PRODUCTION OF BIODIESEL FROM THE FRESHWATER MICROALGAE *Chlorella vulgaris*

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***Abstract*:** Algae have emerged as one of the most promising sources for biodiesel production. Biological materials can be utilized for the production of energy by burning of biomass in the production of biofuels. The lipid and fatty acid elements of microalgae are converted to biodiesel via a transesterification reaction with methanol in the presence of a catalyst, to produce mono alkyl esters, which is generally known as biodiesel or fatty acid methyl esters (FAME). Biodiesel has gained much attention in recent years due to its eco-friendly nature, non-toxic characteristics, biodegradability and lower net carbon cycle compared to conventional diesel fuels.In the current study, carried out at laboratory-scale, potential algal species *Chlorellavulgaris* were collected from Kerala and employed as a feedstock for biodiesel production. The biofuel was determined to confirm the presence of biodiesel by Gas Chromatography - Mass Spectroscopy (GC MS), Fourier-Transform Infrared Spectroscopy (FTIR) and Thin Layer Chromatography (TLC).

Key words: Biodiesel, *Chlorella vulgaris*, FAME, Transesterification, FTIR, GC MS, TLC.

I.INTRODUCTION

Microalgae have been considered as a promising alternative and renewable feedstock source for biofuels. Algal biomass is a fascinating sustainable feedstock for production of biodiesel. Algal biomass have versatile uses in agriculture, such as fertilizers, cattle feed, poultry feed and packing material for mushroom cultivation. It can be also used as food supplement, part of healthy diet, vitamin source, etc. Microalgae are unicellular photosynthetic organisms that use light energy and carbon dioxide (CO_2), with relatively higher photosynthetic efficiency. *Chlorella vulgaris* is one of the most attractive algae species for producing biofuels owing to its fast growth and easy cultivation. However, it is yet to be commercially viable due to its low lipid content. Biodiesel refers to any diesel-equivalent biofuel made from renewable biological materials such as vegetable oils or animal fats by chemical reaction with a short-chain alcohol, such as methanol, ethanol, or butanol and a catalyst (Meher*et al*., 2006), called transesterification. (The study about biofuel from microalgae Yusuf C., 2007), cited that the biodiesel from oil crops are potential renewable and they are carbon neutral alternative to petroleum fuels. Microalgae appears to be the source of renewable biodiesel which is efficient in meeting the global demand for transport fuels.

II. MATERIALS AND METHODS

2.1. Microalgae Strain and Medium

A culture of fresh water microalgae *Chlorella vulgaris* was obtained from the Central Marine Fisheries Research Institute (CMFRI), Cochin, Kerala. BG11 Medium adapted for freshwater algae was used. The stock solutions were prepared (Table 2.1) and stored in the refrigerator at 4 °C.

Table 2.1: The composition of BG11 medium

SL NO	CHEMICAL	FORMULA	1 LITRE
1.	Sodium Nitrate	$NaNO_3$	1.5g
2.	Di-potassium hydrogen phosphate	K_2HPO_4	0.04g
3.	Magnesium Sulphate	$MgSO_4.7H_2O$	0.075g
4.	Calcium chloride	$CaCl_2.2H_2O$	0.036g
5.	Citric acid		0.006g
6.	Ferric ammonium citrate		0.006g
7.	Ethylenediaminetetraacetic acid	EDTA	0.001g
8.	Sodium carbonate	Na_2CO_3	0.02g
TRACE METALMIX			
9.	Boric acid	H_3BO_3	2.86g
10	Manganese chloride	$MnCl_2.4H_2O$	1.81g
11.	Zinc sulphate	$ZnSO_4.7H_2O$	0.222g
12.	Sodium molybdate	$NaMoO_4.2H_2O$	0.39g
13.	Cupric sulphate	$CuSO_4.5H_2O$	0.079g
14.	Cobalt nitrate	$Co(NO_{3)2}$	49.0mg

To prepare BG11 medium, one mL of each stock solution (1–5) was added to one litre of sterilized fresh water. The pH was adjusted to 7.2. The media was autoclaved at 121 °C for 15 min. The stock solution of Trace metal mix was prepared by dissolving 2.86g of H_3BO_3 , 1.81g of $MnCl_2$, 0.22g $ZnSO_4$, 0.39 g of $NaMoO_4$, 0.79g of $CuSO_4$ and 49.0mg of Co $(NO_3)_2$ in 1000 ml of distilled water then autoclaved at 121 °C for 15 min. 10 mL of the trace metal mix solution was added to one litre of sterilized fresh water media.

