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Biodiesel production by microalgal species isolated from water samples in Keffi, Nasarawa state, Nigeria

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Abstract

To counter the effects of climate change due to use of fossil fuels, much attention has been placed into the production of biomass-derived fuels. This investigation was aimed at producing biodiesel from microalgae species isolated from water samples in Keffi, Nasarawa state. Standard microbiological methods were used for isolation and identification of the microalgal isolates. The percentage occurrence of the isolates showed that *Chlorella vulgaris* had a 100% occurrence with *Chlorella lewinii* and *Stigeoclonium tenue* having a 50% occurrence each while *Cladophora glomerata* having a 25% occurrence. The growth curve showed that the micro algal isolates experienced exponential growth under daily sunlight exposure. The effect of temperature on the biomass and lipid extracted showed that *Chlorella vulgaris*, *Chlorella lewinii* and *Stigeoclonium tenue* had the highest biomass yields of 8.01g/l, 12.17 g/l and 15.10 g/l respectively and lipid production of 3.30 µg/l, 3.11 µg/l and 5.11 µg/l respectively at 32 ℃. Further increase in temperature led to decrease in their biomass yields and lipid production. The effects of pH showed that all the microalgal isolates had its highest biomass yields and lipid production in an alkaline pH of 7.5 but when the pH was reduced up to 4.5, the resultant microalgal biomass yields and lipid production of the microalgal isolates decreased. The fatty acid profiles of the biodiesel derived from the microalgal isolates showed that it was composed mainly of palmitic, oleic, Myristic and Stearic acids which made it a viable fuel source.

Keywords: Micro algal; Lipid production; Biomass yields; Fatty acid profiles; Biodiesel derived

1 Introduction

The excessive use of fossil fuels as a direct source of energy has led to its high rate of consumption, which has led the world facing a perceptible oil crisis. Researchers have postulated that fossil fuel consumption would rise by about 60% in the next 25 years [1]. This, together with significant depletion in resources over the next few decades, can result in further economic constraints [2]. In Nigeria, petroleum reserves and petroleum provides 95% of foreign trade earnings and about 80% of budget revenues [3]. The current estimated oil reserve in Nigeria is 37 billion barrels (sixth largest petroleum exporter) with daily production capacity of 2.5 million barrels of crude oil. This implies that at the current rate of utilization, the oil will be depleted by 2053 [4]. In addition, the high rate of Energy-related fossil fuel usage is significantly linked to the effects of global warming and climate change on the planet, accounting for 82% of airborne irrespirable-particulate pollution and greenhouse gas emissions to the atmosphere [5].

To combat these environmental and economic challenges, much attention has been given to the production of biomass-derived fuels. Based on feedstock and method of production, biofuels are classified in different groups named as first, second, third, and fourth generation biofuels [6]. First-generation biofuels use edible biomass such as starch and sugar

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[7] which increases the cost of production and causes inefficient utilization of resources and energy spent in cultivating crops. The second generation of biofuels uses inedible lignocellulosic biomass such as sawdust, low-priced woods, crop wastes, switch grass and municipal wastes [8]. The third generation of biofuels takes advantage of specially engineered crops such as algae as its energy source [9]. Fourth-generation biofuels, in its early developmental stage, makes use bioengineered microorganisms such as bioengineered algae that are genetically altered to consume more CO_2 from the environment. Biofuels are used to produce different fuels including ethanol, butanol, methane, biodiesel, gasoline, isoprene and jet fuel [10, 11].

