This article was downloaded by: [DUT Library]
On: 31 March 2014, At: 02:06
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Environmental Technology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tent20

Improving the feasibility of producing biofuels from microalgae using wastewater

I. Rawat [a] , V. Bhola [a] , R. Ranjith Kumar [a] & F. Bux [a]

[a] Institute for Water and Wastewater Technology, Durban University of Technology , P.O. Box 1334, Durban , 4000 , South Africa

Published online: 08 Oct 2013.

To cite this article: I. Rawat , V. Bhola , R. Ranjith Kumar & F. Bux (2013) Improving the feasibility of producing biofuels from microalgae using wastewater, Environmental Technology, 34:13-14, 1765-1775, DOI: 10.1080/09593330.2013.826287

To link to this article: http://dx.doi.org/10.1080/09593330.2013.826287

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the “Content”) contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

Environmental Technology, 2013
Vol. 34, Nos. 13–14, 1765–1775, http://dx.doi.org/10.1080/09593330.2013.826287

Improving the feasibility of producing biofuels from microalgae using wastewater

I. Rawat, V. Bhola, R. Ranjith Kumar and F. Bux*

Institute for Water and Wastewater Technology, Durban University of Technology, P.O. Box 1334, Durban 4000, South Africa

(*Received 9 April 2013; accepted 6 July 2013*)

Biofuels have received much attention recently owing to energy consumption and environmental concerns. Despite many of the technologies being technically feasible, the processes are often too costly to be commercially viable. The major stumbling block to full-scale production of algal biofuels is the cost of upstream and downstream processes and environmental impacts such as water footprint and indirect greenhouse gas emissions from chemical nutrient production. The technoeconomics of biofuels production from microalgae is currently unfeasible due to the cost of inputs and productivities achieved. The use of a biorefinery approach sees the production costs reduced greatly due to utilization of waste streams for cultivation and the generation of several potential energy sources and value-added products while offering environmental protection. The use of wastewater as a production media, coupled with CO_2 sequestration from flue gas greatly reduces the microalgal cultivation costs. Conversion of residual biomass and by-products, such as glycerol, for fuel production using an integrated approach potentially holds the key to near future commercial implementation of biofuels production.

Keywords: biofuels; microalgae; wastewater; biorefinery

Introduction

The steady increase in the price of crude oil and growing concerns surrounding the increase in anthropogenic greenhouse gases (GHGs) and climate change have signalled a need to diversify energy production. Biofuels have received much attention as they are renewable and sustainable. Suitable alternatives to transportation fuels from renewable feedstocks in the near future are paramount to mitigating climate change. The production of crop-based biofuels, such as biodiesel, is unlikely to meet the production capacity required for liquid fuels using traditional feedstocks (soybeans, rapeseed/canola, palm, various greases and used cooking oils).[1–3] Research initiatives have established that microalgae have great potential as feedstocks for renewable fuels owing to their faster growth rates and higher CO_2 fixation efficiency when compared with terrestrial plants.[1–5] Commercial biofuels production using microalgal biomass has been hampered by the unfavourable process technoeconomics.[6,7]

In addition to serving as a biofuels feedstock, microalgae offer the potential for wastewater treatment. Discharge of wastewater with excessive amounts of nitrogen (N) and phosphorus (P) due to improper or incomplete treatment leads to eutrophication, and thereby damage to ecosystems.[8] Existing chemical- and physical-based technologies for nutrient removal utilize a considerable amount of energy and chemical additives. The high energy demand and cost associated with treatment of wastewaters remain ongoing challenges for industries and municipalities.[6,7]

Microalgae-based treatment has the added benefits of resource recovery and recycling. Microalgae cultivated in wastewater as a feedstock for biofuels production can be achieved in the near future.[9] Microalgal biomass production coupled with wastewater treatment could prove beneficial for both wastewater treatment as well as biomass production for biofuels. Wastewater usage can offset utilization of unsustainable amounts of freshwater and the costs associated with commercial fertilizers that are ordinarily used for microalgae production, and reduced energy consumption from wastewater treatment can offset microalgae production costs.[6] The availability of land for microalgal biomass production in the vicinity of wastewater treatment plants comes strongly into perspective if wastewater is to be used as a substrate for cultivation. Fortier and Sturm [10] found that it is feasible to commercially produce microalgal biofuels in Kansas USA due to the availability, within 1 mile, of most wastewater treatment plants. They have further suggested that nutrients and not land is the limiting factor to microalgal cultivation. Studies of this nature are required to determine the feasibility in other regions.

The biorefinery concept is an emerging field whereby an integrated approach to the production of multiple fuels/products from a single feedstock or its by-products is undertaken.[11,12] A biorefinery approach is key for an economically competitive process of fuel production. Microalgae biorefineries provide a promising technology that mediates between biodiesel production, economic feasibility (bio-based by-products provide additional

---

*Corresponding author. Email: faizalb@dut.ac.za

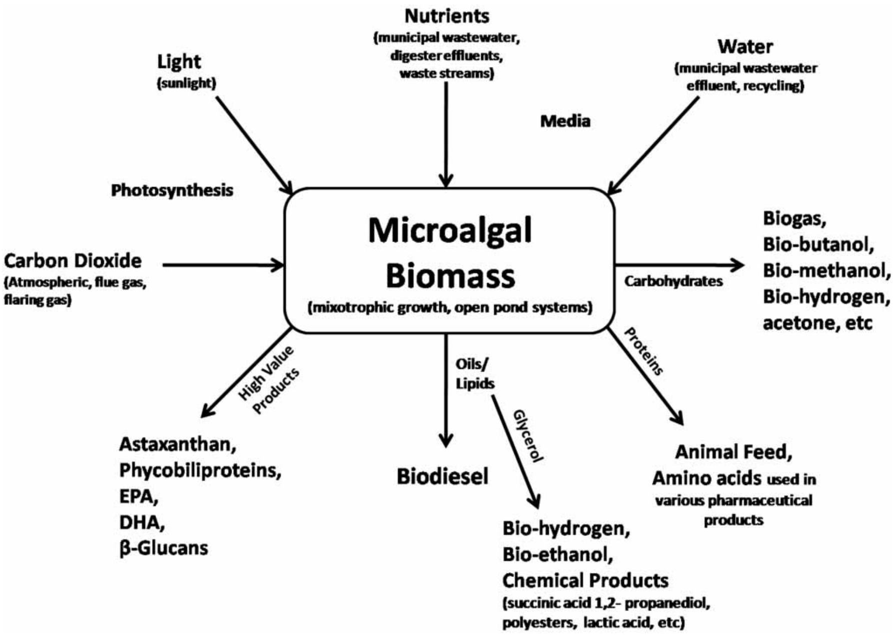

Figure 1. Microalgal biorefinery inputs and potential products.

economy) and positive environmental impacts. A number of studies have been undertaken on economic and technical levels indicating the capacity of feedstock, products and novel technologies to valorize the main biodiesel by-product stream.[7,8,13,14] Microalgae can yield multiple energy sources (Figure 1): value-added resources and other renewable fuels such as biodiesel, biomethane, bioethanol,[4] biohydrogen [15] as well as pigments: chlorophyll,[16] chemicals, fine chemicals,[17] metabolites (protein and carbohydrates), animal feed, organic fertilizers, solvents, pharmaceuticals and more.[18] This review aims to provide avenues via which the overall economic feasibility of the biodiesel production process may be improved by the utilization of wastewater as a substrate, use of appropriate cultivation technologies and the biorefinery approach for the production of valuable co-products.

