HAYATI Journal of Biosciences 24 (2017) 163–167

HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

HAYATI Journal of Biosciences

journal homepage: http://www.journals.elsevier.com/hayati-journal-of-biosciences

Review Article

Biodiesel Production From Algae to Overcome the Energy Crisis

Suliman Khan,[1] Rabeea Siddique,[2] Wasim Sajjad,[3] Ghulam Nabi,[1] Khizar Mian Hayat,[4] Pengfei Duan,[5] Lunguang Yao[5*]

[1] *The Key Laboratory of Aquatic Biodiversity and Conservation of Chinese Academy of Sciences, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei 430072, China.*
[2] *Department of Biomedical Engineering, Huazhong University of Science and Technology, Wuhan 430074, PR China.*
[3] *Key Laboratory of Petroleum Resources, Gansu Province/Key Laboratory of Petroleum Resources Research, Institute of Geology and Geophysics, Chinese Academy of Sciences, Lanzhou 730000, PR China.*
[4] *MOE Key Laboratory of Cell Activities and Stress Adaptations, School of Life Sciences, Lanzhou University, Lanzhou 730000, PR China.*
[5] *Collaborative Innovation Center of Water Security for Water Source Region of Mid-line of South-to-North Diversion Project, College of Agricultural Engineering, Nanyang Normal University, Henan, China.*

ARTICLE INFO

Article history:
Received 14 January 2017
Received in revised form
9 October 2017
Accepted 30 October 2017
Available online 22 November 2017

KEYWORDS:
algae,
biofuel,
fossil fuel,
gene

ABSTRACT

The use of energy sources has reached at the level that whole world is relying on it. Being the major source of energy, fuels are considered the most important. The fear of diminishing the available sources thirst towards biofuel production has increased during last decades. Considering the food problems, algae gain the most attention to be used as biofuel producers. The use of crop and food-producing plants will never be a best fit into the priorities for biofuel production as they will disturb the food needs. Different types of algae having the different production abilities. Normally algae have 20%–80% oil contents that could be converted into different types of fuels such as kerosene oil and biodiesel. The diesel production from algae is economical and easy. Different species such as tribonema, ulothrix and euglena have good potential for biodiesel production. Gene technology can be used to enhance the production of oil and biodiesel contents and stability of algae. By increasing the genetic expressions, we can find the ways to achieve the required biofuel amounts easily and continuously to overcome the fuels deficiency. The present review article focusses on the role of algae as a possible substitute for fossil fuel as an ideal biofuel reactant.

1. Introduction

Energy crisis is among the biggest problems, leading the world to be unsafe and non-peaceful. The demand is increasing day by day. The available resources are rapidly decreasing and indication is, soon will be vanished. In such situations, more attention is needed to be given towards renewable energy sources. Fossil fuels are used on a large scale in the world, but unsustainable because they increase CO_2 level and accumulate greenhouse gases which make the environment unhealthy. To keep the environment clean and maintain sustainability, renewable and environmentally friendly fuels are needed to be produced (Schenk, 2008). Biofuels are defined as the liquid fuels produced from the biomass of different agricultural and forest products and biodegradable portion of industrial waste (Dufey, 2006). Biodiesel is extracted from vegetable oils (Shay, 1993), biobutanol (Dürre, 1997), *Jatropha curcas* (Becker and Makkar, 2008) and algae (Roessler et al., 1994; Sawayama et al., 1995; Dunahay et al., 1996; Sheehan et al., 1998). Brazil, the United States and the European Union are the world's largest biodiesel producers (Balat 2007). Biofuel production has been estimated to be 35 billion litres (O European Commission, 2006).

