

A Comparative study of cyanobacteria and microalgae for biofuel production

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Abstract

The use of algae for producing biofuel is expected to play a critical role as an alternative energy source in the near future, particularly in light of depleting fossil fuel reserves and the adverse environmental impact on fossil fuels. This study discusses the production of biofuel from cyanobacteria and microalgae by various processes. Microalgae and cyanobacteria can produce high amount of lipids and that is the sole reason for they being identified as a potential biodiesel feedstock. They also have the ability to fix CO₂ and have rapid growth rates. It is observed that algae can be converted to oily substances that grows in Carbon-dioxide enriched air. It also can contribute to solve major problems of air pollution resulting from CO₂ evolution. This experiment is undertaken to know the proper trans-esterification, amount of biodiesel production (ester) from cyanobacteria and *microalgae* species. In this experiment, common species such as *Cyanobacterium aponinum* and *Spirogyra sp.* are used to compare the amount of biodiesel production. It is observed that *C.aponinum* can produce high amount of algal oil and biodiesel (ester) than *Spirogyra sp.* However, biomass when calculated was found to be higher in *C.aponinum* compared to *Spirogyra sp.* The paper indicates that biodiesel can be produced from both the species with *C.aponinum* being a better one.

Keywords: Microalgae, cyanobacteria, Biofuel, Renewable energy, Transesterification

INTRODUCTION

Fossil fuel reservoir beneath the earth surface is fixed and non-renewable. According to IEA (International Energy Agency) and BP (British Petroleum) reports, techniques are in progress to discover new depositories. It has been forecasted that this will not be sufficient to satisfy the demand of the exponentially growing population (Radmer et. al. 1996, Clarens et. al. 2010). Thus, due to the shrinkage in the supply of petroleum-based traditional fuel, the need to produce viable renewable fuels becomes utmost necessary. On the other hand this fuel production also reduces greenhouse gas emissions. Microalgae and cyanobacteria both have high growth rates, high oil content, and helps in the reduction of CO₂ by trapping it. Their growth is independent of the different weather conditions. This is the reason for they being recommended as biofuel manufacturers. They can be grown in non-arable lands as well. Microalgae and cyanobacteria utilize nitrogen and phosphorus from waste water source thus helping in waste water bio remediation. They can also be cultured in photo bioreactors and can produce biomass exceeding tenfolds than that of terrestrial crops. According to the International Energy agency, Department of energy, the production of oil by algae is much higher than those produced by other crops as shown in Figure 1.

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Microalgae

The oldest living organisms on the Earth are the Microalgae. Microalgae belongs to Protista group. The size of the microalgae is measured by micrometers. Microalgae do not have roots, stems, and leaves unlike higher plants. Besides, around the reproductive cells of microalgae, there is no sterile cell covering. That is why they are described as thallophytes. Microalgae have a number of advantages and can be referred as a feedstock for biofuel. It does not require arable land and uses less water than other crops.

