# Production of FAME from freshwater microalgae and profiling of fatty acids for biodiesel feedstock

**Ilavarasi, A., MubarakAli, D., Parveez Ahamed, A and N. Thajuddin***

Department of Microbiology, School of Life Sciences, Bharathidasan University,

Tiruchirappalli – 620024, Tamil Nadu, India.

*Corresponding author: E. mail: nthaju2002@yahoo.com

**Abstract**

Due to diminishing fossil fuel reserves, it is necessary to shift for an alternative fuel source that could be sustainable and eco-friendly. Microalgae produce different ratios of lipids, proteins and carbohydrates. The lipid composition of microalgae is similar to vegetable oil which has already been used as biodiesel so that it can potentially be employed for biodiesel production. In present study has demonstrated the total lipid content, fatty acid profile and biodiesel production from a naturally isolated fresh water strain of *Chlorella* sp. and the total lipid content was found to be 8% under normal nutrient conditions. Gas Chromatography of FAME was analyzed, it was found to be the major fatty acids were palmitic acid, stearic acid, oleic acid, linoleic acid and alpha linolenic acid and pH of the biodiesel was found to be 8.1.

***Keywords***: Microalgae, *Chlorella* sp., BG-11, Lipid, FAME, GC, Biodiesel.

***Introduction*:**

Robust population explosion, receding fossil fuel reserve, enhanced GHG, larger emission of flue gases etc have brought the world scientists and biotechnologists to the threshold of exploring alternative energy sources especially biodiesel. Biodiesel is characterized as a renewable, biodegradable and eco-friendly fuel which has attracted wide attention. Most recently, research effort has been aimed at identifying suitable biomass species which can provide high energy outputs to replace the fossil fuels. Many of the researchers have attempted to produce biodiesel from non-edible sources like frying oil, greases, tallow, jatropha, mahua and soy bean oils. Besides these non-edible sources we can use microalgae as feed stock for biodiesel.

Microalgae are oxygenic photosynthetic organisms which inhabit almost all moist and lighted environments. They are veritable miniature biochemical factories, appeared to be more photosynthetically efficient than terrestrial plants and are efficient $CO_2$ fixers. Many of them are exceedingly rich in oil, fast growing and so they are used in biodiesel production. They have the ability to replace the fossil fuel (Chisti, 2007). Even, there is a pressing need to improve the biodiesel production from microalgae and that too on selection of suitable biomass species. The nutrients deprivations would be influence on the lipid accumulation in a dominant indigenenous microalga, *Chlorella* sp., BUM11008 for biodiesel production (Praveenkumar *et al.,* 2012). In the present study, fresh water microalga, *Chlorella* sp. NTAI01 was isolated and identified. The total lipid was characterized both quantitatively and qualitatively in biofuels aspects.

***Materials and Methods:***

***Samples*:**

The microalgal samples were collected from stagnant fresh water body in Bharathidasan University campus, Tiruchirappalli, India.

***Isolation, identification and growth condition of microalgae:***

The collected samples were enriched initially in BG -11broth in conical flasks (250ml) at 24±2°C under 37.5$\mu mol^{-1}m^2s^{-1}$ intensity with 16:8 hours photoperiod for 10 days. Then the enriched culture samples were spread on BG – 11 agar plates and incubated at the above said conditions (Ilavarasi *et al*., 2011). After incubation, individual colonies were picked and transferred to the same media for purification in 250ml conical flask. The culture broth was shaken manually for five to six times a day. The purity of the culture was monitored by regular observation under microscope. The isolated microalgae were identified microscopically using light microscope with standard manual for algae (Shashikant and Gupta, 1998).

***Measurement of growth:***

The growth of the algal biomass was assessed by means of optical density with five days intervals from 5$^{th}$

day upto 25th day at 540nm (Sachez Miron, 2003) using UV-Vis spectrophotometer (OPTIZEN 3220).

***Mass cultivation:***

*Chlorella* sp. was mass cultured in 1000ml conical flasks for further experiments.

***Harvesting the biomass:***

The *Chlorella* sp. was harvested in their stationary growth phase by centrifugation at 4000 rpm for 15 minutes. The harvested biomass was lyophilized and weighed before lipid extraction.

