Research Paper / Makale

Optimum Production and Characterization of Biodiesel from *Spirogyra* Algae

Babatope OLUFEMI[1*], Saleem SULAIMON[1], Adewale ARIKAWE[1]

[1]Department of Chemical and Petroleum Engineering, University of Lagos, Akoka, Lagos, Nigeria.
bolufemi@yahoo.com

Abstract: This work focused on the optimum production and characterization of biodiesel from *Spirogyra.* Parametric optimization with Minitab software was used to design the experiment using Central Composite Design (CCD). Optimum values for the solvent to biomass ratio, extraction temperature and time were 15 ml/g, 100 ^{o}C and 30 minutes respectively. Careful and practicable implementation of the optimum conditions for lipids extraction and biodiesel production was done. The algal oil average yield was 20.25 %. The extracted algal oil was chemically transesterified using ethanolic-NaOH as catalyst. The average yield of the biodiesel was 62.96 %. Fourier Transform Infrared Spectroscopy (FTIR) analysis of biodiesel showed strong intensity of –C=O ester bond. Characterizations done confirmed good suitability with conventional diesel properties and standards.

Keywords: Biodiesel; Spirogyra*;* algae; optimization

Spirogyra Algae Biyodizel Optimum Üretimi ve Karakterizasyonu

Öz: Bu özel çalışma, Spirogyra'dan biyodizelin optimum üretimi ve karakterizasyonuna odaklanmıştır. Minitab yazılımı ile parametrik optimizasyon, Merkezi Kompozit Tasarım (CCD) kullanarak deneyi tasarlamak için kullanıldı. Solvent / biyokütle oranı, ekstraksiyon sıcaklığı ve süresi için optimum değerler sırasıyla 15 ml / g, 100 ^{o}C ve 30 dakika idi. Lipit ekstraksiyonu ve biyodizel üretimi için optimum şartların dikkatli ve pratik uygulaması yapıldı. Alg yağı ortalama verimi% 20.25 idi. Çıkarılan yosun yağı, katalizör olarak etanolik-NaOH kullanılarak kimyasal olarak transesterifiye edildi. Biyodizelin ortalama verimi% 62.96 idi. Biyodizelin Fourier Dönüşümü Kızılötesi Spektroskopisi (FTIR) analizi, güçlü -C = O ester bağının yoğunluğunu göstermiştir. Yapılan karakterizasyonlar, geleneksel dizel özellikleri ve standartları ile iyi uygunluğu doğruladı.

Anahtar Kelimeler: Biyodizel; Spirogyra; yosun; optimizasyon

1. Introduction

Over the years and recently, various efforts had focused on the role of algaes as a possible substitute for the production of biofuels. The possibility of substituting fossil fuel with an ideal biofuel reactant had been reported by Khan *et. al.* [1]. Konga *et al* [2] made use of *Spirogyra* algae using the Soxhlet extraction process. They reported various characteristics such as saponification value, iodine value, calorific value, flash point and acid value. The oil extracted had a calorific value of 3,257 kcal/kg, flash point of 78 ^{o}C, showing that oil produced could serve as a potential fuel substitute. Haq and coworkers had optimized the transesterification of oil from *Spirogyra* in which different parameters such as the alkyl group donor, reaction temperature, stirring time and oil to alcohol ratio were investigated. From their work, the maximum biodiesel conversion of *Spirogyra* was 87.5% with an oil to alcohol ratio of 1 to 8 [3]. Recently Gomaa and fellow researchers in 2020

demonstrated the potential of using microbial mats from desert streams to produce bioethanol, biodiesel and biomethane, which was dominated by the alga *Spirogyra* [4].

Some results that indicated the feasibility of using *Spirogyra* to recover nutrients from multiple municipal wastewater sources with the simultaneous production of biomass that contains value-added biochemical components for bioenergy and biofuel applications had been reported by Ge *et al* [5]. A high yield of biofuel was recorded through a fermentative process for untreated *Spirogyra* biomass when compared to chemically pre-treated biomass, and the yield of the biofuel using the algal biomass was more when compared to that produced from other sources like agrobased raw materials [6]. Accoring to Balat [7], the production of biodiesel is a very modern and technological area for researchers due to the relevance attached to it everyday because of the increase in the petroleum price and the environmental advantages it possesses. It had been observed that microalgae show a higher growth rate, which makes it possible to satisfy the huge demand on biofuels using limited land resources without causing shortage in biomass [8]. In addition, growing of microalgae requires lesser water relatively to terrestial crops, and are able to tolerate high carbon dioxide content in gas streams. Thus, microalgal farming could be more cost effective than conventional farming [8].