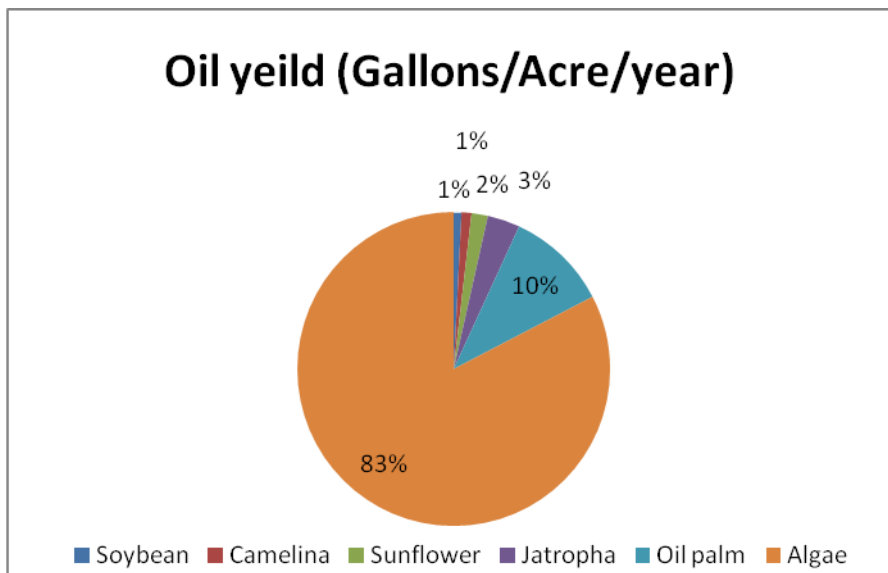


Fig 1: Comparison of oil yield from different sources

It is observed that microalgae can convert more than 60% of its body weight in lipids. According to their pigment kinds, microalgae can be classified. The major classes include Chlorophyceae (green-algae), Bacillariophyceae (diatoms) and Chrysophyceae (golden algae). Microalgae contain chlorophyll-a for photosynthesis. It has been observed that other than any crops based on acreage; microalgae can produce 15-300 times more oil. Microalgae produce lipids that are typically triacylglycerols (TAG). TAG can be converted to fatty acid methyl esters (FAME) in reaction with alcohol, which is a biodiesel.

Cyanobacteria

This is also called **blue green algae**. Cyanobacteria have thylakoids, which are surrounded by internal membranes and look like flattened sacs, in which photosynthesis takes place. As cyanobacteria lack nuclei, the chromosome of it is held in the nucleoid, the region of the cell where DNA is located. It also contains chlorophyll-a for photosynthesis. Some freshwater cyanobacteria contain monogalactosyl diacylglycerols and digalactosyl diacylglycerols. Cyanobacteria are considered to be great potential for biofuel production because of their high growth rate, ability to fix carbon dioxide gas, and also their genetic tractability.

Materials and Method

Site

The samples were collected from local water deposits (22°42'15N 88°23'30E) of Guru Nanak Institute of Pharmaceutical Science and Technology, Sodepur, West Bengal.

Isolation

Desired species are isolated from the sample by serial dilution in specific broth. For cyanobacteria, it is the *Cyanobacterium aponinum* strain and for microalgae the *spirogyra sp.* For obtaining pure culture the strains were subjected to specific media. Obtained strains were grown individually in 125 mL of sterilized BG11 media. In the incubator shaker algal and cyanobacterial culture were rotated with constant shaking of 120 rpm, at $25 \pm 2^\circ\text{C}$ under the light intensity of $50 \pm 5 \mu\text{mol m}^{-2}\text{sec}^{-1}$ and 18:6 (light: dark) duration photoperiod. For photosynthesis, CO_2 in the air was used as carbon source.

Optical density value measurement by Spectrophotometry

Indirect measurements of cell concentrations of *Cyanobacteria and Microalgae* were determined by UV-Vis spectrophotometer. Hemocytometry were applied to determine cyanobacterial and algal cell concentrations. The hemocytometer is composed of nine large squares. They all had same dimensions. *Cyanobacteria and Microalgae* cells were counted in small squares.

Determination of Cell concentration of algal species and cyanobacterial species at exponential growth phase

During exponential growth phase, phototrophic growth were set up to determine individual cell dry weight, size and cell density of *C.aponinum* and *spirogyra sp.* Algal and cyanobacterial cultures were kept in a controlled environment in a conditioned room. In a 100-mL Erlenmeyer flask, the pure strain culture was maintained for each cyanobacteria and algal culture using modified BG11 media. From that culture 1ml of culture was taken and kept in new vessels to start new growth. All the vessels were kept under a light intensity and constant stirring had been done once in a day. Triplicate samples of *Cyanobacterium aponinum* and *spirogyra sp.* were collected on days 4, 6, 8, 10 during their exponential growth phase as shown in Table 1.

Harvesting

This step was done by flocculation. The flocculant used here is NaOH which clustered the growing species within the solution. The biomass is concentrated by further thickening of the solution. After the centrifugation, the pellets are taken for fresh weight measurements.

Dewatering

The samples were subjected to filtration using 0.25 micron filter paper. First, the resulting paste was rinsed with de-ionised water and then the fresh weight was taken. This paste was then dried at 60°C in an oven overnight. This was followed by the dry weight measurement of cyanobacteria and algal samples. The difference of the two weights gives the biomass of the sample as shown in Table 2.

Lipid extraction

For lipid extraction Bligh and Dyer method was used. 1ml algal sample and 3ml solvent mixtures (Chloroform and methanol) were taken. The lipid and sediment layers were allowed to settle and then they were separated by the separating funnel.

