Bionature, 39(1) 2019 : 14-22 ISSN: 0970-9835 (P), 0974-4282 (O)

MICROALGAE DERIVED BIOFUEL AS A PROSPECTIVE TECHNOLOGY IN INDIA

ALIFHA SEVERES[1], LEO D'SOUZA[1] AND SMITHA HEGDE[2*]

[1]Laboratory of Applied Biology, Dr. Küppers Biotechnology Unit, St. Aloysius College, Mangalore – Karnataka 575 003, India.

[2]Nitte University Centre for Science Education and Research (NUCSER), Paneer Campus, Deralakatte, Mangalore, Karnataka, India.

Email: smitha.hegde@nitte.edu.in

Received: 04 January 2019
Accepted: 13 March 2019
Published: 03 April 2019

Mini-review Article

ABSTRACT

India is a large country with diverse landscape and abundant bio-resources. The growing demand for petroleum-based fuels associated with its growing economic status and the ever increasing population presents a great challenge to the energy security of a nation. Hence, alternative fuel resources and processes are becoming essentially impellent. With considerable sunshine, generally warm climate, sources of CO_2 and other nutrients, low-quality water, and marginal lands, India has very favorable conditions to support algae farming for biofuels production. Sustainable algae biofuels production implies that this technology would not put additional demand on freshwater supplies and use low-quality water such as brackish/saline groundwater, recycled water, agricultural drainage waters and other wastewaters. The present article provides an understanding of this magnificent resource and its potential in India, for production of biofuels that promotes a sustainable system based technology as one of the best alternatives to overcome the looming energy crisis.

Keywords: Energy crisis; wastewaters; biofuels; India; sustainable technology.

INTRODUCTION

The rapid population growth has been escalating the demand for energy and resources. However, the fossil based oil reserves are diminishing at an astonishing rate and greenhouse emissions associated to its combustion are having a huge impact on the global climate. Hence, the need for alternative resources and processes is becoming essentially impellent. Algae, the miniature photosynthetic organisms have the ability to transform available nutrients into valuable biomass using sunlight and water.

Due to the fact that the oceans cover 70% of the earth's surface, aquatic algae are major producers of oxygen and important users of carbon dioxide. Being a good source of vitamins, minerals, lipids, proteins and pigments, they represent a promising source of various products that could be employed in the pharmaceutical/ nutraceutical industry and the biofuel industry. All algae are primarily made up of proteins, carbohydrates, fats and nucleic acids in varying proportions. While the percentage can vary with the type of algae, some types of algae are made up of up to 40%fatty acids based on their overall mass. It is this fatty acid that can be extracted and converted into biofuel. Due to the high lipid content, algal strains have gained great interest in the search for sustainable sources for the production of biodiesel.

Algae derived fuel can not only promote an initiative to preserve a healthy global environment but also proves to be a substitute to reduce our dependency on fossil fuels. High lipid content, ease of cultivation and rapid growth rate even on non-arable land are the factors that make microalgae a desired candidate for biofuel production [1]. Over the past decade there have been plenty of advancements in algal technology for biofuel production. However, harnessing algal biomass and its conversion into biofuel is still in its infancy and algae very little explored as potential biorefinery feedstocks.

BIOFUELS AND ITS SOURCES

Biofuels (bioethanol and biodiesel) are solid, liquid or gaseous fuels derived from organic matter. Bioethanol derived from agri-waste like sugarcane bagasse is one of the most widely used alternate transport fuel. However, bioethanol has only approximately 64% energy content of biodiesel and moreover it would need planting crop plants over large hectares of land [2]. Hence considering the production cost of extracting fuels from these sources, microalgae derived biodiesel serves to be a prospective technology.

