

OPTIMUM PRODUCTION AND CHARACTERIZATION OF BIODIESEL FROM *MICROCYSTIS AERUGINOSA* ALGAE

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Abstract: This work examined the optimum production and characterization of biodiesel from *Microcystis aeruginosa* (*M. aeruginosa*). Optimization with Minitab software was used to design the experiment using Central Composite Design (CCD). The found optimum values of the solvent to biomass ratio, extraction temperature and time were 6.5:1, 60 °C and 144 minutes respectively. Validation of the optimum conditions for the lipids extracted was done to produce biodiesel. The average yield of the algal oil was found to be 26.7 %. The extracted algal oil was subjected to chemical transesterification using ethanolic-NaOH as catalyst and the biodiesel yield was 89.76 %. Fourier Transform Infrared Spectroscopy (FTIR) carried out on the produced biodiesel showed strong intensity of –C=O ester bond. Other characterizations done confirmed commendable suitability with conventional diesel properties.

Keywords: *Microcystis aeruginosa*, biodiesel, extraction, production, optimization

1. INTRODUCTION

Energy consumption has significantly and increasingly becoming one of the global issues that requires attention. Fossil fuels are considered to be the most readily available energy source, but the uncertainty of its sustainability is yet to be resolved [1]. Fossil fuels are the preeminent form of energy particularly in the oil-producing nations where activities of oil drilling have invariably created environmental hazards to man. Rapid depletion of fossil fuel necessitates alternative and sustainable fuel, which can replace the conventional fuel for the fulfillment of energy crisis with minimal environmental impact. Researchers are working continually to discover renewable, sustainable and eco-friendly energy sources, which can replace or reduce the excess load on the conventional fuel. Thus, the development of viable and renewable source of fuel is always a burning issue all over the world.

In more recent times, biodiesel has become an acceptable alternative option to researchers for supplementing conventional fuel. The properties of biodiesel are very close to diesel, which makes it possible for biodiesel to be blended in any proportion with diesel and to be used in existing engines without any modification. But at present, biodiesel costs more than diesel due to the higher cost of raw feedstock and unavailability of oil crops that serves as a source of biodiesel production [2]. Microalgae are one of the most prominent alternative sources for the conventional feedstock. Algae has considerable high oil content like some other feedstocks [3]. The yield (per acre) of oil from algae is over 200 times the yield from the best-performing plant/vegetable oils [3]. Biodiesel from algae is renewable, biodegradable, non-toxic, and a potential green alternative fuel for Compression Ignition (CI) engine. Biodiesel from algae has satisfactory combustion and emission profile than petro-diesel by contributing to lower combustion emissions than fossil fuels per equivalent power output [4].

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Biodiesel is a fuel containing long chain alkyl esters (methyl, ethyl or propyl), produced by treating oils or fat feed stocks with alcohol to produce fatty acid methyl esters. Biodiesel can be used in pure form (B100) or can be blended with petro-diesel in the form of B2 (2% biodiesel, 98% petroleum diesel), B5 (5% biodiesel, 95% petroleum diesel), B20 (20% biodiesel, 80% petroleum diesel) and B100 (pure biodiesel). Biodiesel is helping several countries in reducing their dependence on foreign oil reserves as it is domestically produced and can be used in most diesel engine with little or no modification to the engine or the fueling system. Integration of renewable energy resources in the energy sourcing could help in ameliorating the energy crises in the developing countries and seriously reduce the environmental unfriendliness of fossil fuel [5]. It will also help in achieving zero net carbon dioxide balance and improvement in urban atmospheric quality [6]. Several non-edible raw materials could be used to replace edible raw materials such as algae, which could be utilized as a source of biodiesel production [7]. Algae as a raw material in biodiesel production has several advantages with characteristics, which could be explored in Nigeria. Microalgae can be chemically treated easily as a result of their relatively small sizes. They are usually grown under conditions which are unsuitable for conventional crop production, have capability of fixing atmospheric CO₂, thereby facilitating the reduction of ever-increasing atmospheric CO₂ levels, which is a confronting global problem. Other benefits of microalgae are high photosynthetic efficiency due to their simple structure with daily harvesting and short harvesting cycle in comparison with crop plants. Algae have around 80% energy content to that contained by petroleum [2]. Algal cells have about 30% lipid content [8], which is higher than other sources including soybeans and palm oils [9, 10]. Microalgae have 30 - 40% lipid contents by dry weight. *Botryococcus braunii* is a microalgae which has 30 - 40% hydrocarbon content, which can be extracted easily [11].