Conversion to fuel

The conversion of triacylglycerol to fatty acid methyl ester was done by the transesterification process Figure 1. In this reaction methanol was added to lipids. NaOH,

was used as catalyst. The reaction took place with constant stirring and produced FAME and glycerol, as a by product. The volume of FAME was noted.

Results

From the above experiments, it was observed that optical density value, and cell density value of cyanobacteria was higher than microalgal species as shown in Figure 2(A) and Figure 2(B). This resulted in high biomass quantity and lipid content of cyanobacteria than microalgae species as shown in Figure 3 (A). As the lipid content is higher in cyanobacteria it produced high amount of biodiesel than microalgal species as shown in Figure 3(B).

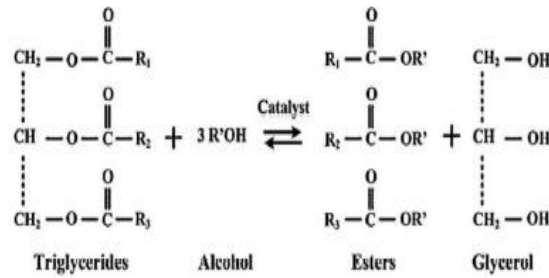


Fig 1: Conversion of TAG to esters

Table 1. OD value measurements of *C. aponinum* and *Spirogyra* sp.

OD	Day 4	Day 6	Day 8	Day 10
<i>C. aponinum</i>	0.67	0.99	1.35	2.5
<i>Spirogyra</i> sp.	0.62	0.85	1.05	1.12

Table 2. Comparison between *C. aponinum* & microalgae according to their Lipid and biodiesel content

Species	Lipid Content (% dry weight biomass)	Biode-sel (g/L)
Cyanobacteria	12.63	10
Microalgae	10.50	6.2

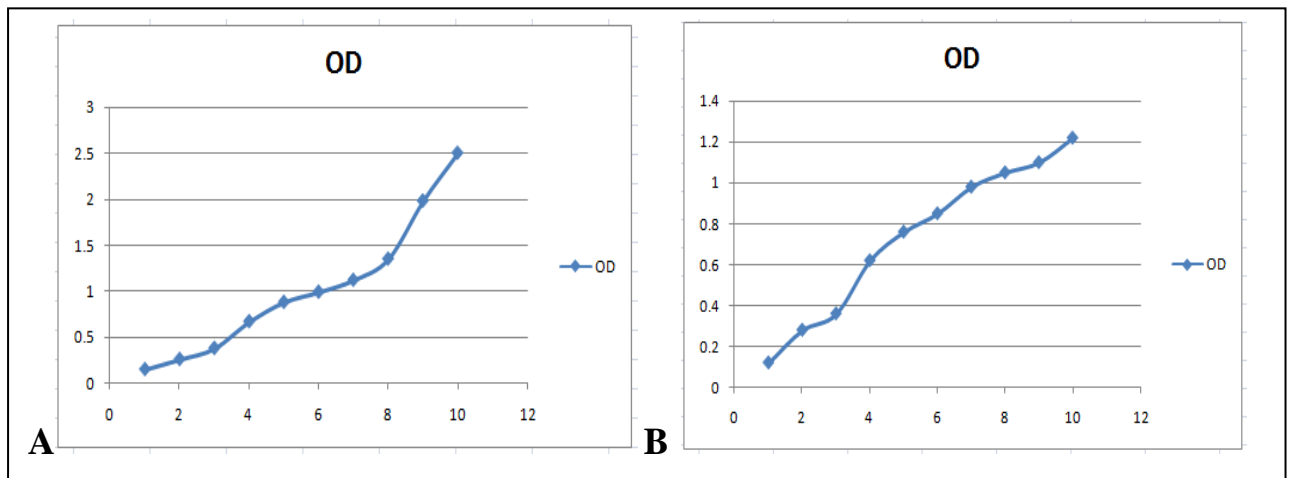


Figure 2(A). OD value of *C. aponinum*. Figure 2(B). OD value of *Spirogyra* sp.

