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Isolation of microalgae species from arid environments and evaluation of their potentials for biodiesel production

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Twenty-five (25) strains of microalgae were isolated and screened for growth, lipid accumulation and biodiesel production from arid environments of North East Nigeria. Isolates that produced biomass concentration (≥ 1.50 g L^{-1} cell dry weight), accumulated high concentrations of lipids (≥ 18% of the cell biomass) and could be purified on agar plates were selected for further studies. Four strains morphologically identified as *Oocystis* IA1, *Chlorella* IA7, *Chlorococcum* KA9 and *Botyrococcus* YA5 were selected. The maximum biomass concentrations (g L^{-1}) and lipid contents (%) were 3.02, 3.92, 3.59 and 2.89, and 57, 67.23, 63 and 65 dry cell weights for *Oocystis* IA1, *Chlorella* IA7, *Chlorococcum* KA9 and *Botyrococcus* YA5, respectively. The highest oil productivities (mg L^{-1} day^{-1}) were 135.28 ± 3.32 (*Oocystis* IA1), 165.22 ± 3.36 (*Chlorella* IA7), 131.76 ± 1.11 (*Chlorococcum* KA9) and 140.37 ± 2.13 (*Botyrococcus* YA5). Maximum chlorophyll contents (g g^{-1} cell) were 34.97, 30.00, 39.71 and 32.27, respectively. Fatty acid methyl ester profiles indicated the presence of C16:0, C16:1, C18:0, C18:1, and C18:2. Oleic acid (C18: 1) was predominant, ranging between 73.3 and 85.6%. Biodiesel properties were within the ASTM standards. The present study suggested that the four isolates are good for biodiesel production.

Key words: Biodiesel, *Chlorella*, *Chlorococcum*, *Botyrococcus* sp., lipid accumulation, microalgae, *Oocystis*.

INTRODUCTION

There is a perceptible problem of oil crisis in Nigeria and many other parts of the world. Researchers have postulated that fossil fuel consumption would rise by about 60% in the next 25 years (Rittmann, 2008). Veziroglu and Sahin (2008) also noted that fossil fuel will deplete significantly over the next few decades. In addition, fossil fuel is associated with global warming and climate change. They are non-renewable, cause pollution, and their prices steadily increase in many countries of the world. Renewable energy, carbon neutral

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Abbreviation: FAME, Fatty acids methyl esters.

and environmentally friendly alternative sought globally is biofuel energy. In this respect, first-generation biofuels made from sugar, starch, vegetable oil or animal fats using conventional technology were practical alternatives. This is however being criticized for diverting food away from human food chain, leading to food shortages and price rises (Rosegrant et al., 2006). In addition, to generate enough biofuel from arable crops that could serve any country for one year, available land area would not support such production (Chisti, 2007). The second-generation biofuel made from the stalks of wheat, corn, and wood, generally referred to as non-food-crops from cellulosic materials are also being explored in search of replacement of fossil fuel with their attendant demerits. The major disadvantage as noticed by Schenk et al. (2008) is the economic non-feasibility and environmental related problems.

Microalgal lipids are potential sustainable biofuel feedstocks in future (Chisti, 2007). The use of microalgae as biofuel feedstock has many advantages. They have rapid growth rate with cell doubling time of 1 to 10 days (Schenk et al., 2008) in some species. They have high lipid content - more than 50% of cell dry weight (Hu et al., 2008). Karpenyuk et al. (2013) reported 80% oil in some microalgae and Metting (1996) reported even 90%. Microalgae need smaller land usage. They have 15 to 300 times more oil production than conventional crops on a per-area basis (Li et al., 2010). They can grow on saline and hyper-saline water, and thus there is less dependency on freshwater (Sing et al., 2013). They have high carbon sequestration rate (Jorquera et al., 2010); Moheimani et al., 2012). Algal biomass production systems can easily be adapted to various levels of biotechnological skills (Vonshak, 1990).

Microalgae-based biofuel however has its own bottlenecks. Biodiesel oil productivity by microalgae is determined by the overall growth rates of the algae and the design of the reactors. In addition, the ability of the algae to efficiently harvest light energy for the synthesis of oil is another major consideration in oil productivity: Further Particulars Algae.doc - SlideShare. (n.d.). Retrieved from http://www.slideshare.net/Pammy98/furtherparticularsalgaedoc. Although, many strains of microalgae have been isolated from various environments and reported to have good potentials for biodiesel oil production, the present cost of microalgae diesel oil is still too high to compete with fossil diesel. There is therefore a need to screen for more oil productive strains.

Moreover, it is desirable to locate microalgae culture ponds in arid areas that are not suitable for conventional agriculture to reduce competition for arable land. In that regard, it is necessary to isolate oil productive strains from such environment since such strains could be better adapted to the arid climates. In this work, therefore, effort was made to isolate oil-producing strains from arid region of North East Nigeria.

