

MODIFICATION OF GROWTH MEDIA FOR *Scenedesmus* and *Nitzschia* spp. FOR FATTY ACID METHYL ESTERS PRODUCTION IN RACEWAY POND

ABSTRACT

The study aimed at modification of growth media for Scenedesmus and Nitzschia spp. in raceway pond for the production of fatty acid methyl esters. Native species of Scenedesmu and Nitzschia were cultivated in raceway pond in three different growth media (N-Sufficient, N-deficient and BG 11) media at different periods to assess their quantitative parameters as well as their fatty acid methyl esters composition. The biomass concentration (g/L), biomass productivity (g/L/d), lipid content (%) and lipid productivity (mg/L/d) were assessed following the methods of Rai et al. (1991), Sharma et al. (2016), modified method of Bligh and Dryer (1959), and Griffiths and Harrison (2009) respectively. The fatty acid methyl esters composition was analysed by Gas chromatography mass spectrometry (GC-MS). The results of the quantitative parameters obtained from Scenedesmus sp. were highest in the BG 11 (control) media as compared to the experimental media. Statistically, there is significant different ($p < 0.05$) in the lipid content obtained from Scenedesmus sp. between the BG 11 media and the experimental media. The biomass concentration and lipid content obtained from Nitzschia sp. were also found to be highest in BG 11 media as compared to the other two media. However, the lipid content obtained from Nitzschia sp. in the three different media showed insignificant difference ($p > 0.05$). Scenedesmus and Nitzschia spp. did not respond to nitrogen deficiency condition. The GC-MS analysis of the FAMES composition revealed the presence of similar compounds in different proportion. The dominant compound of the FAMES 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 C16-C18 carbon chain in Scenedesmus and Nitzschia spp. 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 FAMES in large scale cultivation.

Keywords: Growth Media, *Scenedesmus* sp., *Nitzschia* sp., Fatty Acid Methyl Esters, Raceway Pond

INTRODUCTION

Algae are a large and highly diverse group of organism which can be found in almost all ecosystems (Buijks, 2013). They are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure (Mata *et al.*, 2010). Most algae are photosynthetic, that is they use assimilate inorganic carbon for conversion into organic matter. However, some species are capable to grow on organic molecules either heterotrophically or mixotrophically (Markou *et al.*, 2014). Cultivation of algae is a promising method for producing renewable hydrocarbon feedstock for biofuel production (Ozkan *et al.*, 2012) this is because algae have many characteristics over other feedstock (Scott *et al.*, 2010; Knothe, 2009). According to Pati *et al.* (2008) two thirds of the earth's surface is covered with water, thus algae would truly be renewable option of great potential for global energy needs. 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 jatropa (Attabaniet *et al.*, 2012). Another advantage of making biodiesel from microalgae over terrestrial plant is their metabolic flexibility, which means that a variation in the biochemical composition of the biomass (towards higher lipid, carbohydrates or protein accumulation) can be regulated by varying the cultivation

conditions (Tredici, 2010). These interesting properties lead to potential productivities (in terms of oil production per ha and per year) which are far higher than those of rapeseed or sunflower (Chisti, 2008). Large scale cultivation of algae using sun light energy can either be in the open pond system or the photo bioreactor (Jansen *et al.*, 2003). Open ponds or raceway ponds on the one hand are shallow ponds in which algae are cultivated and are built in different form of sizes and shapes (Adebodeely *et al.*, 2013). They are the oldest, simplest most commonly used systems for mass cultivation of microalgae (Chisti, 2006). Over 90% of the world commercial microalgae production uses shallow open pond (Peng and Zohou, 2014). The four common types of open systems are big shallow ponds, tanks, circular ponds and the raceway ponds (Borowitzka, 1999). The enclosed photobioreactor on the other hand is a reactor design that can be placed outdoors for cultivation of algae (William, 2015). The photobioreactor system comes in several designs such as the tubular, flat-plate, bubble-sparged, vertical column and airlift (Borowitzka, 1999).

Culture medium being the nutrient medium is an important factor that can influence algae growth (Sharma *et al.*, 2016). 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). The main nutrients requirements for growing algae are carbon, nitrogen, phosphorus and certain trace metals (Hannon *et al.*, 2011). Algae growth medium must contribute the inorganic elements that help make up the algal cell, such as nitrogen, phosphorous, iron and sometimes silicon (Grobbelaar, 2004). However, It was reported that nitrogen deficiency and other adverse condition increases the lipid content of some green algae (Illman *et al.*, 2000; and Rodolfiet *al.*, 2009). The use of fresh water that contains nutrients is advantageous in making algae cultivation less expensive. According to Brennan and Owende (2010) and Adebodeely *et al.*, (2013) some microalgae can be cultivated in a large scale with waste water as waste water is a source of water and nutrients for cultivating microalgae.

