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Article *in* Green Chemistry · January 2011

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CRITICAL REVIEW

A critical review on recent methods used for economically viable and eco-friendly development of microalgae as a potential feedstock for synthesis of biodiesel

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Received 10th May 2011, Accepted 2nd August 2011
DOI: 10.1039/c1gc15535k

Microalgae are being considered as a viable feedstock for large-scale production of biodiesel. However, though it may look simpler to obtain lipids from microalgae, the overall process of choosing an algal strain, cultivation, harvesting, dewatering, and extraction of oil is quite complicated and not economically prudent at this time. A thorough understanding of algae and the overall biodiesel production process discussed in this paper is vital so that focused research might lower the costs involved. Various diverse species of microalgae are currently being used as feedstocks for biofuel. Heterotrophic culture may be preferred over photoautotrophic cultivation. For cultivation, specially fabricated photobioreactors (PBRs) have the capability to overcome the constraints and limitations of the open raceway ponds, although the former are cost intensive as compared to the latter. Exergy analysis of algal-biodiesel-carbon dioxide cycle shows the overall process to be renewable and hence should attain global attention.

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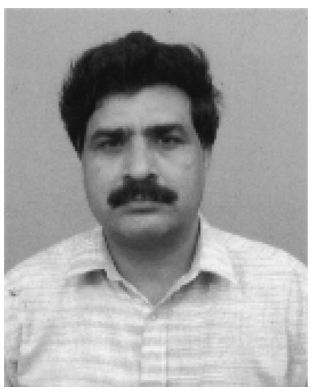
1 Introduction

The scenario of increasing global population growth, increasing energy demands, and diminishing fossil fuel reserves is alarming. It is believed that the proven oil, gas, coal, and uranium reserves will be exhausted in 60–80 years.[1] Even considering a stable population of 9 billion people after 2050, the "ultimately recoverable" reserves are estimated to only last until 2084 to



Yogesh C. Sharma

Yogesh C. Sharma, Ph.D., D.Sc. is a Professor of Applied Chemistry at Institute of Technology, Banaras Hindu University, India. Sharma's current research interests are in renewable energy. His group is a forerunner in India on synthesis and characterization of biofuels, especially biodiesel. He is working on various research aspects of producing algal biomass. India provides good conditions for algal growth. He desires to develop a cluster where carbon dioxide and wastes from various industries could be effectively used to grow algae and thus a strong step in the direction of manufacturing biofuels. This will also generate a job market for the country.

Dr Bhaskar Singh

Dr Bhaskar Singh is presently working as Research Associate (CSIR) in the research group of Prof. Y. C. Sharma on biodiesel development and related aspects. Bhaskar Singh holds an M.Phil. from Pondicherry (Central) University, India with a gold medal (2006). His current interests lie in the application of algal biomass for biodiesel synthesis, development of heterogeneous and green catalysts, and corrosion aspects of biodiesel fuel.

2112 considering the economic growth rates of 1.5 and 3.0% per annum, respectively.

Recent focus on sources of alternative energy includes "first generation" feedstocks for biofuels, which includes fuel derived from the starch and oils in plants like cereals, sugar cane, and oil seeds. However, concern over the low energy efficiency, nutrient and water needs, and the use of fuel crops in place of agricultural crops has caused redirected focus to other feedstock sources. "Second generation" biofuels include non-food biomass of lignocellulosic ("cellulosic") materials such as bagasse or stover from sugar, forest and crop residues, municipal solid wastes, vegetative grass, and short rotation forest crops.[2–3] Another aspect that has led to the emergence of the 2nd generation feedstock is the high cost of bioethanol and biodiesel derived from 1st generation feedstock.[4] The oilseed from which the oil is derived for synthesis of biodiesel becomes a discarded waste, which is comprised of lignocellulosic material that is composed of cellulose, hemicellulose, and lignin and thus finds further application.[5] Gutierrez *et al.*[6] have proposed simultaneous biodiesel and bioethanol production to make the process cost effective. Oil was extracted from palm seeds for biodiesel feedstock and bioethanol was developed from the lignocellulose obtained from the residue of the palm shell kernels.

More recently, algae, particularly microalgae, have been considered as a "third generation" biofuel feedstock for oil (sometimes known as "oilgae") as they grow in water and thus do not compete with land-based food crops.[7] The first generation feedstocks that mostly employ food grains (*viz*. cereals) and edible oils require a high amount of fertilizers and non-renewable energy and result in addition of CO_2 to the atmosphere. However, algae have all the benefits that are possessed by the second generation biofuel feedstock such as reduction in net CO_2 emission and are renewable in nature. A recent comprehensive review of first, second and third generation biofuels is written by Dinjus *et al.*[8] The possibility of biohydrogen and bioelectricity as fourth generation biofuels are also being explored.[9] The added advantages of using algae as a biofuel feedstock are summarized in Table 1.

In 2002, world energy demands were 3.8×10^{18} GJ, of which 81% came from fossil fuels.[10] In 2008, fossil fuels comprised 88% of the global energy consumption.[11] The current production of biofuel is low but has shown a steep increase in recent times. The world biofuel production was 62 billion liters in 2007 and is poised to increase further, provided that a sufficient feedstock is availabile.[12] Hence, if biofuel production is to be enhanced, a tremendous quantity of feedstock will be needed. The feedstocks that are available worldwide are scanty and have competing uses, which is a major constraint in large scale production of biofuels. The alternative is to find a feedstock that can be cultivated in an enormous amount, in less space, and is not consumed by humans. Microalgae appear to satisfy these criteria and have the potential to supply several times more lipids for biofuels than alternative feedstocks.

2 Research activities on production of biodiesel from 1980 to 1996

The National Renewable Energy Laboratory (NREL) has been actively engaged in production of biodiesel from algae since 1980s. A pioneering work on microalgae derived biodiesel has been studied and documented by Sheehan *et al.*[13] A comprehensive report has been prepared by Sheehan *et al.* with focus on aquatic species programme. It is reported that microalgae systems require much less water than oilseed plants. The simple cellular structure in microalgae is helpful in efficient conversion of solar energy to chemical energy. Thus, microalgae are capable of producing 30 times more oil per unit area of land as compared to the terrestrial plants. The algal biomass contains three major components, *viz*. carbohydrates, proteins, and natural oils. Sheehan *et al.*[13] also demonstrated that in an open pond, theoretical productivity of microalgae can go as high as 50 grams of algae per square meter per day. However, it was observed that a fall in temperature was a major deterrent to achieve the high level of productivity and reduced the microalgae productivity to 10 g m^{-2} d^{-1}. When the cultivation was tried in deserts where high temperatures existed in day time, the similar problem of a fall in temperature was encountered during night time. The authors are of the view that tropical nations (such as India, China, Indonesia, South Africa, *etc.*) are more suited to provide high temperature during most months. The authors (Sheehan *et al.*[13]) discussed the efficacy of raceway ponds which are termed so because algae, water, and nutrients circulate around a racetrack with paddlewheels to provide flow. They are designed so that they are shallow to provide a better penetration of sunlight where water and nutrients are constantly fed and then the mature algae that are suspended in water are removed from the other end. It was also observed that the lipid content in various microalgal strains differed substantially and the nature of the lipid has a significant effect on the properties of the fuel developed. Hence, the characterization of the lipid profile in microalgae becomes pertinent. The photosynthetic efficiency of six algal strains, *viz*. *B. braunii*, *Dunaliella primolecta*, *Isochrysis* sp., *Monallanthus salina*, *Phaeodactylum tricornutum*, and *Tetraselmis sueica* was studied. Of these species, *Phaeodactylum tricornutum*, and *Tetraselmis sueica* were found to show a high lipid productivity

John Korstad

John Korstad, PhD, is a Professor of Biology and Director of the Honors Program at Oral Roberts University in Tulsa, OK. His specialty is limnology with specific expertise in nutrient–phytoplankton–zooplankton interactions. He has vast experience in aquaculture, including two sabbatical years doing research at SINTEF in Trondheim, Norway. Dr Korstad's desire is to connect various industries that produce excess CO_2 and wastewater nutrients to cultivate algae for biofuels and high-end bioproducts. This would essentially be a win-win-win situation through air and water quality remediation while producing economically valuable products and local jobs.

