid, can be estimated from the composition of the structure of chemical compounds which have the general formula C$_a$H$_b$O$_c$N$_d$ by use of Equation 1 (Buswells’ formula), where $V_m$ is the normal molar of methane (Sialve et al., 2009).

$$B_0 = \frac{4a + b - 2c - 3d}{12a + b + 16c + 14d} * V_m$$  \hspace{1cm} \text{Equation 1}

Two factors have been found to have significantly inhibitory effects on the anaerobic digestion of algal biomass: ammonium and sodium toxicity (Sialve et al., 2009). Acetoclastic methanogen bacteria are the most sensitive to ammonia, with inhibition being observed in the range 1.7 – 14 g/L but following acclimatisation of the bacterial consortium the inhibitory effects were lessened.

Toxicity has been reported with sodium ions above concentrations of 0.5M, although it has been feasible to produce adapted consortia which did not show any inhibition in high salinity conditions such as those experienced in marine water.

In general, toxicity effects of both ammonia and sodium had a greater effect in thermophilic rather than mesophilic conditions (Sialve et al., 2009).

### 6.4 Biobutanol

Butanol is an alcohol with a four carbon chain backbone. This longer chain makes butanol more polar compared to two carbon ethanol, resulting in properties which are more similar to those of petroleum gasoline. Butanol can be used as vehicle fuel in an undiluted form meaning, unlike ethanol, it has the potential to completely replace petroleum gasoline.

Butanol is immiscible in water, leading to a lessening of the corrosion and water absorption problem anticipated with ethanol in fuel distribution systems. However, there are technical obstacles that need to be overcome (mainly related to biobutanol’s relatively high viscosity) before it is acknowledged as a suitable fuel by engine manufacturers.

Biobutanol is produced commercially by the anaerobic conversion of carbohydrate into acetone, butanol and ethanol by *Clostridium Acetobutylicum*. This process is known as

Despite the interest of large industrial players, there are limited publications on the production of biobutanol from algal biomass. DuPont, citing commercial competitive reasons, have not disclosed how much butanol they expect to produce from microalgae (Maron, 2009), but have entered into R&D projects with several energy providers.

6.5 Other uses of ‘waste’ products

Unless major advances can be made in the productivity of algal biomass and efficiency of processing techniques, it is likely that production of transport fuel from algal biomass will only become economically viable following increases in the price of crude oil with associated increases in gasoline-derived transport fuels.

The traditional transesterification reaction by which biodiesel is produced from triacylglycerides leads to the production of glycerol as a waste product. Although glycerol is used in soap manufacture, there is a large quantity already on the market due to increased production of biodiesel from terrestrial crops (Packer, 2009). As such, alternative uses of glycerol need to be developed to match the increased production of biodiesel.

Work by Siles et al. (2010) used glycerol containing waste and wastewater from biodiesel production from used-cooking oil for biogas production. Using a mixture containing 15% glycerol and 85% wastewater, close to 100% biodegradation was obtained, giving a methane yield of 310 ml CH\textsubscript{4}/gCOD removed at 1 atm and 25°C.

This use of glycerol is synergistic with the approach taken by Sialve et al. (2009), which used anaerobic digestion to recover maximum energy from algal biomass and identified the need to co-digest with a nitrogen poor substrate.

6.6 Fuel distribution

The most economically viable option for transportation of biofuels involves the use of the existing distribution and storage infrastructure. There may be potential issues in this regard dealing with pipeline ownership (Sivakumar et al., 2010).

Where a pipeline and/or storage tank is located underground, it is inevitable that infiltration of water will occur. With traditional gasoline, this is removed through a series of ‘traps’ where water and gasoline are allowed to separate due to differences in density.

Unlike traditional gasoline, ethanol is hydroscopic and potentially corrosive meaning that it cannot be transported in existing pipelines (Sivakumar et al., 2010). Alternative transport methods, such as rail or truck, significantly add to the net energy cost.
7 Conclusion

Commercially viable algal biofuels are not yet a reality. Research has delivered a significant body of work since the oil crises of the 1970s in response to the threat of dwindling reserves and high crude oil prices, demonstrating the potential of micro and macro algae resources. The majority of this work has tended to focus on single operations along the supply chain, often failing to address how these technologies will function in real life.