MATERIALS AND METHODS

Microalgae and culture condition

Microalgae were obtained from different locations around North East Nigeria from water bodies between June and December 2009 and were cultivated in BG-11 medium. The medium contains $NaNO_3$ 1.5 g, $K_2HPO_4.3H_2O$ 0.04 g, $KH_2PO_4.3H_2O$ 0.2 g, disodium EDTA 0.001 g, Fe ammonium citrate 0.001 g, citric acid 0.006 g, Na_2CO_3 0.02 g and 1 ml of trace metal solution per litre, pH 7.3. The trace metal solution contains H_3BO_3 2.85 g, $MnCl_2$. $4H_2O$ 1.8 g, $ZnSO_4.7H_2O$ 0.02 g, $CuSO_4.5H_2O$ 0.08 g, $CoCl_2$.$6H_2O$ 0.08 g and $Na_2MoO_4.2H_2O$ 0.05 g per litre. Each isolate was cultivated in a 75 ml sterile medium contained in a 200 ml capacity sterile transparent Roux bottle capped with urethane foam. Pond water containing microalgae was inoculated (inoculum ratio = 25%) and incubated near windows in our Laboratory at room temperature (30±2°C) under atmospheric CO_2. The incubation lasted between two and three weeks depending on the isolate. Purification of the isolates involved successive decantation of the upper growing layer into a freshly prepared medium followed by plating on the BG-11 medium solidified with 1% agar-agar. Growth on the agar plates lasted also for about 3 weeks for some of the cultures. The emergent colonies were re-inoculated into a sterile BG-11 agar medium with repeated sub-culturing. Thereafter, the colonies were transferred into a fresh sterile BG-11 medium.

Microscopic identification of the isolates

Microscopic identifications of the isolates were based on cell morphology and colonial characteristics (APHA, 1985). Cell micrographs were prepared using a Microscope Digital Camera model DCM310.

Experimental set-up for the growth of the isolates

Experimental set-up for the growth of the isolates involved cultivation of each isolate in a 2000 mL transparent Teflon bottle containing 1000 mL of sterile BG-11 medium at an initial pH of 7.3. Each bottle was inoculated with one week-old pure culture (inoculum ratio = 15%), capped with urethane foam and incubated at room temperature (30±2°C) for 12 days. They were either naturally illuminated (about 4000 lux) or by using 2-feet fluorescent tubes arranged in parallel with maximum light intensity of 5000 lux. The photoperiod was 14 h (14 h light followed by 10 h dark). The light intensity was measured at the centre of the culture bottle with a digital light meter (model LX-1000, Custom Limited, Japan). All the cultures were shaken twice at 120 rpm for 3 min in a Gallenkamp orbital shaker (Gallenkamp Limited, UK) every day and samples were taken every two days for analyses. All the experiments were carried out in triplicates and the results presented as average values ± standard deviation.

Analytical procedures

Cell dry weight

Samples were harvested by centrifugation at 3,000 rpm in a bench-top centrifuge for 15 min and washed three times with distilled water. This was thereafter transferred to a pre-weighed filter paper (w_1) and dried to a constant weight in a hot air oven at 70°C overnight. They were left in desiccators for 5 h before weighing (w_2).

$$\text{Cell concentration (g/L)} = \frac{\text{W2} - \text{W1}}{\text{V}} \times 1000$$

Where, w_2 = weight of filter paper and dried cells (g), w_1 = weight of filter paper (g), v = volume of culture (ml).

Measurement of chlorophyll content

The chlorophyll contents were measured according to the procedure reported by Becker (1994) using methanol and water.

Lipids extraction

Lipids were extracted in a chloroform-methanol-water system by the two-step method of Bligh and Dyer (1959). The lipid fraction was transferred into a pre-weighed vial. The chloroform was evaporated by heating in a 55°C water bath under a constant stream of nitrogen gas. After 1 h in a 105°C oven, vials were weighed again. The extracted lipid was expressed as % lipid. Lipid productivity was calculated as the product of average lipid content and biomass productivity in gram per litre per day (Griffiths and Harrison, 2009).

$$\text{Plipids } (\text{gL}^{-1}\text{d}^{-1}) = \frac{\text{Total microalgae biomass production (g)} \times \text{lipid content (\%)}}{\text{working volume (l)} \times \text{cultivation time}}$$

Transesterification of the microalgal oil

Transesterification of the microalgal oil was done according to the methods reported by Kywe and Oo (2009) and Ojolo et al. (2011), using methanol (6:1) and 1% NaOH as the catalyst. The reaction temperature was 65°C while the reaction time was 1 h.

