International Journal of Agriculture Sciences
ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 10, Issue 8, 2018, pp.-5817-5821.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Review Article

MICROALGAE: THIRD GENERATION ENERGY FEEDSTOCK FOR BIODIESEL PRODUCTION IN DEVELOPING COUNTRIES

KARMAKAR A.*, KARMAKAR S. AND MUKHERJEE S.
Department of Post-Harvest Engineering, Faculty of Agricultural Engineering, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, 741252 Nadia, West Bengal, India
Department of Farm Machinery & Power, Faculty of Agricultural Engineering, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, 741252 Nadia, West Bengal, India
*Corresponding Author: Email - karmakar.ani@gmail.com

Received: April 16, 2018; Revised: April 20, 2018; Accepted: April 21, 2018; Published: April 30, 2018

Abstract- In developing countries like India where import accounts for 80 percent of the crude oil supplies, alternative high yield sources are given utmost priorities for sustaining renewable energy generation. Discrepancies between expectations and realities of different non-edible biodiesel feedstocks are pushing for feedstock diversification. Microalgae, having extremely high photosynthetic efficiency are drawing attention as rich source of third generation renewable energy. The main hurdle of microalgal biodiesel production is lowering the cost to make it competitive with petroleum derived fuels. The problem can be solved by developing process technologies for harvesting and drying of algal biomass, extraction of oil, transesterification and downstream processing. India in spite of being one of the major producers of algae is yet to start such type of research and development activities on algal biodiesel. This paper reviews the potential of microalgae to produce a multitude of biofuel including biodiesel, bioethanol, biomethane (biogas), producer gas etc with integrated approach for energy, environment and agriculture.

Keywords- Microalgae, Biodiesel, Transesterification, Integrated approach, Tubular photobioreactors

Citation: Karmakar A., *et al*., (2018) Microalgae: Third Generation Energy Feedstock for Biodiesel Production in Developing Countries. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 10, Issue 8, pp.-5817-5821.

Introduction

Despite awareness drives and governmental initiatives towards popularizing and production of non-conventional energy for environmental sustainability, petroleum remains the primary energy source in most of the countries. In developing countries like India where close to 80% of country's petroleum needs are met by import, energy security issues putting strain on the country's economy and overall growth in view of spiralling price of petroleum fuels. This strongly advocates for sustainable production of renewable energy. Biodiesel is a biodegradable, clean-burning, renewable fuel made from vegetable oils and animal fats. Biodiesel is produced from edible and non-edible oilseeds across the world. In developing countries like India, biodiesel is produced only from non-edible oils as the edible oils are in short supply. Unfortunately, biodiesel from non-edible oil crops cannot even satisfy a small fraction of the existing demand for transport fuel. Microalgae on the other hand happen to be the only source which can meet the complete demand of transport fuels. Algae farming in less than 2-3 percent of India's total land can make the country self-sufficient in liquid fuel [1]. The growth rate is much faster than the other terrestrial crops, oil yield is estimated to be 20000 to 80000 litres per acre per year [2]. Algae can be grown on degraded land that is not suitable for conventional agriculture. Hence, using microalgae for biodiesel production will not compete with food and fodder production. Moreover, while extracted microalgal lipids can be utilized as a potential feedstock for biodiesel production microalgal carbohydrates can be used as a carbon source in fermentation industries to replace conventional carbohydrate sources like simple sugars or treated lignocellulosic biomass [3]. Algae are also used to treat wastewater in many facilities worldwide [4]. Algal biomass cultivation absorbs nutrients from the water and carbon dioxide from the air which results in cleaned wastewater and reduced atmospheric carbon levels. Microalgal bio-fixation of CO_2 in photobioreactors is a promising approach for higher biomass and biofuel production [5]. Production of 1kg of biodiesel from algae can fix 1.83kg of CO_2 [6].

Microalgae consist of a huge group of photosynthetic, auto/heterotrophic organism which has an amazing potential for cultivation as energy crops. Important considerations during selecting suitable microalgae species for biofuel production include cell biomass, lipid content, lipid quality, growth rate, response to factors such as light, temperature and nutrient input, growth medium etc. Microalgae produce and store lipids in the form of fatty acids, phospholipids, glycolipids and it can be used as feedstocks for biodiesel production by transesterification reaction in the presence of acid or base with methanol [7]. However, slow growth rate, low biomass yield, low and expensive harvesting efficiency are some of the challenges for economical biodiesel production from microalgae [8]. The problem can be solved by developing process technologies for harvesting and drying of algal biomass, extraction of oil, transesterification and downstream processing.

