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# Biofuel Production from Microalgae for Energy Applications

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## Abstract

In today's scenario, algae are widely documented as a promising source of renewable energy. This is because of having many advantages like high growth rates, high oil content and ability to grow on waste water. Microalgae, like *Chlorella* is known to exceedingly rich in oil. In our present study, *Chlorella* was grown autotrophically in BG-11 medium as a batch culture. After proper incubation of algae, harvesting, drying and extraction of lipid were carried out with the help of standard procedure i.e. Bligh & Dyer's method. Extracted lipid was 16.66% and it was further converted into biodiesel with the help of base catalyzed trans-esterification reaction. Produced biofuel was characterized by FTIR and GC–MS techniques. Analysis of FTIR spectrum revealed that esters were produced and hence confirming the production of biodiesel. Composition of biofuel was further analyzed by gas chromatography-mass spectroscopy (GC-MS). GC-MS results revealed that Palmitic acid methyl ester ($C_{17}H_{34}O_2$), 9-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$) and Linoleic acid methyl ester ($C_{19}H_{34}O_2$) were major contents of this biofuel.

**Key words**: Bio-fuel, Microalgae, FTIR, GC-MS, Chlorella and Lipid.

## Introduction

Global atmospheric concentrations of carbon dioxide, methane and nitrous oxide have increased markedly due to high production and consumption rate of fossil fuel. Energy shortage, pollution and global warming issues force us to look an alternative and environment friendly fuel i.e. Biofuel. Biofuel is a fuel that is derived from plant or animal lipids. Haas et al. explained that using biodiesel instead of conventional petro-diesel can reduce emissions of total hydrocarbons, particulates and carbon monoxide up to 55%, 53%, and 48% respectively (Hass et. al, 2001). Other advantage of biodiesel is rapid biodegradability over conventional diesel (Zhang et al., 1998). Forty times higher oil production from algae compared to terrestrial oilseed crops such as soy and canola has been reported under controlled conditions (Brown et. al., 1990; Sheehan et al., 1998). Microalgae based fuel is renewable and environment friendly (Mata *et al*., 2010). Although biodiesel production from macroalgae like Oedogonium and Spirogyra (Shariff Hossain et al., 2008) has been reported but today's mostly research is focussed on microalgae based fuel because of their high lipid content. The most extensive research into the development of biofuels from algae was performed by the National Renewable Energy Laboratory (NREL) from 1978 to 1996 (Sheehan et al. 1998). Algal culturing in photobioreactors (Chen et. al., 2011) and their genetic engineering are tools for economical biodiesel production from microalgae (Chisti, 2007).

Extraction of lipid from algae can be done through various solvent extraction methods like chloroform/methanol (Bligh and Dyer, 1959), hexane/isopropanol (Gunnlaugsdottir & Ackman, 1993), hexane/ethanol (Fajardo et al., 2007). Bligh and Dyer method was best reported for extraction of lipid from *Nannochloropsis* microalgae (Long and Abdelkader, 2011). Produced biodiesel through transesterification from *Nannochloropsis* was characterized through ATR-FTIR and GC–MS (Prafulla D. Patil et.al, 2011). Mojaat *et al*. explained the importance of proper algal strain and their lipid profile for higher oil production (Mojaat et al., 2008)

*Chlorella* sp. is a green microalga and can grow photoautotrophically in BG-11 medium. Higher lipid productivity under autotrophic condition of algae shows its potential for bioenergy applications. The present study reports the oil extraction from the algae and their conversion into fatty acid methyl esters (FAME) which is requisite biodiesel by simple chemical process known as "transesterification". Produced biodiesel was characterized by GC-MS and FTIR techniques. To our knowledge, there is little data available on FAME analysis of produced biodiesel from the algae but it is mandatory to design the economical path for biofuel production.

## Materials and Methods:

### Organism and growth conditions:

The culture of green alga *Chlorella sp.* was procured from Indian Agricultural Research Institute (IARI), New Delhi, India. Microscopic analysis of culture was done through scanning electron microscope (SEM) as shown in Fig1. The cultures were grown in 5 litres Erlenmeyer flasks with 3 litres BG-11 medium at $30^0$C temperature in the photo-incubator. Cultures were

grown under 3–4 Klux intensity of fluorescent white light at 16 h/8 h alternate cycles of light and dark. The cultures were hand shaken three to four times daily to avoid sticking. Glass wares and media were always sterilized prior to inoculation. Experiments were carried out in triplicates.