Microalgal cultivation

Wastewater as a nutrient source

An appropriate growth medium providing essential nutrients in adequate amounts is necessary for the cultivation of microalgae.[19] Microalgal cells sense their environment for suitable nutrients and energy sources, which they store. In doing so, they are able to optimize the efficiency of resource consumption.[20] Algae use organic and inorganic N for the synthesis of amino acids while P, preferentially inorganic, is used for cellular processes related to energy transfer and synthesis of nucleic acid.[21]

Large-scale microalgal cultivation requires large amounts of N and P. Microalgae of typical composition ($C_{1.06}H_{1.81}O_{0.45}N_{0.16}P_{0.01}$) undergoing phototrophic growth require the addition of fertilizer with a N:P ratio of 16:1. This is, however, highly variable depending on algal species and nutrient availability. Ratios can vary from about to 4:1 to almost 40:1.[22] These are generally supplied in the form of chemical or organic fertilizer which is generally supplied in excess.[23] The use of chemical fertilizer is favoured due to its enabling of recycling of water.[23] The sustainability of fertilizer use comes into question due to the high energy consumption and GHG emissions associated with chemical fertilizer production. This accounts for up to 50% of the energy use and GHG emissions of microalgal cultivation when cradle to the grave life cycle analysis is employed.[24] Fossil diesel production uses 2.5 times less energy than current algal biodiesel production.[25] This is due to direct and indirect energy inputs required for the production of fertilizer, ponds, harvesting facilities and transport.[25,26] The cost associated with the supply of chemical media is estimated to be approximately \$3000 ton^{-1} of biomass produced based on the production of 100 ton per annum.[4] The price of mined P is ever increasing due to diminishing supply. At the current rate of agricultural use, the world's mineral P reserves are expected to last only 50–100 years.[27] The production of nitrogen fertilizer is an energy-intensive process utilizing 60 kJ/g N produced. This has an impact on the overall energy balance of the process thereby reducing sustainability.[28] The production of low-value products, such as biofuels, is thus not economically feasible using conventional media.[29] Production of microalgae using freshwater and chemical fertilizer has higher environmental impacts in terms of energy use, GHG emissions and water consumption as compared with biofuels feedstocks such as corn, canola and switchgrass. These environmental impacts are driven by upstream processes. An additional drawback to large-scale microalgal cultivation using chemical fertilizer is the consumption of up to 10 times the N requirement of palm cultivation.[26]

Wastewater is rich in organic and inorganic materials. Treatment is essential before discharge in order to protect receiving waters from eutrophication and accumulation of other nutrients. N and P are the primary constituents that require removal before wastewater can be discharged. Wastewater treatment for N removal is most commonly carried out in the form of biological treatment mainly by bacterial action in order to reduce the organic load to within discharge standards.[8] P is generally mitigated by physicochemical dephosphatization.[21] Both these processes have high-energy consumption and operational costs. Various authors have suggested the utilization of wastewater for nutrient supply.[3,7,8,23,30–33] Recent findings have shown that wastewater may be economically viable and sustainable for the production of microalgal biofuels.[8,33] N and P content promote microalgal growth while simultaneous nutrient removal occurs before discharge.[33] Ammonia N and phosphates in secondary-treated wastewater are generally in the range of 20–40 mg L^{-1} and 10 mg L^{-1}, respectively. These are deemed sufficient to support highly productive growth of most freshwater microalgae.[26] Table 1 shows the typical compositions of wastewater at different strengths. Effluents containing higher nutrient levels than that of secondary-treated effluents, such as centrates, have also been successfully utilized for the cultivation of microalgae.[34]

Coupling wastewater treatment with biofuels production is a very attractive option for energy production, while reducing freshwater and fertilizer demand.[3,28] Martínez et al. [35] showed that wastewater serves as a complete medium from a kinetics standpoint equivalent to chemical media. Cultivation of microalgae using wastewater has the potential to use 90% less freshwater and reduce the N requirement by up to 94%. Wastewater utilization as a replacement for freshwater can totally negate the need for the addition of potassium, magnesium and sulphur.[36] *Botyrococcus braunii* and *Scenedesmus obliquus* have been successfully used to remove N and P with significant removal efficiencies. In some cases, 98% P and 100% ammonia removal was achieved.[7]

Wastewater treatment with the production of microalgae for biofuels significantly improves the economics of biomass production as well as reduces the cost of treatment that would normally be incurred for nutrient removal by conventional methods.[6,23] The cost of conventional N and P removal are reported to be \$4.4 kg^{-1} N and \$3.05 kg^{-1} P removed.[14] Medium strength domestic wastewater in the USA (Table 1) contains sufficient N and P to produce a total of 77.6 million kg day^{-1}.[6] Zamalloa et al. [14] showed that a 70–110 ton ha^{-1} $annum^{-1}$ facility using wastewater can result in a saving of

Table 1. Typical composition of domestic wastewater.