The algae are now becoming the main source of biofuel production in the world. They are considered as the safer, non-competitive and rapidly growing organisms among those could be used for biodiesel production. They have the abilities to grow without much care on waste nutrients (Roberts, 2013), and are considered the better source of biodiesel production as other sources can cause food problems as they are mainly including those plants which are used for food (Patil et al., 2008). Moreover,

---

* Corresponding author.
Peer review under responsibility of Institut Pertanian Bogor.

https://doi.org/10.1016/j.hjb.2017.10.003

biodiesel contents of crops are less sustainable and less in quantity as compared with algae (Charles et al., 2007). Algae have around 80% energy content to that contained by petroleum (Chisti, 2007, 2013). Algal cells have 30% lipid content (Lam and Lee, 2012), which is higher than other sources including soybeans and palm oils (Lam and Lee, 2011; Kligerman and Bouwer, 2015). Microalgae have 30%—40% lipid contents by dry weight and this value raises to 85%. *Botryococcus braunii* is a microalga which is having 30%—40% hydrocarbon content which can be extracted easily (Mirza et al., 2008). Algae can efficiently remove the toxic components from water, so playing a role in waste water treatment. Their remediating role in waste water treatment and rich sources of biodiesel make them suitable sources to be grown on large scale (Pittman et al., 2011; Kligerman and Bouwer, 2015).

Micro as well as macro algae can be cultivated on large scale in short period. Micro algae are photosynthetic and heterotrophic having the potential to be grown as energy crops. They have the ability to produce certain economically important compounds including fats and oils (Rawat et al. 2011; Pittman et al. 2011). Algal biofuel does not have harmful chemicals, so environment can be kept clean after the combustion.

Pakistan state oil has currently started work on biodiesel production and successfully cultivated *J. curcas* plant to produce biodiesel. Drying needs energy in the case of crops and food-producing plants; but in the case of algae, it is economical to dry them with sun light. Thermo chemical drying process is also easy in algae in comparison with other plants (Banerjee et al. 2002; Tsukahara and Sawayama, 2005). Biomass is treated with anaerobic microorganisms to produce biogas which is having high content of methane, so it can be used as an alternate source of energy (Mes et al. 2003). Algae cells have the bio fuel contents inside the cells, and the cell wall retains these components intact, so the cell wall must be broken; and in most cases, anaerobic digestion is preferred, which is the most prominent method of extracting these components from the cells (Reith, 2004). The algae with high biofuel contents and easily cultivatable are preferred. The objective of this review article was to critically describe various aspects of algae as an ideal target for biofuel productions.

2. The Processes for Biofuel Production Using Algae

Algae have oil contents with different compositions depending on the specie types. Some species were identified that they have good fatty acid values. In the same way, some algae have more components of fatty acids by their dry masses. Micro algae can grow in different conditions even in availability of fewer nutrients. They are best to be chosen for cultivation. The collection of sample needs care so that the whole biofuel contents could be obtained through careful handling of the instruments. The growth is also affected by different environmental factors which are not specifically known for every region, so the process needs careful attention accordingly (Richmond, 2004).

The simple method of fatty acids extraction and separation of biodiesel is the blending method on small or experimental scale. This process consists of several steps which have been shown in Figure 1.

It is also necessary to know about the cultivation unit of the algal cultivation, whether it is good to choose the closed system or open system. The process either batch or continuous is confirmed depending on the conditions and facilities, including pH, temperature, type of algal specie and the amount of algal biomass (Kaewpintong, 2004; Chojnacka and Marquez-Rocha, 2004; Chojnacka et al. 2005). Harvesting techniques are finalized based on the location and conditions (Grima et al. 2003). Most favourable harvesting techniques suggested are based on settling pond or sedimentation tank. Density and moisture adjustment is required during the whole process of biodiesel production. The drying technique mostly used is spray drying, drum drying was also

