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ISOLATION, CHARACTERIZATION OF ALGAE AND PRODUCTION OF BIODIESEL

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ABSTRACT

Algae sample was collected from a pond and was indentified as *Spirogyra* spp. It was grown in BG11 media in the required conditions. The algae was dried, powdered and used for further extraction processes. The lipid content was 16%, carbohydrate 50 mg/ml and protein 7.5 mg/ml. Algae oil was extracted from *Spirogyra* spp. to compare their conversion into biodiesel. The acid value of fats was estimated to be 7.17. It was transesterified and conversion percentage was found to be 88.9%.The obtained crude biodiesel was purified and purity of biodiesel was checked using Fourier Transform Infrared Spectrometer. Biodiesel as a fuel not only helps reducing the pollution, it also reduces health hazards and gives our society – A CLEANER AND GREENER TOMMOROW.

KEYWORDS: Biodiesel, algal oil, spirogyra, BG11 media, FTIR

INTRODUCTION

Renewable biofuels are needed to displace petroleum-derived transport fuels, which contribute to global warming and are of limited availability. Biodiesel and bioethanol are the two potential renewable fuels that have attracted the most attention. As demonstrated here, biodiesel and bioethanol produced from agricultural crops using existing methods cannot sustainably replace fossil-based transport fuels, but there is an alternative. Biodiesel from microalgae seems to be the only renewable biofuel that has the potential to completely displace petroleum-derived transport fuels without adversely affecting supply of food and other crop products. Most productive oil crops, such as oil palm, do not come close to microalgae in being able to sustainably provide the necessary amounts of biodiesel. Similarly, bioethanol from sugarcane is no match for microalgal biodiesel (Yusuf Chisti, 2010).

MATERIALS AND METHODS

Isolation, identification and growth of algae:

The algae sample was collected from a pond in Tambaram and cultivated in BG-11 media in the plant tissue culture racks which have the fluorescent lamps as an artificial light source. Air pump was fixed in the containers for aeration. The algae was identified by microscopic observation.

Extraction and estimation of total lipids, carbohydrates and proteins:

Lipids from the algae were extracted and estimated by Folsch`s method while carbohydrates were estimated using Phenol Sulphuric acid method. The proteins were estimated by Lowry`s method.

Extraction of oil from the algae – *Spirogyra* spp

The oil in the algae was extracted using the Soxhlet apparatus with hexane as a solvent. Acid value estimation and transesterification of the oil from the algae. The acid value of the oil extracted from the algae was estimated by titrimetric method. The transesterification of the oil was carried out using an alkali-KOH and methanol. The transesterified oil was separated as FAME (fatty acid methyl esters) and glycerol.

Purification of Biodiesel:

The FAME obtained is blended with 50% tannic acid, centrifuged at 10,000 rpm for 10 minutes and the upper layer of purified FAME was collected and used.

Different parameters for conversion of *Spirogyra* spp oil to biodiesel:

the parameters like temperature, retention time and catalyst concentration

Blends of Biodiesel and applications:

Biodiesel was blended with petrodiesel in different proportions and then it was used for burning spirit lamps in the laboratory. FT-IR was used for the verification of the purity and to quantitate the biodiesel samples.

RESULTS AND DISCUSSION

Isolation, identification and growth of algae

A small amount of the algae sample was added to the BG11 culture medium for the growth of algae. Light only penetrates the top 3 to 4 inches (76–100 mm) of the water. As the algae grow and multiply, the culture becomes so dense that it blocks light from reaching deeper into the water. Direct sunlight is too strong for most algae, which need only about 1/10 the amount of light they receive from direct sunlight. Thus the cultures were kept in the window shade for minimum sunlight. Some of the cultures were placed in tissue culture racks providing artificial light source.The growth of algae-Spirogyra spp. was observed in 1 month. A small amount of the algae was taken and observed under a microscope identification. The multicellular filamentous green alga covered by a mucilagenous sheath was identified to be *Spirogyra* spp. *Spirogyra* spp. occurs in freshwater bodies such as ditches,ponds,lakes etc.It is commonly called water silk or pond scum because of slippery touch of its thread like filaments. Each filament is unbranched and consists of cylindrical cells placed end to end. The cell wall is two layered and is made up of cellulose and pectin. Cytoplasm lies in the periphery of cell enclosing a vacuole in the centre. One or more ribbon shaped spirally arranged chloroplasts are present in the cytoplasm. Each chloroplast bears pyrenoids. Single nucleus is stranded in the centre of the vacoule by cytoplasmic strands. After growth the algae was filtered, sun dried and grinded to a powdered form (Fig I).