		Concentration		
Contaminants	Units	Weak	Medium	Strong
Total solids	mg L^{-1}	350	720	1200
Total dissolved solids	mg L^{-1}	250	500	850
Fixed	mg L^{-1}	145	300	525
Volatile	mg L^{-1}	105	200	325
Suspended solids	mg L^{-1}	100	220	350
Fixed	mg L^{-1}	20	55	75
Volatile	mg L^{-1}	80	165	275
Settleable solids	mg L^{-1}	5	10	20
BOD5. 20C	mg L^{-1}	110	220	400
TOC	mg L^{-1}	80	160	290
COD	mg L^{-1}	250	500	1000
Total nitrogen	mg L^{-1}	20	40	85
Organic	mg L^{-1}	8	15	35
Free ammonia	mg L^{-1}	12	25	50
Nitrites	mg L^{-1}	0	0	0
Nitrates	mg L^{-1}	0	0	0
Phosphorus	mg L^{-1}	4	8	15
Organic	mg L^{-1}	1	3	5
Inorganic	mg L^{-1}	3	5	10
Chlorides	mg L^{-1}	30	50	100
Sulphates	mg L^{-1}	20	30	50
Alkalinity (as $CaCO_3$)	mg L^{-1}	50	100	200
Grease	mg L^{-1}	50	100	150
Total coliforms	No/100 mL	$10^{-6} - 10^{-7}$	$10^{-7} - 10^{-8}$	$10^{-7} - 10^{-9}$
Volatile organic compounds	μg/L	<100	100–400	>400

Source: Rawat et al.[8]

\$48 400–\$74 800 ha^{-1} $annum^{-1}$ for N removal and \$ 4575–\$ 7625 ha^{-1} $annum^{-1}$ for P removal. The combination of cost reduction from wastewater treatment and microalgae production is thus a win–win strategy.[23] Besides the income generated from biodiesel production, anaerobic digestion of the residual biomass can produce biomethane for electricity generation.[37] Menger-Krug et al. [28] analysed the use of integrated microalgal systems for the production of biogas and treatment of wastewater. They concluded that energy savings as compared with the conventional wastewater treatment can be as high as 120%. Pittman et al. [3] and Christenson and Sims [6] stated that the potential of microalgae as a source of renewable energy, and based on current technologies was able to conclude that without the utilization of wastewater, microalgal cultivation for biofuels production is unlikely to be economically viable or provide a positive energy return. Christenson and Sims [6] considered several approaches towards microalgae-based biofuels production coupled with wastewater treatment and suggested that only those studies that gave emphasize to wastewater treatment were able to yield cost competitive biofuels. From these findings, they were able to conclude that large-scale algae biofuels production would not be feasible in the near future without wastewater treatment as a primary goal.

Despite the favourable outlook of wastewater-mediated biomass production, no routine large-scale commercial cultivation of microalgae using wastewater is evident and much of the research has been done at laboratory scale thus making it is essential to determine the real potential in practice at large scale. Wastewater is susceptible to viral and bacterial contamination, and generally has inconsistent nutrient compositions. These factors as well as the presence of inhibitory substances could impede the growth of microalgae.[24] High nutrient concentrations are also known to be inhibitory to certain microalgal strains. Excess ammonia is inhibitory to microalgae, resulting in decreased photosynthesis and thus growth rate. These factors cannot be controlled, and close monitoring and adjustment of nutrient levels by augmentation or dilution may be required depending on the type of inhibitory substance and pretreatment of wastewater used. Utilization of wastewater will further necessitate frequent cleaning of the culturing system.[24]

Water footprint

Water footprint, in terms of algal biorefineries, is defined as the water used for microalgal cultivation and biomass processing into products and co-products. Water related to microalgal growth is directly related to the biomass and lipid productivity in that, higher productivity requires less water to achieve the target production.[11,24] The impact of large-scale biofuels production on water utilization has generated great debate. Microalgal cultivation requires relatively large amounts of water for growth and various processes. Generation of 1 kg microalgal biomass (freshwater species) requires 3715 L freshwater if cultivation is carried out without recycling.[26] Freshwater use can be reduced by up to 90% if sea water, brackish water or wastewater is used for microalgae culturing.[8,32] The water footprint of large-scale microalgal cultivation utilizing seawater or wastewater is significantly smaller than that of crop-based biofuels production.[20,32]

In open pond systems, replenishment of water is required due to losses incurred by evaporation and harvesting. In the absence of a water recycle, 84.1% of the water is discharged post-harvest. Evaporative losses from open pond systems can be as high as $10\,L\,m^{-2}\,day^{-1}$, and consequently losses of up to 410 kg water per kg biomass produced can occur.[30] Recycling of water has the potential to reduce nutrient addition by up to 55%.[32] The drawback to recycling is the accumulation of inhibitory metabolites produced by certain microalgae and cyanobacteria.[38] Recycling of water concentrates contaminants and inhibitory substances and should thus be carried out after taking relevant precautions, in terms of screening for inhibitory substances/metabolites. Wastewater utilization reduces the requirement of freshwater while providing a source of nutrients. This improves economic viability of the process and offers an eco-friendly means to the production of renewable microalgal biomass.[7]

The requirement of freshwater cannot be totally negated as some degree of water is required for the prevention of excessive changes to osmoregularity and to compensate for evaporative losses.[32] This can further be reduced by the utilization of treated wastewater. Factors such as the microalgal species choice and cultivation system further impact the water footprint. Photobioreactors require less than $1/3$ of total amount of water required for raceway pond cultivation to attain the same quantity of biomass.[39] *Chlorella vulgaris* requires less than 17% the total amount of water required for the cultivation of *Chaetocerosgracilis*, *Cyclotellacryptica* and *Nannochloropsis* sp. to attain the same level of production.[32]

Biomass productivity

Utilization of carbon

Microalgae possess a greater capacity to fix CO_2 as compared with terrestrial plants owing to the photosynthetic apparatus and chlorophyll being present within a single cell, thus permitting rapid biomass generation.[1,40] Typical sources of CO_2 that promote growth of microalgae include: atmospheric CO_2, CO_2 present in industrial exhaust gases such as flue and flaring gases, and chemically fixed CO_2 from soluble carbonates ($NaHCO_3$ and Na_2CO_3). Carbon dioxide concentration present in the gaseous phase is not an accurate indication of the actual CO_2 taken up by microalgal cells during dynamic liquid suspension. In an aqueous solution, dissolved CO_2 almost always coexists with H_2CO_3, HCO_3^- and CO_3^{2-}. The concentration of each of these chemical species is dependent upon the pH and temperature of the environment. Carbonic acid is preferred as a microalgal carbon source. HCO_3^- uptake is favoured by microalgal cells over atmospheric CO_2, despite CO_2 being a better carbon source.[41]