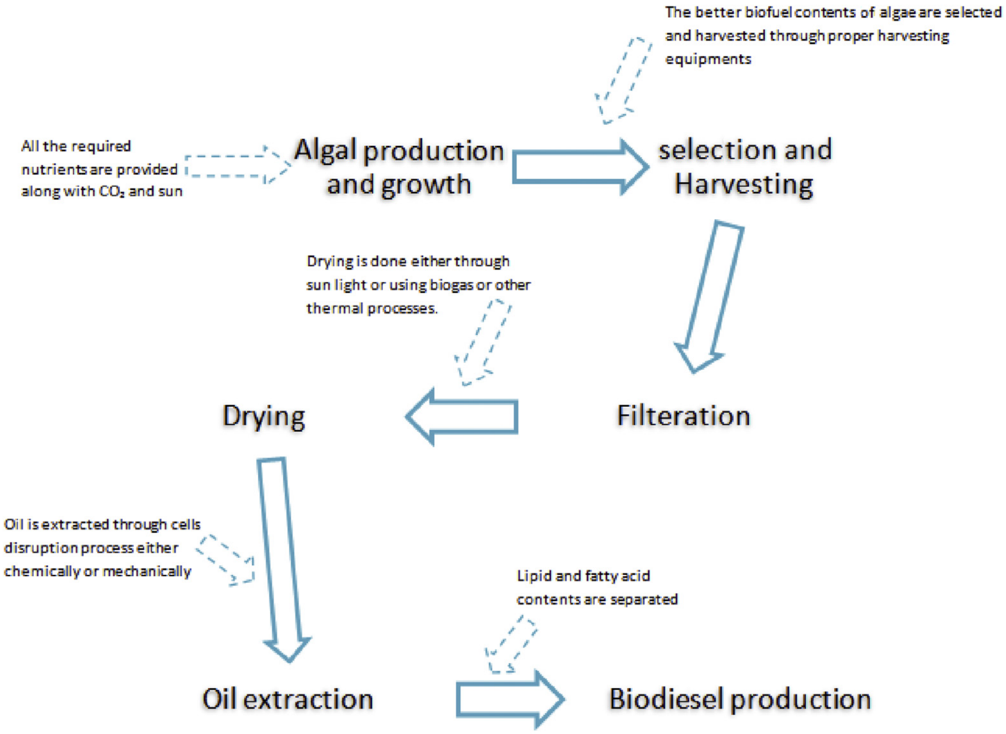

Figure 1. This figure illustrates the general process of biodiesel production from algae on small scale and for experimental purpose. This method could be used to compare different algal species for the oil contents. Dotted arrow indicates the addition to specific steps which have been highlighted with bold letters and full lined arrows.

suggested. The disruption process through mechanical handlings is considered the most favourable. The other requirements are the use of solvents such as hexane and ethanol are required for active process. Ultrasound and microwave-based extraction methods can also be of benefit if other sources are not available (Richmond, 2004).

3. Cultivation Systems

Two main cultivation systems are open system and closed system. The open system is normally a pond system, where the algae can grow naturally or their growth can be enhanced with human interactions. According to Kaewpintong (2004), closed system is preferred as the process is easy and energy saving along with least chances of the interference by environmental factors. Also, the factors and nutrients can be controlled easily in the closed system; but in some cases, the open system gains priority. In a study regarding algae production, Ugwu (2008) concluded that algae could be grown either in open culture systems or closed systems, but on larger scale production open system is preferred. Open system does not require the huge amount of energy and man power, and also economical. Open system cultivation also helps in the treatment of waste water sources, and the production process continues without disruptions. Open ponds are easy to construct and operate. However, major limitations in open ponds include poor light utilization by the cells, evaporative losses, diffusion of CO_2 to the atmosphere and requirement of large areas of land. The closed system has some advantages over open system. In closed systems (photo bioreactors), higher biomass products are obtained, and contamination can easily be prevented. Because of these advantages, the closed system also obtains attention.

Besides these major systems, the other cultivation systems or methods include water stabilization pond, advanced integrated waste water pond system and algal high-rate ponds. Cost-effectiveness, larger production, commercial scale growth, minimization of sludge accumulation, low energy demands, well suited for tropical and subtropical countries and removal of Biological Oxygen Demand (BOD) are some of the advantages these systems have, but they also have some serious disadvantages, including light penetration limitation, windy weather disruptions, hard to take care and the chances of destroying are higher (Kligerman and Bouwer, 2015).