From the works of Baig *et al.* [9], *Spirogyra* collected from Chashma, Achuzai and Quetta in Pakistan had been used for biodiesel production. The oil from the *Spirogyra* was extracted using n-Hexane as a solvent and the effects of n-Hexane to oil ratio, size of algal biomass and contact time on the percentage yield of extracted oil was studied, analyzed and reported. They concluded that maximum amount of oil was extracted from the *Spirogyra* species by using a greater ratio of solvent to algal biomass, maximum contact time and smaller algal biomass size. It had been reported that oils from microalgaes can be arranged in the decreasing order of oxidation stability index according to Kumar and Sharma [10]. In their order, Pithophora > *Spirogyra* > Hydrodictyon > Cladophora > Tolypothrix > Rhizoclonium based on their oxidizability, which signified that Pithophora takes more time to oxidize, followed by *Spirogyra* and are best suited for biodiesel production in that order. As the order decreases, the other oils lower in the list needs antioxidants to increase their stability in the production of biodiesel. ExxonMobil and Synthetic Genomics Inc. on March 6, 2018 announced a new phase in their joint algae biofuel research program that could lead to the technical ability to produce up to 10,000 barrels of algae biofuel per day by year 2025 [11].

Reddy and Majumder [12] had reported another technique of oil recovery from *Spirogyra* under an optimized condition at 50% ammonium sulphate concentration using tert-butanol (in 1 : 1, v/v ratio). It was observed that a presonicated and papain treated algal suspension produced 24% (w/w, dry weight) oil within few hours which was realized to be ten times higher compared to the oil obtained by Soxhlet extraction using hexane and two times higher than the oil obtained without using the protease. About 3.333 g of oil had been extracted from 15 g dry weight of *Spirogyra* algae in comparison with some others. It was found out that pH variation was insignificant among the species considered [13]. Another work reported 14.82% of oil extracted from *Spirogyra* and comparison made with oil from other algae did not show significant change in pH values [14]. In another effort in a comparative study, results showed that high-yield of biofuel was obtained from untreated *Spirogyra* biomass when compared to chemically pretreated biomass [15]. It had been reported that *Spirogyra* is the most frequent genus of freshwater green algae [16]. In this inexhaustible and interesting field, being a very promising, sustainable, environmentally friendly and renewable area of study, various efforts are being used scientifically and technologically to produce biodiesel from *Spirogyra* and another methodology using pyrolysis was used to produce biodiesel from *Spirogyra* [17].

This particular study is to optimally produce biodiesel from *Spirogyra* algae species obtained at a location in Lagos State, Nigeria. Estimate the algal lipids yeild extracted to produce biodiesel by

chemical transesterification of algal oil through parametric variation of algal lipid extraction parameters (temperature, reaction time, solvent to biomass ratio) and give a comparative analysis with physicochemical properties with respect to biodiesels as well as conventional petro-diesel products to ascertain the produced biodiesel suitability.

2. Materials and Method

2.1. Materials

The materials used in this work are Magnetic Heating Stirrer (TOPTION, MS-H280-Pro, China), Electronic Oven (Gallenkamp, BS300, England), Sensitive Weighing Balance (Mettler Toledo, PLS202-S, USA), Chloroform (Merck, 02487, Germany), Phenolphthalein, Hydrochloric acid, Methanol (EMSURE, ACS, ISO, Reag. Ph. Eur, Germany), Ethanol (EMSURE, ACS, ISO, Reag. Ph. Eur, Germany), Sodium Hydroxide (Merck, 2815 11 00, Germany), pH meter (HANNA instruments pHep*), Sulphuric acid, Distilled water, Thermometer (-2 to 300 ^{o}C, -2 to 400 ^{o}C), Measuring Cylinder (100 ml, 250 ml) (Pyrex, England), Hydrometer (700-750 kg/m^{3}, 750-800 kg/m^{3}, 800-850 kg/m^{3}, 850-900 kg/m^{3}, 900-950 kg/m^{3}), Refrigerator, Pensky Martens Flash Point tester (AntonPaar PMA5, Austria, Brookfield Viscometer (DV2T, USA), Test jar, Cork, USA manufactured Bruker Fourier Transform Infrared (FTIR) Tensor 27 (platinum ATR) model: Alpha Laser Class: 1 Spectrophotometer, Centrifuge (Generic, 80-2 laboratory centrifuge, USA), Glass wares (Pyrex) – Conical flasks, beakers, separating funnel, stirrer, Porcelain Mortar and Pestle, Blender (Panasonic, MX-AC300, USA) and Electric water bath (Wine Light Analytical system)

