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Review Article on

Microalgae: Future biofuel

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Uses of non renewable sources like petroleum increase the production of green house gases and global warming. To overcome these problems use of renewable sources is necessary. Microalgae are a renewable source of energy. We can produce biofuels like lipid, methane, bioethanol and hydrogen from them; they are more efficient than conventional plants and can be cultivated using simple methods. Main focus of this review article is to point out the role of microalgae in the production of biofuels, their biosynthesis and comparison of algal biofuel with other sources.

[**Key words:** Petroleum, Microalgae, Biofuel, Methane, Bioethanol]

Introduction

Microalgae are classified as the most primitive form of plants. The mechanism of photosynthesis in microalgae is similar to that in higher plants, but they are usually more efficient converters of solar energy because of their simple cellular structure. They normally grow in suspension within a body of water [1]. They can double every few hours during their exponential growth period[2].They commonly double every 24 hrs. During the peak growth phase, some microalgae can double every 3.5 hrs[3]. Microalgae are veritable miniature biochemical factories, and appear more photosynthetically efficient than terrestrial plants [4] and are efficient CO_2 fixers [5].The ability of algae to fix CO_2 has been proposed as a method of removing CO_2 from flue gases from power plants, and thus can be used to reduce emission of GHG. Many algae are exceedingly rich in oil, which can be converted to biodiesel. The oil content of some microalgae exceeds 80% of dry weight of algae biomass [6].The development of biofuels as a substitute fuel to supplement or replace conventional diesel is receiving great attention among researchers and policy makers for its numerous advantages such as renewability, biodegradability and lower gaseous emission profile. Also, concerns over increasing energy demand, continuous global warming effects, declining petroleum reserves, petroleum price hike and scarcities have raised the need to search for alternative renewable fuels[7-11].

This article will focus on the characterization, benefit and future applications of microalgae as an eco friendly and economically viable platform for the production of biofuels.

Biofuels: Definition, Classification and Characterization

The term biofuel is referred to a solid, liquid or gaseous fuel that is predominantly produced from biorenewable feedstocks[10]. The most common renewable liquid transportation fuels are bioethanol and biodiesel. These biofuels can replace gasoline and diesel respectively, in today cars with little or none modifications of vehicle engines. Biofuels can be classified based on their production technologies and feedstock: first generation biofuels (FGBs), second generation biofuels (SGBs), third generation biofuels (TGBs), and fourth generation biofuels (FoGBs). FGBs are produced from sugar, starch, vegetable oils or animal fats, while SGBs are made from non-food crops, like wheat straw, corn and wood. Fuels produced from algae, also called algal oils, are classified as TGBs. Finally, FoGBs include crops that are genetically engineered to consume more CO_2 from the atmosphere than the amount of CO_2 that will be produced during their combustion as biofuels. Moreover, some fourth generation technology pathways include: pyrolysis, gasification, upgrading, solar-to-fuel, and genetic manipulation of organisms to secrete hydrocarbons. In addition, fourth generation technologies are based in the conversion of vegetal oil and biodiesel into biogasoline, using more advanced technology[12]. Algae are cost-effective and provide a relatively high yield of biofuel. Their main advantage is that they are eco- friendly and not a burden on the environment.

Characteristics of microalgae and benefits of micro algae

Microalgae are prokaryotic or eukaryotic photosynthesizing microorganisms that are characterized by rapid growth; most often live in acid environment and have a unicellular or simple multicellular structure. Examples of prokaryotic microorganisms are Cyanobacteria (*Cyanophyceae*) and of eukaryotic microalgae is green algae (*Chlorophyta*) and diatoms (*Bacillariophyta*) [13,14].

They can be grown on land that would not be used for traditional agricultural, and are very efficient at removing nutrients from water. Thus, not only would production of algae biofuels minimize land use compared with biofuels produced from terrestrial plants but, in the process of culturing these microalgae, waste streams can be remediated. Potential waste streams include municipal wastewater to remove nitrates and phosphates before discharge, and flue gas of coal or other combustible-based power plants to capture sulfates and CO_2 [15-17].

Various species of microalgae can adapt to living in various environmental conditions. Therefore, it is possible to find the most specific species of algae and to grow them under local conditions, which is not possible in the case of other biodiesel raw materials (like soybean, rapeseed or palm seed oil). Microalgae are also characterized by higher rate of growth and productivity compared to the yielding of traditional crops and require significantly smaller growing areas than other substrates of biofuels of agricultural origin, therefore, in the case of algae grown for energy, the competition for arable soils with other crops, especially those grown for food, is greatly limited[18].