Fatty acids methyl esters (FAME) analyses

The fatty acids profiles were determined using Shimadzu Gas Chromatograph (Shimadzu, Japan, Model GCMS-QP2010 Plus). The analytical method involved the following details. Number of rinses with both pre and post solvents were 4 each; number of rinses with sample was 3; plunger speed (suction, injection) and syringe insertion were high. The injection mode was normal, pumping times was 4, and injection port dwell time was 0.3 s. There was no terminal air gap, plunger-washing speed was high, washing volume was 0.8 µL, and both syringe suction position and syringe injection position were 0.0 mm. The column oven temperature was 70°C, injection temperature was 250°C, injection mode was split, and the flow control mode was linear velocity while the pressure was 116.9 kPa. Total flow was 40.8 mL/min, column flow was 1.80 mL/min, linear velocity was 49.2 cm/s and purge flow was 3.0 mL/min while split ratio was 20.0. High-pressure injection, carrier gas saver and splitter hold were all turned off. The GC program of the GCMS-QP2010 Plus ion source temperature was 200.0°C, interface temperature was 250°C, solvent cut time was 2.50 min, detector gain mode was relative and detector gain was 0.00 kV while threshold was 2000. The MS table start time was 3.00 min, end time 24.00 min, ACQ mode was scan, event time was 0.50 s, scan speed was 666 and start m/z was 30.00 while end m/z was 350.00.

Properties of the biodiesel from the microalgal oils

To determine the properties of the microalgal methyl esters, the volume of the algal methyl ester was measured in a graduated cylinder and density obtained by dividing the mass by the volume. Viscosity was measured with a viscometer (Anton Paar DSA 3000 M Stabinger Viscometer instrument). The kinematic viscosity was calculated from the equation , where v was the kinematic viscosity, µ the dynamic viscosity, and ρ the density. A crucible, a thermometer and a hot plate were used for flash point determination. The acid value of the biodiesels was measured by AOCS Method (Ca 5a-40). Alcoholic KOH (0.5 M) was standardized using 1 M H_2SO_4. An aliquot (1 g) of biodiesel was weighed into a conical flask, followed by the addition of 10 ml of hot neutralized ethanol and the mixture boiled on a water bath. The solution was then titrated whilst hot to neutrality with 0.5 M alcoholic KOH using phenolphthalein indicator. The titre values were taken after triplicate runs, the mean value recorded, and their acid values calculated using a conventional formula.

$$\%\text{FFA (as oleic)} = \frac{\text{ml alkali} \times \text{N of alkali} \times 28.2 \text{ mg}}{\text{sample weight (g)}}$$

$$\text{acid value} = \%\text{FFA (as oleic)} \times 1.99$$

Acid value conversion factors for lauric and palmitic were 2.81 and 2.19, respectively. The iodine values determined by AOAC (1990) Method 920.159 (18) required tetrachloromethane, potassium iodide, sodium thiosulphate, and Wij's solution. An aliquot (0.15 g) of the biodiesel was weighed into a conical flask followed by the addition of 15 ml of tetrachloromethane and 25 ml of Wij's solution. This mixture was then placed in a stoppered conical flask, swirled gently and placed in a dark cupboard for 1 h after which 20 ml of potassium iodide solution and 150 ml of distilled water was added. After gentle shaking, liberated iodine was titrated with 0.1 M sodium thiosulphate solution until a yellow colour appeared. The starch indicator (1 ml) was added and titration continued until the blue colour disappeared even after vigorous shaking. The titre values were taken after triplicate runs and the mean values recorded. Thereafter, a blank was prepared in which distilled water was added in place of biodiesel. The iodine value was calculated according to the formula below:

$$\text{iodine value} = \frac{(\text{B} - \text{S}) \times \text{N} \times 12.69}{\text{sample weight (g)}}$$

Where, S = sample titration, B = blank titration, N = normality of $Na_2S_2O_3$ solution, 12.69 was used to convert from meq. thiosulphate to g iodine; M. W. iodine is 126.9.

Determination of the peroxide values of the biodiesels was done according to AOAC (1990) method 965.33 (18). An aliquot (1 g) of biodiesel was weighed into a conical flask containing 6 ml of glacial acetic acid: chloroform solution (3:2 v/v). Saturated potassium iodide solution (0.1 ml) was added and the solution swirled in the dark for 1 min after which 6 ml of distilled water was added. The mixture was titrated with 0.01 M sodium thiosulphate with vigorous shaking until all the yellow colour disappeared. Thereafter, starch indicator (0.1 ml) was added and titration continued until all the blue colour disappeared. The above procedure was repeated for the various samples. A blank experiment was carried out in which 1 ml of distilled water was added in place of biodiesel. The titre values for the various samples and the blank were determined after each triplicate runs and the mean values recorded. These were used to calculate the peroxide values of the biodiesels according to the formula:

$$\text{Peroxide value} = \frac{(\text{S} - \text{B}) \times \text{N} \times 1000}{\text{sample weight (g)}}$$

Where, S = sample titration, B = blank titration and N = normality of $Na_2S_2O_3$ solution.

Saponification values of the oils were determined according to AOAC (1990) Method 920.160 (18). A 1.0 g quantity of the algal diesel was weighed into a conical flask containing 12.5 ml of 0.5 M alcoholic KOH and the mixture refluxed for 30 min. The reflux condenser was removed and the reflux mixture titrated with 0.5 M H_2SO_4 using phenolphthalein indicator. A blank experiment was also titrated without adding the oil and without refluxing. The titre values for the sample extracts and the blank were taken each after triplicate runs and the mean values recorded. These were used to calculate the saponification values of the oils according to the formula:

$$\text{saponification number} = \frac{(S - B) \times N \times 56.1}{\text{sample weight (g)}}$$

Where: S = sample titration, B = blank titration, N = normality of the HCl, 56.1 = the M. W. of KOH.

