



Microalgae Cultivation Systems for Biodiesel Production: A review

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Abstract Microalgae represent a sustainable biofuel source because of their high biomass productivity and ability to sequester carbon dioxide from the air and remove water born pollutants. This paper reviews metabolic pathways, the current status of microalgae cultivation systems, including the advantages and disadvantages of both open and closed systems. The key barriers to commercial cultivation of microalgae and the way forward is also discussed.

Keywords Biofuel, Cultivation systems, Metabolic pathways, Microalgae

1. Introduction

There is increased interest in biofuels due to fluctuating crude oil prices and recognition of the fact that the global fossil fuel reserves are getting exhausted fast. Also, concerns about environmental pollution and resultant environmental change due to fossil fuel emissions. Furthermore, there is need for provision of alternative outlets for agricultural producers.

Global biofuel production has been increasing rapidly over the last decade, but the expanding biofuel industry has recently raised pertinent concerns. In particular, the sustainability of many first-generation biofuels; fuels made from food and feed crops and mainly vegetable oil, has been increasingly questioned over concerns such as reported displacement of food crops, effects on the environment and climate change [1]. In general, there is

growing consensus that if significant emissions reductions in the transport sector are to be achieved, biofuel technologies must become more efficient in terms of net lifecycle greenhouse gas emission reduction while at the same time be environmentally and socially sustainable. It is increasingly understood that most first-generation biofuels, except sugarcane ethanol, will likely have a limited role in the future transport fuel mix [2].

Biodiesel is a mixture of fatty acid alkyl monoesters (FAMES) derived from vegetable fats and oils. It can be used as a replacement of petro-diesel because of their structural similarity. Biodiesel is produced using vegetable oil, plant oil, and animal fat. Biodiesel is an alternative fuel for diesel and most diesel engines can use 100% biodiesel [1]. The main feedstock currently used for biodiesel production includes palm oil, sunflower, rapeseed, soybean, and canola seed. A great challenge of



using vegetable oils for biodiesel production is the availability of crop land for oil production to produce enough biodiesel that significantly replaces the current fossil fuel consumption [3]. According to [3], it would take approximately 24% of the existing crop land in the US to grow oil palm that is considered as a high yield oil crop or over three times of the current cropland in the US to grow soybean to produce enough biodiesel that would replace 50% of the transportation fuel in the US. Several studies have been conducted on using alternative oils such as waste oils from restaurants and kitchens and microalgal oils for biodiesel production [1]. In [4] investigation was done with restaurant waste oil as a precursor for sophorolipid and biodiesel production and [5] shows evaluation of the biodiesel production from waste cooking oil including economic analysis. Furthermore, [6] shows studies on biodiesel production from heterotrophic microalgal oil. A great advantage of using microalgal oil over vegetable oils for biodiesel production is that the production of algal oil does not need cropland and has much higher oil yield per acre of land because the microalgae can be grown in 3 dimensions in photobioreactors [1]. However, a big challenge of biodiesel production using algal oil is that the cost of algal oil production is extremely high [1].

The paper is organized as follows. Microalgae as a feedstock for biodiesel production is presented in Section II. Section III displays metabolic pathways: photoautotrophic, heterotrophic and mixotrophic. Microalgae cultivation systems are presented in Section IV and challenges for commercialization in Section V. Section VI develops future perspectives of microalgal biodiesel production. Conclusion is done in Section VII.

2. Microalgae as a Feedstock for Biodiesel Production

Microalgae are a diverse group of aquatic photosynthetic microorganisms that grow very fast and have the ability to yield large quantities of lipids adequate for biodiesel production [7]-[8]. Algae as a potential source of fuel was initially investigated during the gas scare of the late 1970s [8]. The National Renewable Energy Laboratory (NREL) started its algae feedstock studies in the late 1970s, but their research program was halted in 1996. Recent renewed interest has led the NREL to restart their research in algae [9]. The potential for microalgae to provide biomass for biodiesel production is now widely accepted [10]. Further, microalgae are recognized among the most efficient for this purpose, and some studies, for instance by [3], it was asserted that microalgae is the “only source

