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Utilization of microalgae and accumulation of lipids for biodiesel production from grey-water in Makurdi area of Benue State, Nigeria

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ABSTRACT: This study was conducted to investigate the utilization of some microalgae for simultaneous remediation of grey-water and accumulation of lipid for biodiesel production. Isolation was achieved by serial dilutions of the algal suspension in Bold's Basal medium (BBM). The algal DNA was isolated using cetyltrimethyl ammonium bromide (CTAB). Morphological analysis established that the shape, size, and colour of various isolates matched those of members of the genus *Chlorella.*Four pure algal cultures were isolated from the grey-water channel and designated *Chlorella lewinii* KU220 JNI1, *Chlorella vulgaris*. NEIST JNI3, *Chlorella lewinii* KU217 JNI6, and *Chlorella* spp. NC JNI8. The *Chlorella* strains were confirmed to the genus level by ITS2 rDNA as shown by BLAST. The FT-IR characterization of the biodiesel indicated peak region at 1745 cm^{-1} *Chlorella* spp. were isolated from grey-water which produced biodiesel with high yield per algae biomass 50 g of *Chlorella* spp. biomass generated 5.1 g of lipid which was converted to biodiesel from simple trans-esterification reaction. FT-IR spectrum revealed that *Chlorella* spp. lipids were converted to**fatty acid**methyl ester (FAME) at 1745 cm^{-1} stretch, while the standard value for ester bond formation was obtained at 1744 cm^{-1} stretch. The physicochemical properties of the biodiesel produced from *Chlorella* spp. analyzed were all in accordance with the ASTM standard.

Keywords: Biodiesel, grey-water, lipids, Makurdi area, microalgae

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INTRODUCTION

The improper management of the grey-water is a major challenge in most of the developing countries. The discharge of these wastes into the environment constitutes many adverse effects on the natural biodiversity. Conversely, the characteristics of the grey-water in terms of the nutrients and elements make these wastes a suitable production medium for the generation of biomass. Among several types of the microorganisms which might be cultured in the grey-water, the microalgae are the most appropriate organisms because they have chlorophyll which can obtain the required energy from the light by the process of photosynthesis. This process is used by the microalgae cell to convert light and CO_2 into glucose which is the main substrate in the anabolic pathways and production of biomass (Shekhawat *et al.*, 2012). The generation of microalgae biomass and their application in the different sectors of life has been started since the 1970s. However, the applications such as biofuel production and bio-generation of bio-products such as a source of valuable chemicals, food additives and pharmaceuticals have increased significantly since 2008 (Pahazri *et al.*, 2016). In the recent years, several companies are working on producing microalgae biomass in the marine and freshwater (Jais *et al.*, 2017). The most common microalgae species used are *Botryococcus Sudeticus Dunaliella*sp. *Chlorella vulgaris*, *Haematococcus*

Pluvialis, *Nannochloropsis Oculata* and *Spirulina platensis*. It has been estimated that the total amount of the *Haematococcus Sp.* is 30 tones/years and that for *Spirulina* sp. is 20 tones/year. It is estimated that more than 6000 L of water are required to produce biomass yield enough to generate one litre of algal oil based on the conventional systems of cultivation, which indicates that the use of a large-scale algal cultivation in freshwater is not an economically suitable option due to the problems of water shortage in many of the developing countries (Ozkan *et al*., 2012). In this work, the grey-water might provide the alternative source of the water. The choice of microalgae species is an important parameter to be considered, the ability of microalgae to survive under harsh environmental conditions reflect its potential to grow in the grey-water and thus results to overproduction of biomass. In this case, the best option to consider is to use indigenous microalgae species isolated from the surrounding environment. It is estimated that the microalgae species are more than 200,000 types; many of these species have the ability to survive in extreme conditions (Kalin *et al*., 2005). The ability of the microalgae to survive in different environmental conditions is attributed to their rapid rate of acclimatization to the surrounding environment even with low concentrations of the nutrients. This process is called the natural selection process. However, the selected microalgae species in the biomass production should be non-pathogenic and should not have the potentials to produce toxins, since these toxins in the biomass might limit the application of biomass as fish or animal feeds or as soil fertilizers. Extraction of microalgal lipids and its conversion to biodiesel have proven to be challenging and are associated with environmental and economic concerns.

Statement of the problem

Little attention is given to conventional discharge of grey-water into drains in terms of environmental sanitation when compared with toilet waste and solid waste disposals. In individual village house areas in Makurdi, bathroom grey-water is most often discharged untreated into water drains. These discharges can cause unpleasant odour, breed mosquitoes, flies, aesthetics of the environment is disturbed and add nutrients (nitrogen and phosphorus) in the drain. An excess nutrient in bathroom grey-water causes eutrophication. This discharge of untreated bathroom grey-water in an uncontrolled manner to the main drain leads to excess nutrients flow into the rivers. Rivers are the main source of water supply in Makurdi. Therefore, eutrophication of rivers with enriched nutrients will rapidly grow algae. The decaying algal plant may result to death of fish and other aquatic lives. In addition, the rapid increase of pollutants (nutrients and pathogens) will occur actively and result in an unhealthy environment for humans or animals with dangerous diseases.