***Lipid extraction and preparation of Fatty Acid Methyl Ester (FAME):***

To extract the lipid, 0.5g of culture (dried cells) was initially ground in mortar and pestle followed by chloroform: methanol extraction (Folch *et al*., 1957) for the control cultures at their stationary growth phase. Meanwhile, the cell residues were observed under microscope for inclusive destruction to ensure efficient lipid extraction. The extracted lipid was dried in a rotary evaporator, weighed and stored for FAME preparation. The total lipid content was estimated using the following formulae:

$$Total\ lipid\ content\ (\%) = \frac{Dry\ Cell\ Weight}{Lipid\ Weight} X100$$

The entire process of FAME preparation was done following the protocol of Rasoul-Amini *et al*., 2009. Then, the oily substance was further subjected to Gas Chromatography analysis.

***Gas Chromatography (GC) analysis:***

The fatty acid compositions were determined by Gas Chromatography (GC) analysis. The oily substance obtained was analyzed by Shimadzu GC 2014 (Japan), equipped with FAME WAX column (RESTEK column, USA). The column details were 30m x 0.3mm ID x0.25µm thickness. The injection port temperature was 250℃. Here, Flame Ionization Detector (FID) was used with temperature of 260℃. Nitrogen was used as a carrier gas kept at a constant rate of 22.2 ml/min. Fatty acid composition was calculated as percentage of the total fatty acids present in the sample determined from the peak areas.

***Results:***

Microalgae offer potential source for sufficient production of renewable fuels to impact consumption of fossil fuels. In our present investigation, totally seven microalgal strains were isolated. The morphology of all the isolates was studied under bright field microscope. Out of that, *Chlorella* sp. NTAI01 was further exploited as it exhibits higher growth rate than the rest of the isolates. The isolated strain was measuring about 7µm in diameter, spherical in shape, without flagella, single-celled green algae, belonging to the phylum Chlorophyta and it was tentatively identified as *Chlorella* sp Fig: 1.

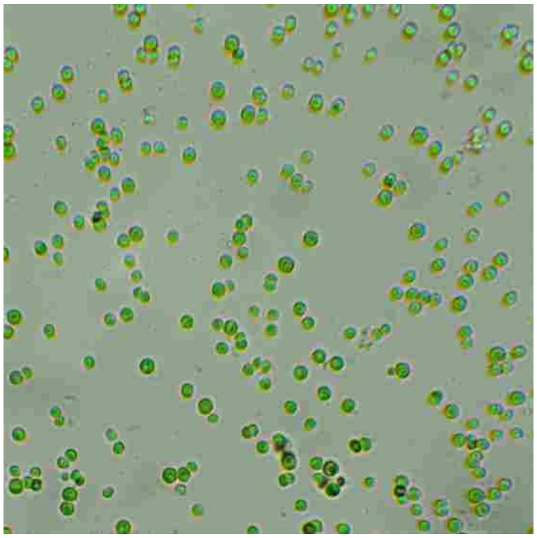


**Fig: 1. Microphotograph of *Chlorella* sp.**

Moreover, the growth rate was deliberated based on the optical density (OD) measurement and the experiment lasted for 25 days. It was found that the growth of the microalgae (*Chlorella* sp. NTAI01) has been slowly increased from 5th day till 25th day. The average increase in OD value has indicated the notable growth of *Chlorella* sp. which has been depicted in Fig:2.