of biodiesel that has the potential to completely displace fossil diesel” (Table I). The superiority of microalgae as a feedstock for biodiesel production compared with the other conventional oil crops such as soybeans are : (1) microalgae have simple structures, but high photosynthetic efficiency with a growth doubling time as short as 24 h. Moreover, microalgae can be grown all year round [11]. (2) The species abundance and biodiversity of microalgae over a broad spectrum of climates and geographic regions make seasonal and geographical restrictions much less of a concern compared with other lipid feedstocks [11]. Microalgae may be cultivated on fresh water, saltwater lakes with eutrophication, oceans, marginal lands, deserts, etc., hence reducing or eliminating the competition for arable land with conventional agriculture [12] and opening economic opportunities in arid or salinity affected regions of the world [13]. (3)Microalgae can effectively remove nutrients such as phosphorus and nitrogen, and heavy metals from wastewaters [11]. (4)Microalgae sequester a large amount of carbon dioxide from the atmosphere via photosynthesis, for example, the CO₂ fixation efficiency of *Chlorella vulgaris* was up to 260 mg.L⁻¹.h⁻¹ in a membrane photobioreactor [14]. Utilization of CO₂ from thermal power plants by large-scale microalgae production facilities can reduce a great deal of the greenhouse gas emissions blamed for global warming [14]. (5)The production and use of microalgae biodiesel contribute near zero net CO₂ and sulfur to the atmosphere. (6)Microalgae can produce a number of valuable products, such as proteins, polysaccharides, pigments, animal feeds, fertilizers, and etc. In summary, microalgae are a largely untapped biomass resource for renewable energy production [11].

However, commercialization of microalgae biomass and biofuel production is still facing significant obstacles due to high production costs and poor efficiency. In face of these challenges, researchers are undertaking profound efforts to improve microalgae biomass production and lipid accumulation and lower downstream processing costs [11].



Table I. Comparison of some sources of biodiesel [3]

Crop	Oil yield(L.ha ⁻¹)
Corn	172
Soybean	446
Canola	1,190
Jatropha	1,892
Coconut	2,689
Oil palm	5,950
Microalgae (70% oil in biomass)	136,900
Microalgae (30% oil in biomass)	58,700

3. Metabolic Pathways

Microalgae may use one or more of the three main metabolic pathways depending on carbon conditions and light: photoautotrophy, heterotrophy, and mixotrophy [15]. Most microalgae are capable of photoautotrophic growth. Photoautotrophic cultivation in open ponds is a simple and low-cost method for large-scale production; however the biomass density is low because of contamination by other species or bacteria, limited light transmission and low organic carbon concentration [16]. Some microalgae can make use of organic carbons and oxygen to undergo rapid propagation through heterotrophic pathway. Heterotrophic cultivation has drawn increasing attention and it is regarded as the most practical and promising way to increase the productivity [36]-[38]. Currently, research on heterotrophic cultivation of microalgae is mainly focused on *Chlorella* [11]. Cell densities as high 104.9 g.L⁻¹(dry cell weight, *Chlorella pyrenoidosa*) have been reported [20]. Microalgae can adapt to different organic matters such as sucrose, xylan, glycerol and organic acids in slurry after acclimatization [21]. The ability of heterotrophic microalgae to utilize a wide variety of organic carbons provides an opportunity to reduce the overall cost of microalgae biodiesel production since these organic substrates can be found in the waste streams such as municipal and animal wastewaters, effluents from anaerobic digestion, food processing wastes, etc. [11]. On the basis of heterotrophic cultivation, researchers have carried out studies of mixotrophic cultivation which can greatly enhance the growth rate because it realizes the combined effects of photosynthesis and heterotrophy [11]. After examining the biomass and lipid productivities characteristics of fourteen microalgae [22], it was found that lipid and biomass productivities were boosted by mixotrophic cultivation. In [23], effects of molasses concentration and

light levels on mixotrophic growth of *Spirulina platensis* was studied. It was found that biomass production was stimulated by molasses, which suggested that this industrial by-product could be used as a low-cost supplement for the growth of this species. According to [24], it was established that the mixotrophic growth of *Chlamydomonas globosa*, *Chlorella minutissima* and *Scenedesmus bijuga* resulted in 3-10 times more biomass production compared with that obtained under phototrophic growth conditions. The maximum lipid productivities of *Phaeodactylum tricorutum* in mixotrophic cultures with glucose, acetate and starch in medium were 0.053, 0.023 and 0.020 g.L⁻¹.day⁻¹. These were respectively, 4.6-, 2.0-, and 1.7-fold of those obtained in the corresponding photoautotrophic control cultures [25].