2.2. Method

Figure 1 shows the workflow of the methodology adopted in this work. The practical approach adopted in this work are presented as follows with averages taken after three runs for the purpose of reproducibility of values.
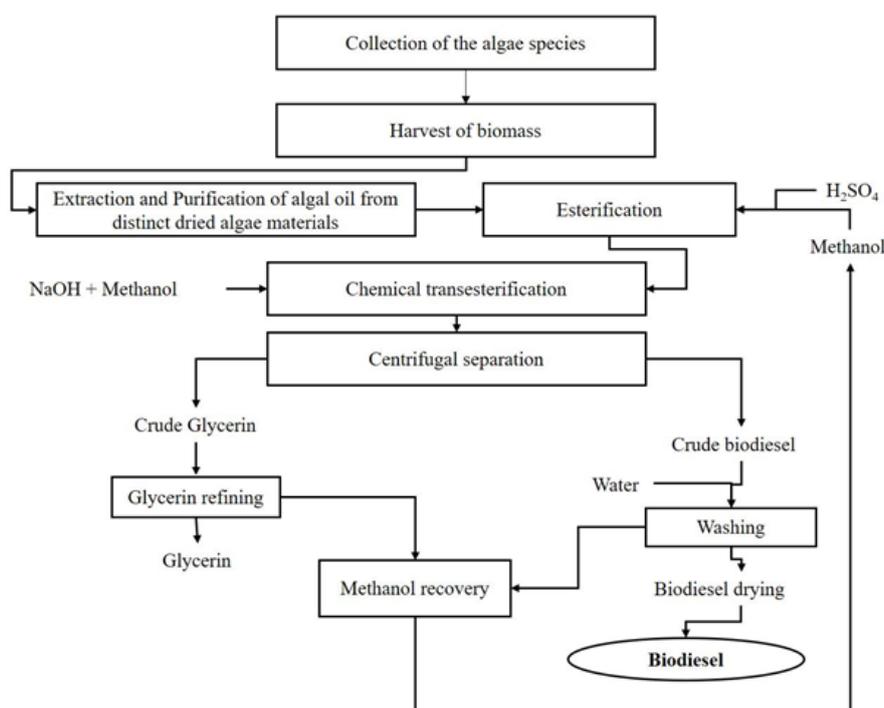
Figure 1. Workflow of adopted methodology

2.2.1. Collection of Microalgae Spirogyra Species

The *Spirogyra* species was collected from a residential area in the University of Lagos, Akoka, Lagos, Nigeria.

2.2.2. Preparation of the Reagents

For this purpose, alcoholic NaOH (0.5 M alcoholic sodium hydroxide) was prepared by dissolution of NaOH pellets (20 g) in a 1000 ml conical flask using absolute ethanol, while the solution was made up to 1 L with the same solvent. After, hot neutralized ethanol was prepared by heating 10 ml of absolute ethanol in a water bath to 40 °C, after then 2 – 3 drops of phenolphthalein were added. The hot ethanol was titrated with 0.1 M NaOH to give neutral solution. Phenolphthalein indicator was also prepared by dissolving 0.2 g of phenolphthalein in a quantity of ethanol after which the solution was made up to the 20 ml mark using ethanol solvent. Diluted 0.5 N sulphuric acid (H_2SO_4) was also prepared by mixing 1 ml of 18 M H_2SO_4 with 35 ml volume of distilled water.

2.2.3. Harvesting of the Biomass

The algal biomass of *Spirogyra* was harvested in the solid state from the collection source. The pellet sediment from its washing solution was filtered using muslin cloth, and then followed by filtration with a filter paper.

2.2.4. Extraction of the Algal oil

The residue from the filtration process was collected together and sun-dried for 24 hrs. After drying, the dried algae were ground properly and 10 g (above the optimun predicted value) of the dried algae species was measured. The algal oil extraction was carried out following a modified method of Bligh and Dyer [18]. A combined sample of the dry algae powder with a mixture of chloroform, methanol and distilled water (1:1:0.8, v/v) in a beaker was made and the content was left 24 hours. Afterwards the contents were centrifuged to separate the biomass, while the biomass was washed with chloroform to extract the residual lipids. The extracts were then washed with 1 % aqueous sodium chloride solution and later separated using a separation funnel. The solvent layer was later heated in a water bath to obtain the algal oil from it.

2.2.4.1. Optimization of Process Parameters to Obtain Maximum Lipid Yield From Spirogyra

To guide the extraction experiment, MINITAB Copyright © 2019 was used to analyze the influence of three extraction parameters on the lipid extraction yield, as well as to specify the optimum extraction conditions. Table 1 presents the actual factor levels corresponding to the coded factor levels. Twenty (20) experiments were designed in total.

Table 1. Parameters Levels and their Corresponding Real Values

Parameter code	Parameter	-1.68	-1	0	1	1.68
X1	Solvent to Biomass Ratio (ml/g)	5	7	10	13	15
X2	Temperature (^{o}C)	50	60	75	90	100
X3	Extraction Time (min)	30	60	120	180	210

2.2.4.2. Experimental Extraction of Oil from Spirogyra

This stage involved a careful possible implementation of the optimization results specified. The biomass was washed using water for purification and then sun-dried for 5 hours. The dried biomass

was then ground using a blender and sieved to obtain evenly-sized fine powder. Exactly 8.05 g of *Spirogyra* biomass powder was weighed and poured into a 250 ml beaker. Using the modified Bligh and Dyer [18] method, 150 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 (so as not to destroy the biomass thermally) for 3 hrs (well above the optimum required) using a magnetic heat stirrer. Up to 40 ml of 1% NaCl-water solution was added to the agitated mixture. Phase separation was facilitated by 15 min of centrifugation at 3,000 rpm and the lower phase (chloroform layer) was recovered for further analysis. The chloroform solvent was then removed from the chloroform-oil layer by vaporization. The chloroform-oil layer was later heated to vaporise the chloroform until the liquid contained in the beaker had no change in mass. The residue from the vaporisation process was the required lipid extracted. The extracted lipid was weighed to be 1.63 g.