Ambient CO_2 levels (approximately 0.036%) are generally insufficient to sustain high microalgae growth rates and biomass productivities required for a full-scale biofuels production plant. However, waste gases released from various combustion processes usually contain $>15\%$ (v/v) CO_2. Theoretically, this percentage suggests that combustion processes could supply ample amounts of CO_2 to support large-scale microalgae cultivation.[1] Microalgal cells can only tolerate CO_2 up to a certain level. Increases in CO_2 beyond these levels damage cells due to a decrease in culture pH. Microalgal cells are unable to function at a low pH which eventually leads to a decrease in biomass productivity.[1] Tolerance to CO_2 concentrations varies with algal species. Chiu et al. [42] reported that 2% (v/v) of CO_2 was optimum for the growth of *Chlorella* whereas at 10% (v/v) concentration of CO_2; specific growth rate became severely retarded. However, the experiment conducted by Maeda et al. [43] for the sequestration of CO_2 from flue gas emitted by a coal-fired thermal power plant confirms that *Chlorella* sp. T-1 can tolerate up to 50% CO_2 concentration. The maximum growth rate was obtained at 10% CO_2 concentration and no significant decrease in growth rate was observed up to 50% CO_2. From these results, they were able to conclude that pre-adaptation of cells with lower percentage of CO_2 concentration leads increased tolerance of cells to higher percentages of CO_2. Flue gases containing CO_2 concentrations ranging from 5% to 15% (v/v) have been introduced directly into ponds and bioreactors of varying configurations.[1] CO_2 from power plant flue gases contains NO_x and SO_x, which may add the requirement of scrubbing and could prove costly. Flue gases emitted from power plants generally consist of 4–20% CO_2 and up to 200 ppm of NO_x and SO_x depending on the combustion process.[41,43] Some researchers argue that the presence of NO_x in flue gases pose little or no problem to microalgal growth, whereas the difficulty arises in the presence of SO_x, which decreases the pH due to the formation of sulphurous acid.[41] Others, however, argue that some strains are not inhibited by CO_2 with <50 ppm SO_x, but can be inhibited by CO_2 when NO_x are also present.[40] The use of CO_2 produced by anaerobic digestion/co-digestion provides an easily accessible source of CO_2 as digesters are commonplace at wastewater treatment sites. Biogas from digesters typically contains 30–40% CO_2. Carbon dioxide present in the biogas can be collected by covering an existing anaerobic digester.[44]

Mode of cultivation

Certain microalgae are able to utilize organic and inorganic carbon sources for metabolic synthesis. Metabolic shifts are common as responses to changes in environmental conditions. Several species, such as *C. vulgaris*, *Chlorella protothecoides, Chlorella sorokiniana*, *Chlamydomonas debaryana*, *Micractinium* sp. *Haematococcus pluvialis*, *Scenedesmus* sp. and *Spirulina platensis* are able to grow under photoautotrophic, heterotrophic and mixotrophic conditions [2,45] whereas other strains, (*Selenastrum capricornutum* and *Scenedesmus acutus*), can only grow either photoautotrophically, heterotrophically or photoheterotrophically.[20] Phototrophic cultivation utilizes sunlight and CO_2, as an inorganic carbon source, for energy production and growth.[2] This form of growth is particularly attractive due to freely available CO_2 from the atmosphere and flue gases. Following photosynthesis, this energy is stored in the form of chemical energy (Adenosine triphosphate). Phototrophic growth is a popular cultivation technique and is frequently employed on the basis of ease of scale-up. Microalgal biomass can eventually be upgraded to value-added products post lipids extraction, thus beneficiating a waste substrate.[2,20] Phototrophic microalgal cultivation is less prone to contamination as compared with other cultivation modes. Depending on the microalgal species, a large variation in lipid content (5–68%) is often noted during phototrophic cultivation.[20]

Heterotrophic cultivation utilizes organic carbon sources under dark conditions for growth. This gives rise to higher lipid productivities than phototrophic growth utilizing sources such as glucose, acetate, glycerol, fructose, sucrose, lactose, galactose and mannose.[20,46] Light limitation which is often the rate-limiting step of phototrophic cultivation is avoided by this cultivation mode. Due to higher cell densities and lipid productivity, this form of cultivation may translate to better economic viability.[47] Scale-up costs are considerably lower than some types of phototrophic cultivation. Reactor set-up costs are at a minimal and the process is very well understood.[7] This is, however, offset by the high cost of carbon sources. This high cost has peaked the interest in the search of less expensive sources of organic carbon.[20,48] A study by Liang et al. [48] showed that corn powder hydrolysate used as a substitute for sugars, yielded favourable results in terms of biomass productivity ($2\,g\,L^{-1}\ d^{-1}$) and lipid content ($932\,mg\,L^{-1}\,d^{-1}$). The highest lipid productivity of $3700\,mg\,L^{-1}\,d^{-1}$ was achieved by Chen et al.,[46] who used a 5 L fermenter under heterotrophic cultivation giving a 20-fold increase in lipid when compared with phototrophic cultivation. When considering the higher lipid productivity, this technology has the potential to be an attractive technology of the future.[20] Miao and Wu [49] achieved a four-fold increase in lipid accumulation for *C. protothecoides* for heterotrophic condition (55.2%) compared with 14.57% achieved under phototrophic conditions. The major disadvantage associated with heterotrophic cultivation systems is their vulnerability to contamination.[20] This is a major concern, especially at large scale, as this results in the utilization of substrate by undesirable organisms thus increasing production cost. Besides the risk of contamination, the cost of organic carbon sources is a concern from a commercial perspective. Furthermore, the numbers of species that undergo heterotrophic growth are limited.[47] Mixotrophic cultivation occurs when microalgae have the ability to utilize both organic as well as inorganic carbon sources for growth. Organisms that are able to undergo mixotrophic growth have the ability to photosynthesize or use organic substrates as a carbon source. Mixotrophic cultivations result in higher biomass and lipid yields than autotrophic cultivation but lower than heterotrophic yields. Carbon dioxide released via respiration will be trapped and reused under phototrophic growth.[46] Mixotrophic production reduces photoinhibition and decreases the loss of biomass due to dark phase respiration.[7] This is highly beneficial as organic carbon may available from wastewater used as a substrate.

Culturing systems

The selection of culturing system must take into account the intrinsic properties of the microalgal species to be cultivated. Species with high biomass and lipids productivities as opposed to lipid storage potential are preferred for cultivation.[50,51] Natural climatic conditions and the cost of land and water availability also play major roles in the determination of culturing system to be utilized.[47] Use of marginal and non-arable land is a major advantage. Large-scale cultivation of microalgae for biofuels production is generally carried out in open raceway ponds due to the low set-up and operational costs. This generally limits the number of strains that can be used. Strains that have a competitive advantage, such as cultivation in systems with high salinity, pH or other factors that limit contamination by non-target microalgae and other contaminants, are ideal for open raceway ponds. Photobioreactors are generally utilized when the culture to be grown produces higher value products for neutroceutical or pharmaceutical products.[52] Maintenance and cleaning of open systems is easier and less energy intensive than photobioreactors.[7] The overall energy input for raceway pond operation is lower than that for photobioreactors and results in the potential for a higher ratio.[53]