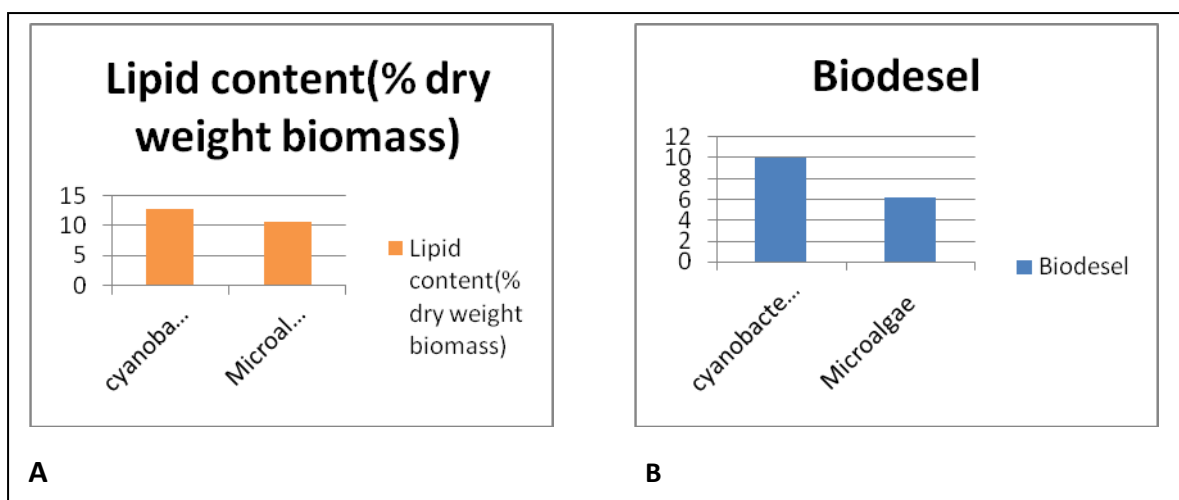


Figure 3(A) Lipid content of cyanobacteria & Microalgae; Figure 3(B). Biodiesel content of cyanobacteria & Microalgae

Table 3. Different biofuel sources

Source	Biofuel	Current yield of biofuel	Maximal theoretical yield
Palm Oil	Biodiesel	5,500 L	22000L
Cyanobacteria	Biodiesel	50,000 L	168,000 L
MicroAlgae	Biodiesel	13,125 L	58,700 L

Microalgae is a very strong source for the new energy generation by offering noteworthy advantages over higher plants as shown in Table 3. This is because their life span is very short and they can double their biomass within 24hrs. Within a very short time period, their biomass increases. The optimum temperature for their growth is 29.2°C.

Cyanobacteria on other hand has a mean growth rate of 0.92¹. In the comparison with the terrestrial crops micro-organism can be cultivated throughout the year and that is why they can produce much higher oil yield/area. Oil content of microalgae varies usually between 20-50% of their dry weight. For the terrestrial crops the maximum oil content is about 5% of their dry weight. Microalgae is a source of non-toxic and highly biodegradable biofuels. Thus unique properties of microalgae and cyanobacteria make them a promising model for the production of biofuel.

Conclusion

One of the economically, environmentally sustainable safe sources of energy is biofuel. Microalgae and cyanobacteria offers new potential for the improvement of the present biofuel production. Conversion of solar energy to chemical energy are done by the process of photosynthesis but oil from microalgae and cyanobacteria are useful for the production of biofuels. As microalgae produces large amounts of oil, they are considered to be the highest potential triacylglycerol resources. On the other hand, cyanobacteria has both the character of prokaryotes and plants and this makes them highly tolerant to foreign genes. Here cyanobacteria and microalgae have been cultured in a BG11 media for the production of

biofuel and their growth is observed in the exponential phase. It is found that the OD value of *C. aponinum* was higher than micro *spirogyra sp.* Biomass and lipid concentration of cyanobacteria and microalgae are also measured and was found that cyanobacteria contain higher concentration of biomass (5.025%) and lipid (25%). Biofuel (Fatty acid methyl ester) was also obtained in higher concentrations in cyanobacteria (10ml) than that microalgae species (6.2ml). Therefore, cyanobacteria holds much more potential than microalgae in the production of third generation biofuels.

Acknowledgement

I avail this opportunity to express my sincere thanks and gratitude to Maulana Abdul Kalam Azad University of Technology for providing support through my work.

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