Methods of energy extraction from microalgal biomass: a review

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Introduction

The potential of micoalgal biomass as a source of liquid and gaseous biofuels is a highly topical theme, with over 70 years of sometimes intensive research, and more than US$ 2 billion of private investment since 2000 (Service 2011). The process operations for microalgal biofuel production can be grouped into three areas: growth, harvesting and energy extraction. A wide range of unit operations can be combined to form a microalgal biofuel production system, but as yet there is no successful commercial system producing such biofuel. This suggests that there are major technical and engineering difficulties to be resolved before economically viable microalgal biofuel production can be achieved.

The harvesting of microalgae (Milledge and Heaven 2013) and the exploitation of microalgal biomass for non-fuel uses (Milledge 2011) have been previously reviewed in this journal. This review addresses the topic of how microalgal biomass can be exploited for fuel.

Many types of microalgal biofuel have been considered and energy may be extracted from microalgal biomass by:
a. direct combustion
b. pyrolysis
c. gasification
d. liquefaction
e. hydrogen production by biochemical processes in certain microalgae
f. fuel cells
g. fermentation to bioethanol
h. trans-esterification to biodiesel
i. anaerobic digestion

Table 11 summarises the form of the energy outputs (heat, electricity, liquid, gaseous or solid fuel) from the various potential methods of producing useful energy from microalgae, together with the requirement for biomass drying after harvesting and the capacity to exploit the entire biomass in each case.

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Table 1 Methods of energy extraction from microalgal biomass

	Utilises entire organic biomass	Requires biomass drying after harvesting	Primary energy product
Direct Combustion	Yes	Yes	Heat
Pyrolysis	Yes	Yes	Primarily liquid by fast pyrolysis
Gasification	Yes	Yes [b] (conventional)	Primarily Gas
Liquefaction	Yes	No	Primarily Liquid
Bio-hydrogen production	Yes	No	Gas
Fuel Cells	Yes	No	Electricity
Bioethanol production	No [a]	No	Liquid
Biodiesel production	No	Yes [c]	Liquid
Anaerobic digestion	Yes	No	Gas
[a] Currently restricted to fermentable sugars as no large-scale commercial production of fuel bioethanol from lignocellulosic materials			
[b] Supercritical water gasification (SCWG) an alternative gasification technology can convert high moisture biomass			
[c] No current commercial process for the wet trans-esterification of wet microalgal biomass			

Direct combustion

Direct combustion is, historically and currently, the main method by which biomass energy is utilised (Demirbas 2001), but does not appear to have been greatly explored for the production of energy from microalgae. Biomass direct combustion can provide heat or steam for household and industrial uses or for the production of electricity (Demirbas 2001). Many industries devote a considerable amount of energy to the production of steam, with the pulp and paper industry using 81 % of its total energy consumption for this purpose (Saidur et al. 2011). The lumber pulp and paper industry uses biomass to provide 60 % of its energy needs (Demirbas 2001). The efficiency of biomass direct combustion for the

production of electrical energy is between 20 - 40 % with the highest efficiencies being achieved in large plants of ≥100 MW and in the co-combustion of biomass and coal (McKendry 2002; Demirbas 2001). The co-combustion of biomass in coal fired plants is considered an especially attractive option for biomass exploitation due to its higher efficiencies (Demirbas 2001; McKendry 2002; Saidur et al. 2011). The co-generation of heat and electricity can significantly improve the economics of biomass combustion, but requires that there is a local demand for heat (Demirbas 2001). The co-firing of a power plant with microalgae grown using the carbon dioxide emissions of the power plant appears conceptually to be an elegant method of reducing the GHG emissions per unit of electrical power generated by the plant, but unfortunately may not be practicable. A Life Cycle Assessment of co-firing of coal and solar dried microalgae found that, although GHG emissions and air acidification could be reduced by co-firing with microalgae grown on the power plant emissions, the depletion of natural resources and eutrophication potential increased and the use of microalgae was "obviously more expensive than coal" (Kadam 2002).

The moisture content of biomass can reduce the available heat compared to that from dry biomass by 20 % (Demirbas 2001) and the direct combustion of biomass is feasible only for biomass with a moisture content of less than 50 % (McKendry 2002; Varfolomeev and Wasserman 2011). Large biomass plants can be as efficient as fossil fuel plants, but the higher moisture content of biomass results in additional costs (Demirbas 2001). Microalgal biomass will need considerable further drying after harvesting before it could be used for direct combustion. Generally high moisture content biomass is considered better suited to biological conversion, by anaerobic digestion or fermentation, to other more readily useable fuels (McKendry 2002).

Ash content can be a considerable problem in direct combustion due to fouling of the boilers, thus restricting the use of high ash content biomass (Demirbas 2001). Wood has a typical ash content of 0.5 - 2 % (Saidur et al. 2011; Misra et al. 1993), but the ash content of microalgae can be high: *Spirulina* has been reported as having an ash content of 7 - 10 % (Tokusoglu and Unal 2003), diatoms as containing 9 - 35 % ash due to the silica outer wall (Brown and Jeffrey 1995) and

Pleurochrysis carterae a coccolithophorid, as containing 10 % calcium carbonate (Moheimani and Borowitzka 2006; Moheimani 2005). Biomass from marine algae is likely to have a high salt content, contributing to corrosion and ash deposition, unless energy-demanding washing processes are carried out (Miles et al. 1996; Björkman and Strömberg 1997)

The fine particulate nature of dry microalgae may be advantageous in co-combustion with pulverised coal as no additional particle size reduction is required. Non-microalgal biomass particles are normally much bigger than pulverised coal and the amount of energy required for grinding of the biomass to a diameter of less than 1 mm (2-3 % of the heating value) is almost double that required for coal pulverisation (0.9-1.2 % of the heating value). The energy requirements for biomass pulverisation increase significantly (>20 % of the heating value) for fibrous and or moist biomass (Belosevic 2010). The combustion of biomass can generate fine particle emissions which can be harmful to health, with the amount, type and quantity of particulate emissions being influenced by biomass type, combustion technology and emission control equipment (Sippula 2010). If microalgal biomass is to be a used in direct combustion, extensive research will be required to optimise combustion or co-combustion and to reduce emissions.

Using direct combustion of microalgae it is possible to produce both heat and electrical energy; however, the problem of drying the microalgae together with challenges of ash and emission control may make the direct combustion of microalgae impractical on a commercial scale.

Pyrolysis

Pyrolysis is the thermal decomposition of the organic component of dry biomass by heating in the absence of air, producing, as its primary product, a hydrocarbon rich liquid (bio-oil or bio-crude) (McKendry 2002; Saidur et al. 2011). The exploitation of pyrolysis products dates back to ancient times with the ancient Egyptians using the pyrolysis of wood to produce tars for use in embalming (Demirbas 2001). Pyrolysis can produce high volumes of fuel relative to the

biomass feed and the process can be modified to favour the production of bio-oil, syngas or solid char (Miao et al. 2004). Bio-oil is perhaps a more attractive end product than char or syngas as it has a higher energy density and is easily transport and stored (Jena and Das 2011).