The purpose of this study is to carry out the production of biodiesel using microalgae from wastewater located in different locations in Nasarawa state, Nigeria. This is because microalgae are considered a very promising choice for the production of biodiesel and a variety of microorganisms can be used for this purpose. Microalgae have been found to synthesize a large variety of fatty acids and lipids [12, 13], the composition of which often reflects adaptation to environmental conditions [14]. Both microalgae autotrophs and heterotrophs can be used for biodiesel production but vary in their biodiesel yield [15]. Because microalgae are photosynthetic (*i.e.* CO_2-fixing) organisms, their use mitigates the effects of greenhouse gases thus benefiting the environment. Depending on the species, microalgae can grow on fresh, brackish, sea, or even waste water and can accumulate up to 60% oil per dried weight under stress conditions [16]. Therefore, microalgae have attracted increasing attention for its ability to produce biodiesel or other lipid-based biofuels [17, 1].

2 Material and methods

2.1 Samples and Sample Collection

Water samples were collected from 4 different locations in Keffi town. Keffi is a town located within Keffi Local Government Area in Nasarawa State, Nigeria which is located within Lat 8°, 51 and 9° 25' N and Long 7°52' 80401 E . It has an area of 138 km^2 and a population of 92,664 at the 2006 census. It is bound by Karu in the West and Kokona and Nasarawa Local Government Areas in the South [18]. Samples were collected using plastic bottles. Three drops of Lugol's iodine solution was added to the samples for the purpose of preserving them and taken to the Microbiology Laboratory for analysis

2.2 Media Preparation

A modified BG-11 medium was used for the isolation and cultivation of samples collected. The composition of the modified medium include 1.5g of $NaNO_3$, 0.04 g of K_2HPO_4, 0.075 g of $MgSO_4.7H_2O$, 0.036 g of $CaCl.2H_2O$, 0.006 g of glucose, 0.006g of yeast extracts, 0.001 g of EDTA (disodum salt), 0.02 g of Na_2CO_3 and 1 ml of trace metal solution per liter of distilled water. The trace metal solution contained 2.86g of $H3BO_3$, 1.81 g of $MnCl_2.4H_2O$, 0.222 g of $ZnSO_4.7H_2O$, 0.39g of $NaMoO_4.2H_2O$, 0.079 g of $CuSO_4.5H_2O$ and 49.4 g of Co(NO3)2.6H2O per litre of distilled water. BG-11 medium contains 1.5 g $NaNO_3$, 0.04 g $K_2HPO_4.3H_2O$, 0.2 g $KH_2PO_4.3H_2O$, 0.001 g Disodium EDTA, 0.001 g Ferric Ammonium Citrate, 0.006 g Citric Acid, 0.02 g Na_2CO_3 and 1 ml of trace metal solution per liter. The trace metal solution contains 2.85 g H_3BO_3, 1.8 g $MnCl_2$. $4H_2O$, 0.02 g $ZnSO_4.7H_2O$, 0.08 g $CuSO_4.5H_2O$, 0.08 g $CoCl_2$.$6H_2O$ and 0.05 g $Na_2MoO_4.2H_2O$ per liter.

The Modified Liquid BG-11 medium was prepared by adding the chemical components into 1 liter of distilled water and covered and autoclaved. It was then allowed to cool and stored at refrigerated temperature. Solid BG-11 agar was made first by adding 7.2 g of Nutrient agar in 200ml of distilled water. The agar solution was then autoclaved and allowed to cool. 4.8 g of Sodium Thiosulfate was then added to the agar solution and the solution was then added to 200 ml of already autoclaved liquid BG-11 medium and mixed properly.

2.3 Isolation and Identification of Samples

The samples were then inoculated in plates containing BG-11 agar media and incubated near windows in the presence of sunlight under room temperature for 7 days. Care must be taken to ensure that the isolates are not exposed to direct sunlight. Isolation of the samples was carried out by streaking sample across the agar medium. The emergent colonies was then re-inoculated in a sterile BG-11 agar medium with repeated sub-culturing [19]. The identification of the microalgae species was carried out through examination under a light microscope. Microalgal species would be identified based on cell shape, cell size and arrangement of cells and connecting strands within the colonies as described by [20, 21]. These were compared with reference atlas from [22].

