MODIFICATION OF GROWTH MEDIA FOR *SCENEDESMUS*AND *NITCHZER*SPECIESFOR FATTY ACID METHYL ESTERS PRODUCTION IN RACEWAY POND

ABSTRACT

The aim of this studywas to modifythe growth media for Scenedesmus and Nitchzia speciesfor fatty acid methyl esters production in raceway pond.Native Scenedesmus and Nitchziaspecieswere cultivated in raceway pond in three different growth media (Nitrogen-sufficient, Nitrogen-deficient and Blue Green 11) media at different periods to assess their quantitative parameters(biomass concentration (g/L), biomass productivity (g/L/d), lipid content (%), and lipid productivity (mg/L/d) as well as their fatty acid methyl esters composition. The quantitative parameters were determined following standard methods and the fatty acid methyl esters composition was analysed by Gas Chromatography Mass Spectrometry (GC-MS). The results of the quantitative parameters obtained from Scenedesmusspecies were highest in the Blue Green 11 (control) media as compared to the experimental media. Statistically, there is significant difference (p<0.05) in the lipid content obtained from Scenedesmusspecies between the Blue Green 11 media and the experimental media. The biomass concentration and lipid content obtained from Nitchziaspecies were also found to be highest in Blue Green 11 media as compared to the other two media. However, the lipid content obtained from Nitchziaspecies in the three different media showed insignificant difference (p>0.05). Scenedesmus and Nitchziaspecies did not respond to nitrogen deficiency condition. TheGas Chromatography Mass Spectrometryanalysis of the fatty acid methyl esterscomposition revealed the presence of similar compounds in different proportion. The dominant compound of the fatty acid methyl esters of the two species in the three different media are Hexadecanoic acid methyl ester ((palmitic acid)), 9-octadecenoic acid (Z) methyl ester ((oleic acid) and 9, 12-octadecadienoic acid methyl ester (linolenic acid) among others. The Carbon16-Carbon18(C16-C18) chain in Scenedesmus and Nitchziaspecies ranges from 74.14-61.84% and 63.81-61.98% respectively. These could suggest that both species have a great potential for the production of fatty acid methyl esters in large scale cultivation.

Keywords: Growth Media, Scenedesmusspecies, Nitchzerspecies, Fatty Acid Methyl Esters, Raceway Pond

INTRODUCTION

The urgent need for sustainable energy sources is becoming increasingly pressing as the world grapples with the challenges posed by dwindling fossil fuel reserves, rapid industrialization and environmental degradation. A report from Yusuff and Owolabi (2019) and Ibrahim *et al*. (2020) stated that “the quest for alternative and sustainable energy resource is gaining worldwide attention due to the depletion of fossil fuel reserves, high energy demands arising from an increase in industrialization and mechanization, energy insecurity and environmental degradation. The global energy crisis has sparked a renewed focus on exploring alternative energy sources that can help mitigate these issues and provide a more stable, eco-friendly future”.

“Microalgae and other feed stocks have emerged as promising sources for the production of biofuels and various other materials that are essential for both humans and animals. Numerous research studies have documented the potential of using different feedstock in generating bioenergy and other valuable products, demonstrating the viability of using microalgae as a sustainable alternative to conventional fossil fuels and raw materials[58-61]. Microalgae have gained a lot of attention as a renewable feedstock due to their high productivity, high lipid content and use of non- arable land among others.Algae grow much faster than food crops, and can produce hundreds times more oil per unit area than conventional crops such as rapeseed, palms, soy beans, or jatropha” (Attabani*et al*., 2012). According to Zhang *et al.* (2014) “microalgae can yield higher lipid content of between 15-300 times for biodiesel production compared to the traditional crops on an area and are non-toxic and highly biodegradable”.

In addition to being a promising source of biofuel and other valuable products, microalgae can play a vital role in the treatment of wastewater. Microalgae can be utilized to remove pollutants and nutrients from wastewater, making it safe for discharge or reuse. More than 50 years ago Cadwell (1946) and later