Table 1 Benefits of using algae for biofuels

i. Non-competitive with food crops and land use (as long as ponds are not built on agricultural land).

ii. By most, but not all, estimates, prospective/proven oil content from algae/biomass is orders of magnitude higher than from other feedstocks like corn, sugar cane, jatropha, *etc.*

iii. Algae need CO_2 to photosynthesize and can be used to sequester CO_2 from industrial sources of flue and flaring gas.

iv. Algae-based fuels are C-neutral or even more C-capturing than releasing.

v. Algae can be used to remediate high-nutrient water sources such as sewage treatment plant and agricultural runoff.

vi. End-product can be biofuel and/or other higher value feed (protein), pharmaceutical, and health-related products.

vii. Different species of algae can be grown in polluted, saline, brackish, and freshwater.

viii. Algae can be used as one component of an aquaculture-centered system where fish like Tilapia are grown for food in a recirculating and hydroponic system.

ix. Algae do not contribute to acid deposition (no SO_2 emissions).

x. Some species of bluegreen algae utilize N_2 to produce NO_3 (a process called "biological nitrogen fixation"), thus sequestering atmospheric nitrogen and eliminating or at least lessening the need for nitrogen fertilizers. This can help reduce the need for nitrogen fertilizers and, more importantly, help confront problems such as the expanding "Dead Zone" in the Gulf of Mexico.

xi. Algae biofuels are thus renewable, sustainable, and environment "friendly".

of 4.34 and 4.47 g lipid m^{-2} d^{-1}, respectively. Among the various strains of algae, diatoms were found to be promising for neutral lipid production. The lipid content of the biomass was found to increase when the microalgae was cultured under nutrient-limited conditions (*i.e.* deprivation of nitrogen and silicon). However, the nitrogen deprivation was not favorable for a few species like *Euglena* and *Nannochloropsis* strains as it blocked their cell division. The complex mechanism that leads to lipid accumulation in some algal species when the cells are deprived of nitrogen is still unexplained. The general hypothesis is that the nutrient (nitrate or silica) deprivation 'targets' the excess fixed carbon into storage lipids. It is also hypothesized that lipid accumulation is an indirect consequence of inhibition of a stage in the cell cycle. Also, when the divisions are blocked, the rate of neutral lipid utilization by the cell is slower than its rate of synthesis and thus triglycerides get accumulated in the cells. The light saturation limits the productivity to below 50 mt ha^{-1} per year due to limited penetration of light and other factors. The productivity range that was common lay between 15 and 25 g m^{-2} d^{-1} during the 8 month growing season and ranges higher than this were rare.

3 About algae and their feasibility as feedstock for biodiesel

3.1 Microalgae species

Algae can be arbitrarily separated into two groups – prokaryotic bluegreen algae (cyanobacteria) and eukaryotic algae (which are non-vascular plants without complex reproductive organs).[14] The diverse taxonomic groups of eukaryotic algae are normally distinguished by key cell wall or other chemical differences, particularly accessory pigments that often contribute to their distinct coloration and lipid properties.[15] The eukaryotic algal species are grouped into nine divisions. Among these divisions, only six divisions are prominent with the largest being Chlorophyceae (green algae), followed by Phaeophyceae (brown algae), Pyrrophyceae (dinoflagellates), Rhodophyceae (red algae), Chrysophyceae (yellow-green algae, and also called golden-brown algae) and Bacillariophyceae (diatoms).[16,17] Microalgae are commonly found in freshwater and saltwater habitats, have growth rates at 1–2 cell divisions per day in optimum conditions. Their size varies from very small (3–30 μm) to giant kelps that are 70 m long. Macroalgae (commonly known as "seaweed" or "kelp", and in the Chlorophyceae, Phaeophyceae, and Rhodophyceae) are more commonly found in saltwater habitats, can grow up to 50 cm day^{-1}, and can reach lengths of 70 m. Different species of algae can thrive in varied habitats, including fresh, salt, brackish, pristine, polluted, and other types of waters.[18] As autotrophs, algae are at the base of aquatic food chains.[10]

3.2 Potential of microalgae for biodiesel production

Microbes that accumulate more than 20% of their cellular dry weight in lipid-like oils are called oleaginous organisms. Yeast, fungi, and algae have the potential for accumulation of lipids in the form of triacylglycerol. Lipid obtained from microalgae, yeast, or fungi can be categorized in three parts: crude lipids, neutral lipids, and total lipids. Crude lipids include neutral lipids and pigments. Neutral lipids are comprised of triglycerides, free fatty acids, hydrocarbons, sterols, wax and sterol esters, and free alcohols. Total lipids comprises of pigments, phospholipids, glycolipids, and the neutral lipids.

Escherichia coli and *Saccharomyces cerevisiae* can be converted into oleaginous organisms by genetic engineering.[19] *Rhodosporidium toruloides*, a yeast, has been reported to accumulate up to 67.5% (w/w) of cellular lipid content cultured in a high-density fermentation in a 15 L stirred tank bioreactor in 134 h. The lipid productivity of the microalgae was 0.54 g l^{-1} h^{-1}. The fatty acids that were prevalent in the oil were C16 and C18 carbon atoms. The major fatty acid constituents

were oleic acid, palmitic acid, stearic acid and linoleic acid.[20] However, the composition of neutral lipids in yeast and the potential for conversion of lipids to biodiesel is still unexplored for their suitability as a potential feedstock oil. Excellent reviews are available on the scope of microalgae for synthesis of biodiesel.[11,21–28] The major saturated fatty acid in algae is palmitate (palmitic acid), the most common saturated fatty acids in animals and plants. In comparison to cyanobacteria, eukaryotic algae contain more unsaturated fatty acids. Like other photoautotrophs, their requirements for photosynthesis and growth include sunlight, CO_2, inorganic nutrients, and water.[24] Thus, sequestering carbon dioxide is another advantage they bring in the overall process of their growth.

Recently, Ahmed *et al.*[29] and Rodolphi *et al.*[30] reviewed various microalgae strains for effectiveness as feedstock for biodiesel. Ahmed *et al.*[26] looked at diatoms, green algae, eustigmatophytes, prymnesiophytes, and red algae and reported lipid content in them ranged from 9.5% (red algae) to 39.8% (diatoms). The lipid productivity (amount of lipid produced per volume of algae per day) ranged from 17.4 to 60.9 mg L^{-1} day^{-1}. Researchers are optimistic that microalgae may one day prove to be a reliable and large-scale alternate feedstock for production of biodiesel. Bioethanol contains only around 64% of the energy content of biodiesel.[31] Hence, biodiesel as a fuel is favorable to bioethanol considering the EROEI (energy return on energy invested) that is discussed later in this review. Jansson and Northen[32] report that cyanobacteria and other microalgae can be used for carbon capture to mitigate climate change.

Algae may be single-celled individuals or multi-celled colonies. Up to half of microalgal dry weight biomass is principally comprised of carbon, which is obtained from carbon dioxide. Around 100 tons of algal biomass in the process of photosynthesis fixes approximately 183 tons of CO_2.[31] Microalgae have the ability to synthesize lipids through photosynthesis. Macroalgae are composed of sugars and other carbohydrates and thus may be converted to other biofuels such as biogas and bioethanol. The photosynthetic efficiency (efficiency to capture light) of microalgae is more than 10%, whereas the same for terrestrial plants is less than 0.5%.[33] An important aspect with algae is that they can accumulate more lipids in stressed conditions in comparison to the optimum conditions. When microalgae are deprived of a nutrient such as nitrogen, excess carbon is assimilated in the cells of algae and converted to triglycerides. However, reduced nitrogen supply results in non-proliferation of algal cells and lipids have to be stored in the existing cells. Nitrogen deprivation, though, has varying effects on different species of microalgae. *Chlorella* sp. can accumulate total lipids up to 85% of their dry weight, while the total lipid content decreases for *Dunaliella* sp. when deprived of nitrogen.[34] Though the lipid productivity from the microalgae is low as compared to that obtained from yeast (0.54 g l^{-1} h^{-1}), the microalgal oil mainly contains neutral lipids (*i.e.* triglycerides and free fatty acids) that can undergo saponification and esterification and hence can be converted to biodiesel to meet the specifications of American Society for Testing and Materials (ASTM).[34] However, the neutral lipids (if they are not subject to the usual vegetable oil refining processes) also are comprised of hydrocarbons, wax and sterol esters, free alcohols, and sterols which cannot be saponified. The effect of their presence in biodiesel is still unexplored and warrants a holistic approach before they can be used as a source of fuel. Methyl ester production from oil derived from yeast is also unexplored at present. Packer *et al.*[35] have developed a mathematical model for growth and neutral lipid production of a green microalgae, *Pseudochlorococcum* sp. in batch culture. The model with low nitrogen concentration fitted well with the data. However, the model overestimated the biomass with high nitrogen concentration which was an indication that there are limiting factors other than nitrogen.