Fig. I Algal Powder

In our laboratory conditions the algae was cultivated in bottles containing BG11 media with the help of air pump for aeration. Thus the indoor cultivation of algae showed positive results. The amount of algae powder obtained was 500 grams.

The algal samples were collected from the blooms in fresh water ponds in Mahabalipuram in same type of research work . The algal cells were microscopically examined and it was found to be *Botryococcus* sp. Cultivation of algae was done in race way pond. Modified Chu13 media was prepared and poured into the raceway pond.The paddle wheel was set to 15 rpm to provide aeration. Sunlight was the source of light. Cultures were monitored for their growth for two weeks in outdoor condition and the biomass was harvested (C.Dyananda et al., 2010).

Extraction and estimation of total lipids, carbohydrates and proteins

Estimation of lipids in Antartic sea ice microalgae by Nile Red Flourescence was done (John.C.Priscu *et al*., 2000). Lipid content of the microalgae-*Chlorella* spp. was estimated by Nile Red Flourescence technique. Flourescence of the sample was read at an excitation wavelength of 475 nm and an emission wavelength of 580 nm. Lipid fluorescence was read at 570nm- 580 nm. This shows the high level of lipid content in *Chlorella* spp.

In present work the lipid content of *Spirogyra* spp. was estimated by Folsch`s method and it was estimated to be 16%. This method is an easier method when compared to the Flourescence method, as it method requires a large amount of sample and it is costly. The concentration of carbohydrates in the *Spirogyra* spp. was found to be 50 mg/ml. A rapid and simple spectrophotometric method was done for the estimation of microgram quantities of algal polysaccharides present in *Ulva rigida* spp. The absorbance of carbohydrates was read at 664 nm (Q Jiao & Q Liu, 2000). However, the total carbohydrates of the algal sample was estimated by Phenol sulphuric acid method.

The concentration of protein in the *Spirogyra* spp. was found to be 7.5 mg/ml. Biochemical composition of seaweeds (marine algae) *Enteromorpha intestinalis* has been reported. The total protein was estimated using Biuret method and it was reported to be 4.5 mg/ml (K. Manivannan *et al*., 2008)

Extraction of oil from the algae – *Spirogyra* spp.

Oil from Spirogyra spp. was extracted by Hexane solvent using soxhlet apparatus. It is better than the mechanical methods as it extracts maximum amount of oil from the algae. Similar work was done on microalgae Botryococcus spp (Paula Mercer & Roberto E. Armenta , 2011). The oil was extracted from *Botryococcus* spp. through mechanical methods such as Expeller and Ultrasonic assisted extraction. The normality of KOH was standardised andv found to be 0.071 N. Acid value is the milligrams of KOH required to neutralize the free fatty acid present in 1 g of fat. Transesterification is the process of exchanging the organic group R" of an ester with the inorganic group R' of an alcohol. These reactions are often catalyzed by the addition of an acid or base catalyst. In this process the oil from Spirogyra spp. is made to react with methanol and a catalyst. The transesterification was done using the catalyst sodium hydroxide.

In a work done in alkali catalysed sunflower oil transesterification (Dario Frascaria *et al*., 2010) sunflower oil was made to react with methanol and a catalyst (potassium hydroxide). Crude biodiesel and glycerol was obtained. In the present work sodium hydroxide was used as a catalyst and similar results were obtained. The upper layer has FAME (crude biodiesel) while the lower layer has glycerine with methanol and catalyst.

Purification of Biodiesel:

Biodiesel, regardless of the quality of reaction or of oil has contaminants. The contaminants include (primarily) soaps, a small amount of left over methanol and some free glycerine. Washing the fuel with water removes these impurities. Washing also has two additional advantages- it stops the very slow remaining reaction that sometimes occurs. It provides some quality control feedback.

Washing of crude biodiesel can be done by bubble washing where forming air bubbles in the water is carried out (Rickdatech, 2010). The process can lead to the formation of soap. Hence the settling of Crude biodiesel and water is a better option.

After washing the biodiesel, it will usually have a slightly hazy or cloudy appearance to it, this is because there are micro droplets of water still in suspension in just washed fuel, biodiesel has an ability to keep this water in suspension and up to 1500 parts per million is allowable by international quality specifications, but even at these levels biodiesel should look clear. There are several ways to remove the excess water which causes this cloudiness but the main objective of them all is to expose the biodiesel to as much air as possible to allow evaporation of the water, this can be achieved by just leaving the biodiesel in a container with it's top open to air exposure, but depending on the temperature of the air and how much it is circulating over the container it can take from days to weeks to evaporate the water off and for the biodiesel to lose it's haze, biodiesel dries more quickly or more slowly depending on how well washed it is(i.e. less soap = quicker drying).

