

Review of promising strategies for zero-waste production of the third generation biofuels

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Abstract. Biodiesel obtained from microalgae is considered a promising alternative to conventional diesel fuel. However, it has been proposed that cultivation of algae for the sole purpose of making biodiesel is neither economically efficient nor sustainable. Nevertheless, there are several ways in which microalgae can be utilized to their full potential. One possibility is to view the cultivation and utilization of microalgae as a complex process that includes wastewater treatment, carbon dioxide sequestration, production of nutritional supplements, biofuels etc. The aim of this paper is to review the most promising possibilities of combining different cultivation strategies/technologies with the coproduction of high value products (e.g. Ω -fatty acids) and biofuels (algal diesel, ethanol and biogas).

Keywords: microalgae, microalgal biodiesel, microalgal bioethanol, coproduction.

INTRODUCTION

Due to increasing energy demand and environmental issues associated with fossil fuels, there has been a rising interest in alternative energy sources such as solar, wind and hydro energy (Razzak et al., 2013). In the transportation sector, biomass derived biofuels have received great attention (Christenson & Sims, 2011). Biofuels have potential to replace fossil-based fuels (Pittman et al., 2011). First generation biofuels, based on vegetable oil or bioethanol, have however many disadvantages including competition with food crops for arable land, high water requirements and indirect environmental impacts from pesticide and fertilizer use (Lardon et al., 2009). Second generation biofuels from lignocellulosic biomass could address some of the issues above by utilising agricultural by-products and biomass waste however, the conversion of the biomass into ethanol is still relatively costly (Sander & Murthy 2010; Tutt & Olt 2011; Tutt et al., 2013).

Microalgae have been considered a viable biofuel feedstock. Although algae require neither arable land nor pesticides and can be cultivated in wastewater, production of algal biodiesel has received a lot of critique because of the high costs of algal biomass production and downstream processing (van Beilen 2010; Petkov et al., 2012).

In order for the microalgal production to be economically viable, it needs to be less expensive than conventional diesel fuel. Therefore, the costs of algae cultivation, biomass harvesting, and processing must be minimal (Vlysidis et al., 2011). Production of 1 kg of biodiesel requires approximately 12 kg of algal biomass (Petkov et al.,

2012). If grown in fresh water without water recycling, then an estimated 3,726 kg of water, 0.33 kg of nitrogen and 0.71 kg of phosphate is needed to produce 1 kg of biodiesel (Yang et al., 2011). Energy input for algae cultivation and downstream processing are high with current technologies. The energy used in these processes is mostly fossil fuel-derived and thus, needs to be minimized and optimized. Biodiesel from microalgae cannot be obtained at the expense of more energy than is provided by the biofuel (Scott et al., 2010). To produce feedstock sufficient for a continuous supply of biodiesel, a suitable strain of algae needs to be used. There are oil-rich algae species e.g. *Botriococcus braunii* with reported oil content up to 70% lipids, however it must be highlighted that these species grow too slowly to meet the expectation of continuous biodiesel supply (Petkov et al., 2012).

In order for microalgal biodiesel to be economically feasible, the costs of algae cultivation, biomass harvesting, and processing must be minimal. Furthermore, by-products of biodiesel refining such as glycerol as well as the residual biomass from lipid extraction must be utilised (Vlysidis et al., 2011; Zhu 2013).

Aim of this paper is to review the potential approaches and methods to make algal biofuel production economically viable and environmentally friendly by using wastewater for algae cultivation and recycling the by-products and waste.

Algae cultivation in wastewater with CO₂ supplementation

In order to lower the costs of algal cultivation, wastewater could be used as a growth medium. In fact, some authors have stated that production of algal biofuels without utilising wastewater for cultivation is unlikely to be economically viable (Christenson & Sims, 2011; Pittman et al., 2011). It has been estimated that coupling wastewater treatment with biomass and biofuel production has a positive energy balance, assuming that nutrient removal with algae compensates for the cost of biological nutrient removal by 24–55% (Sturm & Lamer 2011). Furthermore, the use of wastewater as a culture medium could reduce fresh water requirement by 90% and eliminate the need for nutrients, except phosphate (Yang et al., 2011).

Conventional wastewater treatment plants use activated sludge for organic carbon, N and P removal. Activated sludge treatment is an aerobic process and requires aeration, which is energy demanding and accounts for 45–75% of a wastewater treatment plant's total energy costs. An estimated 1 kWh of electricity is needed for aeration per each 1 kg of BOD (biochemical oxygen demand) removed by activated sludge, resulting in 1 kg of CO₂ emissions (Razzak et al., 2013). Wastewater treatment has been estimated to consume 60 tWh of electricity per annum worldwide (Williams, 2011). Algae on the other hand do not require oxygen and have the potential to reduce the costs of wastewater treatment. A low-cost algal-bacterial wastewater treatment system has been proposed, where oxygen released from algal photosynthesis reduces the need for aeration and in return, algae utilise the CO₂ released by the bacteria thus, reducing the need for CO₂ supply (Munoz & Guieysse 2006; Cabanelas et al., 2013). Additionally, the cultivation of algae does not produce sludge by-products and allows recycling of nutrients, since microphytes incorporate nitrogen into their biomass instead of removing it in gaseous form like nitrifying/ denitrifying bacteria (Christenson & Sims, 2011; Pittman et al., 2011).

Wastewater is an abundant, nutrient rich resource. It has high concentrations of nitrogen and phosphorus, which are essential for algal growth. High load of these

elements in the discharged wastewater leads to eutrophication and may cause groundwater pollution (Christenson & Sims, 2011; Razzak et al., 2013). Urban and agricultural wastewater can be utilized for algae cultivation. The use of industrial wastewaters may be limited due to low N and P concentration and high concentration of growth-inhibiting organic contaminants and heavy metals (Munoz & Guieysse, 2006; Pittman et al., 2011).

The concentrations of total N and total P may be 10–100 mg L⁻¹ in municipal wastewaters and > 1,000 mg L⁻¹ in agricultural wastewaters (Pittman et al., 2011). Discharge thresholds for total N and P from urban wastewater treatment plants, established by the European Directive 98/15/EC are 10–15 mg L⁻¹ and 1–2 mg L⁻¹, respectively with a minimum removal of 80% of N and 70–80% P (DIRECTIVE, 1998). In the conventional wastewater treatment, the removal of phosphorus is particularly challenging. Species of *Chlorella* and *Scenedesmus* have been shown to effectively remove > 80% of total nitrogen and total phosphorus from secondary treated wastewater, *Chlorella vulgaris* has been shown to reduce total N and P by > 90% and 80% respectively from primary treated sewage water (Pittman et al., 2011).

Although there are sufficient nutrients in wastewater to support high algal productivity, a certain ratio of nitrogen and phosphorus is required. The optimal ratio of these nutrients is N16 : P1 and imbalance may result in growth limitation. Thus, it may be necessary to supplement the wastewater with additional nutrients (Christenson & Sims, 2011; Olguin, 2012). Additional nutrients may be supplied from anaerobic digestion effluents, which have an appropriate N/P ratio and additionally contain carbon in the form of bicarbonate (Olguin, 2012; Zhu, 2013). Nitrogen in the wastewater may be in the form of ammonia, especially in animal wastewater. Although algae can take up ammonia, high concentration of ammonia can significantly inhibit algal growth and therefore, a pre-treatment to reduce ammonia concentration in the wastewater may be required (Pittman et al., 2011; Ji et al., 2013).