Methods of pyrolysis

Pyrolysis processes can be classified by temperature and reduced processing time. While there are no formal definitions, slow pyrolysis is characterised by long residence times (> 5 s for volatiles, and from minutes to days for solids) at low reactor temperatures (< 400 ^{o}C) with very low rates of heating (0.01 - 2 ^{o}C s^{-1}) and of product quenching (Peacocke and Joseph ND). Fast pyrolysis is characterised by short gas / vapour residence times (< 2 s), high reactor temperatures (> 450 ^{o}C) and high heating rates (>1000 ^{o}C s^{-1}) with rapid product quenching (< 0.04 s); while intermediate pyrolysis lies between these values (Peacocke and Joseph ND). Slow pyrolysis results in higher yields of char rather than the liquid or gaseous products (Ghasemi et al. 2012; Brennan and Owende 2010). Fast pyrolysis is considered a more effective process than slow pyrolysis (Ghasemi et al. 2012), with the capability of achieving greater liquid product and gas yields of around 70- 80 %, compared to 15 - 65 % for slow pyrolysis (Brennan and Owende 2010; Varfolomeev and Wasserman 2011). The properties of bio-oil from fast pyrolysis have also been reported to be more suitable for refining to liquid fuels (Miao et al. 2004). Fast or flash pyrolysis also covers a range of newer technologies with temperatures above 500 °C and residence times of a few seconds or less (McKendry 2002; Ghasemi et al. 2012). These are considered as having future potential for the commercial production of biofuel from biomass (Brennan and Owende 2010).

Pyrolysis of microalgae

The pyrolysis of dry *Chlorella* has been found to give higher yields and better quality bio-oil (higher calorific value) than from macroalgae or moss. (Demirbas 2010). The optimum pyrolysis reaction range for *Chlorella,* in terms of the proportion of biomass converted, was found to be close to the conditions used in

flash pyrolysis (Bhola et al. 2011). The maximum bio-oil yield from pyrolysis of dry *Chlorella* was found to be between 50.8 - 57.9 % of the weight of the original dry biomass (Ozkurt 2009) and (Miao et al. 2004; Miao and Wu 2004; Ozkurt 2009; Demirbas 2010).The Higher Heating Values (HHV) of *Chlorella* and the microalgal bio-oil were 23.6 MJ kg^{-1} and 39.7 MJ kg^{-1} respectively (Demirbas 2010). The conversion of 50.8 % w/w of biomass to bio-oil thus gives an energy yield of 85 % of the initial energy in the microalgal biomass. The lipid content of microalgae is believed to influence the pyrolysis only energy balance (i.e. not including the biomass cultivation, harvesting or preparation stages) with higher lipid content microalgae having an improved energy balance and producing bio-oil that requires less refining (Bhola et al. 2011).

It is suggested that the energy required to produce bio-oil by pyrolysis from microalgae would be similar to the narrow range 200 - 400 kJ kg^{-1} reported in the published literature for a 'diverse' range of dry biomass feedstocks (Maddi et al. 2011). The energy input for pyrolysis has been quoted as 1.3 - 2.7 % of the calorific value of the microalgae, but no estimate of the energy to dry the microalgae, which can be very significant, was given(Maddi et al. 2011). The major challenge for pyrolysis of microalgal biomass is that it requires dry biomass. When drying of microalgal biomass was included pyrolysis used more energy than was produced as usable solid, liquid and gaseous fuels (Jena and Das 2011). The Energy Consumption Ratio for the production of bio-oil from microalgae, a ratio of the energy input for thermochemical treatment to the energy in the bio-oil, was found to be 0.44 to 0.63 for hydrothermal liquefaction and 0.92 to 1.24 pyrolysis due to the requirement for the moisture to be evaporated prior to pyrolysis (Vardon et al. 2012); again indicating that pyrolysis can use as much, or more, energy than is generated as biofuels.

Although pyrolysis is carried out at atmospheric pressure and is a well-established and 'simpler' process than hydrothermal liquefaction (Babich et al. 2011), the ability of other processes such as hydrothermal liquefaction to use wet biomass would appear to give them an advantage over conventional pyrolysis. As with direct combustion the need to dry the microalgae prior to pyrolysis may preclude it as an energetically and economically viable method of producing bioenergy.

Techniques such as microwave or microwave-assisted pyrolysis may provide a solution capable of dealing with high moisture content biomass. A new experimental microwave assisted method of pyrolysis has been laboratory tested on *Chlorella* yielding up to 22 % bio-oil (Du et al. 2011) ; but further development of these approaches is required before they can be regarded as suitable for full-scale application (Lam and Chase 2012).

Refining of pyrolysis bio-oils

The bio-oils from pyrolysis are normally highly oxygenated complex mixtures of organic compounds resulting in a mixture than can be polar, viscous, corrosive, unstable and unsuitable for use in conventional fuel engines unless refined (Peng et al. 2000). Bio-oil from microalgae pyrolysis has been reported to have a lower oxygen content and viscosity and higher heating value than bio-oil from wood or other terrestrial plant material (Miao et al. 2004; Miao and Wu 2004; Du et al. 2011), and could be more suitable for refining into liquid fuels. However bio-oil from both macroalgae and microalgae may contain nitrogen compounds that bring additional fuel refining costs (Maddi et al. 2011). It is highly probable that bio-oil from microalgae will need to be refined requiring additional energy input prior to the production of a readily useable fuel. The optimum process for energy return from pyrolysis alone may therefore differ from that which produces the best energy return after refining. The use of a catalyst (sodium carbonate) during pyrolysis has been suggested to upgrade the bio-oil products, reducing or eliminating the need for further refining; and was found to improve the bio-oil quality by reducing acidity and oxygenation, from the pyrolysis of *Chlorella* without a reduction in yield (Babich et al. 2011).

Gasification

Gasification is the conversion of organic matter by partial oxidation at high temperature (800 -1000 °C) mainly into a combustible gas mixture (syngas) with small quantities of char and bio-crude, typically 10 % and 5 % respectively (Demirbas 2001; McKendry 2002; Saidur et al. 2011; Bain 2004) . The syngas has

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a calorific value of 4-6 MJ m^{-3}, around half that of natural gas (McKendry 2002), and is a mixture of hydrogen (30-40 %), carbon monoxide (20-30 %) methane (10-15 %), ethylene (1 %), nitrogen and water vapour (Demirbas 2001; Saidur et al. 2011). The gas can be burnt to produce heat or converted to electricity in combined gas turbine systems (Demirbas 2001; McKendry 2002), or can be used to produce methanol and hydrogen as a fuel for transport and other uses (McKendry 2002; Saidur et al. 2011).

Conventional biomass gasification processes require dry feedstock (Guan et al. 2012a), but supercritical water gasification (SCWG) is an alternative gasification technology for the conversion of high moisture biomass and it is suggested that in well-engineered systems it can be net energy positive (Guan et al. 2012b). Chakinala et al. (2010) found that higher temperatures, low microalgal concentrations, and longer residence times improved the efficiency of SCWG of *Chlorella vulgaris*. The enthalpy change needed to take ambient liquid water to a low-density supercritical state (400 °C and 250 bar) is similar to that required to vaporise liquid water at ambient temperature, but the advantage of the SCWG process is that much of the energy invested in reaching a supercritical state can be captured and used again, with the hot effluent from the gasification reactor being used to preheat the wet biomass feed stream (Guan et al. 2012a).

