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Biofuels from Microalgae

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Abstract

Biofuels are the most awaited products of scientific research. The fossil fuels are being exhausted, and pollution is increasing globally. Algal biofuels are one of the promising options. They are wonderful tiny factories that yield a variety of substances that have the property to act as sources of ecofriendly fuels. More attention has been focused on microalgae-derived biomass for generating diverse renewable energy sources. The distinct features that microalgae possess include high biomass yield, abundant oil content, no requirement for land and easy cultivation in wastewaters coupled with carbon dioxide mitigation. Microalgae are tiny reservoirs of a plethora of biofuels. The diverse algal biofuels range from biodiesel, straight vegetable oil (SVO), lipids, ethanol and hydrogen. Biofuels are the need of today, and researchers around the globe are exploring the options for biological fuel production.

Keywords: microalgae, biofuels, renewable energy, algal biomass, biorefinery

1. Introduction

The choice for the most suitable energy carrier to be produced from algae is a promising option. Algae have been explored for their unique potential to yield a variety of biofuels concomitantly with generation of value-added products and phycoremediation of wastewater (**Figure 1**). Many algal strains like *Chlamydomonas*, *Chlorella*, *Scenedesmus*, *Botryococcus braunii*, and so on have been reported to produce biofuels (**Table 1**). The selection of algal strains is exclusively dependent on various factors like oil content, production yield and downstream processing and also on adaptability of microalgae toward high oxygen concentration, temperature variations and water chemistry [1].

The algal metabolism consisting of photosynthetic potential, which makes it unique in comparison to other microorganisms when it comes to processing sugars from cellulosic sources

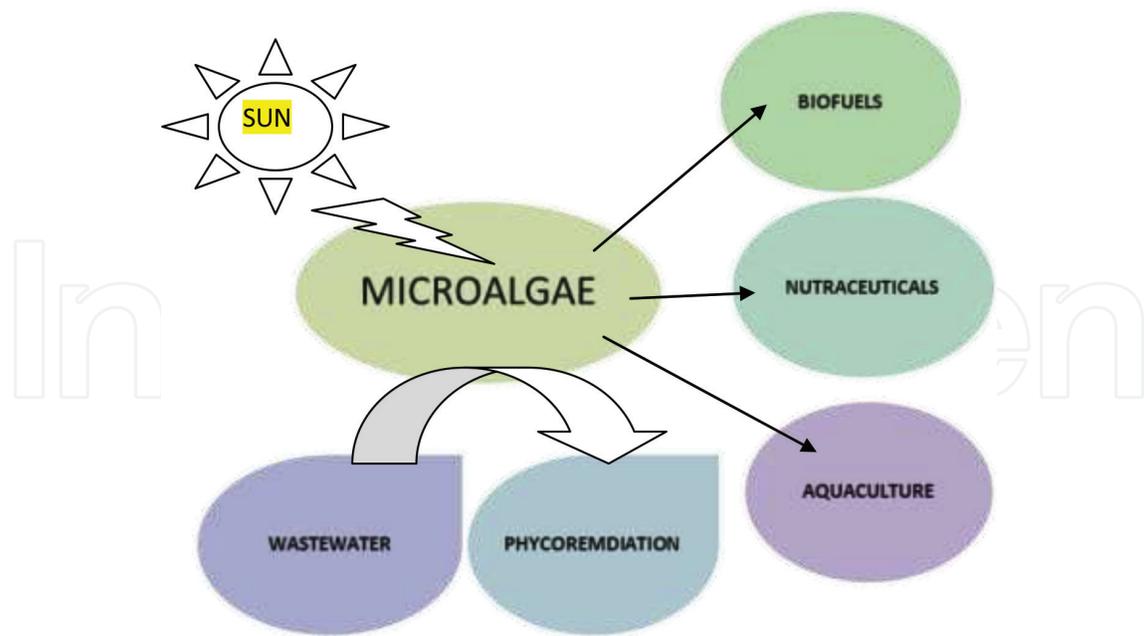


Figure 1. Potentials of microalgae.

