Biodiesel production from marine cyanobacteria cultured in plate and tubular photobioreactors

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Carbon (neutral) based renewable liquid biofuels are alternative to petroleum derived transport fuels that contribute to global warming and are of a limited availability. Microalgae based biofuels are considered as promising source of energy. *Lyngbya* sp. and *Synechococcus* sp. were studied for the possibility of biodiesel production in different media such as ASNIII, sea water enrichment medium and BG11. The sea water enrichment medium was found superior in enhancing the growth rate of these microalgae. Nitrogen depletion has less effect in total chlorophyll *a* content, at the same time the lipid content was increased in both *Lyngbya* sp. and *Synechococcus* sp. by 1.4 and 1.2 % respectively. Increase in salinity from 0.5-1.0 M also showed an increase in the lipid content to 2.0 and 0.8 % in these strains; but a salinity of 1.5 M has a total inhibitory effect in the growth. The total biomass yield was comparatively higher in tubular LED photobioreactor than the fluorescent flat plated photobioreactor. Lipid extraction was obtained maximum at 60 °C in 1:10 sample: solvent ratio.

GC-MS analysis of biodiesel showed high content of polyunsaturated fatty acids (PUFA; 4.86 %) than saturated fatty acid (SFA; 4.10 %). Biodiesel production was found maximum in *Synechococcus* sp. than *Lyngbya* sp. The viscosity of the biodiesel was closely related to conventional diesel. The results strongly suggest that marine microalgae could be used as a renewable energy source for biodiesel production.

**Keywords:** Biodiesel, LED photobioreactor, Lipid, *Lyngbya* sp., Salinity, *Synechococcus* sp.

The effective use of fossil energy resources in an economic way still remains as a major challenge. The main drawback of fossil fuel is that it is a finite non-renewable resource and will be depleted in the near future or one day be exhausted. Since the last few decades, fossil fuels have become an integral part of day to day human lives. Specifically, these fuels are burned to produce energy for transportation and electricity generation, as these two sectors have played a vital role in improving human living standard and accelerating advance technological development. Biodiesel is a transportation fuel that has gained popularity over the past decade. With the dwindling reserves of fossil fuels, it is now more important than ever to search for transportation fuels that can serve as alternatives to crude oil-based fuels such as gasoline and diesel. Biodiesel decomposes well and reduces toxic exhaustion gases and dust hence is environmental friendly and has a high flash point and is easy to handle or store.

Among primary feedstocks for biodiesel, microalgae are currently considered to be one of the most promising alternative resources. Microalgae are microscopic photosynthetic organisms that are found in both marine and fresh water environments. Microalgae have attracted attention for bioenergy production because they can produce oil in the cell body. Unlike oil crops, microalgae grow rapidly and many are exceedingly rich in oil. Although the growth of the microalgae varies depending on the characteristics of the species, they mostly double their biomass within 24 h. Biomass doubling time during exponential growth is commonly as short as 3.5 h. Oil content in microalgae ranges from 20-50% and it can exceed 80% by weight of dry biomass. Microalgae with high oil productivities are desirable for biodiesel production. Interestingly biomass production in microalgae may be combined with direct bio-fixation of waste CO₂ to the value of 1.8 kg per kg of dry algal biomass. They are, therefore considered as promising candidates for the industrial level production of biodiesel.

The composition and fatty acid profile of lipids extracted from a particular species is further affected
by the microalgal life cycle and the cultivation conditions, such as medium composition, temperature, illumination intensity, ratio of light/dark cycle and aeration rate. The fatty acids determined from algal oil were found to contain 36% oleic (18:1), 15% palmitic (16:0), 11% stearic (18:0), 8.4% iso-17:0, and 7.4% linoleic (18:2). Marine cyanobacteria could be cultivated in open pond system or using photobioreactor (PBR) system. Photobioreactors have higher efficiency of biomass concentration (2–5 g/L), shorter harvest time (2–4 weeks) and higher surface to volume ratio (25–125/m) than open ponds. Photobioreactor gives a better control on most of the parameters compared to open pond systems. Growth of algae in PBRs reduces the risk of contamination, improves the reproducibility of cultivation conditions, provides control over hydrodynamics, temperature and suitably technical designed.