2.2.5. Acid-catalyzed Esterification of Extracted Oil

A volume of 50 ml of the extracted oil was poured into a conical flask and heated at a temperature of 55 °C to remove moisture. The required volume of methanol and sulphuric acid mixture was heated in a separate flask and then poured slowly into the algal oil. A typical acid catalyst esterification reaction of the extracted oil is shown in Fig. 2.
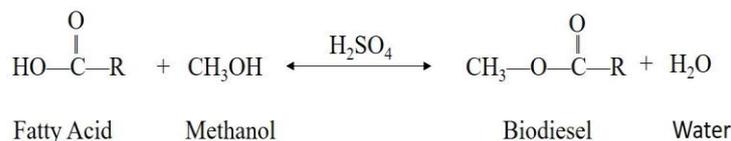
Figure 2. A typical acid catalyst esterification reaction of extracted oil

2.2.6. Transesterification Process of Esterified Oil

The oil that was esterified was poured into a conical flask and heated at 55 °C temperature. Inside a separate flask, the NaOH catalyst was dissolved with methanol using various concentrations and molar ratios. The ethanoic-NaOH solution was heated to 55 °C and mixed with the oil that was esterified. The resulting mixture was allowed to react for 2 hours and allowed to settle for 18 hours in a different separating funnel so as to separate the glycerol and biodiesel layer. The upper layer was the biodiesel and the lower layer was glycerol. The upper layer was then collected and washed with distilled water to purify, while the lower layer of glycerol was discarded. The acid catalyzed transesterification reaction is shown in Fig. 3.
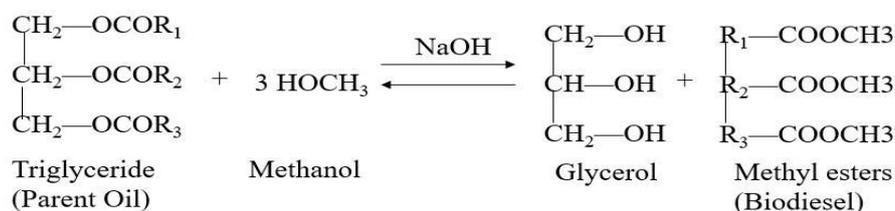
Figure 3. The acid catalyzed transesterification reaction

Afterwards a volume of 5 ml of the extracted oil was poured into a beaker and heated at a temperature of 55 °C. Inside a conical flask, the 10 g NaOH catalyst was dissolved in 250 ml of ethanol to get a 0.5 M ethanolic NaOH solution. Afterwards, 18 ml of methanol was poured into the reactor with 9 ml of ethanolic NaOH and then properly mixed and also heated to 55 °C. The

methanol-ethanolic NaOH mixture was mixed later with the extracted oil and allowedbfor a reaction time of 150 minutes. The resulting product was then poured into a separating funnel so as to settle for 30 minutes and separate the biodiesel from the glycerol layers. The upper layer of biodiesel was collected and washed with distilled water for purification. The purified biodiesel obtained was heated at 50 ^{o}C to remove any moisture, which gave biodiesel of an average weight of 3.62 g, with an average volume of 4.3 ml.

2.2.7. Analytical Calculations

A parameter called the average lipid productivity, which is an indication of oil produced on basis of volume and time was calculated according to Equation (1);

$$V = \frac{c_i}{t} \tag{1}$$

where,
C_1 = Concentration of lipids at the end of each batch process (mg/L)
t = Time run of the process (day)
V = Lipid productivity for each batch (mg/L-day)
The average biodiesel yield was determined with Equation (2).

$$Average\ yield\ of\ biodiesel = \frac{Weight\ of\ biodiesel\ produced\ (g)}{Weight\ of\ algal\ oil\ sample\ used\ (g)} x100\% \tag{2}$$

2.2.8. Determination of the Density of Produced Biodiesel

The density of biodiesel produced was determined by the hydrometer method as prescribed by the American Society for Testing and Materials (ASTM) procedure in ASTM D1298.

2.2.9. Flash Point Determination of Produced Biodiesel

The flash point was determined with the Pensky-Martens closed-cup apparatus temperature measuring device and ignition source (Gas flame). The gas pressure did not exceed 3 kpa in accordance with ASTM D93.

2.2.10. Determination of Viscosity of Biodiesel Produced

The viscosity of the biodiesel formed was determined according to ASTM D-445 using Brookfield Viscometer. For a 5 ml of the sample, the analysis was carried out at 40 °C.

2.2.11. Determination of Biodiesel Distillative Properties

As a requirement for composition, vapor pressure, expected Initial Boiling Point (IBP) or expected Final Boiling Point (FBP), or their possible combination, each sample was placed in one of the five groups. The apparatus arrangement, temperature of the condenser and other operational variables are as defined by the group in which the sample fell. Under required conditions, a 100 ml of the sample was distilled for the group in which the sample belong to. The distillation was carried out in accordance with the tests methods specified by ASTM D86.

