# Enhancement of Biodiesel Production from Different Microalgae Species

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***Abstract - Continently rising petroleum fuel cost, polluting environment by releasing toxic gases upon combustion, driving an attention to search alternative sustainable fuel. Production of biodiesel is one of the most efficient ways to reduce petroleum fuel use and environmental pollution. Dependence on petroleum based fuels is not sustainable due to increasing fuel costs, steady depletion of crude oil, and the environmental consequences associated with the use of fossil fuels (Chisti, 2007; Demirbas and Fatih Demirbas, 2011; Schenk et al., 2008). One option for the production of renewable liquid fuels is biodiesel from microalgae to offset usage of crude oil based diesel (Demirbas and Fatih Demirbas, 2011). There exist many different algae strains with high oil content e.g. Botryococcus brauni, Schizochrytrium sp., Nannochloropsis sp., Neochloris oleoabundans, Nitzschia sp..Two algal species (Botryococcus brauni and Cladophora sp. ) were used for the production of biodiesel using two extraction solvent systems (Hexane/ether (1:1, v/v)) and (Chloroform/methanol (2:1, v/v)). In both algae species Hexane/ether (1:1, v/v) extraction solvent method resulted in low lipid recoveries compare to chloroform/methanol (2: 1, v/v) extraction solvent method. The green colonial algae Botryococcus sp.. extracts the highest lipid and biodiesel yield then filamentous green algae Cladophora sp., whereas Cladophorasp., was produce higher quantity of biomass and sediments than Botryococcus sp.. No prominent difference in pH of biodiesel was found.***

***Key words: Microalgae, Transesterification, Hexane, ether, Chloroform, total lipids, biodiesel.***

## I. INTRODUCTION

In India, The basic sources of energy are petroleum, natural gas, coal, hydro-electrical and nuclear. The need of energy is increasing continuously due to the increase in population and industrialization. The continued use of petroleum sourced fuels is now widely recognized as unsustainable because of the depletion supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment leading to increase of global warming. Biomass-derived fuels, namely biodiesel and ethanol, are considered by the Indian government as one option to substitute petroleum-based products—diesel and gasoline, respectively. Diesel consumption in 2008–2009 was 52 million tons (Mt) about 40% of the total petroleum products and it grows by about 6%–8% annually.

Biodiesel is primarily produced from oil crops, animal fat and cooking oils cannot realistically satisfy but it is now synthesizing mainly from microalgae. Algae (macro and micro) have recently received a lot of attention as a new biomass source for the production of renewable energy. Microalgae possess advantageous characteristics that warrant its consideration as a source of alternative oil for biodiesel production, as well as a feedstock for the production of additional biofuels and bioproducts (Christenson and Sims, 2011; Mata et al., 2010). Biodiesel production from algae source is not new (Chisti, 1980 – 1981; Nagle and Lemke, 1990; Sawayama et al., 1995), but it is now being taken seriously because continuous increasing price of petroleum and global warming effect due burning fossil fuels (Gavrilescu and Chisti, 2005).

The mass of oil produced per unit volume of the microalgae broth per day depends on the algal growth rate and the oil content of the biomass. All algal are not satisfactory for high oil contain microalgae. Production of algal oils requires ability to low cost produce large quantities of oil rich microalgae. Microalgal oils differ from most vegetables oils in being rich in polyunsaturated fatty acids with greater than four double bond. Production of bio diesel from micro algae involve simple steps such uas cultivation, harvesting, cell disruption and oil extraction. Lipids and fatty acids are converted in bio diesel by transesterification process.

Biodiesel can be made from almost any source of oil or fat when reacted with alcohol; the major components of these sources are triacylglycerol molecules. It is often made through a catalyzed chemical reaction known as transesterification between these oils/fats and an alcohol, usually methanol shown in Figure 1.

Strong bases such as sodium hydroxide (NaOH) or potassium hydroxide (KOH) are commonly used as catalysts in large scale production of biodiesel. In the laboratory scale, acid-catalyzed transesterification is often used because it does not produce the soaps that occur when using a base as the catalyst. The glycerol backbone of the triglyceride is a waste product after completion of the reaction; it must be washed out of the biodiesel before the fuel is ready for use. Biodiesel originally from pure vegetable oils has even been standardized based on the parameters defining their quality by the ASTM, available in publication ASTM D 6751.