such as grass and wood chips. After algal biomass degradation into sugar, there are substances like lignin associated with it, which are toxic to microorganisms. Removal of lignin is, thus, essential to promote further microbial growth leading to processing of sugar. Algae are tolerant to the presence of lignin, which makes the processing convenient coupled with reduction in the economic cost. In addition to this, there are other applications of algae like aquaculture, high-value products and nutraceuticals, which can be extracted from algae [2]. The microalgae require minimal inputs for metabolic processes—namely sunlight, CO_2 and water, with few required mineral nutrients. Sunlight is the most readily available and inexpensive source of energy on earth. The efficiency of microalgae in converting captured solar energy into biomass exceeds the potential of terrestrial plants. Microalgae do not compete with terrestrial plants for land or water supply as they can be grown in wastewater, leading to their remediation coupled with biomass production. The acumen of microalgae to inhabit diverse habitats could be exploited to allow for the production of compounds near the site of use, which could reduce the transportation costs [3].

1.1. Advantages of microalgae as source of biofuels

Microalgae are one of the most promising candidates for plethora of biofuels owing to their easy, inexpensive and simple cultivation system. They grow easily with basic nutritional requirements like air, water and mineral salts with light as the only energy source. They grow on liquid media, so diverse wastewater can also be utilized, which can be efficiently remediated by algae coupled with biofuel production.

The optimal use of light energy through photosynthesis is very efficiently executed by microalgae. They possess higher photosynthetic levels and growth rates and can be used for the production of desired biofuels. They can contain considerable amounts of lipids that are mainly

Species	Product	Yield	
<i>Chlamydomonas reinhardtii</i> (CC124)	Biohydrogen	102 mL/1.2 L	
		0.58 mL/hL	
		0.30 mol/m ²	
		0.6 mL/L h	
<i>Chlamydomonas reinhardtii</i> (Dang 137C mt+)		175 mL/L	
		4.5 mmol/L	
		71 mL/L	
<i>Chlorella vulgaris</i> MSU 01		26 ml/0.5L	
<i>Scenedesmus obliquus</i>		3.6 ml/μgChl a	
<i>Platymonas subcordiformis</i>		11,720 nL/h	
		7.20 mL /h	
		0.339 mL/hL	
<i>Dunaliella tertiolecta</i>	Bio-oil	43.8%, 34 MJ/Kg	
		42.6%, 37.8 MJ/Kg	
		25.8%, 30.74 MJ/Kg	
<i>Chlorella protothecoides</i>		52%	
		57.9%	
<i>Chlorella sp</i>		28.6%	
<i>Chlorella vulgaris</i>		35.83%	
<i>Nannochloropsis sp.</i>		31.1%	
<i>Chlorella vulgaris</i>	Biogas	0.63-0.79 LCH ₄ /gVS	
<i>Dunaliella salina</i>		0.68 LCH ₄ /gVS	
<i>Euglena gracilis</i>		0.53 LCH ₄ /gVS	
<i>Scenedesmus</i>		140 LCH ₄ /KgVS	
<i>Scenedesmus</i> (Biogas from lipid-free biomass)		212 LCH ₄ /KgVS	
<i>Scenedesmus</i> (Biogas from amino acids-free biomass)		272 LCH ₄ /KgVS	
<i>Scenedesmus obliquus</i>		0.59-0.69 LCH ₄ /gVS	
<i>Botryococcus braunii</i>		Lipid content for	25-75%
<i>Chlorella sp.</i>		Biodiesel	28-32%
<i>Chlorella vulgaris</i>			56%
<i>Cryptocodinium cohnii</i>	20%		
<i>Monallanthussalina</i>	20-70%		
<i>Nannochlorisis sp</i>	20-35%		
<i>Nannochloropsis sp</i>	31-68%		
<i>Neochlorisoleo abundans</i>	35-54%		
<i>Nitzschiasp</i>	45-47%		
<i>Scenedesmus dimorphus</i>	6-40%		
<i>Scenedesmus obliquus</i>	11-55%		
<i>Schizochytrium sp.</i>	77%		
<i>Chlorella pyrenoidosa</i>	Carbohydrates		26%
<i>Chlorella vulgaris</i>			content for
<i>Dunaliella salina</i>	Bioethanol		32%
<i>Scenedesmus obliquus</i>			10-17
<i>Porphyridium cruentum</i>			40-57%
<i>Euglena gracilis</i>			14-18%