Production methods and biomass productivity

Algae are most commonly cultured either in open pond systems or closed photobioreactors. The typical aerobic ponds for wastewater treatment are large and shallow without internal mixing, and are not optimized for algal growth (Munoz and Guieysse, 2006). A preferable alternative for nutrient circulation enhancement and increase of algal biomass productivity are high rate algal ponds (HRAP) or raceway ponds (Christenson and Sims, 2011). HRAPs are shallow, 10–30 cm deep ponds with a paddle wheel mixing system. HRAPs can treat up to 35 g BOD (biochemical oxygen demand) m⁻² d⁻¹ and have a typical biomass productivity of 10–20 g m⁻² day⁻¹ (Munoz & Guieysse, 2006). Although high rate algal ponds are relatively inexpensive to build and operate, they require a large surface area and are prone to contamination by other protozoa, fungi and bacteria. High evaporation rate, ineffective use of CO₂ and poor mixing are other disadvantages for algal cultivation (Christenson & Sims, 2011).

Closed photobioreactors provide better control over temperature and evaporation, reduce the risk of culture contamination, and provide better mixing and utilization of CO₂. Consequently, the biomass productivity is higher compared to open ponds and range from 20 to 40 g m⁻² day⁻¹ (Christenson & Sims, 2011). There are several closed photobioreactor designs (Borowitzka 1999; Molina et al., 2001; Molina Grima et al., 1999; Ugwu et al., 2008), but the easiest ones to scale up are tubular bioreactors, which

have a large surface area per unit of occupied land and therefore exhibit higher efficiency of light utilization compared to other bioreactor designs (Munoz & Guieysse, 2006).

Other promising approaches for algal cultivation are immobilized cultures such as matrix-immobilized cultures and algal biofilms. Although matrix-immobilized cultures show increased lipid content and efficient nutrient removal from wastewater, the high cost of immobilization matrixes makes this approach less suitable for large scale wastewater treatment and biofuel production compared to biofilm systems (Christenson & Sims, 2011; Munoz & Guieysse, 2006). Algal productivity in a biofilm may be greater compared to suspended algae and because of simpler harvesting and dewatering operations compared to suspended algal cultures, using algal biofilms can reduce the costs of downstream processing (Christenson & Sims, 2011; Christenson & Sims, 2012). Depending on the design, algal species, water source and attachment materials used, biomass productivities between $\sim 3.5 \text{ g m}^{-2} \text{ day}^{-1}$ and $\sim 75 \text{ g m}^{-2} \text{ day}^{-1}$ have been reported (Gross et al., 2013). Rotating Algal Biofilm Reactors (RABR) have also been shown to sequester more total phosphorus compared to suspended reactors. RABRs can be coupled with raceway ponds for effective wastewater treatment and biomass growth. The lipid productivity and the potential fatty acid methyl ester yield in algal biofilms have been reported to be comparable with lipid productivity in suspended algae. Optimization of biofilm reactors may improve lipid productivity of such systems (Christenson & Sims, 2012).

Algal biomass productivity in wastewater depends on various factors, including the type of bioreactor used, wastewater composition and nutrient availability, illumination, pH, carbon dioxide supply etc. It has been estimated that 1,443 m³ of wastewater can be treated to produce 1 ton of biomass (Feng et al., 2011a). Although the mean biomass productivity may be significant (up to 13 g dry weight m⁻² d⁻¹) for algae grown in wastewater with high nitrogen concentration, lipid productivity may remain relatively low (< 11%) (Dalrymple et al., 2013). To induce and increase lipid production, a two phase approach has been used, consisting of a growth phase with high N concentration and a starvation phase with low nutrients concentration (Prathima Devi et al., 2012). The nitrogen deprivation enhances lipid productivity and leads toward unsaturation. Increasing lipid production via two phase approach can be applied for up-scale wastewater treatment in a cost-effective way (Prathima Devi et al., 2012).

CO₂ supplementation and flue gas as a carbon source

To enhance algal productivity, CO₂ should be supplied to the reactors. It has been estimated that at a photosynthetic efficiency of 9% microalgae could produce up to 280 tons of dry biomass ha⁻¹ year⁻¹ while consuming approximately 513 tons of CO₂ (Sydney et al., 2010). Inorganic carbon in the water can exist in the form of CO₂, HCO₃⁻, CO₃²⁻ and H₂CO₃. In mediums with a common pH for algal growth (6.4–10.3), the dominant carbonate species is bicarbonate (HCO₃⁻) (Van Den Hende et al., 2012, Zhao & Su, 2014). Algae can utilize CO₂ and HCO₃⁻, which is converted into CO₂ before it can be used by Rubisco (ribulose 1.5-bisphosphate carboxylase/oxygenase) enzyme for organic compounds assimilation (Van Den Hende et al., 2012).

Flue gas from power plants and other industries, which contains up to 20% (v/v) CO₂, has received a lot of attention as a potential source of concentrated carbon dioxide. Although some algal species can tolerate high concentrations of CO₂, flue gas

also contains supplementary gases such as SO_x , NO_x and other compounds, which can strongly inhibit algal growth (Sudhakar et al., 2011; Ono & Cuello 2003).

The inhibiting effects of SO_2 on algal growth are mainly attributed to the reduction in pH of the growth medium (Zhao & Su, 2014). SO_2 has a high solubility in water and forms H_2SO_3 , which can be further oxidized to H_2SO_4 . SO_3 , also present in flue gas (2–4%), similarly forms H_2SO_4 (Van Den Hende et al., 2012). Moreover, the reaction of NO_2 with SO_2 forms SO_3 , which may further reduce the pH of the medium (Van Den Hende et al., 2012). Certain levels of dissolved sulphur dioxide in the form of SO_4^{2-} and HSO_4^- have been shown to have a direct toxic effect on the algal cells (Ronda et al., 2014), although the toxicity levels of sulphur are species specific (Van Den Hende et al., 2012).

The presence of NO_x (90–95% NO and 5–10% NO_2) is associated with changes in algal cell physiology (Zhao & Su, 2014). Low concentrations of dissolved nitric oxide form primarily nitrite (NO_2^-) in water (Ignarro et al., 1993), which can be further oxidized to NO_3^- . Both forms can be absorbed and utilised by the cells as a source of nitrogen nutrition (Chiu et al., 2011). However, concentrations higher than 300 ppm have been shown to cause a decrease in microalgal growth (Zhao & Su, 2014). Additionally, NO species (NO , NO_2 and very small amounts of N_2O_2 , N_2O_3 , N_2O_4 , N_2O_5 present in flue gas) form nitrous acid (HNO_2) and nitric acid (HNO_3) in water (Van Den Hende et al., 2012). However, NO has a low solubility in water and therefore the pH effects are lesser compared to SO_2 .

Contrarily, it has been reported that changes in medium pH and flue gas composition do not appear to affect the photochemical yield of microalgal cultures, and different microalgal strains exhibit a substantial ability to withstand a wide range of pH and flue gas composition (Olaizola, 2003). The toxic effects of NO_x and SO_x on algae can be overcome by using cultures with higher initial cell densities, adjusting the pH of the culture medium, or by using mutant or acidophilic algal strains (Chiu et al., 2011, Van Den Hende et al., 2012) or SO_x and NO_x tolerant species isolated from a close proximity of power plants (Randmann et al., 2011). Nevertheless, the use of flue gas as a carbon source for algal mass cultivation is likely to remain limited because of the inhibiting effects of SO_x and NO_x and additional inhibiting compounds found in flue gas such as particulate matter, halogen acids and heavy metals (Van Den Hende et al., 2012). Furthermore, the flue gas needs to be transported from the power plant to the location of algal cultivation, which would account for additional costs and indirect fossil fuel consumption (Christenson and Sims, 2011).