Oswald *et al*. (1953) suggested "the use of massive microalgae cultures for wastewater treatment and protein production simultaneously". "Microalgae can be used for tertiary wastewater treatments to achieve better effluent quality through a low energy cost mechanism, where nutrients that were being discharged are nowadays be utilized and metabolized to biomass of high commercial value" (Rodriguez-Palacio *et al.*, 2022). This biomass can become as source of fertilizers as well as energy in the first instance(Rodriguez-Palacioet al., 2018), Vanthoor-Kaoopmans*et al*., (2014).
"Microalgae are simple microscopic autotrophic and/or heterotrophic organisms that can be grouped into either unicellular or multi-cellular forms" (Ajala*et al*., 2021).Most algae are photosynthetic, that is they use assimilate inorganic carbon for conversion into organic matter. A report from Enamala*et al.* (2018) stated that "microalgae effectively utilize CO_2, light (energy source) and water in a photosynthesis process to synthesize phospholipids, proteins, nucleic acids and carbon-rich lipid".**"**However, some species are capable to grow on organic molecules either heterotrophically or mixotrophically" (Markou*et al*., 2014). Algae are an incredibly diverse group of eukaryotic organisms commonly found in marine and freshwater systems (Daneshvar, *et al*., 2018) and also in all environments including extreme habitat (Merino *et al*, 2019).
Algae contain some amount of oil or lipids that can be converted into various biofuels. A report from William (2015) stated that "majority of algae species possess oils that are capable of being converted into biodiesel, however, very few species of algae contain enough oil to be suitable candidate for biofuel production". "Microalgae differ in their quantitative parameters, depending on the type of algae species, growth conditions, availability of nutrients and other factors" (Maishanu and Muhammad, 2024).
"The main nutrients requirements for growing algae are carbon, nitrogen, phosphorus and certain trace metals" (Hannon *et al*., 2011). "Successful mass cultivation of microalgae requires adequate light, temperature and nutrients such as nitrogen and phosphorus along with a variety of microelements" (Markou*et al*., 2014).**"**However, it was reported that nitrogen deficiency and other adverse condition increases the lipid content of some green algae" (Illman*et a*l., 2000; and Rodolfi*et al*., 2009).
"Generally, there are two methods of cultivating microalgae, the open pond and the closed systems" (Shah, *et. al.* 2014). "Open culture systems are the oldest and simplest systems for mass cultivation of microalgae" (Rakesh *et al*., 2017)."Certain microalgae species are quite suitable for growth in open culture conditions in the local environmental conditions where they use natural sunlight for photosynthesis. Outdoor cultivation requires the selected algal strain not only to have a strong ability to accumulate oil but also to adapt to the external environment" (Huo*et al.,* 2017). "Over 90% of the world commercial microalgae production uses shallow open pond" (Peng and Zohou, 2014). This system of cultivating microalgae has been regarded as the most cost-effective (Ajala*et al*., 2021) due to its simplicity and low capital investment, although it has some drawbacks."The closed photobioreactor on the other hand is a reactor design that can be placed outdoors for cultivation of algae" (William, 2015).
The choice of culture medium is a key factor in algal growth. Culture medium being the nutrient medium is an important factor that can influence algae growth (Sharma *et al*., 2016). Different algal species have varying nutrients requirements and the composition of the growth medium must be tailored to meet its needs.Blue Green 11 medium is one of the most preferred growth medium as it can be used for both fresh water and marine microalgal growth but is more suitable for freshwater microalgae (Pandy*et al*., 2023).
"Industrial cultivation of microalgae is restricted by the high cost of nutrients for microalgae production" (Zuliani*et al,* 2016). "Developing cost-effective media for microalgae is crucial for the continued development of microalgae-based biodiesel production. However, it was reported that wastewater can be a potential culture medium for cultivating microalgae due to many collateral benefits such as biological treatments of polluted water, production of biomass and biofuels" (Rodriguez-Palacio*et al*., 2018)**.** According to Adebodeely*et al.* (2013) "some microalgae can be cultivated in a large scale with wastewater as wastewater is a source of water and nutrients for cultivating microalgae".A report from Carvalho*et al.* (2019) stated that "using industrial or municipal wastewaters for microalgae cultivation is a trend in the development of microalgae"."Wastewater might be the ideal nutrient source for balancing the culture medium for algae cultivation" (Valverde-Perez *et al.* 2015). A report from Halfhide*et al.* (2015) noted that "nitrogen and phosphorus were sufficiently present in wastewater sources that could potentially be utilized for algae cultivation". "Some research documented the possibility of using urban wastewater with anaerobic digestion for the Production of*Nannochloropsisgaditana* (Ledda*et al*., 2015) or *Nannochloropsissalina"* (Sheets *et al*., 2014). In this study, the nutrients-rich agricultural runoff water from ShellaFadama Pond was modified and utilized as an alternative to the more expensive commercial medium to reduce the cost of cultivating microalgae**.**

An important factor for the overall success of biofuel production is the selection of the microalgae strain that should perform optimally in the production of algal biomass and be able to accumulate triacylglycerides and adapt to extreme environments among others Arenas *et al*. (2017). This highlights the importance of pursing innovative approaches that can meet the ever-growing energy demands while using low cost media. A preliminary investigation was conducted for some native algae species using Blue Green 11 media under the laboratory conditions, the results of the study indicated that *Scenedesmus* and *Nitchzia* species exhibited impressive biomass and lipid accumulation making them promising candidates for biodiesel production (Maishanu and Muhammad, 2024). However, Information about a low cost culture media for outdoor cultivation of these species for high biomass and lipid productivity is not documented.*Therefore, agricultural runoff from ShellaFadama Pond was modified and used as an enrichment media for the growth of the selected microalgae species for fatty acid methyl esters production in a raceway pond.*

Materials and Methods

Study Areas

The study was conducted in the main campus of UsmanuDanfodiyo University Sokoto. The algae cultivation was carried out at the Sewage Treatment Plant (STP) of UsmanuDanfodiyo University Sokoto. Sokoto lies in the extreme Northwest of Nigeria between the longitudes and latitudes of 4° 10 05'E to 6 ^{o} 35 42$^{'}$ E and 11° 32 51N to 13°53 14'E respectively and altitude of 272 meters. Sokoto is a hot area, with variable temperatures during the dry and rainy seasons. The warmest months are from February to April when daytime temperatures can exceed 45°C (113.0F). The rainy season is from June to October which showers is a daily occurrence and mean annual rain fall ranges from 500mm-1300mm.