In recent times, the concept of producing biodiesel from microalgae has gained interest by researchers due to the high oil yield derived from algal feedstock compared to other sources which produce lesser amounts of oil and are found to be less economical to use in biodiesel production. In March 6, 2018, ExxonMobil and Synthetic Genomics Inc. announced a new phase in their joint algae biofuel research program that could lead to the technical ability to produce 10,000 barrels of algae biofuel per day by 2025 [12]. Biofuel annual production has been estimated to be 35 billion liters [13]. Algae are now becoming the main source of biofuel production in the world. They are considered as the safer, non-competitive and rapidly growing organisms among those that could be used for biodiesel production. They have the abilities to grow without much care on waste nutrients [14], and are considered the better source of biodiesel production as other sources can cause food problems [15]. Moreover, biodiesel contents of crops are less sustainable and less in quantity as compared with algae [16]. *M. aeruginosa* are species of freshwater cyanobacteria (blue-green algae) which can form harmful algal blooms of economic and ecological importance. They are the most common toxic cyanobacterial bloom in eutrophic fresh water. In another study, *M. aeruginosa* was selected according to its sufficient oil content and simple cell structure, which can be easily ruptured during oil extraction using chemical methods according to El-Mekki and other co-researchers in year 2020 [17]. Cyanobacteria produce neurotoxins and peptide hepatotoxins, such as microcystin and cyanopeptolin.

This particular study aim is to produce biodiesel from *M. aeruginosa* algae species and give an optimum analysis on the algal lipids extracted to produce biodiesel by chemical transesterification of algal oil through optimization of algal lipid extraction parameters (temperature, reaction time, solvent to biomass ratio) and give a comparative analysis with physicochemical properties of conventional diesel products.

2. MATERIALS AND METHOD

2.1. Materials

All the chemical substances used in this work are of analytical grade, manufactured by Merck Germany. These include Gas Chromatograph (GC) ≥99.9% chloroform, EMSURE American Chemical Society (ACS) ISO Reag. Ph. Eur. ≥99.8% (GC) methanol, EMSURE ACS ISO, Reag. Ph. Eur. ≥99.8% (GC) ethanol, ≥97.0% sodium hydroxide, ≥98.0% sulphuric acid, distilled water, ACS, Reag. Ph Eur 0.5 wt.% in ethanol: water (1:1) Phenolphthalein indicator, ACS, ISO, Reag. Ph Eur ≥ 99.99% aluminum sulphate and ACS, ISO, Reag. Ph Eur ≥ 99.5% sodium chloride.

The apparatus used in this work are United States of America (USA) manufactured Generic model number 80-2 laboratory centrifuge, Pyrex glass wares (conical flasks, beakers, separating funnel), stirrer, porcelain mortar and pestle), USA manufactured Panasonic model number MX-AC300 blender, China manufactured TOPTIAN model number MS-H280-Pro Magnetic Heating Stirrer, England manufactured Gallenkamp model number BS300 electronic oven, USA manufactured Mettler Toledo model number PLS202-S Sensitive Weighing Balance, USA

manufactured Thermo Fisher Scientific model number Precision CIR 35 circulating water baths, USA manufactured Bruker Fourier Transform Infrared (FTIR) Tensor 27 (platinum ATR) model: Alpha Laser Class: 1 Spectrophotometer, USA manufactured Thermo Fisher Scientific Thermco ACC011C mercury in glass thermometers, England manufactured Pyrex measuring cylinders, Switzerland Linetronic Technologies model 312H – 317H hydrometers (700-750 kg/m³, 750-800 kg/m³, 800-850 kg/m³, 850-900 kg/m³, 900-950 kg/m³), China manufactured model RD-21DC4SA refrigerator, Austria manufactured Anton Paar Pensky Martens model number PMA5 Flash Point tester, USA manufactured model number DV2T Brookfield viscometer, rubber corks and USA manufactured Thermo Fisher Scientific model number 1210K19 Test jar.