A more holistic view of the production processes involved is required for the development of commercially viable algal biofuels. This is necessary to ensure use is made of existing infrastructure and promote adoption of new fuels by the public.
## 8 Literature review

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<tr>
<td>(Wang et al., 2010)</td>
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<tr>
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<td>Eliminating the need for drying of biomass reducing energy requirements.</td>
</tr>
<tr>
<td>(Patil et al., 2011)</td>
<td>Single-step supercritical process for extraction and transesterification, biodiesel</td>
<td>N/A</td>
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<td>N/A</td>
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<td>Single-step supercritical process for extraction and transesterification for biodiesel.</td>
<td>Eliminating the need for drying of biomass reducing energy requirements.</td>
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<tr>
<td>Reference</td>
<td>Study Title</td>
<td>LCA and review on biodiesel.</td>
<td>N/A</td>
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<td>(Lardon et al., 2009)</td>
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<tr>
<td>(Stephenson et al., 2011)</td>
<td>Theoretical Review on Algal Cultivation, exploiting possibility of improving photosynthesis through genomics.</td>
<td>N/A</td>
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<tr>
<td>(Kumar et al., 2010)</td>
<td>Determine best total ammonia nitrogen level for optimal culture growth.</td>
<td>Anaerobically digested piggery effluent/ Pure mineral media.</td>
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<td>12L Plastic bags</td>
<td>N/A</td>
<td>N/A</td>
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<td>(Chojnacka &amp; Noworyta, 2004)</td>
<td>Algae cultivation, effects of light and glucose on autotrophic, heterotrophic and mixotrophic algae.</td>
<td>Zarrouk liquid medium</td>
<td><em>Spirulina sp.</em></td>
<td>Spectrophotometer / working vol. 1 L</td>
<td>2 L Photobioreactor / working vol. 1 L</td>
<td>N/A</td>
<td>N/A</td>
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Highest algal growth rate at 20 mg/L TAN. Piggery effluent good feed for algal growth. Supplementation of specific nutrients may increase GR.