2.2.12. Determination of Higher Heating Value (HHV) of Biodiesel Products

Determination of the higher heating value of the biodiesel was done using empirical correlations. The HHV is an important property defining the energy content and indirectly

efficiency of fuels. The HHV was determined from the values of the viscosity (v), density (ρ) and flash point (FP) according to Demirbas [19] as well as Sivaramakrishnan and Ravikumar [20].

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

$$HHV = 0.4625v + 39.450 \qquad (3)$$

The relationship between density and higher heating value of biodiesel produced is given in Equation (4):

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

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

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

2.2.13. Determination of the Cloud Point of Biodiesel Produced

The biodiesel sample was filled into a test jar up to a mark and then placed inside a cooling bath. The temperature at the bottom of the test jar, at which the biodiesel start to form a cloud was taken as the cloud point.

2.2.14. Determination of Biodiesel Pour Point

After heating the sample and cooled at a specified rate, it was examined at intervals of 3 $^{\circ}$C for flow characteristics. The lowest temperature at which slight movement of the specimen took place was recorded as the pour point.

2.2.15. FTIR Analysis

The FTIR analysis was done 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. Optimisation Result of Process Parameters to Obtain Maximum Lipid Yield

Analysis of the data in Table 1 yeilded the following second order polynomial in Equation (6), which was expressed in terms of coded values fitted to the results of the optimization.

$$Y(g) = 0.649X1 - 1.081X2 + 2.303X3 + 4.365 \qquad (6)$$

Where, Y represents the lipid extraction yield (gram of the dry weight); X1, X2 and X3 are already defined in Table 1.

As a requirement to check the adequacy of the polynomial model, the statistical significance of Equation (6) was obtained with a Correlation Coefficient R^2 value of 0.9414, which indicated that 94.14% of the data in the Central Composite Design (CCD) could be explained perfectly by the response surface model. The model therefore revealed the effects of important variables on the

response value and also predicted the maximum response value in subsequent optimization experiments. The Fisher's F-value of 9.36 showed the significance of the model. It is required that a F-value hgher than zero indicated that the model is significant. Futhermore the p-value which was 0.003 confirmed the fitness of the proposed model. The Fisher's F value by definition is a ratio of the variance between groups to the variance within groups and the probability factor p-value should be less than 0.05 in the 95% confidence interval in the Analysis of Variance (ANOVA) analysis. The calculated pure error of the fit was 0.3656%. From the foregoing analysis, these results clearly showed that the model could be used to represent the data very well. From the optimization, the corresponding response values obtained from each run are given in Table 2, as a result of the input from Table 1.

Table 2. Results and Experimental Layout in Central Composite Design

Run	Solvent to biomass ratio (ml/g)	Extraction Temperature (^{o}C)	Extraction time (min)	Experimental lipid yield (g)
1	15	90	120	2.37
2	13	50	180	5.03
3	15	50	30	5.50
4	10	75	120	6.42
5	13	75	180	5.79
6	15	100	30	6.70
7	5	75	180	3.87
8	10	50	30	7.33
9	10	100	180	6.72
10	7	50	210	1.46
11	15	100	120	5.03
12	10	90	120	6.03
13	7	60	30	4.27
14	15	75	60	5.12
15	7	90	30	4.89
16	7	100	60	4.60
17	15	100	60	5.56
18	10	50	210	2.72
19	7	75	30	5.28
20	10	50	60	6.19

From the optimization, the linear coefficient indicated that the ratio of solvent to biomass (X1) temperature (X2) and time (X3) have varying significance impacting on extraction yield. The higher their values, the more the extraction yield as observed. The extraction time (X3) seems to exert the largest influence on the extraction yield, implying that an increase in the extraction time could improve the amount of lipid extracted more than the other variables.

3.2. Parametric Variation of Variables from the Model on Extraction Yield Optimization

The optimum extraction conditions obtained is given in Table 3. The ratio of the solvent to biomass was 15:1 (ml/g) at 100 ^{o}C for 30 min of extraction time. The lipid extraction yield predicted obtainable from *Spirogyra* was 6.228 g of lipid. The composite desirability value of 0.8123 further confirmed storng influence of the independent variables on the optimum yeild.

Table 3. Optimization Conditions

Solution	X1 (ml/g)	X2 (^{o}C)	X3 (min)	Fit, Y (g)	Composite Desirability
1	15	100	30	6.228	0.8123