To effectively exploit the commercial potential of algae, a cheap, durable, reliable and highly efficient light source is needed. Instead of fluorescent lamps, Light Emitting Diodes (LED) may lead to a 50% decrease in power consumption and a lower cost per unit time in PBRs. Among the currently available light sources, only the LEDs are able to meet the preceding criteria. LED lights are small enough to be fitted into virtually any photobioreactor and they are advantageous because they have a longer life-expectancy, lower heat generation, higher conversion efficiency and a greater tolerance for switching on and off. In addition, LEDs have narrow light emission spectra between 20 and 30 nm, which can be suited for photosynthetic needs and the absorption wavelength of blue and red LED are around 450–470 and 645–665 nm, respectively. The highest specific growth rate and biomass production were obtained by using red LED in the photoautotrophic cultivation of *Spirulina platensis*. The optimal wavelength required could vary from species to species. Illumination with red LED is suitable for microalgal growth, replacing fluorescent lamps by multi-LED light source resulted in a 50% decrease in electricity consumption (from 40.32 to 20.16 kW-h).

The energy density of biodiesel is comparable to petroleum diesel. The higher heating value of petroleum diesel is 42.7 MJ/kg, where as values for biodiesel range from 34 to 48 MJ/kg, depending on the source of biomass. Hence, the present study has been undertaken to produce biodiesel from marine cyanobacteria by selecting one filamentous (*Lyngbya*) and one single cellular (*Synechococcus*) species by culturing them in plate and tubular photobioreactors, in order to compare the biomass yield and oil production in short duration.

**Materials and Methods**

The marine cyanobacterial strains (*Lyngbya* sp. and *Synechococcus* sp.) were obtained from the National Facility for Marine Cyanobacteria (NFMC), Bharathidasan University, Tiruchirappalli.

*Cultivation of cyanobacterial strains*—The cyanobacterial strains (*Lyngbya* sp. and *Synechococcus* sp.) were grown autotrophically and axenically of 3600 lumen in BG11, ASNIII and seawater enrichment medium. The cultures were mildly shaken by hand on alternate days in shake flask. They were also cultured in nitrogen depleted ASNIII medium and varying salinity ASNIII medium (0.5, 1.0 and 1.5 M) to study the effect of growth and lipid accumulation.

*Cultivation of cyanobacteria in indoor photobioreactor*—The indoor photobioreactors (Flat plate and tubular photobioreactor; Figs 1a and b) were designed with the operating volume of 5 and 2.5 liters respectively. The reactors were illuminated with fluorescent CFL tube (850 lm) and LED light (1000 lm) respectively. The culture was mixed by submerged circulating pump for proper mixing, nutrient utilization or preventing the settling of the biomass. The axenic culture of *Synechococcus* sp. was grown in the photobioreactors and its growth was studied by estimating Chlorophyll *a* and Carotenoid and dissolved oxygen accumulation.
Lipid extraction from biomass—Lipid was extracted from the biomass of cyanobacterial strains (Lyngbya sp. and Synechococcus sp.), cultured in different media. A known volume (3 g) of cyanobacterial cell pellet was ground using a mortar and pestle by adding pulverized glass powder and extraction solvent (2:1 chloroform:methanol). The extract was filtered through Whatmann No.1 filter paper where three volumes of distilled water were added to remove water-soluble impurities. Then the filtrate was vortexed and allowed to stand for separation of two layers and the lower lipid layer was separated by the addition of sodium sulfate crystals and dried. Weight of the lipid and its content in the algal sample was calculated by the following equations:

\[
\text{Weight of lipid} = (\text{weight of container + extracted lipid}) - \text{weight of the container}
\]

\[
\text{Lipid content (\%) } = \frac{\text{amount of lipid extracted (g)}}{\text{weight of original sample (g)}} \times 100
\]

Determination of optimum extraction temperature and sample-solvent ratio—Cyanobacteria cultured in 1.0 M ASNIII medium (1.0 M salinity) were used to determine the optimum extraction temperature and sample solvent ratio. Extraction of lipid was performed in a single-stage extraction at room temperature for 30 min with four variations of sample-solvent ratios. Solvent measuring 15, 30, 50 and 250 mL were placed in 5 g of cyanobacterial samples in order to prepare mixtures with sample-solvent ratios of 1:3, 1:6, 1:10 and 1:50 (w/v) respectively. Extraction was also carried out at two different temperatures (30 and 60 °C) to determine the optimum extraction temperature.