The unicellular structure of microalgae allows the easy conversion of solar energy in chemical energy.[36] When compared with terrestrial plants, microalgae are more efficient in conversion of sunlight to chemical energy and thus provide more energy than needed during their processing.[31] Also, there are high losses of water during photosynthesis in terrestrial plants. Cooper *et al.*[37] have discussed means of expanding algae oil production by various methods. These include isolation of new strains of algae to produce larger quantities of lipids, identification of environmental conditions suitable for fast growth of oil producing algae, and studying the life cycle of algae for optimized harvesting. Ehimen *et al.*[38] have successfully optimized the variables affecting the transesterification reaction using microalgal lipids. A high methanol to oil molar ratio of 315 : 1 was found to be optimum for the synthesis of biodiesel. An asymptotic curve for decrease in specific gravity (an indication of high biodiesel product) was obtained in 2 h at 60 °C. Microalgae can double their biomass within 24 h and the doubling time during exponential growth is just 3.5 h. Microalgae commonly have an oil content in the range 20–50%, and may exceed 80% for selected species.[39] Microalgae have the potential to produce around 100 times more oil per hectare than terrestrial plants in a comparable growing area.[24] Demirbas and Demirbas[31] estimated that algae could yield oil in the range 8093.71 to 32 374.85 l per hectare which is 7–31 times more than that which could be obtained from a high yield terrestrial crop such as palm. The high volume of oil accumulated by microalgae is the result of their efficiency to capture solar energy, which is 10 to 50 times more than the terrestrial plants.[40] However, not all microalgae have good prospects for feedstocks for synthesis of biodiesel. For example, *Dunaliella*, despite being cultivated on a large scale and able to grow in hypersaline environments, is not suitable for biodiesel feedstock as it can bear oil content of only 23% of the dry weight of the microalgae as compared to other microalgae that can bear high oil content.[41]

Microalgae have the potential to displace fossil fuels owing to their fast biomass productivity and high lipid content.[42] Algal oil has been shown to bear low acid value (acid number, 0.71) and a high conversion (90.20%) of fatty acid methyl esters (FAME) using base-modified titania as catalyst.[43] A low acid value of microalgal oil will allow for a single step transesterification using a super base catalyst which otherwise would have necessitated a two-step process (*i.e.* esterification followed by transesterification). The chemical formula for FAME is $C_nH_{xn}O_2CH_3$, where $n = 14–18$ and $x = 2$. Its energy content of 32.6 MJ L^{-1} is close to that of petroleum diesel (35.0 MJ L^{-1}).[44] Jordan and Gutsche[45] developed a continuous process for the production of FAME by transesterification from natural triglycerides (fats and oils). Chi *et al.*[46] reported

conversion of glycerol (obtained from biodiesel production) to docosahexaenoic acid (DHA) through fermentation using the microalga *Schizochytrium limacinum*. The quantitative analysis of the lipid content has been determined by time-domain nuclear magnetic resonance (TDNMR) and was found to be simpler than the gravimetric method of analysis.[42]

3.3 Other uses of microalgae

Harun *et al.*[36] report on the renewable fuels and other useful resources that can be obtained from microalgae. These include biodiesel, bioethanol, biomethane, fine organic chemicals, food, and food products. Algae such as *Nostoc* and edible cyanobacteria have been used as a food by Chinese people for over 2000 years. The commercial applications of eukaryotic algae have only been developed in the last 50 years.[17] Algae have been used in the aquaculture industry as enhanced feed for invertebrate "start food" for fish larvae.[47] Microalgae have also been considered as a rich source of antioxidants.[48] Brown and red algae have been harvested for synthesis of valuable products such as agar and alginic acid.[49] There are some varieties of algae that are edible and are also rich in dietary fiber, minerals, and proteins.[48] A group of green algae have been used to decolorize the toxic azo dyes.[50] Marine plants produce C_{18}, C_{20}, and C_{22} polyunsaturated fatty acids (PUFAs) that are essential nutrients of many animals and human beings.[51,52] COGNIS (Whyalla and Hutt Lagoon, Australia),[53] is engaged in large scale production of carotenoids from the algae *Dunaliella salina*.

The separation of such precious by-products is crucial for the economics of microalgae culture for extraction of oil. The Bio Fuel Systems (BFS) Blue Petroleum Organization is engaged in production of artificial oil from CO_2 emissions and in the process neutralizes 938 kg of CO_2 per barrel. The biofuel that is produced has calorific value of 9700 kcal kg^{-1}. The phytoplankton production is linked to a cement plant that supplies CO_2. The process utilizes solar energy, photosynthesis, and electromagnetic fields to capture and accelerate the conversion of CO_2 to oil. The biomass created by this biochemical conversion is subjected to catalytic and thermo-chemical conversion yielding middle-distillate hydrocarbons. The biofuel thus developed is free from sulfur and heavy metals besides reducing atmospheric CO_2 to significant level. The organization reports that out of 2168 kg of CO_2 utilized during the process of synthesis of biofuel, 938 kg will not return back to the environment.[54]

4 Choosing an appropriate algal strain

There are approximately 300 000 species of microalgae, so the possibilities of selecting appropriate species for cultivation for biofuels appears vast. Prominent species currently used for biofuel feedstock include *Chlamydomonas reinhardtii*, *Dunaliella salina*, *Botryococcus braunii*, *Chlorella* spp., *Phaeodactylum tricornutum*, *Thalassiosira pseudonana*, *Nannochloropsis*, and *Isochrysis* spp.

Two important factors that should be considered in selection of appropriate algal species are their cell biomass and lipid content. Liu *et al.*[55] reported that different species of the green alga *Chlorella* can grow photoautotrophically, mixotrophically, and heterotrophically with high biomass concentration. Based on the lipid content, *Chlorella vulgaris* and *Chlorella protothecoides* can be chosen for synthesis of biodiesel in photoautotrophic or heterotrophic culture conditions. The oil obtained from heterotrophically grown cells bears properties similar to that of fossil fuel oil in terms of oxygen content, heating value, density, and viscosity. Another advantage of heterotrophic growth is that the process does not require light and thus reduces the overall cost of the product. However, its disadvantage is that it needs sugar (preferably glucose) for the growth of cells which enhances the production cost of oil from microalgae. The fed-batch cultivation (controlled fermentation system) was found to perform better than the batch culture where various carbon sources, *e.g.* glucose, lactose, sucrose *etc.*, was used to feed the cell. A lipid content and lipid yield of 0.52 g g^{-1} and 5.27 g L^{-1} was obtained with the batch culture using glucose as substrate, whereas in the fed-batch cultivation system, an exponential growth phase of the cell was observed with the lipid yield of 20.7 g L^{-1}.

Growing conditions must be optimized for triglyceride production by the microalgae.[56] Diatoms can accumulate a high amount of lipids as energy storage molecules, but they include a considerable amount of phospholipids that cannot be converted to biodiesel by the commonly employed method of transesterification. The constituents of fatty acids in microalgae show that they are rich in polyunsaturates, which is desirable for good cold flow properties in the biodiesel fuel. For example, 62.8% of the biomass of *Chlorella protothecoides*, a commonly used microalgae for biofuel production, are polyunsaturated fatty acids.[31]

Liu *et al.*[55] investigated the production potential of *Chlorella zofingiensis* for production of biodiesel using various organic carbon sources. *C. zofingiensis* was earlier found to grow without light using glucose as a sole source of carbon energy.[57] It was found that batch fermentation enhanced the lipid production in *C. zofingiensis* to a high yield of 20.7 g L^{-1} and a productivity of 1.38 g d^{-1} L^{-1}. However, glucose is cost intensive and accounts for 60% of the overall production cost of biodiesel. The cost could be reduced by other sources of sugar from industrial and agricultural waste such as molasses, which is a by-product of the sugar industry and constitutes 40–50% (w/w) sugar.