The UsmanuDanfodiyo University sewage treatment plant (STP) is located near the ShellaFadama UDUS and is on latitude 13°12'10.8'' N, longitude 5°12'10.8''E and an area of 0.251km (global position system (GPS) Trex Garmin model) (Figure 1).

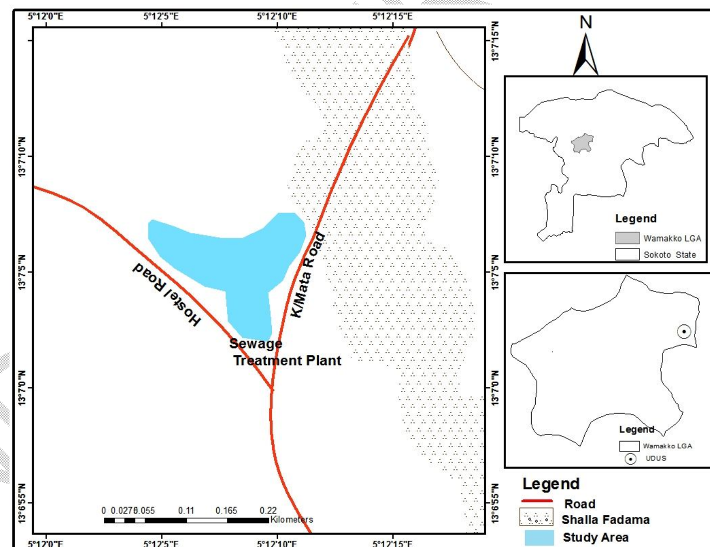

Figure 1: Map of UsmanuDanfodiyo University Showing the Sewage Treatment Plant.
Source: Geographic Information System Laboratory, UDUS (GIS lab) (2018).

The Open Raceway Pond Construction

The algal raceway pond was constructed in the sewage treatment plant of UDUS. The pond design was in consistent with industrial standard as described by Richmond (2004). The open raceway pond is an outdoor pond which was built in a raceway configuration, in which the area/land was divided into a rectangular grid (6ft by 3ft). The pond was dug at 1 foot depth (Chisti, 2007) built in concrete blocks on a 10cm thick sole. A partition wall was constructed in the middle of the pond and an outlet was provided for water passage to enable cleaning of the pond. Tiles were used to cover the concrete to decrease roughness, avoid biomass attachment (Lardon *et al*., 2009) and to prevent the ground from soaking up the liquid (Chisti, 2007).An eight-bladed paddle wheel with flat blades was used to drive water flow

continuously around a circuit (Chisti, 2013). A net was constructed around the open raceway pond to prevent predators such as birds and insects from interfering with the culture media. Transparent polythene was used to cover the top of the net to avoid interruption with the rain and toreduce the amount of solar radiation from the sun getting inside.

Media preparation, Experimental Set up, Variation in Temperature, Solar sunlight duration and pH variation

Media preparation
Blue Green 11 media
Composition of the media: In g/L
Sodium nitrate : 1.500, Dipotassium hydrogen phosphate : 0.04, Magnesium sulphate : 0.075, Calcium chloride dehydrate : 0.036, Sodium carbonate :0.02 : disodium magnesium EDTA : 0.001 : Citric acid : 0.006 :Ferric ammonium citrate: 0.006 and I ml trace metal solution per litre.

Trace metal solution (g/L)
H_3BO_3: 2.86, $Mncl_2.4H_2O$: 1.81, $ZnSO_4.7H_2O$: 0.22, $CuSO_4.5H_2O$: 0.08, $Na_2MoO4.2H_2O$: 0.39
And CO $(NO_3)_2.6H_20$: 0.05 (Ilavarasi*et al.*, 2011)

For Nitchzia sp. 1ml/L of $Na_2sio_3.9H_2o$ was added to the BG 11 media.

The media was prepared according to the manufacturer`s specification. All of the powdered media was suspended into a flask containing 1 L distilled water. The content of the flask was heated in a water bath to dissolve the medium. The pH was adjusted with 1M NaOH or HCl depending on the species requirements and was filter sterilized with 0.2 µm filter.

The Nitrogen Sufficient and Nitrogen Deficient Media
The Shellafadama water sample was collected and some of the physico-chemical parameters were recorded. About 40 litres of the water sample was treated and used for each pond as N-sufficient and N-deficient media to compare the growth of the algae with the Blue Green 11 Media (B-11 media). Potassium nitrate (KNO_3) and diammonium phosphate (DAP) were added to the pond water to make the concentration of nitrate and phosphate to reach 1.0g/L and 0.04g/L respectively in the Nitrogen-sufficient media. For the Nitrogen-deficient media, concentration of nitrate and phosphate were made to be 0.5g/L and 0.04 g/L respectively. Each media was filter sterilized with 0.2 µm filter to remove any microorganisms that might be present in the water and treated with chlorine (1-2 mg/l, incubated for 24 h without aeration, followed by aeration for 2 h to remove residual chlorine) (FAO, 2006). It was supplemented with 0.5g/l glycerol and added to 40 litres of Blue Green 11 media (modified method of Hena*et al.*, 2015). The pH was adjusted with 1 M NaOH or HCl depending on the species` requirements.