GC–MS analysis of biodiesel—The composition of the biodiesel was subjected to gas chromatographic–mass spectrometric analysis. Gas chromatography was performed on a 0.25 mm (id) × 30 m fused silica column lined with a 0.25 µm film of polyethylene glycol. Samples (1.0 µL) were injected in split mode (split/column flow ratio 30:1). The column head pressure of the carrier gas (helium) was 3 Kpa at the initial oven temperature and its flow rate 1.0 mL min⁻¹. The injection temperature was 290 °C; the oven temperature was 100 °C for 2 min, rose to 300 °C over 20 min and was held at this temperature for 20 min (total run time 42 min). The GC–MS apparatus was connected to a PC for running software for data acquisition and processing (SGS India Pvt. Ltd. Chennai).

Transesterification of triglyceride—Transesterification and purification tests were carried out using standard protocol of National Biodiesel Board (2002) India. The Fuel property tests like density (IS1448 part 16), viscosity (IS1448 part 25), aniline point (IS1448 part 3), API gravity and Cetane No. (IS1448 part 16 and part 9), flash point and fire point (IS1448 part 20), hydrocarbon ratio (IS1448 part 16), calorific value (IS1448 part 7) and acid value (IS1448 part 2) were carried out with Indian Standards 1448.

Results

Effect of media composition on cyanobacterial growth—The cyanobacterial cultures were grown in BG11, ASNIII and seawater enrichment media. The chlorophyll a content of the cultures was estimated at an interval of every 7 days for 35 days. Two cyanobacterial strains grew well in seawater enrichment medium in exponential order till 21 days after that these strains start decline in the chlorophyll content. The effect of nitrogen source on cyanobacterial growth was studied by culturing the strains in nitrogen depleted ASNIII medium. Nitrogen deficiency did not affect the chlorophyll a content in either of the species. Carotenoid content increased in both the strains by 10.9 to 12 and 10.2 to 11.5 µg/mL. The cultures were grown in ASNIII medium with 0.5, 1.0 and 1.5 M salinity. The growth of the strains were inhibited at 1.5 M salinity but not affected in 1.0 and 0.5 M salinity.

Growth of Synechococcus sp. in indoor photobioreactor—Synechococcus sp. was cultured in the indoor photobioreactors (Flat plate and tubular photobioreactor) with continuous mixing by a submerged circulating pump. Synechococcus sp. showed high biomass growth rate within 14 days in tubular photobioreactor than Flat plate photobioreactor. The dissolved oxygen accumulation in tubular photobioreactor was low when compared to flask cultures.

Lipid extraction from biomass—The lipid content of Lyngbya sp. and Synechococcus sp. were 6.7 and 9.0% respectively. The amount of lipid extracted from cyanobacterial species was high when compared to various plants such as Jatropha sp. (0.56%), Azadirachta sp. (0.12%), Acanthophoenix sp. (0.34%) and Pongamina sp. (1.4%). The lipid content of Lyngbya sp. and Synechococcus sp. in nitrogen depleted medium (9.2
Microalgae appeared to be the only source of renewable biodiesel capable of meeting the global demand for transport fuels, with the potential to completely displace the use of fossil fuels. The growth characteristics and composition of microalgae are known to significantly depend on the cultivation conditions. The results of the present study showed that the physiological adaptability of cyanobacterial strains was high under nitrogen stress. The nitrogen deficiency in culture medium caused a complete loss of phycocyanin, with no considerable change in the chlorophyll content. The total inhibition of Synechococcus sp. and Synechococcus sp. growth was observed at 1.5 M salinity. Similar conditions were reported by with Synechococcus sp. PCC7942.

Sodium nitrate was the most favourable nitrogen source for cell growth and lipid production for the two tested cyanobacterial strains. It was observed that lipid contents decreased with increase of sodium nitrate in the medium with a range of 3-20 mM. It was also observed that under high salinity condition there is an increase in TAG content in the marine...
cyanobacterial strains. An initial NaCl concentration of 1.5 M suppressed the growth but 1.0 M concentration showed an increase in higher intracellular lipid content about 9.8% in *Synchococcus* sp. and 8.0% in *Lyngbya* sp. which was comparatively higher than 0.5 M NaCl concentration which showed 9.0% in *Synchococcus* sp. and 6.7% in *Lyngbya* sp. The salt concentration increased the lipid content of *Dunaliella* from 60 to 70% by the addition of 0.5 M to 1.0 M NaCl\(^{21}\).

