

Biodiesel Synthesis from Modern Energy Fuel Crop Green Algae *Chlorococcum humicola* and Its Chemical Parameters

Sonawane Swati¹, Dalvi Sanjaykumar² and Pokharkar Raghunath¹

¹ Department of Chemistry, S. N. Arts, D. J. M. Commerce & B. N. S. Science College, Sangamner, Dist. Ahmednagar, Pin- 422605, India

² Department of Physics, S. N. Arts, D. J. M. Commerce & B. N. S. Science College, Sangamner, Dist. Ahmednagar, Pin- 422605, India

Abstract- The present paper study is on fresh water microalgae from region of Ahmednagar district in Maharashtra. Fresh water microalgae are modern biomass for the production of biodiesel fuel due its faster growth, highest biomass productivity and high lipid content with various conversion methods into biofuel. The biodiesel has very mimic property like petroleum diesel. The study emphasis on fresh water algae strain in the class of Chlorophyceae and Chlorococcum *humicola* species isolated and convert it into biodiesel by base catalysed transesterification method. The chemical properties of product analyze by standard method Infrared spectroscopy and Gas Chromatography Mass Spectroscopy.

Index Terms- Biodiesel, Chlorococcum *humicola* , Infrared spectroscopy, GCMS.

I. INTRODUCTION

Energy demand worldwide continues to increase at a rapid pace with the negative environmental impacts of burning of fossil fuel energy which has been drawn significant attention to renewable liquid fuels as a way to replace petroleum based fuels [12]. Biodiesel is common term for long chain of alkyl ester, is renewable, biodegradable and non-toxic biofuel that shows great promise. Biodiesel is obtained by transesterification of mono-, di- and triglycerides of naturally occurring biological lipid such as plant oils and an animal fat [16-17]. Biodiesel has a vital potential to be a carbon neutral fuel after combustion it produce low levels of environmental pollutants like sulfur compounds, particulate matter and volatile organic compounds [7][22][26]. Recent study has reported that an increase in production of biofuels on arable land lead to deforestation which releases more CO₂ in the atmosphere [6][23]. The increase in production of biofuel from traditional crop is not enough land to meet the demand for liquid fuel [2]. The cost of biofuel production is high due high price of refined oils, which make 80% of the cost of production [5][9-10]. Hence, it is necessary to find the new renewable energy resources for lipid with fast productivity and minimum farmland use, which make economically feasible bio-liquid.

Microalgae are modern biomass containing lipid, starch, cellulose, protein hence it producing variety of renewable fuel such as biodiesel, bioethanol and biohydrogen [3]. The higher potential of lipid productivity was attaining the microalgae than the land crop; microalgae are feasible for synthesis of long chain

alkyl ester liquid fuel [2]. The growth of microalgae and lipid productivity is depend on growth medium composition e.g. types of carbon source, vitamins, salts and nutrients; physical parameters such as pH, temperature and light intensity and type of metabolism like phototropic, heterotrophic and mixotropic growth [18]. Microalge produce biomass at a rate 50 times faster than growing terrestrial land plant [15]. The microalgae produce high lipid content about 1-85% lipid by dry weight biomass [2][21][24-25] Freshwater microalgae strains in the class of Chlorophyceae have been isolated, most which are having fast growth rate and higher lipid content [11][14][20]. Hence, microalgae's high lipid content and growth rate would make to possible satisfy demand of liquid fuel.

In this present study, the isolation and identification of microalgae species, medium and culture cultivation condition, algae growth study, transesterification reaction, the Infrared Spectroscopy and Gas Chromatography Mass Spectroscopy analysis of components of product.

II. MATERIAL AND METHOD

2.1 Isolation and Identification of Microalgae

Microalgae were collected by plankton net (20 µm pore size) from Nizarneshwar, Ahmednagar (Maharashtra), India. The microalgae sample were collected in clean plastic container from sampling location and labeled. The sample was immediately brought for chemical and algal studies. The water sample was observed on the spot in natural condition. Part of sample was preserved in 4% formalin solution and Lugol's solution for detail study in laboratory The morphology of pure strains was regularly examined under an optical microscope and identified with the help of standard literature and monographs: Fritsch (1935), Smith (1950), Prescott (1951), Desikachachary (1959), Iyengar (1940), Sarode and Kamat (1984), Pal (1990), Philipose (1967).