4. Microalgae Cultivation Systems

Annual oil production from high-oil microalgae can be in the range of 58 700 to 136 900 litres per hectare [3]. If this microalgal oil is used for biodiesel production, it would take approximately 1.0 – 2.5% of the current cropland in the US to meet 50% of the US transportation fuel needs. This is much more feasible than the current oil crops [1]. Commercially growing microalgae for value-added products is usually conducted in open ponds (raceways) or closed photobioreactors (PBRs) under autotrophic (making complex organic nutritive compounds from simple inorganic sources by photosynthesis) or heterotrophic (cannot synthesize its own food) conditions at relatively warm temperature (20 – 30 °C) [1]. In autotrophic microalgal cultivation, the microalgae need sunlight (energy source), CO₂ (carbon source) and nutrients (P, N and minerals) for their photosynthesis and generate oxygen. The main difference of growing heterotrophic microalgae from autotrophic ones is the carbon source. The former requires organic carbon source such as glucose to support its growth. Normally autotrophic microalgae are grown for biodiesel production, mainly because they use CO₂ as their carbon source for growth [1]. Therefore, the whole cycle of growing microalgae for biodiesel production and combustion of biodiesel as fuel would generate zero net carbon dioxide emission to the atmosphere. However, sometimes heterotrophically grown microalgae can make much more oil than autotrophic ones. In [6], it was shown that heterotrophic growth of *Chlorella protothecoides* resulted in a significant increase of oil content of



microalgae from 14.5% under the original autotrophic growth to 55.2% (dry weight).

In a photobioreactor microalgal growth system, pure high-oil microalgae are grown in closed glass or plastic tubular bioreactors. Nutrient water is circulated in the bioreactors for keeping the microalgae from settling and for the growth of the microalgae. Natural sunlight is usually the energy source for microalgal growth [1]. Although artificial illumination to the photobioreactors is viable, it is much more expensive than natural illumination. Pure microalgal culture can be maintained in the photobioreactors. Heat exchanger is usually necessary to maintain an adequate temperature in the photobioreactors. A high concentration of microalgal biomass can be achieved in photobioreactors. In that case high dissolved oxygen may inhibit the microalgal growth, so degassing system is usually necessary to release oxygen from the water [1].

Open (raceway) ponds are similar to oxidation ditches used in wastewater treatment systems. They are large, open basins of shallow depth and a length of at least several times greater than that of the width [26]. Raceway ponds are typically constructed using concrete shell lined with polyvinyl chloride. Dimensions range from 10 to 100 m in length and 1 to 10 m in width with a depth microalgal growth of 10 to 50 cm [26]. Ponds are kept shallow as optical absorption and self-shading by the algal cells limits light penetration through the algal broth [27]. Wastewaters from animal operations and municipalities can be used for growing microalgae. Water recirculation or agitation is necessary to keep the microalgae from settling. Microalgal biomass concentration in the ponds is usually low compared to the photobioreactors. Wild algae and/or bacterial contamination is normally challenging in the open ponds (Table II) [1], [26]. According to [28], it was found that the open pond was the most viable method of combining algal cultivation and wastewater treatment in the 1950s.

Table II. A comparison of growing microalgae in open ponds and photobioreactors [1], [26]

	Raceway Pond	Photobioreactor
Estimated productivity (g.m⁻².day⁻¹)	11	27
Advantages	Low energy Simple technology	Pure algal culture High volumetric productivity

	Inexpensive	High controllability
	Well researched	Small area required
		Concentrated biomass
Disadvantages	Low productivity	High energy
	Contamination	Expensive
	Large area required	Less researched
	High water use	
	Dilute biomass	

Photobioreactors (PBRs) are more commonly used for growing algae for high value commodities or for experimental work at a small scale. Recently, however, they have been considered for producing algal biomass on a large scale as they are capable of providing optimal conditions for the growth of the algae [29]- [30]. A closed reactor allows species to be protected from bacterial contamination and shallow tubing allows efficient light utilization. Bubbling CO₂ provides high efficiency carbon uptake and water loss is minimized. PBRs provide very high productivity rates compared with raceway ponds. In their life-cycle assessment (LCA) study [29], volumetric productivity was estimated to be at least eight times higher in flat-plate and tubular PBRs. The reason why PBRs have not become popular is due to the energy and cost intensity of production and operation. PBRs require a far higher surface area for the volume of algal broth compared with alternative infrastructure. Much higher volumes of material are therefore required which in turn requires a higher capital energy input and increases environmental impacts [30]. During operation, algal biomass must be kept in motion to provide adequate mixing and light utilization. These increase productivity but also require additional energy for pumping. So far in comparison to raceway ponds the benefits of PBRs do not outweigh the necessary energy requirements identified in the LCA study published [29]. A net energy ratio (*i.e.*, energy produced/energy consumed) of 8.34 has been reported for raceway ponds as compared to a net energy ratio of 4.51 and 0.20 for flat-plate and tubular photobioreactors, respectively [29]. It is likely that ponds will continue to provide the most effective infrastructure for algal cultivation due to their low impact design and low energy input requirement. PBRs will continue to be important however, for laboratory work, developing cultures and producing biomass with high economic