Microalgae and energy Chain

First generation biofuels are produced from organic matters like starch, sugars, animal fats and vegetable oils. These are essentially food materials and if too much fuel is produced from these raw materials, the food prices may rise drastically. There is also doubt that the second-generation raw materials such as wood, straw, grasses and wastes from the wood processing industry can partially satisfy the requirement of sustainable, environment friendly fuel in an inexpensive manner. In this perspective algae have been identified as a potential third generation biofuel feedstock due to its tremendous energy generation potential in multiple platforms. The requirements for establishing microalgae as an environmentally and economically viable, an emphasis should be given on combining fuel production with production of co-products [9]. There is also a possibility of establishing microalgal refinery which aims at obtaining biofuels, energy and high value products through biomass transformation [3]. With an integrated planning and arrangement, a variety of bioenergy other than biodiesel can also be synthesized from algae. Different ways to convert microalgal biomass

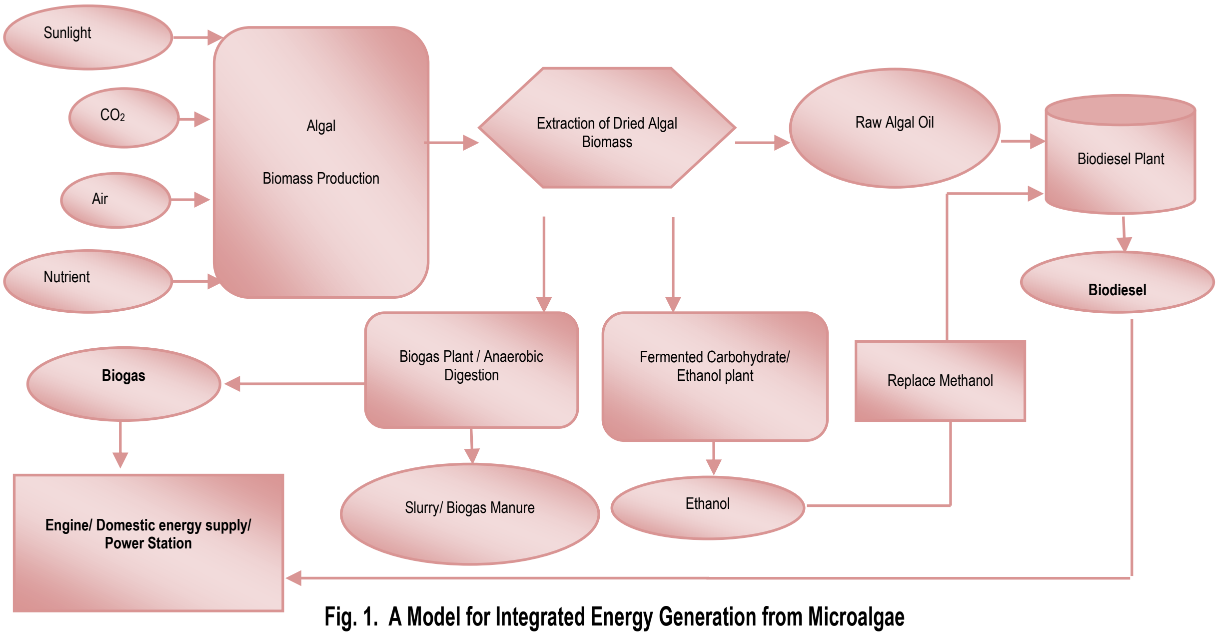

Fig. 1. A Model for Integrated Energy Generation from Microalgae

to biofuel include biochemical conversion, chemical reaction, direct combustion and thermochemical conversion. Biochemical conversion includes biomethane production through anaerobic digestion and ethanol production through fermentation [10] chemical conversion includes biodiesel production through transesterification of extracted algal lipids [11] Thermo-chemical conversion includes pyrolysis, gasification and liquefaction [12] Biomass generated in bioreactors can also be gasified or pyrolysed to produce a range of biofuels and can act as a complement in CO_2 sequestration strategy.