Biodiesel, a fuel comprised of mono-alkyl esters of long chain fatty acids derived from triacylglycerol (TAG), can be produced from first generation feedstock such as vegetable oil of oleaginous plants like soyabean, sunflower, jatropha, canola, palm oil or animal fat [3,4,5]. The second-generation biofuels are the cellulosic-based biofuels obtained from non-food crops materials (wood, leaves, straw, etc.) while the third-generation biofuel sources are microorganism’s (yeast, fungi) and algae-based fuels like jet-fuels, biohydrogen, biodiesels [6,7].

Since the first and second generation biofuels rely on arable land and create food-fuel conflict, microalgae as the third generation C- neutral biofuel resource proves to be a promising technology.

MICROALGAE AS A SOURCE OF BIOFUEL

Microalgae comprise of over 50,000 species representing a range of forms and sizes that can exist from unicellular microscopic organism (microalgae) to multicellular large size (macroalgae) present in not only aquatic but also terrestrial environments, that possess the capability to produce renewable oil at rates much faster than land based plants and crops. [8,9]. They are claimed to be 6-8% more photosynthetically efficient than terrestrial plants (1.8 – 2.2%) and have very short doubling time [10]. In addition, microalgae can be grown in non-arable land, under wide range of environmental conditions and wastewaters without requiring significant maintenance.

Microalgae can produce a substantial amount of triacylglycerols as a storage lipid under photo-oxidative stress or other adverse environmental conditions. The oil content of microalgae ranges from 15 to 75% (dry weight). From a taxonomic perspective, the most widely utilized species of microalgae for various biotechnological applications including biodiesel belong to the group Chlorophyceae [11,12]. The representative genus *Chlorella* is a focus of efforts for commercial production of biodiesel and other industrial applications [13,14]. *Chlorella* strains like *C. protothecoides, C. vulgaris, C. emerson'u and C. sorokiniana* may be suitable for diesel replacements [15]. Also, *Botryococcus braunii* (80% lipid dry weight) due to its rich quantities of hydrocarbons, *Nannochloropsis salina* for its high quantities of esters and *Dunaliella salina* for its high fatty acid content are promising species [16]. The microalgae *C. vulgaris, C. pyrenoidosa and Scenedesmus obliquus* are reported to have great potential for CO_2 biofixation and biodiesel. The National Renewable Energy Laboratory (NREL) in United States affirms that *Spirulina*, *Dunaliella*, *Scenedesmus* and *Chlorella* are the most popular strains that have been produced at commercial or large-scale. Most common microalgae *Botryococcus, Chlamydomonas, Chlorella, Dunaliella, Neochloris, Chaetoceros, Scenedesmus, Chlorococcum sp.,* etc. can have oil levels between 20 and 75% by weight of dry biomass. The annual oil production from such oleaginous microalgae could be estimated in the range of 58,700–136,900 L/ha. However, the economic feasibility of algal biomass cultivation for biodiesel production greatly depends on the high biomass productivity and high quality lipid yields. The molecular identification for most algal production strains is deficient and among the vast diversity of algae, only a few thousands are kept worldwide in algal culture collections out of which only few hundred are investigated for their chemical composition [17]. Thus, the isolation and characterization of algae from unique aquatic environments is a continuing effort for screening the biodiesel potential strains [12].

MICROALGAL BIODIESEL

Algal lipids can be grouped into, storage lipids (non-polar lipids) and structural lipids present in the membranes (polar lipids). Structural lipids typically have a high content of polyunsaturated fatty acids (PUFAs), which are also essential nutrients for aquatic animals and humans and have a role in responding to changes in the environment [18]. Storage lipids are mainly in the form of tri-acyl glycerols (TAGs) accumulated in large amounts during photosynthesis as a mechanism to endure adverse environmental conditions [19]. These are made of predominately saturated FAs and some unsaturated FAs which can be transesterified to produce biodiesel [11]. However, some oil-rich species have demonstrated a capacity to accumulate high levels of long-chain polyunsaturated fatty acids (PUFA) as TAG [20].