5 Cultivation of microalgae

The steps involved in biodiesel production from algae are growing algae in an engineered pond or photobioreactor, harvesting of the biomass, extracting the oil from algal biomass, and lastly, synthesis of biodiesel from the algal biomass.[58] Broadly, four types of microalgae cultivation techniques are available, including photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic. Of these, the most commonly used methods are the first two.[26]

Photoautotrophic cultivation is the most commonly employed technique utilizing sunlight or artificial light. There is a large variance in the lipid content of the microalgae grown this way, ranging from 5–68%. The highest lipid content is sometimes obtained by restricting the nitrogen content, resulting in higher lipid content but reduced biomass productivity. Nitrogen availability to the microalgae should be balanced so that it optimizes lipid and biomass production. The highest lipid productivity

Table 2 Comparison of oil content and biodiesel obtained from some microalgae species

Microalga	Reactor for cultivation	Oil content	Biomass productivity	FAAE (%)	Reference
Dunaliella tertiolecta	Drum & high density polyethylene totes	NL = 5.6 ± 1.0%	2.77 g m^{-2} d^{-1}	19.0–20.9	34
Wild freshwater microalgae	Natural pond	NL = 4.5 ± 0.2%	3.75 g m^{-2} d^{-1}	31	
Chlorella; acid value = 10.21 mg_{KOH} g^{-1}	Photoautotrophic culture	0.276 g g^{-1} of biomass	—	92.22	38
Chlorella protothecoides	Cultivation done in shaking flasks at 28 °C at 220 rpm for 4 to 8 days	38.20 to 61.00%	—	—	42
Algae oil; acid number = 0.71	—	—	—	90.20	43
Chlorella protothecoides	Batch culture (using carbon sources) Fed-batch cultivation (controlled fermentation system)	L = 52% of the dry biomass; TAG = 72.1% of the total lipids (in batch culture)	LY = 20.7 g L^{-1} Productivity = 1.38 g d^{-1} L^{-1} (in fed-batch cultivation)	—	55
Tetraselmis suecica	Heterotrophic cultivation	L = 53.8%	CC = 28.88 g L^{-1}	—	59
Chlorella protothecoides	Photosynthesis-fermentation model	L = 69.32%	CG = 23.9 g L^{-1} d^{-1} LP = 11.8 g L^{-1} d^{-1}	—	60
Chlorella sp.	Semi-continuous mode cultivation (batch mode was converted into semi-continuous mode by withdrawing a fraction of working volume and replacing it with fresh nutrients)	0.448 g g^{-1} of biomass	LP = 0.139 g L^{-1} d^{-}	—	61

FAAE: fatty acid alkyl ester; NL: neutral lipids; L: lipids; LY: lipid yield; LP: lipid production; CC: cell concentration; CG: cell growth; rpm: rotation per minute

reported through photoautotrophic cultivation is 179 mg^{-1} L^{-1} d^{-1} by *Chlorella* sp. with 2% CO_2 and 0.25 vvm (gas volume flow per unit of liquid volume per minute) aeration.[26]

Heterotrophic cultivation of microalgae refers to a situation where organic carbon is used as both energy and carbon source. The simple and cheap carbon source such as glucose, acetate, and glycerol may be used. Azma *et al.*[59] cultivated the green microalgae, *Tetraselmis suecica* in heterotrophic conditions in total darkness in natural sea water. The cell concentration obtained by this method of cultivation (*i.e.*, 28.88 g L^{-1}) was 2–3 times higher than that obtained from the photoautotrophic culture (*i.e.*, 8.40 g L^{-1}). Xiong *et al.*[60] developed a model to maximize the growth of *Chlorella protothecoides* coupled with heterotrophic fermentation for maximizing cell density and increasing lipid accumulation. The photosynthesis-fermentation model (PFM) was found to perform better than the individual photosynthesis model (PM) and fermentation model (FM). Cell growth rates of 23.9 g L^{-1} d^{-1} and lipid production rates of 11.8 g L^{-1} d^{-1} were obtained with the PFM. The dry cell weight (DCW) obtained in the PFM and FM were 33 g L^{-1} and 18 g L^{-1} respectively. Whereas, the oil concentration in PFM and FM were 14 and 9 g L^{-1} respectively. The higher conversion ratio of glucose to oil in PFM in comparison to FM has been attributed to enhancement of carbon efficiency in the fermentation stage of PFM, which occurred as a result of Rubisco-involved sugar metabolism. Garcia *et al.*[28] advocated the possibility of switching from photoautotrophic cultivation to heterotrophic cultivation by genetic transformation.

Mixotrophic cultivation of microalgae is done by photosynthesis using both organic compounds and CO_2 as carbon sources for growth. Microalgae in this mode grow under either phototrophic or heterotrophic cultivation or both. Photoheterotrophic cultivation requires light for the growth of microalgae using organic compounds as carbon source. However the latter two methods of microalgae cultivation are uncommon.[26]

Hsieh and Wu[61] cultivated a marine species of *Chlorella* in artificial seawater using various modes of cultivation including batch mode, fed-batch mode, and semi-continuous mode. Urea was used as the nitrogen source and 0.100 g L^{-1} was observed to be optimum for a high specific growth rate (0.0576 h^{-1}) and high lipid productivity (0.124 g d^{-1} L^{-1}). The amount and timing of the urea feed was found to influence the formation of intracellular lipid. Supplying 0.025 g L^{-1} of urea at the late log phase produced a lipid content of 0.387 g g^{-1} in the algal cells. The semi-continuous mode of reactor in which 25% of the feed was harvested and was replaced with further addition of 0.025 g L^{-1} of urea in stationary phase was found to be superior to the other culture conditions. A high lipid content of 0.451 g g^{-1} and lipid productivity of 0.139 g d^{-1} L^{-1} was observed. A comparison of biodiesel obtained from some microalgae species have been depicted in Table 2. It can be seen that *Chlorella* sp. has been the widely utilized species among the microalgae for production of oil to be utilized for production of biodiesel. Among the various types of reactors, the heterotrophic cultivation and photosynthesis-fermentation model resulted in high biomass productivity of 28.88 g L^{-1} and 23.9 g L^{-1} d^{-1} respectively. Though, oil has been obtained depending on the culture conditions and microalgae strain, only few studies have been carried out to utilize the oil for biodiesel production. It is also observed that none of the oils obtained from microalgae could fulfil the ASTM (D 6751) specification for minimum fatty acid alkyl ester (FAAE) content in the biodiesel (*i.e.* 96.5% w/w). This may be attributed to the presence of unsaponifiable matter

such as hydrocarbons, pigments, sterols, wax, sterol esters, free alcohols, phospholipids, and glycolipids in the (crude, unrefined) algal oil.

5.1 Open ponds *vs.* photobioreactors for cultivating algae

Microalgae may be cultured either in open ponds or enclosed airlift type photobioreactors (PBRs). Open "raceway" ponds can be rectangular- or oval-shaped. Cultivation of microalgae in oval-shaped raceways are being used in the USA and China, whereas circular tanks are more common in Japan, Indonesia, and Taiwan.[33] Garcia *et al.*[28] report that open ponds may have problems such as poor diffusion of sunlight with increased depth, contamination by "foreign" species, uncontrollable effects of local weather, costly harvesting of algal biomass, and overall low cell density.

A major constraint with open pond cultivation of microalgae is its contamination with non-native algae, which was observed by researchers at the National Renewable Energy Laboratory (NREL), a pioneer group engaged in microalgal growth.[62] Though the contaminants find their way in open ponds as well as closed photobioreactors during the period of cultivation of microalgae, the contaminants can be better managed in the latter.[1] Schipper[63] reported that commonly used chemicals in the wastewater can adversely affect the alga. For example, Winters *et al.*[64] reported that phenalen 1-one (perinaphthenone) is toxic to green algae. Thus, caution should be taken when using wastewater as a substitute for freshwater for cultivation of microalgae.

PBRs are constructed from plastic or glass, tubular or flat-plate, and horizontal or vertical column types. Of these, the vertical column type PBR can be easily built and need less energy for operation.[65] Nutrient levels, temperature, light, dissolved carbon dioxide, evaporation, and pH can be more easily controlled in PBRs.[36] Demirbas[27] reports that the operating cost of open ponds are less as compared to PBR. However, the biomass concentration will be more in PBR. While oxygen inhibition is more in open ponds, contamination risk will be higher in PBR. Other parameters such as water loss, loss of carbon dioxide, process control, and space required are comparable for the two.

An airlift, tubular photobioreactor was favored by Chisti[66] for high density algal production. However, irrespective of the type of PBR, the overall material and operational costs are higher than that of open pond systems.[44,67] Das and Obbard[67] reported that energy consumed for mixing cultures in PBRs is higher than the calorific value of biomass produced and, hence, is unsuitable for production of microalgae for biofuel. However, an incremental energy supply for culture mixing and illumination could reduce the overall energy consumption by 44%.[67] The biomass productivity of phototrophically cultured microalgae (*Nannochloropsis* sp.) in PBRs can be as high as 10 g L^{-1}.[67] At present, no perfect design currently exists for optimized cost, energy usage, and longevity of PBRs but their continued redesigning may provide a feasible model in a few years.[68] The abiotic factors that control the growth of microalgae are temperature, light, nutrient concentration, CO_2, pH, and salinity.[69]

5.2 Temperature

The temperature of water is an important factor in the culture of microalgae as it determines the growth rate as well as the length of the growing season.[66] The control of temperature is difficult to maintain in an open raceway pond system.[70] The optimum water temperature needed for cultivation of microalgae ranges from 15 to 30 °C.[22,63] Beyond this temperature range microalgal cell damage or death may result. Yue and Chen[71] reports 25 °C to be optimum temperature for growth of freshwater microalgae belonging to genus *Chlorella* with a growth rate of 1.099 g L^{-1} day^{-1} and cell concentration of 5.814 g L^{-1} cell after 6 days. Xu *et al.*[72] cultivated *Chlorella protothecoides* by heterotrophic method maintaining the temperature at 28 ± 1 °C. Kitaya *et al.*[73] observed temperature range of 27–31 °C to be optimum for high multiplication rate of microalgal cells of *Euglena gracilis*. Uduman *et al.*[74] tried cultivation of microalgae by flocculation using polymer and observed that increase in temperature resulted in increase in the microalgal recovery. The reason attributed is that with increase in temperature, the collision between the polymer and microalgae cell caused increased mobility of the cellular particles which improved the flocculation rates.