Cultivation of microalgae

Microalgae cultivation is the first production step in the energy production system. How to produce the best quality microalgae feedstocks is the main task in the cultivation process. According to the study of characteristic and function of microalgae, some scientific microalgae cultivation methods have been concluded. At present, the cultivation methods of the microalgae production are commonly using in these two ways: The open pond system and Close photo bioreactors system[19].

Comparison of other sources of biodiesel with microalgae

Two of the most common biofuels currently produced are ethanol produced from corn or sugarcane and biodiesel produced from a variety of oil crops such as soybeans and oil palm. Ethanol production has flourished in the US, rising 25% between 2000 and 2008 due to its use as a gasoline additive and due to federal mandates and tax incentives to fuel blenders. Today 30% of the corn currently grown is used for ethanol production. If corn ethanol was the sole source used to achieve the 2020 federal mandates for renewable fuel, than 100% of the corn currently available in the US would be required. To meet

Lipid content of some microalgae species

Table 1:[3, 15, 20-22]

Microalgae species	Lipid content (% dry weight biomass)
Ankistrodesmus sp.	24–31
Botryococcus braunii	25–75
Chaetoceros muelleri	33
Chlamydomonas reinhardtii	21
Chlorella emersonii	25–63
Chlorella minutissima	57
Chlorella protothecoides	14–57
Chlorella sorokiniana	19–22
Chlorella sp.	10–48
Chlorella vulgaris	5–58
Crypthecodinium cohnii	20–51
Dunaliella salina	6–25
Dunaliella primolecta	23
Dunaliella tertiolecta	16–71
Dunaliella sp.	17–67
Euglena gracilis	14-20
Ellipsoidion sp.	27
Haematococcus pluvialis	25.0
Isochrysis sp.	7–33
Monodus subterraneus	16
Monallanthus salina	20–22
Nannochloris sp.	20–56
Nannochloropsis oculata	22–29
Nannochloropsis sp.	12–53
Neochloris oleoabundans	29–65
Pyrrosia laevis	69.1
Pavlova salina	30
Prostanthera incisa	62
Prymnesium parvum	22-39
Pavlova lutheri	35
Phaeodactylum tricornutum	18–57
Scenedesmus obliquus	11–55
Skeletonema costatum	13–51
Scenedesmus dimorphus	16-40
Schizochytrium sp.	50-77
Thalassiosira pseudonana	20
Isochrysis galbana	7-40
Zitzschia sp.	45-47

these mandates and maintain today's 30% corn crop utilization
would require an increase in corn harvest by 423%, a number unlikely to be achievable in the next 10 years. One source of these biofuels may be ethanol derived from sugarcane; however, although the domestic production cost of sugarcane ethanol is 24% lower than corn ethanol, the transportation cost and the coproduct credits associated with corn ethanol make sugarcane ethanol 17% more costly. Thus, sugarcane ethanol is also an unlikely candidate for the displacement of significant amounts of fossil fuels[23-25].

The high cost of sugarcane ethanol and the competition with food from corn ethanol leave a large gap between the current feasible production levels of ethanol and the fuel requirements for the RFS2 mandate. To overcome these limitations, lignocellulosic feedstocks are also being developed as sources for the production of ethanol[26]. Lignocellulosic feedstocks come in a wide range of different plants, including agricultural waste products such as corn stover, woody sources such as aspen and dedicated energy crops such as hybrid poplar and switchgrass. Currently, no commercial scale cellulosic ethanol plants are in operation largely due to the high price of production, almost twice that of corn ethanol[27, 28]. Another source of biodiesel that has recently had a lot of media exposure is Jatropha curcas. J. curcas is a small tree that is considered drought-tolerant and produces seeds that contain 20–40% nonedible oil, therefore being noncompetitive with food sources and agricultural land[29].

In recent years, algae have become a focus in both academic and commercial biofuels research. These photosynthetic organisms are known to produce high oil and biomass yields, can be cultivated within non freshwater sources including salt and wastewater, can be grown on nonarable land, do not compete with common food resources, and they very efficiently use water and fertilizers for growth[30].

Algae can tolerate and adapt to a variety of environmental conditions, and are also able to produce several different types of biofuels. Bioethanol from algae holds significant potential due to their low percentage of lignin and hemicellulose as compared to other lignocellulosic plants[31].