Gasification of microalgae

Conventional gasification of microalgae has been found to yield syngas containing hydrogen (5- 56 %), carbon monoxide (9-52 %) and methane (2-25 %), together with a smaller quantities (0.1- 20 %) of bio-char, bio-crude and ash (Suali and Sarbatly 2012). Increasing the gasification temperature and using a catalyst, such as nickel or potassium carbonate increases the yield of hydrogen, and reduces tar production in the bio-crude. SCWG in the presence of a catalyst can considerably increase the proportion of methane in the syngas with 60-70 % of the heating value from the microalgal biomass being recovered as methane (Murphy et al. 2013; Suali and Sarbatly 2012).

Experimental laboratory studies of the steam gasification of dry *Chlorella* and moss found yields of syngas were higher for the microalgae than for both moss and macroalgal biomass, with maximum syngas yields of 40.6 % of the weight of the original biomass and a hydrogen gas content of up to 48.7 % by volume (Demirbas 2010). The syngas yield increased with increasing temperature from 302-652 °C, in agreement with a recent model of the kinetics of SCWG that indicated that higher temperatures favour production of intermediates which are more easily gasified together with the production of gas at the expense of char (Guan et al. 2012b).

Gasification of *Spirulina* to a methane-rich syngas using supercritical water and ruthenium catalysts has been predicted to yield up to 60-70 % of the heating value contained in the microalgal biomass (Stucki et al. 2009). Experimental studies on SCWG at 550 °C of *Nannochloropsis* found energy conversion of biomass to syngas of up to 60 % (Guan et al. 2012a). A theoretical study of the production of methanol from *Spirulina,* using the maximum theoretical yield of 0.64 g methanol g^{-1} dry microalgal biomass, gave a ratio of produced energy to required energy of 1.1, with the gasification and methanol synthesis process using some 25 % of the total process energy (Hirano et al. 1998). As the energy content of methanol at 23 MJ kg^{-1} is similar to that of microalgae this would equate to an energy conversion of 60-70 %. Although the energy balance is slightly positive, the evaluation assumes the maximum theoretical yield and that wet microalgal biomass with 79 % moisture content can be successfully treated using gasification. Using a novel method of catalytic gasification of wet *Chlorella* biomass (87 % water)*,* at lower temperature (350 °C) and higher pressure (18 MPa) than conventional gasification, up to 70% of maximum theoretical syngas was produced (Minowa and Sawayama 1999). The study also attempted a brief energy evaluation comparing gasification to direct combustion of microalgae. Although the energy evaluation showed gasification to be superior to direct combustion the assumptions made included: the maximum theoretical yield of syngas, recycling of nutrient only in gasification and a halving of the calorific value of microalgal biomass for combustion, but no similar allowance for combustion of the syngas. The energy for gasification was given as 5.95 MJ kg^{-1} of dry cells, or approximately 28 % of total energy in the original biomass. It has been suggested

that for every 4.5 J of energy in the syngas gas, 1.0 J of unrecovered heat energy is required for SCWG (Guan et al. 2012a), equivalent to an energy input of 22 % of the syngas or 21 % of the calorific value of the original microalgal biomass.

A recent review concluded that there is little data available on the gasification of microalgae and in particular on the energy balance and the need for drying of microalgae prior to gasification (Brennan and Owende 2010). If gasification of microalgae can be achieved with wet biomass it would possibly become more economic and energetically attractive.

The economics and energy balance of gasification could also potentially be improved by recycling of nutrients in the microalgal biomass for the growth of new microalgae. It has been found that nitrogen recovered from the aqueous phase after gasification can be used as part of a medium to successfully grow microalgae (Minowa et al. 1995).

Combustible gas can also be produced from wet microalgal biomass by anaerobic digestion at much lower temperatures than gasification. Both gasification and anaerobic digestion have been suggested as promising methods for exploiting bioenergy from biomass in India (Singh and Gu 2010). Anaerobic digestion of microalgal residues, however, has been shown to have a higher net energy return and much lower GHG emissions than gasification (Delrue et al. 2012). Although gasification is generally a more rapid process than anaerobic digestion it would appear than the energy input needed to achieve the temperatures required will make it uncompetitive with anaerobic digestion in terms of energy ratio, unless a much higher yield of combustible gas can be achieved than that from anaerobic digestion.

Liquefaction and hydrothermal upgrading

Liquefaction is a low temperature high pressure process where biomass is converted into a stable liquid hydrocarbon fuel (bio-oil) in the presence of a catalyst and hydrogen (McKendry 2002; Demirbas 2001). In hydrothermal upgrading the biomass is converted to partially oxygenated hydrocarbons at high

pressure in the presence of a catalyst in a wet environment (McKendry 2002; Demirbas 2001). In practice the terms liquefaction, hydroliquefaction and hydrothermal liquefaction are used for processes where wet biomass is converted to bio-oil by temperature and pressure in the presence of a catalyst, with and without the presence of gaseous hydrogen. Reviews of thermal treatments of biofuel have concluded that commercial interest in liquefaction is low due to the more complex feed systems and higher costs than pyrolysis and gasification (McKendry 2002; Demirbas 2001); but hydrothermal upgrading has the advantage of the conversion taking place in an aqueous environment and drying of biomass after harvesting may not be required prior to liquefaction and hydrothermal upgrading (Minowa et al. 1995; Sawayama et al. 1999; Brown et al. 2010). The ability of hydrothermal treatments to handle wet biomass make them some of the most interesting methods of producing biofuel from microalgae (Torri et al. 2012). *Dunaliella* with a moisture content of over 78 % has been treated by hydrothermal upgrading (termed liquefaction by the authors) at a laboratory scale (20 g of wet microalgae) yielding 37 % oil, based on the dry organic weight of the microalgal biomass (Minowa et al. 1995); but hydrothermal liquation of biomass with a moisture content above 90 % is believed to have an unfavourable energy balance (Vardon et al. 2012).

Hydrothermal carbonisation is a process in which biomass is heated in water under pressure to create char rather than liquid products. In an experimental study of various microalgae a char with an energy content similar to bituminous coal and containing 55 % of the carbon from the original biomass was produced by hydrothermal carbonisation (Heilmann et al. 2010). The hydrothermal carbonisation process requires a 10 % solids concentration and thus drying of microalgal biomass may not be required prior to conversion. The hydrothermal carbonisation process can produce char with a calorific value of 12.01 MJ from 1kg of dry microalgal biomass, but to heat a system contain 1kg of dry microalgal biomass and 9 kg of water from ambient to 203 °C will require, 7.31 MJ. With insulation and temperature control, however, no significant additional energy was needed to maintain reaction temperature for 2 hours (Heilmann et al. 2010). It is suggested that hydrothermal carbonisation gives a better return on energy investment than direct combustion as, the microalgae do not need to be dried after

harvesting; and heat recovery of the type employed in any industrial process would result in additional improvements to the energy balance. Lack of production of char is generally considered to be an advantage of hydrothermal treatment as bio-oil is a more useful product than both char and syngas (Jena and Das 2011), and the application of hydrothermal carbonisation for biofuel production may therefore be more limited than hydrothermal liquefaction.