Algal biomass of *M. aeruginosa* was harvested by flocculation using aluminum sulphate as flocculant. The pellet sediment was filtered using muslin cloth, followed by filtration carried out with filter paper.

2.2. Method

The practical approach adopted in this work are stated as follows with averages taken after three runs for the purpose of reproducibility of values.

2.2.1. Collection of microalgae species

The *M. aeruginosa* species was collected from an open pond at the lagoon front in the University of Lagos, Lagos, Nigeria.

2.2.2. Preparation of reagents

Alcoholic NaOH (0.5 M alcoholic sodium hydroxide) was prepared by dissolving NaOH pellets (20 g) in a 1000 mL conical flask using absolute ethanol and the solution was made up to 1 L using the same solvent. Hot neutralized ethanol was prepared by heating 10 mL of absolute ethanol in a water bath to 40 °C after which 2 – 3 drops of phenolphthalein were added. The hot ethanol was then titrated with 0.1 M NaOH to neutrality. Phenolphthalein indicator was prepared by dissolving 0.2 g of phenolphthalein in a given quantity of ethanol after which the solution was made up to the 20 mL mark using the same solvent (ethanol). Diluted 0.5 N sulphuric acid (H₂SO₄) was prepared by mixing 1 mL of 18 M H₂SO₄ with 35 mL of distilled water.

2.2.3. Algal oil extraction

The residue from the filtration process was collected and subjected to sun-drying for 24 hrs. The dried algae were ground thoroughly, and 10 g of each dried algae specie was measured. The algal oil extraction was carried out according to a modified method of Bligh and Dyer [18]. A sample of the dry algae powder was combined with a mixture of chloroform, methanol, and distilled water (1:1:0.8) in a beaker and the content was left to stand overnight for about 24 hours. After, the contents were centrifuged to separate the biomass and the biomass was washed with chloroform to extract the residual lipids present. The extracts were washed with 1% aqueous sodium chloride solution and then separated with a separation funnel. The solvent layer was heated in a water bath to obtain the algal oil.

2.2.3.1. Optimization of process parameters to obtain maximum lipid yield

In the experiment, MINITAB Copyright © 2019 was employed to analyze comprehensively the influences of three extraction parameters on the lipid extraction yield and determine the optimum extraction conditions. Table 1 shows the actual factor levels corresponding to the coded factor levels. In total, 20 experiments were designed.

Table 1. Levels of Parameters with their corresponding real values.

Parameter code	Parameter	-1.68	-1	0	1	1.68
X ₁	Solvent to Biomass Ratio (mL/g)	10	15	20	25	30
X ₂	Temperature (°c)	50	60	75	90	100
X ₃	Extraction Time (min)	30	60	120	180	210

2.2.3.2. Experimental extraction of oil from *M. aeruginosa*

A careful implementation of the optimization results was carried out. Since *M. aeruginosa* floats on water, the biomass was scooped into a 250 mL beaker and sun-dried to form an algal paste. The wet algal biomass was weighed up to 10 g to extract oil from it. Solvent extraction was used for the lipid extraction process using modified Bligh and Dyer [1] method where 210 mL chloroform-methanol mixture in the ratio 2:1 (v/v) was poured into a beaker containing the algal biomass. The mixture was agitated at 60 °C for 150 mins using a magnetic heat stirrer.

Up to 56 mL of 1 % NaCl-water solution was added to the agitated mixture. Phase separation was facilitated by 15 min of centrifugation at 3000 rpm and the lower phase (chloroform layer) was recovered for analysis. The chloroform solvent was removed from the chloroform-oil layer by vaporization. The chloroform-oil layer was heated to vaporize the chloroform until the liquid contained in the beaker had no significant change in mass. The residue after the vaporization process was the required lipid extracted.

2.2.4. Acid-catalyzed esterification

A quantity of 50 mL of the extracted oil was poured into a conical flask and heated at 55 °C for moisture removal. An appropriate volume of methanol and sulphuric acid mixture was heated in a separate flask and then poured into the algal oil slowly. A typical acid-catalyzed esterification is shown in Figure 1.