Raceway ponds are the most common cultivation system used.[7,52] Construction, material and operational costs of raceway ponds are significantly lower than that of photobioreactors.[8,19] They are therefore regarded as the most cost-effective method of biomass production.[54] One of the main limitations of raceway pond cultivation is low biomass productivity due to a number of factors that cannot be controlled.[4,19,47,52] CO_2 transfer rates and light limitation due to increased culture density are among the largest contributors to low productivity. These may be partially alleviated by limiting the depth of raceway ponds and effective mixing.[7] Evaporation from open raceway ponds not only causes water loss and a larger water footprint but also results in change of ionic composition of the culture medium potentially negatively affecting growth. Contamination by undesirable organisms further affect the stability and productivity of open pond systems which tend to become contaminated fairly quickly.[19,54]

The limitations of open pond culture have led to much research into photobioreactors, as a method of primarily overcoming low productivities and limiting contamination.[7,20] Photobioreactors allow a greater degree of process control and have the capability of achieving 13 times the productivity of raceway or open ponds.[50] Due to the level of control, the resultant product is more consistent in terms of quality and composition.[47] Despite the benefits of photobioreactor cultivation, the high cost of materials and high operational costs in terms of energy consumption make the use of photobioreactor economically prohibitive for fuels production alone.[20,39] Photobioreactors can cost up to 10 times that of raceway ponds to construct.[55] Scale-up presents engineering and design challenges.[7]

Hybrid systems combine photobioreactors and raceway ponds at different stages of production.[54] The utilization of hybrid systems is regarded as a logical step for cost-effective microalgal production. They are generally two-stage systems whereby biomass is grown, contaminant free, in large-scale photobioreactors to a high density and thereafter stressed using raceway ponds.[7] Hybrid systems can produce as much as 20–30 ton ha^{-1} of lipid annually (climate dependant).[53]

Biomass production rates vary between species and strain and are dependent on the level of inputs. Raceway ponds and photobioreactors generally produce up to $1\,\text{g L}^{-1}$ and $4\,\text{g L}^{-1}$ dry cell weight (DCW) biomass, respectively.[39] Theoretical biomass productivities are estimated to be 77–96 $\text{g DCW m}^{-2}\,\text{day}^{-1}$, with productivities in the order of 27–62 $\text{g DCW m}^{-2}\,\text{day}^{-1}$being regarded as reasonable.[14,54] In practice, researchers were able to achieve average biomass productivities ranging between $8.2\,\text{g DCW m}^{-2}\,\text{day}^{-1}$–and $25\,\text{g DCW m}^{-2}\,\text{day}^{-1}$ in open raceway ponds with peak productivities ranging from 12 to $40\,\text{g DCW m}^{-2}\,\text{day}^{-1}$.[7,55,56] Reported productivities for photobioreactors range from 20 to $40\,\text{g DCW m}^{-2}\,\text{day}^{-1}$.[6]

Microalgal biofuels and by-products

Lipids production for biodiesel

Nitrogen is one of the most important macronutrients for microalgal growth and lipid regulation in microalgae. Nitrogen constitutes about 7–10% of microalgae cell dry weight.[56] Nitrogen constitutes amino acids and proteins essential for microalgae growth and can be supplied in the form of ammonia, nitrate and urea. Nitrogen limitation to the point of becoming a growth-limiting factor is generally signified by an accumulation of lipid above 40% g^{-1} DCW. Under these conditions, excess carbon is channelled into lipids and/or starch as storage products.[56,57] Illman et al.

[58] showed that the reduction in N increases the lipid content in *Chlorella* strains, including *C. emersonii*, *C. minutissima* and *C. vulgaris*, gained increases in lipid content of 63%, 56% and 40% biomass by dry weight, respectively. Nitrogen sources are readily available in domestic wastewater, and thus can substantially reduce the cost and induce N limitation due to their limited amounts depending on the stage of treatment from which the wastewater is utilized.[8]

Glycerol by-product reuse

The process of tranesterification of triglycerides to produce biodiesel produces raw glycerol as a by-product. This crude glycerol contains many impurities and cannot be feasibly purified due to cost. Crude glycerol is generally found to be 65–80% of glycerol.[15] Disposal of the glycerol is not looked upon favourably due to ecological and economic implications. It is, thus, vital to establish effective utilization methods that can also improve the economics of biodiesel production. On-site utilization on glycerol reduces the energy costs associated with water disposal and off-site transportation.[27] Biodiesel-derived glycerol has an average carbon content of approximately 25% and potential trace amounts of elements such as Na, Ca, K, Mg, P and S. Crude glycerol offers a multitude of uses as substrates for chemical or biological conversion. Biological utilization of glycerol for hydrogen and ethanol production offers another avenue for adding value to waste glycerol. Other valuable chemical products that can be produced include 1,2-propanediol, 1,3-propanediol, succinic acid, polyesters, lactic acid and polyglycerol as dihydroxyacetone, succinic acid, propionic acid, citric acid, pigments, polyhydroxyalcanoate and biosurfactants. Glycerol may also be used as solvents or fuel additives after undergoing etherification with alcohols.[13,15] Glycerol use as a carbon substrate for the microalgal production of high-value metabolites, such as asthaxanthan and beta-carotene, has also been suggested.[59]

Glycerol-based energy fuels production

Utilization of glycerol as a feedstock for hydrogen production has gained much interest in recent years due to the trend towards renewable carbon neutral fuels. Much of the world's current hydrogen production (95%) is based on fossil fuel feedstocks. Hydrogen may be formed chemically by the processes of steam reforming, partial oxidation, auto-thermal reforming and aqueous-phase reforming and supercritical water. Hydrogen can be used for electricity generation using a gas turbine or for fuel cell production.[15] Hydrogen and ethanol may also be produced using *Enterobacter aerogenes* HU-101 with a glycerol substrate. Glycerol can be co-digested or co-gassified to produce biogas at concentrations of up to 20% (wt) without affecting the quality and quantity of gas yield.[15] Heterotrophic fermentation of glycerol can be used for further production of lipids.[27]

Biogas production from residual biomass

After lipid extraction, large quantities of residual algae biomass containing enriched nutrients, such as biologically bound N, P, K and CO_2. Recovery/recycling of nutrients present in the residual biomass are essential and could be used as a fertilizer for crop plants or further microalgae growth.[12] These nutrients are stored as secondary metabolites in the form of carbohydrates and proteins that are essential for the production of high-energy biogas via fermentation technology. Table 2 represents carbohydrates and proteins content of microalgae species. Biogas production is essential to promote the expansion and optimization of the entire biofuels production process at low cost and may be achieved by improving technology using essential microbes.