The surface plot for Y as functions of X2 and X3, X1 and X2 as well as X1 and X3 are shown in Figures 4, 5 and 6 respectively.
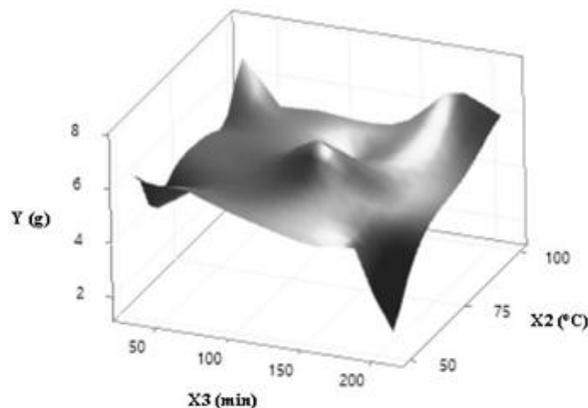
Figure 4. Surface Plot of Y (g) with X2 (^{o}C) and X3 (min)
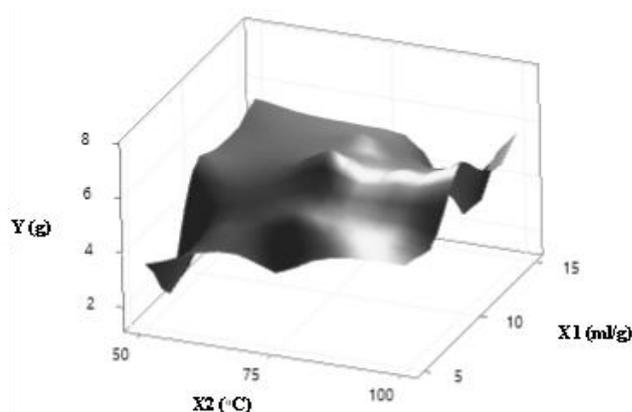
Figure 5. Surface Plot of Y (g) with X1 (ml/g) and X2 (^{o}C)
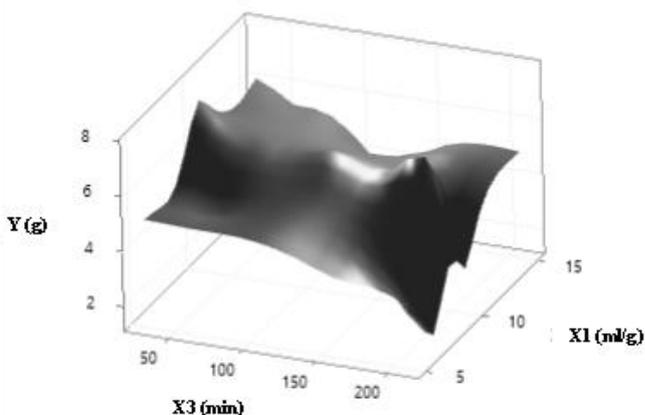
Figure 6. Surface Plot of Y (g) with X1 (ml/g) and X3 (min)

3.3. Experimental Determination of Average Lipid Content from Spirogyra

The average lipid content from Spirogyra is calculated by using equation (2) as

$$\frac{1.63}{8.05} \times 100\% = 20.25\%.$$

3.4. Determination of Average Yield of Produced Biodiesel

The average yield was calculated with Equation (2). The average weight of the biodiesel produced = 3.62 g. The average weight of algal oil used (from *Spirogyra*) = 5.75 g.
The result;

$$\text{Average biodiesel yield} = \frac{3.62}{5.75} \times 100\% = 62.96\%$$

3.5. Comparison of Some Useful Properties of Produced Biodiesel with Conventional Diesel Using ASTM and European Standards

As it can be seen in Table 4, all the determined properties are close to expected ranges specified by the two standards [21] considered as well as that of the conventional petro-diesel.

Table 4. Comparison of the properties of produced biodiesel with petro-diesel, ASTM and EN standards [21]

Fuel Property	EN 14214	ASTM D6751	Biodiesel from *Spirogyra*	Petro-diesel
Higher heating value (MJ/kg)	-	-	63.7537	43.3-46.7
Kinematic Viscosity at 40 ^{o}C (mm^2/s)	3.5-5.0	1.9-6.0	3.48	1.9-3.8
Density (g/l)	0.86-0.9	-	0.86	0.84-0.86
Flash Point (^{o}C)	140^{min}	140^{min}	140	139
Initial Boiling Point (^{o}C)	160	160	170	160
10%	205	205	209	-
50%	254	255	259	-
90%	305	304	317	-
Final Boiling Point (^{o}C)	-	-	343	-
Cloud Point (^{o}C)	-	-3 to 15	4	-1
Pour Point (^{o}C)	-	-5 to 10	9	-

Viscosity serves as a major important biodiesel propery because it affects the operation of fuel injection equipment, especially at low operating temperatures where the increase in viscosity adversely affects the flow of the fuel. This is because high viscous fuel causes poorer atomization of the fuel spray and leads to less accurate operation of the fuel injector engines. The kinematic viscosity of the *Spirogyra* produced biodiesel at 40 ^{o}C specification was 3.48 mm^2/s. This value fell within the range of values obtained by Kumari *et. al*. [22] when various blends of diesel and safflower oil were utilized in some diesel engines and estimated values from 2 to 6.24 cSt were obtained. The flash point of the *Spirogyra* produced biodiesel was 140 ^{o}C, exactly the same value of 140 ^{o}C minimum requirment. The Cloud and pour points were 4 and 9 °C respectively, which was also within the ranges specified. Another parameter known as the higher heating value refers to the measure of the energy content in the fuel. Usually, the lower the heating value of the produced biodiesel, the lower the engine power achieveable from it. The *Spirogyra* produced biodiesel has a heating value of 63.7537 MJ/kg. This value was desirably higher than that of petro-diesel products

(43.3 - 46.7 MJ/kg) obtained. This higher value is an encouraging property of the produced biodiesel.