**Cell growth analysis:**

Optical density measurement at 660 nm was used to monitor cell growth by UV/visible spectrophotometer (Labomed UVS-2700). Initial culture's OD was 0.1. When the algal cells attained a good proliferation and optimum growth rate then they were subject for further experiments. Algal cells were harvested by centrifugation at 10,000 rpm for 10 minutes and the pellet was then washed twice with distilled water to remove any debris and salts and dried at 80°C to determine the cell dry weight (expressed as g/l).Biomass concentration and biomass productivity were calculated according to the formula:

Biomass concentration ($g/m^3$) = mass of culture/volume

Biomass productivity ($g/m^3.d$) = mass of the culture/volume x days

**Lipid extraction procedure:**

Bligh & Dyer method was used for extraction of lipids from microalgae culture **(**Bligh and Dyer, 1959). The cells were harvested by centrifugation at 10,000 rpm for 10 min. Re-centrifugation of cells with distilled water was done to remove salts. The centrifuged cultures were further subject of wet weight estimation and then dried in oven at 80°C. Methanol and chloroform were added to the dried algal powder in 2: 1 ratio and left it on room temperature for 18 hrs. After 18 hrs, centrifuged the chemical treated culture and recovered the solvent phase. The pellet was again treated with the same chemicals as mentioned above for second extraction. The supernatants were pooled; chloroform was added and then mixed properly. Distilled water was added for phase separation. Organic layer with the lipids was transferred to a clean pre-weighed tube (W1). Evaporation of solvents in the sample was carried out in rotary evaporator. The dried lipid extract was weighed (W2) and lipid content and lipid productivity was calculated as:-

Lipid content (%) = Mass of lipid (in grams) X 100/ Mass of algae culture (in grams)

Lipid production (grams/$m^3$) = Mass of lipid (in grams) / Volume ($m^3$)

**Fatty Acid Methyl Ester (FAME) formation:**

Trans-esterification process for fatty acid methyl ester formation can be carried out in a number of ways by using different catalyst i.e. alkali catalyst, acid catalyst and biocatalyst (Fjerbaek et. al. 2009, Lin et. al. 2009). But conversion speed of oil into FAME was higher in case of base-catalyzed transesterification (Freedman et al., 1986). In our study extracted algal lipid was also esterify through base catalyzed transesterification reaction. We had taken 2ml Methanol and added slowly 0.035 g Sodium hydroxide into the glass blender pitcher while blender was set on its lowest setting point. Before pouring 10 ml extracted oil into glass blender mixture the sodium hydroxide should be completely dissolved. This blending (on low speed) should continue for at least 30 minutes. After completion of this reaction, the mixture was transferred into sterile container. After 2-3 hrs the mixture was separated out into two layers. The top and bottom layers were of biodiesel and glycerine. Further biodiesel was subject for multiple washing with distilled water for removing remaining traces of alcohol, catalyst and glycerol (A. Abbaszadeh et.al, 2014). After 2 to 3 washing of FAME with distilled water, organic yellowish layer was collected and dried in rotary evaporator. The methyl esters were then dissolved in hexane for gas chromatography-mass spectroscopy (GC-MS) analysis.

**FAME analysis by GC-MS**

Fatty acid methyl ester (FAME) composition was determined using GC-MS. The column oven temp was set on 140.0 ° C and the injection temperature was 260 ° C. The sampling time was 1.0 min. Column flow was set on 1.21 ml/min. Fatty acid methyl esters (FAMEs) were identified by comparison of the retention times with the standard of FAME (Sigma-Aldrich Co.)

**FTIR analysis**

Fourier transforms infrared spectrometer based on OPUS software (Bruker Co.) was used to analyze the samples. The samples were analyzed in transmission mode in 400-4000 $cm^{-1}$wave number range.

**Results and discussion:**

For healthy cells, autotrophic *Chlorella* sp. was observed under the scanning electron microscope. After 45 days of incubation, the cell cultures were centrifuged and dried. Biomass productivity and lipid productivity were 0.3 g/l and 0.05 g/l respectively (on the basis of cell dry weight). The experimentally calculated lipid content in form of percentage for this alga was 16.66% which had shown similarity with existing record for lipid content for this alga in the literature i.e. 14-50% (Li P et.al.2011, Francisco et.al.2010, Nigam et.al.2011, Ogawa et.al 1981, Liang et.al 2009, Chen et.al. 2011, Wang et. al. 2012). Extracted oil was converted into biodiesel or fatty acid methyl ester (FAME) through the base catalyzed trans-esterification

reactions and further analyzed through the gas chromatography- mass spectroscopy. The obtained results were shown below in the Table1.