Justification of the work

In the context of a densely populated Makurdi city, full wastewater treatment and re-use is rarely feasible at the household level due to the high space requirement and technological complexity of grey water purification and phosphorus which are normally generated by activities such as bathing. It is anticipated that the utmost source of nitrogen is urine, as some people pass urine in the shower rooms or from body washing and bathing through the use of protein-rich shampoos and conditioners. Additionally, nutrient loads may come from washing babies, children after defecation, diaper changes or diaper washing. Phosphorus is mainly found in detergents. The nutrients represent the key factors for the microalgae growth. The grey-water has a content of nitrogen, phosphorus and trace elements in the range required for microalgae growth and production of biomass to accumulate lipids for biodiesel production (Pahazri *et al*., 2016).

Objectives of the study

The objectives of the study were to:

i.Monitor the conditions for microalgae biomass production in grey-water.
ii.Characterize the microalgal species.
iii.Produce biodiesels from lipids found in the microalgal species.
iv.Determine chemical profile of the biodiesel.

MATERIALS AND METHODS

Area of study

The study area was within the Makurdi metropolis in Benue State, it is between latitude 7^0450^1 N and 7^0500^1 N and longitude 8^0000^1 E, and 9^0000^1 E (Odoemena and Igomu, 2017). Map of the study area is presented in (Figure 1).

Sample collection

Grey-water samples were collected from discharge points of bathrooms in sterile plastic containers (two liters) from households in Makurdi. The containers containing the samples were covered and labeled to avoid errors and taken to Benue State Water Board for physiochemical analysis.

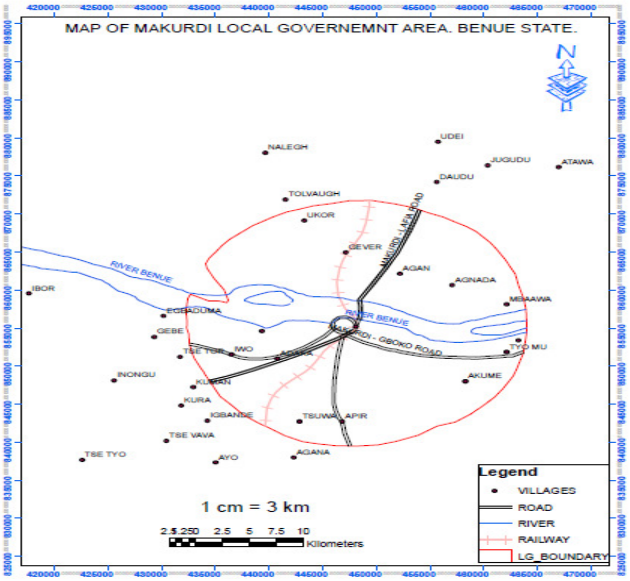

Figure 1: Map of Makurdi Showing the Study Area; Source: Odoemena and Igomu (2017).

Growth of microalgae in grey-water

The grey-water in which the microalgae was grown were channeled from an open bathroom outlet and allowed to stand for one month. The microalgae samples were collected during the onset of rainy season in May, 2021.

Growth on media

The microalgal mass grown in grey-water was collected into clean water containers and transported to the laboratory on ice packs. The isolated strains were acclimatized for a day in conditions similar to their natural environment and then maintained under indoor setting at 27 ± 2 °C. Isolation was achieved by serial dilutions of the algal suspension in Bold's Basal medium (BBM) (Nichols, 1973), followed by inoculation of 40 µL algal suspension on Petri dishes containing BBM prepared with 15 gL^{-1} agar (Difco, USA). The cultures were supplied with artificial light at 150 µmol photons $m^{-2}S^{-1}$ and photoperiod of 14:10^{-h} light-dark cycle.

Algal DNA extraction and characterization

The algal DNA was isolated using cetyltrimethyl ammonium bromide (CTAB) (Doyle and Doyle, 1990) and the concentration of the DNA extract was determined by measuring the absorbance at 260 nm and the 260/280 nm and 230/260 ratios using a NanoDrop ND - 1000 spectrophotometer (Thermo Scientific).

The internal transcribed spacers ITS1 and ITS 2 were amplified from the isolated DNA by the polymerase chain reaction (PCR) using the PTC-100 Programmable Thermal controller thermo cycler (MJ Research, Inc.). Exactly 10 µL DNA sample was amplified in a final volume of 50 µL (500 mM KCl, 100 TIM Tris-HCl, 1 % Triton X-100, 1.2 mM Mg^{++} 20 ng μL^{-1} BSA, 0.2 mM dNTP, 0.5 units thermostable.

