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Research Paper

Isolation, extraction of oil and production of biodiesel from microalgae

Thankyou Saturday Okpabi[1*], Uche Joy Nzube[2], Robert Chioma Lilia[3]
[1*,2,3]Biology Department, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education, Rumuolumeni Port Harcourt, Rivers State Nigeria.

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ABSTRACT

This work is aimed at producing biodiesel from microalgae using Bold Basal Medium. After a month of cultivation, isolation, and harvesting, the microalgae were processed (oil was extracted using a chemical extraction procedure including alcohol with a 96% alcohol content and n-hexane). Transesterification was employed to produce the biodiesel from the extracted algal oil, and the resulting fuel was characterized. The results show that Chlorella sp, Desmid sp and Ankistrodesmus sp were isolated, density, viscosity, acid value, saponification, free fatty acid, peroxide value, iodine value, impurity level, and moisture content of Chlorella sp, Desmid sp and Ankistrodesmus sp oil were (0.805, 0.702, 0.716), (4.92, 4.45, 4.22), (1.51, 1.61, 1.62), (179.8, 178.61, 186.60), (0.86, 0.87, 0.88), (28.6, 28.9, 28.8), (0.31, 0.35, 0.33), (0.90, 0.80, 0.88) and (0.30, 0.36, 0.24) respectively, biodiesel had cetane number (126,125,127), density (0.79, 0.88, 0.87), flash point (126, 127, 125), calorific value (38.39, 38.68, 38.72), and viscosity (2.43, 2.66, 2.33) respectively, the quantity of biodiesel produced varied across all the replicates as the mean difference were less than the standard mean of 2.074. The production of biodiesel is practically possible in Nigeria, and the government should set up companies so as to diversify the oil sector and reduce pollution. The major limitation in this research work was using the traditional method for the isolation and identification of dominant microalgae instead of the molecular method.

Corresponding Author:
Thankyou Saturday Okpabi
Biology Department, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education, Rumuolumeni Port Harcourt, Rivers State Nigeria.
Email: okpabis@gmail.com

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1. INTRODUCTION

The energy and transportation sectors are the main human-caused sources of greenhouse gas (GHG) emissions. GHG, in addition to contributing to global warming, also exerts a significant influence on the environment and the well-being of individuals [1], [2], [3], [4], [5]. Annually, approximately one-third of the carbon dioxide emitted by human activities is absorbed by the oceans. As the atmospheric concentration of CO2 rises, there will be a corresponding increase in the dissolution of CO2 in the seas, leading to a gradual decrease in the pH of the water [6], [1]. [7] Argue that multiple measures are necessary to mitigate global warming, which has adverse effects on the environment and various aspects of human life. One aspect of the issue is the reduction of crude oil reserves and challenges associated with extraction and processing, resulting in an increase in the price of gasoline [8]. The lack of viable alternatives to fossil fuels makes this problem particularly urgent in the transportation industry [9]. Discovering sustainable and environmentally friendly energy sources will pose a significant and complex challenge for humanity in the foreseeable future [10]. The aforementioned concerns are closely linked to the expansion of the economy and well-being, the quality of life, worldwide stability, and the involvement of stakeholders [11]. A crucial goal in the effort to decrease emissions related to transportation is the gradual substitution of fossil fuels with sustainable energy sources, and biofuels are regarded as essential contributors to achieving this goal [12]. Similarly, [8], [13], stated that the advancement of biofuels is expected to offer opportunities for expanding fuel supplies and revenue sources, generating employment in rural areas, mitigating greenhouse gas emissions, expediting the decarbonization of transportation fuels, and enhancing energy supply security. Biodiesel and bio-ethanol are the two primary biofuels that can serve as alternatives to petrol and diesel, as stated by [14]. [8] Found that biodiesel demonstrates favourable lubricating qualities and cetane ratings in comparison to low-sulfur diesel fuels. According to [12], biodiesel exhibits enhanced lubricity and more complete combustion, which improves engine performance and partially compensates for the higher energy density of petroleum diesel. According to [15], [16], biodiesel can vary in hue from golden to dark brown, with the specific shade depending on the method of manufacture.