Production of microalgal biomass for biofuels production

Microalgae are projected to be grown in those areas where lignocellulosic or oilseed crops will not grow well. The yield of biomass per acre from microalgae is threefold to fivefold greater than the yield from typical crop plant acreage. Algal growth is influenced by factors such as sun exposure, temperature, and pH [13] The choice of cultivation systems significantly affects the efficiency and cost effectiveness of microalgal biofuel production process. Three most widely used systems for microalgae cultivation are namely raceways, photobioreactors and fermenters. Selection of a particular production system depends on the cultivation process, economic factors, genetic engineering aspects like type of strains etc. In this perspective, major systems have been reviewed as under.

Open pond culture

Open ponds are useful for little number of algal species that can tolerate extreme environmental conditions. The capacity, shapes and sizes of open pond can be varied depending upon the natural resources mainly water and land. However, an optimum depth of 15-30 cm which facilitates uniform distribution of sunlight to the bottom is required for maximum biomass yield. Generally, fast-growing algal species such as *Chlorella*, *Spirulina*, and *Dunaliella*, which thrive in highly alkaline or saline environments are suitable for open pond cultivation [11]. Algae cultivation by open pond culture system depends on seasonal variation. Two species of microalgae - *C. luteoviridis* and *P. Hussii* showed highest growth rate, productivity and remediation characteristics during summer and spring in comparison to winter [14]. Generally, open ponds are of four different types namely unmixed open ponds, raceway ponds, circular ponds and thin layer inclined ponds. Unmixed open ponds have lowest productivity (<1gm-2d-1) in comparison to other ponds. Raceway ponds are the most widely used for algal culture. Circular ponds have productivities ranging between 8.5 gm−2 d−1 and 21 gm−2 d−1 and are mainly used in Asian countries [15]. Thin layer inclined ponds show productivity upto 31 gm−2 d−1 [16]. Microbial contamination is inevitable because open ponds or raceways cannot be sterilized or kept under axenic conditions. Studies have shown that economic fuel production will require the microalgae to be grown in intensive culture in large outdoor ponds. An effective system could be like a raceway-shaped pond with a paddlewheel for circulating the water. Today, most of the large scale algal biomass production facility is based on Raceway ponds and this accounts for nearly 95% of the total worldwide algal production [17]. Raceway ponds require about 5x the volume that photobioreactors do to produce equivalent masses of algae [18] It was reported that using a specialized membrane for medium recycling algae cultivation in open pond were the most techno economically feasible and profitable option [19].

Photobioreactors

Even though open pond systems are economical due to their low capital-cost, closed systems offer better control over contamination, mass transfer and other cultivation conditions. In addition, photobioreactors permit culture of single-species for a prolonged duration [20]. So, to enable microalgal growth at the maximum productivity level to enhance economic competitiveness, the design of photobioreactors for efficient and cost-effective phototrophic cultivation is one of the most important issues in practice [21]. Photobioreactors of different types such as tubular photobioreactors, vertical bubble columns and airlift reactors, combined bubble column and inclined tubular reactors, helical photobioreactors, and flat plate photobioreactors have been developed and tested [22-25]. Tubular photobioreactor is most widely used for algal cultivation. It consists of an array of straight transparent tubes having diameters 10 mm to a maximum of 60 mm and the length as long as several hundred meters [26]. The tubes act as solar collector. The availability as well as intensity of light affects the success of algae culture. The growth of algae culture increases with increase in light intensities.The performance of a photobioreactor depends on several factors like the concentration of the biomass, the light intensity, the flow rate, and the concentration of oxygen at the entrance of tube [27]. Sometimes it becomes necessary to inject carbon dioxide to prevent excessive increase of pH [28]. Large tubular photobioreactors have been placed within temperature controlled greenhouses [29], but doing so is prohibitively expensive for producing biodiesel.

Fermenters

Even though most algae grow phototrophically, there are some species that are capable of heterotrophic growth. They use organic substrates as the sole carbon and energy sources. Generally, heterotrophic cultivation has been found to increase the total lipid content in algae compared to phototrophically grown cells [30, 31]. This mode of algal cultivation has got several advantages like non-requirement of light, lower harvesting cost, not dependant on weather and climate conditions, consistent reproducible production etc. The heterotrophically grown *Chlorella* cells accumulated lipids to about 55.2% of the cellular dry weight as opposed to 14.6% in phototrophically grown cells [30]. Heterotropically grown algae requires oxygen in sufficient quantity for catabolism of organic substrates.