Determination of chemical composition of grey-water

Total protein content of grey-water was determined according to the method of Lowry *et al*., (1951) with slight modification by Moheimani *et al*. (2013). This method measured the total soluble protein using Biuret reagent as working solvent. Two and a half milligram (2.5 mg) freeze-dried biomass was released by bead milling; the samples were dissolved in the Biuret reagent solution. The mixture was incubated at 100°C for 1 h in a water bath before addition of 0.5 mL of Folin-Ciocalteu reagent. The mixture was incubated at room temperature for 30 min. Protein standard concentrations were prepared with bovine serum albumin and used to prepare calibration curves on each day of protein quantification. The absorbance was measured with a spectrophotometer at 660 nm. Carbohydrate analysis was based on Dubois *et al.* (1956). Two and a half milligram (2.5 mg) freeze-dried biomass was extracted by bead milling, treated with 0.5 mL 1 M H_2SO_4 (5 mL) to digest the complex carbohydrates to sugar monomers and incubated at 100 °C for 60 min in a water bath. Then 0.5 mL phenolic solution was added while stirring the mixture and 5 mL of concentrated sulfuric acid was also added while mixing. Calibration curve was obtained using D-glucose as a standard reference. The optical density was measured at 485 nm using a densitometer. The Bligh and Dyer method (Bligh and Dyer 1959) was used to extract total lipids from 2.5mg freeze-dried microalgal culture. Briefly, the sample was desalted by rinsing with 5 mL of 0.01 M HCl and maintained at – 80 °C until lipid analysis (Moheimani *et al*., 2013). The thawed biomass was quickly bead milled in a tissue lyzer to release crude lipid content. The extract was homogenized with a mixture (5.7 mL) of methanol: chloroform: water (2:1:0.8 v/v) at room temperature. The mixture was separated by centrifugation at 4000 × g for 10 min and the supernatant collected in a 20 - mL centrifuge tube while the pellet was further homogenized with the extraction mixture (5.7 mL). Again, the mixture was centrifuged at 4000 × g for 10 min and supernatant collected while the pellet was discarded. The supernatant (11.4 mL) was treated with deionized water (3 mL), vortexed, and chloroform (3 mL) added to separate the solution into two layers. While the upper layer methanol and water was removed, the lower layer of chloroform containing the lipid was treated with

acetone (drops) to expel cell debris. The chloroform was evaporated under a stream of ultra-pure nitrogen gas at 38°C on a heating plate while the crude algal lipid extract was estimated gravimetrically and recorded as a percentage of dry weight (Hempel *et al.,* 2012; Moheimani *et al*., 2013).

Lipid= Weight of lipid/cell dry weight X 100

The lipids extracted were converted to biodiesel using the Canakei and van Gerpen (1999) method.

FT-IR spectrum analysis of the biodiesel

The NEAR FT-IR machine was used for the analysis of the biodiesel. The stage of the FT-IR machine was thoroughly cleaned using a cotton bud that had been dipped in petroleum ether. The machine was then test run to ensure no impurities remained on the stage. The sample (biodiesel) was then loaded onto the stage when the machine had finished the test running process and was declared ready for use as displayed on the computer screen. A drop of the sample was loaded on the equipment by the use of a clinical syringe. The peak values of the analysis were marked and the reading was recorded accordingly (Indabawa, 2014).

Physicochemical characterization of the biodiesel

Physico**chemical properties** of *Chlorella* sp. biodiesel (Iodine value, acid value, saponification value, flash point, fire point, cloud point, pour point, density, **refractive index** and pH) were determined in order to compare the biodiesel produced with standards.

Determination of iodine value [ASTM D974 (01)]

Iodine value [ASTM D974 (01)] was achieved using the method employed by Indabawa (2014).

Determination of acid value and free fatty acid value (ASTM D974)

Two grams of oil was measured and poured in a beaker. Then 50 mL of a neutral solvent prepared by the mixture of ethanol and petroleum ether were taken and poured into the beaker containing the sample. The resulting mixture was stirred vigorously for 30 min. About 0.56 g of potassium hydroxide pellet was measured and placed in a conical flask and 0.1 M potassium hydroxide was prepared, 3 drops of phenolphthalein indicator was added to the sample and titrated against 0.1 M potassium hydroxide until a pink coloration was observed. The acid value (AV) was calculated using the following relations as employed by Indhumathi *et al*. (2014).

$$AV = \frac{(56.1 \times A \times N)}{Woil}$$

where:

A = Volume of standard alkali used

N = Normality of standard alkali used

Woil = Weight of oil used

Determination of saponification value (ASTM 5558-95)

A freshly prepared solution of alcoholic potassium hydroxide was made by dissolving potassium hydroxide pellet in ethanol. About 2 g of oil was measured and poured into a conical flask. About 25 mL of the ethanolic potassium hydroxide was then added to it. The sample was covered and placed in a steam water bath and was allowed for 30 min, while shaking the sample periodically, 1 mL of phenolphthalein indicator was added to the mixture. The mixture was titrated against 0.5 M Hydrogen chloride (HCl) and the titer value was noted. The saponification value (SV) was then obtained using the following relations according to (Indhumathi *et al.,* 2014).

$$SV = \frac{(B-A) \times 28.92}{Woil}$$

where:

B = Volume of HCl used in titration with the blank

A = Volume of HCl used in titration with the oil

Woil = Weight of oil used

B = 18.4

A = 5.10

Woil = 2 g

SV = 192.61 mg g^{-1}

Determination of flash point and fire point (ASTM D6751)

An ignition was placed on a measured sample in an open flame. A thermometer was placed in order to record the temperature changes. When the source of ignition was removed, the vapor ceased to burn and this temperature was recorded as the flash point. The flash point was measured as the temperature for which the vapor continued to burn for at least 5 sec after the source of ignition was removed at an open flame (Indabawa, 2014).