value. As research continues it may also be possible to develop infrastructure that will provide the benefits of both PBRs and open ponds together. PBRs are of different configurations including flat plate, column and tubular [31]. In both open and closed microalgae culture systems, light source and light intensity are vital for the performance of phototrophic growth of microalgae. The development of optical trapping system, light delivery and lighting technologies, which improve the distribution and absorption and the advent of some new photobioreactors, will improve the efficiency of photosynthesis [32]. In addition, gas-liquid mass transfer efficiency is another critical factor affecting CO₂ utilization and thus the phototrophic growth [31]. According to [33], a 10 L photobioreactor integrated with a hollow fiber membrane module increased the gas bubbles retention time from 2 s to more than 20 s. This increased the CO₂ fixation rate of *Chlorella vulgaris* from 80 to 260 mg.L⁻¹.h⁻¹.

5. Challenges for Commercialization

In principle, producing biodiesel from microalgae has been proven to be economically viable. Biodiesel production from microalgal biomass or the advanced biodiesel technology has a potential for biofuel production to substitute fossil fuel without serious competition for arable land against food and feed production [1]. However, the prime challenge of the advanced biodiesel production is its high cost. The present microalgae production and the separation of the microalgal biomass from the growing media are too costly. An estimated cost to produce a kilogram of microalgal biomass with a mean oil content of 30% is \$2.95 and \$3.80 for photobioreactors and open ponds, respectively, assuming that carbon dioxide is available and free [3]. Taking account of 30% oil content in the microalgal biomass and the cost of oil extraction from the microalgae, the cost to produce a kilogram (approximately 1.14 liters) of crude microalgal oil is more than three times of that of producing a kilogram microalgal biomass [1]. This cost is much higher than vegetable oil production, e.g. the market price for crude palm oil which is possibly the cheapest vegetable oil was only \$0.52/liter in the US in 2006. It would be more disheartening if compared with petrodiesel production cost (the retail price of petrodiesel including taxes in the US in 2006 was only between \$0.66 and \$0.97 per liter) [1]. As of late 2008, it was indicated that seven US

government laboratories, thirty US universities, and around sixty biofuels companies were conducting study in this area [34]. Passionate efforts are also taking place in other parts of the world including (among many others) Australia, Europe, the Middle East, and New Zealand [35].

6. Future Perspectives of Microalgal Biodiesel

Production

To improve the economics of microalgal biodiesel production, more research and development are compulsory to reduce the costs of growing microalgae and the separation of microalgal biomass from the growth media, and to competently control culture contamination when grown in open ponds. The research and development efforts probably need to focus on the following areas: (1) Selection and development of high-yield, oil-rich microalgae: Oil-rich microalgal species can be enhanced through cultivation and genetic engineering to increase the oil content in their biomass without compromising the biomass production rate [3]. (2) Enhancement of the tolerance oil-rich microalgae to high and/or low temperatures. Most microalgae prefer to grow at the temperatures of 20-30 °C. When the temperature is higher than 30 °C, which happens very frequently during the sunny days in photobioreactors, heat exchangers have to be operated to cool down the microalgal culture to sustain a high microalgae growth. Installation and operation of the heat exchangers significantly add cost to the whole microalgal biomass production. Selection and modification of microalgae to aid them grow fast at high temperatures would probably eradicate the heat exchangers and contribute to the cost reduction of microalgal biomass production [3]. (3) Enhancement of the tolerance of oil-rich microalgae to the high concentration of oxygen. When microalgae grow under autotrophic conditions, they produce oxygen that dissolves in water to yield a super saturated dissolved oxygen concentration in the media, sometimes 4-5 times of the air saturation value. A combination of high dissolved oxygen with intense sunlight impedes the growth of the microalgae and destroys the microalgal cells. To prevent the inhibition and damage to the microalgae, a degassing system is necessary to keep the dissolved oxygen at a suitable level in the growth media. Increasing the tolerance of the microalgae to the high dissolved oxygen concentration in the media could also decrease the cost of microalgal biomass production [3].