The growth rate of algae observed in designed photobioreactor was higher because of decreased dissolved oxygen accumulation when compared to flask culture. Using microalgae for CO\(_2\) fixation in PBRs is an effective and promising method\(^{22,23}\). In comparison with open systems, closed PBRs are characterized via regulated and well-controlled cultivation, with the additional benefits of low contamination risk, high CO\(_2\) fixation efficiency, high metabolic flexibility and controllable hydrodynamics\(^{24}\). The criteria for a good microalgal-CO\(_2\) fixation PBR system are good mixing, gas transfer and light distribution\(^{25,26}\). The reason is that the vertical photobioreactor allows greater mass transfer and decrease in energy usage, while the available horizontal reactor is more scalable, but requires a large area of land\(^{27}\). Flat-plated photobioreactors are made of transparent (acrylic) material. The large surface area illumination allows

<table>
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<th>Peak No.</th>
<th>Ret. Time (min)</th>
<th>Type</th>
<th>Width (min)</th>
<th>Area (pA*S)</th>
<th>Area (%)</th>
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<td>11- Eicosenoic acid ME C20:1c</td>
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Fig. 2—GC-MS analysis of biodiesel and GC-MS profile of biodiesel produced from cyanobacteria. The bold numbers and names indicate the unsaturated and other minor fatty acids.
high photosynthetic efficiency but, low accumulation of dissolved oxygen concentration and immobilization of algae. Extraction of oil from algal biomass is difficult and expensive. Currently there is no defined lipid extraction method to scale up the process. Most of the known extraction methods are high cost associated with water removal and difficulties with disrupting the cells to make lipids sufficiently accessible. Among all the solvent systems examined for microalgal lipid extraction, chloroform–methanol system provided the highest extraction efficiency. Hence, lipid was extracted by using chloroform and methanol (2:1 v/v). One of the drawback of this system is the flammability and toxicity of the solvents used. In order to avoid less non-lipid products the lysate was washed with aqueous sodium sulfate. Lipid productivity was quantified in maximizing biomass and also under nitrogen starvation conditions, which triggers lipid accumulation. The lipid content was enhanced by nutrient stress. Lipid content in cyanobacteria reported under N deficient condition was 10.4 and 9.2% for Synechococcus sp. and Lyngbya sp. respectively.

Acid catalyzed process was adapted only in oils with more than 10 wt% free fatty acids (FFA). The alkali catalyzed transesterification process was chosen because in this process high purity and yield of biodiesel product can be achieved in a short time. Biodiesel (methyl ester) production was found maximum in Synechococcus sp. and minimum in Lyngbya sp. Moreover, sediment (glycerine, pigments and other elements) were higher in Synechococcus than in Lyngbya sp. The viscosity of the biodiesel decreased because of the removal of glycerol content. The viscosity of biodiesel has been found to be closely related to that of diesel. It produced a much smaller drop which burns clear. The fire and flash point of the biodiesel was higher than diesel. So it could be very effective and suited for all motor engines. It was generally not volatile. Therefore it was safer to handle at higher temperatures than diesel. Biodiesel is reportedly produces three times more energy than conventional diesel.

Typically, biodiesel derived from oil seeds, such as rapeseed or soybean produces, 39.5 MJ/kg, while biomass derived from algae yields 41 MJ/kg. The optimal temperature for microalgal cultures is generally between 20 and 24 °C, although this may vary with the composition of the culture medium, the species and strain. Most commonly cultured species of microalgae could tolerate temperatures between 16 and 27 °C. Microalgae have different requirements of pH for growth too and at high pH levels, the availability of CO2 may become limiting to the growth and photosynthesis of microalgae. The pH range needed for the growth of most algal species is between 7 and 9, with an optimum range of 8.2–8.7. Tubular PBRs are one of the most suitable types of outdoor mass cultures.

The advantages of biofuels over traditional fuels include greater energy security, reduced environmental impact, foreign exchange savings and socioeconomic issues. The industrial viability of microalgae based biofuels hinges upon the economics underlying the process; whatsoever advances may arise in terms of technological innovations, the market will not exhibit an enthusiasm for funding capital-intensive energy projects unless the risk-return ratio is acceptable.

Conclusion

The massive need for sustainable energy has led to an increased interest in new resources for energy. Microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels. Major breakthroughs are still needed towards the design and development of photobioreactors that can reduce the production costs while increasing yields and upgradation of lipid extraction, at the same time fine chemical production from the spent biomass. Microalgae are definitely a promising resource towards meeting the fuel demand of the growing population.

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References