A more convenient source of carbon dioxide is from bioethanol fermentation and anaerobic digestion of lipid extracted algal waste biomass (Harun et al., 2009; Zhu, 2013). Recycling carbon dioxide from these sources has the potential to make algal biofuels carbon neutral.

Harvesting

Due to the small size of the algal cells and the relatively dilute solutions, large water volumes need to be processed to harvest the biomass. In addition to processing large flows, harvesting has to enable recycling of the separated water and needs to be time and cost effective (Jonker & Faaij, 2013). With currently used technologies harvesting and dewatering are energy consuming and have been estimated to contribute 20–30% of the total cost of microalgal biomass (Hanotu et al., 2012; Razzak et al., 2013). In this section, potentially energy and cost effective harvesting techniques are discussed.

The most common harvesting method used is centrifugation. Although centrifugation is a relatively easy method with high harvesting efficiency, it is estimated to consume from 3.3 to 4.5 MJ m⁻³ of electricity and it is therefore cost-prohibitive for large scale algae harvesting (Jonker & Faaij, 2013). For suspended algal cells, tangential flow filtration has been used. However, on a large scale this method is not economically suitable because of high costs of membrane replacement and high energy requirements (Munoz & Guieysse, 2006; Christenson & Sims, 2011).

Different flotation methods have been successfully used in wastewater treatment. Flotation employs microbubbles that attach to hydrophobic particles, lifting them up to the surface where they can be collected (Hanotu et al., 2012). Flotation methods differ in the method of producing bubbles and the size of the produced bubbles. Generally, the efficiency and harvesting rate increase with decreasing bubble size. Smaller bubbles have higher surface to volume ratio and therefore a slower rising velocity, which enables better contact with the flocules (Hanotu et al., 2012).

In dissolved air flotation (DAF), water is saturated with air at a high pressure and bubbles ranging from 30 to 100 µm are formed upon realising the pressure. DAF is one of the most widely used flotation method in industrial effluent treatment (Rubio et al., 2002). This method also has a high yield of algae recovery, but is unfortunately energy intensive due to the high pressure required for air dissolution (Hanotu et al., 2012).

A less energy consuming method is the dispersed or induced air flotation, where a continuous air stream is forced through a porous material generating bubbles (Hanotu et al., 2012). Usually a high-speed mechanical agitator is used, creating bubbles 700–1,500 µm in diameter (Rubio et al., 2002). Size of the bubbles is relatively large, thus making the harvesting less effective (Hanotu et al., 2012).

Promising method for large scale algae harvesting is microflotation. Fluidic oscillator is used to produce bubbles roughly 10 times smaller and consuming 2–3 times less energy compared to DAF. Minute size of the bubbles enables effective harvesting (Hanotu et al., 2012). Unfortunately, there has been very little research regarding this method.

As individual algal cells are very small (5–50 µm), a pre-concentration step is needed prior to flotation to aggregate the small algal cells into larger flocules. Microalgal cells are negatively charged which prevents self-aggregation (Vandamme et al., 2012). In order to neutralize the cells, chemical coagulants or flocculants, typically electrolytes and synthetic polymers are used (Christenson and Sims, 2011; Hanotu et al., 2012). The most commonly used inorganic flocculants for charge neutralization are metal salts such as aluminium sulphate (Al₂(SO₄)₃) and ferric chloride (FeCl₃), which have a harvesting efficiency of > 90% (Vandamme et al., 2013). However, high doses of metal salts (120–1,000 mg L⁻¹) are required for

effective flocculation (Granados et al., 2012). Moreover, because the added flocculating chemicals remain in the harvested biomass, the use of the biomass as animal feed may be prohibited (Vandamme et al., 2013). A high concentration of metals in the biomass may also inhibit the activity of methanogenic and acetogenic bacteria in the downstream processing of the biomass (Christenson & Sims, 2011).

Alternatively natural polymers such as chitosan and cationic starches may be used as to avoid secondary pollution (Christenson & Sims, 2011; Razzak et al., 2013). Chitosan is a polymeric polyelectrolyte derived from the chitin of shellfish, which though effective, is expensive and works best at low pH values, while algal cultures require relatively high pH in the growth medium (Schlesinger et al., 2012; Vandamme et al., 2013). Positively charged cationic starches work over broader pH range and are relatively cheap (Vandamme et al., 2013). Unfortunately, cationic starches are generally less effective compared to metal salts (Granados et al., 2012). On the other hand cationic starches are not toxic and are biodegradable, which is advantageous if algal biomass production is coupled with wastewater treatment or is used as an animal feed supplement. Starches remaining in the biomass after harvesting may be hydrolysed to sugars and fermented after the lipid extraction process (Gerde et al., 2013).

In addition to natural flocculants a process referred to as autoflocculation may be used as a low cost alternative. However, the term autoflocculation is misleading, because it is a kind of chemical flocculation, which occurs in the presence of calcium or magnesium at high pH values (Vandamme et al., 2012; Wu et al., 2012). Out of the two, magnesium has been found to be more effective in flocculating freshwater algae with removal efficiency between 90 and 100% at pH 10.5–12. For a relatively safe and cost-effective increase of pH, calcium hydroxide, slaked lime and dolomite can be used (Schlesinger et al., 2012). The raise in pH also effectively kills pathogens and sterilises the biomass, which is a beneficial aspect in wastewater treatment (Vandamme et al., 2012).

Besides chemical flocculation, several physical methods have also been proposed such as electrolytic or electrocoagulation flocculation, ultrasound-aided flocculation and magnetic separation. The latter is a promising harvesting method with a cost lowering potential, which uses magnetite (Fe_3O_4) nanoparticles to flocculate the algal cells (Xu et al., 2011; Cerff et al., 2012). The method does not employ chemical flocculants, is rapid and relatively simple in operation and can be applied on a large scale. In addition the magnetite nanoparticles are reusable. Recovery efficiency of 95–98% was achieved depending on pH, nanoparticle dose, algal species (Xu et al., 2011) and growth medium composition. For example the presence of di- and trivalent ions such as Ca^{2+} , PO_4^{3-} and Mg^{2+} in the growth medium significantly enhances flocculation and the harvesting efficiency (Cerff et al., 2012). Although magnetic separation has been used in wastewater and water treatment systems (Zaidi et al., 2013), its application in microalgae harvesting has been limited. Further research needs to be done on the nanoparticle removal.

Extracting high value products

Microalgae produce high value products such as omega 3 fatty acids (Ω -3), including DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid), carotenoids (e.g. astaxanthin, lutein), α -tocopherol (vitamin E), and omega 6 polyunsaturated fatty acids (Ω -6) (arachidonic and γ linoleic acid) as part of their metabolism (Koberg et al., 2011). These products have use as nutraceuticals and food supplements. Algae such as *Chlorella* sp and *Scenedesmus* sp are rich in Ω -3 and Ω -6 fatty acids which can be extracted prior to lipid extraction for biodiesel production. The lipid composition of *Chlorella* sp and *Scenedesmus obliquus* are provided in table 1 (Makarevičienė et al., 2011).

