

A Method for Production and Characterization of Biodiesel from Green Micro Algae

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Abstract

Biofuels are one of those renewable fuel sources developed to fuel the transportation sector to replace conventional petroleum based fuels. Due to widespread research and development programs from last two decades, significant progress has been made in the field of biofuels. The first and second generation biofuels are produced from various food crops and plant seed oils. Algae has been identified as a the potential source to produce biofuels, which can replace transportation fuels derived from fossil energy sources due to various advantages than the previous two generation feed stocks. The ability to produce high amounts of oil, efficiency in harvesting solar energy, adaptability to different climatic conditions and capability to produce valuable byproducts makes algae as a potential source for renewable energy source. In the present work the key concentration is directed towards producing renewable biofuels from algal biomass by transesterification process. Its Characterization study reveals that algal based biodiesel is an alternative source of diesel.

Keywords: *Algae, Lipid, Biodiesel, Fuel properties*

1. Introduction

Biodiesel is the name given to fuel for Diesel engines created by the chemical conversion of animal fats or vegetable oils. Pure vegetable oil works well as a fuel for Diesel engines itself, as Rudolf Diesel demonstrated in his engine at the 1900 world's fair with peanut oil as the fuel.

The United State Fueling stations make biodiesel readily available to consumers across Europe and increasingly in the USA and Canada. This is an indication that biodiesel can operate in compression ignition engines like petroleum diesel without requiring no essential engine modifications [1-3]. Moreover it can maintain the payload capacity and range of conventional diesel unlike fossil diesel, pure biodiesel is bio-degradable, nontoxic and essentially free of sulphur and aromatics [4-6].

A growing number of transport fleets use it as an additive in their fuel. Biodiesel is being used in both public and private fleet vehicles due to environmentally friendly and the facts that it offers a reduction in some emissions without requiring any modifications to the vehicle of biodiesel [1]. It is worth of mentioning that the production of this alternative energy source (biodiesel), which is a variety of ester-based oxygenated fuels, can be produced from different feedstock, such as vegetable oil, animal fats and non edible oils [7-11]. Hence, the production of biodiesel is sensitive to feedstock; care must therefore take in selecting the feed stock for the production of biofuels.

The advantages of biodiesel as perfect alternative energy sources, emission of NO_x is one of the setbacks of biodiesel. The temperature within the cylinders of a vehicle fuelled with

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biodiesel would increase due to the enhanced combustion as a result of high oxygen content of biodiesel. This increase in temperature stimulates the production of NO_x from the reaction with nitrogen in the air, which results in a small increase in NO_x emission compared to those produced from conventional diesel fuel [12]. Aside from the formation of NO_x by the engine powered with biodiesel, the chemical contents of biodiesel is also a fatty acid methyl ester when the alcohol used during transesterification is methanol or fatty acid ethyl ester in case of ethanol. These ester molecules are susceptible to hydrolytic and oxidization reactions resulting in the formation of polymers. This makes the biodiesel unstable on storage and hence cannot sit on the shelf for long time as it develops mould when it gets old [13]. Exploring the means of producing biodiesel that will compete well with the existing petroleum diesel is of much interest in the recent biodiesel research, especially for those methods concentrating on minimizing the raw material cost. Biodiesel as alternative energy source can be produced from different sources.

This work involves the study of biodiesel production from green micro algae. The choice of green micro algae as a feedstock for the production of biodiesel is also favoured by the abundantly available in all areas. Hence, Production of biodiesel from algae oil will lead to proper utilization of vegetable oils and it also includes characterization of produced biodiesel.

2. Materials and Methods

2.1 Algal Samples and Oil Extraction

The algal samples collected and analyzed were identified as green algae-*chlorella vulgaris*, dried algal biomass (5g) was taken in solvent mixture (100 ml) of acetone and the content were refluxed for 4 hrs. After the extraction, the contents were cooled and filtered (or centrifuged) to separate the biomass and washed the biomass with 25 ml of acetone twice to extract the residual lipids present in the biomass. The extracts were pooled and taken in a separating funnel and washed with 1% aqueous sodium chloride solution (50 ml) twice. The solvent were removed by using rota-evaporator under vacuum to get the algal oil. The weight of algal oil was taken to determine the oil content in biomass. If the biomass is available in smaller quantities, the content may be reduced accordingly.