Statistical analysis

Statistical analysis was by Multiple-Sample Comparison using STATGRAPHICS Centurion XVI Version 16.1.05 (32-bit). All the experiments were carried out in triplicates and data were presented using descriptive statistics.

RESULTS AND DISCUSSION

Screening of the isolates for growth and lipid accumulation

All the 25 isolates grew on BG-11 medium (Table 1) with variation in growth and lipid accumulation rates. Isolates AA7, GA8, GA10, IA1, IA7, IA8, KA9, UA11, UA8 and YA5 grew well and produced biomass concentrations greater than or equal to 1.50 g L^{-1} dry cell weight. Isolates GA1, GA8, GA12, IA1, IA2, IA6, IA7, IATM, KA9 and YA5 grew and had lipid contents up to 18% of the cell biomass (Table 1). The predominant fatty acid composition of each isolate is oleic acid (C18.1). Out of the 25 isolates, sixteen (AA3, AA7, GA1, IA1, IA2, IA6, IA7, IA8, IA9, IA25, IAMAN, KA9, KA11, UA8, UA11 and YA5) could be purified on agar plates (Table 1). While isolates GA8, GA10 IA8 UA11, UA8 and AA7 showed good growths, they had insignificant lipids. Isolate GA1 had significant lipid but showed relatively low growth. The most effective lipid producers that showed good growth and could be purified on agar plates, were morphologically identified as *Oocystis* IA1, *Chlorella* IA7, *Chlorococcum* KA9 and *Botyrococcus* YA5 and were used for further studies (Figure 1). The microalgae *Oocystis* IA1, *Chlorella* IA7, *Chlorococcum* KA9 and *Botyrococcus* YA5 were superior to other isolates of this same study on the ground of sustained growth and lipid accumulating capabilities. During the screening processes, some of the isolates did not show good growth in the media. Some did not withstand the isolation conditions and showed orange to brownish coloration during their cultivation and subsequently bleached and died. Some isolates had insignificant lipid accumulating capabilities whereas purification of some on agar plates was difficult. Isolation and screening of native species for biodiesel production are important because they are already adapted to their local environment (Wu et al., 2012).

Growth of the four isolates on BG-11 medium

The cultures attained the stationary phase between 10th to 12th days of incubation (Figure 2). The overall highest biomass concentration of 3.92 g L^{-1} was obtained by *Chlorella* IA7 on the 10th day of cultivation (Figure 2). *Chlorococcum* KA9 also had its highest biomass of 3.55 g L^{-1} on the 10th day of cultivation. The highest biomass concentrations for *Oocystis* IA1 and *Botyrococcus* YA5 were 3.02 and 2.89 g L^{-1}, respectively (Figure 2).

Comparison of biomass concentrations, oil contents, productivities and chlorophyll contents of the isolates

Comparisons of maximum biomass concentrations, oil contents, productivities and chlorophyll contents of the isolates are presented in Table 2. The highest oil productivity of 165.22 ± 3.36 mg L^{-1} day^{-1} was achieved by *Chlorella* IA7. The highest oil productivities obtained for *Oocystis* IA1, *Chlorococcum* KA9 and *Botyrococcus* YA5 were 135.28 ± 3.32, 131.76 ± 1.11 and 140.37 ± 2.13 mg L^{-1} day^{-1}, respectively (Table 2). Maximum lipid content got from *Oocystis* IA1, *Chlorella* IA7, *Chlorococcum* KA9 and *Botyrococcus* YA5 were 57, 67.23, 63 and 65% dry cell weights, respectively (Table 2). Maximum chlorophyll contents (g g^{-1} cell) of the isolates were 34.97, 30.00, 39.71 and 32.27 for *Oocystis* IA1, *Chlorella* IA7, *Chlorococcum* KA9 and *Botyrococcus* YA5, respectively. For the production of biodiesel from microalgae, biomass and lipid contents and compositions remain critical issues (Chisti, 2007; Griffiths and Harrison, 2009). The present study suggests that *Chlorella* IA7 was the best candidate for high-density culture and lipid productivity followed by *Botyrococcus* YA5 while *Chlorococcum* KA9 had the highest chlorophyll content (Table 2). Mata et al. (2010) reported lipid content value of 10.5% for *Oocystis pusilla*, which is lower than that obtained in the present work for *Oocystis* sp. *Chlorella* IA7 of the present study, with its final biomass of 3.92 g L^{-1} was higher than 2.58 ± 0.07 g L^{-1} reported by Praveenkumar et al. (2012), and 1.65 ± 0.07 reported for *Chlorella vulgaris* (Abou-Shanab et al., 2011). The final biomass concentration reported by Cheirsilp and Torpee (2012) for marine *Chlorella* sp was supported by the present study, however, the final lipid content and productivity varied.