Polar lipids containing long chain PUFA have good fluidity properties while TAG in lipid storage bodies typically contain mostly saturated fatty acids which have a high energy content. As long as the algal oil is low enough in moisture and free fatty acids, biodiesel is typically produced from TAG with methanol using base-catalyzed transesterification as fatty acid methyl esters (FAME) [11]. Principally, microalgal oil can be directly used as fuel feedstock, based on the conventional process of biodiesel production, provided the fatty acid profile is favorable.

The type and amount produced of lipid produced by an algal species, e.g. chain length, degree of saturation and proportion of total lipid made up by triglycerides is an essential characteristic to determine its suitability for biodiesel production. Properties such as cetane number, kinematic viscosity, cold flow, and oxidative stability also determine the suitability of biodiesel as a fuel [21]. The majority of lipid-producing algal species have a similar lipid profile, generally equivalent to vegetable oil from land plants suitable for biodiesel production, *Botryococcus braunii* as a notable exception [22,23]. The most common fatty acid methyl esters present in biodiesel are palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid. Saturated fatty acids have significantly higher melting points than unsaturated fatty acids and so, in a mixture, saturated fatty acids crystallize at a higher temperature. Higher content of polyunsaturated fatty acid esters in the feedstock oil causes deterioration in the quality of biodiesel upon storage due to oxidation triggered by air, light, heat, peroxides, trace metal, or even the structural features of the fatty acids. Hence, characterization of fatty acid profile is an essential step.

INDIA – A RICH SOURCE OF ALGAL BIORESOURCE / SUSTAINABLE CULTIVATION

The Indian subcontinent is regarded as a home to three of the world's bio-diversity hotspots namely the Western Ghats, the eastern Himalayas and the Indo-Burma region. However, there is a lack of studies explicitly addressing the exploration of oleaginous microalgae from each of these regions. India has been bestowed upon with vivid wetland resources that provide habitat and an environment conducive to support a rich diversity of fresh water microalgae, which remain unexplored and untapped [24].

The Western Ghats ranging from Tapti River in Southern state of Gujarat to the south tip of Kerala state is enriched with a diversity of flora at various taxonomical levels comprises of around 300 species of known algae which could be tapped for a number of potential applications. One of the biosphere hotspots of the Western Ghats in Karnataka- the Kudremukh National Park spreads over an area of 600.32 km^2, encompassing regions in the districts of Dakshina Kannada, Udupi and Chikmagalur. Parts of the river flowing in different regions of Dakshina Kannada serve accessible points for microalgal distribution studies.

Algal growth is greatly influenced by climate and weather conditions. While nutrient status of the water could be managed, issues such as light intensity, water temperature, evaporation loss from the algal pond surface are influenced by climate [25]. The high salinity found at Kutch, Gujarat, India could facilitate growth of selected halotolerant oleaginous algae. Earlier studies have addressed to some extent, the C capture ability of marine planktonic microalgae as *Nannochloropsis salina* and *Isochrysis galbana* as well as macroalge *Gracilaria corticata*, *Sargassum polycystum* and *Ulva lactuca* under laboratory conditions with high biomass productivities [26].

Another type of land quite suitable for algae cultivation in India are the constantly flooded paddy lands where even after the cultivation of a paddy crop there is a 30–60day window with adequate water for cultivation of algae. Algal growth is often high in areas which receive runoff from paddy cultivation. Thus, under such circumstances when algae are cultivated only for the first 60 days of crop life, there is a potential for nearly 16–18 Mha of algae cultivation.

On a more sustainable approach, much of current algal biofuel research envisages growing algae on wastewaters. Using wastewater as the primary feedstock helps to generate substantial quantities of algal biomass as alternative energy sources as well as treat wastewaters in an inexpensive and eco-friendly manner. One of the major factors that make biodiesel production in India more attractive is the potential to cultivate cheap feedstock [27].