2.2. Culture Conditions

The cultivation of microalgae was done in laboratory scale according to Halim*et al*., (2012). The fresh water microalgae *Chlorella vulgaris* was grown at the laboratory using sterilized BG11 medium. The culture temperature was fixed at 18-20°C. Fluorescent light was used to supply constant light intensity for the culture which was not less than 2000 lux on a 16:8 light to dark cycle Cheirsilp*et al*., (2012). For this experiment, the microalgae was grown in 2L flasks. The cultures were thoroughly mixed regularly and monitored their growth.

2.3. Microalgae Harvesting

The samples were then transferred to petri dishes. In order to determine the dry weight of the fresh water *Chlorella vulgaris*, the dewatering of algae culture can be done by using centrifuge. The microalgal concentrate can be subjected to cell disruption process, achieved by alkali chemical lysis. The micro algal concentrate can be completely dried and then crushed to fine powder form.

Biodiesel Production from Chlorella vulgaris

2.4. Lipid Extraction

Lipids were extracted using hexane/isopropanol(3/2 v/v) organic solvent mixture system. The solvents were added simultaneously to the microalgal biomass (in the form of dried powder). The cell debris formed during the lipid extraction process was removed by filtration process and complete removal of extraction solvent and residual water content of lipid was done with vacuum evaporation method.

2.5. Transesterification.

The oil extracted from *Chlorella vulgaris* was converted to biodiesel using transesterification. According to Chisti (2007), in lab-scale experiments only less amount of microalgal lipids are produced, large amount of methanol is often added to quantitative transesterification. In this process, crude lipids are mixed with methanol (alcohol) and converted to fatty acid alkyl esters with an alkali KOH as catalyst for transesterification.After transesterification process, the reaction mixture contained biodiesel, glycerol, excess amount of methanol, and un-transesterified lipids. Then it undergoes post tranesterification purification the other by products and contaminants. It is followed by two steps: the reaction mixture kept for settlement of phase partitioning. Two phases were formed - upper and lower phase. Upper phase contained biodiesel/un-transesterified lipids and

lower or bottom phase contained glycerol. Then the upper phase, was poured out and washed with water several times to eliminate the presence of any catalyst and excess of methanol.

2.6. Fatty Acid Methyl Esters (FAMEs) Analysis

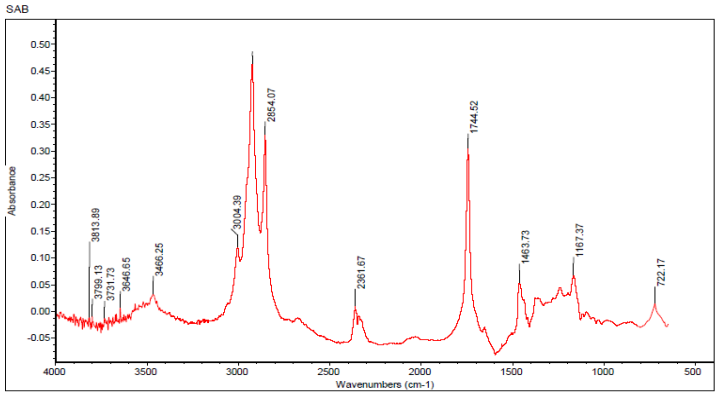
The analysis of Fatty acid methyl esters of purified biodiesel or un-transesterified lipids of *Chlorella vulgaris* were carried out using as Fourier Transformation Infrared spectroscopy (FTIR) and Gas Chromatography (GC) and Mass Spectroscopy(MS) system and Thinlayer chromatography (TLC). The FTIR analysis was carried out by Potassium bromide pellet method and spectrum was recorded in Thermo Fischer Nicolet Impact 500 FT-IR spectrometer at The South Indian Textile Research Association (SITRA), Coimbatore using diffuse reflectance mode. Fatty acid methyl ester (FAME) composition was determined using gas chromatography-mass spectrometry (GC-MS). The fatty oils were esterified with methanol prior to GC-MS analysis to make the fatty oils more volatile and to avoid the acidic attack to the stationary phase/column. 1 µl sample was injected in spilt less mode. The inlet temperature was set at 260˚C and oven temperature was programmed as 70˚C (0 min); 150˚C (5 min); 200˚C (15 min); 220˚C (5 min). Total run time was 38.833 minute and column flow rate 0.6 ml/min helium gas. Thin layer chromatography was carried out according to Shah G C *et al*., (2012). Prepared 202 ml of solvent system (Hexane:Ether:Acetic acid 60:40:1) in a 500 ml of conical flask. Mix and pour~150 ml into the chamber saturate while loading the plates. With a capillary pipette, spot 1-2µl of phospholipid standard onto the TLC plate. The spots should be smaller than 4mm in diameter. After the spots have dried, repeat loading each standard approximately reaches to 10µl. Let dry the spots, make sure that loading area is above the solvent. Place the plates in the chamber to develop the process. Immediately close the cover and allow to run for approximately 30 minutes, until the solvent front has reached the upper line. Remove the plate and leave to dry in the rack in the fume hood.

