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Comparative Study of Catalyst for Biodiesel Synthesis from Microalgae Chlorella *vulgaris*

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Abstract: ***The present paper objective was to compare methanolic acid and base catalyst for direct transesterification of Chlorella vulgaris sp. dried microalgae. The microalgae were produce higher lipid content and it was applicable for synthesis of long chain alkyl ester as biodiesel liquid fuel. The microalgae sample was collected from a fresh water river resource in Maharashtra. The algae cultivation method is important part for the faster growth and high lipid productivity. The characteristic functional groups of biodiesel identified by FT-IR spectroscopy and retention time, molecular property were studied by GCMS.***

Keywords: Biodiesel, Chlorella *vulgaris*, Transesterification, FT-IR, GC-MS

1. Introduction

An evaluating the clean energy sources are one of the vital needs for the future of every developing country. The demand of energy is increases continuously due to rapid industrialization and population. The basic non-renewable energy sources are petroleum, coal, natural gas; hydro and nuclear [12].The petroleum sources after combustion creates atmospheric pollution and contribute for the green house gas emission (GHG). This emission comprises the air pollutants like NOx, SOx, CO, particulate matter and volatile organic compounds [11]. Hence the biomass energy is one of the key solution for minimize environmental hazards and better replaceable input for fossil fuels [7]. The utilization of biomass energy in large extent provides sustainable development which link to global stability, economic balance and quality of life [22].

Biodiesel is the example of biomass energy which is renewable, non-toxic and biodegradable fuel that derived from renewable sources. Biodiesel is liquid fuel which obtained by transeterification of triglyceride oil with monohydric alcohol. The non-edible oil seeds and algal oil alkyl esters can be use in diesel fuel as additive [21]. The combustion of biodiesel does not affect on atmospheric CO_2 balance because the emitted CO_2 fixed by plants during photosynthesis [8] [15]. The macro algae and microalgae have higher photosynthetic efficiency than the terrestrial crop plant. Algae have large oil productivity than terrestrial plant with simple extraction of oil [20]. The 'microalgae biodiesel' is not very resent term but due to increase in petroleum prices, burning of fossil fuels and environmental harms, it taken as seriously[4][10][18].

Chlorella *vulgaris* sp. is fresh water microalgae in the class of Chlorophyceae, which are commercially possible for cultivation due to its higher lipid content and fast growth rate [9] [14] [19]. The cultivation method and other parameters like growth medium composition e.g. types of carbon source, vitamins, salts and nutrients, pH, temperature and light intensity and type of metabolism such as phototropic, heterotrophic and mixotropic growth influencing the growth and lipid productivity of microalgae [17]. Chlorella *vulgaris* having 14-56% of lipid on dry matter basis [1] [13], hence chlorella *vulgaris* sp. is best for biodiesel production.

The present paper study was collection of Chlorella *vulgaris* strain, lipid extraction and product was obtained by acid, base transesterification method, product analyzed by standard method FT-IR Spectroscopy and Gas Chromatography Mass Spectroscopy.

2. Method and Material

Sample Collection and Cultivation Method

The water samples for microalgae isolation were collected aseptically from sites that appeared to contain algal growth in a fresh water river Godawari at Kopargao, Maharashtra, India. The water sample and floating algae on the water were collected separately in clean plastic container from sampling location and labeled. Fresh as well as preserved algal forms were observed under research microscope and identified with the help of standard literature.

The Chu·s10 Medium consisted trace elemental solution of Ca $(NO_3)_2$ (0.04); K_2HPO_4 (0.01); $MgSO_4.7H_2O$ (0.0250); Na_2CO_3 (0.02); Na_2SiO_3 (0.025); $FeCl_3$ (0.008) in mg per liter fresh water. Ten ml of water samples were transferred to a 500 ml conical flask containing 200 ml of sterilized Chu·s10 Medium and then incubated on a rotary shaker at 27 ˚C and 150 rpm under continuous illumination using white fluorescent light at intensities of 40 µmol /m^2/ s. Every two days, the flasks were examined for algal growth using an optical microscope.

Harvesting, Drying and Extraction of Oil

Harvesting methods depends primarily on the type of algae. Microalgae can be harvested by using centrifugation method. The algae were dried under solar radiation at 25˚C for 3 days. Solar radiation provides energy to vaporize water. For a given amount of incoming solar radiation, as air temperature increases, more of that radiation goes to vaporization of water. The lipid content of microalgae was

extracted by using the Bligh and Dyer method [2]. After cell drying, algal powder was mix with chloroform–methanol (1:2) solvent for 30 min. Algal solid was removed by centrifugation at 3000 rpm for 5 min. The residual solid and lipid separated by solvent extraction procedure.