The fire point was assumed to be almost 10°C higher than the flash point.

Determination of cloud point (ASTM D2500)

A sample of the biodiesel was placed in a test tube and then placed in a cooling bath after it had been heated to about 40 °C. When the biodiesel started to form cloud below the test tube, its temperature was quickly measured and taken as the cloud point. This procedure was repeated 3 times and the mean value was recorded (Indhumathi *et al.,* 2014).

Determination of pour point (ASTM D97)

A sample of the biodiesel was placed in the freezer for 24 hours. The biodiesel was removed after the period and placed in a beaker containing warm water of about 10 °C to melt. The temperature at the bottom of the test tube at which the biodiesel starts to pour is recorded as the pour point of the biodiesel (Indabawa, 2014).

Determination of specific gravity/density (ASTM D1298) by hydrometer method

This procedure was used by Indhumathi *et al*. (2014) to evaluate the specific gravity of the biodiesel.

Determination of refractive index

This was done using an Abti refractometer as employed by Indabawa (2014).

Determination of pH

A portable pH meter was used to determine the acidity or basicity of the oil.

RESULTS

The Chemical Composition of the Isolated Microalgal Strains

Chlorella lewinii KU220 JNI1, *Chlorella vulgaris*. NEIST JNI3, *Chlorella lewinii* KU217 JNI6, and *Chlorella* sp. NC JNI8 showed significant differences in lipid content ($p < 0.05$) across the tested growth temperatures (Table 1). *Chlorella* sp. NC JNI8 had the highest lipid content at 20 °C (Table 1). There was no significant difference ($p > 0.05$) between the carbohydrate content of strain C. lewinii KU220 JNI1 at 25 °C and 30 °C, while there was substantial increment at 20°C. Values presented are mean values from triplicate sample standard deviation are < 10 % of the mean values.

Different superscript within a row shows significant differences (ANOVA $P < 0.05$) between temperatures for each species. A similar trend was recorded in *Chlorella* sp. NEIST JNI3 for carbohydrate content while there was significant reduction in carbohydrates content of *Chlorella* sp. NC JNI8 at 20 °C compared to 25 °C and 30 °C. At 30 °C, the *Chlorella lewinii* KU220 JNI1 and *Chlorella* sp. NC JNI8 strains showed significantly higher ($p < 0.05$) protein contents compared to 20°C and 25°C (Table 1). The lowest percentage of protein was recorded at 20 °C in *Chlorella* sp. NC JNI8 cultures.

Microalgae strain isolates and identity

After the microalgae were grown for one month in grey-water channeled from an open bathroom outlet in Makurdi Benue State, four pure algal cultures were isolated from the grey-water, and designated *Chlorella lewinii* KU220 JNI1, *Chlorella vulgaris*. NEIST JNI3, *Chlorella lewinii*KU217 JNI6, and *Chlorella* sp. NC JNI8. They were isolated based on rapid growth in the initial evaluation of growth and biomass production. Preliminary morphological identification of microalgal cultures by microscopic analysis revealed the four isolates belonged to the genus *Chlorella*. The cells were solitary, 3–6 µm in diameter and spherical, ellipsoidal, or globular in shape. They lacked flagella, having a parietal and cup-shaped chloroplast with a single pyrenoid surrounded by a thin cellulose wall and no mucilaginous sheath was observed (Table 2).

Physicochemical qualities of biodiesel

The 11 physicochemical qualities of biodiesel produced from *Chlorella* sppall fell within the ASTM acceptable limit with saponification value of 192 mg KOH g^{-1}, an acid value of 0.64 mg KOH mg^{-1} and iodine value of 85 mg g^{-1} mI_2. Also, the biodiesel had a refractive index of 10, fire point of 105°C and pour point of 4 °C. A pH of 7.2 was obtained while ASTM value is 7.0 and density at 40 °C was obtained to be 848 g mL^{-1}. Cloud point of +2 °C was observed while specific gravity at 40 °C was measured to be 0.855 g cm^{-3} with a flash point of 115 °C (Table 3). The genomic DNA extract was used as biomarkers for strains description by PCR amplification of the ITS-2 rDNA sequence. Single-DNA bands of amplified ITS-2 rDNA products were successfully attained from the isolates. BLAST hits generated close matches of deposited sequences for *Chlorella* in the NCBI GenBank and the accession numbers of the sequences are shown in (Table 2).

FT-IR characterization of biodiesel

The NEAR FT-IR characterization of the biodiesel

Table 1: Comparison percentage of dry weight of four *chlorella* strains grown at different culture temperatures.