(4) Improvement of the competitiveness of oil-rich microalgae against wild algae and bacteria. In open pond microalgae production, the contamination of wild algae and bacteria is very challenging. If the growth media is contaminated by wild algae and/or bacteria, the wild algae and bacteria will devour the nutrients in the media and significantly diminish the yield of the desired microalgae. Improving the competitiveness of the oil-rich microalgae against the wild algae and bacteria and deterring the wild algal and bacterial activities in the media for growing the microalgae also has a potential to reduce the cost of microalgal biomass production [3]. (5) Improvement of the engineering of the microalgae growth systems. Both microalgae growing systems presently used for microalgal biomass production, photobioreactors and open ponds have rooms for improvement. When microalgae grow in tubular photobioreactors, some of them stick on the wall of the tubes, significantly decreasing the penetration of light to the growth media and resulting in a lower yield of the microalgal biomass. Cost-effective materials which inhibit the microalgae from attaching the surface should be explored to maintain a high growth rate of the microalgae. The main drawback of growing microalgae in open ponds is contamination. Greenhouse ponds can be an effective system to avert contamination and to increase the microalgal density in the growth media [3]. (6) Development of cost-effective microalgae harvesting systems. Harvesting microalgal biomass contributes markedly to the total costs of the biomass production. Current technologies ordinarily involve coagulation, filtration and centrifugation, which are costly. Innovative cost-effective harvesting systems need to be explored to significantly reduce the cost of microalgal biomass harvesting [3]. (7) Application of the biorefinery model to microalgal biodiesel production system. Microalgal biomass contains lipids (oil), carbohydrates, proteins and other minor components such as minerals and vitamins. Oil is used for biodiesel production. Other constituents can be processed into value-added products. After oil extraction, the residues which are rich in carbohydrates, proteins and minor nutrients can be used to produce animal feed. They can also be utilized for biogas production through anaerobic digestion. Special high-value organic chemicals could be extracted from the residues and should be explored to increase the revenue of the microalgae-to-biodiesel process. All these byproducts have capabilities to improve the economics of the microalgae-to-biodiesel process [3]. (8) Combine microalgae cultivation with wastewater treatment. The microalgae could therefore provide a

means of improving the water quality of raw or partially treated effluent as well as providing livestock feed and/or biomass for energy generation.

7. Conclusion

Microalgae are diverse, grow very fast and have the ability to yield large quantities of lipids. They have simple structures and can be grown all year round. The species are abundant and can be grown over a broad spectrum of climates and geographic regions. They may be cultivated on fresh water, saltwater lakes with eutrophication, oceans, marginal lands, deserts. They can effectively remove nutrients such as phosphorus and nitrogen, and heavy metals from wastewaters and sequester a large amount of carbon dioxide from the atmosphere via photosynthesis.

Microalgae may use one or more of the three main metabolic pathways depending on carbon conditions and light: photoautotrophy, heterotrophy, and mixotrophy. In photoautotrophy, the microalgae need sunlight (energy source), CO₂ (carbon source) and nutrients (P, N and minerals) for their photosynthesis and generate oxygen. Heterotrophy requires organic carbon source such as glucose to support its growth and heterotrophic cultivation is regarded as the most practical and promising way to increase the productivity. Mixotrophy combines both photoautotrophy and heterotrophy.

Commercially growing microalgae for value-added products is usually conducted in open ponds (raceways) or closed photobioreactors (PBRs). In a PBR microalgal growth system, pure high-oil microalgae are grown in closed glass or plastic tubular bioreactors. Nutrient water is circulated in the bioreactors for keeping the microalgae from settling and for the growth of the microalgae. Natural sunlight is usually the energy source and artificial illumination may be used though it is much more expensive than natural illumination. Pure microalgal culture can be maintained in the PBRs. Heat exchanger is necessary to maintain an optimum temperature in the PBRs. A high concentration of microalgal biomass can be achieved in PBRs. A degassing system is necessary to release the generated oxygen from the water. Open ponds are large, open basins of shallow depth and a length of at least several times greater than that of the width. They are constructed using concrete shell lined with polyvinyl chloride. Dimensions range from 10 to 100 m in length and 1 to 10 m in width with a depth microalgal growth of 10 to 50 cm. Biomass concentration in the ponds is usually low compared to the PBRs. Wild algae and/or bacterial contamination is normally challenging in the



open ponds. PBRs provide very high productivity rates compared with raceway ponds.

The prime challenge of the advanced biodiesel production is its high cost. Therefore to improve the economics of microalgal biodiesel production, research should focus on: (1) Selection and development of high-yield, oil-rich microalgae, (2) Enhancement of the tolerance oil-rich microalgae to high and/or low temperatures, (3) Enhancement of the tolerance of oil-rich microalgae to the high concentration of oxygen, (4) Improvement of the competitiveness of oil-rich microalgae against wild algae and bacteria. (5) Improvement of the engineering of the microalgae growth systems, (6) Development of cost-effective microalgae harvesting systems, and (7) Application of the biorefinery model to microalgal biodiesel production system.

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