Transesterification Reaction and Fatty Acid Analysis

The acid- base catalyzed transesterification carried for the preparation of fatty acid methyl ester. The acid catalyzed reaction carried according to Garces and Mancha (1993) [6]. The methanolic HCl is efficient catalyst for biodiesel preparation. The mixture of 1.09M HCl and MeOH was added in round bottom containing microalgae powder. The reaction mixture was continuously stirring. The reaction carried out at 60˚C for 60 minute.

The base catalyzed reaction according to Dalvi et al. (2012) and methanolic KOH catalyst used [5]. The mixture of KOH and MeOH was added in round bottom containing microalgae powder. The reaction mixture was continuously stirring. The reaction carried out at 60˚C for 60 minute. At room temperature both reactions mixture were cooled and separate the algal retardant and product separately each. Products were washing with 5% water to remove water soluble impurities and heat the product at 85˚C. The fatty acid methyl esters of both acid – base catalyzed products were analyzed by Infrared spectroscopy and gas chromatography mass spectroscopy.

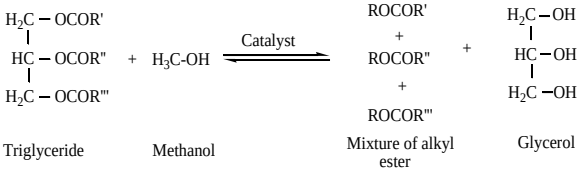

Figure 1: Transesterification reaction of Triglyceride.

3. Result and Discussion

Infrared Spectroscopy

The IR Spectroscopy is the method to determine the function group of present in obtained product. The microalgae Chlorella vulgaris oil and its fatty acid methyl ester are analyzed by infrared spectroscopic method (Figure 2, 3, 4). The spectrum Chlorella *vulgaris* algae oil (Figure 2)shows the υ (O-H) stretching of hydroxyl group of fatty acids at 3366.89 cm^{-1} , υ_{as} (sp^3 C-H) stretching of hydrocarbon at 2924.12 cm^{-1}, υ (C=O) stretching of carbonyl group from fatty acids at 1743.29 cm^{-1}, methylene (CH_2) groups have a characteristic bending absorption at 1461.06 cm^{-1}. The methyl δ_s (CH_3) group have characteristic bending absorption is 1375.68 cm^{-1}, the υ (C-O) stretching of alkyl carbon and oxygen from fatty acids are at 1158.32 cm^{-1} and δ_r (CH_2) bending (rocking) motion associated with four or more methylene (CH_2) groups in an open chain of fatty acid occurs at about 720.15 cm^{-1}.

Infrared Spectrum of Acid catalyzed transesterification of Chlorella *vulgaris* Biodiesel (Figure 3) shows the υ_{as} (sp^3 C-H) stretching of ester group of fatty acid methyl ester at 2922.78 cm^{-1} , υ (C=O) stretching of ester at 1743.61 cm^{-1} , δ_s (CH_2) bending of methylene group at 1460.21cm^{-1} , δ_s (CH_3) bending of methyl group absorption frequency at 1372.48 cm^{-1}, υ (C-O) stretching of alkyl carbon and oxygen from fatty acid methyl ester at 1160.01 cm^{-1}, δ_r (CH_2) bending of methylene group was observed at 721..21cm^{-1}.

Infrared Spectrum of Base catalyzed transesterification of Chlorella *vulgaris* Biodiesel (Figure 3) shows the υ_{as} (sp^3 C-H) stretching of ester group of fatty acid methyl ester at 2922.06 cm^{-1} , υ (C=O) stretching of ester at 1743.61 cm^{-1} , δ_s (CH_2) bending of methylene group at 1458.65 cm^{-1} , δ_s (CH_3) bending of methyl group absorption frequency at 1369.59 cm^{-1}, υ (C-O) stretching of alkyl carbon and oxygen from fatty acid methyl ester at 1158.10 cm^{-1}, δ_r (CH_2) bending of methylene group was observed at 720.51cm^{-1}.

The FT-IR analysis is carried out both Chlorella *vulgaris* oil and its biodiesel, the hydroxyl group (–OH) of fatty acid is disappear which not observed in IR spectrum of obtained product biodiesels, the appearance of peak in algal oil and its biodiesel was different.