Chemical component	Temperature (^{0}C)	Micro algal strains			
		***Chlorella lewinii* KU220JN11**	***Chlorella vulgaris* NEISTJN13**	***Chlorella lewinii* KU217JN16**	***Chorella*sp. NCJN18**
% Dry Weight					
Lipid	20	16.21 ± 3.00^c	25.49 ± 2.12^c	14.01 ± 1.76^c	31.45 ± 1.75^c
	25	12.96 ± 2.56^b	15.69 ± 1.86^b	24.44 ± 2.12^b	26.47 ± 2.43^b
	30	20.21 ± 1.50^a	22.25 ± 2.10^a	21.94 ± 1.74^a	18.87 ±2.02^a
Carbohydrate	20	31.96 ± 2.08^b	46.44 ± 4.22^c	26.32 ± 1.43^b	40.31 ± 2.06^a
	25	20.60 ± 2.65^a	39.56 ± 1.98^b	18.49 ± 1.76^a	51.11 ± 1.54^b
	30	19.21 ± 2.87^a	32.86 ± 2.56^a	18.41 ± 2.34^a	41.90 ± 2.21^a
Protein	20	40.28 ± 2.23^a	30.61 ± 2.54^c	35.50 ± 2.54^b	27.83 ± 2.43^b
	25	39.76 ± 2.05^a	39.28 ± 1.78^b	42.35 ± 2.32^a	29.91 ± 2.54^b
	30	41.17 ± 2.65^a	31.94 ± 2.12^a	40.01 ± 2.65^a	35.06 ± 2.32^a

Table 2: Identified Microalgal Isolates with the Blast Analysis, Colour, Query Length, Organism, Accession Number and Maximum Identity.

Isolates	Colour	Query Length	Organism	Accession number	Maximum Identity
JN1	Green	781	*Chlorella lewinii* KU220JN11	KM061464.1	96
JN2	Green	734	*Chlorella vulgaris* NEISTJN13	MG799374.1	94
JN6	Green	778	*Chlorella lewinii* KU217JN16	KU217KM061462.1	96
JN8	Green	732	*Chorella sp.* NCJN18	MG762094.1	98

Table 3: Physicochemical Properties of Biodiesel Compared with American Society for Testing and Materials (ASTM) Standard.

Physicochemical property	Biodiesel	ASTM Standard
Saponification value (mgKOHg^{-1})	192.61 mgg^{-1}	191 – 202 mgg^{-1}
Acid value (mgKOHg^{-1})	0.64 gg^{-1} of oil	< 0.8 gg^{-1} oil
Iodine value (mgL$_2$g^{-1})	85	82 – 98 mgL$_2$g^{-1}
Refraction index	10	12
Fire point (^{0}C)	105^{0}C	140 – 215^{0}C
Pour point (^{0}C)	4^{0}C	-15 to +10^{0}C
PH	7.2	7.0
Density at 40 ^{0}C	848 gmL^{-1}	< 920 kgmm^{-3}
Cloud point	+2	+2
Specific gravity at 40 ^{0}C	0.855 gCm^{-3}	0.9
Flash point (^{0}C)	115^{0}C	100 – 170^{0}C

indicated highest peak region of 1745 wave number (cm^{-1}) at 50.922 absorbance followed by 2924 cm^{-1} at 55.190 absorbance which was as a result of C-H stretch. Carbonyl stretch C=O of esters appears however; as aliphatic from 1750-1735 cm^{-1}. The peak band of 3010 cm^{-1} was olefinic =C-H stretch. The bands between 600 and 1200 cm^{-1} indicated the presence of cis-alkene. The peak 2855 was formed as a result of C-H stretch. C–H bend or scissoring from 1470-1450 cm^{-1} indicated the presence of saturated hydrocarbons (alkanes). The peak 1380 indicated C-H rock methyl. The band 724 indicated the presence of long chain methyl bonds while the peak 1100 indicated the presence of unsaturated C–O bond (Figure 2).

DISCUSSION

Environmental changes affect the growth and chemical composition of microalgae. Temperature is recognized as a major environmental condition affecting the microalgae culture system and biomass yields. As temperature varies over time and location and shapes the ecology of living organisms, microalgae must be well adapted to