WASTEWATERS - LOW COST GROWING MEDIA

The concept of sustainability is based on the principle of reuse of water, recovery of potentially polluting nutrients, removal of potentially toxic and undesirable elements and recycling of useful nutrients into the food production system etc. Algal cultivation systems depend on physicochemical variables like pH, growth medium temperature, nutrient concentrations primarily C, N and P and other nutrients, light availability and gas exchange.

Wastewaters generated, from industrial operations or urban wastes and domestic and municipal discharge lead to nutrient overload (especially N and P) in aquatic systems causing eutrophication and environmental damage due to algal blooms. Utilizing this concept, algal growth could be contained along with simultaneous wastewater treatment. Urban wastewaters are generally rich with high concentrations of C, N and P compared to the other water sources. The algal communities in an urban lake at Bangalore were reported to be responsible for about 76% N removal and 60% C removal [28,29]. Similar findings also showed that *Chlorella* and *Scenedesmus* could tolerate several wastewater conditions and were efficient at uptake of nutrients from wastewater [30,31,32,33,34].

In a study wherein *Chlorella sp.* was grown in specific combination of N: P: K a four-fold increase in the cell number was observed within a weeks' time. Most municipal wastewaters are rich in ammonia (NH_3), phosphate (PO4 -), and other essential nutrients that are required to support microalgal biomass production. A 6 day batch cultivation of *Auxenochlorella protothecoides* in municipal wastewaster showed high growth rate (0.490/day), high biomass productivity (269 mg/L/day) and high lipid productivity (78 mg/L/day) [35]. Algae efficiently utilize nutrients from agricultural and livestock slurries rich in N and P. A bountiful growth of *Botryococcus braunii* was reported in piggery wastewater comprising 800ppm nitrates and showed ~80% removal of nitrate-N. Studies on *Botryococcus brauni* in secondary treated municipal wastewater showed higher lipid content (>18%) than conventional growth media (11%).

In a study conducted by Wang et al. [36], municipal wastewaters derived from different process stages of municipal wastewater treatment plant showed that the algal growth was significantly enhanced (more than 10 times higher) in centrate wastewater probably due to its much higher levels of COD, N and P compared with other wastewater streams. Li et al. [37] suggested that CO_2- supplemented algae cultures can simultaneously remove dissolved nitrogen and phosphorus to low levels while generating a feedstock potentially useful for liquid biofuels production.

CHALLENGES IN LARGE SCALE CULTIVATION

While large scale microalgae cultivation appears to be an environmentally sustainable option for alternative fuel production, the technology has not been able to challenge fossil based fuels in an economically competitive way. Parameters such as pH, salinity, nutrient composition could be controlled so a certain extent. However temperature, irradiation and contamination pose a significant challenge.

On a larger scale, two cultivation systems could be used: open raceway ponds and closed photobioreactors. Widely used open pond systems, bear the risk of attracting competing contaminating agents while closed PBR's incur expenses in setup and monitoring. Hence, both systems require optimization of complex factors that satisfy high level production and cultivation [38,39]. Algal cultivation for the purpose of biofuels could compromise on contaminants to a certain extent while focusing on large scale biomass and lipid productivities. Hence, taking into account the ample sunlight and suitable climatic conditions in our country, algae farming could be taken seriously in order to meet the rising energy demands.

However, large-scale cultivation of microalgae is still in its infancy with a number of factors that need to be optimized and researched. Firstly, robust, highly productive strains with high growth rates and high oil content must be identified. These cultures need to be stabilized since "culture crashes" have a severe toll on the overall productivity. Nutrient sourcing and recycle can be critical factors to the play with the technology economics. The overall cost of cultivation systems, especially the capital infrastructure costs also need to be considered. Further, downstream processes also pose significant challenges. Finally, the management of water that is, sourcing water for cultivation systems, makeup water to replace losses due to evaporation, and treatment of incoming and outgoing water streams (e.g., removing nutrient residues, organics, heavy metals, salt, and live organisms) needs a systematic design.