Biogas production processes require high moisture content and organic waste for anaerobic digestion.[61] Algae (after lipid-extracted wet biomass) are thus highly suitable candidates due to high moisture and organic waste content (80–90% moisture). Inorganic nutrients sources,

Table 2. Microalgae metabolites protein and carbohydrates amounts from various microalgae on a dry matter basis (%) modified from Becker.[60]

Name of the organism/group	Protein (% dwt)	Carbohydrate (% dwt)
Anabaena cylindrical/ cayanophyceae	43–56	25–30
Chlamydomonas rheinhardii/Chlorophyceae	48	17
Chlorella pyrenoidosa/ Chlorophyceae	57	26
C. vulgaris/Chlorophyceae	51–58	12–17
Dunaliella bioculata/ Chlorophyceae	49	4
Dunaliella salina/Chlorophyceae	57	32
Euglena gracilis/Euglenaceae	39–61	14–18
Porphyridium cruentum/Porphyridiaceae	28–45	40–57
Prymnesium parvum/Prymnesiaceae	28–45	25–33
S. obliquus/Chlorophyceae	50–56	10–17
Scenedesmus quadricauda/Chlorophyceae	47	–
Scenedesmus dimorphus/Chlorophyceae	8–18	21–52
Spirogyra sp./Chlorophyceae	6–20	33–64
Spirulina maxima/ Caynophyceae	28–39	13–16
S. platensis/Caynophyceae	52	8–14
Synechoccus sp./Caynophyceae	46–63	15
Tetraselmis maculate/Caynophyceae	52	15

such as iron, cobalt and zinc, are also present. These are known to stimulate anaerobic digestion to produce energy feedstocks.

Biogas production using fermentation technology has been carried out for more than 50 years.[62] The astonishing biodiversity of the microbial world has enabled the production of biogas primarily and lesser quantities of CO_2 from the fermented biomass. Over the years, many projects have been carried out, focused extensively on selection of promising microalgal species for biogas production. Biogas production involves the breakdown of organic matter through the sequential process of hydrolysis, fermentation and biogas production. During the process, nutrient rich (N, P and K) organic biomass is broken down into soluble sugars by hydrolysis. These are further converted into alcohols, acetic acid, volatile fatty acids and biogas by bacterial fermentation. Fermentation technology of biogas production is a well-developed method of energy conversion of biomass to combustible gasses. Biogas produced from microalgal biomass consists mainly of methane (55–75%) and CO_2 (25–45%), with trace amounts of other gases, such as hydrogen sulphide (below the standard limit). Table 3 represents the percentage of methane produced from different microalgae. Microalgae have the potential for superior quality methane production by anaerobic digestion, as the cell wall comprises lipids and protein with little cellulose and almost no lignin content.[62] Methane production has received much attention recently as there is a potential to recover high volumes of energy from the microalgal biomass post lipid extraction.[37] Biogas generation from microalgal biomass has a number of limitations. High protein content leads to release of ammonia which inhibits acetoclastic methanogen bacteria and thus reducing biogas yield. The use of marine microalgae further adds the limitation of high sodium (Na) content which is also inhibitory to the process. These limitations may be overcome by the utilization of wastewater for algal cultivation thereby reducing overall Na content and reduced protein concentrations due to conversion to lipids. Co-digestion of microalgal biomass with a high carbon containing waste, such as municipal sludge, effectively improves the C/N ratio. Co-digestion delivers higher biomass yields that either substrate digested individually.[64] The production of a secondary energy product, such as biogas, is necessary to effectively reduce the cost of biodiesel production as it may be used to run processes, and the electricity generated in excess may be sold for additional revenue generation.[4] Furthermore, the use of algae grown on waste gases from anaerobic digestion allows for increased revenue generation in the form of carbon credits gained by negating CO_2 emissions from fossil-based resources.[14] However, for methane production to become economically feasible at large scale in the near future, research would need to focus on the elucidation of the effects of organic loading, retention time, pH, temperature and necessary characteristics for purification of the biogas.

Table 3. Theoretical methane productions from the biomass of various microalgae species.

Microalgal species	CH_4 ($L\,g^{-1}$ VS)
E. gracilis	.53–.8
C. Reinhardtii	.69
C. Pyrenoidosa	.80–.80
C. vulgaris	.63–.79
D. salina	.68–.8
S. maxima	.63–.74
S. platensis	.47–.69
S. obliquus	.59–.69

Source: Adapted from Singh and Olsen.[63]

Biohydrogen

Singh and Gu [12] utilized microalgae for CO_2 fixation, bio-treatment of wastewater and biohydrogen production as a promising alternative biofuel. Hydrogen is a clean energy source and can be achieved via the implementation of various processing technologies, including anaerobic digestion,[65] pyrolysis, gasification, catalytic cracking and enzymatic or chemical transesterification.[15] Hydrogen production by a unique process has been developed for renewable energy that combines microalgal biomass with a consortium of bacteria. This is subjected to anaerobic fermentation which produces hydrogen and CO_2.[65] Unicellular microalgae have the ability to capture ample solar energy which is used to spilt water to produce molecular oxygen as well as evolved H^+ and e^- that are combined to produce biosolar hydrogen.[66] Melis and Happe [67] have reported that photosynthetic biohydrogen production is a two-stage process, consisting of an aerobic and an anaerobic stage. The first stage involves microalgae grown photosynthetically (accumulation of carbohydrates) under normal conditions, while the second stage involves fermentation of microalgae (carbohydrates) by sulphur deprivation. Physiological reactions take place after 60 h of fermentation for consistent hydrogen production. Theoretical maximum yields of hydrogen using green microalgae are approximated to be $198\,\mathrm{kg\,H_2\ ha^{-1}\,day^{-1}}$. This process does not produce any toxic or harmful products and can provide value-added by-products.[67] High-energy containing, clean burning, biohydrogen is potentially one of best alternatives to fossil fuels and other conventional fuels.[65]

Demirbas [65] noted that anaerobic hydrogen production proceeds photofermentatively, in the absence of light. During the biological process anaerobic microbes utilize organic substances which are present as a sole source of electrons and energy, converting them into hydrogen. The following reactions are involved in hydrogen production (Equations (1) and (2)) are rapid and these processes do not require solar radiation:

$$\text{Glucose} + 2H_2O \longrightarrow 2\,\text{Acetate} + 2CO_2 + 4H_2, \quad (1)$$

$$\text{Glucose} \longrightarrow \text{Butyrate} + 2CO_2 + 2H_2. \quad (2)$$

Chlamydomonas reinhardtii has the remarkable ability to produce hydrogen via hydrolysis of water during illumination [68] and the ability to produce biosolar hydrogen (H_2) under anaerobic conditions. Some cyanobacteria are also a good source for hydrogen production. Block and Melody [69] reported that the cost of photobiologically producing hydrogen is considerably less (US\$25 m^3) than photovoltaic splitting of water (US\$170 m^3). Aside from potential use for transportation fuels, microalgal biohydrogen also offers potential in domestic applications. In order for microalgae biohydrogen to become a feasible option of the future, research needs to focus on high biohydrogen yielding strains.