2. RELATED WORK

According to [17], who studied the extraction of bio-oils from microalgae, conventional techniques for dewatering, extracting, and recovering bio-oil from oil seeds are not very useful for microalgae. They also noted that dewatering present's significant challenges for any technology processing biofuels from microalgae, meaning that determining the best routes for the right extraction technology is highly dependent on the type of microalgae and its state of cultivation.

[18], Examined the algal oil extraction of selected monoculture microalgae species of Botryococcus braunii, Nannochloropsis sp., Arthrospira platensis and mixed cultures microalgae from South Coast of Yogyakarta, Indonesia and among all species of microalgae studied, Nannochloropsis sp. was found to have the highest algal oil yield (0.0346 g dry algal oil/g dry microalgae) and theoretical calorific value (187.69 kcal/kg dry microalgae).

[19], Determined the microalgae-based biodiesel production and its challenges and future opportunities where advanced cultivation techniques, such as photobioreactors and open pond systems, microalgae were grown efficiently and in large quantities. Furthermore, various methods, such as lipid extraction and transesterification were employed to convert the lipid-rich microalgae biomass into biodiesel and equally reported several challenges, such as higher production costs, reduced productivity rates, and potential environmental impacts.

[20], Reviewed biodiesel production from microalgae and reported that microalgae have emerged as a promising renewable feedstock for biodiesel. Many species contain high lipid concentrations and require simple cultivation, including reduced freshwater and land area needs compared to traditional crops used for biofuels. [21], examined the extraction of oil from microalgae for biodiesel production and indicated that microalgae cultivation can be done in open-culture systems called "ponds" or in highly

controlled closed-culture systems called "photobioreactors, or PBRs." After algal growth, there are many methods for harvesting microalgae, such as centrifugation, filtration, and gravity sedimentation, which may be preceded by a flocculation step. The choice of the suitable harvesting method depends on the algal species, growth medium, algae production, end product, and production cost. [22] Reviewed biodiesel production from microalgae: processes, technologies, and recent advancements and revealed that the physicochemical properties of lipids extracted from microalgae, the properties of the produced biodiesel fuel, and the transesterification process depend on the method used.

[23] Identified "the oil: alcohol mole ratio as a crucial factor influencing the output of biodiesel production because the stoichiometric ratio for transesterification necessitates the use of one mole of triglyceride and three moles of alcohol to produce three moles of fatty acid alkyl esters and one mole of glycerol, and transesterification is a reversible process that requires a significant surplus of alcohol to shift the reaction towards the production of the desired product. The main aim is to isolate, extract oil, and produce biodiesel from microalgae, and the above aim is supported by the following objectives to;

1. isolate and identify microalgae species for oil production;
2. extract and characterize algal oil produced from identified microalgal strains;
3. Produce and characterize biodiesel from the algal oil;
4. Determine the quantity of biodiesel produced.

3. METHODOLOGY

- **Sample Collection:** Water samples containing microalgae were aseptically collected into a clean beaker from the Ignatius Ajuru University of Education drainage and taken to the laboratory for culturing.
- **Isolation of Microalgae:** Sterile needles were used to pick three dominant isolates into three 1000 ml each of the bold basal medium and incubate them under natural sunlight for one month.
- **Identification of Microalgae:** The microalgae were identified using the traditional method with a microscope and a standard taxonomical key.
- **Nitrogen and Carbon Sources:** A liquid maximize fertilizer was used as a source of nitrogen and phosphorus with the following compositions: nitrogen (33.33%), phosphorus (16.67%), potassium (20.0%), calcium (20.0%), and magnesium (10.0%). A gasoline generator with a frequency of 50 H3 was used as a source of carbon, and an air pump was also used for mixing to prevent sedimentation.
- **Harvesting:** A Rotofix 32 centrifugation machine was used at 4000 rpm for 10 minutes. The samples were loaded into a centrifugation tube and centrifuged, and the sediments were collected in a beaker while the supernatant was poured away. The sediments were dried under the sun and weighed Figure 1 shows the centrifugation process used for algal oil extraction. The sediments were dried under the sun and weighed.