5.3 Light

Light energy is considered to be an important aspect that has to be taken into consideration during cultivation of algae. The amount of light energy received and stored by the microalgal cells has a direct relationship with the carbon fixation capacity and thus influencing the biomass productivity and cell growth rate.[75] An insufficient amount of light might lower the growth rate. Hsieh and Wu[76] recently developed an open transparent rectangular chamber (TRC) reactor for microalgal cultivation and observed a better penetration of light in the reactor particularly at high cell concentration.

The outdoor light intensity during midday in a normal sunny day in an equatorial region is around 2000 μE m^{-2} s^{-1} which is a major deterrent for culture in open ponds. Whereas, the light saturation constants for common microalgae is much less (*i.e.* 185 μE m^{-2} s^{-1} for *Phaeodactylum cruentum* and 200 μE m^{-2} s^{-1} for *Porphyridium cruentum*). Beyond a certain value of light intensity, a reduction in biomass growth rate is observed due to photoinhibition. Photoinhibition (generally reversible damage) results from damage to the photosynthetic apparatus because of excessive light.[66] Hence, artificial illumination in photobioreactors, though expensive, is preferable over natural illumination.[77] Hence, use of photobioreactors is preferable over common bioreactors for the culture of microalgae. Kitaya *et al.*[73] used fluorescent lamps as a source of continuous lighting for 24 h in the range of 20–200 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF) for culture of *Euglena gracilis*. The specific growth rate of the microalgae was found to be three times more at 50 μmol m^{-2} s^{-1} as compared to that obtained at 20 μmol m^{-2} s^{-1}. The specific growth rate was observed to be maximum at 100 μE m^{-2} s^{-1} and decreased when the PPF was increased beyond 150 μE m^{-2} s^{-1}. Lopes *et al.*[75] also used 150 μmol m^{-2} s^{-1} of photon flux density for culture of cyanobacterium *Aphanothece microscopica Nageli*. Yoo *et al.*[78] also used continuous

illumination of 150 μmol $m^{-2}s^{-1}$ incubation of *Chlorella vulgaris* and *Scenedesmus* sp.

5.4 Use of wastewater as an alternate to freshwater for growth of algae

Of the 11 species of microalgae selected for lipid production by Xin *et al.*,[79] they observed that the freshwater microalga *Scenedesmus* sp. LX1 possessed biomass (dry weight) of 0.11 g L^{-1} and lipid content of 31–33% which was highest among the species selected for study. The *Scenedesmus* sp. grew effectively in polluted water and removed >98% of the total nitrogen and total phosphorus from the effluent wastewater. Hence, though the biomass obtained from *Scenedesmus* sp. is low as compared to that from a typical microalgae in the conventional conditions, the benefit of treatment of pollution water makes it an desirous alternative. Garcia *et al.*[28] advocated that cultivation of microalgae will be cost effective only when it is grown in wastewater which will be remediated in the process. Chinnasamy *et al.*[80] studied 27 species of green algae, 20 species of cyanobacteria, and 8 species of diatoms in treated as well as untreated wastewater from a carpet mill. The selected strains of microalgae removed >96% of the nutrients from the wastewater in 72 h. The biomass production potential from the microalgae was estimated to be ~9.2–17.8 tons ha^{-1} $year^{-1}$. Biomass productivity was optimized at elevated levels of CO_2 (6%) and 15 °C, and also at ambient levels of CO_2 and 25 °C.

Pitman *et al.*[81] tried to reduce the cost of microalgae production by growing it in wastewater. High biomass productivity was observed along with treatment of the wastewaters from municipal sewage wastewater, agricultural wastewater, industrial wastewater, and artificial wastewater. Powell *et al.*[82] studied phosphorus removal by microalgae in waste stabilization ponds. Lower light intensity and a temperature of 25 °C augmented high removal rates. The treatment efficiency of phosphorus (10 mg L^{-1}) increased with the microalgal solid concentration from 0 to 300 mg L^{-1} in the waste stabilization ponds. The minimum and maximum percentages of phosphorus in microalgae biomass were 0.4% and 3.2%, respectively. The microalgae were easily removed from the ponds by dissolved air floatation, microfiltration, sand filtration, and sedimentation. Sydney *et al.*[83] cultivated twenty strains of *Botryococcus braunii* and *Chlorella vulgaris* in photobioreactors. *Botryococcus braunii* LEM 14 was found to be effective in treatment of wastewater and could remove 79.63% of nitrogen and 100% of phosphorous after 14 days of culture at 25 °C.

Yang *et al.*[84] conducted a life cycle analysis (LCA) of biodiesel from microalgae and emphasized the application of seawater and wastewater over freshwater for cultivation of microalgae to reduce freshwater use by 90% in open pond systems. Yang *et al.*[84] also advocated the recycling of the harvested water for microalgae cultivation and found that 100% recycling resulted in 55% decrease in nutrient requirement. They estimated that, without recycling, 3726 kg of water, 0.33 kg of nitrogen, and 0.71 kg of phosphate is required to generate 1 kg of biodiesel. Using recycled water, the water and nutrient requirements were reduced by 84% and 55%, respectively.

5.5 Carbon dioxide

The carbon source accounts for 60% of the total cost of nutrients.[33] Microalgae are unicellular microorganisms and assimilate CO_2. One kg of dry biomass sequesters about 1.7 kg of CO_2. For an optimum growth of microalgae, the atmospheric content of CO_2 must be at least 1%. Ota *et al.*[85] used the green alga *Chlorocuccum littorale* and studied the effect of high carbon dioxide concentration on growth rate and productivity of fatty acids in algae. The alga showed high tolerance to carbon dioxide in the presence of inorganic carbon and nitrate. At 5% CO_2 concentration, the fatty acid concentration obtained from the algae was 34%. Yoo *et al.*[78] cultured three microalgal species (*Chlorella vulgaris*, *Scenedesmus* sp., and *Botryococcus braunii*) in high concentration (*i.e.* 10%) of CO_2 using the flue gas. The biomass and lipid productivity obtained with *Scenedesmus* sp. was found to be 217.50 and 20.65 mg L^{-1} d^{-1}, respectively. The growth rate and lipid accumulation was influenced by nitrogen concentration. Fulke *et al.*[86] studied the photosynthetic potential of various microalgae, taking into consideration their CO_2 fixation rates, calcite formation, and lipid production ability.[86] Among the 27 algal strains selected, *Chlorella* sp., *Chlamydomonas* sp., and *Synnecococcus* sp. were found to have a higher growth rate. The lipid productivity of the microalgal species obtained were 0.322 g day^{-1} at CO_2 concentration of 3%. Even in the ambient air, which contains approximately 0.03% CO_2, lipid productivity of 0.190 g day^{-1} was achieved which can be considered a significant CO_2 sink.

When CO_2 levels become low in open ponds or PBRs, supplemental CO_2 may be added from nearby sources such as coal-based power plants, pulp and paper mills, cement factories, sugar refineries, fertilizer manufacturers, steel mills, and distilleries. "High rate" ponds employ the transfer of atmospheric CO_2 to the bottom water of the pond by direct bubbling.[87] However, the efficiency of the process is low. Putt *et al.*[87] designed a carbonation column coupled with a raceway pond system for CO_2 supplementation with 90% efficiency, which was twice that obtained from direct bubbling. The carbonation column coupled with a raceway pond system was observed to perform best at pH 8 to 9. Pfromm *et al.*[88] suggested that CO_2 used for cultivating microalgae should be supplied from biomass-based ethanol production instead of power plants or fertilizer production in an attempt to make biodiesel production from algae more sustainable.