Conclusion

The use of wastewater as a nutrient and water source is essential for the success of fuels production due to the drastic cost savings. Furthermore, the generation of electricity and environmental implications in terms of GHG production make the use of wastewater the only viable method for microalgal biofuels production in the near term. The implementation of wastewater utilization for microalgal cultivation however must be considered on a case-by-case basis dependent on the land availability on nutrient availability. Existing infrastructure, such as anaerobic digesters, in wastewater treatment plants serves to improve the capital cost expenditure. The feasibility of microalgal biofuels production from an economics standpoint resides firmly in the utilization of a biorefinery for the production of multiple biofuels and/or co-products. The production of biogas, biomethane and biohydrogen as co-products using an integrated process has immense potential of decreasing the total cost of microalgal biofuels production thereby improving feasibility. The specific biorefinery approach adopted is dependent on a number of factors and a high-level feasibility study is required dependent of factors, including microalgal strain/consortium of choice, climatic conditions, existing infrastructure, logistic considerations as well as overall availability of waste resources in sufficient quantity.

References

[1] Kumar A, Ergas S, Yuan X, Sahu A, Zhang Q, Dewulf J, Malcata FX, van Langenhove H. Enhanced CO(2) fixation and biofuel production via microalgae: recent developments and future directions. Trends Biotechnol. 2010;28(7):371–380.
[2] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. Renew Sustain Energy Rev. 2010;14:217–232.
[3] Pittman JK, Dean AP, Osundeko O. The potential of sustainable algal biofuel production using wastewater resources. Bioresour Technol. 2011;102:17–25.
[4] Chisti Y. Biodiesel from microalgae. Biotechnol Adv. 2007;25:249–306.
[5] Chisti Y. Biodiesel from microalgae beats bioethanol. Trends Biotechnol. 2008;26:126–131.
[6] Christenson L, Sims R. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol Adv. 2011;29:686–702.
[7] Brennan L, Owende P. Biofuels from microalgae – a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev. 2010;14:557–577.
[8] Rawat I, Ranjith Kumar R, Mutanda T, Bux F. Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Appl Energy. 2011;14:3411–3424.
[9] Boelee NC, Temmink H, Janssen M, Buisman CJN, Wijffels RH. Nitrogen and phosphorus removal from municipal wastewater effluent using microalgal biofilms. Water Res. 2011;88:5925–5933.
[10] Fortier MO, Sturm BS. Geographic analysis of the feasibility of collocating algal biomass production with wastewater treatment plants. Environ Sci Technol. 2012;46:11426–11434.
[11] Subhadra BG, Edwards M. Coproduct market analysis and water footprint of simulated commercial algal biorefineries. Appl Energy. 2011;88:3515–3523.
[12] Singh J, Gu S. Commercialization potential of microalgae for biofuels production. Renew Sustain Energy Rev. 2010;14:2596–2610.
[13] Vlysidis A, Binns M, Webb C, Theodoropoulos C. A techno-economic analysis of biodiesel biorefineries: assessment of integrated designs for the co-production of fuels and chemicals. Energy. 2011;36:4671–4683.
[14] Zamalloa C, Vulsteke E, Albrecht J, Verstraete W. The techno-economic potential of renewable energy through the anaerobic digestion of microalgae. Bioresour Technol. 2011;102:1149–1158.
[15] Leoneti AB, Aragão-Leoneti V, de Oliveira SVWB. Glycerol as a by-product of biodiesel production in Brazil: alternatives for the use of unrefined glycerol. Renew Energy. 2012;45:138–145.
[16] Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. J Biosci Bioeng. 2006;101:87–96.
[17] Harun R, Singh M, Forde GM, Danquah MK. Bioprocess engineering of microalgae to produce a variety of consumer products. Renew Sustain Energy Rev. 2010;101:1037–1047.
[18] Pulz O, Gross W. Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol. 2004;65:635–648.
[19] Zeng X, Danquah MK, Chen XD, Lu Y. Microalgae bioengineering: from CO2 fixation to biofuel production. Renew Sustain Energy Rev. 2011;15:3252–3260.
[20] Amaro HM, Guedes AC, Malcata FX. Advances and perspectives in using microalgae to produce biodiesel. Appl Energy. 2011;88:3402–3410.
[21] Martinez ME, Jimenez JM, El-Yousfi F. Influence of phosphorus concentration and temperature on growth and phosphorus uptake by the microalga *Scenedesmus obliquus*. Bioresour Technol. 1999;67:233–240.
[22] Putt R, Singh M, Chinnasamy S, Das KC. An efficient system for carbonation of high-rate algae pond water to enhance CO2 mass transfer. Bioresour Technol. 2011;102:3240–3245.
[23] Lam MK, Lee KT. Microalgae biofuels: a critical review of issues, problems and the way forward. Biotechnol Adv. 2012;30:673–690.
[24] Lam MK, Lee KT. Potential of using organic fertilizer to cultivate *Chlorella vulgaris* for biodiesel production. Appl Energy. 2012;30:303–308.
[25] Shirvani T, Yan X, Inderwildi OR, Edwards PP, King DA. Life cycle energy and greenhouse gas analysis

for algae-derived biodiesel. Energy Environ Sci. 2011;4: 3773–3778.

[26] Olguín EJ. Dual purpose microalgae-bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a Biorefinery. Biotechnol Adv. 2012;30:1031–1046.

[27] Chowdhury R, Viamajala S, Gerlach R. Reduction of environmental and energy footprint of microalgal biodiesel production through material and energy integration. Bioresour Technol. 2012;108:102–111.

[28] Menger-Krug E, Niederste-Hollenberg J, Hillenbrand T, Hiessl H. Integration of microalgae systems at municipal wastewater treatment plants: implications for energy and emission balances. Environ Sci Technol. 2012;46: 11505–11514.

[29] Park JB, Craggs RJ, Shilton AN. Wastewater treatment high rate algal ponds for biofuel production. Bioresour Technol. 2011;102:35–42.

[30] Sheehan J, Dunahay T, Benemann J, Roessler P. A look back at the US Department of Energy‘s aquatic species program – biodiesel from algae. Golden, CO: National Renewable Energy Laboratory; 1998. (Report NREL/TP-580-24190).