Figure 1. Centrifugation Process of Algal Oil Extraction

- **Oil Extraction:** Solvent extraction techniques were used where 95% alcohol and n-hexane were mixed together in a ratio of 1:1 (50% each), then added to the dried algae isolates and stirred using an electromagnetic stirrer for two hours, as shown in Figure 2. The supernatant was filtered into a beaker and kept open to allow evaporation of the solvent used for extraction. After complete evaporation of the solvent, the extracted algal oil was obtained and collected for further characterization, as shown in Figure 3.

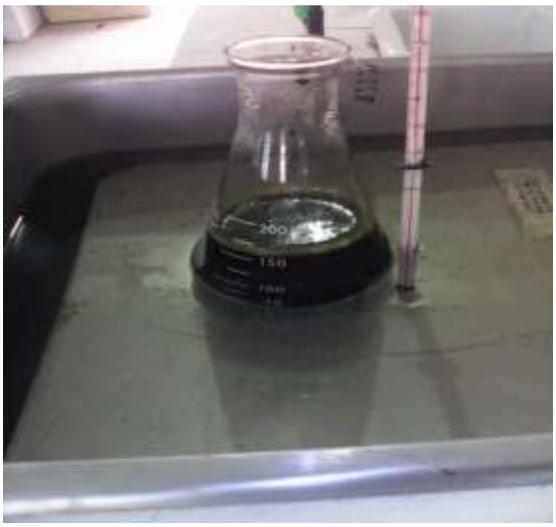

Figure 2. Chemical Extraction of Algal Oil (Stirring Process)



Figure 3. Algal Oil after Extraction

3.1. Algal Oil Analysis

Density: A 50ml density bottle was heated at 105^{0}C in an oven for one hour, cooled and weighed empty bottle. Then the bottle is then filled with oil sample and capped with the cover and placed on the balance to obtain the weight of the oil. The density of the oil is obtained by;

$$\text{Density} = \frac{\text{mass of oil}}{\text{Volume of oil}}$$

Viscosity: The ubb hole all glass viscometer apparatus was used to determine the viscosity of the oil.

Acid Value and Free Fatty Acid: About 1g of the oil product was weighed into a 250ml conical flask. 20ml of the 95% alcohol was added to the mixture. The solution was then titrated against 0.05M sodium hydroxide solution with constant shaking until a pink colour appeared that persists for 30 seconds.

$$\%\text{ free fatty acid (FFA)} = \frac{\text{Ml NaOH x M of NaOH x 28.2mg}}{\text{Weight of oil}}$$

FFA x 1.99 = Acid value

Saponification: About 25ml of alcoholic KOH was added to 1gm of the oil product and refluxed with a condenser for 30 minutes. The refluxed mixture was back-titrated using 0.1M HCl, using phenolphthalein as an indicator. A blank titration without the oil was also done.

Saponification number = S – B) x mx 56.1
Sample weight

Where S = sample titration
B = Blank titration
M = Morality of Hcl
56.1 = M.W of KOH

Peroxide Value: Exactly 5.0g of oil product was dissolved in 30ml of glacial acetic acid-chloroform mixture in ratio 3.2v/v. 0.5ml of saturated KI solution was added (to librate I_2). The mixture was then titrated against 0.1m sodium thiosulphate using starch as an indicator.

Peroxide value = (S – B) x M x 100
(Meq peroxide/kg oil) Sample weight

Where S = Sample titration
B = Blank titration
M = Mobility of thiosulphate

Iodine Value: Exactly 10ml Wij reagent (a mixture of iodine monochloride + glacial acetic acid) was added to 5ml of the oil product in cyclohexane. The mixture was allowed for halogenation to take place 5ml of KI was added to reduce the excess iodine monochloride ice to free iodine. The liberated iodine was titrated with a 0.1M sodium thiosulphate solution using starch as an indication.