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° 35' 42" E and 11° 32' 51"N 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 Shella Fadama 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).

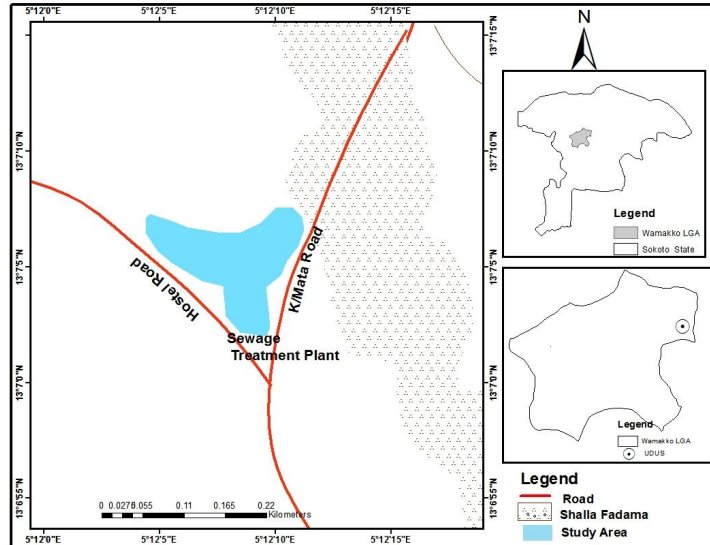


Figure 1: Map of Usmanu Danfodiyo University Showing the Sewage Treatment Plant.
Source: Geographic Information System Laboratory, UDUS (GIS lab) (2018).

The Open Raceway Pond Construction

The algal raceway pond was constructed in the sewage treatment plant of UDUS. The pond design was 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 to reduce 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

BG-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 1 ml trace metal solution per litre.

Trace metal solution (g/L)

H_3BO_3 : 2.86, $MnCl_2 \cdot 4H_2O$: 1.81, $ZnSO_4 \cdot 7H_2O$: 0.22, $CuSO_4 \cdot 5H_2O$: 0.08, $Na_2MoO_4 \cdot 2H_2O$: 0.39
And $CO(NO_3)_2 \cdot 6H_2O$: 0.05 (Ilavarasiet *al.*, 2011)

For *Nitzschia* sp. 1ml/L of $Na_2SiO_3 \cdot 9H_2O$ was added to the BG 11 media.

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 Shella fadama 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 (BG-11 media). Potassium nitrate (KNO₃) 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 N-sufficient media. For the N-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 BG 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 *Nitzschia* collected from different location of UDUS (Muhammad, H. 2021). *Scenedesmus* and *Nitzschia*spp. were 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 autotrophically using atmospheric carbon dioxide for photosynthesis, and in the treatments the algae were mixotrophically 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.

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* spp. 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)°C. *Nitzschia*spp. 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) °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). *Scenedesmus* sp. grows well in pH range of 7.5-10 and for *Nitzschia* sp. 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, Stopped 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 (W₁) 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 (W₂).

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

V

Where W₁ = the weight of the filter paper (g)
W₂ = 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:

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

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 (%) =
$$\frac{\text{Volume of CHCl}_3 \text{ extract (ml)} \times \text{total lipid conc. in extract (ml/mg)} \times 100}{\text{Mass of biomass extracted (mg)}} \dots \dots \dots (3)$$

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 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 (Indhumatiet *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 (Indhumatiet *al.*, 2014).

Determination of Lipid Productivity

The lipid productivity was calculated using the following equation

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

(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 of the FAME. 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

Table 2: The Quantitative Parameters Obtained from *Nitichzia* 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

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.β.,5.α,6.β) 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-1-en-3-one-yl	C ₁₉ H ₃₀ O ₃	15.278	1.49	Z-2-Tridecen-1-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-1-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 ₅₀	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,	C ₁₈ H ₃₄ O ₂