Table 1. Biofuel yields from microalgae [27].

present in the thylakoid membranes. Their biofuels are nontoxic and highly biodegradable. They are essentially free-living chloroplasts and are the pinnacle of minimizing structural component. They have high carbon dioxide sequestering efficacy thereby, reducing GHG emissions.

They reduce nutrient load in wastewater as they can utilize nitrogen and phosphorous present in agricultural, industrial and municipal wastewater owing to their phycoremediation acumen. They can be cultivated in areas like seashore, desert, and so on, which is not suitable for agricultural plants and not competing with cultivable land. Their cultivation is independent of seasons as they can be cultivated round the year and have minimal environmental impact. The cultures can be facilitated to produce high yields through technological interventions of genetic engineering, synthetic biology, metabolic engineering, and so on as algal systems are readily adaptable.

The biofuels from algae are diverse in nature. Carbohydrate component of biomass is used for bioethanol production, while algal oil for biodiesel and the residual biomass can be utilized for methane, fuel gas or fuel oil production. The biomass after biofuel production can further be used as source of many value-added products like eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), nutraceuticals, protein supplements, therapeutics, biocontrol agents, fertilizers, animal feed and aquaculture.

1.2. Biofuels derived from microalgae

A plethora of biofuels are derived from microalgae by virtue of their unique potential (Figure 2). The biofuels include alcohols, which are produced through fermentation, processing of algal biomass through dual approach of hydrolysis and fermentation, traditional method of transesterification, gasification of biomass or Fischer-Tropsch synthesis [4].

1.2.1. Biodiesel

Biodiesel has comparable engine performance to petroleum diesel fuel, while reducing sulfur and particulate matter emissions [5, 6]. Biodiesel is a biodegradable alternative fuel derived from renewable sources and is nontoxic in nature [7]. During the manufacturing process, triacylglycerols (TAGs) are transesterified with an acid or alkali catalyst to produce biodiesel and glycerol [8].

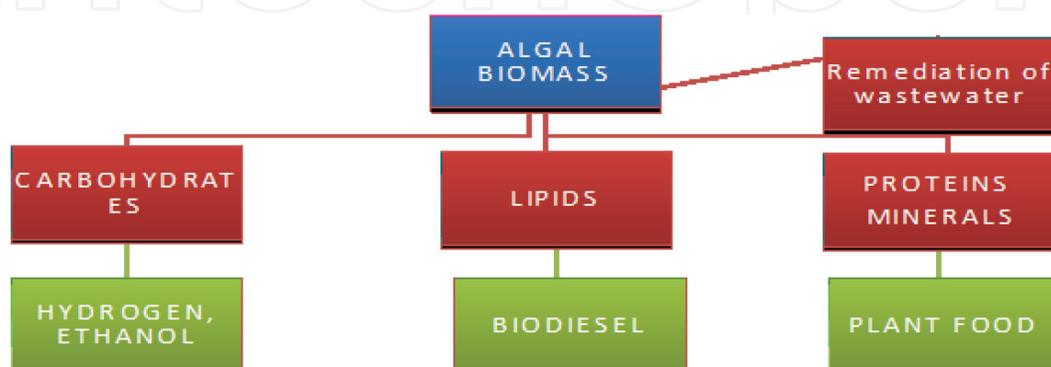


Figure 2. Biofuels derived from microalgae.

The algal biodiesel production processes fatty acid methyl esters (FAME). The chemical composition of biodiesel is generally produced by transesterification of algal oil in the presence of acid or alkali as a catalyst [5]. The biodiesel from algae can be derived directly from transesterification of algal biomass [9]. Alternately, it can also be produced by two-step process wherein the lipids are initially extracted and later on transesterified, though either of the processes involves lipid extraction through solvents and alcohols like methanol, isopropanol and petroleum ether [8, 10]. The process of direct transesterification is fast and cost-effective technology. Biodiesel generated from microalgae can be an excellent alternative to current diesel crisis, but in order to efficiently produce biodiesel from microalgae, strains with a high growth rate and oil content have to be selected [11].