Iodine value = (B – S) x m x 12.69
Sample weight

B = Blank titration
S = Sample titration
M = Molarity of $Na_2S_2O_3$

12.69 is used to convert from meq thiosulphate to g iodine. Mw of iodine is 12.69.

Impurity: About 10g of oil product was heated on a hot-plate for 10 minutes and poured into sintered filter funnel and filtered through with the aid of sunction pump. 10ml of petroleum spirit 60-80^{0}C boiling range was used to wash out the filter, leaving the residue on the filter sintered funnel. The clean oil was obtained by evaporating the petroleum spirit off on a hot plate.

% impurity = weight of pure oil x 100
Weight of impure oil 1

Moisture Content: About 5g of oil sample was placed in the oven set of 105^{0}C and heated for 30 minutes, weighted at every 30 minutes intervals until a constant weight is obtained.

% moisture = weight loss x 100
Original weight 1

Fatty Acid Methyl Ester Analysis (Using Gas Chromatography): About 50g of the extracted fat content of the sample was saponified (esterified) for five minutes at 9500 °C with 3.4 ml of 0.5 M KOH in dry methanol. The mixture was heated for five minutes at 900 °C to achieve a complete methylation process. The fatty acid methyl esters were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated to 1 ml for gas chromatography analysis, and 1 µl was injected into the injection port of the GC.

Biodiesel Production: The extracted oil was boiled for 30 minutes to reduce the moisture content and increase the average kinetic molecules of the oil, then 40 ml of methanol and 20 ml of sodium hydroxide in a ratio of 2:1 were added to the oil in a conical flask in a water bath while the temperature was set at 40°C

using a thermometer, and after 15 minutes, the contents were frequently decanted to separate the biodiesel formed, as illustrated in Figure 4.



Figure 4. Production of Biodiesel

3.2. Biodiesel Analysis

- **Density:** Density was measured using a hydrometer (ASTM D941) at a temperature of 312k.
- **Flash Point:** The flash point of the biodiesel was measured in the temperature range of 65-180^{0}C by an automated pensky – marten's apparatus (ASTM D93) enclosed cup.
- **Calorific Value:** Calorific value was measured in a bomb calorimeter according to ASTD 240 standard method.
- **Viscosity:** This was done using a redwood viscometer (ASTM D445).
- **Cetane Number:** The cetane number was measured using ignition quality fester (ASTMD 613)
- **Statistical Analysis:** ANOVA was used to analyse the data.

4. RESULTS AND DISCUSSION

Chlorella sp, Desmid sp and Ankistrodesmus sp were isolated.

Table 1. Physio- Chemical Characterization of Algal Oil Produced

Oil Parameters	Chlorela Sp	Desmid Sp	Ankistrodesmus Sp
Density(gm/ml)	0.805	0.702	0.716
Viscosity (mm^2/sec)	4.92	4.45	4.22
Acid value (mg/g)	1.51	1.61	1.62
Saponification(mg/g)	179.8	178.61	186.60
Free fatty acid(1gm)	0.86	0.87	0.88
Peroxide value(meq/kg)	28.6	28.9	28.8
Iodine value (gm)	0.31	0.35	0.33
Impurity level (%)	0.90	0.80	0.88
Moisture content (%)	0.30	0.36	0.24

Table 1 revealed that the density, viscosity, acid value, saponification, free fatty acid, peroxide value, iodine value, impurity level, and moisture content of Chlorella sp, Desmid sp and Ankistrodesmus sp were (0.805, 0.702, 0.716), (4.92, 4.45, 4.22), (1.51, 1.61, 1.62), (179.8, 178.61, 186.60), (0.86, 0.87, 0.88),

(28.6, 28.9, 28.8), (0.31, 0.35, 0.33), (0.90, 0.80, 0.88) and (0.30, 0.36, 0.24) respectively. Which means that Chlorella sp was more concentrated, followed by Ankistrodesmus sp, and Desmid sp, Chlorella spp had the highest viscosity, followed by Desmid sp and Ankistrodesmus sp, Chlorella sp had the lowest acid value, followed by Desmid sp and Ankistrodesmus sp, Ankistrodesmus sp had the highest saponification value. Chlorella sp has the lowest free fatty acid content; three other oils have similar peroxide values. Desmid sp has the highest iodine value, suggesting a higher degree of unsaturation. Desmid sp had the lowest impurity level, followed by Ankistrodesmus sp and Chlorella sp. Ankistrodesmus sp had the lowest moisture content, followed by Chlorella sp and Desmid sp.