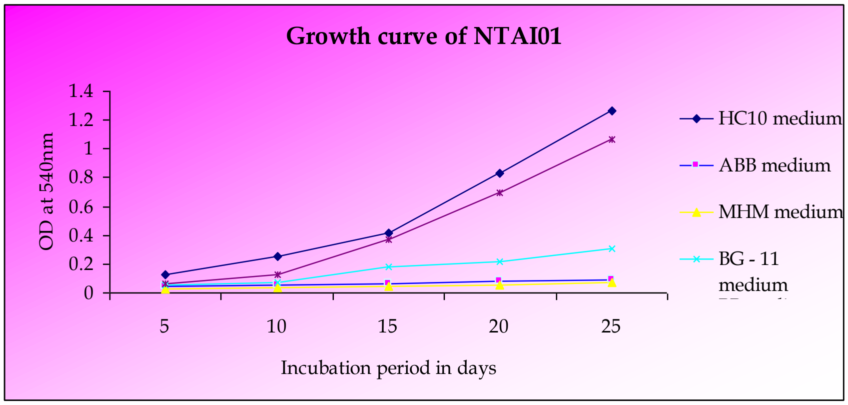
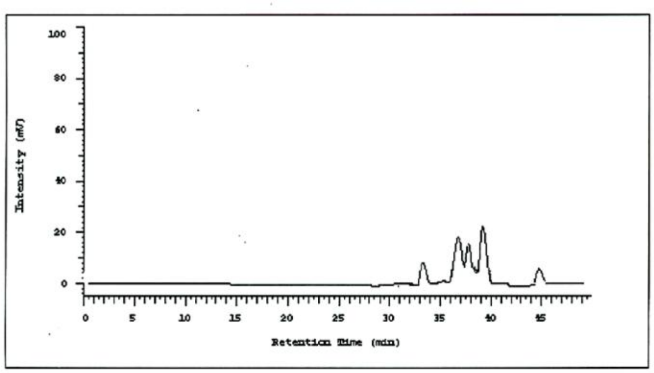



**Fig:2. Growth curve of *Chlorella* sp. NTAI01**

The fresh water isolate *Chlorella* sp.NTAI01 was harvested and lyophilized for further analysis. It exhibited total lipid content of about 8% to their dry cell weight. The Gas Chromatographic analysis revealed the fatty acid profile of *Chlorella* sp. NTAI01. The major fatty acids were found to be palmitic acid, stearic acid, oleic acid, linoleic acid and alpha linolenic acid (Fig: 3). Furthermore, the pH of the transesterified oil was found to be 8.1 which was similar to that of existing biodiesel.




| No. | COMPONENT NAME | CARBON | RT | AREA |
|---|---|---|---|---|
| 1 | PALMITIC ACID | C 16 | 30.6 | 512.4 |
| 2 | STEARIC ACID | C 18 | 33.7 | 125.600 |
| 3 | OLEIC ACID | C 18 1 | 35.7 | 320.700 |
| 4 | LINOLEIC ACID | C 18 2 | 37.4 | 465.600 |
| 5 | ALPHA LINOLENIC ACID | C 18 3 | 40.4 | 1376.700 |
| 6 | BEHENIC ACID | C 22 : 0 | 46.2 | 117.6 |

**Fig: 3. Chromatogram of detected fatty acids of *Chlorella* sp. NTAI01**

***Discussion:***

In recent years, microalgae have garnered interest for producing valuable molecules ranging from therapeutic proteins to biofuels. They are considered to be the potential source of a number of biofuels (Pirt, 1986; Wolf *et al*., 1985; Oswald, 1988 and Benemann, 1997) and because of their small size and dry powdered microalgae such as *C. vulgaris* could be used as a fuel supplement in a diesel engine. The growth efficiency of *Chlorella* sp. NTAI01 was higher when compared with rest of the isolates and it was supported by previous studies on growth of microalgae (Chisti, 2007).

In fuel point of view, total lipid content of *Chlorella* sp. NTAI01 was estimated from the dried algal cells. The drying temperature during lipid extraction from algal biomass was found to affect not only the lipid composition but also the lipid content. It was reported that drying at higher temperature decreased the content of tricacylglyceride. In order to avoid that trouble, here lyophilization was carried out to obtain dried cells (Widjaja *et al*., 2008). The total amount of lipid was found to be 8% of their dry cell weight. The total lipid content was found to vary from species to species. It also depends on the environmental condition at which the isolate survives. The ability of algae to survive or proliferate over a wide range of environmental conditions, to a large extent, reflected in the tremendous diversity and sometimes unusual pattern of cellular lipids as well as the ability to modify lipid metabolism efficiently in response to changes in environmental conditions (Guschina and Harwood, 2006; Thompson, 1996; Wada and Murata, 1998). In the present investigation, the lipid content was about 8% under normal nutrient conditions, which may be increased when these strain exposed to stress conditions.