1.2.2. Biogas

The anaerobic digestion of organic matter leads to formation of fuel called biogas or biomethane. Biogas is mainly formed by methane (55–75%) and CO₂ (25–45%). There are four stages of anaerobic digestion [12], which are described as follows:

- Biopolymer hydrolysis to monosaccharaides mediated by hydrolytic bacteria.
- Conversion of monosaccharaides to acids via fermentation.
- Action of acetogenic bacteria leading to the formation of acetate.
- Methane and carbon dioxide formation by methanogenic bacteria.

Microalgae has been reported to produce biogas as source of fuel, although the yield of biogas formation is quite low because of the sensitivity of algal cells to bacterial degradation and low carbon and nitrogen (C:N) ratio, which leads to the formation of inhibitor (ammonia). In *Scenedesmus* spp., residual biomass free from lipids and amino acids was investigated for biogas production, and results exhibited that residual biomass gives better biogas yield compared to raw biomass [13].

1.2.3. Hydrocarbons

The microalga species are capable of producing hydrocarbons, which can further be converted to diesel, kerosene and gasoline. The microalga, *Botryococcus braunii*, has been reported to produce hydrocarbons with excellent oil yield [14]. The habitat of *B. braunii* is freshwater, which can be one of the factors leading to its adaptation to varied salt concentration. In addition to this, the hydrocarbons from *B. braunii* get deposited outside the cell, thus the extraction becomes relatively easier and convenient [15–17].

1.2.4. Hydrogen

Microalgae can directly produce hydrogen from sunlight and water, only in the complete absence of oxygen. Hydrogen is a promising future energy source because it does not emit greenhouse gases and releases water as a by-product [18]. There are limitations existing regarding the large-scale production of hydrogen as fuel. At present, hydrogen is produced by steam reformation, photofermentation [19] and photolysis of water mediated by photosynthetic algae

[20]. Purple non-sulfur bacteria derive hydrogen from diverse substrates, while green sulfur bacteria get hydrogen gas from hydrogen sulfide (H_2S). Other microalgae can make hydrogen directly from sunlight and water, although only in the complete absence of oxygen.

Exploring new organisms for hydrogen production, optimization of growth conditions and use of biotechnological techniques can open new doors in making hydrogen a viable fuel for future [21, 22].

1.2.5. *Biosyngas*

Biosyngas is produced by the biomass gasification in presence of oxygen, water vapor or air, produces carbon monoxide, hydrogen, methane, water, other hydrocarbons and ashes. For gasification, high temperature (800–1200°C) is essential, and the feedstock needs to have not more than 20% water content in the biomass [23]. Electricity can be produced by burning in boilers and turbines and subsequently, kerosene, wax, naphtha and gasoline can be obtained [24].

1.2.6. *Ethanol*

Ethanol production from or by microalgae has very interesting prospects, but is currently only in the preliminary phase of research. Bioethanol can be used as a biofuel, which can replace part of the fossil-derived petrol. More development is needed to analyze a full-scale production system. Currently, bioethanol is produced by fermenting sugars, which in the case of corn are derived from hydrolyzing starch. Microalgae species with starch content of over 50% have been reported. With new technologies, cellulose and hemicellulose can be hydrolyzed to sugars [25]; thereby, facilitating formation of ethanol from major part of dry algal biomass. Compared to the traditional use of woody biomass, microalgae hold better options some of which are enlisted below [26]:

- Microalgae lack lignin, so the processing becomes easier.
- The microalgal cellular composition is very simple and biomass can be utilized readily.
- Microalgal cells consist of copious amounts of polysaccharides, which can be converted to sugar.
- Microalgae can be genetically engineered to produce ethanol.