Gas Chromatography-Mass Spectroscopy Analysis

Gas Chromatography is used to separate and identify the chemical ingredients in the biodiesel. The Figure 5 and 6 shows the various obtained peak. In acid catalyzed Biodiesel of Chlorella *vulgaris*, four types of ester obtained at retention time (min) at 25.378, 27.444, 27.508, and 27.757; similarly for base catalyzed Biodiesel of Chlorella *vulgaris*, four types of ester obtained at retention time (min) at 25.378, 27.445, 27.508, and 27.759. The product of biodiesel components shows the base peak at m/z 74.05[16]. The experimental test results and the ester were confirmed with MS library. Table 1 and 2 shows the peak obtained with various retention time, percentage area, name of compound and molecular weight.

In acid catalyzed product of biodiesel contain higher content of 9-Octadecenoic acid methyl ester (37.22%), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (28.27%), Hexadecanoic acid methyl ester (27.95%) and Octadecanoic acid, methyl ester (6.56%) while in base catalyzed product of biodiesel contain higher content of 9-Octadecenoic acid methyl ester (20.37%), Hexadecanoic acid methyl ester (15.33%) ;9,12-Octadecadienoic acid (Z,Z)-, methyl ester (14.66%), and Octadecanoic acid, methyl ester (2.78%).

4. Conclusion

The present paper was evaluated comparative study of acid and base catalyzed single step direct transesterification for biodiesel synthesis from Chlorella *vugaris* microalgae. The hydrochloric acid and potassium hydroxide, both catalysts were cost effective and appropriate for methylation fatty acid methyl ester. The characterization of biodiesel liquid fuel is done with FT-IR and GC-MS technique. FT-IR shows characteristic peak of ester at 1743.61 cm^{-1}. The fatty acids methyl esters were identified ranging between C17 to C19 and verified with standard data of MS library. In acid catalyzed biodiesel of Chlorella *vugaris* contain 34.51% saturated fatty acid methyl ester and 65.49% unsaturated fatty acid methyl esters. Similarly, in base catalyzed biodiesel of Chlorella *vugaris* contain 18.11% saturated fatty acid methyl ester and 35.03% unsaturated fatty acid methyl esters.

Polyunsaturated fatty acids are susceptible to oxidation during storage which reduces the acceptability of microalgal oil for biodiesel production [3]. The GC-MS study (Figure 5 & 6) demonstrated that the biodiesel from Chlorella *vugaris* contains mainly 19-35 % saturated fatty acid methyl ester and 35-65% unsaturated fatty acid methyl esters, advocates its higher oxidative stability. Thus, Chlorella *vugaris* could be considered as potential algae for biodiesel production.

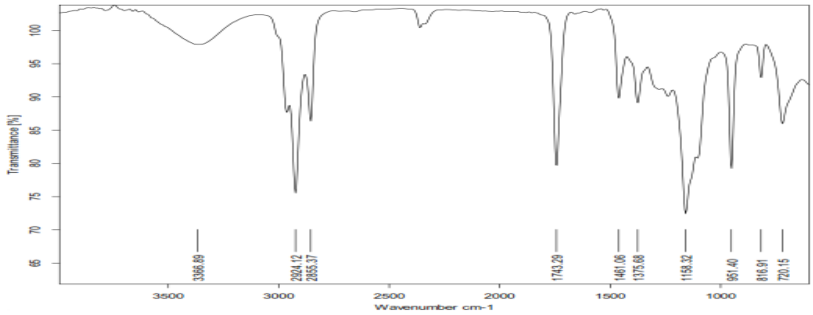

Figure 2: Infrared Spectrum of Chlorella vulgaris oil

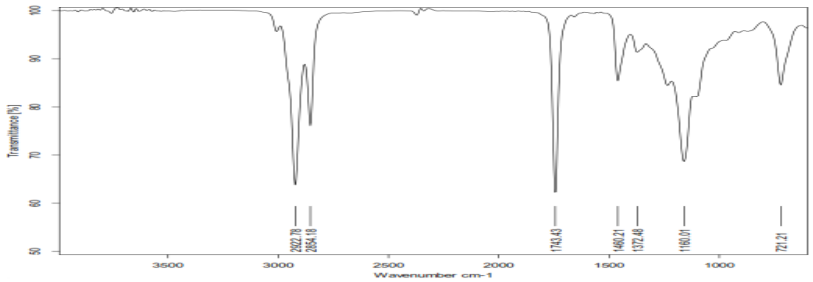

Figure 3: Infrared Spectrum of Acid catalyzed transesterification of Chlorella *vulgaris* Biodiesel