Although biomass liquefaction has been extensively researched, microalgae have seldom been studied despite the fact that they should decompose and hydrolyse more easily than lignin containing biomass (Yang et al. 2011a). The yield of bio-oil from the liquefaction of *Dunaliella* has been reported to be as much as 87 % of the weight of original microalgal organic matter (Yang et al. 2011a), although this appears unrealistically high: the calculated energy is over 140 % of that in the original biomass and there appears to be ~ 24 % more carbon in bio-oil than in the original biomass. This may be due to a simple error in assessment methods and calculations, or may be due to a reaction between the microalgal biomass and the large quantity of ethanol (9 times the weight of wet biomass) used in the liquefaction. The maximum bio-oil yield from the liquefaction of *Microcystis* has been reported as 33 % of the weight of the organic matter and 40 % of the energy in the microalgal biomass. (Yang et al. 2004). Bio-oil yield from the liquefaction of wet *Nannochloropsis* (79 % moisture content) has been found to be a maximum of 43 % of the biomass dry weight with recovery of 80 % the carbon and 90 % of the energy in the *Nannochloropsis* organic material (Brown et al. 2010). Bio-oil yields from hydrothermal liquefaction, as a percentage of the mass of original dry microalgal biomass, have been reported as: up to 41 % for *Spirulina* (Jena and Das 2011), between 24 - 45 % for *Scenedesmus* (Vardon et al. 2012) and up to 49 % for *Desmodesmus* or 75 % recovery of the energy in the microalgal biomass as bio-oil (Alba et al. 2012). The cell wall of *Desmodesmus* has been found to be resistant to hydrothermal liquefaction (Alba et al. 2012).

The energy needed to heat the wet microalgal biomass to operating temperature for liquefaction has been estimated at between 65-85 % of the total energy available in the bio-oil produced (Minowa et al. 1995). A more recent estimate reported the energy required for hydrothermal liquefaction of microalgal biomass

with a moisture content of 80 % as between 44-63 % of the bio-oil energy produced (Vardon et al. 2012). Although the liquefaction of microalgal biomass can be a net energy producer and the bio-oil has a high calorific value and can be readily refined into a variety of liquid fuels, it would appear that a complete process for the production of microalgal biofuel from liquefaction using existing microalgal growth and harvesting techniques may use more energy than is produced.

The ability of liquefaction to use wet biomass and to convert the vast majority of the chemical energy into readily refined liquid fuels may make it worthy of further study, but the heat energy required for the process is the major challenge. The enthalpy of compressed water at 200 °C and 15.55 bar, the least energetic conditions found in the literature (Brown et al. 2010), is 852 kJ kg^{-1} (Mayhew and Rogers 1972), equivalent to an increase of around 768 kJ kg^{-1} from water at 20 °C and atmospheric pressure. As outlined in a review of harvesting (Milledge and Heaven 2013) a moisture content of 75 % appears to be the minimum achievable by current techniques without drying. A kilogram of microalgal slurry with 75 % moisture content contains 0.25 kg of dry microalgal biomass: if this biomass had a 20 % lipid content its calorific value would be approximately 1.5 kWh (Milledge 2010b). The enthalpy change from atmospheric temperature and pressure to the minimum requirements for microalgal liquefaction (200 °C and 15.55 bar) would be approximately 0.16 kWh or under 11 % of the calorific value of the microalgal slurry. The enthalpy change of the dry microalgal biomass is not known, but if it is assumed to be similar to that of water the total energy required to increase the enthalpy of the entire slurry would still be below 15 % of the calorific value of the microalgal biomass. It may therefore be possible considerably to reduce the energy requirements of liquefaction making it potentially viable as a system for net energy production.

The hydrothermal liquefaction of microalgae can produce bio-oil of similar or higher calorific value to that from pyrolysis, but the chemical composition is different and hydrothermal liquefied oil can have higher viscosity and a greater percentage of higher boiling point compounds (Vardon et al. 2012; Jena and Das 2011). As with bio-oil from pyrolysis, bio-oil from hydrothermal liquefaction will

probably need further refining to produce a commercially useable biofuel. The lower quantity of low boiling point compounds in hydrothermal bio-oil will make it less desirable for light fuel applications (Vardon et al. 2012).

The recycling of nutrients particularly nitrogen and phosphorus, could reduce the net energy inputs for microalgal biofuel production, and the nitrogen and phosphorus dissolved in the aqueous phase from hydrothermal liquefaction is believed to be capable of reuse as a growth medium for microalgae (Alba et al. 2012)

Liquefaction and hydrothermal upgrading can handle wet biomass eliminating the need for drying after harvesting, but the process is more complex and has higher costs than pyrolysis and gasification. Large amounts of energy are required to heat and compress the wet biomass, and processes such as anaerobic digestion that can operate at lower temperatures will have a lower energy input and potentially higher return on energy investment.

Bio-hydrogen production

Hydrogen is considered a particularly attractive replacement for fossil fuel as its combustion produces water vapour rather than greenhouse gases. Fuel cells and other technologies to exploit hydrogen are commercially available, and vehicles using hydrogen as fuel are already in operation, for example Honda's FCX. Although, hydrogen is believed to be beginning to move from a "fuel of the future to an energy carrier of the present" (Benemann 2000), the major challenge remains producing renewable hydrogen at an affordable and competitive cost.

The production of hydrogen by cyanobacteria has been known since the late 19th century (Benemann 2000). Gaffon is generally credited with the first scientific study of bio-hydrogen from microalgae in research on *Scenedesmus* in the late 1930s and early 1940s (Benemann 2000; Varfolomeev and Wasserman 2011; Kruse et al. 2005). Species of *Chlamydomonas, Chlorella* and *Scenedesmus* have now been reported as able to produce bio-hydrogen (Levin et al. 2004; Rashid et al. 2013; Healey 1970), as have several species of cyanobacteria, of which the

most studied is *Anabaena variabilis* (Levin et al. 2004). A variety of metabolic pathways for the production of bio-hydrogen have been studied (Ljunggren 2011; Kruse and Hankamer 2010; McKinlay and Harwood 2010) with hydrogenase, the enzyme mainly responsible for hydrogen production, being widely found in both prokaryotic organisms and eukaryotic plants and with all five major taxonomic groups of cyanobacteria containing hydrogenase genes (Kruse and Hankamer 2010). Detailed discussion of the metabolic pathways for hydrogen production is beyond the scope of this paper, but further details can be found e.g. in papers by Benemann (2000), McKinlay and Harwood (2010), Rashid et al. (2013) and Srirangan et al. (2011).

Considerable research on bio-hydrogen has been carried out since the 1970s, and over US$ 100 million had been spent on research up to 2000, but with "little progress toward the goal of a practical and commercial process" (Benemann 2000). Research has continued since 2000, but yields of energy from bio-hydrogen production systems have been low at 0.3-1.3 % of the total light energy arriving at the surface of the reactor, while 5 % is required for an economically viable system (Kruse and Hankamer 2010). It has been suggested, however, that the maximum practical efficiency of conversion of solar energy is 1 %, with cyanobacteria only able to achieve this conversion rate for a short period in a pure argon atmosphere, and outdoor systems achieving an average of only 0.05% (Sorensen 2012). The considerable challenges of oxygen inhibition and scale-up also still need to be overcome (McKinlay and Harwood 2010; Varfolomeev and Wasserman 2011; Kruse and Hankamer 2010). Substantial research effort appears to be being directed at the genetic modification of microalgae (McKinlay and Harwood 2010; Gressel 2008; Varfolomeev and Wasserman 2011), but although this may overcome the challenges of yield and oxygen inhibition it may produce fresh issues of containment and public acceptability.