Table 2. Fatty Acid Composition of Algal Oil Extracted

Fatty Acid	Structure	Conc. (%) Chlorella	Conc. (%) Desmid	Conc. (%) Ankistrodesmus
Caprylic acid methyl ester	C8:0	0.00	0.00	0.00
Capric acid methyl ester	C10:0	0.00	0.00	0.00
Lauric acid methyl ester	C12:0	1.35	1.35	1.35
Myristic acid methyl ester	C14:0	6.25	5.41	6.52
Palmitic acid methyl ester	C16:0	26.09	27.20	26.69
Plamitoleic acid methyl ester	C16:1	3.28	2.17	3.28
Margaric acid methyl ester	C17:0	0.09	1.20	0.09
Stearic acid methyl ester	C18:0	6.02	6.02	6.02
Oleic acid methyl ester	C18:1	40.21	38.21	40.21
Linolenic acid methyl ester	C18:2	13.20	15.20	13.20
Linoleic acid methyl ester	C18:3	0.13	0.13	0.13
Arachidic acid methyl ester	C20:0	2.07	2.07	2.07
Arachidonic acid methyl ester	C20:2	0.00	0.00	0.00
Behenic acid methyl ester	C20:0	0.92	0.92	0.92
Erucic and methyl ester	C22:1	0.09	0.09	0.09
Lignoeric acid methyl ester	C24:0	0.02	0.02	0.02
Saturated		43.31	44.42	43.31
Unsaturated		56.69	55.58	56.69

Table 2 indicates the total percentage of saturated fatty acids calculated for each type of oil: Chlorella (43.31%), Desmid (44.42%), and Ankistrodesmus (43.31%). The total percentage of unsaturated fatty acids calculated for each type of oil is: Chlorella (56.69%), Desmid (55.58%), and Ankistrodesmus (56.69%). The concentrations of most fatty acids were similar across the three oils, indicating a comparable fatty acid profile. The fatty acid composition analysis provides insights into the types and proportions of fatty acids present in Chlorella, Desmid, and Ankistrodesmus oils. The balance between saturated and unsaturated fatty acids is crucial for understanding the nutritional and functional properties of these oils. Figure 5, Figure 6, and Figure 7 illustrate the relationship between the profile acids of Chlorella sp, Desmid sp, and Ankistrodesmus sp respectively, while Figure 8, Figure 9, and Figure 10 present the corresponding acid profile diagrams for each species.

Table 3. Characterization of Biodiesel Produced

Properties	Chlorella Spp	Desmid Spp	Ankistrodesmus Spp
Cetane Number	126	125	127
Density (Kg/m)	0.79	0.88	0.87
Flash Point (^{0}C)	126	127	125
Calorific Value (MJ/Kg)	38.39	38.68	38.72
Viscosity (mm^2/s)	2.43	2.66	2.33

Table 3 showed that biodiesel produced from Ankistrodesmus sp (127) has the highest cetane number, followed by Chlorella sp (126) and Desmid sp (125). Biodiesel produced from Chlorella sp has the lowest density (0.79), followed by Ankistrodesmus sp (0.87) and Desmid spp (0.88). Biodiesel produced from Desmid sp (127) having the highest flash point and Ankistrodesmus sp (1125) having the lowest. Biodiesel produced from Ankistrodesmus sp having the highest calorific value while biodiesel produced from Chlorella sp has the lowest viscosity, followed by Ankistrodesmus sp (38.72) and Desmid sp (38.68). These properties provide information about the combustion quality, density, flammability, energy content, and flow characteristics of the three oils.