2.1.1 Alkali Catalyzed Transesterification

50 ml of oil was measured and poured into a 150ml conical flask and heated to a temperature of 45°C using a water bath. A solution of sodium methoxide was prepared in a 250ml beaker using 0.25g of NaOH pellet and 10.5mls of anhydrous methanol. The solution was properly stirred until the NaOH pellet was completely dissolved in it. The sodium methoxide solution was then poured into the warm oil and stirred vigorously for 90minutes using a magnetic stirrer and the mixture was left to settle for 24hours in a separating funnel. After settling, the upper layer which was biodiesel was decanted into a separate beaker while the lower layer which comprises of glycerol and soap was collected from the bottom of the funnel. The quantity of biodiesel collected was measured and recorded.

2.1.2 Washing and Drying

Biodiesel must be washed to remove any remaining methanol, glycerin, catalyst, soaps and other impurities. Water used is warmed to about 45°C and is passed through the esters to allow soluble material, excess catalyst and other impurities to stick to the water and be settled to the bottom of the vessel. The water is removed from the vessel periodically until the wash water drained out is clear or the pH of the biodiesel becomes relatively neutral.

The biodiesel washing sometimes leaves the biodiesel looking a bit cloudy. This means there's still a little water in it. It was heated slowly to 100°C and held there until all moisture present was evaporated.

2.2 Physical and Chemical Characterization of the Biodiesel

The production of biodiesel from green micro algae (*Chlorella vulgaris*), and its characterization various analysis such as pH, Specific gravity Density, Flash point, Fire point, Cloud point, Pour point, Viscosity, Cetane number, Acid value, free fatty acid value, Saponification value, Calorific value, moisture ash content, Carbon residue, Na and K, Water sedimentation and Copper corrosion test.

2.2.1 Determination of Acid Value/Free Fatty Acid (FFA)

2g of the oil was measured and poured in a beaker. A neutral solvent (a mixture of petroleum ether and ethanol) was prepared and 50ml of it was taken and poured into the beaker containing the oil sample. The mixture was stirred vigorously for 30minutes. 0.56g of potassium hydroxide (KOH) pellet was measured and placed in a separate beaker and 0.1M KOH was prepared, 3drops of phenolphthalein indicator was added to the sample and was titrated against 0.1M KOH till the color change observed turned pink and persisted for 15minutes.

$$AV = \frac{56.1 \times A \times N}{W_{oil}}$$

Where; V= volume of standard alkali used;
N= normality of standard alkali used;
W_{oil} = weight of oil used

$$FFA = \frac{AV}{2}$$

2.2.2 Determination of Saponification Value

The alcoholic KOH was freshly prepared by dissolving KOH pellet in ethanol. 2g of oil was measured and poured into a conical flask. 25ml of the alcoholic KOH was added to it, a blank was used. The sample was well covered and placed in a steam water bath for 30minutes shaking it periodically, 1ml of phenolphthalein was added to the mixture and titrated against 0.5M HCl to get the end point.

$$SV = \frac{56.1 \times B - A \times N}{W_{oil}}$$

Where; B= volume of standard ethanol potassium hydroxide used in blank titration;
A=volume of standard ethanol potassium hydroxide used in titration with the oil;
N=normality of standard acid; W_{oil}= weight of oil used.

2.2.3 Determination of Moisture Content

In other to determine the moisture content in the oil (%), 48.15g of oil was weighed in a moisture pan, the weight of the pan and oil was taken and was put inside an oven for 3hours at a temperature of 450°C. After every 1hour, the sample was cooled and weighed until the weight before and after was approximately equal.