Table 2-Comparison of some sources of biodiesel[3]

Crops	Oil yield(L/ha)	Land area needed(M ha)*
Corn	172	1540
Soybean	446	594
Canola	1190	223
Jatropha	1892	140
Coconut	2689	99
Oil palm	5950	45
bMicroalgae	1,36,900	2
cMicroalgae	58,700	4.5

b70% oil (by wt) in biomass; c30% oil (by wt) in biomass

Biofuels from microalgae

Biomethan

Microalgae are considered valuable substrates for biogas production due to their high content of lipids, carbohydrates and proteins and low amount of lignin, i.e. recalcitrant compounds, up to 5% of dry wt. [32,33]. In addition, also the residual algal paste after lipids extraction (also defined as lipids-extracted algal, LEA, biomass) can be treated by anaerobic digestion. Lipids-extracted algal biomass contains mainly carbohydrates and proteins residuals from the original feedstock. Biomass contains mainly carbohydrates and proteins residuals from the original feedstock[34,35].

Bioethanol and biobutanol

Microalgal biomass can contain significant amount of carbohydrates (about 40-50% dry wt.) with no structural biopolymers, such as lignin and hemicelluloses[36-38]. Under specific conditions, microalgal carbohydrates can be degraded via hydrolysis and then fermented to bioethanol with yeast[39]. In the case of microalgae-based fermentation, it is possible to avoid chemical and enzymatic pre-treatments, which are energy intensive processes necessary for ligno-cellulosic feedstocks, to release the sugars contained in the algal cells. However, mechanical pre-treatments are still needed to break down the algal cells, e.g. disruption by high pressure homogenizer or collision plate. Microalgae-based bioethanol production yields range between 0.240 and 0.888 g of ethanol/g of substrate, at the temperature of 25-30 °C[40-42].

Biohydrogen from microalgae

Microalgae also received growing interest as a feedstock for hydrogen production. Production of hydrogen from different microalgal strains can occur via dark-fermentative process using a pure or mixed culture of hydrogen-producing bacteria. Alternatively, hydrogen from microalgae can be produced via light-driven process, i.e. photofermentation, under anoxic conditions These processes can either employ water or specific bacteria related to the fermentative conversion of biomass to hydrogen. Recent studies indicate that, under dark fermentation, the potential degradability of various microalgae species can range between 2-28% [37,40,43,44]. Hydrogen yields resulting from experimental tests can vary from 13 to 48 ml of H2/g of algal substrates, depending on the composition of the selected species and operating conditions (such as pH, temperature, substrate/inoculums ratio)[45]. Biohydrogen yields, which were obtained during experimental tests, correspond to only 25-30% of the theoretical biohydrogen potentials calculated considering the degradability of all carbohydrates contained in the biomass.

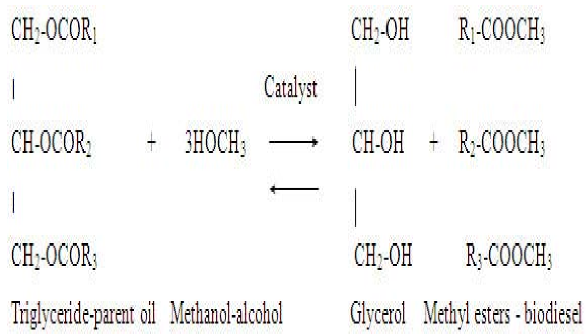
Bio-oil (or bio-crude)

Microalgae can be suitable feedstocks for producing bio-oil (or "biocrude") via thermochemical conversion pathways, such as pyrolysis and hydrothermal liquefaction (HTL)[46]. As opposed to previous processes, which focused on converting specific components of algae, the thermochemical processes can convert the whole biomass into bio-oil[47-49].

Biodiesel

Besides biogas, bioethanol and biobutanol, macroalgae can be converted into biodiesel, bio-oil and bio-hydrogen via appropriate biochemical and thermochemical conversion technologies [43,50,51]. Transesterification method is used to convert algal oil into biodiesel. Transesterification can be performed by using acid and base catalysts, or a combination of both is used. Base catalysis gives rapid reaction than acid catalysis, but it is also depend upon selection of the types of lipids that are transesterified[52,53]. Ttransesterification process of biodiesel production is several steps chemical reaction in which triglycerides first converted to diglycerides, diglycerides to monoglycerides, and monoglycerides to free fatty acids and glycerol (as byproduct); If a lipase is used, hydrolysis and esterification may take place simultaneously, but the thermal liability of that enzyme does not crate industrial scale[54,55].