Cooksey and colleagues[89] at Montana State University have recently reported that baking soda ($NaHCO_3$) added a critical time during the algae culturing process and can significantly enhance overall productivity. In their report that more than two times the oil can be produced in half the time can be shown to work in large-scale, this may be a significant achievement. Sydney *et al.*[83] used *Botryococcus braunii* LEM 14 microalgae and found its CO_2 uptake efficiency to be 144.91 mg_{CO_2} $g^{-1}_{biomass}$ L^{-1} day^{-1}.

6 Harvesting methods for algal biomass

Harvesting of algal biomass poses a challenge owing to their small size and high water content.[90] The typical initial step for algal harvesting is solid-liquid separation which involves

concentration and drying processes. Concentration processes include coagulation, flotation, centrifugation, filtration, and gravity sedimentation.

Filtration technique can be done by screen or membrane. For example, Zhang *et al.*[90] harvested *Scenedesmus quadricauda* by ultrafiltration membranes. This membrane technique, combined with air-assisted backwash, resulted in 150-fold concentration of microalgae with a harvesting efficiency of 46 gm^{-2} h^{-1}. However, all of the harvested microalgae biomass cannot be converted to biodiesel. The yield for a common microalgae, *Chlorella vulgaris*, extraction is around 70%.[84] Godos *et al.*[91] opted for harvesting of algal-bacterial biomass from the effluent of pig wastewater using coagulants and polymeric flocculants. Two coagulants ($FeCl_3$ and $Fe_2(SO_4)_3$), and five polymeric flocculants, augmented biomass removal of 66–98% at coagulant and flocculant concentrations of 150–250 mg L^{-1} and 25–50 mg L^{-1} respectively.

7 De-watering and concentrating algae

The large-scale production of microalgae requires continuous agitation to prevent the biomass from settling.[66] Flocculation, centrifugation, and filtration are used to ultimately dewater and concentrate algal biomass. The common methods employed for extraction of oilseeds are oil press or expeller and may be applied for microalgae. However, the process is time consuming and more suitable methods for extracting microalgal oil include solvent extraction, supercritical fluid extraction, and ultrasound.[36] Downstream processing plays a significant role in the energy consumption for culture and harvest of the microalgae. Biodiesel developed from *Chlorella vulgaris* in a raceway pond needed an energy input of 1.66 MJ for 1 MJ of energy obtained in form of biodiesel if biomass is dried before extraction. However, 1.23 MJ of energy may be recovered from algal waste. This could be made possible by the anaerobic digestion of waste biomass to methane and liquid fertilizer.[56]

7.1 Cell breakage

Microalgal cells can be harvested as intact or first broken down by various methods including microwave pulses, bead-beating, sonication, or other means.[92] Cells disrupted using 'microwave oven method' gave the highest productivity of oleic acid at 5.7 mg L^{-1} d^{-1}.[93]

8 Extraction of oil from algal biomass

After harvesting, oil has to be extracted from the algae, usually following the same pathway as that of any oilseed obtained from terrestrial plants – through expeller or press, solvent extraction with hexane, or supercritical fluid extraction.[31] Microalgae have high water content, so the biomass conversion processes must be suitable for such feedstock (discussed further in the next section). About 70–75% oil can be extracted through the expeller press and the remaining oil can be extracted through solvent extraction with an appropriate solvent like hexane. By adopting supercritical fluid extraction using CO_2, 100% oil could be extracted by this single method but the process is cost intensive. Wahlen *et al.*[94] developed a single-step process of simultaneously extracting and converting total lipids from microalgae, cyanobacteria, and their mixed culture for synthesis of biodiesel by optimizing reaction time, temperature, concentration of reactants, and concentration of sulfuric acid as catalyst. The overall '*in situ* process' comprised addition of alcohol that acted as solvent in extracting lipid from the biomass and thereafter acting as reactant for conversion of fatty acid to fatty acid methyl esters (FAME). Additional methanol was added to samples with higher water content to facilitate FAME yield.

Bae *et al.*[95] tried bio-oil production by pyrolysis from the brown macroalgae *Undaria pinnatifida* and *Laminaria japonica*, and the red macroalgae, *i.e. Porphyra tenera*, and reported 500 °C to be the optimum temperature requirement for yield ranging from 37.5–47.4 wt%. However, the bio-oil was found to contain nitrogen content in the range 1.5–6.13 wt% and thus is a major constraint in the applicability of macroalgae as an alternate fuel. Nitrogen and sulfur have been lowered by the synthesis of algal (*Nannochloropsis* sp.) bio-oil in supercritical water using hydrothermal liquefaction.[96] The "upgraded" synthesized algal bio-oil using platinum catalyst loaded on carbon (Pt/C) possessed high calorific value (MJ kg^{-1}) and a low acid value essential for fuel grade oil.

8.1 Hydrothermal and pyrolytic processing

Since microalgae have high moisture content, Ross *et al.*[97] tried hydrothermal liquefaction to extract oils, which involves processing by hot compressed water either with or without a catalyst. The species selected were *Chlorella vulgaris* (a green algae) and *Spirulina* (a bluegreen algae), which both possess low lipid content and high protein content. The bio-crude obtained was composed of various aromatic hydrocarbons such as toluene, ethyl-benzene, styrene, substituted phenols, pyrrolidinones, and indoles, along with fatty acids, fatty acid amides, and alcohols. Around 40% of the crude had a boiling point below 250 °C. Pinnarat and Savage[68] and Savage[98] have summarized the supercritical reaction with and without catalysis, and Brown *et al.*[99] have tested the effectiveness of hydrothermal processing to produce crude bio-oil and aqueous products from *Nannochloropsis* sp., a marine microalga. They found that "nearly 80% of the carbon and up to 90% of the chemical energy in the microalga can be recovered as either bio-oil or gas products." The future of hydroprocessing and use of sub- and supercritical water to extract organics from algae, wastewater sludge, and other sources to produce bioenergy along with bioremediation benefits seems promising.

8.2 Lipid Levels

Of the various *Chlorella* strains tested by Huang *et al.*,[100] *Chlorella vulgaris* was selected as the species with the highest lipid accumulation. The lipid content was quantified with a gravimetric method and a spectrofluorometer method using Nile Red, a hydrophobic dye. The spectrofluorometer method was faster (2 days *vs.* 5 days) and more sensitive (0.2 mg *vs.* 10 mg sample required) compared to the gravimetric method. The spectrofluorometer Nile Red method could distinguish differences of 0.1% of lipid content.[100]

The oil extracted from the microalgae used by Chinnasamy *et al.*[80] was mainly composed of unsaturated fatty acids ranging from 66–81% of the total. The algal oil possessed a high acid value of 50% and the free fatty acids were converted to biodiesel by acid esterification had a >95% conversion and 71% yield. Final yield obtained after alkaline transesterification was ~64%, but went down to ~39% after purification.

Kanad and Li[101] used dimethyl ether for extracting crude oil from *Microcystis*, a bluegreen algae containing 91% water content. The method is reported to be economical and feasible compared to other methods of extraction because it alleviates drying, cell breakage, and heating the solvent in a one-step extraction process. Krohn *et al.*[34] used continuous catalytic processing for lipid production in *Dunaliella tertiolecta* and *Nannochloropsis oculata*. The gravimetric analysis using chloroform/methanol extraction was followed for the determination of lipid content in biodiesel.

As all lipids are not saponifiable (lipids with an ester functional group that can be hydrolyzed under basic conditions), non-saponifiable lipids cannot be converted to biodiesel on transesterification.[34] Table 3 lists the neutral and total lipid content from various types of microalgae. The FAME (*i.e.*, biodiesel) content ranged from 3.3% in *Nannochloropsis*, 20.9% in *Duniella*, and 31% in wild (mixed) algae.

9 Biodiesel from microalgal oil

After extraction of oil from microalgae, the transesterification process can develop biodiesel. Vijayaraghavan and Hemanathan[102] synthesized biodiesel from the microalgal oil using ethanol as reactant and KOH as catalyst. The fuel properties of the biodiesel were investigated and observed to be of high calorific value (40 MJ cal^{-1}) and high cetane number (52). Although the flash point of 98 °C was lower than that specified by ASTM (*i.e.* ≥130 °C), it still was higher than the mineral diesel. All other properties were within the ASTM specifications (Table 4). Chart 1 depicts the stages of production of biodiesel from a selected microalgae.

9.1 Southeast Asian aspect

Being mostly agrarian economies, Southeast Asian countries have good potential for synthesis of liquid biofuels (*i.e.*, bioethanol and biodiesel). The feedstocks that can be utilized for synthesis of bioethanol are of plant origin such as wheat, sugar beet, corn, straw, and wood.[103] Phalan[104] suggested that consideration for the best feedstock must include social and environmental impacts such as phytoremediation of wastewater.