4. Comparative Analysis of Algal Growth and Their Oil Contents Production

Mitsuo et al. (1989) worked on *spirulina*, which is a blue-green alga. They developed the cultivation method of this alga in the closed system. They were developing this method to sue this algal specie for space. This development was beneficial to keep the conditions for growing of algae homogenous. This was a good step to obtain the products of algae. The system was made in such a way the carbon dioxide and oxygen control was made beneficial. Moreover, by the separation of algae and biomass, product isolation was made easy. Leghari (2002) carried out the ecological study of algal flora of the Jhelum River in Azad Kashmir during the period of January to June 1998. This included the research on 134 species, belonging to 68 genera from seven groups of algae. The basic idea of the study was to assess the population of this algal flora as they play an important role in a balanced ecological system. Water pollution has increased the population of the flora to dangerously high levels, inducing oxygen-depletion in water, which unbalances the ecosystem as is hazardous for aquatic life. Scarcity of such plants may also be dangerous as they are the primary producers and their scarcity may unbalance the aquatic food chain. Thus, for a balanced ecosystem, a balanced population of the algal flora is necessary. Leghari (2004) carried out the comparative ecological study of algal genera and useful aquatic weeds of various localities. Twenty-nine genera of algae belonging to five classes, 47 aquatic weeds belonging to 21 families of five groups and 35 physicochemical parameters were recorded. Lakes are rich in primary productivity and fish production. The study revealed to search the algal flora and aquatic weeds, water analysis to observe physicochemical properties, comparative study to explore species either rare or common to observe and are beneficial or toxic in use.

Ali (2007) reviewed the limnological studies, keeping in view their strong impact on wetland's biodiversity. The results revealed that among total 103 species of planktons, 51 were found in Uchalli, 47 in Khabbaki and 39 in Jahalar Lake, and 31% species were phytoplankton, whereas 69% were zooplankton. Chisti (2008) revealed that because of the higher photosynthetic efficiency of algae, higher biomass productivities, a faster growth rate than higher plants (which was also important in the screening step), highest CO_2 fixation and O_2 production, growing in liquid medium which can be handled easily, algae can be grown in variable climates and non-arable land including marginal areas unsuitable for agricultural purposes (e.g. desert and seashore lands), in non-potable water or even as a waste treatment purpose, use far less water than traditional crops and do not displace food crop cultures; their production is not seasonal and can be harvested daily. Fernandez et al. (2008) studied viability of marine algae as a source of renewable energy to laboratory scale. They evaluated the anaerobic digestion of *Macrocystis pyrifera, Durvillaea antarctica* and their blend 1:1 (w/w) in a two-phase system and observed methane in biogas yield of algae blend, but a lower biogas yield was obtained. They concluded that methane could be produced in a two-phase digestion system using either algae species or their blend. Mirza et al. (2008) discussed that attention had been given to the use of municipal and industrial wastes for power generation. The government had been financing many projects related to biomass energy development in the country, but still lot more efforts were being needed for harnessing full potential and taking maximum benefit out of this important renewable energy resource.

Gouveia (2009) investigated the screening of microalgae (*Chlorella vulgaris*, *Spirulina maxima*, *Nannochloropsis* sp., *Neochloris oleoabundans*, *Scenedesmus obliquus* and *Dunaliella tertiolecta*), which was performed to choose the best one(s), in terms of quantity and quality as oil source for biofuel production. *N. oleoabundans* (fresh water microalga) and *Nannochloropsis* sp. (marine microalga) proved to be suitable as raw materials for biofuel production because of their high oil content (29.0% and 28.7%, respectively). Both microalgae, when grown under nitrogen shortage, showed a great increase (»50%) in oil quantity. If the purpose is to produce biodiesel only from one species, *S. obliquus* presents the most adequate fatty acid profile, namely in terms of linolenic and other polyunsaturated fatty acids. However, the microalgae *N. oleoabundans, Nannochloropsis* sp. and *D. tertiolecta* can also be used if associated with other micro algal oils and vegetable oils. Sharif et al. (2008) studied the common species *Oedogonium* and *Spirogyra* to compare the amount of biodiesel production. Algal oil and biodiesel (ester) production was higher in *Oedogonium* than *Spirogyra* sp. However, biomass (after oil extraction) was higher in *Spirogyra* than *Oedogonium* sp. Sediments (glycerin, water and pigments) were also higher in *Spirogyra* than *Oedogonium* sp. There was no difference of pH between *Spirogyra* and *Oedogonium* specie. These results indicate that biodiesel can be produced from both species and *Oedogonium* is better source than *Spirogyra* specie.