Similarly, the final lipid productivity of *C. vulgaris* ESP-31 (144 mg L^{-1} d^{-1}) was lower but biomass concentration of 5 g L^{-1} (Yeh and Chang, 2012) was higher than that

Table 1. Screen characteristics of the isolates for growth and lipid accumulations.

Isolate designation	Max. cell dry weight g L^{-1}	Isolation source	Lipid content (%)	Predominant fatty acid composition	Purification on agar plate	Cultural characteristics
AA3	1.65	Fresh water	8.65	C18:1	+	Long cylindrical and striated
AA7	1.88	Fresh water	10.26	C18:1	+	Long cylindrical and striated
ALS	0.22	Fresh water	10.21	C18:1	-	Long cylindrical and striated
AUSA1	1.00	Fresh water	7.14	C18:1	+	Motile, Euglenoid
GA1	1.40	Fresh water	18.36	C18:1	+	Coccoidal
GA8	1.60	Fresh water	19.92	C18:1	-	Coccoidal
GA10	2.20	Fresh water	15.32	C18:1	-	Coccoidal
GA12	0.84	Fresh water	20.29	C18:1	-	Coccoidal
IA1	2.46	Fresh water	26.18	C18:1	+	Oval, conspicuous nucleus
IA2	0.30	Fresh water	20.00	C18:1	-	Coccoidal
IA6	1.00	Fresh water	26.13	C18:1	+	Coccoidal
IA7	1.87	Fresh water	45.59	C18:1	+	Oval, conspicuous nucleus
IA8	2.55	Fresh water	11.20	C18:1	+	Coccoidal
IA9	1.36	Fresh water	8.07	C18:1	+	Coccoidal
IA11	1.00	Fresh water	10.12	C18:1	-	Coccoidal
IA25	1.60	Fresh water	12.14	C18:1	+	Coccoidal
IAMAN	1.40	Fresh water	14.35	C18:1	-	Coccoidal
IATMU	0.80	Fresh water	19.62	C18:1	+	Coccoidal
KA9	1.62	Fresh water	31.60	C18:1	+	Coccoidal, motile and minute
KA11	1.00	Fresh water	18.61	C18:1	+	Long cylindrical and striated
UA8	1.19	Fresh water	7.95	C18:1	+	Long cylindrical and striated
UA11	1.93	Fresh water	9.30	C18:1	+	Coccoidal
UOA4	1.18	Fresh water	10.33	C18:1	-	Coccoidal and motile
UOA8	1.00	Fresh water	17.82	C18:1	-	Motile, Euglenoid
YA2	1.00	Fresh water	16.96	C18:1	-	Circular
YA5	1.80	Lake chad	47.82	C18:1	+	Coccoidal and motile

- = could not be purified; + = could be purified on agar plate.

obtained in the present study. Liu et al. (2011) reported a range of dry biomass (g L^{-1}) of 0.55±0.21 to 5.75±0.45, lipid content (%) of 18.67±2.94 to 52.08±2.37 and lipid productivity (mg L^{-1} d^{-1}) of 13.74±2.94 to 194.27±1.56 for *Chlorella* sp which were corroborated by the present study.

In addition, Mata et al. (2010) reported a maximum lipid content and productivity for different strains of *Chlorella* sp. as 63.0%, and 121.4 mg L^{-1} d^{-1}, which were similar to the values in the present study. Moheimani (2013) reported maximum biomass and lipid productivity of 407±5.5 mg biomass L^{-1} day^{-1} which was higher than the values of the present study and 99±17.2 mg lipid L^{-1} day^{-1} for a *Chlorella* sp.

Liu et al. (2011) and Mata et al. (2010) reported lower values of biomass productivity, lipid content and lipid productivity for *Chlorococcum* sp than the value of the present study. Report by Mata et al. (2010) for the range of lipid content obtained for *Botryococcus braunii* (25.0 to 75.0) was similar to the values obtained in the present study.

However, the final cell dry weight of 4.55 g L^{-1} obtained by Zhang et al. (2011) for *B. braunii* was higher than the 3.2 g L^{-1} of the present study. Similarly, Ruangsomboon (2012) obtained lipid content for *B. braunii* which was supported by the present work, however; the highest biomass concentration of 1.91 g L^{-1} (Ruangsomboon, 2012) was lower than that obtained in the present study.