A major advantage of bio-hydrogen production is that hydrogen does not accumulate in the culture (Ghasemi et al. 2012) and harvesting and energy extraction costs could thus be reduced; but despite extensive research commercially viable production appears to be some way off.

Microalgal fuel cells

In microbial fuel cells (MFCs) electrical current is generated from oxidation-reduction reactions that occur within living microorganisms with the oxidation of an organic compound at the anode generating electrons that produce an electric current (Powell et al. 2011; De Schamphelaire and Verstraete 2009). Microalgae have been used as a source of organic material for bacterial oxidation at the anode. With marine plankton as a substrate 80 % of organic carbon was removed in a MFC (De Schamphelaire and Verstraete 2009). Using *Chlorella* as a substrate around 60 % of the chemical oxygen demand was removed, but conversion of chemical energy to electrical energy was low at between 10 - 25 % (Velasquez-Orta et al. 2009). The electrical yield from MFCs has been quoted at 2.5 kWh kg^{-1} of dry *Chlorella* biomass (Velasquez-Orta et al. 2009), but this appears to be over stated by a factor of 10 due to the use of an incorrect conversion factor of 2.77 kWh MJ^{-1} rather than 0.277 kWh MJ^{-1} (Perry and Chilton 1973). When the data presented are corrected, however, the energy produced from dry microalgal biomass by a fuel cell is approximately a quarter of that achieved by either direct combustion or anaerobic digestion. The maximum power generation of MFCs oxidising biomass is currently only up to 1 W m^{-2} (Howe 2012; Thorne et al. 2011) and could only produce power equivalent to 3.8 tonnes of oil (toe) ha^{-1} considerable below that anticipated from growth of microalgae for the production of biodiesel (Howe 2012).

A recent development in MFCs is a photo-microbial fuel cell or bio-photovoltaic fuel cell where photosynthetic microalgae growing at the anode generate the electrons, thereby removing the need for an organic substrate (Thorne et al. 2011; Howe 2012). Bio-photovoltaic fuel cells currently have very low efficiency, however, and energy production is between 1 and 1.5 orders of magnitude lower than in MFCs oxidising biomass (Howe 2012).

Photosynthetic microalgae can also act as electron acceptors at the cathode (Powell et al. 2011). A power density 0.95 mW m^{-2} was achieved in a coupled MFC, with *Chlorella* growing at the cathode and yeast growing heterotrophically on glucose as an electron donor (Powell et al. 2011): this is considerably below

the best output of MFCs. In a unique design combining anaerobic digestion and a coupled MFC, bacteria growing on the waste from the anaerobic digestion of microalgae biomass act as the electron donor, with microalgae, grown as the biomass for anaerobic digestion, acting as the electron acceptor, but power output was low at an average of 12 mW m^{-3} (De Schamphelaire and Verstraete 2009).

The energy output of MFCs can be low and is currently many times lower when live microalgae are used either as an electron donor or acceptor. It is clear that there is a need for a considerable improvement in microalgal fuel cell efficiencies before they can be considered as a commercial option for exploiting microalgae for biofuel. There will also be significant problems in the scale-up of MFCs, in particular those requiring the growth of photosynthetically active microalgae (Rosenbaum et al. 2010). Microalgal fuels cells, if they have a future in the production of microalgal biofuels, may be limited to exploiting additional energy production opportunities generated by their use in conjunction with other methods of microalgal biofuel production, principally anaerobic digestion and fermentation where microalgal fuel cells may have the capability of giving incremental energy output gains.

Bioethanol production

First generation bioethanol, such as that produced from corn in the USA and sugar-cane ethanol in Brazil, is now widely used (Yang et al. 2011b) and there is considerable interest in producing second generation bioethanol from cellulosic biomass (Balat et al. 2008). Bioethanol can be readily used in current technology, with 86 % of cars sold in Brazil in 2008 capable of using ethanol or a mixture of ethanol and fossil fuel petroleum (Walker 2010). Bioethanol accounted for more than 94 % of global biofuel production in 2008, with the majority coming from sugar-cane (Balat et al. 2008). Bioethanol has been suggested as having better development potential than conventional biodiesel (Lee 2011). Bioethanol does have disadvantages, however, which include: "lower energy density than gasoline, corrosiveness, low flame luminosity, lower vapour pressure (making cold starts difficult), miscibility with water, and toxicity to ecosystems" (Balat et al. 2008). The energy balance of corn ethanol is probably marginal (Beal 2011) and it has

been suggested that "at present, bioethanol produced from sugar-cane in Brazil is the only credible example of a biofuel that exhibits a significant net energy gain" (Walker 2010). Ethanol from sugar-cane has a reported energy return on energy investment (EROI) of between 1.25 - 8 and corn ethanol between 1 - 1.34 (Beal 2011; Clarens et al. 2011; Mulder and Hagens 2008; Hall and Klitgaard 2012; Twidell and Weir 2006). The growth of crops such as sugar-cane and corn for the production of sugar for bioethanol will be considerably constrained, as these compete directly with food production.

In grain crops about half of the above-ground biomass (straw) is 'wasted'. Worldwide 2 billion tonnes of cereal straw are produced annually (Gressel 2008) and there is now considerable interest in exploiting straw and other lignocellulosic materials for ethanol production (Balat et al. 2008). In sugar and ethanol production from sugar-cane considerable quantities of bagasse are produced which can be burnt to produce heat to distil bioethanol, but there are concerns about the environmental effects of this and it may be more beneficial to convert bagasse to bioethanol (Gressel 2008). The total potential worldwide bioethanol production from crop residues and wasted crops has been estimated at 491 billion litres year^{-1}, about 16 times higher than the current world bioethanol production (Balat et al. 2008). Cellulosic ethanol was expected to play a large role in meeting the goals of the US Energy Independence and Security Act of 2007 for renewable biofuels (Ferrell and Sarisky-Reed 2010); but despite extensive research and the availability of low cost lignocellulosic biomass there is, as yet, no large-scale commercial production of fuel bioethanol from lignocellulosic materials (Balat et al. 2008). One of the problems encountered with production of bioethanol from straw is that biodegradation of hemicelluloses and cellulose by cellulases can be inhibited by lignin, found in many terrestrial sources of second generation biofuel biomass (Gressel 2008). Microalgae do not normally contain lignin and therefore may hold out a prospect of the cellulosic components being more readily converted to sugars by cellulases.

Microalgal bioethanol

Microalgae can contain significant quantities of carbohydrates and proteins that can be converted to bioethanol via fermentation (Harun et al. 2010) with e.g. *Chlorella* containing up to 50 % w/w of starch under favourable growth conditions (Doucha and Lívanský 2009). Other microalgae are also known to contain up to 50 % w/w of carbohydrates that can be fermented to bioethanol (Singh and Olsen 2011). The yield of ethanol from the fermentation of microalgal biomass has been found to be up to 38 % of the dry microalgal biomass (Harun et al. 2010). An advantage of fermentation of microalgae may be that wet biomass could be used, but a recent study found that the sugar released from fermentation of dried biomass was 55 % higher than that from wet microalgal biomass (Miranda et al. 2012). If the microalgal biomass needs to be dried prior to conversation to fermentable sugars for bioethanol production it is likely that bioethanol production will not be energy efficient or economic.