Park et al. (2011) reported that algae could use growth nutrients such as nitrogen and phosphorous from a variety of wastewater sources (agricultural run-off, concentrated animal feed operations and industrial and municipal wastewater), thus providing a sustainable bioremediation of these waste water for economic benefits).

Prem et al. (2009) reviewed that worldwide, many nations impose blending of their transport fuels with biofuels, approximating 10% globally by 2020, to contribute to energy security while reducing emission of greenhouse gases. Pienkos and Darzins (2009) concluded that the comparison of one unique aspect of algae with other advanced feedstock was the spectrum of species available for amenability for biofuel production. Various species may be selected to optimize the production of different biofuels. Algae offer a diverse spectrum of valuable products and pollution solutions such as food, nutritional compounds, animal feed, energy sources (including jet fuel, aviation gas, biodiesel, gasoline and bioethanol), organic fertilizers, biodegradable plastics, recombinant proteins, pigments, medicines, pharmaceuticals and vaccines. A number of microorganisms belonging to the genera of algae, yeast, bacteria and fungi have ability to accumulate neutral lipids under specific cultivation conditions. The microbial lipids contained high fractions of polyunsaturated fatty acids and had the potential to serve as a source of significant quantities of transportation fuels (Liam and Owende, 2010). Subhadra and Edwards (2010) studied that algal biomass provided viable third generation feedstock for liquid transportation fuel that does not compete with food crops for cropland. However, fossil energy inputs and intensive water usage diminishes the positive aspects of algal energy production. An integrated renewable energy park approach was proposed for aligning renewable energy industries in resource-specific regions in the United States for synergistic electricity and liquid biofuel production from algal biomass with net zero carbon emissions.

5. Role of Gene Technologies in Biodiesel Production

Genomics can play a key role in solving the world energy crisis. Through genomics, scientists can develop a better approach of how to harness various renewable sources of energy, such as cyanobacteria, microalgae and lignocellulosic biomass. One day on a global scale, the development of sustainable biofuel through genetic engineering of an enzyme will replace fossil fuels (Davidson, 2008). Cellulose biomass has the capability to meet the demand for liquid fuel, but for high-scale deployment of biomass-to-biofuel technologies, process inefficiencies and land-use requirements are the main obstacles. Thus, the genomics knowledge of microorganisms can break down the biomass, and potential energy crops will be vital for improving the prospects of important cellulosic biofuel production (Rubin, 2008). The genomic information of bioenergy crops will also accelerate the domestication of these plants, and even will improve their growth on poor agricultural lands to avoid competition with other food crops (Rubin, 2008). Further genetic underpinning, such as cell wall chemistry, stem thickness, branching habit, competition for light and growth rates, will maximize biomass yield per unit land area (Tuskan et al. 2006; Rubin, 2008). Currently, intensive global researches, for increasing and modifying the accumulation of energy rich compounds, such as polysaccharides, hydrocarbons, alcohols, lipids and other energy rich chemical compounds in photosynthetic organisms, bacteria and yeast *via* genetic engineering have been carried out with good results. Although many advances through genetic engineering in photosynthetic microorganisms have been achieved, because of their capability to secrete or store energy-rich hydrocarbons, superior growth rates, diverse metabolic abilities and high photosynthetic conversion efficiencies (Radakovits et al., 2010). Still, further studies are needed to extend these advances to industrially relevant microorganisms.

6. Conclusion

Although much work has been performed, identification of suitable specie having good characteristics not only production wise but also growth wise is needed. The growth of algae needs suitable environment, and work on its cultivation and harvesting is needed to be developed that a large amount could be grown. Technical experience and technological development are also required. More advancement in genetically engineering species growth is needed to produce required amounts of biodiesel in short time and less energy utilization.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgement

The authors are thankful to CAS-TWAS president's fellowship program and State Key Laboratory of Freshwater Ecology and Biotechnology (Grant No. Y119011F01) for financial support.

References

Ali Z, Ahmad SS, Akhtar M, Khan MA, Khan MN. 2007. Ecology and diversity of planktons in lakes of Uchalli wetlands complex. *Pak J Anim Plt Sci* 17:1–2.