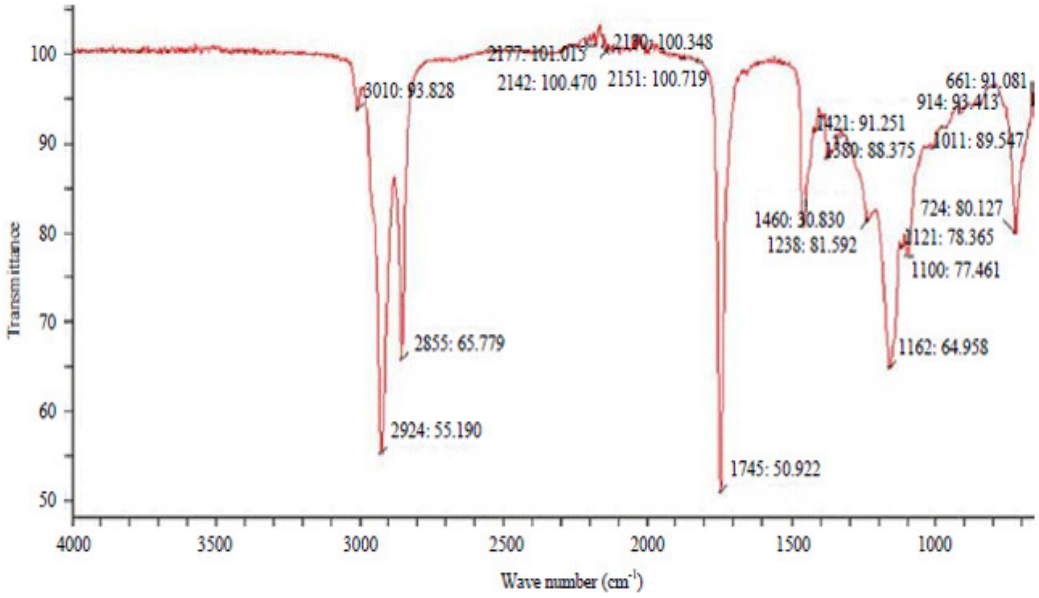

Figure 2: FT-IR Spectrum Analysis of the *Chlorella* sp. Biodiesel.

changes in temperature to be suitable candidates for the production of high-value products. In the present study, most physical characteristics of the isolation sites remained relatively steady during sample collections but temperature varied between 25 and 30°C, and the pH between 6.5 and 8.4. Morphological analysis clearly established that the shape, size, and color of various isolates matched those of members of the genus *Chlorella* (Luo *et al*., 2010). The *Chlorella* strains were confirmed to the genus level by ITS2 rDNA as shown by BLAST, thereby canceling out the 30 °C. Similarly, increased biomass was obtained in cultures treated in association with morphological heterogeneity due to environmental effect on the taxa. Cell density of four isolates increased with culture temperature from 20 to with elevated temperature. Maximum growth was recorded within the upper boundary and higher at 30 °C, which is the temperature of the drainage system from which the strains were isolated. Microalga *Chlorella* sp. NC JNI8 demonstrated highest cell density in all temperature treatments. Consistent with these findings, an earlier study observed increased growth rates of three tropica Australian algae *Cryptomonas* sp., Rhodomonas sp. and Prymnesiophyte NT19 as temperatures were ramped from 25 to 30 °C but decreased at temperatures above 30 °C (Renaud *et al*., 2002).

Classically, the growth of microalgae can be enhanced due to predictable accelerated metabolic activity of elevated temperature. As temperature increases, demand for carbon is higher which enhances the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (a key photosynthetic enzyme) to accelerated cell division, thus producing more biomass (Teoh *et al.,* 2020). Perhaps, this is one of the reasons why these strains yielded high biomass at elevated temperature compared to low temperature. Chlorophyll a and carotenoids contents were increased in *Chlorella* cultures at higher temperature (30 or 25 °C). Increased chlorophyll a content under elevated temperature shows increased energy provided via light harvesting process of photosynthesis (Geider *et al.,* 1997). Similarly, high temperature stresses microalgae by activating over-production of reactive oxygen species (ROS). In an attempt to prevent the damaging impacts of increased ROS, increased chlorophyll and carotenoids serve as antioxidants (Ras *et al*., 2013; Teoh *et al.,* 2020). Nevertheless, pigment content response to different culture temperatures, varies from one species to another. Generally, the chemical constituent for each tropical *Chlorella* strain varies with change in temperature.

Carbohydrate content of all the strains increased with a declined in temperature, reaching maximum at 20 °C. This observation agrees with the proposal that increased carbohydrate content are achieved at lower temperatures (20 and 25 °C) compared to high temperature (30 °C) in the diatom Chaetoceros cf. wighamii (DeCastro and Garcia, 2005). However, an opposite trend was noticed when *Spirulina platensis* M^2 was cultured at higher temperature (40 °C) where the carbohydrate content increased significantly (30 %) (Yaakob *et al.,* 2014). These findings show that there is no uniformity in the response to variation in culture temperature per algal

carbohydrate composition. Isolate *Chlorella* sp. NC JNI 8 of our collection stored high content of carbohydrates above 50 % (AFDW) at 25 °C. Some species such as *Dunaliella, Chlamydomonas, Scenedesmus*, and *Chlorella* have been declared to accumulate more than 50 % carbohydrate based on their dry cell weight and therefore, microalgae are considered potential substrates for bioethanol formation through microbial fermentation (Chen *et al.,* 2013). The protein contents followed the same trend as carbohydrates content in response to varying temperature conditions. Data collected showed that most strains accumulated higher percentage of cellular protein at 25 °C than at 30 °C. This finding supports past research that revealed a significant decrease in percentage of cellular protein per cell of four Australian microalgal species cultivated at temperatures higher than 27 °C (Renaud *et al*. 2002). All Chlorella strains synthetized less lipids at high temperature (30 °C), while there was no consistent trend in lipid storage when cultured at 20 °C and 25 °C. Similar results were obtained by Converti *et al.* (2009) when culture temperature was increased from 25 to 30 °C resulting in a decrease in lipid content of *C. vulgaris* from 14.71 to 5.90 % (Converti *et al.,* 2009). On the other hand, it has been reported that the lipid content in *Nannochloropsis Oculata* doubled with increased growth temperature from 20 to 40 °C (Bounnit *et al.,* 2020). Characteristically, the chemical composition of a rapid-growing photosynthetic cell (microalgae) is usually linked with increased protein and reduced carbohydrates and lipid content. However, when microalgae culture is under stress (elevated suboptimal temperature or nutrient depletion), its growth becomes stunted, and additional photo-assimilated carbon is diverted to either abundant carbohydrates or lipid (Zhu *et al.,* 1997). Also, it is common to observe variable responses of microalgae as a function of changing temperature and strain or species type (Teoh *et al.,* 2010).