Oil Potential of microalgae

Considering the present situation, it is evident that conventional oil crops are not going to significantly contribute to replace petroleum derived liquid fuels in the foreseeable future. This scenario could be changed dramatically when microalgae are used to produce biodiesel. Oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops. It is also possible to increase the lipid concentration of microalgae up to 80% by optimizing the growth determining factors. Microalgal oils differ from most vegetable oils in being quite rich in polyunsaturated fatty acids with four or more double bonds [32]. Studies show that biodiesel can be produced from macro algae also even though the lipid content (1.3-7.8%) of macro algae is less than micro algae [33]. Also, the lipid production rate (mg/L/day) in green microalgae is much higher than those of red or blood-red microalgae [5]. Several species of microalgae are capable of accumulation of lipids which contributes to high oil yields. The lipid content of microalgae varies within the range 1-70% which may increase upto 90% for certain varieties. Microalgae with high oil content are especially desirable for biodiesel production [11].

Extraction of oil from algal biomass

Oil can be extracted from microalgal biomass by various extraction methods like mechanical extraction using hydraulic or screw press, chemical extraction using different organic solvents, ultrasonic extraction and supercritical extraction using carbon-di-oxide.

Enzymatic extraction

Enzymatic extraction process is costlier than any other extraction process and this fact limits its utilisation for large scale production. In enzymatic extraction process water is used as solvent for enzymes to open up cell walls and facilitate easy and mild fractionation of oil, proteins and hulls. When the cell wall surrounded by oil and protein is removed with enzymatic degradation process, down-stream processing makes fractionation of the components possible to a degree which cannot be reached when using the conventional technique like mechanical pressing [34].

Chemical extraction

In chemical extraction method oil is extracted from solid materials by an organic solvent usually n-hexane or petroleum ether using an apparatus called Soxhlet apparatus. The process is called solvent extraction process. In this method large amount of extraction is possible for limited amount of solvent. This method is viable for large scale extraction. This process has got some disadvantages like poor extraction of polar lipids, long extraction time and hazardous boiling of solvent etc.

Ultrasonic extraction

The ultrasonic extraction of algae oil involves intense sonication of liquid which generates sound waves that propagate into the liquid media resulting in alternating high-pressure and low-pressure cycles [34]. During high pressure cycle, ultrasonic waves facilitate the diffusion of solvents in cell structure. Ultrasound exerts cavitation shear force on the cell walls and that results in breakage of cell walls and transfer of lipids into the solvent. After the oil is fully dissolved in solvent, the pulp is removed by filtration and the remaining solution is distilled to separate the oil from the solvent. This process is not cost effective for large scale production.

Biodiesel production from microalgae

Biodiesel is produced by a process called transesterification in which vegetable oil reacts with alcohol to produce biodiesel and glycerol. The reaction proceeds in stages: triglycerides are first transformed into diglycerides, then to monoglycerides and next to glycerol. The amount of FFA and water present in the oil affects the reaction rate and biodiesel yield. The higher amount of FFA will lower biodiesel yield by formation of soaps. All microalgal oils are not preferred for biodiesel production but suitable oils occur commonly and are known to produce many different kinds of lipids, hydrocarbons, and other complex oils) [35, 36]. The amount of long chain poly unsaturated fatty acids (greater than C18) is mostly common in microalgal oils which are not produced in sufficient quantities in plant oils. However, different factors like microalgal species, nutrient, environmental, and developmental conditions in which the cells are cultured and harvested affect the lipid composition.

Fatty acid composition of parent oil determines the different physicochemical properties of biodiesel. Biodiesel produced from feedstocks with high concentrations of saturated fatty acid commonly show better stability. But biodiesel produced from poly unsaturated oils has a lower melting point and thus, possess excellent cold flow properties compared to biodiesel from saturated fats (which tends to gel at ambient temperatures [37, 38]. To meet the biodiesel fuel quality standards, the extent of unsaturation in algal oils can be reduced by partial catalytic hydrogenation [39]. Biodiesel was produced from two species of algae and it was reported biodiesel production was maximum in *Odeogonium* species and minimum in Spirogyra species [33]. Biodiesel produced from 14 algae strains from the genus of *Chlorella, Haematococcus, Scenedesmus, Chlamydomonas* and *Chloroccum* by a one step extraction-transesterification method was quite comparable to diesel [15]. Biodiesel was produced from extracted algae oil by transesterification of fatty acids from algae biomass which followed fractioning on chromatographic columns [40].