Figure 1. Trans esterification of oil to biodiesel. R1-R3- hydrocarbon groups [56]



Future perspectives of Algal Biofuel

Algal biofuel is an ideal biofuel candidate which eventually could replace petroleum-based fuel due to several advantages, such as high oil content, high production, less land, etc. Currently, algal-biofuel production is still too expensive to be commercialized. Due to the static cost associated with oil extraction and biodiesel processing and the variability of algal-biomass production, future cost-saving efforts for algal-oil production should focus on the production method of the oilrich algae itself. This needs to be approached through enhancing algal biology (in terms of biomass yield and oil content) and culture-system engineering. In addition, using all aspects of the microalgae for producing value-added products besides algal fuel—such as in an integrated biorefinery—is an appealing way to lower the cost of algal-biofuel production. Indeed, microalgae contain a large percentage of oil, with the remaining parts consisting of large quantities of proteins, carbohydrates, and other nutrients[57]. This makes the residue after oil extraction attractive for use as animal feed or in other value-added products.

Conclusion

Algal biofuels provide natural, sustainable and environmentally clean fuel for the future. Culturing algae provide several benefits such as biomass for the production of biofuels, removing the nutrients from the wastewater and it is also use as food. This review focus on the benefit and future demand of algal biofuel, though it is economically not feasible but provide more benefits than fossil fuels. Algal biofuels can become a substitute of fossil fuel in near future.

References

1. Chang, H., Marine Biodiversity and Systematics. Research Programmes Retrieved April 14 (2007), 2009
2. Metting, F. B., Biodiversity and application of microalgae. Journal of Industrial Microbiology & Biotechnology 17(5-6) (1996): 477-489.
3. Chisti, Y., Biodiesel from microalgae. Biotechnology Advances, , **25(3),** (2007):294-306.
4. Pirt, S.J. The Thermodynamic Efficiency (Quantum Demand) and Dynamics of Photosynthetic Growth. New Phytol. (1986), 102, 3-37.
5. Brown, L.M.; Zeiler, B.G. Aquatic Biomass and Carbon Dioxide Trapping. Energy Convers. Manage (1993), 34, 1005-1013.
6. Banerjee, A.; Sharma, R.; Chisti, Y.; Banerjee, U.C. Botryococus Braunii: A Renewable Source of Hydrocarbons and Other Chemicals. Crit. Rev. Biotechnol. (2002), 22, 245-279.
7. Sahoo, P.K., Das, L.M., Babu, M.K.G., Naik, S..N.,Biodiesel development from high acid value polanga seed oil and performance evaluation in a CI engine. Fuel 86(2007).:448-454.
8. Basha, S.A., Raja, Gopal, K., Jebaraj, S., A review on biodiesel production, combustion, emissions and performance. Renew. Sustain. Energy Rev. 13(2009):1628-1634.
9. Refaat, A.A., Different techniques for the production of biodiesel from waste vegetable oil. Int. J. Environ. Sci. Technol. 7(1) (2010):183-213.
10. Demirbas, M.F., Biorefineries for biofuel upgrading: A critical review. Appl. Energy 86(2009), S151eS161.
11. Yang, C.Y., Li, Z.F.B., Long, Y.F.. Review and prospects of Jatropha biodiesel industry in China. Renew. Sustain. Energy Rev. 16 (2012):2178-2190.
12. Demirbas, A., Demirbas, M.F., Algae Energy: Algae as a New Source of Biodiesel (Green Energy and Technology).,(2010) Springer.
13. Li, Q., Du W., Liu, D., Perspectives of microbial oils for biodiesel production. Appl. Microbiol. Biotechnol. 80(2008): 749-756.
14. Li Y, Horsman, M., Wang, B, Wu, N., Lan, C.Q., Effects of nitrogen sources on cell growth and lipid accumulation of green alga Neochloris oleoabundans. Appl. Microbiol. Biotechnol. 81: (2008) 629-636.
15. He P, Xu, S, Zhang, H., et al. Bioremediation efficiency in the removal of dissolved inorganic nutrients by the red seaweed, *Porphyra yezoensis*, cultivated in the open sea. Water Res. (2008); 42(4–5):1281–1289. [PubMed: 17959220].
16. Fierro, S, Sanchez-Saavedra, Mdel, P, Copalcua, C., Nitrate and phosphate removal by chitosan immobilized *Scenedesmus*. Bioresour Technol.,(2008); 99(5):1274–1279. [PubMed: 17531478]
17. Douskova, I, Doucha J, Livansky K, et al. Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. Appl Microbiol Biotechnol., (2009); 82(1):179–185. [PubMed: 19096837].
18. Mata, T.M., Martins, A.A., Ceatano, N.S., Microalgae for biodiesel production and Rather applications: A review. Renew. Sustain. Energy Rev. 14(2010): 217-232
19. Demirbas, M.F., Biofuels from algae for sustainable development. Appl. Energy 88(2011): 3473-3480.
20. Mata, T.M., Martins, A.A., Caetano, N.S., Microalgae for biodiesel production and other applications: A review. Renew. Sustain. Energy. (2009)Rev. doi:10.1016/j.rser.2009.07.020.
21. Sialve, B., Bernet N, Bernard O., Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Boitechnol. Adv., 27: (2009). -416
22. Um, BH, Kim, Y.S., Review: A chance for korea to advance algal-biodiesel technology. J. Ind. Eng. Chem., 15(2009): 1-7.
23. Blanco, L, Isenhouer, M: Powering America: the impact of ethanol production in the Corn Belt states. Energ Econ (2010), 32:1228-1234.