Experimental Set Up
The experiment was setup using 2x3 factorial designs. The algae species studied are native species of *Scenedesmus* and*Nitchzia* collected from different location of UDUS (Muhammad, H. 2021). *Scenedesmus*and *Nitchzia*specieswere cultivated in raceway pond in triplicate using three different growth media at different periods for 21 days under the local environmental condition.Eighty (80) litres of the desired media was poured into each of the pond and about 0.1g/L of the algal strain was inoculated into each of the pond to initiate growth. In the control setting, the algae were grown autotropically using atmospheric carbon dioxide for photosynthesis, and in the treatments the algae were mixotropically grown by using both atmospheric carbon dioxide and supplemented with 0.5g/l glycerol as a carbon source. An eight bladed paddle wheel was manually used for mixing of the culture media at the speed of 15 rpm/minutes, three times for 30 minutes daily. About 10mg/l glycerol was further supplemented to the treatments after two weeks.
After three weeks of inoculation, all the algae that have been grown were collected and the pond water was flushed in order to control the development of bacteria and also to avoid accumulation of toxic or inhibiting compounds (Lardon *et al.*, 2009). The biomass concentration (g/L), biomass productivity (mg/L/d), the lipid content (%), and lipid productivity of each of the algal specie was analysed and the fatty acid profiles of the algal species were also determined.

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Variation in Temperature, Solar Sunshine Duration and pH Variation

In order to develop an efficient and cheap way to cultivate algae, the algae species were cultivated in raceway pond using the natural environmental temperature based on the species' temperature requirement. *Scenedesmus*spcies grow well in high temperature environments and was cultivated between the period of March-May when the water temperature range was between (37.12 ±0.25) to (35.25±0.96)^{o}C.*Nitchzia*species. required low temperature for its growth and was cultivated between the periods of December-February, when the water temperature was between (27.02±0.34) to (23.55±0.06) ^{o}C.

The solar sunshine duration during the study period was in the range of 7.0 to 7.7 hrs in March to May and 8.3 to 9.3 hrs in December to February (Information supplied by Nigerian Meteorological Agency (NIMET) Abuja). *Scenesdesmus*species grows well in pH range of 7.5-10 and for*Nitchzia* species the pH of the media was regulated to 7.0. The pH of the media was regulated by either adding 1 M NaOH when the pH is low or by the addition of 1M HCl acid when the pH is high.

Determination of the Quantitative Parameters of the algal species.

These parameters of the algal species were determined as follows;

The biomass concentration was determined gravimetrically according to the method of Rai *et al.* (1991). The algal samples were placed in a centrifuge tube, Stoppered and centrifuged at a speed of 5,000rpm for 10 minutes. It was washed three times with distilled water and centrifuged again. This was then 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 hours before weighing (W_2).

$$\text{Biomass concentration (g/L)} = \frac{W_2 - W_1}{V} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where W_1 = the weight of the filter paper (g)

W_2 = the weight of the filter paper and dried cells (g).

V = the volume of culture (l)

Determination of Biomass productivity

The biomass productivity P (g/L/d) was calculated by the following equation:

$$P = \frac{W_2 - W_1}{T} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Where, W_1 = the initial biomass concentration

W_2 = the biomass concentration at the last day of cultivation.

T = the cultivation time (days)

(Sharma *et al*., 2016)

Determination of Lipid Content (Algae Oil Extraction)

This was carried out using modified method of Bligh and Dryer (1959). All samples were extracted with 3 ml of chloroform/methanol at a ratio of 1:2 volumes for volume (V/V) by vortexing for 1 minute and centrifugation at 8,000rpm for 15 minutes at room temperature. The supernatants were collected and residues were re-extracted thrice with 2ml of chloroform/methanol (1/1, V/V) by centrifuging as stated above. All the supernatants were pooled together, filtered with Whatman No. 1 filter paper and washed with 2 ml distilled water, followed by centrifugation at 8,000rpm for 5 minutes. The lower organic phases were collected and evaporated to dryness. The oil removed was measured and stored in air tight container at 4°C. The percentage of total lipids in the biomass was determined using the measured volume of the chloroform extract, the total lipids concentration in the chloroform extract and the amount of the dried biomass used in the extraction as follows:

Total lipids (%) =

$$\frac{\text{Volume of } CHCl_3 \text{ extract (ml)} \times \text{total lipid conc. in extract (ml/mg)}}{\text{Mass of biomass extracted (mg).}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Alkali Catalyzed Trans-esterification

For the trans-esterification, 50ml of oil was measured and poured into a 150ml conical flask and heated to a temperature of 45°C using a water bath. A solution of sodium methoxide was prepared in a 250ml beaker using 0.25g of NaOH pellet and 10.5mls of anhydrous methanol. The solution was properly stirred

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until the NaOH pellet was completely dissolved. The sodium methoxide solution was then poured into the warm oil and stirred vigorously for 90 minutes using a magnetic stirrer. The mixture was left to settle for 24 hours in a separating funnel. After settling, the upper layer which was the biodiesel was decanted into a separate beaker while the lower layer which comprises of glycerol and soap was collected from the bottom of the funnel. The quantity of biodiesel collected was measured and recorded (Indhumati*et al*., 2014).