CONCLUSION

Out of the 328,730,300 ha of land in India, 16.8% is wasteland; while in Karnataka itself, out of the 19, 179,100 ha, 7% is a wasteland [40]. According to the survey by MPNG [41] around 1.7% of the total area in Karnataka could be used in order to displace the state's diesel consumption. Hence, these non-arable wastelands provide a suitable area that could be used for mass scale cultivation of algae.

Most of our country meets the climate requirements for growing algae: sufficient solar radiation, sunshine hours, and warm temperatures. A large number of thermal power plants, steel plants, cement plants, fertilizer plants, refineries, and petrochemical plants scattered throughout the country could provide opportunities for co-cultivation of algae farms and recycling the CO_2 into a useable liquid fuel. Considering a population of over 1.3 billion along with agricultural activities, human and animal organic wastes an enormous source of N and P could be made available for large scale algal production. Also, livestock drainage water, brackish/ saline groundwater sources could be used for algal biomass production. Since almost every state in India is capable of generating wastewaters from industrial, municipal and agricultural activities, there is a strong opportunity for either co-cultivation of algae or dedication algal cultivation for the sole purpose of biomass generation.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author AS wrote the first draft of the manuscript and managed the literature searches. Authors LD and SH revised the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGEMENT

The authors greatly acknowledge the financial support by the UGC-MANF and the Mangalore Jesuit Educational Society and also the support and encouragement by the fellow members of Laboratory of Applied biology.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Alam F, Mobin S, Chowdhury H. Third generation biofuel from algae, Procedia Eng. 2015;105:763-8.
2. Chisti Y. Biodiesel from microalgae beats Bioethanol. Elsevier; 2008.
3. Fukuda H. Biodiesel fuel production by transesterification of oils. J Bioscience and Bioengineering. 2001; 92:405–416.
4. Khalid K, Khalid K. Transesterification of palm oil for the production of biodiesel. Am. J. Applied Sci. 2011;8: 804-809.
5. Marc V. Production of biodiesel from microalgae; 2010. Available:www.intechopen.com
6. Fatih D. Biorefineries for biofuel upgrading: A critical review. Applied Energy. 2009;86(1):151-161.
7. Nigam PS, Singh A. Production of liquid biofuels from renewable resources. Progress in Energy and Combustion Science. 2011;37(2):52-68.
8. Ugoala E, Ndukwe GI, Mustapha KB, Ayo RI. Constraints to large scale algae biomass production and Utilization. J. Algal Biomass Utilization. 2012;3(2):14-32.
9. Richmond A. Handbook on microalgal culture: Biotechnology and applied phycology. Iowa State Press, Blackwell Publ. Iowa. 2004;37:125-172.
10. Khan SA. Prospects of biodiesel production from microalgae in India, Renew. Sustain. Energy Rev. 2009; 13:2361-2372.
11. Chisti Y. Biodiesel from microalgae. Biotechnology Advances. 2007;25: 294–306.
12. Hu Q. Microalgal triacylglycerols as feedstocks for biofuel production: Perspectives and advances. Plant J. 2008;54:621–639.
13. Widjaja A. Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. J. Taiwan Inst. Chem. Eng. 2009;40:13–20.
14. Liu ZY. Effect of iron on growth and lipid accumulation in *Chlorella vulgaris.* Bioresour. Technol. 2008;99: 4717–4722.
15. Illman A, Scragg A, Shales S. Increase in Chlorella strains calorific values when grown in low nitrogen medium. Enzyme Microb. Technol. 2000;27:631–635.
16. Deng X. Microalgae: A promising feedstock for biodiesel. African Journal of Microbiology Research. 2009;3(13):1008-1014.
17. Olaizola M. Phototrophic: Efficient alternatives to land based crops for biofuels. Current opinion in Eng. 2003;459-466.
18. Kalpesh KS. High lipid induction in microalgae for biodiesel production. Energies. 2012;5:1532-1553.
19. Van TD. Microalgae isolation and selection for prospective biodiesel production. Energies. 2012;5:1835-1849.
20. Bigogno C. Accumulation of arachidonic acid-rich triacylglycerols in the microalga Parietochlorisincisa (trebuxiophyceae, chlorophyta), Phytochemistry. 2002;60:135–143.
21. Adam MS. Metabolic response of the halotolerant green alga *Dunaliella bardawil* to nitrogen: Phosphorous

ratios in batch culture. Folia Microbiol. 1997;42:357–360.