III. RESULT AND DISCUSSION

The mass culture of algae can be produced in BG11 medium in 2L conical flask under the artificial illumination provided with fluorescent tubes approximately 2000 lux for 16 hours in light and 8 hours in dark incubation. Observed the good quality of mass production on 20th day of incubation. Mixing is carried out by stirring by daily. The water content of algal culture was removed by the centrifugation method. Centrifuged the sample at 10000 rpm for 10 minutes. Pellets were observed. Then the cells were disrupted using alkali lysis method. After the pre-treatment of cell disruption process, the microalgal concentrate was subjected to drying. Drying was carried out in an Oven with temperature of 40˚C for 48 hours. The microalgal concentrate was completely dried and then it was crushed into fine powder. The samples were crushed thoroughly by using a pestle and motor. This dried biomass (figure 3.1) was used for the lipid extraction from microalgal culture, such as *Chlorella vulgaris.* Biodiesel produced from the extracted lipids by transesterification process (figure 3.2).

The microalgal biofuel from *Chlorella vulgaris* was subjected to IR Spectroscopy which showed the presence of functional groups. The functional groups were identified the absorption frequency and also transverse frequency of infra-red waves in wavenumber cm^{-1}. The absorption and transverse frequencies of each of the functional groups varied from one another. The peak values are depicted in Fig 3.3, and identified functional groups are shown in (Table 3.5).

Figure 3.3: FTIR Spectrum of biofuel of *Chlorella vulgaris*



The biofuel compounds were analyzed to confirm the presence of biodiesel by Gas chromatography and Mass Spectroscopy (GC MS), Fourier-Transform infrared spectroscopy (FTIR) Jones *et al.*, *(*2012). The result of biofuel sample of *Chlorella* indicates that (C-H) Alkanes stretching at 3004.39 cm-1 and the peak intensity is strong. The strong intensity is due to stretching vibration of alcohols, carboxylic acid and ether occurs at 1167.37 cm^{-1}. The aromatic (C-C in-ring) stretching at 1463.73 cm^{-1}, where intensity is medium. The alkene (=C-H) stretching at 3004.39 cm^{-1}, which intensity is medium (Table 3.5). This biofuel sample consist of Alkane, Alkenes, ether, alcohol, aromatic, the presence of these functional groups indicate the presence of hydrocarbons in the bio-fuel.

CONCLUSION

It was concluded that the presence of different functional group of compounds indicate the presence of hydrocarbons in the fuel and also the (O-H) broad peak indicate the presence of water contents in bio-fuel. The broad O-H peak was due to the interaction (hydrogen bonding) among the water contents. This biofuel sample consist of Alkane, Alkenes, ether, alcohol, aromatic, the presence of these functional groups indicate the presence of hydrocarbons in the bio-fuel. In this work, fresh water algae was used for extracting biofuel and its compounds were analyzed to confirm the presence of biodiesel. The FAME profiles determine the chemical properties of biodiesel.

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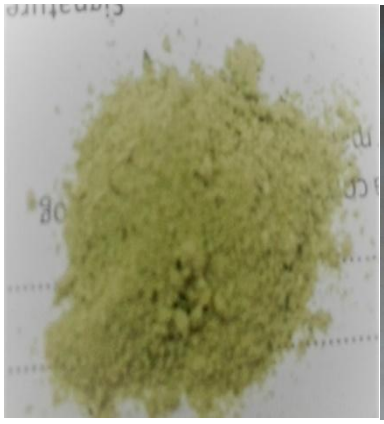
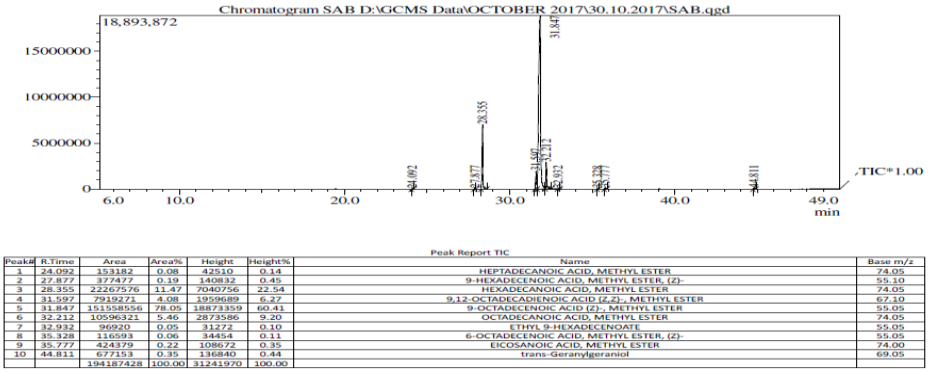