2.2 Culture media and culture condition

The Bold basal medium used for culture of Chlorococcum *humicola* was bold basal medium [13] that consist of (g/l) NaNO₃ (0.75), K₂HPO₄. 3 H₂O (0.075), K H₂PO₄ (0.175), CaCl₂ .H₂O (0.025), MgSO₄.7H₂O (0.075), NaCl (0.025), EDTA (0.000045), FeCl₃.6 H₂O (0.000582), MnCl₂.4 H₂O (0.000246), ZnCl₂.6 H₂O (0.00003), CoCl₂.6 H₂O (0.000012), Na₂Mo₂ H₂O (0.000024). Microalgae samples of about 5 ml were inoculated into 5-ml autoclaved BBM medium in 20-ml test tubes and cultured at room temperature (27 °C) for 2 wk with

cool white fluorescent light. The light intensity was approximately 40 μmol photons/m²/s and the diurnal cycle was 12 h dark/12 h light. The pre-cultured samples were streaked on BBM medium-enriched agar plates and cultured for another 1– 2 wk with cool white fluorescent light using the same light intensity. The single colonies on agar were picked up and cultured in liquid BBM medium, and the streaking and inoculation procedure was repeated until pure cultures were obtained. The morphology of pure strains was regularly examined under an optical microscope.

2.3 Biomass measurement and Lipid content

The growth curves of the *Chlorococcum humicola* strain in BBM media was study by measuring the OD of samples at 680 nm using a UV–VIS spectrophotometer. The microalgae cells were harvested in the stationary phase by centrifugation at 5000 rpm for 5 min and the cells were washed twice using distilled water. The cell pellets were dried at 60°C for 2 days and placed in desiccators until constant weight. Dry weight of cells was obtained using an analytical balance.

The lipid content of microalgae was extracted by using the Bligh and Dyer method [1]. After cell drying, algal powder was mix with chloroform–methanol (1:2) solvent for 30 min. Algal solid was removed by centrifugation at 5000 rpm for 5 min. The residual solid and lipid separated by solvent extraction procedure.

2.4 Biodiesel synthesis and fatty acid analysis

The base catalyzed transesterification reaction carried out for fatty acid alkyl ester synthesis. The reaction is carried in a round bottom flask. The fine microalgae dry powder mixed with methanol then potassium hydroxide in methanol added. The reaction mixture was continuously stirring. The reaction carried out at 60°C for 60 minutes. At room temperature, the algal remnant solid cake with glycerol content & mother liquor were separated by vacuum filtration. Solvent was separated by a rotary evaporator. The biodiesel phase was wash with distilled water to remove water soluble impurities and contaminants by giving 2-3 time water wash to product with heating 85°C. It was preserved in airtight container and used for further analysis [4]. The fatty acid methyl esters were analyzed by standard method Infrared spectroscopy and gas chromatography mass spectroscopy.

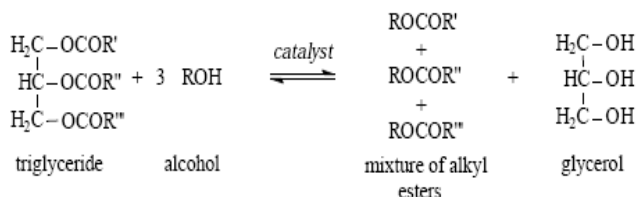


Figure 1: Transesterification of microalgae lipid

III. RESULT AND DISCUSSION

1.1 Characterization of isolated strain

The isolated microalgae strain belonging in the division of Chlorophyta, class of Chlorophyceae, family of Chlorococcaceae, order of Chlorococcales and genus of Chlorococcum.

Under the optical microscope, *C. humicola* (Figure.2) is unicellular with spherical cells from 8 to 20 μm in diameter. Cells form dark green colonies and irregular lumps with mucilaginous outer cover.



Figure 2: Photo of Chlorococcum humicola

1.2 Biomass growth and lipid yield

The growth cycle of *C. humicola* in BBM is shown in Figure 3. Cells in BBM medium 1 day lag period and reached the exponential phase within 3-6 days. By approximately 12-15 day cells reached the stationary phase, after 15 days optical density increases. Lipid content was reported as percentage of lipid to biomass dry weight. Lipid content of *C. humicola* strain in BBM medium was found 43.86%. Lipid productivity is expressed as mass of lipid produced per unit volume of microalgal broth per day. The lipid yield in *C. humicola* was 0.029g/l/d achieved.