Table 1. Lipid composition of *Chlorella* sp, *Scenedesmus obliquus* and rapeseed Makarevičienė et al., 2011)

| Fatty acids | Lipid composition (%) | | |
|----------------------|-----------------------|-----------------------------|----------|
| | <i>Chlorella</i> sp. | <i>Scenedesmus obliquus</i> | Rapeseed |
| Saturated | 48.9 | 51.9 | 5.4 |
| Monounsaturated | 20.9 | 17.5 | 58.3 |
| Polyunsaturated | 23.7 | 27.4 | 36.3 |
| <i>Trans</i> isomers | 4.9 | 2.1 | |
| Omega-3 | 5.0 | 17.7 | |
| Omega-6 | 12.5 | 9.3 | |
| Linolenic | 2.3 | 10.6 | 5.0–13.0 |

According to the requirements of the Estonian Standard EVS- EN 14214, to meet the standard requirements for oxidation stability and iodine value, the contents of linolenic acid methyl ester in biodiesel fuel should not exceed 12 m/m %, polysaturated methyl ester content and polyunsaturated (4 double bonds) methyl ester content must meet the limit of 1 m/m % (EVS-EN 2012). Long-chain fatty acids with double bonds form resins during thermo-oxidation process and therefore cannot be used in motor fuels. These fatty acids are typically saponified during the lipid conversion process and are disposed of as waste. However, the long-chain fatty acids with double bonds (including Ω -fatty acids) can be extracted prior to the production of biodiesel and sold to pharmaceutical companies for further processing. Profits from the realisation of Ω -fatty acids may have a positive effect on the overall economics of algal biodiesel production. Although the chemical company BASF has reported using headspace gas chromatography for extracting Ω -fatty acids from algal cells (BASF, 2013), there is scarce research regarding extracting valuable products from microalgae. Further research is needed to estimate the benefits and potential profits of extraction and vending of high value products from microalgae.

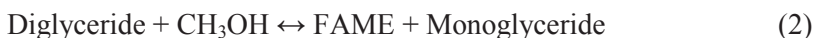
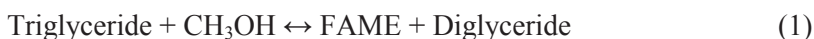
Lipid extraction and conversion to biodiesel

Lipid extraction is an important step in biodiesel production and may amount up to 50% of the production cost (Goettel et al., 2013). Most lipid extraction methods require dewatering and drying of the biomass, which is energy intensive (Schlesinger et al., 2012). Thermal dewatering requires an estimated 3,556 kJ kg⁻¹ and therefore, improvements are needed to reduce this energy use (Sander and Murthy, 2010). Common extraction methods include mechanical pretreatment step to disrupt the algal

cells and chemical extraction using solvents. Pretreatment enhances lipid extraction and helps to reduce the amount of solvent needed. Different mechanical methods for lipid extraction have been investigated, including bead-beating, autoclaving, osmotic shock, sonification, microwaving, supercritical fluid extraction (Lee et al., 2010; Bahadar & Bilal Khan, 2013). Many of the methods above require significant biomass processing such as freezing, are time consuming and difficult to scale-up due to equipment limitations (Lohman et al., 2013). Among these, microwave radiation has been found to be more effective than others in disrupting the cells walls, resulting in higher extracted lipid yields and it can be easily scaled-up (Lee et al., 2010). A promising low-cost method for cell disruption is the application of pulse electric field, which causes cell membrane permeability. This is a low cost method that may be applied on a large scale. Additionally, it does not employ chemicals, which could contaminate or degrade the target products, and does not require drying of the biomass. Although this method is an effective pretreatment method for solvent-extraction, it does not cause the release of lipids from the cells. However, it fosters the release of other intracellular valuables and can be used for a selective extraction of pigments and enzymes, which can be collected prior to solvent extraction of the lipids (Goettel et al., 2013).

Lipids are commonly extracted using different ratios of organic solvents like ethanol, methanol, hexane, chloroform, and methylene chloride. The highest yield of lipids has been obtained using a mixture of chloroform and methanol (2:1 v/v) (D'Oca et al., 2011).

The extraction step is followed by the conversion of the extracted lipids into biodiesel. Biodiesel is a mix of fatty acid methyl esters (FAME), which are mainly produced from triglycerides (Bahadar & Bilal Khan, 2013). Transesterification is a three-step process, where triglycerides react with methanol in the presence of a catalyst to produce FAME and glycerol as final products (1–3) (Vlysidis et al., 2011):



Methanol is used in surplus to shift the reaction toward the products and is recovered and recycled (Vlysidis et al., 2011). However, the high costs of extracting lipids, drying the biomass and using organic solvents make this method disadvantageous. In addition, only triglycerides can be used in the transesterification process (Takisawa et al., 2013).

Direct transesterification on the other hand, can convert phospholipids into FAME in addition to triglycerides (Takisawa et al., 2013). Supercritical methanol and ethanol have been successfully used for direct transesterification. Under supercritical conditions methanol can solvate non-polar glycerides to yield FAME and diglycerides, which are further converted into methyl ester and glycerol. This approach has a relatively short reaction time, allows the use of wet biomass, and yields maximum conversion of triglycerides into FAME (Patil et al., 2011). Microwave-mediated supercritical ethanol conditions have been shown to yield a significant amount of

biodiesel, while consuming less energy compared to conventional heating and reducing the reaction time. If converted into a continuous flow model, this method could be used for large scale biodiesel production (Patil et al., 2013). However, these methods have received a lot of critique due to energy requirements for reaching the supercritical conditions. Nevertheless, since wet biomass is used, the energy requirements may not exceed or be less than in the traditional extraction followed by transesterification method. The method can be improved by adding a co-solvent or co-catalyst, such as SrO (Koberg et al., 2011). Furthermore, utilising a power co-generation unit, direct transesterification under supercritical alcohol conditions has great potential in industrial biodiesel production (Patil et al., 2011; Patil et al., 2013).

Direct transesterification can also be achieved using acid catalysts. However, such reactions are inhibited by water. In order to use wet algal biomass, a hydrolysis using acid and base followed by direct esterification have been investigated (Takisawa et al., 2013). It has been shown that esterification of free fatty acids result in a higher FAME yield than with transesterification of triglycerides and the methylation reactions are not inhibited by water. This method has the potential to lower the cost associated with biomass drying. However, further research is necessary for it to be used on an industrial scale.

There have not been any comparative studies on the two-step (extraction followed by transesterification) and single-step (direct transesterification) biodiesel production. Since direct transesterification allows the use of wet biomass omitting biomass drying and can utilise phospholipids and free fatty acids, it may have more potential in biodiesel production. However, further research is necessary to find a suitable method which would be cost-effective and applicable on a large scale. Lipid extraction and conversion to biodiesel remains a bottleneck in algal biodiesel production.

Utilising the waste – glycerol and lipid extracted waste biomass

Glycerol

It has been proposed that in order to increase the economic sustainability of biodiesel production the by-products should be valorised. Glycerol is a common by-product of biodiesel transesterification and can amount up to 10% w/w of the biodiesel produced (Vlysidis et al., 2011). In 2010 the worldwide production of glycerol was of about 1.8 billion litres with a commercial demand of only 0.8 million per year (Cabanelas et al., 2013). Therefore most of the glycerol is discarded as waste. However, there are several ways to utilise glycerol.