2.4 Cultivation of Microalgae Species

Microalgal isolates were cultivated in 50ml of sterile BG-11 liquid medium that was transfer into 200ml Erlenmeyer flasks. Each flask was inoculated with one week pure culture, capped with urethane foam and incubated for 10 days. They were naturally illuminated with a photoperiod of 7hrs of sunlight. The growth pattern of the culture was measured by absorbance level at 540 nm during the cultivation period using a visible ultraviolet spectrophotometer during the incubation period [19]

2.5 Effects of Temperature and pH

Effect of temperature was carried out by subjecting the isolates cultivated in liquid BG-11 Medium to varying temperatures such as 28 °C, 32 °C, 35 °C, 37 °C and 40 °C. The isolates were thereafter harvested and their lipids extracted with the weight of the dry biomass harvested and the weight of the extracted lipid recorded. Effect of pH was carried out by subjecting the isolates cultivated in liquid BG-11 Medium to varying levels of pH. This was carried out by adjusting the pH levels to 4.5, 5.5, 6.5, 7.5 using 1M HCl and checked using a pH meter. The isolates were thereafter harvested and their lipids extracted with the weight of the dry biomass harvested and the weight of the extracted lipid recorded.

2.6 Harvesting of Microalgal Isolates

Isolates was harvested by centrifugation at 3,000 rpm in a bench-top centrifuge for about 15 minutes. The isolates harvested were then dried in an oven and their weights were then recorded.

2.7 Extraction of Lipids from Harvested Microalgal Cells

Lipids were extracted in a chloroform-methanol-water system by the method of Carbone *et al.* [15]. After placing the dried biomass of the microalgae in an Erlenmeyer flask, 25 ml chloroform, 25 ml methanol, and 10 ml deionized water were poured into the flask. The mixture was shaken for 10 min. The mixture was then transferred to a separator funnel to allow separation of the organic and aqueous layers to get the extracted lipids. The weight of the extracted lipids was then recorded.

2.8 Transesterification of the Microalgal Oil

Biodiesel was obtained from the purification of the mixture of Fatty Acid Methyl Esters (FAME). 0.5 g of NaOH was mixed with 50 ml of methanol in 100 ml Erlenmeyer flask and stirred properly for 20 minutes at 60 °C. Then the mixture of catalyst and methanol was poured into the oil in a Erlenmeyer flask and its mouth was covered with aluminum foil to ensure the transesterification process to occur. The conical flask containing the solution was then stirred for an hour while heated to a temperature of 60^{o}C. After stirring the solution, it was poured into a separator funnel and kept for 20 minutes in order to cool and settle into biodiesel and glycerol sediment layers clearly. The biodiesel and glycerol layers were then collected into separate conical flasks. [23].

2.9 Fatty Acid Methyl Ester (FAME) Analyses

The Fatty Acid Methyl Ester (FAME) of the obtained biodiesel was analyzed through the method called Gas Chromatography Mass Spectrometry (GC-MS). The Gas Chromatography Mass Spectrometry was carried out using a Shimadzu Gas Chromatograph to determine the level of methyl esters present in the biodiesel obtained from the microalgae species.

2.10 Determination of Biodiesel Properties Density

The Density of the biodiesel produced from the microalgal oils was measured as described by [15] by dividing its mass by its volume. The mass of the oil was obtained by weighing it while the volume was obtained through measurement in a graduated cylinder. This test was repeated a further two more times to ensure the accuracy of the results gotten. The density obtained was the mean of the triplicate test and was then compared with the quality density standards worldwide.