Other Biofuels

Bioethanol

The starch content of microalgae is as high as conventional ethanol producing feedstocks like corn, wheat etc. Bioethanol is very important in reducing emissions of greenhouse gases. Two pathways can be used for production of bioethanol from microalgae, first: the fermentation of starch under dark and anaerobic condition within the microalgal cells, second: after oil extraction, fermentation of starch in oil extracted biomass using enzymes and yeast. The extraction of ethanol directly from the culture-media will lower the capital cost as well as energy requirement for ethanol synthesis from microalgae. There have been many efforts towards producing ethanol from microalgae by different processes. Ethanol was produced from *Chlorococum sp*. by fermentation process using yeast [41]. They obtained a productivity level of around 38% which supports the suitability of microalgae as a promising substrate for bioethanol production. However, challenges in bioethanol production from microalgae is imposed by lignin, a recalcitrant substance that cannot be converted to ethanol [42].

Biomethane

Biomethane is considered to be more environment congenial in comparison to petro-chemical fossil fuels. For better economical balance the biomass after lipid extraction needs to be transformed into methane [11]. The energetic value of the methane thus produced can potentially lead to an energetic balance of the microalgae to biofuel process [43]. Microalgae cultivation requires large amount of nitrogen and phosphate which influences the environmental and economic sustainability. It is therefore desirable to recycle the nitrogen and phosphorus contained in the microalgal waste after lipid extraction in order to reduce the use of fertilizers. Anaerobic digestion involving biotechnological approaches which can mineralise algal waste containing organic nitrogen and phosphorus, resulting in a flux of ammonium and phosphate that can then be used again as a substrate for the microalgae [44].

Integrated approach for biofuel production

Burning fossil fuels, primarily in power plants, is the main contributor to excess carbon dioxide. The microalgae essentially recycle the carbon dioxide from the power plant's stack gases into biodiesel [5]. Although this carbon dioxide is eventually released when the fuel is burned, the process effectively doubles the amount of energy generated for a given quantity of carbon dioxide. The process starts with a microalgal production followed by drying and extraction of algal biomass and subsequent processing. The first step of microalgal biomass production requires light, CO_2, water and inorganic nutrients-mainly nitrates, phosphates, iron and some trace elements. Sea water supplemented with commercial nitrate and phosphate fertilizers, and a few other micronutrients, is commonly used for growing marine microalgae. Fresh and brackish water from natural resources like lakes, rivers and aquifers can also be used. The next step is to recover the algal biomass from the produced algal broth. Water and residual nutrients recovered at this stage can be recycled and utilised for biomass cultivation. The concentrated and dried algal biomass is then subjected to oil extraction. Biodiesel is produced from algal oil by transesterification process. The algal biomass residue after oil extraction undergoes anaerobic digestion to produce biogas. This biogas will supply the primary energy needed for most of the production and processing of algal biomass. Assuming average values of biogas energy content and yield, biogas production from microalgal solids, after their 30% oil content has been removed, could provide at least 9360 MJ of energy per metric ton [11]. This substantial amount of energy is sufficient to run the microalgal biomass production process. Additionally, the nitrogen rich compound and water obtained from this process can be used as fertilizer and irrigation water. The fermentation of dried algal biomass produces ethanol that can be used as biofuel. Twostep process developed by [45] involved fermentation of microalgae to produce ethanol that was followed by methane production under anaerobic digestion. Carbon dioxide produced from the first step was used as nutrient for microalgal growth and the remaining algal biomass after second stage was used for electricity production.

Challenges in biodiesel production from microalgae

Continuous research is being carried on to find out the suitable algae species and growing conditions for maximum yield. There remain many challenges in terms of biotechnical, economic and environmental which have to be overcome for achieving the ultimate goal.