Washing and Drying

Biodiesel was washed with water to remove any remaining methanol, glycerin, catalyst, soaps and other impurities. Water used was warmed to about 45°C and was passed through the esters to allow soluble material, excess catalyst and other impurities to stick to the water and be settled to the bottom of the vessel. The water was removed from the vessel periodically until the wash water drained out was clear. The biodiesel washing sometimes leaves the biodiesel looking a bit cloudy. This means there's still a little water in it. It was heated slowly to 100°C and held there until all moisture present was evaporated (Indhumati*et al*., 2014).

Determination of Lipid Productivity

The lipid productivity was calculated using the following equation

$$\text{Lipid productivity} = \frac{\text{Total algal biomass (mg) x lipid content(\%)}}{\text{Working volume (l) x Cultivation time (d)}} \quad \ldots\ldots \quad (4)$$

(Griffiths and Harrison, 2009)

Fatty Acid Methyl Esters (FAMEs) Analysis

The gas chromatography mass spectrometry GC-MS (QP2010 SE Shimadzu, Japan) was used for fatty acid analysis following the manufacturer`s guidelines. The 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 0.3s. 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 0.0mm. The column oven temperature was 70°C, injection temperature was 250°C, injection mode was slit, and flow control mode that is linear velocity was 49.2cm/s and the pressure was 116.9kPa., total flow was 40.8mL/min, column flow was 1.8mL/min and purge flow was 3.0 mL/min and split ratio was 20.0. The GC program of the GCMS ion source temperature was 200°C, interface temperature was 250°C, solvent cut time was 2.50 min. ACQ mode was scan, event time was 0.5s, scan speed was 666 and start m/z was 30.00 while end m/z was 350.00. The MS start time was 6 mins and end time was 22 mins. Helium was used as a carrier gas, and its flow rate was kept at 1.5ml/min.

Statistical Analysis

The data were evaluated with the R statistics version 3.4. One way analysis of variance (ANOVA) was used to compute the quantitative parameters. The fatty acid methyl esters profiles were calculated using descriptive statistics.

Results

Table 1: The Quantitative Parameters Obtained from *Scenedesmus*sp.Cultivated in the Three (3) Different Media

Parameters	Growth Media		
	N-Sufficient	**N-Deficient**	**BG 11 (Control)**
Biomass Concentration (g/L)	0.43±0.00^{b}	0.34±0.02^{a}	0.45±0.02^{b}
Biomass Productivity (g/L/d)	0.31±0.034^{a}	0.32±0.01^{a}	0.36±0.01^{a}
Lipid Content (%)	18.93±0.42^{a}	18.50±0.12^{a}	24.9±0.17^{b}
Lipid Productivity (mg/L/d)	40.77±0.44^{b}	35.17±0.35^{a}	41.60±0.50^{b}

The standard deviation is based on triplicate runs.
Means followed by different superscripts is significant at P<0.05 across the column

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Table 2: The Quantitative Parameters Obtained from *Nitchzia*sp. Cultivated in the Three (3) Different Media

Parameters	Growth Media		
	N-Sufficient	**N-Deficient**	**BG 11 (Control)**
Biomass Concentration (g/L)	0.33 ± 0.06^{ab}	0.26 ± 0.01^{a}	0.43 ± 0.01^{b}
Biomass Productivity (g/L/d)	0.35 ± 0.03^{b}	0.22 ± 0.01^{a}	0.34 ± 0.01^{b}
Lipid Content (%)	19.92 ± 0.10^{a}	19.70 ± 0.12^{a}	20.04 ± 0.18^{a}
Lipid Productivity (mg/L/d)	37.80 ± 0.69^{a}	39.50 ± 0.21^{b}	39.47 ± 0.13^{b}

The standard deviation is based on triplicate runs.
Means followed by different superscripts is significant at $P<0.05$ across the column

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Table 3: The Fatty Acid Methyl Esters (FAMEs) Profile of *Scenedesmus*sp.Obtained from the Three (3) Different Media