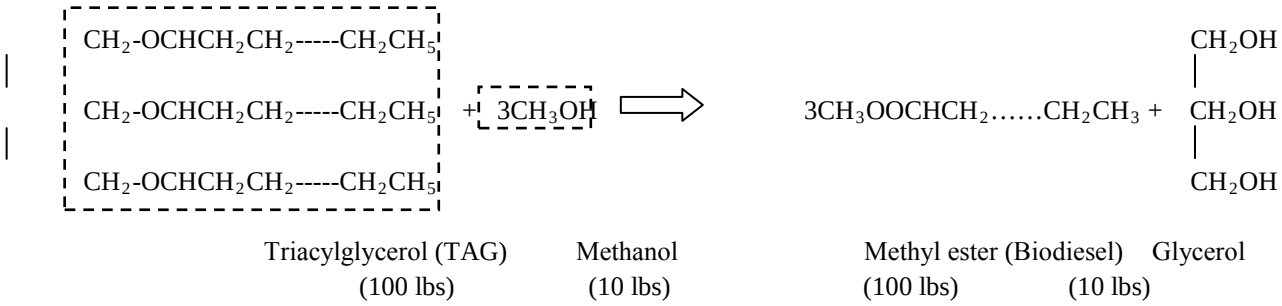


Figure 1: Transesterification reaction to produce biodiesel

## II. MATERIALS & METHODS

The experiment was conducted in the Biotechnology laboratory at Dept. of Biotechnology, Central University of Bihar, Patna, Bihar. The work was designed to isolation and identification of pure algae culture, isolation of lipid content for biodiesel production, effect of two different method for lipid isolation. The algal samples were collected from Sanjay Gandhi Botanic Garden and NMCH pond Patna. The cultures were grown in a thermostatically controlled culture room at 24±20C. Modified Bold's basal medium (Bischoff and Bold, 1963) for Cladophora sp. and Chu 13 medium for Botryococcus brauni were used for providing the optimal nutritional requirements for all the cultures. The cultures were regularly sub-cultured to get sufficient amount of culture, required for various analyses.

Equipment and Chemicals: The some equipment and chemicals is used in the lipids extraction and biodiesel production procedure. Those are Mortar and pestle, Centrifugal Device, test tube, regent bottle (500 ml), conical tubes (100 ml, 250 ml, 500 ml and 1000ml), pure hexane, chloroform, ethanol, ether, acetone, and methanol were provide by Central University of Bihar, and distilled before use.

Preparation of pure culture of Algae : The collected sample cultured on petri-dishes in modified Bold's basal medium (Bischoff and Bold, 1963) and Chu 13 medium (Largeau et al. 1980) with 1.5 gm agar. For the isolation, the required species can be picked up by platinum needle under microscope after serial dilution method and streaked on the surface of the agar plate. After inoculation, these petri-dishes placed in an incubation chamber for 7 -8 days at constant temperature (24±2$^0$C) and 16/8 light/dark cycles under normal tube light. Grown algae species colony transferred to culture tubes by platinum loop. Further it transferred into small conical flasks then larger flasks for mass scale algae grown. After 25 days of each microalgae species was harvested by centrifugation @3000 rpm ad pellets dried at 60°C for 15 min before extraction.

Extraction of oil contain from algae: Extraction of oil was carried out using two extraction solvent systems to compare the oil content in each case and select the most suitable solvent system for the highest biodiesel yield.

Chloroform /methanol (2:1, v/v) method ( Bligh and Dayer (1959)): 15 g of each ground dried algal species was mixed with 150 ml of the extraction solvent mixture; chloroform/methanol (2:1, v/v) for 20 min by the help of shaker. After that added 75 ml of chloroform/water (1:1, v/v) mixture for 10 min. Filtered and the algal residue was extract three to five times by 100 ml chloroform followed by filtration by whatmann filter paper.

Hexane/ether (1:1, v/v) method (Hossain and Salleh (2008)) *:* 15 g of each ground dried algal species was mixed with 150 ml of the extraction solvent mixture, hexane/ether (150 ml, 1:1, v/v). Kept the sample for 24 hrs to settled. After that, filtered the sample with help of whatmann filter paper.

Transesterification and biodiesel production: The extracted oil was kept in rotary evaporator at 40- 45°C to release the solvent mixture solutions. Then, the oil produced from each algal species was mixed with a mixture of catalyst (0.25g NaOH and 24 ml methanol) with stirring properly for 20 min. This process called transesterification. The Mixture was kept for 3hrs in electric shaker at 3000 rpm. (National Biodiesel Board, 2002). After shaking the solution was kept for 16 hours to settle the biodiesel and the sediment layers clearly. The biodiesel layer was separated from sedimentation by micropipettes carefully. Biodiesel was washed by 5% water many times until it becomes clear then Biodiesel was kept under the running fan for 12 hours. The produced biodiesel was measured, ph was recorded and stored for analysis. Quantity of sediments (glycerin, pigments, etc) was measured.

Oil content:

Oil percent of algae(per gram of dry mass) = (weight by difference in algae/original weight of Algae) X 100 .

*Analysis of fatty acids in the produced biodiesel:*

Ideally, there will be two distinct layers: an amber biodiesel layer on top and a darker glycerol layer on the

bottom. Sometimes, there will be a third or fourth layer between the glycerol and the biodiesel. These layers are soap from too much catalyst or water and often appear milky or yellowish. pH of unwashed biodiesel around 9 and washed biodiesel will be closer to 7.