2.10.1 Viscosity

The viscosity of the biodiesel produced from the microalgal oils was measured using a U- Tube viscometer at the constant temperature of 40^{o}C in a water bath. This was done by collecting the oil into the viscometer by inverting it into the sample and applying suction. The viscometer containing the oil was then placed vertically into the water bath and time was allowed at 10 minutes for the oil to come to the temperature of the bath. Suction was applied to the small tube to draw the liquid slightly above the top mark. Time was taken for when the oil passed between the two marks on the viscometer. The kinematic viscosity was calculated by multiplying the time taken for the oil to pass through by the

constant of the calibrated viscometer [24]. This was then repeated two more times with the viscosity being the mean of the triplicate tests. The viscosity obtained was then compared with the quality viscosity standards worldwide

2.10.2 Flash Point

The flash point of the biodiesel produced from the microalgal oils was measured using a Penskey Closed up Flash point tester as described by [25]. The oil was put into the tester cup of the flash point tester and the lid was closed. The thermometer was the attached to the tester with the gas valve switched on and the flame was lit on the tester. The black knob of the tester was turned clockwise for every increase in 30^{o}C. When the flash occurred and puts out the flame, the temperature was recorded. This was then repeated a further two more times with the flash point calculated as the mean of the triplicate tests. The flash point temperature was then compared with quality biodiesel flashpoint values.

2.10.3 Acid Value

The acid value of the biodiesel from the microalgal oils was determined by titrating the oil with a solution of 1M potassium hydroxide as titration solvent as described in [26]. 1g of the biodiesel was weighed in a conical flask and recorded. 125ml of the titration solvent was then added into the flask containing the biodiesel. A few drops of phenolphthalein indicator was then added into the solution. The solution was then swirled to ensure its uniformity. The burette was then filled with the titrant solution with the starting volume of the titrant recorded. The titrant was then carefully introduced into the flask while swirling the solution. The titrant was stopped being introduced when the solution in the flask changed colour to pink. The ending volume of the titrant was then recorded. The acid value was then calculated using the formula seen below:

$$\text{Acid value} = \frac{56.1\text{x V x N}}{\text{W}}$$

Where,

V = Volume in mL of standard potassium hydroxide or sodium hydroxide used,
N = Normality of the potassium hydroxide solution or Sodium hydroxide solution; and
W = Weight in gm of the sample
56.1= Molecular weight of the Potassium Hydroxide

The experiment was then repeated a further two more times and the acid value was calculated as the mean of the triplicate test. The acid value obtained was then compared with the quality biodiesel acid values.

3 Results

3.1 Isolation, Identification and Occurence of Microalgal Species

The cultural and morphological characteristicis of the microlalgae isolated from water samples collected from Keffi are shown in Table 2 respectively. The microalgal species isolated and identified includes: *Chlorella vulgaris*, *Chlorella lewinii*, *Stigeoclonium tenue* and *Cladophora glomerata*. The occurrence of the microalgal species is as given in Table 1. It shows that *Chlorella vulgaris* has the highest occurrence at 100.0% from all locations as 1 was isolated from all of the locations. It is followed by *Chlorella lewinii* which has an occurrence of 50.0% as 2 were isolated from locations I and III. *Stigeoclonium tenue* have a 25.0% occurrence with 1 isolated from location IV while *Cladophora glomerata* has an occurrence of 50 % as it was isolated from locations II and IV.

3.2 Growth curve of the Microalgae species cultivated

The growth curves of the microalgae species cultivated is as shown in Figure 1. The overall highest absorbance was 0.209 which was given by *Stigeoclonium tenue* on the 9th day of cultivation. The highest absorbance value for *Chlorella vulgaris* is 0.178 which was also gotten on the 9th day of cultivation. For *Chlorella lewinii*, its absorbance reading on the 9th day of cultivation was 0.174 while the absorbance reading for *Cladophora* at the same stage of cultivation was 0.190.