Economic challenges for microalgal cultivation

Microalgal biodiesel production process needs to be improved to make it economic and competitive with petrodiesel. Algal biomass harvesting and multiplication techniques together with trans-esterification process determine the economy of algal biofuel production. The choice of cultivation system plays a significant role in making the process cost effective and efficient. Open ponds are cheaper; bioreactors are far more expensive but currently the better method. Bioreactors need constantly pumping algae through a complex array of chambers and pipes, expensive to build, and energy-intensive to run. However, the capital costs for starting an algal biofuel production process include expenses for land, infrastructure establishment, bioreactors, labour and many overhead expenses. Many of the industries engaged in commercial production of microalgal biofuels suggested that for making the process cost-effective, algae to biofuels plants may be effectively developed on land adjacent to power stations (to convert CO2 from exhausts into fuel); in wastewater treatment plants; or in seawater [46]. Besides that, a variety of products like other biofuels, animal feed, and valuable pharmaceutical and cosmetic products may be produced from microalgae other than biodiesel [47]. This makes the production of biodiesel from microalgae a more cost-effective process.

Choice of algal strain

There are about 300,000 species of microalgae, whose diversity is much greater than that of land plants. The factors that should be considered during algae species selection include growth rate, optimal temperature range, lipid accumulation, and response to nutrient deprivation; as these factors affect the performance and productivity of the algae in the proposed culture system. However, high light intensity, nutrient stress, and nitrogen deficiency are considered to be the key factors for satisfactory oil accumulation by microalgae [48]. Rigorous screening is required for identification and selection of strains that can produce and accumulate the desired end product. Green microalgae like *Chlorella* sp., *Chlorococcum* sp. and *Neochlorosis oleabundans* are reported to produce a higher quantity of biofuel in comparison to blue-green algae [5].

Genetic engineering

Genetic engineering plays an important role in manipulating lipid biosynthesis in microalgae, to enhance productivity and to optimize the conditions for their cultivation, harvesting and processing. But unfortunately, this aspect has not been given much importance and genetic engineering of microalgae is still lagging behind than that of bacteria, fungi and other eukaryotes. Genetic and metabolic engineering in microalgae has mostly focused on producing non-oil, high-value bioactive substances [49]. Temperature tolerance of microalgae can be improved to reduce the need for expensive cooling process. There have been several research efforts that have concentrated on applying genetic engineering to microalgae. Potential fuel producing DNA from microalgae has been analyzed chemically and short-term expression of foreign genes such as firefly luciferase was achieved [50]. Progress has also been made with respect to the development of new protocols for introducing foreign genes into microalgae. Photo inhibition is another factor that needs to be addressed by metabolic engineering. Like plants, microalgae experience photoinhibition at high daylight levels, in that photosynthesis slows down once the light intensity has exceeded a certain value. Engineered algae that are either not photoinhibited or have a higher inhibition light threshold would significantly improve biodiesel production [11]. The prospect of growing transgenic microalgae has a great potential. The development of a number of transgenic algal strains boasting recombinant protein expression, engineered photosynthesis and enhanced metabolism encourage the prospects of engineered microalgae [51].

Harvesting and drying

Biomass harvesting cost can be a significant proportion of the total algal production cost and range between 3.3 and 30% of total cost [52]. The two factors that lead to low biomass concentration as well as costs and energy consumption in harvesting process are small sizes of microalgal cells and limited light penetration. Different technologies that are commonly used for harvesting microalgae are chemical flocculation, biological flocculation, filtration, centrifugation, and ultrasonic aggregation. Chemical and biological flocculation are characterised with low operating cost, high processing period and risk of bioreactive product decomposition. Filtration, centrifuge and ultrasonic flocculation on the other hand are more efficient and costly process. Some acceptable results have been obtained on filtration for colonial microalgae, but not for unicellular species [31]. Drying is the most important step that often needs to be considered for extraction of lipid from microalgae. Commonly practised sun drying process is the cheapest and time-consuming method. Some efficient but costly drying technologies include drum drying, spray drying, fluidized bed drying, freeze drying, and refractance window dehydration technology [15]. It is therefore, important to find a balance between harvesting and drying efficiency with the cost effectiveness to maximize the net energy output of the fuels from microalgae.

Conclusion

Microalgae provide the opportunity to satisfy much of the energy demand without competing with other biofuel feedstocks, energy resources and technologies. Microalgae can sustainably meet the existing demand of liquid transport fuel in developing countries which have favourable climatic conditions for algae cultivation, cheap labour and underdeveloped land. But several factors in terms of biotechnical, economic and environmental need to be addressed before commercialization of the process. Appropriate harvesting technologies should be selected based on the value of the target products, biomass concentration and the size of microalgal cells. To make the process cost-effective, different by-products need to be synthesized in an integrated approach. Even though the full access to this technology by the poor may be difficult, foreign investment could lead to revenues for the developing countries. Large-scale facilities are more economically viable but are also more likely to have higher social and ecological impacts.