There are several ways to speed up this drying process:-.Increase the amount of air contact increase the movement of air and increase the temperature of the air as warm air can hold more water vapour. After drying the Biodiesel there will be some water left out which was removed using tannic acid purification step. The obtained biodiesel was centrifuged with 50% tannic acid. Upper layer is the purified FAME. It was separated and stored in a closed container. Acetic acid was used to purify the impurities (M. Berrios et al., 2006). Tannic acid was used can remove soap, methanol and glycerol effectively.

After transesterification the fatty acid present in the oil reacts with the methanol and catalyst and gets converted to fatty acid methyl ester (FAME) which is the crude biodiesel. The conversion was 88.9% for *Spirogyra* spp. oil.

Different parameters for conversion of *Spirogyra* spp oil to biodiesel:

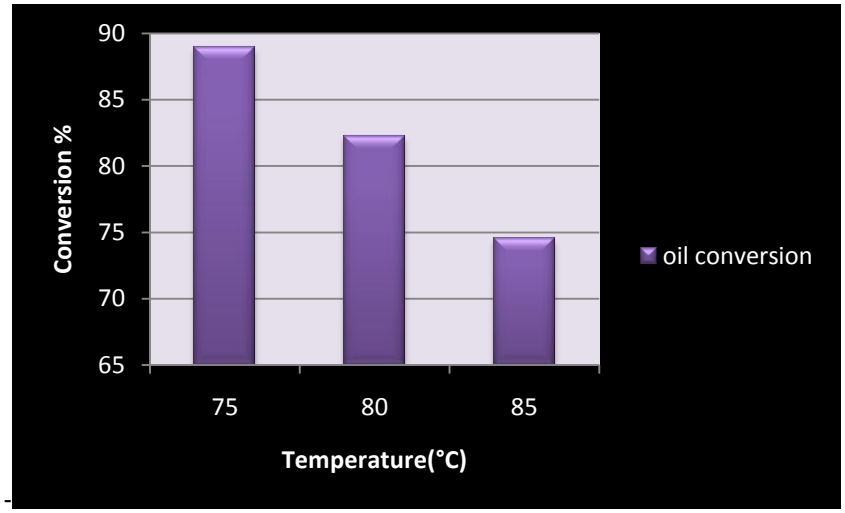

Fig.II Conversion percentage at various temperature

Temperature

When different temperatures were tried for the conversion of oil to biodiesel,the conversion of *Spirogyra* spp. oil was maximum at 75°C.The conversion percentage decreases if there is further increase in temperature (Fig II).

Retention Time:

The reaction was allowed for various periods conversion of *Spirogyra* spp. oil was observed maximum at 1 hour retention time. The conversion % decreases at further increase in retention time, this may be due to feedback inhibition of the product formed, after transesterification Table I.

S.No	Retention Time(in Hours)	Spirogyra spp oil Conversion (%)
1	1/2	60.2
2	1	88.9
3	2	84.6
4	3	79.0

Table I Conversion percentage at various retention time

Concentration of the catalyst:

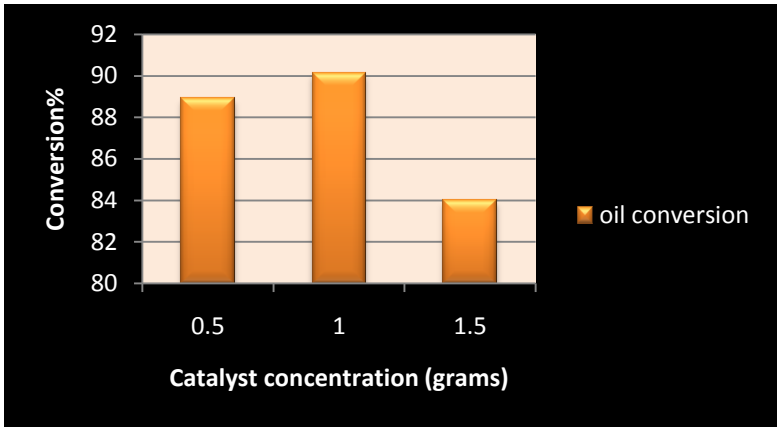

Fig. III Conversion percentage at various concentration of catalyst

The conversion of *Spirogyra* spp. oil was observed maximum at 1 gram catalyst concentration. The catalyst used was KOH. There is no much conversion difference when the catalyst concentration is 0.5 and 1 gram, but the conversion is decreased when the catalyst concentration increased further. Too much catalyst leads to soap accumulation, hence the conversion into FAME decreases. In biodiesel production from soybean oil fatty acid ethyl esters the conversion % of soybean oil to biodiesel was estimated to be 85% for 1 gm catalyst (Ardalla Scabio *et al*., 2009).