2.2.4 Determination of Specific Gravity/Density (ASTM D1298) by Hydrometer Method

This procedure is used to measure of specific gravity of the biodiesels. A clean dry empty 50ml density bottle is to be weighed and the mass recorded as M, it is then filled up with distilled water and subsequently with the samples. The mass of the bottle and water is taken and recorded as M1 and that of biodiesel as M2 respectively hence, the specific gravity is evaluated. This procedure is used to determine the specific gravity of the sample.

2.2.5 Determination of Flash Point: ASTM D 93

A sample of the biodiesel is heated in a close vessel and ignited. When the sample burns, the temperature is recorded, the pensky-martens cup tester measures the lowest temperature at which application of the test flame causes the vapor above the sample to ignite. The biodiesel is placed in a cup in such quantity as to just touch the prescribed mark on the interior of the cup. The cover is then fitted onto the position on the cup and Bunsen burner is used to supply heat to the apparatus at a rate of about 5°C per minute. During heating, the oil is constantly stirred. As the oil approaches its flashing, the injector burner is lighted and injected into the oil container after every 12 second intervals until a distinct flash is observed within the container. The temperature at which the flash occurred is then recorded, it is repeated three times and the average taken.

2.2.6 Determination of Cloud Point and Pour Point (ASTM D 2500,ASTM D 97)

A sample of the biodiesel is placed in a test jar to a mark and then placed inside a cooling bath. The temperature at the bottom of the test jar that is the temperature at which the biodiesel starts to form cloud is taken as the cloud point.

A sample of the biodiesel is kept in the freezer to about 500°C then placed in a heating mantle to melt. The temperature at the bottom of the test jar that is the temperature at which the biodiesel starts to pour is taken as the pour point.

2.2.7 Determination of Kinematic Viscosity (ASTM D 445)

A viscometer is inserted into a water bath with a set temperature and left for 30minutes. The sample is added to the viscometer and allowed to remain in the bath as long as it reaches the test thermometer. The sample is allowed to flow freely and the time required for the meniscus to pass from the first to the second timing mark is taken using a stop watch. The procedure is repeated a number of times and the average value are taken which is then multiplied with the viscometer calibration to give the kinematic viscosity.

2.2.8 Cetane Number (ASTM D 613)

Cetane Number is a measure of the fuel's ignition delay. Higher cetane numbers indicate shorter times between the injection of the fuel and its ignition. Higher numbers have been associated with reduced engine roughness and with lower starting temperatures for engines.

2.2.9 Determination of Distillation Characteristics (ASTM D 86)

The distillation characteristics were studied using distillation apparatus (Model PMD 100) in accordance to the procedure in ASTM D 86. 100ml of the biodiesel was charged into the distillation flask. A thermometer provided with a snug-fitting cork was tightly fitted into the neck of the distillation flask. The flask was then fixed tightly into the condenser tube by raising and adjusting the flask support board of a calibrated distillation batch unit. Systematic observations and recordings of temperature readings at 5mls, 10mls, 20mls, 30mls, 40mls, 50mls, 60mls, 70mls, 80mls, and 90mls respectively, and volumes of condensate were taken and recorded.

2.2.8 Determination of Bottom Water and Sediment (ASTM D 2709)

The water and sediment test was determined according to ASTM D 2709, 50mls of the biodiesel and 50ml of toluene were mixed in a 100ml centrifuge tube with the tube tip having graduation of 0.01ml over the range of 0 to 0.2ml, the centrifuge tube was shaken so that an even distribution of the mixture is observed. The tube was placed in a trunnion cup inside an oil test centrifuge in such a way that the tubes are placed opposite each other to establish a balance in the centrifuge. The centrifuge is then closed and timed for 30 minutes; the samples are then whirled for agitation at a speed of 1800rpm to ensure homogeneity. The combined water and sediment at the bottom of the tube was reported to the nearest 0.005ml.