				Growth Media								
N-Sufficient Media				**N-Deficient Media**				**BG-11 Media**				
No.	RT (Min)	Area %	Name of Compound	Molecular Formula	RT (Min)	Area %	Name of Compound	Molecular Formula	RT (Min)	Area %	Name of Compound	Molecular Formula
1	7.509	0.68	2,4-Dodecadienal, (E,E)	$C_{12}H_{20}O$	7.510	0.85	2,4-Dodecadienal, (E,E)-	$C_{12}H_{20}O$	7.511	0.39	3-Decyn-2-ol	$C_{10}H_{18}O$
2	7.775	1.22	2,4-Dodecadienal, (E,E)-	$C_{12}H_{20}O$	7.779	2.21	2,4-Dodecadienal, (E.E)-	$C_{12}H_{20}O$	7.795	0.88	2,4-Dodecadienal, (E,E)-	$C_{12}H_{20}O$
3	14.896	1.72	Ergost-25-ene-3,5,6,12-tetrol, (3.beta.,5.alpha, 6 beta) $C_{28}H_{48}O_4$	$C_{28}H_{48}O_4$	14.895	1.46	9-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	10.532	0.21	7-Tetradecenal, (Z)-	$C_{14}H_{26}O$
4	15.138	10.08	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{18}H_{32}O_2$	15.135	8.88	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{18}H_{32}O_2$	14.894	1.69	9-Octadecenoic acid (Z)-, methyl ester	$C_{18}H_{34}O_2$
5	15.269	4.44	Acetic acid, 4,4-dimethylcyclopent-l-en-3-one-yl	$C_{19}H_{30}O_3$	15.278	1.49	Z-2-Tridecen-l-ol	$C_{13}H_{26}O$	15.134	10.11	9,12-Octadecadienoic acid, methyl ester	$C_{18}H_{32}O_2$
6	15.847	0.69	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	15.850	0.28	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	15.280	1.75	Z-2-Tridecen-l-ol	$C_{13}H_{26}O$
7	16.671	5.01	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	$C_{25}H_{42}$	16.189	0.56	Tetradecanal	$C_{14}H_{28}O$	16.668	6.89	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eia	$C_{25}H_{42}$
8	16.901	25.90	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	16.667	8.65	Squalene	$C_{30}H_5O$	16.892	27.84	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
9	16.986	19.79	cis-9-Hexadecenal	$C_{16}H_{30}O$	16.893	23.69	Hexadecanoic acid, methyl ester	$C_{17}H_{32}O_2$	16.977	21.57	9-Octadecenoic acid, methyl ester, (E)-	$C_{18}H_{34}O_2$
10	17.033	2.78	9-Octadecenoic acid (Z)-, methyl ester	$C_{18}H_{34}O_2$	16.978	18.82	9-Octadecenoic acid, methyl ester,	$C_{18}H_{34}O_2C_{18}H_{34}O_2$	17.027	3.10	9-Octadecenoi	$C_{18}H_{34}O_2$

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							(E)-				c acid (Z)-, methyl ester	
11	17.262	2.17	Cyclopentanetridecanoic acid, methyl ester	$C_{18}H_{36}O_2$	17.027	2.68	9-Octadecenoie acid (Z)-, methyl ester	$C_{18}H_{34}O_2$	17.258	2.10	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$
12	17.712	0.58	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	17.259	1.89	Cyclopentanetridecanoic acid, methyl ester	$C_{19}H_{36}O_2$	17.711	1.45	cis-9-Hexadecenal	$C_{16}H_{30}O$
13	18.269	0.58	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	17.712	1.35	cis-9-Hexadecenal	$C_{16}H_{30}O$	19.374	2.26	Ethyl iso-allocholate	$C_{26}H_{44}O_5$
14	19.459	3.25	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	19.340	0.94	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	19.644	2.88	9-Octadecenal, (Z)-	$C_{18}H_{34}O$
15	19.649	2.08	E,E-2,13-Octadecadien-1 –ol	$C_{18}H_{34}O$	19.641	3.74	E,E-2,13-Octadecadien-1 –ol	$C_{18}H_{34}O$	20.419	5.50	Z,Z-3,13-Octadecedien-1 –ol	$C_{18}H_{34}O$
16	20.504	10.54	9-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O$	20.422	4.02	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	21.043	2.04	Ethyl iso-allocholate	$C_{26}H_{44}O_5$
17	21.046	2.42	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	21.626	7.29	.gamma.-Tocophenol	$C_{28}H_{48}O_2$	21.625	5.52	.gamma.-Tocopherol	$C_{28}H_{48}O_2$
18	21.295	1.04	7-Hexadecenal, (Z).	$C_{16}H_{30}O$	22.046	11.21	1 –Heptatriacotanol	$C_{37}H_{76}O$	22.055	3.81	1-Heptatriacotanol	$C_{37}H_{76}O$
19	21.631	4.19	Gamma. –Tocophenol	$C_{28}H_{48}O_2$	-	-	-	-	-	-	-	-
20	22.275	0.84	7-Hexadecenal, (Z)-	$C_{16}H_{48}O_{20}$	-	-	-	-	-	-	-	-

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Table 4: The Fatty Acid Methyl Esters (FAMEs) Profile of *Nitchzia*sp. Obtained from the Three (3) Different Media