Balat M. 2007. An overview of biofuels and policies in the European Union. *Energy Sources B Econ Plann* 2(2):167–81.

Banerjee A, Sharma R, Chisti Y, Banerjee UC. 2002. Botryococcus braunii a renewable source of hydrocarbons and other chemicals. *Crit. Rev. Biotech* 22(3): 245–79.

Becker K, Makkar HPS. May 2008. Jatropha curcas: A Potential Source for Tomorrow's Oil and Biodiesel. *Lipid Technol* 20(5):104–7.

Charles MB, Ryan R, Ryan N, Oloruntoba R. 2007. Public policy and biofuels: the way forward? *Energy Policy* 35(11):5737–46.

Chisti Y. 2007. Biodiesel from microalgae. *Biotechnology Adv* 25(3):294–306.

Chisti Y. 2008. Biodiesel from microalgae beats bioethanol. *Trends in Biotechnol* 26(3):126–31.

Chisti Y. 2013. Constraints to commercialization of algal fuels. *Journal of Biotechnol* 167(3):201–14.

Chojnacka K, Chojnacki A, Gorecka H. 2005. Biosorption of Cr 3+, Cd 2+ and Cu 2+ ions by blue–green algae *Spirulina* sp.: kinetics, equilibrium and the mechanism of the process. *Chemosphere* 59(1):75–84.

Chojnacka K, Marquez-Rocha FJ. 2004. Kinetic. Stoichiometric relationships of the energy and carbon metabolism in the culture of microalgae. *Biotechnology* 3(1): 21–34.

Davidson S. 2008. Sustainable bioenergy: genomics and biofuels development. *Nat Educ* 1(1):175.

Dufey. 2006. Biofuels Production, Trade and Sustainable Development: Emerging Issues. London: International Institute for Environment and Development, London.

Dunahay TG, Jarvis EE, Dais SS, Roessler PG. 1996. Manipulation of microalgal lipid production using genetic engineering. *Appl Biochem Biotechnol* 57–58:223–31.

Dürre P. 1997. Biobutanol: an attractive biofuel. *Biotechnol J* 2(12):1525–34., 2007 Dec.

Fernández AV, Vargas G, Alarcón N, Velasco A. 2008. Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. *Biomass and Bioenergy*:338–44.

Gouveia, Oliveira LAC. 2009. Micro algae as a raw material for biofuels production. *J. Ind. Microbial Biotech* 36:269–74.

Grima EM, Belarbi EH, Fernández FA, Medina AR, Chisti Y. 2003. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnol Advances* 20(7):491–515.

Kaewpintong K. 2004. Cultivation of *Haeatococus pluvalis* in Airlift Bioreactor. Master Thesis in Chemical Engineering, Department of Chemical Engineering Faculty of Engineering. Chulalongkorn University.

Kligerman DC, Bouwer EJ. 2015. Prospects for biodiesel production from algae-based wastewater treatment in Brazil: a review. *Renewable and Sustainable Energy Reviews* 52:1834–46.

Lam MK, Lee KT. 2011. Renewable and sustainable bioenergies production from palm oil mill effluent (POME): win–win strategies toward better environmental protection. *Biotechnology Advances* 29(1):124–41.

Lam MK, Lee KT. 2012. Microalgae biofuels: a critical review of issues, problems and the way forward. *Biotechnology Advances* 30(3):673–90.

Leghari MK, Leghari MY, Shah M, Arbani SN. 2004. Comparative ecological study of algal genera and useful aquatic weeds of various localities of Pakistan. *Pak J Water Resources* 8(2):17–28.

Leghari MK, Shah M, Leghari MY. 2002. Ecological study of algal flora of Jhelum river-Azad Kashmir [Pakistan]. *J Drain Water Manag (Pakistan)*.

Liam B, Owende P. 2010. Biofuels from microalgae. A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews* 14(2):557−77.

Mes TZD d, Stams AJM, Reith JH, Zeeman G. 2003. Methane production by anaerobic digestion of wastewater and solid wastes. Bio-methane & bio-hydrogen: status and perspectives of biological methane and hydrogen production. pp. 58−102.