## III. RESULTS AND DISCUSSION

After the preparation of pure algae culture were by serial dilution fallowed by plating only two types on algae are grow in our laboratory. On the microscopic observations bases these algae are Botryococcus brauni and Cladophora sp... These algae were identified microscopic examination but morphological heterogeneity of the alga makes the identification difficult. Botryococcus sp. is a green colonial microalgae which produces high levels of lipids. The sizes of these colonies have a wide range with volume average diameters ranging from 0.05-0.2 mm (Zhang et al. 1998). Komárek and Marvan (1992) proposed the existence of at least 13 species of Botryococcus on the basis of morphological differences by omitting the chemical analyses. Metzger and Largeau (2005) reported that, within in each chemical race and for the same strain the morphology of the alga could vary in relation to age and culture conditions. Cladophora is reticulated filamentous green algae, grows as microscopic thin, hair-like threads. It has multinucleated cells containing oval shaped reticulated, pyrenoid-packed chloroplasts.

Results showed the lipid amounts extracted from algal species by the two extraction methods described in the table 1. The alga has a relatively simple fatty acid profile with myristic acid (C14:0), palmitic acid (C16:0), docosapentaenoic acid (C22:5), and docosahexaenoic acid (C22:6) being the major fatty acids (Pyle, Garcia et al. 2008). When the culture at stationary phase, the green colonial algae Botryococcus sp. produced 52±1 % lipid when extracted using chloroform/ methanol (2:1, v/v), but only 21±1 % by hexane/ ether (1:1, v/v) system. The filamentous green algae Cladophora sp.. produced 9±1 % lipid on chloroform/ methanol (2:1, v/v), but only 4±1 % by hexane/ ether (1:1, v/v) system.

Table 1: lipid percentage (dry wet) produced by algae using hexane/ether(1:1 v/v) and chloroform/methanol (2:1 v/v) extraction method.

| Algae species | Dry weight (g) | Chloroform/Methanol (2:1, v/v) | Lipid % | Hexane/Ether (1:1, v/v) | Lipid % |
|---|---|---|---|---|---|
| *Botryococcus sp.* | 15 g | 7.92 g | 52.8 | 3.2 g | 21.33 |
| *Cladophora* sp.. | 15 g | 1.4 g | 9.33 | 0.63 g | 4.20 |

The obtained results illustrated in tables 1 revealed that the solvent mixture hexane/ether was not suitable system for lipid because hexane/ether solvents were unable to extract polar lipids. hexane/ether method only extract non polar lipid molecules. In the table 2., shown that The *Botryococcus sp.* (4.89 g) 24±1 % produce more biodiesel yield then *Cladophora* sp., (3.34 g) 15.6±1. Berglund *et al*. (2001) reported that both the quantity and quality of lipids produced will vary with the identity of the algal species. Overall, approximately 45 % of the transesterifiable lipids within the algal biomass were isolated for conversion to biodiesel. The fatty acid proportion in algal cell varied and depended on growth conditions (Cohen, 1988; Thompson *et al*., 1990) e.g. environmental or culturing parameters such as light intensity, growth phase, photoperiod, temperature, salinity, CO2 concentration, nitrogen and phosphorous concentration (Wu *et al*., 2011).

The biodiesel esters were characterized for their physical and fuel properties including density, viscosity, iodine value, acid value, cloud value, pure point, gross heat of combustion and volatility and fuel produces slightly lower power and torque, and higher fuel consumption than No.2 diesel fuel (Demirbas, 2008). Biodiesel is better than diesel fuel in terms of sulfur content, flash point, aromatic content and biodegradability (Bala, 2006). Biomass after oil extraction may be used for livestock, ethanol production and also in paper making.

Table 2: total lipid, biodiesel, ph and biomass of sample after biodiesel extraction.

| Algae species | Dry weight (g) | Lipid | Biodiesel yield | Biodiesel yield % | Biomass (g) | pH |
|---|---|---|---|---|---|---|
| *Botryococcus sp.* | 15 g | 7.92 g | 3.62 g | 24.13 % | 10.11 g | 7.1 |
| *Cladophora* sp. | 15 g | 5.34 g | 2.34 g | 15.6 % | 11.8 g | 7.2 |

The growth of algae was not optimized in the present work. The optimization of the growth of algae would certainly enhance the oil yield. Future work could focus on the transesterifiable lipids within the algal biomass were isolated for conversion to biodiesel without drying the harvested biomass, while potentially helping to reduce the need for organic solvents.

## IV. FUTURE SCOPES

In India 70% of the population lives in Rural Areas, and depend on Cow Dung and Firewood as main source of fuel. Edible vegetable oils are in short supply and India needs about 600,000 tons per year. BioDiesel as fuel is best option for fulfill our need. Our future scope to developed new method for more lipid isolation & search new highly micro-alga species in Bihar.

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