2.3 Molecular Weight Determination

According to [sathasiva, (1996)], the saponification and acid value of micro algal oil were determined. The molecular weight of the oil was calculated as [Xu et .al.,2006]

$$MW = \frac{168300}{SV - AV}$$

Where MW-molecular weight of oil

SV- Saponification value of oil

AV- acid value of oil

2.4. Spectral Analysis of Biodiesel

The petro diesel/biodiesel blends were analyzed using a Evaluation 201 spectrophotometer. The soft-ware Lambda 10 (UV Win Lab) served as an interface between the computer and the spectrophotometer. The samples were placed in a 0.5 cm wide square quartz cell. A tungsten lamp was employed for the analysis in the visible region and a deuterium lamp was used for the UV region. The analysis of the samples was performed at a temperature of 25°C. The spectra were collected over the range from 200 - 1100 nm, and 20 scans were performed for each sample, with a resolution of 2 nm.

Liquids are the easiest to study by IR. A pure sample of the liquid (1-2 drops) may be placed between two disks of pure NaCl or KBr and the resulting 'sandwich' placed directly in the sample holder of the spectrometer. Excellent spectra can be obtained in a matter of a few minutes with minimum expense.

Analysis of algal biodiesel was performed through a GC-MS (Perkin-Elmer Clarus 500 model) equipped with GC column (HP-1,30 m X 0.25 mm X 0.25 µm). Sample injection tool place at the oven temperature of 50° C and was maintained at 50°C for 1 min, the temperature was increased up to 325°C at the heating rate of 10°C min⁻¹ and hold at 325°C for 2 min .the GC-MS interface temperature was set to 250°C and helium was used as a carrier gas with a

constant flow of 1.2 ml/min^{-1} . NIST Library was used to identify the compounds representing peaks in the GC spectrum.

2.5. Flue Gas Analyses

The flue gas analyzer kit consists of probe, hand set remote connection, and analyzer. While running the Diesel Engine, with the help of an analyzer kit the emissions such as carbon monoxide (CO), hydrocarbon (HC), nitrogen oxides (NO_x), carbon dioxide (CO₂), and oxygen (O₂) for Biodiesel blends and Diesel fuel can be measured (Table 2).

3. Result and Discussion

The molar ratio of alcohol to oil, catalyst concentration, reaction temperature and time are the main factors affecting alkali-catalyzed esterification. Of which, the molar ratio of alcohol to oil has a great effect on reducing FFA of vegetable oil. Molar ratios between 4.5:1 and 18:1 were reported in the literature [9-12].

Purification processes, including washing and drying, are necessary since untreated biodiesel contains impurities, such as free glycerol, soap, free fatty acid, methanol, catalysts, metals and glycerides. The remaining unreacted methanol in the biodiesel has safety risks and can corrode engine components, the residual catalyst (sodium hydroxide) can damage engine components, and soap in the biodiesel can reduce fuel lubricity and cause injector coking and other deposits [13].

Traditional water wash has been widely used and proved to be effective to remove most impurities. However, there are many disadvantages: production cost increase due to waste water treatment; Emulsion formation when treating biodiesel from waste cooking oil due to soap formation [14]; Methyl esters loss due to retention in the water phase. Dry washes, the use of ion exchange resin, such as magnesol, can remove all these disadvantages. However, Berros et al. [15] found only water washing has purified biodiesel, direct from glycerol separation, to the requirements of EN 14214 Standard. Therefore, in this study, both water and magnesol washes are applied. First use two 20% (v/v, based on methyl ester layer) water wash followed by 0.5% (wt/wt, based on methyl ester layer) magnesol wash.

The Heat of combustion refers to the measure of energy content in the fuel. Heating value of fuels is an important measure of its releasing energy for producing work. So the lower heating value of biodiesel is attributed to the decrease in engine power. The algal biodiesel was observed to record the highest calorific value of 44152 KJ/Kg apart from diesel, whilst the lowest calorific value of 34 MJ/kg was measured with respect to karanja. The energy content of oils depends on the place where they are grown, the season, composition and other factors. The calorific value of vegetable oils was observed to be lower than those of biodiesels. For methyl esters, the heat content increases as the length of the fatty acids chain increases. The presence of a significant amount of oxygen contributes to the lower energy content of biodiesel fuels.