**Table1:** Fatty acid methyl ester analysis of Chlorella sp. grown under autotrophic conditions.
FAME composition was calculated on the basis of specific fatty acid percentage over the total fatty acid of lipids of each sample.

| S. No. | % Area | Fatty Acid Methyl Ester (FAME) |
|---|---|---|
| 1 | 14.71 | Palmitic Acid Methyl Ester ($C_{17}H_{34}O_2$) |
| 2 | 0.83 | 1,2-Benzenedicarboxylic Acid, Diethyl Ester ($C_{12}H_{14}O_4$ ) |
| 3 | 64.08 | 9-Octadecenoic Acid, Methyl Ester ($C_{19}H_{36}O_2$ ) |
| 4 | 8.33 | Linoleic Acid, Methyl Ester ($C_{19}H_{34}O_2$) |
| 5 | 1.28 | 8,11-Octadecadienoic Acid, Methyl Ester ($C_{19}H_{34}O_2$ ) |
| 6 | 6.13 | 3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol ($C_{20}H_{40}O$ ) |
| 7 | 1.02 | 9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester ($C_{19}H_{34}O_2$ ) |
| 8 | 2.07 | Ricinoleic Acid Methyl Ester ($C_{19}H_{36}O_3$ ) |
| 9 | 1.55 | 9-Octadecenoic Acid (Z)-, 2,3-Dihydroxypropyl Ester ($C_{21}H_{40}O_4$) |
| | | |

The GC-MS results have shown that Palmitic acid, Oleic acid and Linoleic acid were major content of algal oil. The FAME (%) identified for *Chlorella* sp. under autotrophic condition was palmitic acid (C-16:0, 14.71%), 9-Octadecenoic acid (C-18:1) and linoleic acid (C-18:2, 8.33%). According to Knothe, the most desirable fatty acids for biodiesel quality were C-16:0 and C-18:1( Knothe et. al., 2008) and these were major contents in produced biodiesel as shown in Table1. Similar kind of FAME profile has been reported for *C. pyrenoidosa* under autotrophic condition was palmitic acid (14.3%) and linoleic acid (8.7%) (Monika et.al, 2013) A FAME profile of 25% palmitic acid methyl ester and 12% oleic acid methyl esters were also reported for autotrophic *Chlorella vulgaris* (Gouveia et. al, 2009). Less work has been done on the FAME profiling of microalgae. The study of lipid profile of various microalgae is mandatory for reduction of green house gases, because microalgae based biodiesel contain less sulphur or nitrogen compare to conventional diesel. Algal lipid profile study is also necessary to get rid off from escalating price of petrol or petroleum based products because it provides better solution for them. This is the reason, why we had made an attempt to study the FAME contents under autotrophic conditions for *Chlorella* sp. FAME profiles study of *Chlorella* sp., represent the species as a potential energy source for future energy needs applications. Transesterified oil was further analyzed through FTIR as shown in Fig2.

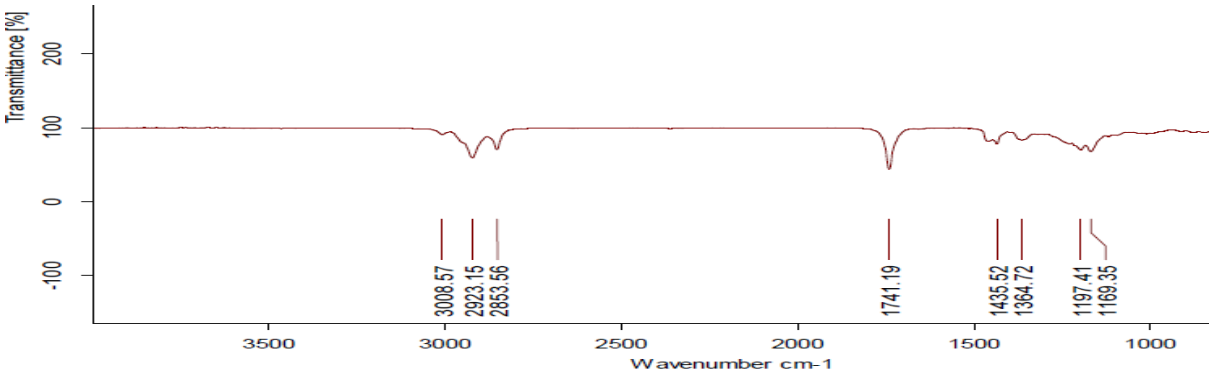


**Fig2: FTIR spectrum of biodiesel**

The IR spectra were obtained using a FT-IR spectrometer of Bruker Company. Biodiesel is mainly mono-alkyl ester, the intense C=O stretching band of methyl ester appears at 1741 $cm^{-1}$ for algal biodiesel which is generally absent in petro-diesel spectra (Prafulla et.al, 2011). The position of the carbonyl band in FTIR is sensitive to substituent effects and to the structure

of the molecule (Pasto et al. 1992). The peak at 1197 cm-1 was attributed to the stretching vibrations of ester bond and the peak observed at 1169 $cm^{-1}$ was attributed to methyl groups near carbonyl groups (Roeges, 1994).

**Conclusion:**

This study provides an effective approach of extraction of lipid from autotrophic *Chlorella* sp. and making it a potential source for biodiesel production. The most desirable fatty acids in biodiesel for quality were C-16:0 and C-18:1 and these were major contents of produced biodiesel. These facts were confirmed through GC-MS and FTIR. Produced biodiesel have broad spectrum of various bio-energy applications in various fields like: automobile, industrial, agricultural and medical fields.

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