Figure 5. A Graph Showing the Relationship between Profile Acids of Chlorella Sp

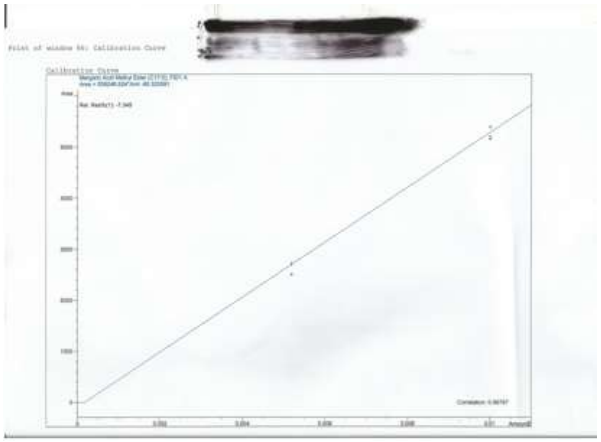

Figure 6. A Graph Showing the Relationship between Profile Acids of Desmid Sp

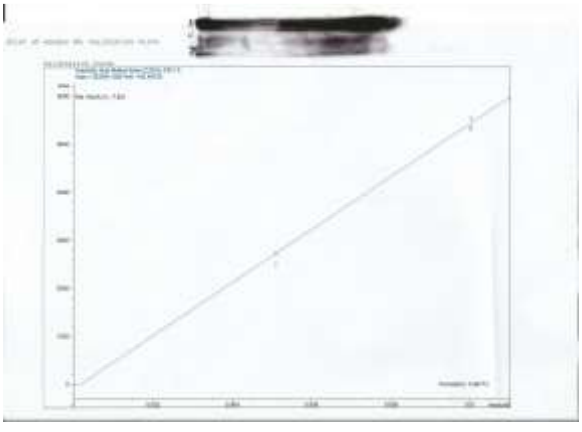

Figure 7. A Graph Showing the Relationship between Profile Acids of Ankistrodesmus Sp



Figure 8. Acid Profile Diagram of Chlorella Sp



Figure 9. Acid Profile Diagram of Desmid Sp



Figure 10. Acid Profile Diagram of Ankistrodesmus Sp

Table 4. Weight of Dried Microalgae

Microalgae	**Replication/Dried Weight of Algae**			
Microalgae	1st	2nd	3rd	4th
Chlorella	25gm	29gm	28gm	21gm
Desmid	27.8gm	17gm	23gm	24gm
Ankistrodesmus	22.5gm	24mg	24.5gm	21gm

Table 4 indicated that Chlorella showed an increase in weight from the 1st to the 2nd replication, followed by a decrease in the 4th replication; Desmid experienced a significant decrease in weight from the 1st to the 2nd replication, followed by an increase in the 3rd and 4th replications; and Ankistrodesmus exhibited fluctuations in weight across replications. The significant drop in weight during the second replication may indicate a challenging growth period, while subsequent replications show recovery.

Table 5. Quantity of Algal Oil Produced by Different Algae

Microalgae	**Replication/Quantity of Oil Produced**			
Algae	1st	2nd	3rd	4th
Chlorella	10ml	10.6ml	10.2ml	12ml
Desmid	9.8ml	6.4ml	5.9ml	8ml
Ankistrodesmus	7.5ml	8ml	7.5ml	7.7ml

Table 6. ANOVA

Sv	**Df**	**Ss**	**Mss**	**Fcal**	**Ftab**
Microalgae	2	25.65	12.83	9.50	5.14
Replicates	3	3.83	1.28	0.95	4.76
Error	6	8.12	1.35		

4.1. LSD

R_1-R_2 (9.1-8.3) = 0.8 < 2.074
R_1-R_3 (9.1-7.9) = 1.2 < 2.074
R_1-R_4 (9.1-9.2) = -0.1< 2.074
R_2-R_3 (8.3-7.9) = 0.5 < 2.074
R_2-R_4 (8.3- 9.2) = -0.9 < 2.074
R_3-R_4 (7.9-9.2) = -1.3 < 2.074