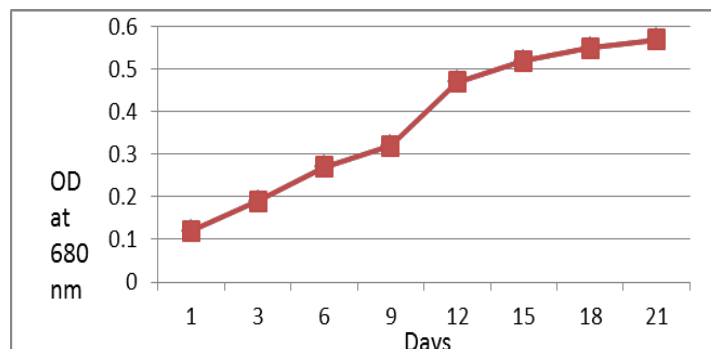


Figure 3: Growth cycle of Chlorococcum humicola in BBM media

3.3 Infrared Spectroscopy analysis

The infrared spectroscopy is standard method to study the functional group of product obtained. The IR Spectrum Chlorococcum humicola biodiesel shows in Figure 4. The vibrational stretching and bending frequency of biodiesel are shown in Table 1.

Band assignment (cm ⁻¹)	Functional groups
~ 2924.00	ν_{as} (sp ³ C-H) Stretching of ester group
~ 1745.66	ν (C=O) Stretching of ester group
~ 1459.96	δ_s (CH ₂) bending of methylene group
~ 1375.90	δ_s (CH ₃) bending of methyl group
~ 1164.49	ν (C-O) stretching of alkyl carbon and oxygen
~ 725.94	δ_r (CH ₂) bending of methylene group

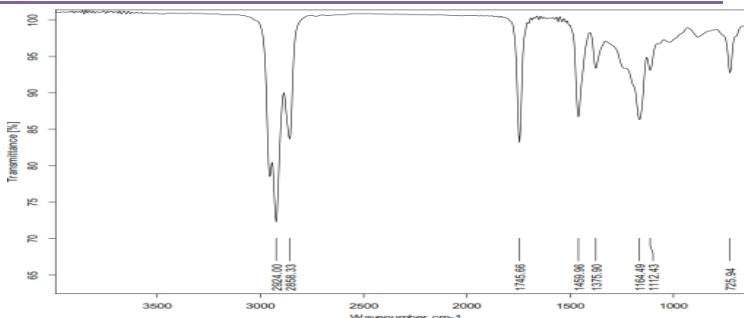


Figure 4: IR spectrum of Chlorococcum humicola Biodiesel
Table 1: FTIR band assignments for Chlorococcum humicola microalgae fatty acid methyl ester

The functional group bond absorption 2924.00 cm⁻¹ presence of hydrocarbon sp³ (C-H). The bond absorption 1745.66

cm⁻¹ presence of ester carbonyl (C=O) group and at 1459.96 cm⁻¹ absorption frequency shows the presence of methylene (CH₂) group. The bond absorption 1164.49 - 1375.90 cm⁻¹ show the presence of ester alkyl carbon oxygen (C-O) bond and methylene (CH₂) groups in an open chain occurs at about 725.94 cm⁻¹.

3.4 Gas chromatography mass spectroscopy analysis

Gas Chromatography mass spectroscopy is method used to separate and identify the chemical component the biodiesel. It was found in (figure 5) that there are eight ester obtained in Chlorococcum humicola microalgae biodiesel as shown Table 2.

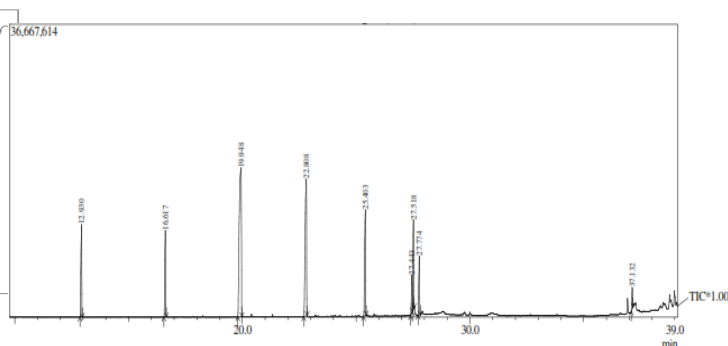


Figure.5: The qualitative peaks of GC-MS of Chlorococcum humicola microalgae Biodiesel