Viscosity is a measure of the internal friction or resistance of oil to flow. As the temperature of oil is increased, its viscosity decreases and it is therefore able to flow more readily. Viscosity is the most important of biodiesel since it affects the operation of fuel injection equipment, particularly at low temperatures when the increase in viscosity affects the fluidity of the fuel. High viscosity leads to poorer atomization of the fuel spray and less accurate operation of the fuel injectors. The viscosity values of vegetable oils are between 27.84 and 52.76 mm²/sec at 40 °C whereas those of vegetable oils methyl esters are between 3.6 and 5.7 mm²/sec. Non edible oils were observed to have high viscosity values about six times more than ASTM limits. Algae were the most viscous with a viscosity of 9.1 mm²/ sec

at 40 °C. The methyl esters and the blends were observed to have viscosity within the ASTM limits.

Density is another important property of biodiesel. It can be observed from the readings that babassu oil sample has the highest value of 0.946 kg/l as compared to all other samples. The sun flower oils methyl ester has a minimum density value of 0.86 kg/l. The algae diesel fuel sample was observed to have a density value of 0.86 kg/l which is lower than edible, non edible oils, methyl esters and its blends. The flash point of vegetable oil methyl esters is much lower than those of vegetable oils. The flash point of sun flower oil was observed to be highest 274 °C among methyl esters rape seed was observed to be lowest with 80 °C. The flash and fire point of algae biodiesel was observed 52°C and 81°C. Cloud and pour point respectively -30 to -10°C. The calculated molecular of weight biodiesel 744. It is similar to GC-MS spectra results.

The ignition quality of a fuel can be deduced through its cetane number. A fuel with good ignition quality has a high cetane number, where the ignition delay period between the start of fuel injection and the onset of auto ignition is short. Cetane number of biodiesel varies with the feed stock used, but it is generally in the higher end of the typical diesel fuel range. The value of cetane number is found to generally increase with increasing carbon chain length. The cetane number of algae biodiesel was observed 44 KJ/Kg.

Ultraviolet – visible (UV) spectroscopic analysis is rapid becoming a common technique for providing analytical data of biodiesel. In these results, the contribution of algal biodiesel component to overall absorption spectrum spectra of pure standard chemical of free fatty acids, methyl, ethyl esters and mono-, di-, triglycerides were measured. Since the all absorption of all components was below 450 nm, one of those components contributed to characteristic maximum of the biodiesel absorption spectra in a visible range. pure aliphatic acids, esters, and glycerides are colorless substance and did not exhibit significant absorption in the visible range, natural fats and oils from plants and animal pigments exhibiting visible absorption. The absorption of vegetable oils in the visible regions is usually due to lipid-soluble pigments such as carotenoids and chlorophylls. Therefore a characteristic absorption of biodiesel in the visible range in Figure 1 is most likely caused by pigments and other pigments.

Characteristic shapes of biodiesel absorption spectra in visible range indicated that the information can be used to detect the biodiesel feedstock. Differences in position of absorption spectra gave the information about biodiesel level with regular diesel.

The volatile product can be found in the all spectrum of microalgal lipids extracts all collected extractions give band at 1014 and 3942 cm^{-1} . An analysis of IR spectrum showed in Figure 2. The main composition stage, reveals the existence of the adsorption bands characteristic of these different bands.

C=O carbonyl compound (aldehydes, acids, etc) are the strong C=O stretching absorption bands in the region of 1870-1540 cm^{-1} . If ester this band appears in the 1705 cm^{-1} and 1658 cm^{-1} . C-O-C (Ethers), these stretching vibrations produce a strong band in the 1200-900 cm^{-1} region. C-H, adsorption bands as an example 2954 cm^{-1} , 2931 cm^{-1} and 2854 cm^{-1} correspond to the asymmetric and symmetric vibration modes of methyl group, ethylene groups respectively, H₂O the adsorption bands of water can be between 1800-1200 cm^{-1} . It referred to as oleaginous algae it has long been hypothesized that algae could be employed as cell factors to produce oils and other lipids for biofuel and other materials.