Table 1 Occurrence of Microalgae species isolated from water samples taken from different locations in Keffi, Nasarawa state

Microalgae species	No. of Sample	Locations				Occurrence
		I	II	III	IV	
Chlorella vulgaris	4	1	1	1	1	4 (100%)
Stigeoclonium tenue	4	0	0	0	1	1 (25%)
Chlorella lewinii	4	1	0	0	1	2(50%)
Cladophora glomerata	4	0	1	0	1	2 (50%)

I - Fish Pond, Nasarawa State University, Keffi; II- GRA Axis, Keffi; III- Old Barracks, Keffi; IV- Stream in Nasarawa State University

Table 2 Cultural and Morphological Characteristics of Microalgae isolated

Cultural Morphology	Morphological Characteristics	Microalgae isolates
Colony appears large and greenish in colour with a brown colour seen reverse of plate	Cells were small and spherical with presence of mucilage. Chloroplast is single and pariental with presence of pyrenoids	*Chlorella vulgaris*
Colonies appear to be light green in colour with a brown colour shown reverse of plate	Branched filamentous cells attached by means of basal cells	*Stigeoclonium tenue*
Colonies appear to be light greenish in color on agar plate with a white pigment growing on the agar plate with a brown color shown reverse of agar plate	Cells were oval shaped. Mucilage was absent. Chloroplast was cup shaped with pyrenoid spherical in shape	*Chlorella lewinii*
Colonies grown on agar plate appear to be greenish in colour with brown colour shown reverse of plate	Cells are branched filaments that are robust and long. Dark green in colour. chloroplast net-like with numerous pyrenoids	*Cladophora glomerata*

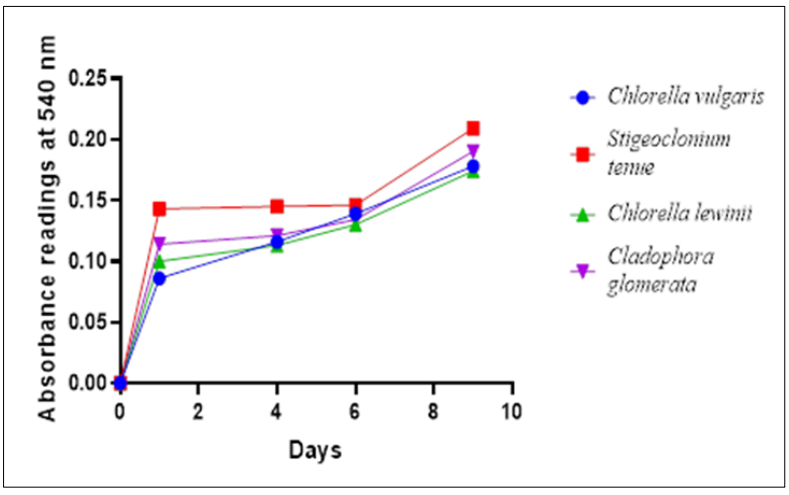

Figure 1 Growth Curve of Microalgal Isolates

3.3 Evaluation of Biomass harvesting and Lipid Extracted

The effect of temperature on the dry biomass of the microalgae harvested and the amount of lipid extracted is as shown in Table 3. *Chlorella vulgaris* produced a high biomass and lipid yield at 32°C with a yield of 8.01g/l and 3.30 µg/l respectively while at 35 °C, it produced a yield of 6.19g/l and 2.17 µg/l; at 37 °C, it gave a biomass and lipid yield of 4.55g/l and 1.13 µg/l respectively while at 40°C, it gave a biomass weight and lipid yield of 3.21 g/l and 0.88 µg/l respectively. *Stigeoclonium tenue* produced the highest biomass weight and lipid yield at 32 °C with a yield of 15.10 g/l