Glycerol has been used for heterotrophic and mixotrophic cultivation of algae as a carbon source (Johnson & Wen, 2009; Cabanelas et al., 2013). Heterotrophic cultivation has several advantages over autotrophic cultivation, namely minimal requirements for light, a good control of the cultivation process, higher growth and better biomass harvesting (Prathima Devi et al., 2012). Adding glycerol to the culture medium has been shown to increase the saturation of fatty acids in algae, which has positive effects on the cetane number, iodine value and oxidation stability (Cabanelas et al., 2013).

Novel bio-routes to produce succinic acid for specialty chemicals and ethanol production using glycerol as a key ingredient have been explored (Vlysidis et al., 2011). Moreover, glycerol can be used for hydrogen and electricity production through microbial bioconversion (Selemba et al., 2009; Feng et al., 2011; Nimje et al., 2011;

Sarma et al., 2012). Several value-added products such as fuel bio-additives and additives for concrete can be derived from glycerol (Pagliaro et al., 2009). Additionally, glycerol can be used for co-digestion of algal waste biomass in anaerobic digestion (Ehimen et al., 2011).

Fermentation of lipid extracted waste biomass

Algal biomass residuals after biodiesel refinery may account up to 65% of the whole biomass (Zhu, 2013). The lipid extracted biomass contains carbohydrates and proteins, which can be converted to bioethanol and biogas. Combining production of ethanol and methane from waste biomass with biodiesel production can improve the sustainability and lower the cost of algal biodiesel (Zhu, 2013).

Theoretically it is possible to obtain up to 0.51 kg ethanol from 1 kg of glucose (Harun et al., 2010). Based on experimental data, it has been estimated that 35 L ton⁻¹ y⁻¹ and 37.5 L ton⁻¹ y⁻¹ of bioethanol can be obtained from the carbohydrates from *Chlorella* sp. and *Botryococcus braunii*, respectively (Cabanelas et al., 2013).

Both starches and cellulose from algal cell walls can be utilised for ethanol production (Zhu, 2013). In order to extract the starches, the algal biomass has to be pretreated either mechanically or enzymatically to disrupt the cell walls (John et al., 2011). The pretreatment contributes significantly to the cost of producing bioethanol (Harun & Danquah, 2011b). However, during lipid extraction, the algal cells are typically pretreated to disrupt the cell walls and aid the release of lipids. Therefore, little or no pretreatment will be required to extract the starch from the algal biomass. Indeed, it has been shown that lipid-extracted algae yielded 60% more ethanol compared to dried and intact algae, due to polysaccharides and carbohydrates released resulting from the cell disruption during the lipid extraction (Harun et al., 2009).

Prior to fermentation, the starch and the carbohydrates from the algal cell walls including cellulose, galactose, arabinose, and xylose have to be converted into simple sugars (Harun et al., 2009). This process is called saccharification and is typically achieved with hydrolysis using acid (H₂SO₄), base (NaOH) or special enzymes (amylases, glucoamylases) (Harun et al., 2011; Chen et al., 2013). Unlike the plant cell walls, algal cells lack lignin and are mainly composed of cellulose, which can be converted into glucose monomers with cellulase enzymes (Harun & Danquah, 2011a; Chen et al., 2013). Following saccharification the sugars from starch and hydrolysed cellulose are fermented into ethanol using suitable yeast strains such as *Saccharomyces cerevisiae*, *S. uvarum*, *S. bayanus* etc (Harun et al., 2009; John et al., 2011).

In order to achieve maximum ethanol yield, the microalgae biomass concentration needs to be optimized. Higher biomass concentration and thus, higher carbohydrate concentration may result in release of toxic chemicals and ethanol at levels, which can inhibit the yeast cells (Harun & Danquah, 2011b). Also the appropriate temperature and pH must be maintained depending on the yeast strain used (Harun et al., 2009).

Production of bioethanol from lipid extracted biomass is an unexplored area and to date there are practically no economical assessments of this process. However, energy recovery from waste biomass is an important step in making the biodiesel production economically feasible.

Anaerobic digestion for methane and electricity production

The waste biomass from ethanol production may be further used as a feedstock for anaerobic digestion for biogas production and nutrient remineralisation (Sialve et al., 2009; Zhu, 2013). It has been estimated that 9,360 MJ metric⁻¹ ton can be recovered as methane from lipid extracted algae (Ehimen et al., 2011). However, there is scarce research on biogas production from lipid extracted and fermented algal biomass. Nevertheless, the expected value of methane recovery will be lower if residues from ethanol production are used.

The biogas yield and its methane content depends on the algal species, biomass composition, and specific conditions of the anaerobic digestion such as temperature, pH and hydraulic retention time (Passos et al., 2013). Different algal species have differences in the cell wall composition, which makes some species more readily digestible than others. Furthermore, some algae may produce extracellular compounds such as bacteriostatic (bacteria inhibiting) and bactericidal (bacteria killing) compounds, which can hinder metabolic activity of the methanogenic bacteria (Mussgnug et al., 2010). Typically, the unprocessed algal biomass requires pretreatment to make the cells more accessible to the fermentative bacteria and enhance methane production. Pretreatment methods such as microwave heating (Passos et al., 2013), thermal (Marsolek et al., 2014) and pressure-thermal pretreatment (Mendez et al., 2014), and ultrasonic disintegration (Park et al., 2013) have been suggested. Effective pretreatment would reduce the hydraulic retention time and thus, the energy consumption by the anaerobic digestion tank reactor. However, as with bioethanol production, the pretreatment required is minimal if processed or lipid extracted biomass is used.

After biodiesel and bioethanol production, the residual biomass is typically poor in carbohydrates and lipids, and has relatively high protein content (Zhu, 2013). The low carbon/nitrogen ratio may result in the production of ammonia, which upon accumulation may lead to the inhibition of the bacterial flora (Prajapati et al., 2014). To lessen the release of ammonia, co-digestion with carbon rich waste, such as shredded paper waste or glycerol can be used (Ehimen et al., 2011; Prajapati et al., 2014). Moreover the addition of glycerol, a common biodiesel production by-product, has been shown to increase CH₄ production yields by 5–8% (Ehimen et al., 2011). The release of NH₄⁺ can also be reduced by decreasing the fraction of unionized NH₃. This can be achieved by increasing the pH using high concentrations of Na⁺, Ca²⁺ and Mg²⁺ (Sialve et al., 2009).

In addition to methane, another major component of biogas is carbon dioxide (Mussgnug et al., 2010). Carbon dioxide separated from the biogas can be recycled for algal growth.

The final waste of the anaerobic digestion contains nutrients such as phosphorus and nitrogen and can be used as a fertilizer.

OUTLOOK AND POSSIBILITIES

Choosing and optimizing suitable methods and technologies for algal cultivation and processing are of great importance to offset the costs associated with microalgal biodiesel. Ways for utilising algae are summarized in Fig. 1.

Urban and animal waste waters can be used as growth medium to reduce the cost of algae mass cultivation with the benefit of treating the wastewater. Harvesting of algae must be effective and cost-efficient. In Fig. 1, flocculation coupled with flotation has been proposed as the harvesting method, however, as the technologies develop, there may be other suitable harvesting methods.