24. Somma, D., Lobokowicz, H., Deason, J.P.: Growing America's fuel: an analysis of corn and cellulosic ethanol feasibility in the United States. Clean Technol Environ Policy (2010), 12:373-380.
25. Crago CL, Khanna M, Barton J, Giuliani E, Amaral W: Competitiveness of Brazilian sugarcane ethanol compared to US corn ethanol. Energ Policy (2010), 38:7404-7415.
26. Dwivedi, P, Alavalapati, J.R.R., Lal, P.: Cellulosic ethanol production in the United States: conversion technologies, current production status, economics, and emerging developments. Energy Sustain Dev (2009), 13:174-182
27. Huang, H.J., Ramaswamy, S, Al-Dajani W, Tschirner U, Cairncross RA: Effect of biomass species and plant size on cellulosic ethanol: a comparative process and economic analysis. Biomass Bioenerg 2009, 33:234-246.
28. 14. Regalobut JR: Cellulosic biofuels — got gasoline? Science 2009, 325:822-824.
29. Srivastava, P, Behera, S.K., Gupta, J., Jamil S., Singh N., Sharma Y.K., Growth performance, variability in yield traits and oil content of selected accessions of Jatropha curcas L. growing in a large scale plantation site. Biomass Bioenerg (2011), 35:3936-3942.
30. Hannon, M., Gimpel, J., Tran M., Rasala, B., Mayfield, S., Biofuels from algae: challenges and potential. Biofuels (2010) 1:763-784.
31. Harun R, Danquah MK, Forde GM: Microalgal biomass as a fermentation feedstock for bioethanol production. J Chem Technol Biotechnol (2010), 85:199-203.
32. M. Dębowski, M. Zieliński, A. Grala, and M. Dudek, "Algae biomass as an alternative substrate in biogas production technologies—Review," *Renewable and Sustainable Energy Reviews,* vol. 27:(2013) pp. 596-604.
33. H. M. El-Mashad, "Biomethane and Ethanol Production Potential of *Spirulina platensis* Algae and Enzymatically Saccharified Switchgrass," *Biochemical Engineering Journal,* (2015).
34. B. Sialve, N. Bernet, and O. Bernard, "Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable," *Biotechnology Advances,* vol. 27(2009), pp. 409-416.
35. E. Ehimen, Z. Sun, C. Carrington, E. Birch, and J. Eaton-Rye, "Anaerobic digestion of microalgae residues resulting from the biodiesel production process," *Applied Energy,* vol. 88 (2011): pp. 3454-3463
36. Algae-based biofuels: A Review of Challenges and Opportunities for Developing Countries" Food and Agriculture Organization of the United Nations,(2010.
37. U.S.DOE, "National Algal Biofuels Technology Roadmap. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program.," Workshop and Roadmap sponsored by the U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Office of the Biomass Program (2010).
38. R. Garofalo, "Algae and aquatic biomass for a sustainable production of 2nd generation biofuels," *AquaFUELs-Taxonomy, Biology and Biotechnology,* pp. 1-258, 2009.
39. C. Enzing, M. Ploeg, M. Barbosa, L. Sijtsma, M. Vigani, C. Parisi, and E. R. Cerezo, *Microalgae-based products for the food and feed sector: an outlook for Europe*: Publications Office.,(2014).
40. L. Zhu, E. Hiltunen, E. Antila, J. Zhong, Z. Yuan, and Z. Wang, "Microalgal biofuels: Flexible bioenergies for sustainable development," *Renewable and Sustainable Energy Reviews,* vol. 30, (2014)pp. 1035-1046..