Lipids are fatty acids and their derivatives as well as substance related biosynthetically or functionally to these compounds. The most typical lipid classes of plant and animal origin consist of fatty acids linked by an ester bond to the trihydric alcohol, glycerol, or to other alcohols such as cholesterol, or by amide bonds to long chain bases, or on occasion to other

amines. Lipid type is important to biodiesel production because the quality and variety of lipids will determine the need for pretreatment before it is converted to biodiesel, as well as the final fuel properties. It was graphically represented in Figure 3.

Gas chromatography is used to identify the chemical ingredients in the biodiesel. It was found Figure 3. and that there are different major esters in the algal oil methyl ester as shown Table 2. The chromatogram shows several compounds at various retention periods. Lipids (C9-C40) were identified using data base library.

The properties of biodiesel are also comparable with diesel properties. The trends of exhaust temperature and combustion efficiency of biodiesel are the same to diesel at different air- to fuel ratios. The CO, NO, SO₂ and CO₂ emissions of lower than diesel. Biodiesel results shows that the acceptable flue gas emission quality values.

4. Conclusion

Biodiesel an alternate diesel fuel is made from renewable biological sources such as algae oil by transesterification reaction using methanol. The purpose of the transesterification process is to lower the viscosity of the oil.

- This process is a potential alternative for the present industrial scale extraction Methods, particularly for algal oil extraction.
- Completely renewable sources of energy such as algae and ethanol were used to produce an 'ecofriendly' fuel.
- It can be concluded that the presence of biodiesel from green micro algae oil blended with petroleum diesel improves the parameter of cetane number.
- This is important since a high cetane number improves the quality of combustion and decreases NO_x emission. Moreover causes less noise and greater durability of the engine.
- Spectroscopic study reveals very useful and rapid technique for characterization of biodiesel.

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Table 1. Characterization of Biodiesel

S. No	Property	Test Method	Limits ASTM D6751	Values of algae Biodiesel
1	pH		7-9	7
2	Specific gravity		0.86-0.89	0.86
3	Density	ASTM D 1298	7.1-7.4	7.3
4	Flash point °C (F)	D93	100-170 (212-338)	52
5	Fire point °C(F)			81
6	Cloud point °C(F)	ASTMD2500	-3 to 15 (26 to 59)	-30 to -60
7	Pour point °C(F)		-5 to 10 (23 to 50)	-10
8	Viscosity 40C mm ² /s	D445	1.9-6.0	9.1
9	Cetane number	D613	47 min.(40-68)	44.
10	Acid value mg KOH/g	D664	-	1.9
11	Free fatty acid value		-	0.9
12	Saponification value		-	192
13	Calorific value KJ/Kg	ASTM D240		44152
14	Moisture content %,		0.050 max.	0.001
15	Carbon residue %,	D524	0.050 max.	0.008
16	Na, ppm	EN14538	5 max.	1.6
17	K, ppm		5 max.	2.1
18	Water sedimentation%,	D2709	0.050 max.	0.001
19	Copper corrosion test 2 h @ 50°C,	ASTM-D445	No. 3	No. 2b
20	Ash content mass %,	D482	0.0001	nil

Table 2. Flue Gas Analysis Result for Biodiesel

S.No	Parameter	Range	Diesel	Biodiesel
1	NO	0-5000 ppm	±5	.0006
2	CO	0-10000 ppm	1	1.5
3	O ₂	0 to 25 %	5	2.2
4	SO ₂	0-5000 ppm	±5	3
5	CO ₂	0-fuel Value %	0.9	0.2
6	NO ₂	0-5000 ppm	-	Nil