The potential for biodiesel was detected using the Fourier transform infrared spectroscopy (FT-IR). The NEAR FT-IR characterization of the biodiesel indicated peak region at 1745 cm^{-1}, this was due to the presence of carbonyl ester. This indicated the presence of aliphatic aldehydes which was in accordance with the findings of Cortes (2010) Esters were present in the biodiesel, the ester present is palmitoleic acid ($C_{17}H_{32}O_2$). Another peak value of 2924 cm^{-1}, which was as a result of C-H stretch at 2924, alkyl was therefore suspected to be present. The values ranging from 2000-2500 cm^{-1} indicated the presence of unsymmetrical internal alkynes (4-octyne). The peak band of 3010 cm^{-1} was due to olefinic = C – H stretch. This indicated the presence of an aromatic compound or alkene while the bands between 600 and 1200 cm^{-1} indicated the presence of cis-alkene. The peak 2855 was formed as a result of C-H stretch. This was suspected to be a carbonyl compound (ketone, H–C=O stretch 2830-2695 cm^{1}) as a result of unsaturated C-H bond. This was also confirmed by Barbara (2004). The 1162 band was due to C-O stretch and this indicated the presence of unsaturated fatty ester oil (Barbara, 2004).

The saponification value was in agreement with standard value for biodiesel set by the American Society for Testing and Materials (ASTM D). The acid value of the biodiesel obtained was within the ASTM range, but higher than an acid value of 0.4 g obtained by Vijayaraghavan and Hemanathan (2009) from freshwater algae and 0.37 mg KOH g^{-1} from *Chlorella protothecoides* in the study by Xu *et al.* (2006). Iodine value also fell within the ASTM range while the fire point obtained was lower than the ASTM D7651 value of 140 – 215 °C. The pour point in this study was in agreement with the ASTM D97, but higher than the pour point observed by Xu *et al*. (2006) in biodiesel produced from *Chlorella protothecoides*. The pH observed in this study was slightly higher than the ASTM standard of 7.0 for biodiesel. Density and specific gravity were in conformity with ASTM D6751 standard. The flash point obtained in this study was similar to the previous findings observed by Xu *et al*. (2006) with a flash point of 115 °C for *Chlorella protothecoides*, but higher than the flash point of 98 °C obtained by Vijayaraghavan and Hemanathan (2006). The flash point in this study was lower than the ASTM standard of 130 °C. The result indicated that majority of the physicochemical properties of biodiesel produced from *Chlorella vulgaris* were in line with the ASTM standard except for the flash point that was 10 % lower and the pH which was slightly higher. This indicated that the trans-esterification process was approximately complete and the quality of the *C. vulgaris* biodiesel obtained from this work was up to standard.

Conclusion

High lipid, carbohydrate, and protein content shown by most isolates were species specific at various temperatures. The strains *Chlorella lewinii* KU220 JNI1, *Chlorella Vulgaris*. NEIST JNI3, *Chlorella lewinii* KU217 JNI6, and *Chlorella* sp. NC JNI8 are potential candidates for high pigment and biochemical content production with a potential for optimization within the investigated temperatures. The ability of the isolated *Chlorella* spp. to acclimate and thrive under temperature fluctuations justify the search for novel strains based on location of collection and the potential to produce high-value products under the current realities of global climate change vis-a-vis rising temperatures. *Chlorella* spp were isolated from channeled grey-water; they produced algal oil with high yield per algae biomass, about 50 g of *C.*

vulgaris biomass generated 5.1 g of lipid which was converted to biodiesel from a simple trans-esterification reaction. FT-IR spectrum revealed that *C. vulgaris* lipids were converted to fatty acidmethyl ester (FAME) the 1745 cm^{-1} stretch, while the standard value for ester bond formation was obtained at the 1744 cm^{-1} stretch.

Recommendations

Based on the findings of this study, the following recommendations are made:

i. FT-IR is recommended for easy and accurate characterization of biodiesel.
ii. *Chlorella* spp is recommended for use as it is economical and viable source of renewable oil for biodiesel production.
iii. Grey-water should be used to grow microalgae as it contains the necessary elements needed for the growth of microalgae.

REFERENCES

Bligh, E.G., Dyer, W.J. (1959). A rapid method for total lipid extraction and purification. *Can J. Biochem Physiol.,* 37:911–917.

Bounnit T., Saadaoui, I., Rasheed, R., Schipper, K., Al Muraikhi, M., Al Jabri, H. (2020). Sustainable production of *Nannochlorisatomus* biomass towards biodiesel production. Sustainability 12:2008.

Canakei and van Gerpen (1999). Biodiesel Production via Acid Catalysis. Transactions of the American Society of Agricultural Engineers, 42(5) 1203-1210.