and 5.11 µg/l. At 35 ℃, it gave a dry biomass weight of 10.61 g/l and a lipid yield of 3.21 µg/l while at 40 ℃, it gave a dry biomass weight and lipid yield of 3.21 g/l and 0.97 µg/l respectively. *Chlorella lewinii* produced a dry biomass weight and lipid yield of 12.17 g/l and 3.11 µg/l at 32 ℃. At 35 ℃, it produced a dry biomass weight of 8.14 g/l and a lipid yield of 2.01 µg/l while at 37 ℃, it produced a biomass weight of 6.20 g/l and a lipid yield of 2.00 µg/l. At 40 ℃, it gave a biomass weight of 5.23 g/l and a lipid yield of 1.00 µg/l. *Cladophora glomerata* produced a high biomass weight of 5.00 g/l at 35 ℃ with its lipid yield for that temperature being 1.11 µg/l. At 35 ℃, the resultant dry biomass weight and lipid yield were 7.70g/l and 1.23 µg/l. At 37 ℃, the dry biomass weight and lipid yield were 5.22g/l and 0.91 µg/l respectively while at 40 ℃, the dry biomass weight was 2.99g/l while the lipid yield was 0.67 µg/l.

Table 4 shows the effect of pH on the dry biomass of the microalgal isolates harvested and the amount of lipids harvested. For *Chlorella vulgaris*, a high dry biomass weight of 12.21 g/l and a high lipid yield of 4.00 µg/l was produced at pH 5.5 with the biomass weight and lipid yield at pH 6.5 was 9.89 g/l and 3.87 µg/l respectively. At pH of 7.5, the dry biomass weight was 4.05 g/l while the lipid yield was 1.43 µg/l. *Stigeoclonium tenue* produced a dry biomass weight and lipid yield of 9.00 g/l and 2.31 µg/l respectively at pH of 5.5 while the biomass weight and lipid yield at pH 6.5 was 14.01 g/l and 4.12 µg/l. At pH 7.5, the dry biomass weight was 5.01g/l while the lipid yield was 0.91 µg/l. At pH 5.5, *Chlorella lewinii* produced a dry biomass weight and lipid yield of 6.67g/l and 3.12 µg/l respectively while at pH 6.5, the dry biomass weight and lipid yield was 5.00 g/l and 1.04 µg/l respectively. At pH 7.5, the dry biomass weight was 4.34 g/l while the lipid yield was 1.00 µg/l. For *Cladophora glomerata*, the dry biomass weight and lipid yield at pH 5.5 was 13.21 g/l and 4.21 µg/l respectively. At pH 6.5, the dry biomass was 7.07 g/l while the lipid yield was 2.31 µg/l while at pH 7.5, the dry biomass weight was 6.12 g/l while the lipid yield was 1.01 µg/l.

Table 3 Effect of Temperature on lipid yield and weight of the dry biomass of the algae

Temp	*Chlorella vulgaris*		*Stigeoclonium tenue*		*Chlorella lewinii*		*Cladophora glomerata*	
℃	Dry Biomass g/l	Lipid yield µg/l	Dry Biomass g/l	Lipid yield µg/l	Dry Biomass g/l	Lipid yield µg/l	Dry Biomass g/l	Lipid yield µg/l
28	4.13±0.01	1.20±0.17	6.11±0.51	1.00±0.27	9.02±1.55	2.15±0.60	3.15±0.34	0.91±0.01
32	8.01±0.31	3.30±0.15	15.10±1.11	5.11±0.09	12.17±1.06	3.11±0.51	5.01±0.00	1.11±0.04
35	6.19±1.29	2.17±0.08	10.61±1.14	3.12±0.81	8.14±1.06	2.01±0.18	7.70±1.45	1.23±0.07
37	4.55±0.13	1.13±0.10	7.11±1.01	2.21±0.02	6.20±0.28	2.00±0.15	5.22±0.16	0.91±0.12
40	3.21±0.00	0.88±0.02	3.21±0.21	0.97±0.07	5.23±0.30	1.00±0.00	2.99±0.18	0.67±0.03