Table.2: Chlorococcum humicola Biodiesel Components with RT, Percentage, Name of the Compound (FAME) and Molecular Formula

Sr. No.	Retention Time (min.)	% Area	Name of the Compound(FAME)	Molecular Formula
1	12.930	8.55	Octanoic acid methyl ester	C ₉ H ₁₈ O ₂
2	16.617	8.00	Decanoic acid methyl ester	C ₁₁ H ₂₂ O ₂
3	19.948	33.93	Dodecanoic acid methyl ester	C ₁₃ H ₂₆ O ₂
4	22.808	20.58	Tetradecanoic acid methyl ester	C ₁₅ H ₃₀ O ₂
5	25.403	10.97	Hexadecanoic acid methyl ester	C ₁₇ H ₃₄ O ₂
6	27.443	3.30	9,12-Octadecadienoic acid (Z, Z) methyl ester	C ₁₉ H ₃₄ O ₂
7	27.518	8.02	9-Octadecenoic acid methyl ester	C ₁₉ H ₃₆ O ₂
8	27.774	4.98	Octadecanoic acid methyl ester	C ₁₉ H ₃₈ O ₂

The Chromatogram shows several compounds at various retention period and base peak found 12.930, 16.617, 19.948, 22.808, 25.403, 27.443, 27.518 and 27.774 is with reference to McLafferty rearrangement process base peak at m/z 74.05. The experimental test results and the ester were confirmed with MS library. The higher concentration of Dodecanoic acid methyl ester, Tetradecanoic acid methyl ester, Hexadecanoic acid methyl ester was obtained. The important peak was identified at m/z 74.05 formed due to carbomethoxy ions and β ion expulsion [19]. A long chain of alkyl group was also found at m/z 74 to m/z 143 helps in identifying the presence of fatty acid methyl ester in the biodiesel. The various esters with respect to their retention time are shown in the Table 2.

IV. CONCLUSION

The fresh water microalgal strain of Chlorococcum humicola was isolated from Nizarneshwar, Ahmednagar (Maharashtra), India. The lipid production in C. humicola was 0.029g/l/d achieved. Infrared spectroscopy show characteristic ester peak at 1745.66 cm⁻¹. In Gas Chromatography mass spectroscopy analysis eight different types of fatty acid methyl ester obtained. C. humicola microalgae biodiesel is mixture of 87.01% saturated fatty acid of methyl ester and 11.32% unsaturated fatty acid methyl ester. The saturated and unsaturated fatty acid methyl ester possesses high oxidative stability [2], hence Chlorococcum humicola species is applicable for biodiesel production.