Ethanol yield has been found to be improved by the removal of lipids from microalgae prior to fermentation (Harun et al. 2010), raising the prospect of bioethanol production being combined with biodiesel production.

In addition to simple carbohydrates and sugars within the cell, more complex carbohydrate associated with the cell wall will need to be broken down into fermentable sugar if the entire microalgal biomass is to be exploited for bioethanol (Harun et al. 2011b). Most microalgal species have cell walls based on cellulose (Harun and Danquah 2011); however there is considerable diversity in microalgal structural polysaccharides. Many algae lack cellulose and have other polymers that provide structure to the cell, while some lack cell walls entirely (Ferrell and Sarisky-Reed 2010; Tarchevsky and Marchenko 1991).

Cell wall disruption is considered essential to release the carbohydrates and sugars to maximise bioethanol yields. A sugar extraction efficiency of 96 % has been achieved by acid hydrolysis of dried *Scenedesmus* biomass (Miranda et al. 2012). Ultrasonic cell disruption followed by enzymatic saccharification released 64 %, of the dry biomass of *Chlorococcum,* as glucose that could be fermented to ethanol (Harun and Danquah 2011). Enzyme pre-treatment of *Chlamydomonas,* in the form of liquefaction by amylase followed by enzymatic saccharification,

yielded 23.5 % w/w ethanol after fermentation (Choi et al. 2010). The alkaline pre-treatment of cells to release fermentable sugars increased the ethanol production from *Chlorococcum* biomass containing 33 % carbohydrate, with a maximum yield of 26 % w/w of the dry biomass (Harun et al. 2011b).

Some microalgae contain cellulases and it may be possible to recover these and other industrial enzymes from the microalgal biomass (Ferrell and Sarisky-Reed 2010). A possible process could be to use extracted microalgal enzymes to produce fermentable sugars from microalgal biomass for bioethanol production, but although elegant this is probably currently uneconomic and will require considerably more research.

Like yeast, some microalgae such as *Chlorella* and *Chlamydomonas* are capable of producing ethanol and other alcohols through heterotrophic fermentation (Ferrell and Sarisky-Reed 2010) , but microalgae are probably of more interest as feedstock rather than as biological means of converting biomass to ethanol as yeast fermentation is an established and extensively researched process.

Continuous fermentation of glucose can produce an ethanol yield of 51 % w/w (McGhee et al. 1982) with an energy conversion of 98 % (glucose HHV 15.6 kJ g^{-1} and ethanol HHV 29.8 kJ g^{-1}). Reported yields of ethanol from disrupted microalgal cells range from 23.5-38 % w/w with the higher figure giving a maximum energy yield of 57 % assuming a HHV of 5.5 kWh kg^{-1} for low lipid content microalgae (Milledge 2010b). The challenge of producing bioethanol from microalgae, as with lignocellulosic biomass, will be to convert the entire organic microalgal biomass to fermentable sugars economically and energy efficiently. There appears to be considerably less research on the production of bioethanol than biodiesel, but bioethanol has been suggested as one of three top targets for future microalgal biofuel production (Gouveia 2011). The fermentation of microalgae has the potential advantage of exploiting the entire biomass, if an economic method of producing fermentable sugars from complex organic material is found. However there are large quantities of complex waste organic matter from agriculture that could potentially provide a lower cost feedstock for fermentation than purpose grown microalgae.

Biodiesel and trans-esterification

"Biodiesel is a fuel that is obtained from a manufacturing process that converts plant oils or animal fats together with alcohol into a fuel that can be used in an internal combustion engine" (Office of Gas and Electricity Markets 2009). In chemical terms it is the alkyl esters of fatty acids which are produced by trans-esterification of triglycerides of fatty acids using an alcohol, normally methanol or ethanol (Knothe et al. 2005).

The high lipid content of some microalgae has led much of the published research work to be focused on the production of biodiesel from microalgal lipids via trans-esterification. The long running and much quoted study by the NREL (Sheehan et al. 1998) focused almost entirely on biodiesel production with relatively little discussion of alternative methods of exploiting the energy within the microalgal biomass.

There are a number of challenges for the production of microalgal biodiesel:
   a. In conventional commercial trans-esterification processes the biomass needs to be dry (Hidalgo et al. 2013)
   b. The cell wall may require disruption to release the lipid which is energetically demanding (de Boer et al. 2012).
   c. Lipid needs to be extracted from microalgal biomass by solvents and other methods (Rawat et al. 2013).

Drying of the biomass prior to oil extraction can use considerable energy and can be the main energy input in the production of biodiesel (de Boer et al. 2012). Wet solvent extraction of lipid from microalgae biomass which eliminates the need for drying and potentially reducing energy input may be possible, but has yet to be proven at industrial scale (de Boer et al. 2012; Lardon et al. 2009; Sills et al. 2012); but for microalgal biofuels to yield net gains in energy lipid extraction methods for wet biomass must be developed (Sills et al. 2012; Delrue et al. 2012).

Intact cell walls hamper lipid recovery and the most effective methods of recovery are from disrupted microalgal cells (Greenwell et al. 2010). Mechanical pressing is the industry standard for oil recovery from oilseeds for both food and biofuel production, but it is ineffective for microalgae (de Boer et al. 2012). A number of cell disruption techniques have been applied to microalgae, but mechanical disruption is generally considered preferable to chemical disruption as it avoids chemical contamination and preserves the functionality of the cell contents (Chisti and Moo Young 1986). The breaking of cell walls can require large amounts of energy, and can be achieved by ultrasound, milling, autoclaving or homogenisation (Mata et al. 2010). Homogenisation can be very efficient, with between 77- 96 % of microalgal cells ruptured per pass (GEA Process Engineering 2011), but to homogenise 10 l of microalgal suspension with algal cell concentrations between 100 - 200 g l^{-1} requires 1.5-2.0 kWh (Greenwell et al. 2010) or 0.75- 2 kWh kg^{-1} of microalgal cells disrupted. It has been suggested that cell disruption and subsequent oil extraction represent the largest energy input in the production of microalgal biodiesel (Razon and Tan 2011). If cell disruption processes could be combined with microalgal harvesting then a considerable reduction could be made in operational energy requirements (Milledge and Heaven 2011).

The high energy demands from microalgal biomass drying, cell disruption and lipids extraction have led to interest in in-situ or direct trans-esterification, where the biomass is directly in contact with the alcohol and catalyst (Hidalgo et al. 2013; Velasquez-Orta et al. 2012), thus reducing the number of unit operations, simplifying the process and potentially reducing energy inputs (Velasquez-Orta et al. 2012; Rawat et al. 2013). Direct trans-esterification of oilseeds using an alkaline catalyst has a high tolerance for water (Velasquez-Orta et al. 2012), but increasing the biomass water content decreases trans-esterification efficiency (Hidalgo et al. 2013). The amount of methanol required for biodiesel production by in-situ trans-esterification is 'extremely high' and will need to be reduced for the process to become economic (Velasquez-Orta et al. 2012). In-situ trans-esterification also faces energetic hurdles, due to the large volumes of water, solvents or reactant that need to be evaporated from the biomass, and these must be overcome if it is to be energetically viable (de Boer et al. 2012).