3.6. Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Biodiesel Produced

Reffering to the FTIR chart in Fig. 7 and interpretation given in Table 5 for the biodiesel obtained from *Spirogyra*, it was discovered that there was a strong presence of –C=O bond. This directly indicates the presence of esters in the product which is the predominant functional group in biodiesel products. The CH and OH functional groups are also represented with their peaks at the corresponding wavenumbers. Also strongly represented are the C-OH and C-F functional groups. The other groups in Table 5 showed medium and weak intensities as depicted in Figure 7.
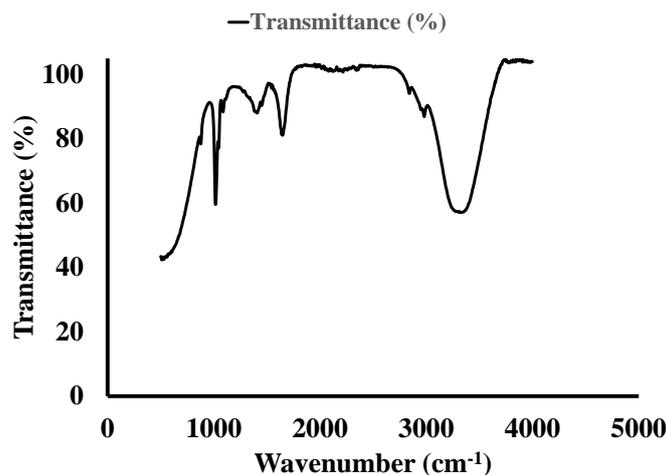
Figure 7. Fourier Transform Infrared Spectroscopy (FTIR) of *Spirogyra* produced biodiesel

Table 5. Functional Group Analysis from FTIR of Produced Biodiesel

Functional Group	Wave Number (cm^{-1})	Intensity
Alcohol OH Stretch	3310.61	Strong
C-H stretch	2979.67	Strong
-	2640.04	-
C=O ester	1657.89	Strong
C=C	1631.19	Weak
C=C aromatic	1408.48	Weak
CH$_3$ bend	1382.38	Medium
C-OH	1085.94	Strong
C-F	1015.76	Strong
-	877.44	-

4. Conclusion and Recommendation

Following the observations made, it can be concluded that it is encouraging to produce biodiesel using *Spirogyra* as the feedstock, due to their availability in our immediate eco-system. It promises to reduce the cost in the source of raw materials for the production of biodiesel. The lipid content of 20.25% reported in this work fell conviniently within the ranges reported in previous works of 14.82% according to Ranjith and Naik [14] in year 2018 and 24% by Reddy and Majumder [12]. The Biodiesel yeild was of 62.96% obtained was also encouraging as it was within 51.39% estimated from the work of Khola and Ghazala [13] and 87.5% yield reported by Haq *et al.* [3]

when they used 50 ^{o}C temperature, oil to methanol ratio of 1:8 and a reaction time of 6 hours. These results established the fact that process conditions backed-up by optimization is very essential for any methodology adopted in obtaining biodiesel from any algae due to the complex interfering nature of the whole process. The higher time used for the production is justified from the results and comparisons made. Temperature and solvent to biomass ration also contributed their impact on the yeild. The accuracy of the model is justified by the statistical parameters reported. The production of biodiesel from microalgae in Nigeria is still in the elementary stage as demonstrated by this work. The adequacy of the biodiesel produced was also satisfactory in performance for engine consumption going by the comparable values of relevant physico-chemical properties considered as set-out by the regulatory bodies. This present study shows the availability of Microalgae species in blooms at a location in Nigeria. Biomonitoring with biofilm colonization techniques demonstrated by Surdem and Dogan [23] could be used to produce and or identify abundant *Spirogyra* algaes to further enhance the sustainable source of feedstock for biodiesel production. In this manner, the use of algae should be adopted as feedstock for renewable energy due to availability, low cost and ease of cultivation. This work was able to assess biodiesel produced from algae (*Spirogyra*), and physicochemical properties characterization to justify performance suitability in engines, which is within a field hardly explored and up till present day still presents some reference materials that contain contradictory results or non-well studied behaviors according to Piloto-Rodríguez *et al.*[24]. Further investigation could focus on optimization of biodiesel from this or other species while varying more parameters such as using different solvent mixtures, reaction time and varying the mixing intensity during the extraction process as well as other discovered relevant parameters.