Application of review

Biodiesel production from micro algae with an integrated approach to utilise the by-products as organic fertiliser.

Review Category: Renewable energy

Acknowledgement / Funding: Author thankful to Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, 741252 Nadia, West Bengal

***Research Guide or Chairperson of research**: **Prof S. Mukherjee**
University: Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, 741252 Nadia, West Bengal
Research project name or number: NIL

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] Bajhaiya A.K., Mandotra S.K., Suseela M.R., Kiran T. and Ranade S. (2010) *Asian Journal of Experimental Biological Sciences*, 1 (4), 728-739.
[2] Demirbas M. F. (2011) *Applied Energy*, 88(10), 3473-3480.
[3] Chew K.W., Yap J.Y., Show P.L., Suan N.H., Juan J.C., Ling T.C., Lee D. J., Chang J.S. (2017) *Bioresource Technology*, 229, 53-62.
[4] Mambo P., Westensee, D., Zuma, B., and Cowan, A. (2014) *Water SA*, 40(2), 385.
[5] Mondal M., Goswami S., Ghosh A., Oinam G., Tiwari O.N., Das P., Gayen K., Mandal M.K., Halder G. N. (2017) *Biotech* 7(2), 99.
[6] Saifullah A.Z.A., Karim M.A., Yazid, A.A. (2014) *American Journal of Engineering Research*, 3, 330-338.
[7] Muthukumar A., Elayaraja S., Ajithkumar T.T., Kumaresan S. and Balasubramanian T. (2012) *Journal of Petroleum Technology and Alternative Fuels*, 3(5), 58-62.
[8] Rashid N., Rehman M.S.U., Sadiq M., Mahmood T., Han J.I. (2014) *Renewable and Sustainable Energy Reviews*, 40, 760-778.
[9] Hannon M., Gimpel J., Tran M., Rasala B. and Mayfield S. (2010) Biofuels 1(5), 763-784.
[10] Spalore P., Cassan C.J., Duran E. (2006) *Journal of Bioscience and Bioenergy*, 101, 87–96.
[11] Chisti Y. (2007) *Biotechnology Advance*, 25(3) 294-306.
[12] Chiaramonti D., Oasmaa A. and Solantausta Y. (2007) *Renewable and Sustainable Energy Reviews*, 11, 1056–1086.
[13] Razzak A.S., Hossain M.M., Lucky R.A., Bassi A.S. and Lasa H.D. (2013) *Renewable and Sustainable Energy Reviews*, 27, 622-653.
[14] Osundeko O., Dean A.P. and Pittman J.K. (2014) *Plant cell and Physiology*, 55(10).
[15] Li Y., Horsman M., Wu N., Lan C.Q. and Dubois-Calero N. (2008) *Biotechnology Progress*, 24(4) 815–820.
[16] Carvalho A.P., Meireles L.A. and Malcata F.X. (2006) *Biotechnology Progress*, 22(6) 1490-1506.
[17] Mendoza J.L., Granados M.R., Godos I., Acién F.G., Molina E. and Heaven S., (2013). *Bioresource Technology*, 137:188–95.
[18] Pruvost J., Van Vooren G., Le Gouic B., Couzinet-Mossion A., Legrand J. (2011) *Bioresource Technology*, 102, 150–158.
[19] Thomassen G., Vila U.E., Dael M.V., Lemmensp B., Passel S.V. (2016) *Clean Technology Environment, Policy*, 1–14.
[20] Das D. (2015). *Algal biorefinery: an integrated approach. New York: Springer*, 73–101.
[21] Yen H.W., Hu I.C., Chen C.Y., Ho S.H., Lee D.J. and Chang J.S. (2013) *Biochemical Engineering Journal*, 78, 1-10.
[22] Oncel S., Vardar S.F. (2008) *Bioresource Technology*, 99(11), 4755–4760.
[23] Ranjbar R., Inoue R., Katsuda T., Yamaji H. and Katoh S. (2008) *Journal of Bioscience Bioengineering* 106(2), 204–207.
[24] Xue S., Zhang Q., Wu X., Yan C., Cong W. (2013) *Bioresource Technology*, 138, 141–147.