Although the lipid content of microalgae can be high this is not the case for all species; and generally the production of lipids, as energy storage compounds, occurs under nutrient stress where the growth rate is reduced. The NREL and others (Illman et al. 2000; Bhola et al. 2011) have shown considerable reductions in microalgal yield under nutrient stress condition that promote high lipid content. Despite the higher lipid content , the actual lipid yield can be lower under nutrient stress than in nutrient replete conditions due to a much lower growth rate (Sheehan et al. 1998; Liu et al. 2013). Unfortunately not all microalgal lipids are suitable for conversion to biodiesel by trans-esterification (Chisti 2007). The presence of lipids other than triglyceride may require energy intensive pre-treatment steps before the alkaline trans-esterification (de Boer et al. 2012).

A considerable number of Life Cycle Assessments (LCAs) have been carried out on the production of biodiesel and it has been concluded that the process may be marginal in terms of energy balance, global warming potential (GWP) and economics. Only in the best case scenarios was microalgal biodiesel found to be comparable to first generation biodiesel and microalgal biodiesel was not "really competitive under current feasibility assumptions" (Lardon et al. 2009). A reworking of the data from 6 LCAs, in what was termed a Meta-model of Algae Bio-Energy Life cycles (MABEL), found that the energy return on energy invested (EROI) ranged from one, no return on the energy invested to two, twice the energy invested (Liu et al. 2011). A recent extensive review and LCA using a Monte Carlo approach to estimate ranges of expected values found that nearly half of all the LCA results had an EROI of less than one (Sills et al. 2012). The Sills (2012) study also showed that methane from anaerobic digestion of defatted microalgae is required for net gains in energy and must be an integral part of microalgal biodiesel production process to yield EROI values that are greater than one.

The anaerobic digestion of microalgal biomass to produce biogas following lipid removal for biodiesel production has been proposed as a means of reducing the cost of biodiesel production by over 40 % through the use of biogas to power parts of the microalgal biofuel process (Harun et al. 2011a). The partial extraction

of energy from the microalgae in the form of biodiesel does not appear to be energetically or commercially viable on its own and anaerobic digestion appears vital to any efficient and economic process for producing biodiesel from microalgae (Milledge 2010a; Stephenson et al. 2010; Zamalloa et al. 2011; Delrue et al. 2012). One study has suggested that when the lipid content is below 40 % the anaerobic digestion of the entire biomass without lipid extraction may be the optimal strategy for energy recovery (Sialve et al. 2009). The Sialve et al. (2009) study contains errors, however, and if lipid is digestible then energetically it is always better to digest microalgae (Heaven et al. 2011).

Microalgal biodiesel production has been shown, in many studies, to produce negative net energy output and where there is a positive output it depends on technology that is not available at an industrial scale and/or the exploitation of the defatted biomass to produce biogas from anaerobic digestion. Anaerobic digestion of the entire microalgal biomass is thus more energy efficient as it utilises the entire wet algal biomass and can exploit microalgae with a wide range of lipid contents (Milledge 2010a; Heaven et al. 2011).

Anaerobic digestion

The advantages of producing biogas from the anaerobic digestion of microalgae are; that wet biomass can be used and there is the potential to exploit the entire organic biomass for energy production. Considerable research has been carried out on the anaerobic digestion of a variety of organic materials and some of the earliest studies on extracting bioenergy from microalgae examined anaerobic digestion (Golueke et al. 1957). Relatively few studies have been carried on the anaerobic digestion of freshwater microalgae, however, and almost none on marine microalgae (Zamalloa et al. 2011; Gonzalez-Fernandez et al. 2012a). Microalgae have been successfully digested to produce methane at a concentration of 1 % dry weight, but higher concentrations are considered more practicable (Oswald 1988). Anaerobic digestion may also allow the recycling of nutrients back to the microalgal growth system (Singh and Olsen 2011), potentially reducing costs and embodied energy inputs, but concerns have been expressed

about pigmentation of microalgal digestate inhibiting light penetration if it is used in growth ponds (Oswald 1988).

The theoretical yield of biogas, calculated from the chemical composition of microalgae using the "Buswell equation" (Buswell and Mueller 1952; Symons and Buswell 1933), can be high. The proportions of carbohydrates, proteins and lipids affect the potential of microalgae as a substrate for anaerobic digestion (Park and Li 2012) with lipid yielding higher volumes of biogas per gram of feed material than both carbohydrate and protein (Weiland 2010; Heaven et al. 2011; Zamalloa et al. 2011). Practical yields from the anaerobic digestion of microalgae are considerably below the theoretical maximum. The destruction of organic volatile solids from microalgae was found to be only 60-70 % of that found in raw sewage (Golueke et al. 1957). Methane yields from the anaerobic production of microalgae have been reported in the range of 0.09-0.34 $l\,g^{-1}$ of volatile solids (Zamalloa et al. 2011; Gonzalez-Fernandez et al. 2012a). There is considerable conjecture about the reason for the relatively low practical methane yields compared to the theoretical values. Is it; the cell wall protecting the contents of the cells from digestion, the relatively high proportion of cell wall to contents, the nature of the cell wall or cell contents?

The low methane production rates from anaerobic digestion have been attributed to the resistance of the microalgal cell wall to digestion even after death (Zamalloa et al. 2011). Microalgal cells walls typically make up 13-15 % of the weight of the cell, but some species may contain up to 40 % (Tarchevsky and Marchenko 1991). The largest variation in cell wall composition is found in microalgae, as if "nature decided to conduct a vast experiment with algae to select from the numerous polysaccharides the one that suits a cell wall best" (Tarchevsky and Marchenko 1991). The degree of polymerisation of cellulose and the diameter of cellulose fibres is higher in microalgae than bacteria and terrestrial plants (Klemm et al. 2005). Could the nature of microalgal cellulose be a factor in low biogas yields? Others have suggested glycoproteins in the cell wall may be a factor in poor biogas yield from microalgae as these are highly resistant to bacterial degradation (Afi et al. 1996; Gunnison and Alexander 1975). The cell walls of microalgae have been shown in many cases not to be a simple micro-fibre

cellulose structure, but a complex structure comprised of several distinct layers, some of which form a highly ordered crystalline lattice (Roberts 1974).

The tough cell wall of some species of microalgae may also prevent the contents being digested to produce biogas and the low conversion of microalgae to methane has been attributed to the resistance of intact microalgal cells to bacterial invasion and destruction (Golueke et al. 1957). Rupturing the cell wall by thermal, chemical and mechanical methods prior to anaerobic digestion has been shown to improve methane yields (Park et al. 2011). Thermal pre-treatment of *Scenedesmus* biomass doubled its anaerobic biodegradability (Gonzalez-Fernandez et al. 2012b). However the energy required for breaking the cell wall will negatively impact on the energy balance unless more energy is released as additional methane than is used to fracture the cells.