Ethanol production from or by microalgae has very interesting prospects, but is currently only in the preliminary phase of research. More development is needed to analyze a full-scale production system. **Table 1** highlights the biofuels produced from different species of microalgae round the globe.

1.3. Cultivation of microalgae for biofuel production

The development of dedicated culture systems for microalgae started in the 1950s when algae were investigated as an alternative protein source for the increasing world population. Subsequently, the diverse products and the bioremediation of wastewater potential of algae

were explored. The initiation of research on algae as a source of renewable energy began by virtue of the energy crisis in the 1970s. The cultivation of algae requires few relatively simple conditions: light, water, carbon source, micro- and macronutrients and optimum temperature. Over the years, different culture systems have been developed keeping in mind the optimum conditions for microalgal growth, although it is a challenging task. The cultivation system for the growth of algae is an important requirement to aid in enhanced production of biofuels which includes open air ponds and closed controlled systems. The development of profitable algae-based fuel generation technology is yet in transition state wherein the final configuration is still to be explored and demonstrated at the industrial scale [29].

The cultivation of microalgae is a significant factor leading to enhanced biofuel production. The choice of cultivation system has to be emphasized because the phycoremediation efficiency and the yield of biofuels and other value-added products would largely depend on it. Broadly, the cultivation systems meant for microalgae are either open systems or closed systems. Hybrid systems, which are a combination of an open system and a closed system, can be used to achieve high biomass productivity with high nutrient removal [28].

1.3.1. Open microalgal systems

The open microalgal pond systems are commonly used for cultivation of microalgae as they have good opportunity to utilize the atmospheric carbon dioxide readily available in the atmosphere. There are several configurations of microalgae cultivation systems for biomass production and enhanced phycoremediation of industrial, domestic and agricultural wastewaters. The most commonly used systems for research and industrial microalgal cultivation are as follows:

- The raceway pond
- The circular pond tank
- The shallow big pond
- The closed pond

For open systems, location is an important criterion keeping in mind, the sufficient sunlight availability and the requirement of the algae to be cultivated. The open ponds can be natural or artificial in nature and usually include natural lagoons, circular ponds, tanks and raceway ponds. Cultivation of *Chlorella* sp. was traditionally done in circular ponds, which are usually made up of concrete. They are also equipped with rotating arm to ensure mixing of the culture and prevention of sedimentation of algal biomass. Generally, the raceway ponds comprise race track or oval channel made up of concrete, and they are meant to circulate nutrients and carbon dioxide regularly to the algal cultures [30].

1.3.2. Closed algal systems

The closed systems (photobioreactors (PBRs)) have well-controlled growth conditions. Generally, these reactors are designed to increase the light accessibility. They also allow

perfect mixing to permit the light to be within an optimum value for cell growth and to improve gas exchange. Since photobioreactors solve many problems of the open cultivation, researchers have focused on designing photobioreactors for large microalgal biomass production [30]. There is a wide variation in the design of the photobioreactor depending upon their geometry and construction. Photobioreactors can be built as bags tanks, and towers. Photobioreactors can be plates or tubular and made up of plastic or glass. Tubular photobioreactors seem to be the most suitable. Bubble columns and airlift photobioreactors can also be considered since they produce a relatively high concentration of microalgal biomass product [31]. An auxiliary tank is used to separate the oxygen produced from the photosynthesis. This is important considering that excessive oxygen can negatively affect the microalgae growth [32]. Despite the advancements in the design of photobioreactors for enhancing biofuel productivity in algae cultivation, bottlenecks are yet to be addressed efficiently considering the cost economics of biofuels and their productivity.

1.3.3. Hybrid algal systems

The hybrid systems are cost-effective and can be used for large algae cultivation [33]. Hybrid systems overcome the limitations of open systems and the high initial and operating cost associated with closed systems. In the hybrid system, the microalgae are initially cultured in closed and controlled photobioreactor system and then shifted to open system in order to enhance the biomass yield [34]. This system offers promising options for algal cultivation toward biofuel production.