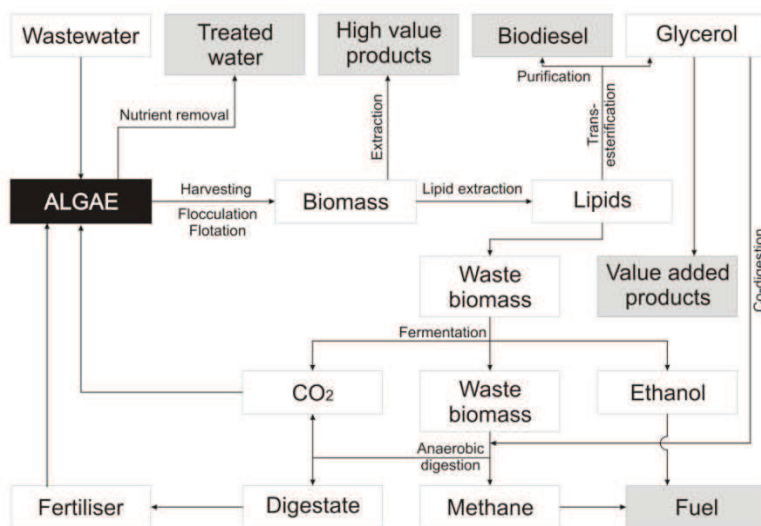


Figure 1. Diagram of hypothetical utilisation of algae and waste products.

Long-chain fatty acids including Ω -fatty acids, which are not suitable for use as fuel and are otherwise saponified in the biodiesel conversion reactions, can be extracted prior to lipid conversion and sold to pharmaceutical companies for further purification and processing. Profits from selling Ω -fatty acids and other high value products are a promising way to help balance the cost of algal biofuel. The waste biomass and by-products can be valorised and recycled. Glycerol, the main by-product of biodiesel production can be utilised in various ways, including for the production of energy and value added products. Utilising glycerol may increase the economic sustainability of biodiesel production (Vlysidis et al., 2011). The lipid extracted biomass can be further processed to obtain bioethanol and biomethane. The energy recovered from ethanol and methane production could potentially power the microalgal cultivation and processing, while the waste CO_2 could be re-circulated for algal cultivation (Zhu, 2013). The final waste from the anaerobic digestion can be used as a fertilizer for algal growth medium supplementation or in agriculture. This may also help to offset the total costs of the whole production chain. It has also been proposed that alternative sources of electricity such as solar and wind energy could be used *in situ* to reduce the consumption of fossil fuel-derived electricity (Collet et al., 2011).

Moreover, the algal cultivation and processing units should be located in close proximity to reduce the costs of transportation.

CONCLUSIONS

Although the production of microalgae derived biodiesel has received a lot of criticism because of its high cost, there are possibilities to offset its high price. Using wastewater as a growth medium, utilising high value products such as Ω -fatty acids and recycling the waste products may help to reduce the production cost of algal biodiesel. Further research, life cycle analysis and economical assessments are required to make algal biodiesel a sustainable fuel.

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REFERENCES

- Bahadar, A. & Bilal Khan, M. 2013. Progress in energy from microalgae. A review. *Renewable and Sustainable Energy Reviews* **27**, 128–148.
- BASF. 2013. Highly concentrated fatty acids for the pharmaceutical, nutraceutical and food sectors. In: Company, B.T.C. (ed) http://www.pharmaceutical-ingredients.com/Documents/ENP/Brochure/EN/03_120802e_Brochure%20Omega-3%20Fatty%20Acids.pdf.
- Borowitzka, M.A. 1999. Commercial production of microalgae: ponds, tanks, tubes and fermenters. *Journal of Biotechnology* **70**, 313–321.
- Cabanelas, T.D., Arbib, Z., Chinalia, F.A., Souza, C.O., Perales, J.A., Almeida, P.F., Druzian, J.I. & Nascimento, I.A. 2013. From waste to energy: Microalgae production in wastewater and glycerol. *Applied Energy* **109**, 283–290.
- Cerff, M., Morweiser, M., Dillschneider, R., Michel, A., Menzel, K. & Posten, C. 2012. Harvesting fresh water and marine algae by magnetic separation: screening of separation parameters and high gradient magnetic filtration. *Bioresource technology* **118**, 289–295.
- Chen, C.-Y., Zhao, X.-Q., Yen, H.-W., Ho, S.-H., Cheng, C.-L., Lee, D.-J., Bai, F.-W. & Chang, J.-S. 2013. Microalgae-based carbohydrates for biofuel production. *Biochemical Engineering Journal* **78**, 1–10.
- Chiu, S.-Y., Kao, C.-Y., Huang, T.-T., Lin, C.-J., Ong, S.-C., Chen, C.-D., Chang, J.-S., Lin, C.-S. 2011. Microalgal biomass production and on-site bioremediation of carbon dioxide, nitrogen oxide and sulfur dioxide from flue gas using *Chlorella* sp. Cultures. *Bioresource Technology* **102**, 9135–9142.
- Christenson, L. & Sims, R. 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology advances* **29**(6), 686–702.
- Christenson, L. B. & R. C. Sims. 2012. Rotating algal biofilm reactor and spool harvester for wastewater treatment with biofuels by-products. *Biotechnol. Bioeng.* **109**(7), 1674–1684.
- Collet, P., Helias, A., Lardon, L., Ras, M., Goy, R.A. & Steyer, J.P. 2011. Life-cycle assessment of microalgae culture coupled to biogas production. *Bioresource technology* **102**(1), 207–214.
- D'Oca, M.G.M., Viêgas, C.V., Lemôes, J.S., Miyasaki, E.K., Morón-Villarreyes, J.A., Primel, E.G. & Abreu, P.C. 2011. Production of FAMES from several microalgal lipidic