22. Xu H. Biodiesel production from heterotrophic microalgal oil. Bioresour Technol. 2006;97:841–846.
23. Banerjee A. *Botryococcus braunii*: A renewable source of hydrocarbons and other chemicals. Crit Rev Biotechnol. 2002;22(3):245–279.
24. Kaur S, Gogoi RB, Srivastava B, Kalita MC. Algal diversity as a renewable feedstock for biodiesel. Current Science. 2009;96(2):182.
25. 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;102:159–65.
26. Kaladharan P, Veena S, Vivekanandan E. Carbon sequestration by a few marine algae: Observation and projection. J. Mar. Biol. Ass. India. 2009;51:107–110.
27. Bajhaiya AK, Mandotra SK, Suseela MR, Toppo K, Ranade S. Algal biodiesel- The next generation biofuel for India. Asian J. Exp. Biol. Sci. 2010;4:728-739.
28. Mahapatra DM, Chanakya HN, Ramachandra TV. Assessment of Treatment capabilities of Varthur Lake, Bangalore. International Journal for Environment, Technology and Management. 2011;14:84–102.
29. Singh P, Singh A. A review paper: Potential of microalgae as source of Biofuel in India. International Journal of Latest Technology in Engineering, Management & Applied Science (IJLTEMAS). 2017;VI(VIII):151-156.
30. Wang ZT, Ullrich N, Joo S, Waffenschmidt S, Goodenough U. Algal lipid bodies: Stress induction, purification, and biochemical characterization in wildtype and starchless *Chlamydomonas reinhardtii.* Eukaryot. Cell. 2009;8: 1856–1868.
31. Voltolina D, Cordero B, Nieves M, Soto LP. Growth of *Scenedesmus sp.* in artificial wastewater. Bioresour. Technol. 1999;68:265–268.
32. Aslan S, Kapdan IK. Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecol. Eng. 2006;28:64–70.
33. Xue F, Miao J, Zhnag X, Tan T. A new strategy for lipid production by mix cultivation of *Spirulina plantensis* and *Rhodotorula glutinis.* Appl. Biochem. Biotechnol. 2010;160:498–503.
34. Xin Li, Hu H, Gan K, Sun Y. Effects of different nitrogen and phosphorus concentration on the growth, nutrient uptake and lipid accumulation of a freshwater microalgae Scenedesmus sp. Bioresour. Technol. 2010;101: 5494–5500.
35. Tam NFY, Wong YS. Wastewater treatment with microorganisms. The commercial Press (H.K.) Ltd. 2D Finnie St. Quarry Bay, Hong Kong; 1995.
36. Wang L. Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. Appl Biochem Biotechnol. 2010;162(4):1174-86.
37. Li Y. Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. Bioresource Technology. 2011;102(8):5138-5144.
38. Thompson GA. Lipids and membrane function in green algae. Biochim. Biophys. Acta. 1996;1302:17–45.

39. Singh K, Kaloni D, Gaur S, Kushwaha S, Mathur G. Current research and perspectives on microalgae-derived biodiesel. Biofuels. 2017;1-18.
40. Ministry of Rural Development (Department of Land Resources) and National Remote Sensing Agency (MRD-NRSA), “Wastelands Atlas of India”; 2005.
41. Ministry of Petroleum and Natural Gas (MPNG), “Basic Statistics on Indian Petroleum and Natural Gas 2008-09” New Delhi, India; 2009.