	Growth Media											
	N-Sufficient Media				**N-Deficient Media**				**BG11 Media**			
No	RT (Min)	Area %	Name of Compound	Molecular Formular	RT (Min)	Area %	Name of Compound	Molecular Formula	RT (Min)	Area %	Name of Compound	Molecular Formula
1	7.512	0.80	2,4-Dodecadienal, (E,E)-	$C_{10}H_{18}O$	7.505	0.33	2,4-Dodecadienal, (E,E)-	$C_{12}H_{20}O$	15.130	8.14	9,12-Octadecadienoic acid, (Z,Z) methyl ester	$C_{18}H_{32}O_2$
2	7.795	1.76	2,4-Dodecadienal,	$C_{12}H_{20}O$	15.132	8.92	9,12-Octadecadienoic acid, methyl ester	$C_{18}H_{32}O$	16.896	40.60	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
3	15.134	12.50	9-Octadecenoic acid, methyl ester, (E)-	$C_{18}H_{34}O_2$	16.903	42.26	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	16.979	25.89	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$
4	16.668	3.69	9-Octadecenoic acid (Z)-, methyl ester	$C_{18}H_{34}O_2$	16.987	29.35	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	17.259	3.28	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$
5	16.907	38.42	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	17.260	4.51	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$	20.400	2.19	2-Dodecen-1-yl (-) succinic anhydride	$C_{16}H_{26}O_3$
6	16.991	26.92	Z-2-Tridecen-l-ol	$C_{13}H_{26}O$	20.435	2.22	7-Hexadecenal, (Z)	$C_{16}H_{30}O$	21.040	1.04	7-Hexadecenal, (Z)	$C_{16}H_{30}O$
7	17.262	4.05	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eia	$C_{25}H_{42}$	21.036	2.32	7-Hexadecenal, (Z)	$C_{16}H_{30}O$	21.401	0.64	3-Chloropropionic acid, heptadecyl ester	$C_{20}H_{39}clO_2$
8	20.405	2.24	9,12-Octadecadienoic acid, methyl ester	$C_{18}H_{32}O_2$	21.621	2.00	12-Methyl-E,E-2,13 octadecadien-1-ol	$C_{19}H_{36}O$	21.625	1.18	Pentadecanal-	$C_{16}H_{30}O$
9	21.627	3.00	7-Tetradecenal, (Z)-	$C_{14}H_{26}O$	22.006	6.51	1-Heptatriacontanol	$C_{17}H_{34}O_2$	21.996	8.23	Pentadeconioc acid	$C_{17}H_{34}O_2$
10	22.005	6.62	9-Octadecenoic acid (Z)-, methyl	$C_{18}H_{34}O_2$	22.578	1.58	Z,Z-3,13-Octadecendien-	$C_{18}H_{34}O$	22.571	8.83	7-Hexadecenal, (Z)	$C_{16}H_{30}O$

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ester	1-ol

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DISCUSSION

One of the most important factors to achieve high biomass productivity and concentration is the nutritional content of the medium (Grobbelaar, 2007). The biomass concentration and biomass productivity obtained from *Scenedesmus* species were highest in the Blue Green 11 media (control media) as compared to those grown in the experimental media (table 1). This may be attributed to the insufficient amount of nitrogen used in the experimental media, as nitrogen is the second most important nutrient in the culture medium of microalgae. When there is a low concentration of nitrogen available for the microalgae, the rate of cellular growth is reduced (Lourenco *et al.*, 2004). It was reported that Blue Green 11 media contained maximum amount of nitrates that support high biomass productivity and concentration (Henrard*et al.* 2015). The result of the biomass concentration obtained from Blue Green 11 media is comparable to the study of Lin and Lin (2011) that reported maximum biomass concentration of 0.4g/L for *Scenedesmusrubescens*.A report from Pandy et al. (2023) found that Scenedesmus species showed better biomass yield of 3.12 ±0.11g/L in Blue Green 11 whereas that obtained from wastewater was 2.50±0.13g/L.However, a report from Wang *et al.* (2015) stated that Chlorella vulgaris growth in wastewater extract could generate sufficient lipid and carbohydrate by replacing nutrients in wastewater.

The lipid content of algae species varies between species and with growing conditions (Borowitzka and Borowitzka, 1988). The lipid content and lipid productivity obtained from *Nitchzia*species was highest in the Blue Green11 media as compared to those cultivated in the experimental media. Statistically, there is significant different ($p<0.05$) in the lipid content obtained from the experimental media and the Blue Green 11 media. There was no significant difference ($p>0.05$) between the Nitrogen-.sufficient and Nitrogen-deficient media. This shows that *Nitchzia*species did not response to nitrogen limitation in the experimental media. This behaviour is peculiar for some green algae and has been reported by other researchers (Illman*et al.* 2000 and Rodolfi*et al.*, 2009). In Tetraselmissuecia and some Dunaliella species, no change in lipids levels has been seen under Nitrogen starvation (Borowitzka, 1999).This result is similar to the result reported from Huo*et al.* (2017) that reported 22% of oil content obtained from *Scenedesmus* species grown in an outdoor raceway pond.A report from Lee *et al.* (2014) reported high levels of cellular dry mass of lipids range from 19.9- 43.6% in *Scenedesmus* strains. However, the lipid productivity obtained from the Nitrogen- sufficient and the Blue Green 11 media showed insignificant difference ($p>0.05$).