Chen, C.Y., Zhao, X.Q., Yen, H.W., Ho, S.H., Cheng, C.L., Lee, D.J., Bai, F.W., Chang, J.S. (2013). Microalgae-based carbohydrates for biofuel production. *Biochem Eng J,* 78:1–10.

Converti, A., Casazza, A.A., Ortiz, E.Y., Perego, P., Del Borghi, M. (2009). Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsisoculata and Chlorella vulgaris for biodiesel production. *Chem Eng Process.,* 48:1146–1151.

DeCastro, A.S., Garcia, V.M.T. (2005). Growth and biochemical composition of the diatom Chaetoceros cf. wighamii Brightwell under different temperature, salinity and carbon dioxide levels. Protein, carbohydrate and lipids. *Aquaculture*, 246:405 – 412.

Doyle, J.J., Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, 12:13–15.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. (1956). Colorimetric method of determination of sugars and related substances. *Anal Chem.* 28:350–356.

Geider, R.J., Macintyre, H.L., Kana, T.M. (1997). Dynamic model of phytoplankton growth and acclimation: responses of the balanced growth rate and the chlorophyll a: carbon ratio to light, nutrient-limitation and temperature. *Mar Ecol Prog Ser.* 148:187–200.

Hempel, N., Petrick, I., Behrendt F. (2012). Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production. *J Appl Phycol*, 24:1407–1418.

Indabawa, I.I. (2014). Production and Characterization of Biodiesel from Microalgae. LAP Lambert Academic Publishing, Germany, 64.

Indhumathi, P., Shabudeen, P.S.S. and Shoba, U.S. (2014). A method for production and characterization of biodiesel from green micro algae*. International Journal Bio-Science,* 6: 111-122.

Jais, N.M., Mohamed RMSR, Al-Gheethi AA, Hashim Amir (2017). Dual role of phycoremediation of wet market wastewater for nutrients and heavy metals removal and microalgae biomass production. *Clean Technol Environ Policy*, 19(1):37–52.

Kalin, M., Wheeler WN, Meinrath G. (2005). The removal of uranium from mining waste water using algal/microbialbiomass. *J. Environ Radioact,* 78:151–177.

Lowry, O.H., Rosebrough, N.J.A., Farr, A.L., Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *J Biol Chem.,* 193:265–275.

Luo, W., Pröschold, T., Bock, C., Krienitz, L. (2010). Generic concept in Chlorella related coccoid green algae *Chlorophyta, Trebouxiophyceae*. 12:545–553.

Moheimani, N., Borowitzka, M., Isdepsky, A., Fon Sing, S. (2013). Standard methods for measuring growth of algae and their composition. In: Borowitzka MA, Moheimani NR (eds) Algae for Biofuels and Energy. Springer, Dordrecht, 265–284.

Nichols H.W. (1973) Growth media – freshwater. In: Stein J (ed) Handbook of Phycological methods, Culture Methods and Growth Measurements. Cambridge University Press, Cambridge, 7–24.

Odoemena, S. O. and Igomu, E. A. (2017). Evaluation of Soil and Climatic Conditions Supporting Maize (Zea mays L.) Production in Makurdi, Nigeria. *Journal of Agriculture and Ecology Research International* 12(2): 1-12, DOI: 10.9734/JAERI/2017/33963.

Ozkan, A., Kinney, K., Katz, L., Berberoglu, H. (2012). Reduction of water and energy requirement of algae cultivation using an algae biofilm photobioreactor. *Biores Technol.,* 114:542–548.

Pahazri, N., Mohamed R.M.S, Al-Gheethi, A.A., Amir, H. (2016). Production and harvesting of microalgae biomass from wastewater, a critical review. *Environ Technol Rev.,* 5(1):39–56.

Ras, M., Steyer, J.P., Bernard, O. (2013). Temperature effect on microalgae: a crucial factor for outdoor production. *Rev Environ Sci Biotechnol*, 12:153–164.

Renaud, S.M., Thinh, LV., Lambrinidis, G., Parry, D.L. (2002). Effect of temperature on a growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture 21:195–214.

Shekhawat, K., Rathore, S.S., Premi, O.P, Kandpal, B.K. and Chauhan, J.S. (2012). Advances in agronomic management of Indian mustard (*Brassica juncea* (L.) Czernj. Cosson). 2(4):13–35.

Teoh, M.L., Anuwar, S., Yap, W.H., Ng, F.L., Phang, S.M. (2020). Effects of elevated temperatures on growth and photosynthetic performance of polar Chlorella. *Adv Polar Sci.,* 31:124–131.

Xu, L., Brilman, D.W.F., Withag, J.A.M., Brem, G. and Kersten, S. (2011). Assessment of a dry and a wet route for the production of biofuels from microalgae: energy balance analysis. *Bioresource Technology*, 102 (8): 5113–22.

Yaakob, Z., Ali E, Mohamad M, Takrif M.S (2014). An overview: biomolecules from microalgae for animal feed and aquaculture. *J Biol Res.,* 21(6):1-10.

Zhu, C.J., Lee, Y.K. (1997). Determination of biomass dry weight of marine microalgae. *J Appl Phycol.,* 9:189–194.