Table 4 Effect of pH on lipid yield and weight of the dry biomass of the algae

pH	*Chlorella vulgaris*		*Stigeoclonium tenue*		*Chlorella lewinii*		*Cladophora glomerata*	
	Dry Biomass g/l	Lipid yield µg/l	Dry Biomass g/l	Lipid yield µg/l	Dry Biomass g/l	Lipid yield µg/l	Dry Biomass g/l	Lipid yield µg/l
4.5	4.05±0.23	1.43±0.07	3.32±0.11	0.89±0.07	2.12±0.15	0.65±0.20	5.05±0.44	1.01±0.32
5.5	7.33±1.11	2.10±0.12	5.01±0.21	0.91±0.02	4.34±0.06	1.00±0.45	6.12±1.76	1.11±0.09
6.5	9.89±1.09	3.87±0.87	9.00±1.31	2.31±0.12	5.00±0.08	1.04±0.04	7.07±1.15	2.33±0.17
7.5	12.21±2.01	4.00±0.65	14.01±2.04	4.12±0.21	6.67±0.66	3.12±0.11	13.21±2.00	4.21±0.10

3.4 Fatty acid composition

The fatty acid composition of the biodiesel produced from the microalgae isolated is as shown in Table 5. *Chlorella vulgaris* had the highest value of Palmitic Acid at 19.5 while both *Chlorella lewinii* and *Cladophora glomerata* had both 12.2 and 12.3 respectively. *Chlorella vulgaris* had the highest value of Stearic Acid at 18.8 followed by *Stigeoclonium tenue* at 15.6 while *Chlorella lewinii* had a value of 14.3. Only *Chlorella lewinii* had presence of Linoleic Acid with a value of 0.98. *Chlorella vulgaris* had the highest value of Myristic Acid with a value of 17.6 followed by *Stigeoclonium tenue* at 15.4 while *Cladophora* had the lowest value of Myristic Acid at 0.78. Biodiesel produced from *Stigeoclonium tenue* had the highest value of Oleic Acid at 18.6 followed by *Chlorella vulgaris* at a value of 14.2 while *Cladophora glomerata* had

the lowest value of Oleic Acid at 0.65. Biodiesel produced from *Chlorella lewinii* had the highest value of Lauric Acid at 15.5 followed by *Chlorella vulgaris* at a value of 13.4

3.5 Properties of Biodiesel

The tested properites of the biodiesel produced from the microalgae oil as compared with standard quality biodiesel parameters is as shown in Table 6. From the density tests carried out, it showed that *Chlorella vulgaris*, *Stigeoclonium tenue*, *Chlorella lewinii* and *Cladophora glomerata* had density values (gml^{-1}) of 0.861± 0.01, 0.867± 0.02, 0.860± 0.02 and 0.870± 0.03 respectively. The viscosity values of the microalgal biodiesel were within the range of 3.75 and 4.14. The flash point values (°C) of the microalgal biodiesel was within the range of 122°C and 126°C while the acid values (mgKOH/g) for the microalgal biodiesel was within the range of 0.23-0.32.

Table 5 Fatty Acid Methyl Ester (FAME) profiles of the microalgal isolates

Fatty Acid Methyl Ester	***Chlorella vulgaris* (µg/l)**	***Stigeoclonium tenue* (µg/l)**	***Chlorella lewinii* (µg/l)**	***Cladophora glomerata* (µg/l)**
Palmitic	19.5	0.00	12.2	12.3
Stearic	18.8	15.6	14.3	0.00
Linoleic	0.00	0.00	0.98	0.00
Myristic	17.6	15.4	0.00	0.78
Oleic	14.2	18.6	0.00	0.65
Lauric	13.4	0.00	15.5	0.00

Table 6 Properties of the biodiesel from the microalgae compared with standard biodiesel

Parameter	***Chlorella vulgaris***	**Stigeoclonium Tenue**	***Chlorella lewinii***	***Cladophora glomerata***	**Standard Biodiesel Values**
Density at 15Oc (gml-1)	0.861±0.01	0.867 ± 0.02	0.860± 0.02	0.870± 0.03	0.860-0.900
Viscosity at 40oC	4.14 ±0.03	3.75±0.01	4.02±0.01	3.91±0.04	3.5 - 5.0
Flash point (oC)	124±0.01	126±0.03	123±0.01	122±0.01	120
Acid Value (mg KOHg-1)	0.32±0.03	0.28±0.01	0.31±0.02	0.26±0.03	0.5

4 Discussion

In this study, the occurrence of microalgal isolates was high which showed a percentage range of 25-100% from all the locations used in the study. This was expected as it showed that the microalgae isolated, especially *Chlorella vulgaris* was able to grow freely in the environment as it was isolated from shallow water bodies with presence of algal growth in them.