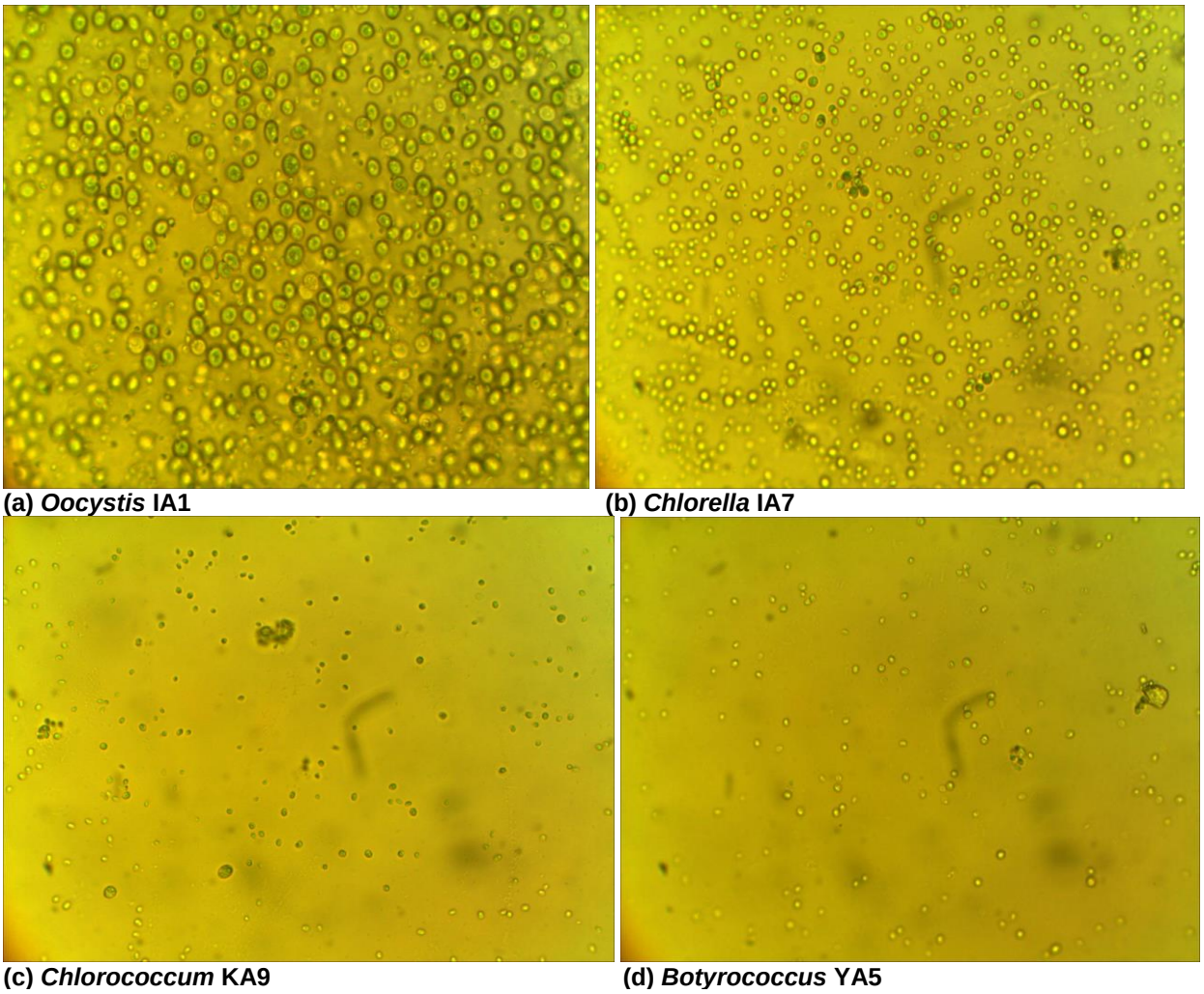

Figure 1. Photos of the representative isolated microalgae (a – d).

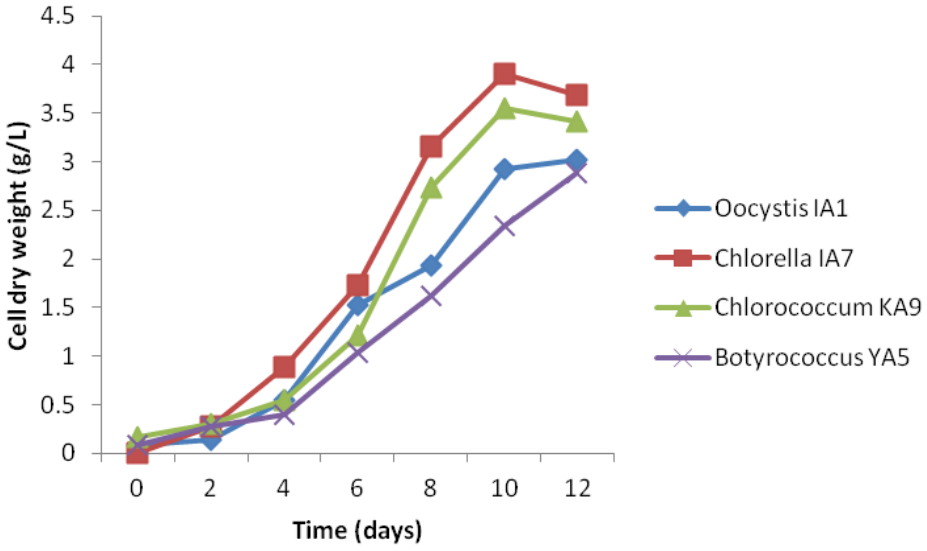

Figure 2. Growth curve of the four isolates on BG-11 medium.