2. Conclusion

Alternate energy sources are to be explored because nonrenewable energy sources are getting depleted, and environmental pollution is increasing globally. The economic viability of microalgal biofuel production should be significantly enhanced by a high-value coproduct strategy, which would, conceptually, involve sequentially the cultivation of microalgae in a microalgal farming facility (CO₂ mitigation), extracting bioreactive products from harvested algal biomass, thermal processing and utilizing residual biomass for extraction of high-value products. The synergy approach toward biofuel production can make the technology more viable and economically more feasible [35].

Enhanced production of these biofuels will help conserve our natural resources and save our environment. Algae technology has enormous potential not only for algae-based biofuels but also for food, feed, renewable chemicals and many other products that are critical for a more sustainable society. Major technological challenges like effective design of photobioreactors, innovative upstream processing and downstream processing ought to be addressed before commercialization of microalgae as a source of biofuels for a better future.

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References

- [1] Thomas Kiran M, Narasimha Reddy P, Archana T. Potential of diatom consortium developed by nutrient enrichment for biodiesel production and simultaneous nutrient removal from waste water. *Saudi Journal of Biological Sciences*. 2017. DOI: 10.1016/j.sjbs.2017.05.011 (in Press)
- [2] Xiao-li L, Thomas Kiran M, Ling T, Archana T, Gu L. A novel growth method for diatom algae in aquaculture wastewater for natural food development and nutrient removal. *Water Science & Technology*. 2017;**75**(12):2777-2783
- [3] Thomas Kiran M, Archana T, Bhaskar MV. A new novel solution to grow diatom algae in large natural water bodies and its impact on CO₂ capture and nutrient removal. *Journal of Algal Biomass Utilization*. 2015;**6**(2):22-27
- [4] Vasudevan PT, Fu B. Environmentally sustainable biofuels: Advances in biodiesel. *Waste and Biomass Valorization*. 2010;**1**:47-63
- [5] Miao XL, QY W. Biodiesel production from heterotrophic microalgal oil. *Bioresource Technology*. 2006;**97**(6):841-846
- [6] Scragg A, Illman A, Carden A, Shales S. Growth of microalgae with increased calorific values in a tubular bioreactor. *Biomass and Bioenergy*. 2002;**23**(1):67-73
- [7] Hossain A, Salleh A, Boyce A, Chowdhury P, Naquiuddin M. Biodiesel fuel production from microalgae as renewable energy. *American Journal of Biochemistry and Biotechnology*. 2008;**4**:250-254
- [8] Johnson M, Wen Z. Production of biodiesel fuel from the microalga *Schizochytrium limacinum* by direct transesterification of algal biomass. *Energy & Fuels*. 2009;**23**(10):294-306
- [9] Lewis T, Nichols PD, McMeekin TA. Evaluation of extraction methods for recovery of fatty acids from lipid-producing micro heterotrophs. *Journal of Microbiological Methods*. 2000;**43**(2):107-116