Temperature had a substantial effect on the dry biomass weight of the microalgae harvested and the corresponding lipids extracted from them. In this study, the highest dry biomass weight and Lipid yield was at 32 °C for *Chlorella vulgaris*, *Chlorella lewinii* and *Stigeoclonium tenue* while *Cladophora glomerata* had its highest dry biomass weight and lipid yield at 37 °C. This showed that temperature played an important role in Biodiesel production. When subjected to higher temperatures at 35 °C, 37 °C and 40 °C, it saw a decrease in the Dry Biomass weight and the corresponding Lipid extracted from the microalgae isolated. This was seen in research done by [27, 28, 29].

The effect of pH is an important parameter to the production of biodiesel from microalgae. In this study, high dry biomass weight and lipid yield was obtained at pH 7.5. A decrease of the pH to 6.5, 5.5 and 4.5 lead to a corresponding decrease in the dry biomass weight and subsequent lipid yield in all the microalgae isolated. This shows that optimal biomass growth and lipid yield values would only grow in a pH that is alkaline and would decrease in an acidic environment. This was seen from research done by [28, 30, 31]

According to Knothe *et al*. [32], palmitic, stearic, oleic and linoleic acids were recognized as the common fatty acids contained in biodiesel. The fatty acid compositions of the biodiesels produced from the microalgae isolated vary differently. It is mainly composed of mixture of fatty acids such as palmitic, oleic, Myristic and Stearic acids. Linoleic and Lauric acids was also present to a small extent. The biodiesel properties tests (Density, Viscosity, Flash value and Acid value) on the microalgal biodiesels is very important to discover if the biodiesel produced have parameters that meets international standards of Biodiesel. The density tests done on biodiesel showed that *Cladophora glomerata* had the highest value of 0.870± 0.03g/ml though all biodiesels produced from all microalgal isolates produced density values that were within the standard biodiesel values. The viscosity test carried out showed that *Chlorella vulgaris* had the highest viscosity value of 4.14 ±0.03 though all biodiesel produced from the microalgal isolates had density values that were within the range that meets the international quality biodiesel standard values. The biodiesel with the highest flash point was *Stigeoclonium tenue* with a value of 126±0.03^{o}C while the highest acid value for the biodiesels tested was found in *Chlorella vulgaris* with a value of 0.32±0.03 mg KOHg^{-1}. In the case of flash point and acid value for all the microalgal biodiesels, they are all within the required standard biodiesel values in respect to flash point and acid value.

5 Conclusion

The production of Biodiesel by microalgal species has been studied in this research. From this study, it showed that microalgae would grow within a temperature of 28-32^{0}C while any further increase of temperature would lead to a decrease in the lipid yield and subsequently the quantity of the biodiesel produced. It also showed that the microalgal cells would grow optimally in an alkaline environment. The *Chlorella* species isolated (*Chlorella vulgaris* and *Chlorella lewinii*) contained palmitic, myristic, oleic and stearic acid predominantly in their fatty acid profiles while *Stigeoclonium tenue* and *Cladophora glomerata* contained myristic and oleic acids predominantly in their fatty acid profiles. The subsequent quality tests proved that the biodiesels produced were within the quality for standard biodiesel. This makes the microalgal isolates viable candidates for quality biodiesel production.

Compliance with ethical standards

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Disclosure of conflict of interest

There was no conflict of interest all through the period of this research work or during the time of drafting of this paper.

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