Autotrophic culture optimum light intensity 30-50 W m⁻², inhibition above 50Wm⁻² Mixotrophic culture light >30 W m⁻² and >0.5g/L glucose Heterotrophic culture >0.5g/L glucose
| (Valderrama et al., 2003) | Chemical (Astaxanthin, Phycocyanin) extraction using supercritical CO$_2$ | N/A | Dried H. Pluviali, & Spirulina maxima sourced externally | N/A | Artificial ponds. Using an extraction unit with natural substances, pure supercritical CO$_2$/ethanol. | N/A | Solvent/feed ratio and the use of cosolvents have great effects on the total substance extracted. |
| (Cravotto et al., 2008) | Simultaneous microwave and ultrasound assisted extraction of methyl esters. | N/A | Milled seaweed externally sourced | N/A | Microwave and ultrasound assisted extraction | N/A | Extraction times increase 10 fold and yields increased 50-500% in comparison to conventional methods of extraction. |
| (Couto et al., 2010) | Evaluate microalgae growth and docosahexaenoic acid production. To compare the supercritical CO$_2$ method with conventional lipid extraction. | f2+NPM medium supplemented with glucose. | C. cohnii | Cylinder-conical 100L fermenter. | Centrifuged, freeze dried. SC-CO2 method used | Transesterification and extraction with hexane to obtain methyl esters. | N/A |
| (Catchpole et al., 2009) | Extraction and fractionation of lipids using near-critical fluids. REVIEW | N/A | N/A | N/A | N/A | N/A | N/A |
| (Harun et al., 2010) | Using microalgal biomass as a substrate for yeast fermentation and bioethanol production | Yeast (Luria broth and Terrific broth)) | Chlorococum sp. | Yeast Growth measured using optical density. | 100L bag photobioreactor outdoors | Lipid extraction using supercritical CO2. | Ethanol production from fermentation of biomass in 500mL Erlenmeyer flasks. | 1. Highest yeast conc. produced highest conc. of ethanol.
2. Algal cells produce more ethanol in fermentation after the lipid extraction process as cell walls are ruptured making more simple sugars available for ethanol production.
3. 30°C best temperature for ethanol production.
4. Algal concentration and medium choice have great effects on ethanol production. |
| (Lee et al., 2011) | Improving the efficiency of galactose fermentation in bioethanol using genetically modified yeast cells. Galactose is an abundant sugar in marine plant biomass such as red seaweed. | Yeast (YP medium, YSC medium) | N/A | Yeast Growth measured using optical density. | N/A | N/A | N/A | 1. The overexpression of a number of genes greatly increased both galactose consumption rate and ethanol productivity.
2. Certain genes may partially alleviate the effects of glucose repression. |
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<td>(Karmakar et al., 2010)</td>
<td>Review focusing mainly on different biodiesel feedstock properties, and briefly on the manufacturing process.</td>
<td>N/A</td>
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<td>(Chisti, 2008)</td>
<td>Review of the prospects of algae derived biodiesels. Production of microalgal biomass. Comparison with bioethanol and biodiesel economics.</td>
<td>N/A</td>
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<tr>
<td>(Demirbas, 2009)(Madras et al., 2004)</td>
<td>Extraction, fractionation and transesterification of two species of algae and microalgae.</td>
<td>N/A</td>
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<td>Cladophora fracta, Chlorella protothecoides obtained from external supplier. Milling and the use of hexane in a Soxhlet extractor for 18 hrs. Transesterification using supercritical methanol</td>
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<td>Madras, Giridhar (2004)</td>
<td>Transesterification of vegetable oil using supercritical methanol and ethanol. Effects of enzyme loading, oil:alcohol ratio, reaction time and temp. Transesterification of vegetable oil using supercritical CO2 and an enzyme.</td>
<td>N/A</td>
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<td>Transesterification using supercritical methanol, ethanol and CO2 (with lipase). High rates of conversion with both supercritical methanol and ethanol at 80-100% conversion. Lower conversion with supercritical CO2 and enzyme at 27-30%.</td>
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<td>Hansen et al., 2005</td>
<td>REVIEW The blending of ethanol and diesel and properties of different blends. Includes, engine performance, durability, emissions information</td>
<td>N/A</td>
<td>N/A</td>
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<td>(Lin et al., 2011)</td>
<td>REVIEW Development of biodiesel, different feedstocks and conversion technologies. Environmental and social impacts of biodiesel, food security, land change, water use.</td>
<td>N/A</td>
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<td>(Lee &amp; Saka, 2010)</td>
<td>REVIEW biodiesel The properties of homogeneous and heterogeneous catalysts. Effects of temp. Pressure, molar ratio, water, free fatty acids and a number of new supercritical techniques.</td>
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<td>(Gheshlaghi et al., 2009)</td>
<td>REVIEW Metabolic pathways of butanol producing bacteria clostridia. Enzymes involved.</td>
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<td>Harvey &amp; Meylemans, 2011</td>
<td>REVIEW Properties of biobutanol as an alternative fuel source and in blends with diesel. Commercialization of biobutanol.</td>
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<td>Sierra et al., 2008</td>
<td>Characterization of a flat panel photobioreactor. Mixing, heat transfer, solar radiation.</td>
<td>N/A N/A N/A N/A N/A N/A N/A N/A</td>
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<td></td>
<td>Major aspects determining productivity is the location, deployment and orientation of the reactor. Flat bioreactors require less power supply than their tubular counterparts.</td>
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<td>Tan et al.</td>
<td>REVIEW Current level of development of China’s biomass resource utilization. Technological level. Resource distribution.</td>
<td>N/A N/A N/A N/A N/A N/A N/A N/A</td>
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<td>Huge potential growth area within China. Large resource base but relatively low exploitation.</td>
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<td>(Stripp &amp; Happe, 2009)</td>
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<td>(Yan et al., 2010)</td>
<td>Coupling of biohydrogen and polyhydroxyalkanoates production through anaerobic digestion of blue algae. Examination of blue algae pretreatments with bases, heat and microwaves.</td>
<td>Mixture of macro and micro-minerals with added sugars, Blue Algae obtained from a eutrophic lake in China and Bascillus cereus isolated from active sludge.</td>
<td>5 L anaerobic digester, 3 L fermenter. Anaerobic digestion. Basification of blue algae before digestion showed higher efficiencies than other pretreatments.</td>
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<td>(Biagini et al., 2010)</td>
<td>Study into different 4 hydrogen generation systems.</td>
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Hydrogen production maximized for the gasification/separation process.
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<td>(Greenwell et al., 2010)</td>
<td>REVIEW</td>
<td>Very extensive focusing on microalgal cultivation, growth technologies, harvesting, conversion of lipids to fuels.</td>
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<td>(Sivakumar et al., 2010)</td>
<td>REVIEW</td>
<td>Looks at a number of different feedstocks for bioethanol and biodiesel production. Starch based, lignocellulose based, plant based, microbial based and algal based.</td>
<td>N/A</td>
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<td>(Festel, 2008)</td>
<td>REVIEW</td>
<td>Economic aspects of biofuel production including first and second generation biofuels.</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Reference</td>
<td>Review</td>
<td>N/A</td>
<td>N/A</td>
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<td>(Larkum, 2010)</td>
<td>REVIEW Compares the potential of solar energy in water organisms vs. land plants. Solar footprint and carbon footprint.</td>
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<td>(Amin, 2009)</td>
<td>REVIEW Describes a number of different microalgal lipid conversion methods including gasification, liquefaction, pyrolysis, hydrogenation, fermentation and transesterification.</td>
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<td>(FitzPatrick et al., 2010)</td>
<td>REVIEW focusing on lignocellulosic feedstocks for the production of valuable chemical compounds and biofuels. Biorefinery. Fractionation.</td>
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</table>