The biomass concentration and biomass productivity obtained from *Nitchzia*speciesare higher in the Blue Green11 and Nitrogen-sufficient media and showed no significant difference ($p>0.05$). This indicates that both media have sufficient nutrients for cellular growth for *Nitchzia*species. Nitrogen starvation in some microalgae species affects not only algal yields, but also shifts anabolic pathways from protein synthesis to carbohydrates or lipids accumulation (Henrard*et al.*, 2015). However, there is significant difference ($p<0.05$) between the Nitrogen-deficient and Blue Green 11 media.

The lipid content obtained from *Nitchzia*species in the three different media showed insignificant difference ($p>0.05$). The reason may be because *Nitchzia*species belongs to the diatoms family and having lipid not starch as their main reserve and therefore do not require nutrient stress to accumulate more lipids. According to Sheehan *et al.* (1998) nutrient limitation (nitrogen or silicon) limitation had less or no significant effect on lipid content of microalgae *Amphora* and *Cyclotella* respectively. The lipid productivity obtained from *Nitchzia*speciesshowed insignificant difference ($p>0.05$) between the Nitrogen- deficient and Blue Green 11 media, but there is significant difference between the Nitrogen-Sufficient and the Blue Green 11 media ($p<0.05$).

The fatty acid profiling of microalgae ultimately affects the quality of the biodiesel (Musharraf *et al.*, 2012). Common fatty acids found in biodiesel are palmitic acid (C16: 0), stearic acid (C18: 0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) (Singh and Singh, 2010). Similar compounds were found in the Fatty Acid Methyl Esters obtained from

the three different media in*Nitchzia*species. In the Fatty Acid Methyl Esters obtained from Nitrogen-sufficient media, Hexadecenoic acid methyl ester (palmitic acid) was found to be high % with low % of 9-octadecenoic acid (oleic acid). However, the Fatty Acid Methyl Esters obtained from Nitrogen- Deficient and Blue Green 11 media revealed the presence of high % of Hexadecenoic acid methyl ester (palmitic acid) and high % of 9-octadecenoic acid (oleic acid). This is similar to the findings of Lee *et al.* (2014) whom reported that the two major fatty acid components in *Scenedesmus* species to be palmitic (C16:0) and oleic acid (C18:1) at 27.3% and 32.7% of the total lipid respectively. The C16-C18 carbon chain of the Fatty Acid Methyl Esters obtained from Nitrogen-sufficient and Blue Green11 media are in the same range. While that obtained from Nitrogen-deficient media was found to be lower. A report from Huo*et al*. (2017) reported the content of Fatty Acid Methyl Esters C16-C18 in the wild strain of *Scenedesmus* to reach 79.68%.

The Fatty Acid Methyl Esters composition of *Nitchzia*species obtained from the three different media were composed of similar compounds and were rich inHexadecanoic acid methyl ester (palmitic acid) and 9-octadecenoic acid methyl ester (oleic acid). However, the Fatty Acid Methyl Esters obtained from Blue Green11 media have the highest percentage of 9-octadecenoic acid methyl ester (oleic acid) which is a rich fatty acid found in biodiesel. Oleic acid is the markers of biodiesel quality (Yoo*et al*., 2010).

Furthermore, the Fatty Acid Methyl Esters obtained from Nitrogen-deficient and Blue Green11 media contain high 9, 12-octadecenoic acid methyl ester (linolenic acid) as compared to that obtained from Nitrogen-sufficient media. While this is very low (2.24%) in the Fatty Acid Methyl Esters obtained from Nitrogen- sufficient media. However, the EN 14214 (2004) and ISO 15607 specifies limit of linolenic acids (C18:3) contents should not be more than 12% for quality biodiesel (Knothe, 2009). An evaluation by Sharma *et al*. (2016) reported maximum linolenic acid (C18:3) in the range of 4.55-14.20% in different *Chlorella* strains. The C16-C18 carbon chain of all the Fatty Acid Methyl Esters obtained from the three different media are similar.According to Knothe (2009) biodiesel enriched with C16-C18 fatty acids have good fuel properties.

Conclusion

The results of the study showed that the parameters obtained from both *Scenedesmus*and *Nitchzia*species from the three different media were found to be highest in the Blue Green 11 media (control media) as compared to the experimental media. However, these parameters obtained from the experimental media are comparable to the Blue Green11 media (control media). Both species did not respond to nitrogen limitation condition in the growth media. The dominant compound found in both Fatty Acid Methyl Esters of the two species in the three different media areHexadecanoic acid methyl ester (palmitic acid), 9-octadecenoic acid methyl ester (oleic acid) and 9,12- octadecadienoic acid methyl ester (linolenic acid) in different proportion. The C16-C18 carbon chain in *Scenedesmus* and *Nitchzia*speciesranges from 74.14-61.84% and 63.81-61.98% respectively. Thus, both species had a great potential as a large scale cultivation strain for Fatty Acid Methyl Esters production.

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