[31] Sturm BSM, Lamer SL. An energy evaluation of coupling nutrient removal from wastewater with algal biomass production. Appl Energy. 2011;88:3499–3506.

[32] Yang J, Xu M, Zhang X, Hu Q, Sommerfeld M, Chen Y. Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. Bioresour Technol. 2011;102:159–165.

[33] Lundquist TJ, Woertz IC, Quinn NWT, Benemann JR. A realistic technology and engineering assessment of Algae biofuel production. Berkeley, CA: Energy Biosciences Institute; 2010. p. 178.

[34] Li Y, Zhou W, Hu B, Min M, Chen P, Ruan RR. Integration of algae cultivation as biodiesel production feedstock with municipal wastewater treatment: strains screening and significance evaluation of environmental factors. Bioresour Technol. 2011;102:10861–10867.

[35] Martínez ME, Sánchez S, Jiménez JM, El-Yousfi F, Muñoz L. Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. Bioresour Technol. 2000;73:263–272.

[36] Yang J, Li X, Hu H, Zhang X, Yu Y, Chen Y. Growth and lipid accumulation properties of a freshwater microalga, *Chlorella ellipsoidea* YJ1, in domestic secondary effluents. Appl Energy. 2011;88:3295–3299.

[37] Vergarafernandez A, Vargas G, Alarcon N, Velasco A. Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. Biomass Bioenergy. 2008;32:338–344.

[38] Stephens E, Ross IL, Mussgnug JH, Wagner LD, Borowitzka MA, Posten C, Kruse O, Hankamer B. Future prospects of microalgal biofuel production systems. Trends Plant Sci. 2010;10:554–564.

[39] Davis R, Aden A, Pienkos PT. Techno-economic analysis of autotrophic microalgae for fuel production. Appl Energy. 2011;88:3524–3531.

[40] Ho SH, Chen CY, Lee DJ, Chang JS. Perspectives on microalgal CO2-emission mitigation systems – A review. Biotechnol Adv. 2011;29:189–198.

[41] Kumar K, Dasgupta CN, Nayak B, Lindblad P, Das D. Development of suitable photobioreactors for CO2 sequestration addressing global warming using green algae and cyanobacteria. Bioresour Technol. 2011;102:4945–4953.

[42] Chiu SY, Kao CY, Chen CH, Kuan TC, Ong SC, Lin CS. Reduction of CO2 by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor. Bioresour Technol. 2008;99:3389–3396.

[43] Maeda K, Owada M, Kimura N, Omata K, Karube I. CO2 fixation from flue gas on coal fired thermal power plant by microalgae. Energy Convers Manage. 1995;36:717–720.

[44] Baylis K, Paulson ND. Potential for carbon offsets from anaerobic digesters in livestock production. Animal Feed Sci Technol. 2011;166:446–456.

[45] Park KC, Whitney C, McNichol JC, Dickinson KE, MacQuarrie S, Skrupski BP, Zou J, Wilson KE, O'Leary SJB, McGinn PJ. Mixotrophic and photoautotrophic cultivation of 14 microalgae isolates from Saskatchewan, Canada: potential applications for wastewater remediation for biofuel production. J Appl Phycol. 2011;24:339–348.

[46] Chen CY, Yeh KL, Aisyah R, Lee DJ, Chang JS. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour Technol. 2011;102:71–81.

[47] Borowitzka MA. Commercial production of microalgae: ponds, tanks, tubes and fermenters. J Biotechnol. 1999;70:313–321.

[48] Liang Y, Sarkany N, Cui Y. Biomass and lipid productivities of Chlorella vulgaris under autotrophic, heterotrophic and mixotrophic growth conditions. Biotechnol Lett. 2009:31:1043–1049.

[49] Miao X, Wu Q. Biodiesel production from heterotrophic microalgal oil. Bioresour Technol. 2006;97:841–846.

[50] Malcata FX. Microalgae and biofuels: a promising partnership? Trends Biotechnol. 2011;29:542–549.

[51] Griffiths MJ, Harrison STL. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. J Appl Phycol. 2009;21:493–507.

[52] Rawat I, Ranjith Kumar R, Mutanda T, Bux F. Biodiesel from microalgae: a critical evaluation from laboratory to large scale production. Appl Energy. 2013;103: 444–467.

[53] Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng. 2009;102:100–112.

[54] Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, Kruse O, Hankamer B. Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenergy Res. 2008;1:20–43.

[55] Demirbas A, Demirbas MF. Importance of algae oil as a source of biodiesel. Energy Convers Manage. 2011;52: 163–170.

[56] Richmond A. Principles for attaining maximal microalgal productivity in photobioreactors: an overview. Hydrobiologia. 2004;512:33–37.

[57] Scott SA, Davey MP, Dennis JS, Horst I, Howe CJ, Lea-Smith DJ, Smith AG. Biodiesel from algae: challenges and prospects. Curr Opin Biotechnol. 2010;21:1–10.

[58] Illman AM, Scragg AH, Shales SW. Increase in *Chlorella* strains calorific values when grown in low nitrogen medium. Enzyme Microbial Technol. 2000;27:631–635.

[59] Abad S, Turon X. Valorization of biodiesel derived glycerol as a carbon source to obtain added-value metabolites: focus on polyunsaturated fatty acids. Biotechnol Adv. 2012;30: 733–741.

[60] Becker E. Microalgae: biotechnology and microbiology. New York: Cambridge University Press; 1994. p. 293.

[61] McKendry P. Energy production from biomass (part 2) conversion technologies. Bioresour Technol. 2002;83: 47–54.

[62] Mussgnug JH, Klassen V, Schluter A, Kruse O. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. J Biotechnol. 2010;150:51–56.

[63] Singh A, Olsen SI. A critical review of biochemical conversion, sustainability and life cycle assessment of algal biofuels. Appl Energy. 2011;88:3548–3555.

[64] Sialve B, Bernet N, Bernard O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol Adv. 2009;63:409–416.

[65] Demirbas A. Use of algae as biofuel sources. Energy Convers Manage. 2010;51:2738–2749.

[66] Kruse O, Hankamer B. Microalgal hydrogen production. Curr Opin Biotechnol. 2010;21:238–243.

[67] Melis A, Happe T. Hydrogen production. Green algae as a source of energy. Plant Physiol. 2001;127:740–748.

[68] Kruse O, Rupprecht J, Bader KP, Thomas-Hall S, Schenk PM, Finazzi G, Hankamer B. Improved photobiological H2 production in engineered green algal cells. J Biol Chem. 2005;240:34170–34177.

[69] Block D, Melody I. Efficiency and cost goals for photoenhanced hydrogen production processes. Int J Hydrogen Energy. 1992;17:853–861.