Good prospects to make better use of cheap and abundant forestry waste.
<p>| (da et al., 2009) | REVIEW Pretreatment of lignocellulosic wastes. Physical, solvent, chemical (acidification and basification), heat, oxidative pretreatments. | N/A | N/A | N/A | N/A | N/A | N/A | Pretreatments play a central role and inefficient pretreatment strategies are an important cost deterrent for the use of lignocellulosic wastes. |
| (Mata et al., 2010) | REVIEW Comprehensive review of the biodiesel production process. Selection of microalgal strains based on lipid content, harvesting, extraction, conversion. Growth technologies. Discussion of numerous secondary applications for microalgal production. | N/A | N/A | N/A | N/A | N/A | N/A | Considerable investment and development of expertise is still needed before the utilization of microalgae for biodiesel production is economically viable. |</p>
<table>
<thead>
<tr>
<th>Reference</th>
<th>Methodology</th>
<th>Organism</th>
<th>Location</th>
<th>Anaerobic process</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Collet et al., 2011)</td>
<td>Life cycle analysis of microalgae culture coupled to biogas production.</td>
<td>N/A</td>
<td>Chlorella vulgaris</td>
<td>Raceway pond</td>
<td>Anaerobic digestion and purified by bubbling through pressurized water. From both an environmental and an economic standpoint, a combination of both lipid extraction, biodiesel production and anaerobic digestion may be preferable.</td>
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<td>(Siles Lopez et al., 2009)</td>
<td>Anaerobic digestion of glycerol by-product of the transesterification of triacylglycerol during the production of biodiesel.</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Anaerobic digestion</td>
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<td>Glycerol-containing waste produces substantial quantities of methane.</td>
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<td>(Fernandez et al., 2010)</td>
<td>Kinetics of mesophilic anaerobic digestion using different initial amounts of municipal solid waste.</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Anaerobic digestion</td>
</tr>
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<td>Higher active biomass and higher coefficient for the production of methane at lower loadings.</td>
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<tr>
<td>Reference</td>
<td>Methodology</td>
<td>Organism</td>
<td>Technique</td>
<td>Results</td>
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<td>(Doukova et al., 2010)</td>
<td>Anaerobic digestion for methane production, ammonium fertilizer production, microalgal by-products harvesting within closed system.</td>
<td>Chlorella Vulgaris</td>
<td>Optical density.</td>
<td>Photobioreactor N/A N/A N/A</td>
<td></td>
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<tr>
<td>(Eckelberry, 2010)</td>
<td>Short booklet showing a number of algal harvesting, dewatering and extraction technologies.</td>
<td>Chlorella Vulgaris</td>
<td>Optical density.</td>
<td>Photobioreactor Polymer flocculation, Decanters/Centrifuges, Hydroclones, Indirect/Direct heat, Fluid bed, microwave, expellers/presses, solvents, supercritical CO2, enzymes, ultrasonification, live extraction.</td>
<td></td>
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</tbody>
</table>
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