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	15.13	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	15.13	8.92	9,12-Octadecadienoic acid, methyl ester	C ₁₈ H ₃₂ O	16.89	40.6	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂
3	15.13	12.5	9-Octadecenoic acid, methyl ester, (E)-	C ₁₈ H ₃₄ O ₂	16.90	42.2	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	16.97	25.8	9-Octadecenoic acid, methyl ester, (E)-	C ₁₉ H ₃₆ O ₂
4	16.66	3.69	9-Octadecenoic acid (Z)-, methyl ester	C ₁₈ H ₃₄ O ₂	16.98	29.3	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	17.25	3.28	Triacontanoic acid, methyl ester	C ₃₁ H ₆₂ O ₂
5	16.90	38.4	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	17.26	4.51	Triacontanoic acid, methyl ester	C ₃₁ H ₆₂ O ₂	20.40	2.19	2-Dodecen-1-yl (-) succinic anhydride	C ₁₆ H ₂₆ O ₃
6	16.99	26.9	Z-2-Tridecen-1-ol	C ₁₃ H ₂₆ O	20.43	2.22	7-Hexadecenal, (Z)	C ₁₆ H ₃₀ O	21.04	1.04	7-Hexadecenal, (Z)	C ₁₆ H ₃₀ O
7	17.26	4.05	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eia	C ₂₅ H ₄₂	21.03	2.32	7-Hexadecenal, (Z)	C ₁₆ H ₃₀ O	21.40	0.64	3-Chloropropionic acid, heptadecyl ester	C ₂₀ H ₃₉ ClO ₂
8	20.40	2.24	9,12-Octadecadienoic acid, methyl ester	C ₁₈ H ₃₂ O ₂	21.62	2.00	12-Methyl-E,E-2,13 octadecadien-1-ol	C ₁₉ H ₃₆ O	21.62	1.18	Pentadecanal-	C ₁₆ H ₃₀ O
9	21.62	3.00	7-Tetradecenal, (Z)-	C ₁₄ H ₂₆ O	22.00	6.51	1-Heptatriacontanol	C ₁₇ H ₃₄ O ₂	21.99	8.23	Pentadecanoic acid	C ₁₇ H ₃₄ O ₂
10	22.00	6.62	9-Octadecenoic acid (Z)-, methyl ester	C ₁₈ H ₃₄ O ₂	22.57	1.58	Z,Z-3,13-Octadecadien-1-ol	C ₁₈ H ₃₄ O	22.57	8.83	7-Hexadecenal, (Z)	C ₁₆ H ₃₀ O

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* sp. were highest in the BG 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 BG 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 BG 11 media is comparable to the study of Lin and Lin (2011) that reported maximum biomass concentration of 0.4g/L for *Scenedesmus rubescens*.

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 *Nitzschia* sp. was also highest in the BG-11 media as compared to those cultivated in the experimental media. Statistically, there is significant difference ($p < 0.05$) in the lipid content obtained from the experimental media and the BG 11 media. But there is no significant difference ($p > 0.05$) between the N-sufficient and N-deficient media. This shows that *Nitzschia* sp. did not respond 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 *Tetraselmis* and some *Dunaliella* species, no change in lipids levels has been seen under N 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 N-sufficient and the BG 11 media showed insignificant difference ($p > 0.05$).

The biomass concentration and biomass productivity obtained from *Nitzschia* sp. are higher in the BG-11 and N-sufficient media and showed no significant difference ($p > 0.05$). This indicates that both media have sufficient nutrients for cellular growth for *Nitzschia* sp. 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 N-deficient and BG 11 media.

The lipid content obtained from *Nitzschia* sp. in the three different media showed insignificant difference ($p > 0.05$). The reason may be because *Nitzschia* sp. 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 *Nitzschia* spp. showed insignificant difference ($p > 0.05$) between the N-deficient and BG 11 media, but there is significant difference between the N-Sufficient and the BG 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 FAMES obtained from the three different media in *Nitzschia* sp. In the FAMES obtained from N-sufficient media, Hexadecenoic acid methyl ester (palmitic acid) was found to be high % with low % of 9-octadecenoic acid (oleic acid). However, the FAMES obtained from N-Deficient and BG 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 with the findings of Lee *et al.* (2014) whom

reported that the two major fatty acid components in *Scenedesmus* sp. 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 FAMES obtained from N-sufficient and BG-11 media are in the same range. While that obtained from N-deficient media was found to be lower. A report from Huo *et al.* (2017) reported the content of FAMES C16-C18 in the wild strain of *Scenedesmus* to reach 79.68%.

The FAMES composition of *Nitcziasp.* obtained from the three different media were composed of similar compounds and were rich in Hexadecanoic acid methyl ester (palmitic acid) and 9-octadecenoic acid methyl ester (oleic acid). However, the FAMES obtained from BG-11 media have the highest % 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 FAMES obtained from N-deficient and BG-11 media contain high 9, 12-octadecenoic acid methyl ester (linolenic acid) as compared to that obtained from N-sufficient media. While this is very low (2.24%) in the FAMES obtained from N-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 FAMES 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 *Nitcziasp.* obtained from the three different media were found to be highest in the BG 11 media (control media) as compared to the experimental media. However, these parameters obtained from the experimental media are comparable to the BG-11 media (control media). Both species did not respond to nitrogen limitation condition in the growth media. The dominant compound found in both FAMES of the two species in the three different media are Hexadecanoic 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 *Nitcziasp.* ranges 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 FAMES production.

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