Table 3 Lipid contents of various types of microalga[34]

	Dunaliella tertiolecta	*Nannochloropsis oculata*	Wild
Neutral lipids	5.6 ± 1.0%	9.0 ± 2.0%	4.5 ± 0.2%
Total lipids	19.0 ± 0.8%	18.0 ± 3.5%	15.8 ± 3.1%
FAME	19.0–20.9%	3.3%	31%

Table 4 Fuel characteristics of biodiesel derived from microalgae[102]

Parameter	Value	ASTM D6751 Specification
Calorific value	40 MJ cal^{-1}	—
Cetane number	52	47 (minimum)
Flash point	98 °C	130 (minimum)
Ash content	0.21 wt (%)	—
Water content	<0.02 vol (%)	0.050 vol% (maximum)

9.2 Energy return on energy invested analysis

Any renewable fuel will be acceptable in the market when the energy that is gained from its combustion is more than that invested in its production. Although various algae species have a high yield-bearing capacity of triglycerides, thermodynamics plays an important role in their growth. Scott *et al.*[56] stated that 8 photons of photosynthetically active radiation, which accounts for 48% of the incident solar flux, are required to fix one molecule of CO_2 into carbohydrate. Hence, the maximum photosynthetic efficiency that can be obtained is 12%. Respiration lowers the efficiency to 9%

Sorguven and Ozilgen[10] reported that thermodynamics should be accounted for in labeling the energy as either renewable or non-renewable. They categorized bioethanol as a sustainable fuel based on its positive energy balance with no net increase in carbon dioxide emission. However, bioethanol derived from corn is non-renewable because of the consumption of non-renewable energy, resource processing, and waste treatment. The exergy (maximum energy content that can be extracted from a system without violating the laws of thermodynamics) that is consumed during the process has been reported to be 5 times larger than the work that can be produced by ethanol. Sorguven and Ozilgen assessed the algal-biodiesel-carbon dioxide cycle by thermodynamic calculations and found it to be renewable. The total energy produced in the whole cycle was 12 MJ. Out of this, 9 MJ is the energy required for restoration of the environment to its initial state and thus, the net work gained is 3 MJ. The renewability indicator factor was calculated to be 0.27, which is considered to increase further by using renewable energy such as hydropower for electricity.

9.3 Life cycle analysis of biodiesel from microalgae

Life cycle analysis (LCA) of any product (biodiesel in this case) is an important aspect and should be kept in consideration for its feasibility in usage. However, the LCA of a biofuel is a rather complex process and involves a detailed modeling of processes such as microalga growth, dewatering, lipid extraction, conversion to biodiesel, and distribution. Singh and Olsen[105] reports that 3726 kg of water is required to generate 1 kg biodiesel from microalgal feedstock if the freshwater is used without recycling. However, with the usage of seawater or wastewater as an alternate of freshwater can reduce the lifecycle freshwater usage by 90%. Batan *et al.*[106] selected *Nannochloropsis salina*, which has the potential to attain a lipid content of 60 wt% of biomass and a growth rate of 260 mg L^{-1} h^{-1} or 150 gm^{-2} day^{-1}. The process, which consumes a large amount of energy, was examined and they found that 99% of the electrical energy used during cultivating the microalga was for compressing air for bubbling (sparge). About 76% of the energy consumed during the extraction process was for solvent recovery, while other processes such as recycling of the media accounted for

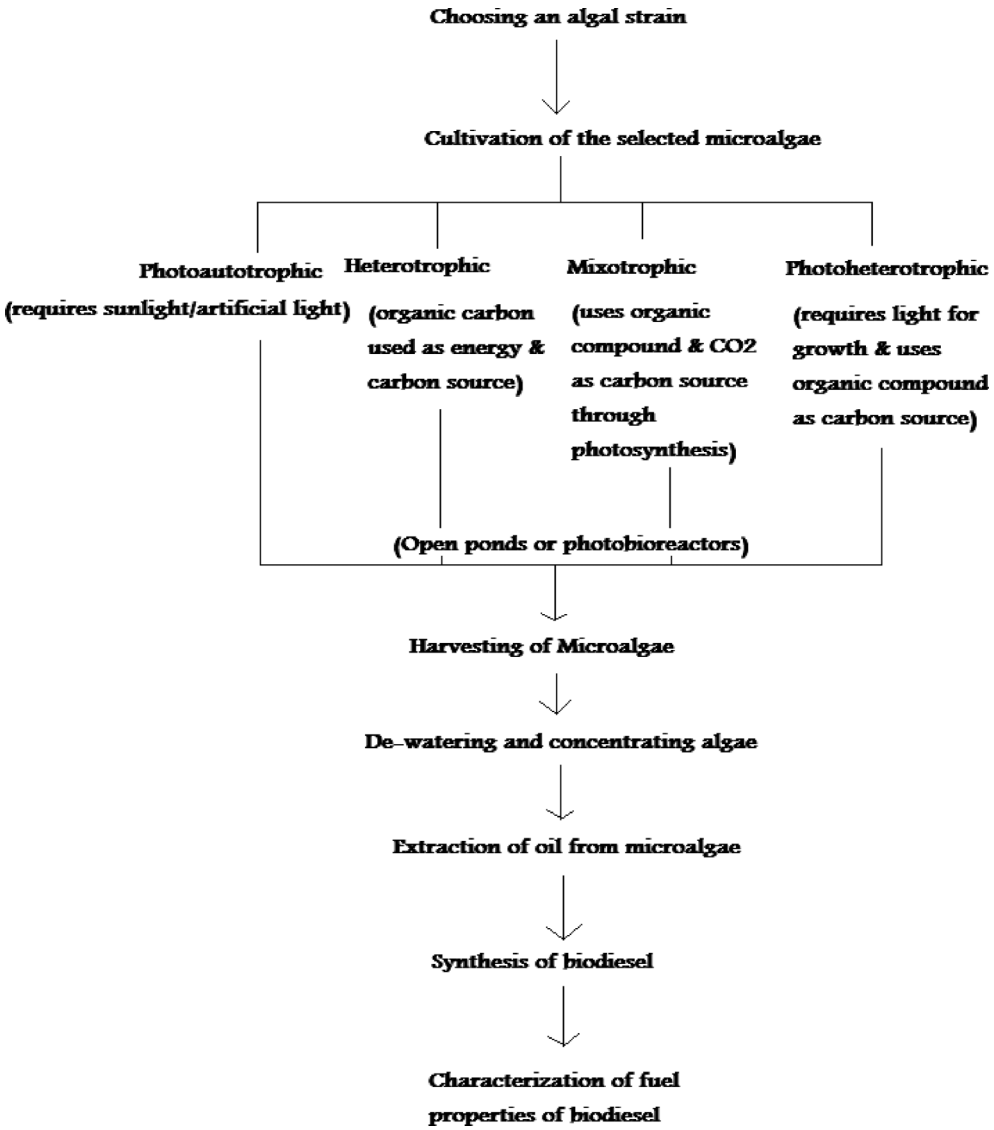


Chart 1 Stages involved in production of biodiesel from a microalgae.

<1% of the energy. Batan *et al.* concluded that the microalgal-based biodiesel accounted for 30% less input energy per unit of product than the conventional biodiesel produced from soybean oil. Another significant finding of Batan *et al.* was that net emission of green house gases was 5% less with microalgae compared to soybean-based biodiesel. Their study should be extended to non-edible oilseeds, which would provide interesting facts and feasibility of various feedstocks for synthesis of biodiesel.

Lardon *et al.*[107] studied the LCA of *Chlorella vulgaris* and reported that switching from dry to wet extraction of the oil from the alga enhanced the sustainability of the product biodiesel. This comes from the fact that nearly 90% of the energy consumption during the dry extraction process is reduced to 70% with wet extraction.

High lipid content along with high growth rate of microalga will also enhance the suitability and feasibility of biodiesel from microalga. The nitrogen deficiency that enhances the lipid production in some microalgae studies is a deterrent in their growth rate and productivity. Table 5 depicts the comparison of annual lipid production of algae compared to terrestrial feedstocks.

Table 5 Annual productivity of lipid-bearing feedstocks[34]

Oil	Annual productivity (liter oil/hectare)
Soybean	450
Canola	1200
Palm	6000
Algae	90 000

9.4 Economics of microalgal biodiesel

At present, the production of microalgal biomass is more expensive than to grow plants.[27] The cost of microalgal oil increases due to various factors, *viz*. requirement of light, CO_2,

water, inorganic salts, and maintaining the temperature of the reactor within a narrow range (15 to 30 °C). The emergence of closed photobioreactors which can control these factors adds to the overall cost of the process. Stephens *et al.*[1] have reported that though microalgal biofuel systems theoretically boast to counter the issues of food *versus* fuel, and also forest *versus* fuel, to date none of microbial strain has attained economic viability. However, the enthusiasm to make microalgal biofuel viable for commercial operation has been witnessed from the increased funding from the institutional bodies to explore this area.