Table 2. Comparison of maximum biomass concentrations, maximum oil contents and productivity and maximum chlorophyll contents of the isolates.

Isolate	Max. Chlorophyll a + b (mg/g cell)	Specific growth rate μ(d^{-1})	Maximum biomass concentration (gL^{-1})	Biomass productivity ($gL^{-1}day^{-1}$)	Max. oil content (%)	Max. oil productivity ($mgL^{-1}day^{-1}$)
Oocystis IA1	34.97	0.37 ± 0.02	3.02 ± 0.20	0.48 ± 0.07	57.00 ± 1.15	135.28 ± 3.32
Chlorella IA7	30.00	0.44 ± 0.02	3.92 ± 0.10	0.65 ± 0.03	67.23 ± 3.99	165.22 ± 3.36
Chlorococcum KA9	39.71	0.35 ± 0.01	3.59 ± 0.19	0.60 ± 0.01	63.00 ± 3.32	131.76 ± 1.11
Botyrococcus YA5	32.27	0.32 ± 0.01	2.89 ± 0.03	0.44 ± 0.10	65.00 ± 2.72	140.37 ± 2.13

Table 3. GCMS analyses results showing fatty acid methyl ester (FAME) profiles of the four microalgae.

Sample designation	Lauric acid C12:0	Myristic acid C14:0	Palmitic acid C16:0	Stearic acid C18:0	Oleic acid C18:1	Linolenic acid C18:3	Elaidic acid C19:	Erucic acid C22:1
Oocystis IA1	0.80	5.12	-	9.34	76.90	1.21	-	2.12
Chlorella IA7	-	4.40	-	8.26	84.31	1.15	-	0.96
Chlorococcum KA9	5.4	0.6	-	9.69	73.15	1.58	-	3.64
Botyrococcus YA5	-	3.66	7.48	-	85.64	-	1.25	1.91

Fatty acid methyl ester (FAME) profiles of the four microalgae

The main fatty acids in *Oocystis* IA1 were myristic acid (C14:0), stearic acid (C18:0), oleic acid (C18:1) and linolenic acid (C18:3). Among these, stearic acid and oleic acid were the two most predominant fatty acid components, accounting for over 80% of the total fatty acids present in the microalgal lipids (Table 3). For *Chlorella* IA7, the main fatty acids were myristic (C14:0), stearic acid (C18:0), oleic acid (C18:1) and linolenic acid (C18:3). Among these, stearic acid and oleic acid were the two most predominant, accounting for up to 90% of the total fatty acids in the alga. *Chlorococcum* KA9 contained, in addition, lauric and erucic acid. However, the predominant fatty acids were stearic acid and oleic acid accounting for about 82% of the total fatty acids in the alga methyl ester. *Botyrococcus* YA5 contained predominantly palmitic and oleic acids (Table 3). Six different fatty acids were found in *Oocystis* IA1 and *Chlorococcum* KA9 methyl esters; whereas, *Chlorella* IA7 and *Botyrococcus* YA5 methyl esters contained five fatty acids each. Similar fatty acid compositions were observed in the four microalgal methyl esters of this study. The predominant fatty acid was oleic acid (C18: 1) signifying that the isolates are appropriate for the production of excellent quality biodiesel. Oleic acids are the markers of biodiesel quality (Yoo et al., 2010). The composition and structure of fatty acid esters determine the properties of biodiesel and addition of methyl oleate improves its properties such as its oxidative stability and low melting temperature (Knothe, 2008). Similarly, palmitic, stearic, oleic and linoleic acid were the most common fatty acids contained in biodiesel (Knothe, 2008).

In a similar study, Chader et al. (2011) noted that the major fatty acid composition of *Chlorella sorokiniana* was a mixture of unsaturated fatty acids, such as oleic (18:1), linoleic (18:2) and linolenic acid (18:3) and trace amounts of saturated fatty acids, palmitic (16:0) and stearic (18:0). Biodiesel feedstock standards require high total fatty acid and low PUFA content (Doan and Obbard, 2012). For example, the International Biodiesel Standard for Vehicles (EN14214) requires the level of linolenic acid methyl ester (18:3n6, 18:3n3) and PUFA in final biodiesel fuel to be less than 12 and 1% (m/m), respectively. The present research is consistent with this requirement since linolenic acid was either not detectable or is lower than this range.

Properties of the oils and fatty acid methyl esters (biodiesel) from the four microalgae

Properties of the oils from the four microalgae are presented in Table 4 with values not greatly differing from the FAME values of Table 5. The densities of the microalgae FAME are within the range of 0.867 to 0.882 g ml^{-1}, the viscosity values fell within 3.55 and 4.50, and flash points between 148 ± 0.22 and 165 ± 1.22°C. The range of iodine values (10^{-4} Pa s), acid values (mg $KOHg^{-1}$)

Table 4. Properties of the oils from the four microalgae.