[25] Hall D.O., Fernandez F.G., Guerrero E.C., Rao K.K. and Grima E.M. (2003) *Biotechnology Bioengineering*, 82, 62–73.
[26] Posten C. (2009) *Engineering in Life Sciences*, 9(3), 165–77.
[27] Huang Q., Jiang F., Wang L. and Yang, C. (2017) *Engineering*, 3, 318-329.
[28] Berzin I. (2005) *United States Patent Application, Pub. no.: US2005/0260553 A1, USA, Publication date: Nov. 24, 2005.*
[29] Pulz O., (2001) *Applied Microbiology and Biotechnology*, 57(3), 287–293.
[30] Tredechi M.R. (1999) *Encyclopaedia of Bioprocess technology: Fermentation, biocatalysis and bioseparation Wiley*, 395-419.
[31] Grima, E.M., Belarbi, E.H. Fernandez, F.G.A., Medina, A.R. and Chisti, Y. (2003) *Biotechnology Advance*, 20(7-8), 491-515.
[32] Choi H.J. and Lee S.M. (2015) *Environmental Engineering Research*, 20(1), 25-32.
[33] Hossain Sharif A.B.M., Salleh, A., Boyce, A.N. and Chowdhury P. (2008) *American Journal of Biochemistry and Biotechnology* 4(3) 250-254.
[34] Mohan T.C., Raghuramudu K., Prasad P.A., Sandya C.R., Shanthanna P and Kumar, P. R. (2017) *International Journal of Fisheries and Aquatic Studies*, 5(6), 41-46.
[35] Banerjee A., Sharma, R., Chisti, Y. and Banerjee, U.C. (2002) Critical *Reviews in Biotechnology Chemicals*, 22, 245-79.
[36] Guschina, I.A., Harwood, J.L. (2006) *Progress in Lipid Research*, 45, 160–186.
[37] Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewite, M., Seibert, M. and Darzins, A. (2008) *Plant Journal*, 54, 621– 639.
[38] Schenk P.M., Thomas-Hall S.R., Stephens E., Marx U.C., Mussgnug J.H., Posten C., Kruse C.O. and Hankamer B. (2008) *Bioenergy Resource* 1, 20–43.
[39] Dijkstra A.J. (2006) *European Journal of Lipid Science Technology*, 108(3), 249–264.
[40] Belarbi E.H., Grima E.M. and Chisti. Y. (2000*) Enzyme Microbial Technology*, 26, 516-529.
[41] Harun R., Danquah M.K., Gareth M.F. (2010) *Journal of Chemical Technology and Biotechnology* 85, 199–203.
[42] Ragauskas A.J., Charlotte K.W., Brian H.D., George B., John C., Charles E., William J.F., Jason P.H., David J.L., Charles L.L., Jonathan R.M., Richard M., Richard T. and Timothy T. (2006) *Science*, 311, 484– 489.
[43] Sialve B., Bernet N. and Bernard O. (2009) *Biotechnology Advance*, 27(4), 409-416.
[44] Phang S.M., Miah M.S., Yeoh B.G. and Hashim M.A. (2000) *Journal of Applied Phycology*, 12(3), 395–400.
[45] Ueda R., Hirayama S., Sugata K. and Nakayama H. (1996) *US Patent* 5578,472, 1996.
[46] Behera S., Singh R., Arora R., Sharma N. K., Shulka M. and Kumar, S. (2015) *Frontiers in Bioengineering and Biotechnology*, 2, 90.
[47] Scott S.A., Davey M.P., Dennis J.S., Horst I., Howe C.J., Lea Smith D.J. and Smith A.G., (2010) *Current Opinion in Biotechnology*, 21, 277–286.
[48] Solovchenko A., Goldberg I.K., Didi-Cohen S., Cohen Z. and Merzlyak M. (2008) *Journal of Applied Phycology*, 20, 245-251.
[49] Bhatia S.C. (2014) *Advanced Renewable Energy Systems*, 610.
[50] Jarvis E.E., Dunahay T.G. and Brown L.M. (1992) *Journal of Phycology*, 28, 356-362.
[51] Rosenberg J.N., Oyler G.A., Wilkinson L., Betenbaugh M.J. (2008) *Current Opinion in Biotechnology*, 19, 430–436.
[52] Rajkumar R., Yaakob J. and Takriff M.S. (2014) *Bioresources*, 9(1), 1606-1633.