Table 3. GC-MS Spectra Result for Biodiesel

S.No	Peak	Name	Molecular weight
1	C8	Octanoic acid	130
2	C10	Decanoic acid	154
3	C10	Dodecanoic acid	186
4	C11	2H-1Benzopyran 2-on	174
5	C12	Dodecanoic acid	196
6	C13	Octadecane	254
7	C14	Marist oleic acid	222
8	C14	Hexenoic acid	238
9	C16	Hexadecadienoic acid	251
10	C16	Palmitoleic acid	252
11	C17	Styrene	230
12	C20	Eicosadienoic acid	292
13	C21	Pentadecene	296
14	C21	Butanoic acid	386
15	C21	Heneicosane	296
16	C23	Docosahexanoic acid	382
17	C24	Nerronic acid	416
18	C25	Pentacosane	352
19	C26	Hexacosane	366
20	C32	Dotriacontane	450
21	C44	Tetracontane	618

Red- Diesel
 Green - Biodiesel

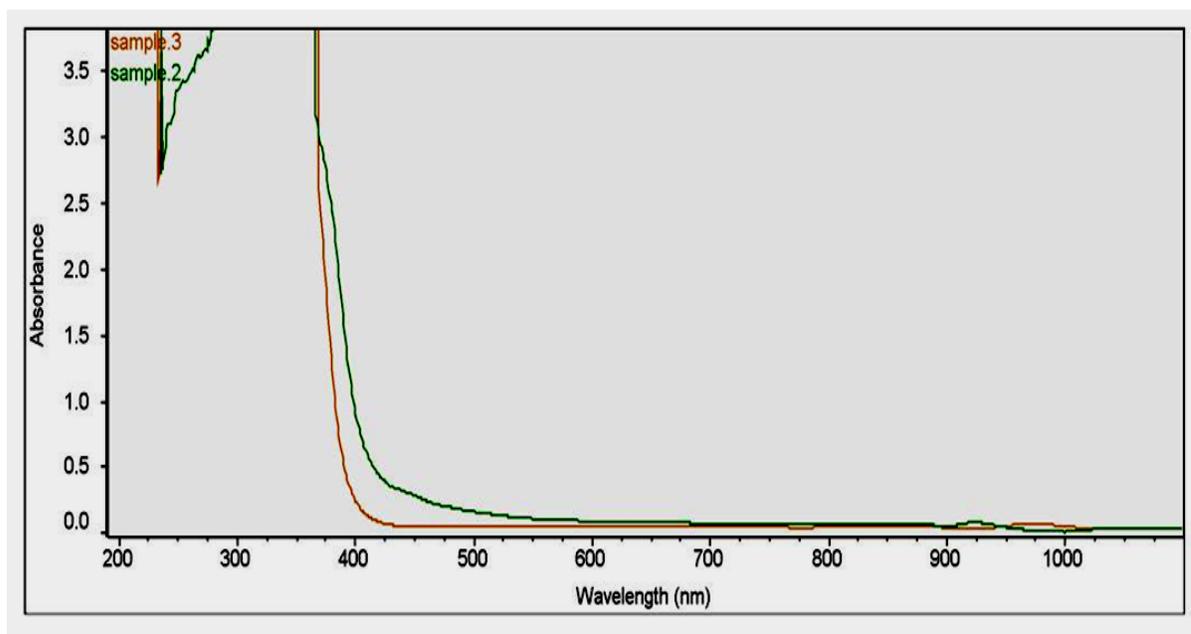


Figure 1. UV –Visible Spectra for Diesel and Biodiesel

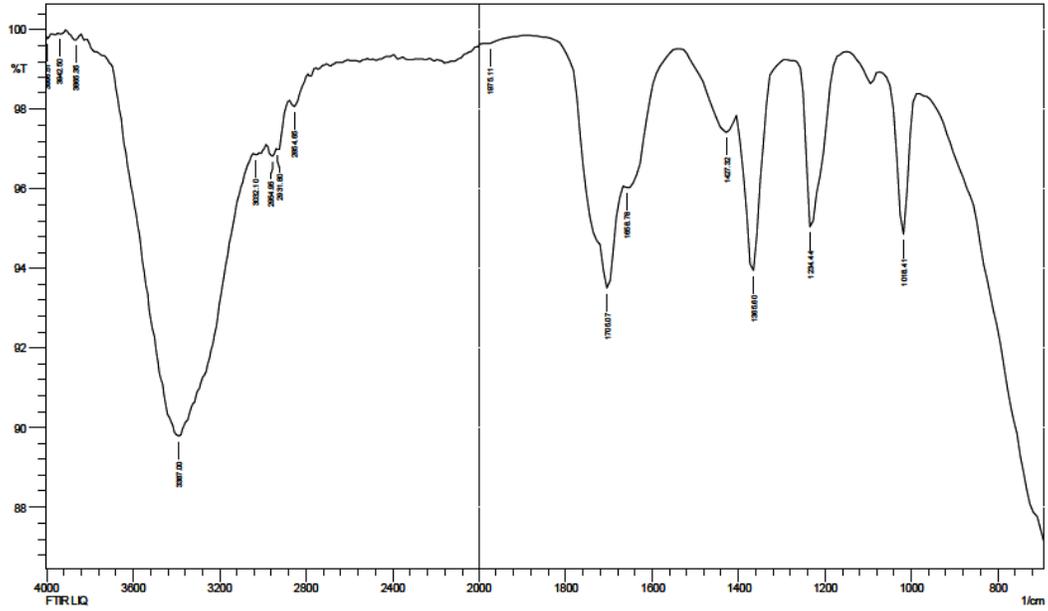


Figure 2. FTIR Spectra for Algal Biodiesel

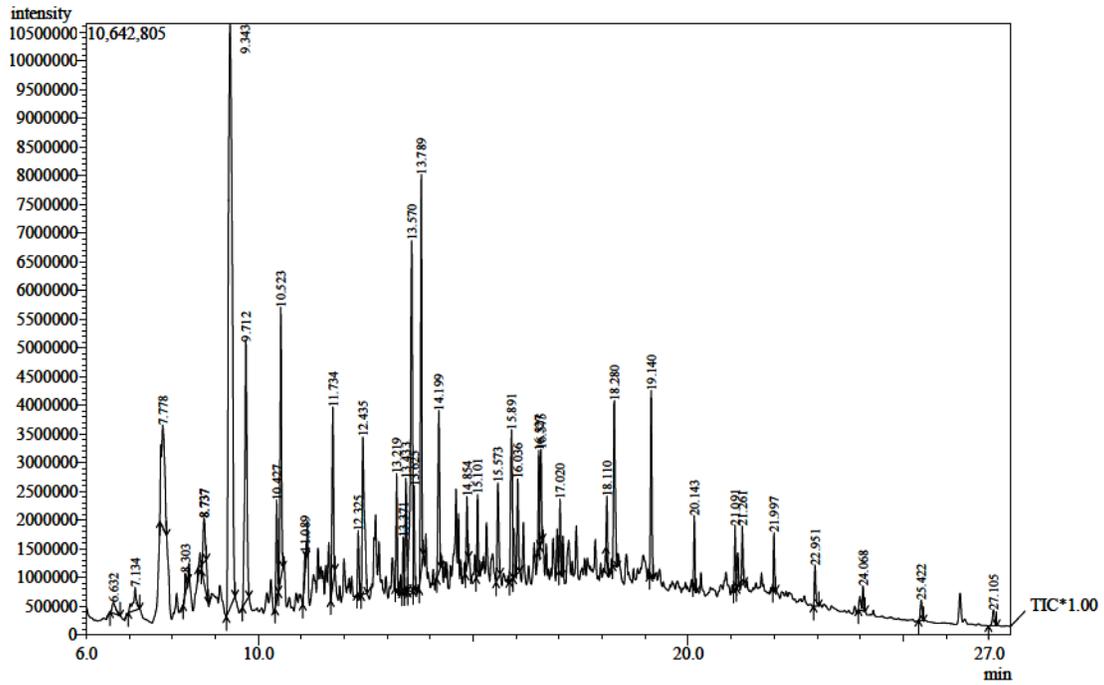


Figure 3. GC-MS Spectra for Algal Biodiesel