Table 5 indicates that Chlorella shows an increase in oil production from the 1st to the 2nd replication, a decrease in the 3rd replication, followed by an increase again in the 4th replication. The fluctuations in oil production may be indicative of variations in growth conditions or the metabolic activity of Chlorella. Desmid experienced a significant decrease in oil production from the 1st to the 2nd replication, followed by a slight increase in the 4th replication. The observed changes may be influenced by factors such as nutrient availability, environmental conditions, or the specific metabolic characteristics of Desmid, while Ankistrodesmus shows relatively consistent oil production across replications, with a slight increase in the 4th replication. The stability in oil production may be attributed to the growth conditions or metabolic characteristics of Ankistrodesmus, and the variations in oil quantity suggest that each microalgae species has unique characteristics influencing its oil production. Table 6 showed that there is a significance difference in the replicates as the F_{cal} (0.95) is less than the F_{tab} (4.76). The LSD also shows that the difference varied in all the replicates as their mean difference is less than the standard mean of 2.074, while there is no significance difference among the microalgae biodiesel produced.

Table 1 revealed that the density, viscosity, acid value, saponification, free fatty acid, peroxide value, iodine value, impurity level, and moisture content of Chlorella sp, Desmid sp, and Ankistrodesmus sp were (0.805 gm/ml, 0.702 gm/ml, 0.716 gm/ml), (4.92 mm2/s, 4.45 mm2/, 4.22 mm^2/), (1.51 mg/g, 1.61 mg/g, 1.62 mg/g), (179.8 mg/g, 178.61 mg/g, 186.60 mg/g), (0.86 gm, 0.87mg, 0.88gm), (28.6 meq/kg, 28.9 meq/kg, 28.8 meq/kg), (0.31gm, 0.35gm, 0.33gm), (0.90%, 0.80%, 0.88%) and (0.30%, 0.36%, 0.24%) respectively. This result differs slightly from the results of [24], [13] in which moisture content (0.07%) for Chlorella Vulgaris and mixed algae (0.05%), iodine value (62 gm), acid value (31.5 mg/g), and free fatty acid (2.6gm) were higher in value while saponification (173mg/g) was lower due to the types of species, growth medium, source of nutrients, method of culturing, geographical location, and the crude method of oil extraction.

The fatty acid methylester analysis in Table 2 reveals that the fatty acids in chlorella, desmid, and ankistrodesmus are both saturated (C8:0, C10:0, C14:0, C16:0, C17:0, C18:0, C20:0, C22:0, and C24:0) and unsaturated (C16:1, C18:1, C18:2, C18:3, C20:2, and C22:1). Ten of the fatty acids in Table 2 were saturated, whereas six were unsaturated. For Chlorella, 44.42% (saturated) and 55.58% (unsaturated), Desmid 43.31% (saturated) and 56.91% (unsaturated), and Ankistrodesmus 43.31% (saturated) and 56.69% (unsaturated). This result differs from that of [12] who revealed that unsaturated fatty acid content was found to be higher in C. vulgaris (78.09%), R. hieroglyphicum (77.5%), and mixed algae culture (77.99%), but agrees with that of [7], [25], [17], [18].

Table 3 showed that biodiesel produced from Ankistrodesmus sp has the highest cetane number, followed by Chlorella sp and Desmid sp. Biodiesel produced from Chlorella sp has the lowest density, followed by Ankistrodesmus sp and Desmid spp. Biodiesel is produced from Desmid sp having the highest flash point and Ankistrodesmus sp having the lowest. Biodiesel produced from Ankistrodesmus sp has the highest calorific value, while biodiesel produced from Chlorella sp has the lowest viscosity, followed by Ankistrodesmus sp and Desmid sp. This result tallies with that of [26], [8] except for the calorific value which was observed to be lower. Also, [19], [20], [21], [27], [23] supported the idea that biodiesel production is feasible from microalgae, implying that the quality and quantity produced depend solely on the method employed. They also posited that green algae have higher productivity compared with other algal types such as red, blood-red, and yellow-green. Figure 11 shows a sample of the biodiesel produced from the algal oil after trans esterification.