Figure 3.1: Biomass of *Chlorella vulgaris*

Figure 3.2: Biodiesel production

Table 3.5 : Fourier Infrared Spectroscopy (FTIR) analysis for biofuel of*Chlorella vulgaris*

SL NO	FREQUENCY	BOND	FUNCTIONAL GROUP	INTENSITY
1.	722.17	C-H rock	Alkanes	Medium to strong
2.	1167.37	C-O stretch C-H wag(CH_2X)	Alcohols, carboxylic acids, esters, ethers. Alkyl halides	Strong, broad
3.	1463.73	C-C stretch (in-ring) C-H bend	Aromatics Alkanes	Medium Strong
4.	1744.52	C=O stretch	Esters, Saturated aliphatic	Strong
5.	2854.07	C-H stretch	Alkanes	Strong
6.	2924.21	C-H stretch	Alkanes	Strong
7.	3004.39	=C-H stretch C-H stretch	Alkenes Alkanes	Medium Strong
8.	3466.25	O-H stretch, H-bonded	Alcohols, Phenols	Strong, broad



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	24.092	153182	0.08	42510	0.14	HEPTADECANOIC ACID, METHYL ESTER	74.05
2	27.877	377477	0.19	140832	0.45	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	55.10
3	28.355	22267576	11.47	7040756	22.54	HEXADECANOIC ACID, METHYL ESTER	74.05
4	31.597	7919271	4.08	1959689	6.27	9,12-OCTADECADIENOIC ACID (Z,Z)-, METHYL ESTER	67.10
5	31.847	151558556	78.05	18873359	60.41	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	55.05
6	32.212	10596321	5.46	2873586	9.20	OCTADECANOIC ACID, METHYL ESTER	74.05
7	32.932	96920	0.05	31272	0.10	ETHYL 9-HEXADECENOATE	55.05
8	35.328	116593	0.06	34454	0.11	6-OCTADECENOIC ACID, METHYL ESTER, (Z)-	55.05
9	35.777	424379	0.22	108672	0.35	EICOSANOIC ACID, METHYL ESTER	74.00
10	44.811	677153	0.35	136840	0.44	trans-Geranylgeraniol	69.05
		194187428	100.00	31241970	100.00		

Figure 3.4 : Fatty acid methyl esters (FAME) compostion of biofuel of *Chlorella vulgaris*(GC MS)

Table 3.6: GC MS analysis for the biofuel of *Chlorella vulgaris*

SL NO	RT (Min)	COMPOUND NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT	AREA (%)
1.	24.092	HEPTADECANOIC ACID, METHYL ESTER	$C_{18}H_{36}O_2$	284.477	0.08
2.	27.877	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	$C_{17}H_{32}O_2$	268.435	0.19
3.	28.355	HEXADECANOIC ACID, METHYL ESTER	$C_{17}H_{34}O_2$	270.451	11.47
4.	31.597	9,12-OCTADECADIENOIC ACID (Z,Z)-, METHYL ESTER	$C_{19}H_{34}O_2$	294.472	4.08
5.	31.847	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	$C_{19}H_{36}O_2$	296.488	78.05
6.	32.212	OCTADECANOIC ACID, METHYL ESTER	$C_{19}H_{38}O_2$	298.504	5.46
7.	32.932	ETHYL 9-HEXADECENOATE	$C_{18}H_{34}O_2$	282.461	0.05
8.	35.328	6-OCTADECENOIC ACID, METHYL ESTER, (Z)-	$C_{19}H_{36}O_2$	296.488	0.06
9.	35.777	EICOSANOIC ACID, METHYL ESTER	$C_{21}H_{42}O_2$	326.557	0.22
10.	44.811	TRANS-GERANYLGERANIOL	$C_{20}H_{34}O$	290.483	0.35