Parameter	*Oocystis* IA1 oil	*Chlorella* IA7 oil	*Chlorococcum* KA9 oil	*Botyrococcus* YA5 oil
Density (at 15°C) (gml^{-1})	0.881 ± 0.01	0.878 ± 0.02	0.882 ± 0.04	0.877 ± 0.02
Viscosity at 40°C	3.85 ± 0.05	4.60 ± 0.09	4.50 ± 0.15	4.70 ± 0.04
Flash point (°C)	148 ± 0.22	155 ± 0.02	157 ± 0.11	165 ± 1.22
Iodine value (10^{-4} Pa s)	120.0 ± 1.09	109.9 ± 1.01	118.5 ± 0.05	116.1 ± 0.92
Acid value (Mg $KOHg^{-1}$)	0.490 ± 0.05	0.388 ± 0.01	0.510 ± 0.06	0.533 ± 0.04
Saponification value (Mg $KOHg^{-1}$)	161 ± 1.02	154 ± 1.01	160.0 ± 0.92	161.5 ± 0.85

Results are means of triplicate tests.

Table 5. Properties of the biodiesel from the four microalgae in comparison with diesel fuel and standard biodiesel.

Biodiesel properties	FAME *Oocystis* IA1	FAME *Chlorella* IA7	FAME *Chlorococcum* KA9	FAME *Botyrococcus* YA5	Diesel fuel	Standard biodiesel values	Test methods
Density (at 15°C) (gml^{-1})	0.867 ± 0.02	0.861 ± 0.04	0.872 ± 0.01	0.867 ± 0.02	0.838	0.860-0.900	EN ISO 3675
Viscosity at 40°C	3.15 ± 0.12	4.10 ± 0.09	4.20 ± 0.15	4.15 ± 0.04	9 - 4.1	2.0 - 5.0	EN ISO 3104 ASTMD445
Flash point (°C)	126 ± 1.20	125 ± 0.77	127 ± 0.18	125 ± 1.05	75	100 min	ISO 3679 ASTM D93
Iodine value (10^{-4} Pa s)	110.0 ± 1.22	113.5 ± 0.55	112.4 ± 0.36	111.2 ± 1.08		130 max	EN 14111
Acid value (Mg $KOHg^{-1}$)	0.210 ± 0.01	0.226 ± 0.03	0.190 ± 0.02	0.214 ± 0.01	Max. 0.5	0.5 max.	ASTM D664, D974
Saponification value (Mg $KOHg^{-1}$)	182.2 ± 0.11	178.5 ± 0.98	180.0 ± 1.02	181.5 ± 1.11			

Results are means of triplicate tests.

and saponification value (mg $KOHg^{-1}$) were 109.9 ± 1.0 to 120.0 ± 1.09, 0.388 ± 0.01 to 0.533 ± 0.04 and 154 ± 1.01 to 161.5 ± 0.85, respectively, (Table 5). Although, the values varied slightly amongst the different fatty acid methyl esters, there was no significant difference between them. Biodiesel properties namely density (at 15°C), gml^{-1}; viscosity at 40°C; flash point (°C); iodine value (10^{-4} Pa s); acid value (mg $KOHg^{-1}$); and saponification value (mg $KOHg^{-1}$) obtained in the present study were within the ASTM standard. The densities of the four-microalgal methyl esters were lower than their oil (Tables 4 and 5).

Lang et al. (2001) noted that the original crude oil, the refining steps and weather influence biodiesel density. Compared with the density of petro diesel, the four-microalgal methyl esters had higher densities. Conversely, the densities obtained in the present work are lower than those obtained for most vegetable oil methyl esters and from animal fats (Canakci and Sanli, 2008). In this study, even though the *Chlorococcum* KA9 methyl ester density was slightly higher than the densities from the other three, there was no significant difference among them.

The viscosities of the four microalgae methyl esters were significantly lower than their respective oils. The viscosities obtained in the present work are lower than those obtained for most vegetable oil and animal fat methyl ester (Canakci and Sanli, 2008). In this study, even though the *Oocystis* IA1 oil and methyl esters viscosities were apparently lower than those obtained from the remaining three, they were not statistically different from each other. The flash points of the oils were higher than their respective methyl esters for the four microalgae.

Compared to petro diesel, the values obtained in this study were higher than those reported of petro diesels. In the present study, the flash points of both the microalgal oils and their respective methyl esters were lower than that reported for vegetable oils and their respective biodiesels (Canakci and Sanli, 2008).

Iodine values obtained in this study for algal oil and their respective methyl esters were not significantly different from each other. This supported the observation previously made by Lang et al. (2001) that iodine values of biodiesel esters made from the same oil were similar. Acid values obtained in this study are statistically similar and there is no significant difference between the acid value of the methyl esters and their respective oils. The saponification value also agreed with that ASTM values for biodiesel.

Conclusion

In this study, four strains of microalgae isolated from the arid environments of North East Nigeria have high potentials for biodiesel oil production. Isolation of native species that could produce biodiesel is important because the isolates are already adapted to the local environment. Characterization and further optimization of their culture conditions are currently under investigation.

Conflict of interests

The authors did not declare any conflict of interest.

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