REFERENCES

- [1] Bligh E.G., Dyer W.J. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol Pharmacol* 1959;37:911-917.
- [2] Chisti, Y. Biodiesel from microalgae. *Biotechnology Adv.* 2007; 25 (3), 294-306.
- [3] Chisti, Y. Biodiesel from microalgae. *beats biotechnol. Trends Biotechnol.*2008; 26,126-131.
- [4] Dalvi S. N., Sonawane S. R. and Pokharkar R. D., Preparation of Biodiesel of Undi seed with In-situ Transesterification. *Leonardo Electronic Journal of Practices and Technologies.* 2012. Issue 20: 175-182.
- Demirbas, A. Importance of biodiesel as transportation fuel. *Energy Policy* 2007; 35, 4661-4670.
- [5] Fargione, J., Hill J., Timan, D., Polasky, S., Hawthorne, P. Land clearing and the biofuel carbon debt. *Science* 2008; 319, 1235-1238.
- [6] Graboski, M. J., McCormick, R.. Combustion of fat and vegetable oil derived fuel in diesel engines. *Prog. Energy. Combust.Sci.* 1998; 24,125-164.
- [7] Haas, M. J., McAloon, A.J., Yee, W. C., Fogila, T. A. A process model to estimate biodiesel production costs. *Bioresour. Technol.* 2006;97, 671-678.
- [8] Hill, J., Nelson, E., Tilman, D., Polasky, S., Tiffany, D. Environmental, economic and energetic costs and benefits of biodiesel and ethanol biofuel. *PNAS* 2006;103, 11206-11210.
- [9] Ho SH, Chen WM, Chang JS. *Scenedesmus obliquus* CNW-N as a potential candidate for CO2 mitigation and biodiesel production. *Bioresour Technol* 2010;101:8725-30.
- [10] Huntley, M.E., Redalji, D.G. CO2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. *Mitigation Adapt. Strateg. Global Change* 2007;12, 573-608.
- [11] Kanz T, Bold HC. In: *Physiological studies, morphological and taxonomical investigation of Nostoc and Anabaena in culture.* Austin, TX: University of Texas, Publication No. 6924; 1969.
- [12] Li X, Hong-ying H, Jia Y. Lipid accumulation and nutrient removal properties of a newly isolated freshwater microalga, *Scenedesmus* sp. LX1, growing in secondary effluent. *New Biotechnol* 2010;27:59-63.
- [13] Li, Y., Horsman, M., Wu, N., Lan, C.Q., Dubois-Calero, N. Biofuels from microalgae. *Biotechnol. Prog.* 2008;24, 815-820.
- [14] Lopez, D.E., Goodwin, J. G., Bruce, D.A., Furuta, S. Transesterification of triacetin with methanol on solid acid and base catalysts. *Appl. Catal. A Gen.* 2005;44, 97-105.
- [15] Ma, F., Hanna, M. A. Biodiesel production: a review. *Biosour. Technol.* 1999; 70, 1-15.
- [16] Mata, T.M., Martins, A.A., Caetano, N.S. Microalgae for biodiesel production and other applications: a review. *Review. Sustainable energy Rev.* 2010;14, 217-264.
- [17] McLafferty . 'Mass spectrometric analysis, molecular rearrangement'. *Anal. Chem.* Vol 31, 1959; pp. 82-87.
- [18] Phukan MM, Chutia RS, Konwar BK, Katak R. Microalgae *Chlorella* as a potential bio-energy feedstock. *Appl Energy* 2011;88:3307-12.
- [19] Rodolfi, L., Zittelli, G., Bassi, N., Padovani, G., Bonini, N., Bonini, G., et al. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low cost photobioreactor. *Biotechnol. Bioeng.* 2009; 102, 100-112.
- [20] Schuchardt, U., Sercheli, R., Vargas, R.M. Transesterification of vegetable oils: a review. *J. Braz. Chem. Soc.* 9, 1998; 199-210.
- [21] Searchinger, T., Heimlich, R.A., Dong, F., Elobeid, A., Fabiosa, J., et al. Use of U.S. croplands for biofuels increase greenhouse gases through emission from land-use change. *Science* 2008, 319, 1238-1240.
- [22] Sheehan, J.T., Dunahay, T., Benemann, J., Roessler, P. A look back at the U.S. Department of energy's aquatic species program: biodiesel from algae. 1998; NERL/TP-580-24190.
- [23] Spoehr, H.A., Milner, H.W. The chemical composition of *Chlorella*: effect of environmental conditions. *Plant Physiol.* 1949; 24, 120-149.
- [24] Swanson, K.J., Madden, M.C. Biodiesel exhaust: the need for health effects research. *Environ. Health Perspect.* 2007;114, 496-499.

AUTHORS



First Author – Swati Sonawane, Ph. D. Scholar at Department of Chemistry, S. N. Arts, D. J. M. Commerce & B. N. S. Science College, Sangamner, Qualification : M.Sc., Email id: swatis2003@yahoo.co.in



Second Author – Dr Sanjaykumar Dalvi, Director, Board of Students Welfare, Savitribai Phule Pune University, Pune. , Qualification: M.Sc. Ph.D., Email id: snd_rang@yahoo.co.in



Third Author – Dr Raghunath Pokharkar, Emeritus, Professor, Department of Chemistry, S. N. Arts, D. J. M. Commerce & B. N. S. Science College, Sangamner, Qualification: M.Sc. Ph.D, Email id: raghunathpokharkar@rediffmail.com

Correspondence Author – Swati Sonawane, Ph. D. Scholar. Department of Chemistry, S. N. Arts, D. J. M. Commerce & B. N. S. Science College, Sangamner, Ahmednagar, Maharashtra, India-422605, Email id: swatis2003@yahoo.co.in, Alternate email id: swati05051985@gmail.com, Contact number: +91 9326710125.