Figure 11. Biodiesel

5. CONCLUSION

According to the study, microalgae are effective and quickly growing organisms for the production of biodiesel since they may be grown in prepared conditions. As a result, if algal oils are used properly, they can become a dependable supply of energy, which might considerably aid Nigeria in resolving its energy crisis and also reduce unemployment since more people would be encouraged to work in agriculture. Currently, as demonstrated, it appears that biodiesel produced from microalgae is the only renewable fuel with the potential to completely replace fossil fuels because microalgae are a promising feedstock for biodiesel production since biodiesel is a sustainable and environmentally beneficial energy source. The fuel created by the esterification of algae oil has a short-chain alcohol, produces no carbon dioxide, and lowers pollution. Nevertheless, the transesterification reaction is greatly affected by the molar ratio of alcohol, reaction temperature, reaction time, and catalyst concentration.

5.1. Recommendations

Based on the findings, the following are recommended that;

1. The production of biodiesel from the algal oil is achievable therefore, the government, multinational companies, or individuals should thinker towards investing since it is ecofriendly and renewable.
2. Industries should be established by government where biodiesel can be produced in large quantities as a way of gradually replacing the fossil fuel. This is because the gases and particulates matters being released daily from industrial plants and transportation industries are contributors to various health challenges experienced in our society today.

Limitation: The use of conventional methods for isolation and identification of dominant microalgae rather than molecular techniques, as well as the absence of contemporary scientific apparatus in the laboratory, were the main limitations in this research endeavour.

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Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Thankyou Saturday Okpabi	✓	✓	✓	✓		✓		✓	✓	✓	✓			
Uche Joy Nzube	✓	✓		✓		✓		✓	✓	✓		✓		
Robert Chioma Lilia	✓		✓	✓				✓	✓	✓	✓			✓

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Informed Consent

All participants were informed about the purpose of the study, and their voluntary consent was obtained prior to data collection.

Ethical Approval

Not Applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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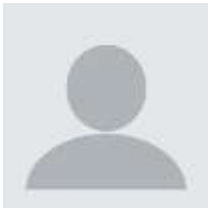
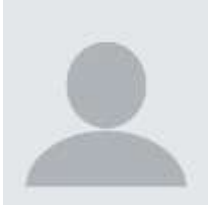
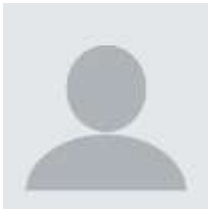
BIOGRAPHIES OF AUTHORS

	**Thankyou Saturday Okpabi,** is a dedicated academic and researcher in the Department of Biology, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rivers State, Nigeria. With a strong foundation in biological sciences, Okpabi has contributed actively to research and scholarship in the natural sciences. His academic work reflects a commitment to advancing scientific knowledge within the Nigerian higher education landscape, and he serves as a corresponding author, demonstrating leadership in scholarly communication and research dissemination. Email: okpabis@gmail.com
	**Uche Joy Nzube,** is a researcher and academic staff member in the Department of Biology, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rivers State, Nigeria. Her scholarly interests are rooted in the biological and applied sciences, where she actively participates in research initiatives aimed at expanding scientific understanding. Nzube is committed to academic excellence and collaborative research, contributing meaningfully to the faculty's scientific output and fostering innovation in biological studies. Email: spectabilisjoy70@gmail.com

	**Robert Chioma Lilia,** is an academic and researcher affiliated with the Department of Biology, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rivers State, Nigeria. Lilia brings a focused commitment to biological research and education, contributing to the academic growth and scientific advancement of the institution. His work supports the broader goals of natural and applied sciences education in Nigeria, and he remains dedicated to producing impactful research that addresses contemporary biological challenges. Email: liliachioma@gmail.com