- extracts and direct transesterification of the *Chlorella pyrenoidosa*. *Biomass and Bioenergy* **35**(4), 1533–1538.
- Dalrymple, O.K., Halfhide, T., Udom, I., Gilles, B., Wolan, J., Zhang, Q. & Ergas, S. 2013. Wastewater use in algae production for generation of renewable resources: a review and preliminary results. *Aquatic Biosystems* **9**(2), 1–11.
- DIRECTIVE. 1998. COMMISSION DIRECTIVE 98/15/EC. In: OJ (ed) L 67.
- Ehimen, E.A., Sun, Z.F., Carrington, C.G., Birch, E.J. & Eaton-Rye, J.J. 2011. Anaerobic digestion of microalgae residues resulting from the biodiesel production process. *Applied Energy* **88**(10), 3454–3463.
- EVS-EN, 2012. EVS-EN 14214: 2014. Liquid petroleum products – Fatty acid methyl esters (FAME) for use in diesel engines and heating applications – Requirements and test methods.
- Feng, Y., Yang, Q., Wang, X., Liu, Y., Lee, H. & Ren, N. 2011. Treatment of biodiesel production wastes with simultaneous electricity generation using a single-chamber microbial fuel cell. *Bioresource technology* **102**(1), 411–415.
- Gerde, J.A. Yao, L., Lio, J., Wen, Z. & Wang, T. 2013. Microalgae flocculation: Impact of flocculant type, algae species and cell concentration. *Algal Research* **3**, 30–35
- Goettel, M., Eing, C., Gusbeth, C., Straessner, R. & Frey, W. 2013. Pulsed electric field assisted extraction of intracellular valuables from microalgae. *Algal Research* **2**(4), 401–408.
- Granados, M.R., Acien, F.G., Gomez, C., Fernandez-Sevilla, J.M. & Molina Grima, E. 2012. Evaluation of flocculants for the recovery of freshwater microalgae. *Bioresource technology* **118**, 102–10.
- Gross, M., Henry, W., Michael, C. & Wen, Z. 2013. Development of a rotating algal biofilm growth system for attached microalgae growth with in situ biomass harvest. *Bioresource technology* **150**, 195–201.
- Hanotu, J., Hemaka Bandulasena, H.C. & Zimmerman, W.B. 2012. Microflotation Performance for Algal Separation. *Biotechnology and Bioengineering* **109**(7), 1663–1673.
- Harun, R. & Danquah, M.K. 2011a. Enzymatic hydrolysis of microalgal biomass for bioethanol production. *Chemical Engineering Journal* **168**(3), 1079–1084.
- Harun, R. & Danquah, M.K. 2011b. Influence of acid pre-treatment on microalgal biomass for bioethanol production. *Process Biochemistry* **46**(1), 304–309.
- Harun, R., Danquah, M.K. & Forde, G.M. 2009. Microalgal biomass as a fermentation feedstock for bioethanol production. *Journal of Chemical Technology & Biotechnology* **85**, 199–203.
- Harun, R., Jason, W.S.Y., Cherrington, T. & Danquah, M.K. 2011. Exploring alkaline pre-treatment of microalgal biomass for bioethanol production. *Applied Energy* **88**(10), 3464–3467.
- Harun, R., Singh, M., Forde, G.M. & Danquah, M.K. 2010. Bioprocess engineering of microalgae to produce a variety of consumer products. *Renewable and Sustainable Energy Reviews* **14**(3), 1037–1047.
- Ignarro, L.J., Fukuto, J.M., Griscavage, J.M., Rogers, N.E., Byrns, R.E. 1993. Oxidation of nitric oxide in aqueous solution to nitrite but not nitrate: Comparison with enzymatically formed nitric oxide from L-arginine. *Pharmacology* **90**, 8103–8107.
- Ji, M.-K., Abou-Shanab, R.A.I., Kim, S.-H., Salama, E.-S., Lee, S.-H., Kabra, A.N., Lee, Y.-S., Hong, S. & Jeon, B.-H. 2013. Cultivation of microalgae species in tertiary municipal wastewater supplemented with CO₂ for nutrient removal and biomass production. *Ecological Engineering* **58**, 142–148.
- John, R.P., Anisha, G.S., Nampoothiri, K.M. & Pandey, A. 2011. Micro and macroalgal biomass: a renewable source for bioethanol. *Bioresource technology* **102**(1), 186–193.

- Johnson, M.B. & Wen, Z. 2009. Production of Biodiesel Fuel from the Microalga *Schizochytrium limacinum* by Direct Transesterification of Algal Biomass. *Energy & Fuels* **23**(10), 5179–5183.
- Jonker, J.G.G. & Faaij, A.P.C. 2013. Techno-economic assessment of micro-algae as feedstock for renewable bio-energy production. *Applied Energy* **102**, 461–475.
- Koberg, M., Cohen, M., Ben-Amotz, A. & Gedanken, A. 2011. Bio-diesel production directly from the microalgae biomass of *Nannochloropsis* by microwave and ultrasound radiation. *Bioresource technology* **102**(5), 4265–4269.
- Lardon, L., Helias, A., Sialve, B., Steyer, J.P. & Bernard, O. 2009. Life-Cycle Assessment of Biodiesel Production from Microalgae. *Environmental Science and Technology* **43**(17), 6475–6481.
- Lee, J.Y., Yoo C., Jun, S.Y., Ahn, C.Y. & Oh, H.M. 2010. Comparison of several methods for effective lipid extraction from microalgae. *Bioresource technology* **101**(Suppl 1), 75–77.
- Lohman, E.J., Gardner, R.D., Halverson, L., Macur, R.E., Peyton, B.M. & Gerlach, R. 2013. An efficient and scalable extraction and quantification method for algal derived biofuel. *Journal of microbiological methods* **94**(3), 235–244.
- Makarevičienė, V., Andrulevičiūtė, V. Skorupskaitė, V. & Kasperovičienė, J. 2011. Cultivation of Microalgae *Chlorella* sp. and *Scenedesmus* sp. as a Potential Biofuel Feedstock. Environmental Research. *Engineering and Management* **3**(57), 21–27.
- Marsolek, M.D., Kendall, E., Thompson P.L. & Shuman, T.R. 2014. Thermal pretreatment of algae for anaerobic digestion. *Bioresource technology* **151**, 373–377.
- Mendez, L., Mahdy, A., Demuez, M., Ballesteros, M. & González-Fernández, C. 2014. Effect of high pressure thermal pretreatment on *Chlorella vulgaris* biomass, Organic matter solubilisation and biochemical methane potential. *Fuel* **117**, 674–679.
- Molina, E., Fernandez, J., Acien, F.G. & Chisti, Y. 2001. Tubular photobioreactor design for algal cultures. *Journal of Biotechnology* **92**, 113–131.
- Molina Grima, E., Acien Fernandez, F.G., Garcia Camacho, F. & Chisti, Y. 1999. Photobioreactors: light regime, mass transfer, and scaleup. *Journal of Biotechnology* **70**, 231–247.
- Munoz, R. & Guieysse, B. 2006. Algal-bacterial processes for the treatment of hazardous contaminants: a review. *Water research* **40**(15), 2799–2815.
- Mussgnug, J.H., Klassen, V., Schluter, A. & Kruse, O. 2010. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. *J Biotechnol.* **150**(1), 51–56.
- Nimje, V.R., Chen, C.Y., Chen, C.C., Chen, H.R., Tseng, M.J., Jean, J.S. & Chang, Y.F. 2011. Glycerol degradation in single-chamber microbial fuel cells. *Bioresource technology* **102**(3), 2629–2634.
- Olaizola, M. 2003. Microalgal removal of CO₂ from flue gases: Changes in medium pH and flue gas composition do not appear to affect the photochemical yield of microalgal cultures. *Biotechnology and Bioprocess Engineering* **8**(6), 360–367.
- Olguin, E.J. 2012. Dual purpose microalgae-bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a biorefinery. *Biotechnology advances* **30**(5), 1031–1046.
- Ono, E. & Cuello, J.L. 2003. Selection of optimal microalgae species for CO₂ sequestration. *Second National Conference on Carbon Sequestration [Online]* **5**.
- Pagliaro, M., Ciriminna, R., Kimura, H., Rossi, M. & Della Pina, C. 2009. Recent advances in the conversion of bioglycerol into value-added products. *European Journal of Lipid Science and Technology* **111**(8), 788–799.
- Park, K.Y., Kweon, J., Chantrasakdakul, P., Lee, K. & Cha, H.Y. 2013. Anaerobic digestion of microalgal biomass with ultrasonic disintegration. *International Biodeterioration & Biodegradation* **85**, 598–602.