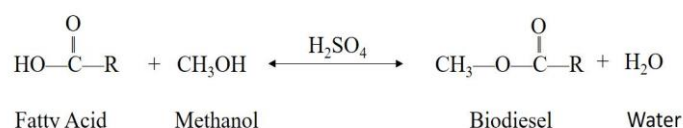


Fig. 1. A typical acid-catalyzed esterification reaction.

2.2.5. Transesterification of esterified oil

After esterification, the yield was improved upon by transesterification. The esterified oil was poured into a conical flask and heated at about 55 °C. In a separate flask, the catalyst NaOH was dissolved in methanol at various concentrations and molar ratios. The ethanolic-NaOH mixture was then heated to 55 °C and mixed with the esterified oil. The mixture was allowed a reaction time of 2 hours and settling time of 18 hours in a different separating funnel to separate the glycerol and biodiesel layer. The upper layer contained the biodiesel and the lower layer contained glycerol. The upper layer was collected and washed with distilled water for purification while the lower layer containing glycerol was discarded. A typical acid-catalyzed transesterification reaction is shown in Figure 2.

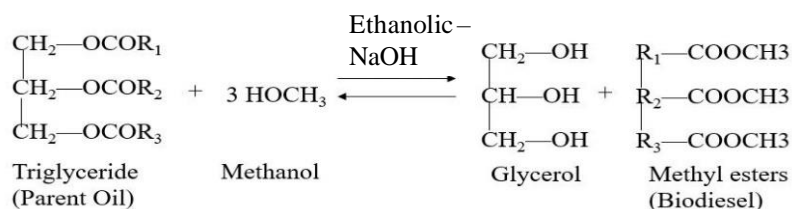


Fig. 2. A typical acid-catalyzed transesterification reaction.

A measured quantity of 6 mL of the extracted oil was poured into a beaker and heated at about 55 °C. In a conical flask, the catalyst 10 g NaOH was dissolved in 500 mL of ethanol to obtain 0.5 M ethanolic-NaOH solution. Then 18 mL of methanol was poured into the reactor containing 9 mL of ethanolic-NaOH and then thoroughly mixed and heated to 55 °C. The methanol-ethanolic-NaOH mixture was mixed with the extracted oil and allowed to react for 150 minutes. The product was poured into a separating funnel and allowed to settle for 30 minutes to separate the biodiesel and the glycerol layers. The upper layer (biodiesel layer) was collected and washed with distilled water for purification. The purified biodiesel was heated at 50 °C to remove moisture and the resulting biodiesel had an average weight of 4.91 g with an average volume of 5.4 mL.

2.2.6. Analytical calculations

The average lipid productivity is an indication of the oil produced. It was calculated with Equation (1).

$$V = \frac{C_i}{t} \quad (1)$$

where: C_i are concentration of lipids at the end of each batch process (mg/L), t are time to run the process (day), V are lipid productivity for each batch (mg/L-day).

The average biodiesel yield was calculated using Equation (2).

$$\text{Average yield of biodiesel} = \frac{\text{Weight of biodiesel produced (g)}}{\text{Weight of algal oil sample used (g)}} \times 100\% \quad (2)$$

2.2.7. Determination of biodiesel density

The density of biodiesel formed was determined by the hydrometer method given by the American Society for Testing and Materials (ASTM) procedure in ASTM D1298-12 [19].

2.2.8. Flash point determination

The flash point test was carried out using the Pensky-Martens closed-cup apparatus temperature measuring device and ignition source (Gas flame). Gas pressure did not exceed 3 kPa which was in accordance with ASTM D93-19 [20].

2.2.9. Determination of biodiesel viscosity

The viscosity of the biodiesel formed was determined according to ASTM D445-19a [21] using Brookfield Viscometer. For a 5 mL of the sample, the analysis was carried out at 40 °C.

2.2.10. Determination of distillation properties

Based on the composition, vapor pressure, expected Initial Boiling Point (IBP) or expected Final Boiling Point (FBP), or combination thereof, each sample was placed in one of five groups. The arrangement of apparatus temperature of the condenser and other operational variables are defined by the group in which the sample fell. Under necessary conditions, a 100 mL specimen of the sample was distilled for the group in which the sample fell. The distillation was performed following the test's methods specified by ASTM D86-20 [22].