41. R. P. John, G. Anisha, K. M. Nampoothiri, and A. Pandey, "Micro and macroalgal biomass: a renewable source for bioethanol," *Bioresource technology,* vol. 102(2011), pp. 186-193.
42. M. Dębowski, M. Zieliński, A. Grala, and M. Dudek, "Algae biomass as an alternative substrate in biogas production technologies—Review," *Renewable and Sustainable Energy Reviews,* vol. 27(2013), pp. 596-604
43. C. Sambusiti, M. Bellucci, A. Zabaniotou, L. Beneduce, and F. Monlau, "Algae as promising feedstocks for fermentative biohydrogen production according to a biorefinery approach: A comprehensive review," *Renewable and Sustainable Energy Reviews,* vol. 44:(2015), pp. 20-36.
44. 83A. Jacob, A. Xia, and J. D. Murphy, "A perspective on gaseous biofuel production from micro-algae generated from CO2 from a coal-fired power plant," *Applied Energy,* vol. 148: (2015), pp. 396-402.
45. M. Ras, L. Lardon, S. Bruno, N. Bernet, and J.-P. Steyer, "Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*," *Bioresource technology,* vol. 102: (2011)pp. 200-206.
46. D. López, Barreiro, W. Prins, F. Ronsse, and W. Brilman, "Hydrothermal liquefaction (HTL) of microalgae for biofuel production: State of the art review and future prospects," *Biomass and Bioenergy,* vol. 53(2013), pp. 113-127.
47. R. M. Handler, D. R. Shonnard, T. N. Kalnes, and F. S. Lupton, "Life cycle assessment of algal biofuels: Influence of feedstock cultivation systems and conversion platforms," *Algal Research,* (2014).
48. D. López Barreiro, W., Prins, F., Ronsse, and W., Brilman, "Hydrothermal liquefaction (HTL) of microalgae for biofuel production: State of the art review and future prospects," *Biomass and Bioenergy,* vol. 53: (2013), pp. 113-127.
49. E. P. Bennion, D. M. Ginosar, J. Moses, F. Agblevor, and J. C. Quinn, "Lifecycle assessment of microalgae to biofuel: Comparison of thermochemical processing pathways," *Applied Energy,* 2015.
50. S. Kraan, "Mass-cultivation of carbohydrate rich macroalgae, a possible solution for sustainable biofuel production," *Mitigation and Adaptation Strategies for Global Change,* vol. 18(2013), pp. 27-46.
51. H. Chen, D. Zhou, G. Luo, S. Zhang, and J. Chen, "Macroalgae for biofuels production: Progress and perspectives," *Renewable and Sustainable Energy Reviews,* vol. 47(2015), pp. 427-437.

52. Al-Zuhair, S., Production of Biodiesel: Possibilities and challenges, *Biofuels Bioprod Bior*, 1(1) (2007), 566.
53. Nagle N and Lemke PR, Production of methyl–ester fuel from microalgae, *Appl Biochem Biotechnol,* 24(5) (1990), 355– 361.
54. Amaro, Helena, M., Macedo, Ângela, C. and Malcat,a F., Xavier Microalgae: An alternative as sustainable source of biofuels?, *Energy,* 44(2014)**:** 158-166.
55. Meier, R.L., Biological cycles in the transformation of solar energy into useful fuels, In: Daniels F, Duffie JA, editors, Solar energy research, Madison (WI), University of Wisconsin Press, (1955) 179–83.
56. Sheehan, J., Dunahay, T. et al., A Look Back at the U.S. Department of Energy's Aquatic Species Program-Biodiesel from Algae, *National Renewable Energy Program*, (1998).
57. Spolaore, P.C.Joannis-Cassan,et al., Commercial application of microalgae., *Journal of Bioscience and Bioengineering,(*2006), 101, 87–96.