References

[1].   Khan S, Siddique R, Sajjad W, Nabi G, Hayat KM, Duan P, Yao L., Biodiesel production from algae to overcome the energy crisis. HAYATI Journal of Biosciences, 2017, 24(4): 163-167.
https://doi.org/10.1016/j.hjb.2017.10.003

[2].   Konga A. K, Muchandi A. S, Ponnaiah G. P., Soxhlet extraction of *Spirogyra* sp. algae: an alternative fuel. Biofuels. 2017, 8(1): 29-35.
DOI:10.1080/17597269.2016.1196328

[3].   [3]   Haq I, Muhammad A, Hameed U. Comparative assessment of Cladophora, *Spirogyra* and Oedogonium biomass for the production of fatty acid methyl esters. Applied biochemistry and microbiology. 2014, 50(1): 69-72.
DOI: 10.1134/S0003683813060094

[4].   Gomaa M. A, Al-Kindi A, Abed R. M., Desert stream phototrophic mats: a promising source for biofuel production. Biofuels. 2020, 11(3): 339-349.

[5].   Ge S, Madill M, Champagne P, Use of freshwater macroalgae *Spirogyra* sp. for the treatment of municipal wastewaters and biomass production for biofuel applications. Biomass and Bioenergy, 2018, 111: 213-23.

[6].   Eshaq F. S, Ali M. N, Mohd M. K., *Spirogyra* biomass a renewable source for biofuel (bioethanol) production. International Journal of Engineering Science and Technology, 2010, 2(12): 7045-7054.

[7].   Balat M., Potential alternatives to edible oils for biodiesel production–A review of current work, Energy conversion and management, 2011, 52(2): 1479-1492.

[8].   Bajpai P., Production of Biofuel from Microalgae. InThird Generation Biofuels, Springer, Singapore, 2019: 5-66.

[9].   Baig R. U, Malik A, Ali K, Arif S, Hussain S, Mehmood M, Sami K, Mengal A. N, Khan M. N, Extraction of oil from algae for biodiesel production, from Quetta, Pakistan. InIOP Conference Series: Materials Science and Engineering, 2018, 414(1): 012022).
doi:10.1088/1757-899X/414/1/012022

[10]. Kumar M, Sharma M. P, Potential assessment of microalgal oils for biodiesel production: A review. J. Mater. Environ. Sci., 2014, 5(3): 757-766.

[11]. ExxonMobil, ExxonMobil, Synthetic Genomics advance algae biofuels program. http://biomassmagazine.com/articles/15118/exxonmobil-synthetic-genomics advance-algae-biofuels-program, 2018.

[12]. Reddy A, Majumder A. B., Use of a Combined Technology of Ultrasonication, Three-Phase Partitioning, and Aqueous Enzymatic Oil Extraction for the Extraction of Oil from *Spirogyra* sp., Journal of Engineering, 2014, Article ID 740631, 6 pages http://dx.doi.org/10.1155/2014/740631

[13]. Khola G, Ghazala B., Biodiesel production from algae. Pak. J. Bot. 2012, 44(1): 379-381.

[14]. Ranjith Y, Naik TP., Biodiesel production from freshwater algae as a renewable source. Plant Archives, 2018, 18(1): 1024-1025.

[15]. Idris M. N, Owofu M. O., Experimental Studies on the Production of Biofuel (Bioethanol) from *Spirogyra* Biomass, International Journal of Engineering and Modern technology. 2016, 2(1): 16-43.

[16]. Ur Rehman K, Khuram I, Yaseen T, Farooq L, Wali S., Relationship of pollution with distribution of Mermaid's tresses (*Spirogyra*) in different localities of river Kabul, Pure and Applied Biology (PAB), 2019, 8(4): 2128-2134.

[17]. Shah Z, Cataluña Veses R, Silva R. D., GC-MS and FTIR analysis of bio-oil obtained from freshwater algae (*Spirogyra*) collected from freshwater, International Journal of Environmental & Agriculture Research (IJOEAR). Bikaner, 2016, (2)2: 134-141.

[18]. Bligh, E. G., Dyer, W. J., A rapid method of total lipid extraction and purification, Canadian Journal of Biochemistry and Physiology, 1959, (37)8: 911-917.

[19]. Demirbas. A., Relationships derived from physical properties of vegetable oil and biodiesel fuels, Fuel, 2008, (87): 1743-1748.

[20]. Sivaramakrishnan, K., Ravikumar, P., Determination of cetane number of biodiesel and its influence on physical properties. ARPN Journal of Engineering and Applied Sciences, 2012, (7)2: 205-211.

[21]. Sakthivel, R., Ramesh, K., Purnachandran, R., Shameer, P. M., A review on the properties, performance and emission aspects of the third generation biodiesels, Renewable and Sustainable Energy Reviews, 2018, (82): 2970-2992.

[22]. Kumari, A. S., Penchalayya C., Raju, A. S., Performance evaluation of diesel engine with safflower oil, Journal of Engineering Studies and Research, 2013, (19)2: 63-69.

[23]. Surdem, S., Dogan, M., Biomonitoring of Heavy Metal Levels in Izmit Gulf in Marmara Sea with Biofilm Formation in Plexiglass Substrate, El-Cezeri Journal of Science and Engineering, vol. 7, no. 1 (2020): 20-30.

[24]. Piloto-Rodríguez R, Sánchez-Borroto Y, Melo-Espinosa EA, Verhelst S. Assessment of diesel engine performance when fueled with biodiesel from algae and microalgae: An overview. Renewable and Sustainable Energy Reviews, 2017 (69): 833-842.