2.2.11. Determination of higher heating value (HHV)

The higher heating value of the biodiesel was determined empirically. The HHV is an important property defining the energy content and thereby efficiency of fuels. The HHV of the biodiesel produced was determined from their viscosity (ν), density (ρ) and flash point (FP) according to Demirbas [23] as well as Sivaramakrishnan and Ravikumar [24].

The equation between viscosity and higher heating value for biodiesel is given in Equation (3)

$$HHV = 0.4625\nu + 39.450 \quad (3)$$

The equation between density and higher heating value for biodiesel is given in Equation (4):

$$HHV = -0.0259\rho + 63.776 \quad (4)$$

The equation between flash point and higher heating value for biodiesel is given by Equation (5):

$$HHV = 0.021FP + 32.12 \quad (5)$$

2.2.12. Determination of cloud point

The ASTM D2500-17a procedure [25] was adopted.

2.2.13. Determination of pour point

The ASTM D7346-15 method [26] was adopted.

2.2.14. FTIR analysis

The FTIR analysis was carried out on the biodiesel product using a USA manufactured Bruker Fourier Transform Infrared (FTIR) Tensor 27 (platinum ATR) model: Alpha Laser Class: 1 Spectrophotometer.

3. RESULTS AND DISCUSSION

3.1. Optimization result of process parameters to obtain maximum lipid yield

By analyzing the data in Table 1, the following second order polynomial in equation (6) was expressed in terms of coded values fitted to the results from the optimization.

$$Y (g) = 5.38 X_1 + 0.16 X_1 * X_2 + 1.08 X_2 + 2.491 X_3 + 1.878 \quad (6)$$

where, Y are stands for lipid extraction yield (gram of the dry weight), X_1 , X_2 and X_3 are as defined in Table 1.

To check the adequacy of the quadratic polynomial model, the statistical significance of the above equation was calculated with a correlation coefficient R^2 value of 0.9978, indicating that 99.78 % of the data in the Central Composite Design (CCD) could be explained by the response surface model. Therefore, the model revealed the effects of variables on the response value and predicted the maximum response value in subsequent optimization experiments. In addition, the Fisher's F-value of 401.02 demonstrated that the model was significant. A sufficiently large F-value indicated that the model is significant. In addition, the p-value which was less than 0.00001 further supported the fitness of the proposed model. The Fisher's F value is a ratio of the variance between groups to the variance within groups and the probability factor p-value must be less than 0.05 in the 95% confidence interval in the Analysis of Variance (ANOVA) analysis. The pure error of the fit had a small value of 0.76%. Therefore, these results clearly indicated that the model could be used to explain these data very well. The corresponding response values obtained from each run are illustrated in Table 2, based on the input from Table 1.

Table 2. Results and experimental layout in central composite design.

Run	Solvent to biomass ratio (mL/g)	Extraction Temperature (°C)	Extraction time (min)	Experimental lipid yield (g)
1	8.0:1	50	180	4.27
2	6.5:1	60	144	8.36
3	8.0:1	60	144	6.18
4	5.0:1	70	180	2.83
5	6.5:1	60	144	8.33
6	6.5:1	60	108	6.54
7	5.0:1	60	144	4.36
8	6.5:1	50	144	7.55
9	8.0:1	70	180	4.59
10	8.0:1	50	108	3.17
11	5.0:1	50	108	2.04
12	6.5:1	60	144	8.16
13	6.5:1	60	144	8.18
14	6.5:1	70	144	7.73
15	5.0:1	50	180	2.43
16	8.0:1	70	108	3.59
17	6.5:1	60	144	8.31
18	5.0:1	70	108	2.39
19	6.5:1	60	180	7.18
20	6.5:1	60	144	8.34

From the optimization, the linear coefficient indicated that the ratio of solvent to biomass (X_1) was the most significant independent variable impacting on extraction yield. The higher the ratio of solvent to biomass, the more the extraction yield as observed. The extraction time (X_3) also exerted a positive individual influence on the extraction yield, implying that an increase in the extraction time improved the lipid extraction amounts. In addition, the influence of the solvent to biomass ratio and extraction temperature ($X_1 * X_2$) also exerted significant interactive effects.