The culture of microalgae by heterotrophic mode for production of 1 L of oil (from *Chlorella zofingiensis*) using sugar as substrate for the growth of microalgae cells bears cost of $0.9. However, the cost may be reduced if some other source of sugar is used such as from cane molasses that is a by-product of sugar industries.[55] This however remains a challenge as there is a substantial difference in growth rate, cell biomass, lipid content, and lipid yield is high when glucose is taken instead of other form of sugars.

Gallagher[58] studied the economics of biodiesel production from algae and reported its economic viability to depend on the future price of crude oil and the government subsidies that are provided in various nations to encourage the production of renewable fuels. The price of crude oil fluctuates to a large extent ($26 in 2002 and $100 in 2008) owing to various reasons including the global oil demand. Assuming the productivity of algae to be 100 mt/ha/yr, and lipid concentration to be 35% by weight, a biodiesel yield of 10 421 gallons per ha has been visualized. This resulted in capital cost of $112 400 per ha and operating cost to be $39 300 per ha for growing, harvesting, and extracting algal oil cultured in open ponds.

Patil *et al.*[108] report that synthesis of biodiesel using supercritical conditions will reduce the production cost of biodiesel to half the value. They used a single-step supercritical process for transesterification for shorter reaction time, simple purification method, and a high conversion of triglycerides to biodiesel using the Response Surface Methodology (RSM) technique. They selected *Nannochloropsis* sp. with a lipid content of 50% on dry weight basis, of which triglycerides comprised 37.74%, isoprenoids constituted 8.72%, and the combined polars, glycolipids, phospholipids amounted to 3.54%. The optimum reaction conditions for the synthesis of biodiesel from algal oil were 9 : 1 methanol to oil molar ratio at 255 °C in 25 min reaction time.

Tang *et al.*[109] studied the influence of various parameters on the lipid content and fatty acid composition of *Dunaliella tertiolecta*. Increasing the intensity of light increased their growth rate and biomass productivity. White and red light emitting diodes compared equally well to fluorescent lighting for the cell growth of microalgae. CO_2 level between 2 to 6% provided the optimum growth rate of microalgae.

The anaerobic digestion of microalgal oilcakes obtained after oil extraction for biogas generation has been suggested by Lardon *et al.*[107] to derive energy from the process. Presently, biodiesel is more expensive than conventional mineral diesel. However, large-scale production of biodiesel from microalgae with minimal infrastructure can hopefully bring the production cost of biodiesel at par with mineral diesel.

9.5 Additional benefits from microalgae

In addition to being a feedstock for biodiesel and an agent for remediation of waste nutrients and CO_2, microalgae can also be used for other products such as vitamins, pharmaceutical drugs, biogas production by anaerobic digestion, methane from microalgal residues, and other uses.[33,110] Microalgae can remove metals ions from wastewater by passive uptake (metabolism-independent) or active uptake (metabolism-dependent). The passive uptake is termed biosorption which occurs by displacement of monovalent and divalent ions in the cell wall by heavy metal ions by ion exchange. The metal ions may also form complex with functional group present at the cell wall. The active uptake mechanism involves metal ion consumption for the growth of microalgae and/or intracellular accumulation of metal ions in their cell wall.[111] The dead biomass of microalgae (*C. vulgaris*) can also be used for removal of organic pollutants such as dyes at pH = 2.[112] Algae have recently been tried as biosensors for detection of toxic compounds and if found accurate they can provide a new dimension in pollution control technologies.[113]

9.6 Limitations of microalgae as feedstock for biodiesel production

The microalgae has a few limitations that need to be overcome for their utilization as a potential feedstock for biodiesel production. The common limitations are low growth rates of photoautotrophic algae, and low biomass concentrations. The algal species can grow only in a specified temperature range (15 to 30 °C) and fluctuation of temperature beyond the optimum range results in inhibition of growth of the micro alga or its death. To achieve the desired temperature range in open ponds may be difficult as temperature at surface go high to about 40 °C. Hence, closed bioreactors are fabricated for the microalgae culture to minimize temperature fluctuations. However, closed bioreactors too, if operated in hot areas, may observe an increase in temperature which has to be controlled by using water for evaporative cooling, heat exchangers, reflection of infra-red radiation, or light dilutions. These processes make the microalgal biodiesel cost intensive. Synthesis of biodiesel that have been obtained from the microalgae at present is low and need further improvement in the process of cultivation of microalgae. Krohn *et al.*[34] found that though the total lipids comprised 19% of algal dry weight, the synthesized biodiesel from the lipids were only 1% of dry weight. Algal lipids possess high free fatty acid content which is not saponifiable and so transesterification cannot be done with the conventional homogeneous base catalyst.[34] The option available is to reduce the acid value by esterification or employing a solid acid catalyst. The deprivation of nitrogen on the accumulation of lipids in microalgae varies among the various species.

A limitation of synthesis of biodiesel from microalgae is a high alcohol to oil molar ratio (up to the extent of 315 : 1) required during the synthesis process that enhances the production cost of biodiesel.[45] Another major limitation of the oil obtained from the microalgae, yeast, of fungi is the lipid contents (broadly classified as neutral lipids, total lipid). Only a part of the neutral lipid that comprises of triglycerides and free fatty acids can be converted to fatty acid methyl esters (*i.e.* biodiesel) and

many of the microalgae tried as feedstock for oil comprises of constituents that cannot be converted to biodiesel.

10 Conclusions

More recently, algae have received wide attention and popularity as they have the potential of high yield of oil that is used as a feedstock for synthesis of biodiesel. Algae, thus appear to be a significant source for biofuels. Their photosynthetic efficiency (efficiency to capture light) is much higher than that of terrestrial plants (*i.e.*, 10.0% in comparison to 0.5% in terrestrial plants. The ingredients that algae, like all plants, require for growth are sunlight, CO_2, inorganic nutrients, and water. Microalgae can flourish in fresh, marine, brackish, as well as wastewater from industries. The life cycle analysis of biodiesel shows that seawater or wastewater can reduce the life-cycle nutrient usage by 90%. They can be grown in photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic culture conditions. Algae can sequester carbon dioxide and nutrients, which creates a potentially added benefit to use them as a feedstock for biofuels. Microalgae can be cultivated either in open raceway ponds or specially fabricated photobioreactors (PBR). While the open ponds are cost effective, it is difficult to control flow of nutrients, temperature, dissolved carbon dioxide, and pH. These limitations could be overcome in the PBR, though they are cost intensive. The harvesting technique for microalgae involves solid-liquid separation achieved by coagulation, flotation, centrifugation, filtration, and gravity sedimentation techniques. The extraction of lipid from microalgae can be achieved by expeller or press, solvent extraction with organic solvents, or supercritical fluid extraction. After extracting the oil from microalgae, transesterification process is adopted for synthesis of biodiesel.

Some microalgae species have the potential to accumulate lipids at more than 50% of their biomass. Oil obtained from microalgae generally bears a low acid value and hence is quite suitable for synthesis of biodiesel. Lipid productivity among microalgae ranges from 17.6–60.9 mg l^{-1} day. With the numerous strains of microalgae available in nature, it is important to choose the one that is best suited for high lipid content. Among the prominent ones are *Chlorella vulgaris* and *Chlorella protothecoides*.

The exergy analysis of algal-biodiesel-carbon dioxide cycle shows the process to be renewable and the net work gained is 3 MJ. Although at present biodiesel is costlier than conventional mineral diesel, synthesis of biodiesel using supercritical conditions might reduce the production cost of biodiesel to half the value. In addition to generation of lipid, microalgae can also be used for treatment of wastewater. Optimizing the cultivation, harvesting, and conversion of microalgal lipids into biofuels, and maximizing the added benefits, will hopefully make microalgal biofuels economically competitive to fossil fuels within the next decade. As the cultivation of microalgae requires less land area, researchers, policy makers, and industrial groups see this as an economic incentive for growing algae for biofuels. Although it will require more time to effectively judge the real potential of algae to yield oil, and their overall effect on the environment, research has come a long way on the possibilities of their usage as biodiesel feedstock.

Acknowledgements

The authors thankfully acknowledge financial assistance in the form of 'Research Associateship' to BS by Council of Scientific and Industrial Research(CSIR), Govt. of India.

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