In the aspect of biodiesel, the fatty acid profile is considered to be important as that of the total fatty acid content. *Chlorella* sp. (NTAI01) showed 18:3 (alpha linolenic acid) as the major fatty acid followed by 18:2 (linoleic acid), 18:4 (moroctic acid), 18:1 (oleic acid), 16:0 (palmitic acid). It was previously reported that palmitic acid, stearic acid and linolenic acid were recognized as the most common fatty acids contained in biodiesel (Knothe,2008). The fatty acid profile mainly composed of unsaturated fatty acids and also significant percentage of palmitic acid (16:0) was also present. The fatty acid profile of *Chlorella* sp. NTAI01 complies with the existing biodiesel standard.

***Conclusion:***

The present work investigated the efficiency of *Chlorella* sp. NTAI01 as source of biodiesel with respect to total lipid content and lipid profile. The test strain showed moderate lipid content with promising lipid profile. The lipid content of *Chlorella* sp. NTAI01 was found to be 8% under normal nutrient conditions. Moreover, these unicellular microalgae are fast growing strain as well as they are already proved to be suited for genetic manipulation and high-throughput analysis. Thus, the fresh water *Chlorella* sp. NTAI01can effectively exploited as a feedstock for renewable energy.

***Acknowledgement*:**

The first authors is grateful to Department of Science and Technology (DST) for INSPIRE fellowship and all the authors are thankful to Department of Biotechnology (DBT), New Delhi (BT/PR/11316/PBD/26/164/2008) for their financial assistance.

***References***

Benemann, J.R. 1997. Feasibility analysis of photobiological hydrogen production. *Int J Hydrogen Energy*, **22:** 979–987.

Chisti, Y. 2007. Biodiesel from microalgae. *Biotechnol Adv*. **25**: 294-306.

Folch, J, M. Lees, G.H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. 226: 497-509.

Guschina I.A and J.L. Harwood. 2006. Lipids and lipid metabolism in eukaryotic algae, *Prog. Lipid Res.* **45**: 160-186.

Hirano, A, K. Hon-Nami, S. Kunito, M. Hada and Y. Ogushi. 1998. Temperature effect on continuous gasification yield of methanol production and its energy balance. *Catalysis Today*, **45 :**399–404.

Ilavarasi, A., Mubarak Ali, D., Praveen Kumar, R., Baldev, E and N. Thajuddin. 2011. Optimization of various growth media to freshwater microalgae for biomass production. *Biotechnology* 10 (6) 360-365.

Knothe, G. 2008. "Designer" biodiesel: optimizing fatty ester composition to improve fuel properties. *Energy fuel*. **22**: 1358-1364.

Mirón, A.S, M.C.C. García , A.C. Gómez, F.G. Camacho, E.M. Grima and Y. Chisti. 2003. Shear stress tolerance and biochemical characterization of *Phaeodactylum* *tricornutum* in quasi steady-state continuous culture in outdoor photobioreactors. *Biochemical Engineering Journal* 16-3: 287-297.

Oswald, W.J. 1988. Microalgal biotechnology (Borowitzka MA, Borowitzka LJ, editors). Cambridge University Press, 357–95.

Pirt, S.J. 1986. The thermodynamic efficiency (quantum demand) and dynamics of photosynthetic growth. *New Phytol*, **102:**3–37.

Praveenkumar, R., Sameera. K, Mahalakshmi.G, Akbersha, M.A.A and N. Thajuddin. 2012. Influence of nutrient deprivations on lipid accumulation in a dominant indigenous microalga, Chlorella sp., BUM11008: evalution for biodiesel production. Biomass and Bioenergy. 37: 60-66.

Rasoul-Amini, S, Y. Ghasemi, M.H. Morowvat and A. Mohagheghzadeh. 2009. PCR amplification of 18S rRNA, Single Cell Protein production and fatty acid evaluation of some naturally isolated microalgae. *Food Chemistry* 116 (1):129–136.

Shashikant, Puspha Gupta. Algal flora of Ladakh. 1998 Scientific publishers, Jodhpur. India.

Thompson, G.A. 1996. Lipids and membrane function in green algae. *Biochim. Biophys*. *Acta*, **1302**: 17-45.

Widjaja, A, C.C. Chien and Y.H. Ju. 2009. Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. *Journal of the Taiwan Institute of Chemical Engineers*. **40**: 13-20.

Wolf. F.R, A.M Nonomura and J.A Bassham. 1985. Growth and branched hydrocarbon production in a strain of *Botryococcus braunii* (Chlorophyta). *J Phycology*, **21:**388–396.