3.2. Experimental validation of the model

From the analysis, the optimum extraction conditions were obtained as shown in Table 3. The ratio of the solvent to biomass was 6.5:1 (mL/g) at 60 °C for 144 min of extraction time. The extraction yield obtained from *M. aeruginosa* was predicted to be 8.28 g extracted lipid. A surface plot for Y is shown in Figure 3.

Table 3. Optimization conditions.

Solution	X_1 (mL/g)	X_2 (°C)	X_3 (min)	Fit, Y (g)	Composite desirability
1	6.5	60	144	8.28	0.481481

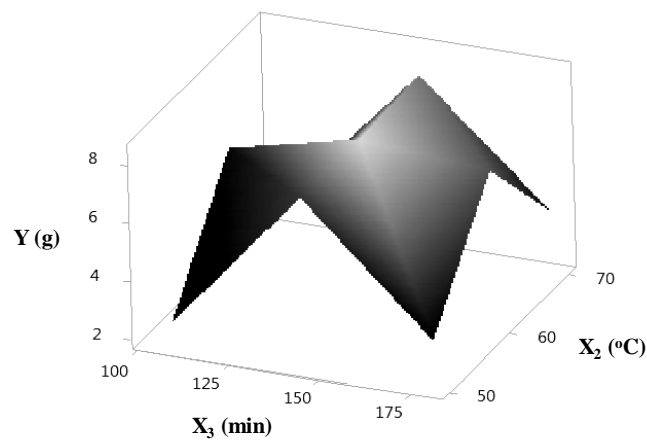
In order, to confirm these conclusions, extraction experiments based on the optimal extraction parameters were performed and the extraction yields were determined as given in Table 4. Three experiments were used to validate the model.

Table 4. Experimental lipid extraction yields using optimum conditions.

Run	Lipid extraction yield (g)	Percentage deviation from the model value (D) (%)
1	8.08	-2.415459
2	8.17	-1.328502
3	8.12	-1.932367

The extraction yields experimentally determined (Y_e) were close to that predicted by the model (Y_m), demonstrating the adequacy of the model as reflected in the low percentage deviation from the model value (D) given in equation (7) as presented in Table 4.

$$D = \frac{(Y_m - Y_e)}{Y_m} \times 100\% \quad (7)$$

Fig. 3. Surface Plot of Y (g) with X_2 ($^{\circ}\text{C}$), X_3 (min).

3.3. Experimental determination of average lipid content from *M. aeruginosa*

The average lipid content from *M. aeruginosa* are:

$$\text{Average Lipid content} = \frac{\text{Mass of oil produced (g)}}{\text{Mass of initial biomass (g)}} \times 100\% = 2.67/10 \times 100\% = 26.7\%$$

3.4. Determination of average yield of produced biodiesel

The average yield was calculated with equation (2).

Average weight of the biodiesel produced = 4.91 g

Average weight of algal oil used (from *M. aeruginosa*) = 5.47 g

$$\text{Average biodiesel yield} = \frac{4.91}{5.47} \times 100\% = 89.76\%$$

Da Rós and co-workers [27] studied the synthesis of biodiesel by enzymatic route using *M. aeruginosa* lipids as feedstock. They found that the lipid content of *M. aeruginosa* biomass was 28.10 ± 1.47 % which closely corresponds to 26.7 % obtained experimentally in this work. Close results was also reported by El-Mekki and fellow researchers in year 2020 [17]. Biodiesel yield was obtained to be 89.76 %, which was also encouraging.

3.5. Comparison of the properties of produced biodiesel with conventional diesel using ASTM and European Standards (EN)

In Table 5, all the determined properties are within expected ranges specified by the two standards [21] considered as well as that of petroleum derived diesel (petro-diesel). Viscosity is one of the most important biodiesel properties 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 kinematic viscosity of the *M. aeruginosa* produced biodiesel was 2.04 mm²/s. This value is within the range obtained by Kumari and co-workers when various blends of diesel and safflower oil were utilized in diesel engines which ranged from estimated values of 2 to 6.24 cSt [28].

Table 5. Comparison of produced biodiesel properties with petro-diesel, ASTM and EN standards [29].