Mirza UK, Ahmad N, Majeed T. 2008. An Overview of Biomass Energy Utilization in Pakistan. pp. 1988−96.

Mitsuo, Oguchi M, Otsubo K, Nitta K, Shimada A, Fujii S, Koyano T, Miki K. 1989. Closed and continuous algae cultivation system for food production and gas exchange in CELSS. *Adv Space Res*:169−77.

O European Commission. 2006. An EU Strategy for Biofuels. Communication from the Commission COM. (2006) 34 final, Brussels: 29.

Park JBK, Craggs RJ, Shilton AN. 2011. Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology* 102(1):35−42.

Patil V, Tran KQ, Giselrød HR. 2008. Towards sustainable production of biofuels from microalgae. *International Journal of Molecular Sciences* 9(7):1188−95.

Pienkos PT, Darzins A. 2009. The promise and challenges of micro-algal derived biofuels. *Biofuel Bioprod Biorefin* 3:431−40.

Pittman JK, Dean AP, Osundeko O. 2011. The potential of sustainable algal biofuel production using wastewater resources. *Bioresour Technol* 102(1): 17−25.

Bindraban Prem S, Bulte Erwin H, Conijn Sjaak G. 2009. Can large-scale biofuels production be sustainable by 2020. *Agric Syst* 101(Iss. 3):197−9.

Radakovits R, Jinkerson RE, Darzins A, Posewitz MC. 2010. Genetic engineering of algae for enhanced biofuel production. *Eukaryotic Cell* 9(4):486−501.

Rawat I, Kumar RR, Mutanda T, Bux F. 2011. Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Appl Energy* 88(10):3411−24.

Reith JH. 2004. Duurzame co-productie van fijnchemicaliën en energie uit microalgen: openbaar eindrapport E.E.T. project K99005/398,510−1010.

Richmond A. 2004. Handbook of Micro Algal Culture: Biotechnology and Applied Phycology. Blackwell Science Ltd.

Roberts GW, Fortier MOP, Sturm BS, Stagg-Williams SM. 2013. Promising pathway for algal biofuels through wastewater cultivation and hydrothermal conversion. *Energy & Fuels* 27(2):857−67.

Roessler PG, Brown LM, Dunahay TG, Heacox DA, Jarvis EE, Schneider JC, et al. 1994. Genetic-engineering approaches for enhanced production of biodiesel fuel from microalgae. *ACS Symp Ser* 566:255−70.

Rubin EM. 2008. Genomics of cellulosic biofuels. *Nature* 454(7206):841−5.

Sawayama S, Inoue S, Dote Y, Yokoyama S-Y. 1995. CO2 fixation and oil production through microalga. *Energy Convers Manag* 36:729−31.

Schenk PM, Thomas-Hall SR, E. S, Marx UC, Mussgnug JH, Posten C, Kruse O, B. 2008. Hankamer second generation biofuels: high-efficiency microalgae for biodiesel production. *Bioenerg Res* 1:20−43.

Sharif HABM, Salleh A, Boyce AN, Chowdhury P, Naqiuddin M. 2008. Biodiesel fuel production from algae as renewable energy. *Am J Biochem Biotech* 4(3):250−4.

Shay EG. 1993. Diesel fuel from vegetable oils: status and opportunities. *Biomass and Bioenergy* 4(4):227−42.

Sheehan J, Dunahay T, Benemann J, Roessler P. 1998. A look back at the U.S. Department of Energy's Aquatic Species Program—biodiesel from algae. Golden, CO: National Renewable Energy Laboratory, Golden, CO.

Subhadra B, Edwards M. 2010. An integrated renewable energy park approach for algal biofuel production in United States. *Energy Policy* 38(9):4897−902.

Tsukahara K, Sawayama S. 2005. Liquid fuel production using microalgae. *J Jpn Pet Inst* 48(5):251.

Tuskan GA, Difazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Schein J. 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* 313(5793):1596−604.

Ugwu CU, Aoyagi H, Uchiyama H. 2008. Photobioreactors for mass cultivation of algae. *Bioresour Technol*:4021−8.