- Passos, F., Solé, M., García, J. & Ferrer, I. 2013. Biogas production from microalgae grown in wastewater: Effect of microwave pretreatment. *Applied Energy* **108**, 168–175.
- Patil, P.D., Gude, V.G., Mannarswamy, A., Deng, S., Cooke, P., Munson-McGee, S., Rhodes, I., Lammers, P. & Nirmalakhandan, N. 2011. Optimization of direct conversion of wet algae to biodiesel under supercritical methanol conditions. *Bioresource technology* **102**(1), 118–122.
- Patil, P.D., Reddy, H., Muppaneni, T., Schaub, T., Holguin, F.O., Cooke, P., Lammers, P., Nirmalakhandan, N., Li, Y., Lu, X. & Deng, S. 2013. In situ ethyl ester production from wet algal biomass under microwave-mediated supercritical ethanol conditions. *Bioresource technology* **139**, 308–315.
- Petkov, G., Ivanova A., Iliev, I. & Vaseva, I. 2012. A critical look at the microalgae biodiesel. *European Journal of Lipid Science and Technology* **114**(2), 103–111.
- Pittman, J.K., Dean, A.P. & Osundeko, O. 2011. The potential of sustainable algal biofuel production using wastewater resources. *Bioresource technology* **102**(1), 17–25.
- Prajapati, S.K., Malik, A. & Vijay, V.K. 2014. Comparative evaluation of biomass production and bioenergy generation potential of *Chlorella* spp. through anaerobic digestion. *Applied Energy* **114**, 790–797.
- Prathima Devi, M., Venkata Subhash, G. & Venkata Mohan, S. 2012. Heterotrophic cultivation of mixed microalgae for lipid accumulation and wastewater treatment during sequential growth and starvation phases. Effect of nutrient supplementation. *Renewable Energy* **43**, 276–283.
- Randmann, E.M., Camerini F.V., Santos, T.D., Costa, J.A.V. 2011. Isolation and application of SO_x and NO_x resistant microalgae in biofixation of CO₂ from thermoelectricity plants. *Energy Conversion and Management* **52**, 3132–3136.
- Razzak, S.A., Hossain, M.M., Lucky, R.A., Bassi, A.S. & de Lasa, H. 2013. Integrated CO₂ capture, wastewater treatment and biofuel production by microalgae culturing. A review. *Renewable and Sustainable Energy Reviews* **27**, 622–653.
- Ronda, S.R., Kethineni, C., Parupudi, L.C.P., Thunuguntla, V.B.S.C., Vemula, S., Settaluri, V.S., Allu, P.R., Grande, S.K., Sharma, S., Kandala, C.V. 2014. A growth inhibitory model with SO_x influenced effective growth rate for estimation of algal biomass concentration under flue gas atmosphere. *Bioresource Technology* **152**, 283–291.
- Rubio, J., Souza, M.L. & Smith, R.W. 2002. Overview of flotation as a wastewater treatment technique. *Minerals Engineering* **15**, 139–155.
- Sander, K. & Murthy, G.S. 2010. Life cycle analysis of algae biodiesel. *The International Journal of Life Cycle Assessment* **15**(7), 704–714.
- Sarma, S.J., Brar, S.K., Sydney, E.B., Le Bihan, Y., Buelna, G. & Soccol, C.R. 2012. Microbial hydrogen production by bioconversion of crude glycerol: A review. *International Journal of Hydrogen Energy* **37**(8), 6473–6490.
- Schlesinger, A., Eisenstadt, D., Bar-Gil, A., Carmely, H., Einbinder, S. & Gressel, J. 2012. Inexpensive non-toxic flocculation of microalgae contradicts theories; overcoming a major hurdle to bulk algal production. *Biotechnology advances* **30**(5), 1023–1030.
- Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., Howe, C.J., Lea-Smith, D.J. & Smith, A.G. 2010. Biodiesel from algae: challenges and prospects. *Current opinion in biotechnology* **21**(3), 277–286.
- Selemba, P.A., Perez, J.M., Lloyd, W.A. & Logan, B.E. 2009. High hydrogen production from glycerol or glucose by electrohydrogenesis using microbial electrolysis cells. *International Journal of Hydrogen Energy* **34**(13), 5373–5381.
- Sialve, B., Bernet, N. & Bernard, O. 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnology advances* **27**(4), 409–416.
- Sturm, B.S.M. & Lamer, S.L. 2011. An energy evaluation of coupling nutrient removal from wastewater with algal biomass production. *Applied Energy* **88**(10), 3499–3506.

- Sudhakar, K., Suresh, S. & Premalatha, M. 2011. An overview of CO₂ mitigation using algae cultivation technology. *International Journal of Chemical Research* **3**(3), 110–117.
- Sydney, E.B., Sturm, W., de Carvalho, J.C., Thomaz-Soccol, V., Larroche, C., Pandey, A. & Soccol, C.R. 2010. Potential carbon dioxide fixation by industrially important microalgae. *Bioresource technology* **101**(15), 5892–5896.
- Zaidi, N.S., Sohaili, J., Muda, K. & Sillanpää, M. 2013. Magnetic Field Application and its Potential in Water and Wastewater Treatment Systems. *Separation & Purification Reviews* **43**(3), 206–240.
- Zhao, B., Su, Y. 2014. Process effect of microalgal-carbon dioxide fixation and biomass production: A review. *Renewable and Sustainable Energy Reviews* **31**, 121–132.
- Zhu, L. 2013. The combined production of ethanol and biogas from microalgal residuals to sustain microalgal biodiesel. A theoretical evaluation. *Biofuels, Bioproducts and Biorefining* **8**, 7–15.
- Takisawa, K., Kanemoto, K., Miyazaki, T. & Kitamura, Y. 2013. Hydrolysis for direct esterification of lipids from wet microalgae. *Bioresource technology* **144**, 38–43.
- Tutt, M., Kikas, T. & Olt, J. 2013. Influence of harvesting time on biochemical composition and glucose yield from hemp. *Agronomy Research* **11**(1), 215–220.
- Tutt, M. & Olt, J. 2011. Suitability of various plant species for bioethanol production. *Agronomy Research* **9**(1), 261–267.
- Ugwu, C.U., Aoyagi, H. & Uchiyama, H. 2008. Photobioreactors for mass cultivation of algae. *Bioresource technology* **99**(10), 4021–4028.
- van Beilen, J.B. 2010. Why microalgal biofuels won't save the internal combustion machine. *Biofuels, Bioproducts and Biorefining* **4**(1), 41–52.
- Vandamme, D., Foubert, I., Fraeye, I., Meesschaert, B. & Muylaert, K. 2012. Flocculation of *Chlorella vulgaris* induced by high pH: role of magnesium and calcium and practical implications. *Bioresource technology* **105**, 114–9.
- Vandamme, D., Foubert, I. & Muylaert, K. 2013. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. *Trends in biotechnology* **31**(4), 233–239.
- Van Den Hende, S., Vervaeren, H., Boon, N. 2012. Flue gas compounds and microalgae; (Bio-) chemical interactions leading to biotechnological opportunities. *Biotechnology Advances* **30**, 1405–1424.
- Williams, S.E. 2011. Energy usage comparison between activated sludge treatment and rotating biological contactor treatment of municipal wastewater. Williams & Works, Inc. 1-11 (tegemist ei ole teaduspublikatsiooniga) Available: <http://williams-works.com/articles/RBC%20v%20AS%20energy%20comparison.pdf> (26.03.2014)
- Vlysidis, A., Binns, M., Webb, C. & Theodoropoulos C. 2011. A techno-economic analysis of biodiesel biorefineries. Assessment of integrated designs for the co-production of fuels and chemicals. *Energy* **36**(8), 4671–4683.
- Wu, Z., Zhu, Y., Huang, W., Zhang, C., Li, T., Zhang, Y. & Li, A. 2012. Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. *Bioresource technology* **110**, 496–502.
- Xu, L., Guo, C., Wang, F., Zheng, S. & Liu, C.Z. 2011. A simple and rapid harvesting method for microalgae by in situ magnetic separation. *Bioresource technology* **102**(21), 10047–10051.
- Yang, J., Xu, M., Zhang, X., Hu, Q., Sommerfeld, M. & Chen, Y. 2011. Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. *Bioresource technology* **102**(1), 159–165.