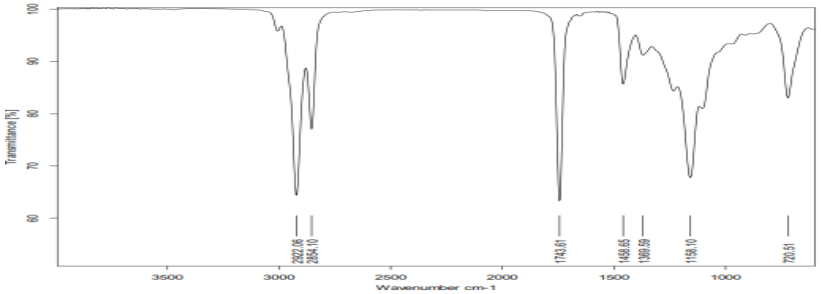

Figure 4: Infrared Spectrum of Base catalyzed transesterification of Chlorella *vulgaris* Biodiesel

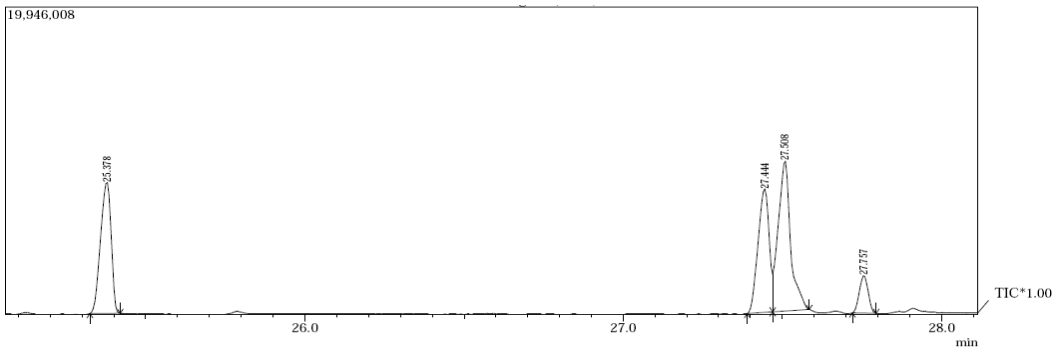

Figure 5: GC spectrum of acid catalyzed FAME of Chlorella *vulgaris*

Table1: Acid catalyzed FAME of Chlorella *vulgaris* with retention time, % area, compound name and molecular formula

Sr. No.	Retention Time (min.)	% Area	Name of the Compound	Molecular Formula
1	25.378	27.95	Hexadecanoic acid methyl ester	C17H34O2
2	27.444	28.27	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2
3	27.508	37.22	9-Octadecenoic acid methyl ester	C19H36O2
4	27.757	6.56	Octadecanoic acid, methyl ester	C19H38O2

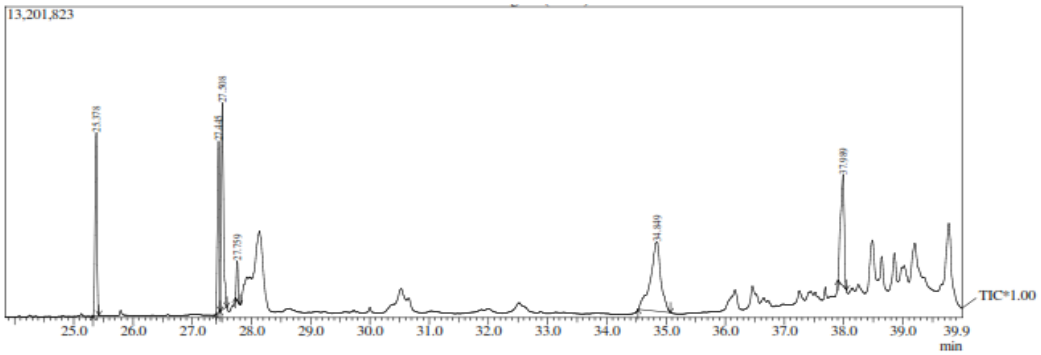

Figure 6: GC spectrum of base catalyzed FAME of Chlorella vulgaris

Table 2: Base catalyzed FAME of Chlorella vulgaris with retention time, % area, compound name and molecular formula.

Sr. No.	Retention Time (min.)	% Area	Name of the Compound	Molecular Formula
1	25.378	15.33	Hexadecanoic acid, methyl ester	C17H34O2
2	27.445	14.66	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2
3	27.508	20.37	9-Octadecenoic acid methyl ester	C19H36O2
4	27.759	2.78	Octadecanoic acid, methyl ester	C19H38O2

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