The microorganisms involved in the production of methane by anaerobic digestion are sensitive to the chemical composition of the feedstock in particular the Carbon to Nitrogen ratio of the substrates (Chen et al. 2008) . The low methane yield of microalgal biomass has been attributed to ammonia toxicity derived from the high concentrations of protein found in many microalgae (Park and Li 2012; Golueke and Oswald 1959; Samson and LeDuy 1983). The co-digestion of organic nitrogen rich microalgae with low nitrogen/ high carbon substrates, such as sewage sludge or glycerol, has been found to produce a synergistic effect with methane yields higher than from either substrate (Samson and LeDuy 1983; Gonzalez-Fernandez et al. 2012a). Methane yields double that of microalgal biomass alone have been achieved by co-digestion with low nitrogen wastes (Samson and LeDuy 1983; Bohutskyi and Bouwer 2013; Ward et al. 2014).

One of the advantages of growing microalgae for biofuel is that many species can be grown in salt water. Low salt concentrations can stimulate microbial growth, but high concentrations (≥ 10 gl^{-1}) are known to inhibit anaerobic systems through an increase of osmotic pressure or dehydration of methanogenic micro-organisms (Hierholtzer and Akunna 2012; Lefebvre and Moletta 2006). The toxicity of salt is predominantly determined by the sodium cation though other light metal ions,

such as potassium, have also been found to be toxic to methanogens at high concentrations (Chen et al. 2008). An optimal sodium concentration for mesophilic methanogens in waste treatment processes of 230 mg Na l^{-1} has been suggested (Chen et al. 2003). Mesophilic methanogenic activity is halved at 14 Na g l^{-1} (Chen et al. 2003; Ramakrishnan et al. 1998), the approximate concentration of sodium found in sea water (El-Dessouky and Ettouney 2002). Anaerobic digesters can be acclimatised to higher salt levels if they are continuously exposed to gradually increasing salt concentration rather than salt shock (Lefebvre and Moletta 2006). Adaptation of methanogens to high concentrations of sodium over prolonged periods of time can allow the anaerobic digestion of high salt concentration wet biomass with the sodium concentration to halve methanogenic activity increasing to 37.4 g Na l^{-1} after acclimation (Chen et al. 2003). It may therefore be possible to produce biogas from microalgae grown in sea water.

A recent energy balance model for the production of microalgal biogas using wastewater as a nutrient source and flue gas as a carbon source has found energy returns on operational energy invested of more than 3 (Milledge 2013a; Milledge 2013b). An EROI of 3 has been suggested as the minimum that is viable to 'support continued economic activity' (Clarens et al. 2011; Hall et al. 2009). Although the model showed that microalgal biogas may be energetically viable the ratio of electrical to heat energy of the microalgal biogas production was very different to that generated from the parasitic combustion of the microalgal biogas in a Combined Heat and Power unit CHP with more electrical than heat energy required. It was concluded that in order for the microalgal biogas to be energy efficient a local source for exploitation of the excess heat generated needs to be found (Milledge 2013a; Milledge 2013b). Finding local uses for excess heat is one of the major operational problems in the current exploitation of CHP, and not just for microalgal fuel production. The study also concluded that for microalgal biogas to be energetically viable requires (Milledge 2013a; Milledge 2013b):
   a. Favourable climatic conditions. The production of microalgal biofuel in UK would be energetically challenging at best.
   b. Achievement of 'reasonable yields' equivalent to ~ 3 % photosynthetic efficiency (25 g m^{-2} day^{-1})

c. Low or no cost and embodied energy sources of CO_2 and nutrients from flue gas and waste water

d. Mesophilic rather thermophilic digestion

e. Adequate conversion of the organic carbon to biogas ≥ 60 %

f. A low dose and embodied energy organic flocculant that is readily digested or microalgal communities that settle readily

g. Additional concentration after flocculation or sedimentation

h. Minimisation of pumping of dilute microalgal suspension

ter Veld (2012) also found a net energy return greater than 3 (3.2) for microalgal biogas with cogenerated heat utilisation, but concluded that maize-based biogas outperforms a prospective microalgae system in terms of net energy return.

Microalgal biomass has shown its potential for the production of various biofuels, although it is clear that there are significant technological hurdles to be overcome before microalgal biofuel is energetically and commercially viable. It is probably too early, at the current stage of biofuel development, definitively to select which method or combinations of methods for exploiting energy from microalgae will be commercial exploited. However anaerobic digestion is relatively simple, has the potential to exploit the entire organic carbon content of microalgae and can utilise wet biomass. It is likely to play a leading role in combination with other methods and could be the major method of biofuel production from microalgae.

Co-production and biorefineries

Current commercially viable exploitation of microalgal products is limited to products other than fuel, and the immediate future for the commercialisation of microalgae may be with non-fuel products (Milledge 2011; Bahadar and Bilal Khan 2013; Schlarb-Ridley 2011). However the lessons learned from non-fuel products, together with their potential for co-production with fuel, may lead to the more rapid commercial realisation of microalgal biofuel. Microalgal co-products "have potential to provide a 'bridge' while the economics of algal biofuels improve" (Hannon et al. 2010).

The cultivation of microalgae simply for biofuels may not currently be profitable and the microalgal industry must take advantage of markets for additional high-value products such as 'nutraceuticals', pigments and vitamins (Milledge 2010a; Milledge 2012; Hannon et al. 2010). Co-production of microalgal bioenergy with high-value products is currently more economically viable than the production of microalgal biofuel alone (Jonker and Faaij 2013; Subhadra and Grinson 2011). A recent economic model of the production of microalgal biofuel found that oil for biofuel production could represent a relatively small portion of microalgae related revenue opportunities (Brown 2009).

The term 'biorefinery' has been used in the literature since the 1980s, and refers to the co-production of a spectrum of high value bio-based products (food, feed, nutraceuticals, pharmaceutical and chemicals) and energy (fuels, power, heat) from biomass (Wageningen University 2011; Taylor 2008; Olguin 2012; Gonzalez-Delgado and Kafarov 2011). The biorefinery concept is an 'emerging research field' (Rawat et al. 2013) and in December 2009 the US Department of Energy announced a US$100 million grant for three organisations to research algal biorefineries (Singh and Ahluwalia 2013).

Biorefineries could allow the exploitation of the entire microalgal biomass. *Dunaliella salina* is grown for the production of β-carotene (Milledge 2011; Ben-Amotz et al. 2009) and is also a source of glycerol for potential use as biofuel and a green chemical feedstock. A recent study has concluded, however, that the production of glycerol for use as biofuel would currently be uneconomic without high value co-products (Harvey et al. 2012). The growth of *Dunaliella* could provide the biomass for a biorefinery. Laboratory studies suggest that *Dunaliella tertiolecta* could also potentially be used as a source; of high value lipids; extracellular polysaccharide, for polymer production; and glucose for bioethanol production (Geun Goo et al. 2013). This type of biorefinery, which produces a variety of products from a single biomass source, may be termed a vertical biorefinery (Milledge 2013b). Although biorefineries could improve the economics of biofuel production (Pires et al. 2012) they are likely to be energy intensive (Rawat et al. 2013; Olguin 2012), and will involve increased energy inputs, process complexity and possibly reduced energy outputs. A biorefinery

plant should operate sustainably with its energy met by biofuels produced (Cherubini 2010). Despite increasing interest, however, it has yet to be established whether microalgal biorefineries can produce more energy than is required by the processes within them.

Although high value microalgal products may allow the commercialisation of microalgae in the short term, the immense potential scale of microalgal fuel production could result in the creation of such large quantities of microalgal non-fuel materials that the market price is dramatically reduced.

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