Blends of Biodiesel and applications:

The purified biodiesel was blended with 50%, 20%, 5% of diesel. Similar work was done in Jatropha biodiesel blends, biodiesel obtained fom Jatropha was blended with petro-diesel and used (Asia Rakesh Sarin *et al*., 2006). Biodiesel obtained from *Spirogyra* spp. was blended with diesel and used for laboratory purposes. The produced biodiesel was either blended or as such used for spirit lamps

FOURIER TRANSFORM INFRARED SPECTROMETER

The Biodiesel IR FAME Analyzer is a total solution for FAME analysis. The commercial biodiesel was used as a standard and the fatty acid methyl ester content was highest at the wave number 3,391.12. In the Biodiesel from algae sample, the fatty acid methyl ester content was highest at the wave number 3,389. This shows that the purity of biodiesel obtained from algae is almost the same of the commercial biodiesel (Fig IV).

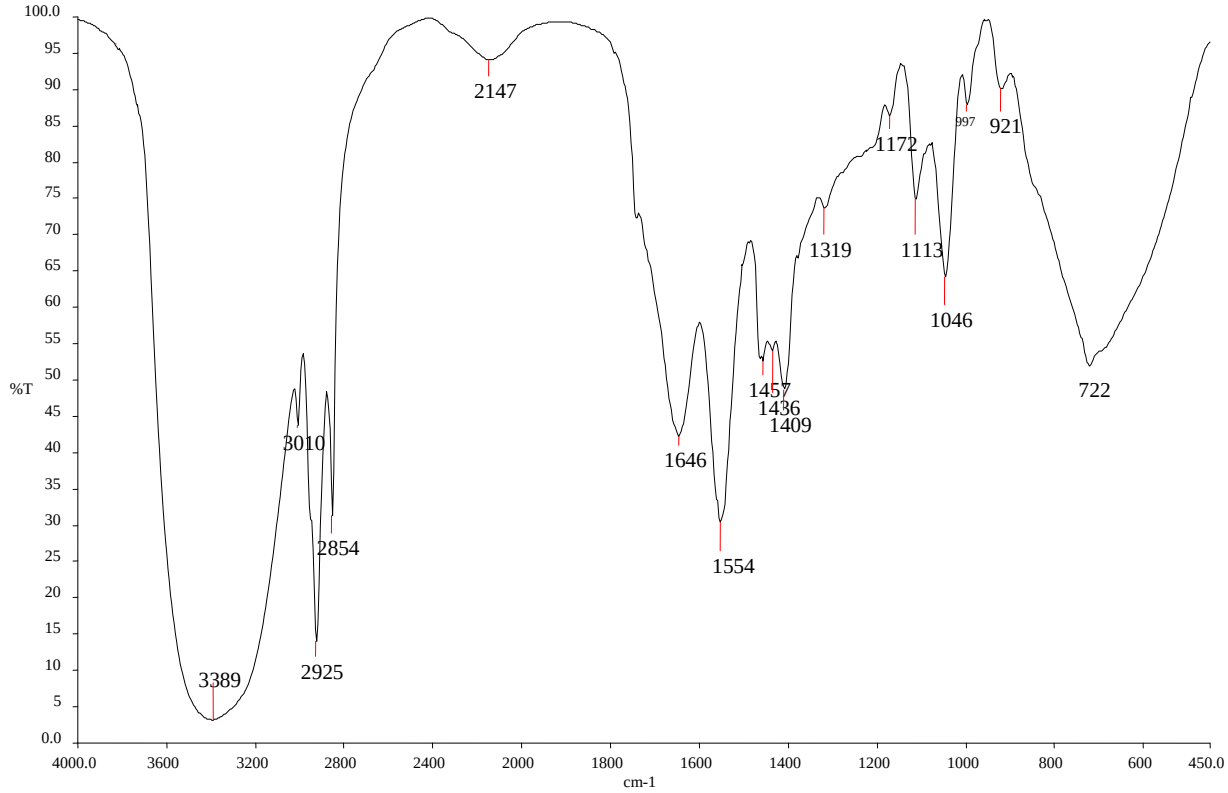

Fig. IV Biodiesel from algae

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