- [10] Mulbry W, Kondrad S, Buyer J, Luthria D. Optimization of an oil extraction process for microalgae from the treatment of manure effluent. *Journal of the American Oil Chemists' Society*. 2009;**86**:909-915
- [11] Xiao-li L, Thomas Kiran M, Ling T, Rong L, Archana T, Gu L. Optimization of growth conditions and fatty acid analysis for three freshwater diatom isolates. *Phycological Research*. 2017;**65**(3):177-187
- [12] Oncel SS. Microalgae for a macroenergy world. *Renewable and Sustainable Energy Reviews*. 2013;**26**:241-264
- [13] Ramos-Suárez JL, Carreras N. Use of microalgae residues for biogas production. *Chemical Engineering Journal*. 2014;**242**:86-95
- [14] Hillen LW, Pollard G, Wake LV, White N. Hydrocracking of the oils of *Botryococcus Braunii* to transport fuels. *Biotechnology and Bioengineering*. 1982;**24**(1):193-205
- [15] Qin J. *Bio-Hydrocarbons from Microalgae: Impacts of Temperature, Light and Salinity on Microalgae Growth*. Barton: RIRDC; 2005
- [16] Wijffels R. *Energie via microbiologie: Status en toekomstperspectief voor Nederland*. Utrecht: SenterNovem; 2006
- [17] RangaRao A, Ravishankar GA. Influence of CO₂ on growth and hydrocarbon production in *Botryococcus braunii*. *Journal of Microbiology and Biotechnology*. 2007;**17**(3):414-419
- [18] Archana T, Anjana P. Cyanobacterial hydrogen production: A step towards clean environment. *International Journal of Hydrogen Energy*. 2012;**37**:139-150
- [19] Kapdan IK, Kargi F. Bio-hydrogen production from waste materials. *Enzyme and Microbial Technology*. 2006;**38**:569-582
- [20] Ran CQ, Chen ZA, Zhang W, Yu XJ, Jin MF. Characterization of photo-biological hydrogen production by several marine green microalgae. *Wuhan LigongDaxueXuebao*. 2006;**28**(2):258-263
- [21] Pandey A, Pandey A, Srivastava P, Pandey A. Using reverse micelle as microreactor for hydrogen production by coupled system of *Nostoc/P4* and *Anabaena/P4*. *World Journal of Microbiology & Biotechnology*. 2007;**23**:269-274
- [22] Melis A, Happe T. Hydrogen production. Green microalgae as a source of energy. *Plant Physiology*. 2001;**127**(3):740-748
- [23] Ghasemi Y, Rasoul-Amini S, Naseri AT, Montazeri-Najafabady N, Mobasher MA, et al. Microalgae biofuel potentials (review). *Applied Biochemistry and Microbiology*. 2012;**48**:126-144
- [24] Cuellar-Bermudez SP, García-Perez JS, Rittmann BE, Parra-Saldivar R. Photosynthetic bioenergy utilizing CO₂: An approach on flue gases utilization for third generation biofuels. *Journal of Cleaner Production*. 2015;**98**:53-65. DOI: 10.1016/j.jclepro.2014.03.034

- [25] Hamelinck CN, van Hooijdonk G, Faaij APC. Ethanol from lignocellulosic biomass: Techno-economic performance in short-, middle- and long-term. *Biomass & Bioenergy*. 2005;**28**(4):384-410
- [26] Deng MD, Coleman JR. Ethanol synthesis by genetic engineering in cyanobacteria. *Applied and Environmental Microbiology*. 1999;**65**(2):523-528
- [27] Cuellar-Bermudez SP, Romero-Ogawa MA, Rittmann BE, Parra-Saldivar R. Algae biofuels production processes, carbon dioxide fixation and biorefinery concept. *Journal of Petroleum & Environmental Biotechnology*. 2014;**5**:185. DOI: 10.4172/2157-7463.1000185
- [28] Shaikh Abdur R, Saad Aldin MA, Mohammad Mozahar H, Hugo L. Biological CO₂ fixation with production of microalgae in wastewater: A review. *Renewable and Sustainable Energy Reviews*. 2017;**76**:379-390. DOI: 10.1016/j.rser.2017.02.038
- [29] FAO. Microalgae-based Biofuels: A Review of Challenges and Opportunities for Developing Countries. 2010; pp. 1-49. www.fao.org/bioenergy/aquaticbiofuels
- [30] Brennan L, Owende P. Biofuels from microalgae: A review of the technologies for production processing and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*. 2010;**14**:557-577
- [31] Ugwu CU, Aoyagi H, Uchiyama H. Photobioreactors for mass cultivation of algae. *Bioresource Technology*. 2008;**99**:4021-4028
- [32] Rawat I, Ranjith Kumar R, Mutanda T, Bux F. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Applied Energy*. 2013;**103**:444-467
- [33] Fon Sing S, Isdepsky A, Borowitzka M, Moheimani NR. Production of biofuels from microalgae. *Mitigation and Adaptation Strategies for Global Change*. 2011;**18**:47-72
- [34] Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, et al. Second generation biofuels: High-efficiency microalgae for biodiesel production. *Bioenergy Research*. 2008;**1**:20-43
- [35] Li YQ, Horsman M, Wang B, Wu N, Lan CQ. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. *Applied Microbiology and Biotechnology*. 2008;**81**(4):629-636