Fuel property	EN 14214 [30]	ASTM D6751-20 [31]	Biodiesel from <i>M. aeruginosa</i>	Petro-diesel
Higher heating value (MJ/kg)	-	-	63.7542	43.3-46.7
Kinematic viscosity at 40 °C (mm ² /s)	3.5-5.0	1.9-6.0	2.04	1.9-3.8
Density (g/l)	0.86-0.9	-	0.84	0.84-0.86
Flash point (°C)	140 minimum	140 minimum	145	139
Initial boiling point (°C)	160	160	175	160
10%	205	205	223	-
50%	254	255	270	-
90%	305	304	330	-
Final boiling point (°C)	-	-	375	-
Cloud point (°C)	-	-3 to 15	5	-1
Pour point (°C)	-	-5 to 10	9	-

The flash point of the biodiesel from *M. aeruginosa* was observed to be 145 °C, which was above the 140 °C minimum required. Cloud and pour point were respectively 5 and 9 °C which was also within specified ranges. The Higher heating value refers to the measure of the energy content in the fuel. The lower the heating value of the produced biodiesel, the lower the engine power derivable from it. The biodiesel produced has a heating value of 63.7542 MJ/kg, which was higher than most other biodiesel products (43.3 - 46.7 MJ/kg). This is an encouraging parameter in the produced biodiesel.

3.6. Fourier Transform Infrared Spectroscopy (FTIR) analysis

From the FTIR chart in Figure 4 and interpretation given in Table 6 for the biodiesel obtained from *M. aeruginosa*, it can be concluded that there is a strong presence of $-C=O$ bond. This indicated the presence of esters in the product which is the predominant functional group in biodiesel. Specific wavelengths of 3320.22, 1641.00, 1066.19, and 1015.36 cm⁻¹ corresponding to various functional groups are presented in Table 6.

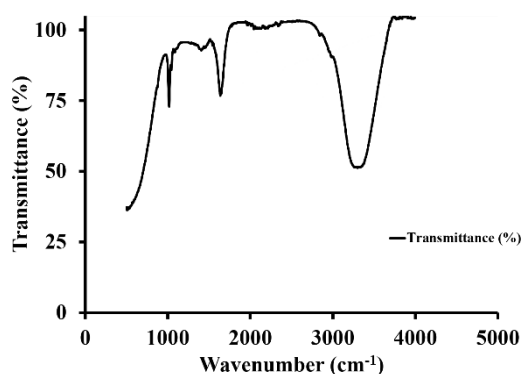


Fig. 4. Fourier Transform Infrared Spectroscopy (FTIR) of Produced Biodiesel.

Table 6. Functional Group Analysis from FTIR of Produced Biodiesel.

Functional Group	Wave Number(cm^{-1})	Intensity
Alcohol OH Stretch	3320.22	Strong
C=O ester	1641.00	Strong
C-OH stretch	1066.19	Strong
C-F	1015.36	Strong

4. CONCLUSIONS

Following the observations made, it can be concluded that it is encouraging to produce biodiesel using *M. aeruginosa* as feedstock. This is based on their availability in our immediate environment, which reduces the cost in purchasing feedstock for the purpose of the production of biodiesel. It can be concluded that the higher the solvent to biomass ratio, the higher the extraction yield. Also, the higher the time allotted for extraction, the higher the extraction yield. Temperature has little effect on the extraction yield. The extraction process could seemingly be carried out at room temperature. The extraction yields experimentally determined was close to that predicted by the model. Therefore, it is concluded that the model is accurate and valid. Biodiesel production from microalgae in Nigeria is still in the infant stage. The lipid content ranged from 20-29%, which is a reasonable amount of lipid present in the algae. As a result, the biodiesel produced from this process is adequate enough to be used for machines and vehicles because the fuel meets desired specifications set by some regulatory bodies. Therefore, it can be said that the process is a newly discovered alternative convincingly. The present study shows the availability of microalgae species in blooms at a location in Nigeria. In this manner, the use of algae should be adopted as feedstock for renewable energy due to availability, low cost, and ease of cultivation. Further investigation could focus on optimization of biodiesel from these species while varying more parameters such as using different solvent mixtures and varying the mixing intensity during the extraction process.

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