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Effects of light intensity and photoperiod on growth, lipid accumulation and fatty acid composition of *Desmodesmus subspicatus* LC172266 under photoautotrophic cultivation

I. O. Ogbonna [1*], J. Ikwebe[2], J. C. Ogbonna[3], C. N. Eze[4], J. B. Ndrimbula[5]

[1]Department of Microbiology, College of Science, Federal University of Agriculture, Makurdi, PMB 2373, Nigeria
[2] Department of Biochemistry, Federal University Wukari, 200 K/Ala Road, P.M.B 1020, Wukari, Taraba State, Nigeria
[3] Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria
[4] Department of Science Laboratory Technology, University of Nigeria, Nsukka, Enugu State, Nigeria
[5] Department of Crop Protection, University of Maiduguri, Borno State, Nigeria

Abstract

Microalgae oil accumulation in their natural growing states hardly reaches the quantities needed to replace fossil-derived diesel. Therefore, for algae to be used as biofuel feedstock, their growths are manipulated with the aim of achieving high cell density and high lipid accumulation. Two major factors affecting microalgal biomass and lipid productivity are light intensity and photoperiod. In the present study, *Desmodesmus subspicatus* was grown photoautotrophically under varying light intensities and photoperiods with a view to assessing the growth, lipid accumulation potential and fatty acid composition. Whereas the optimal light intensity for biomass production of the microalga was 5000 lx, that for lipid productivity was 3500 lx. At 5000 lx and 18: 6 h light/dark cycle, biomass yield, lipid content and lipid productivity were highest, at values of 1.92 ± 0.03 g/L, 53% and 118.80 ± 2.04 $mgL^{-1}day^{-1}$ respectively. The major fatty acid of the alga was oleic acid irrespective of light changes. The quantities of lipid accumulated and the properties of the fatty acid methyl esters showed that *Desmodesmus subspicatus* LC172266 is an ideal feedstock for biodiesel production.

Keywords: *Desmodesmus subspicatus*, light intensities, biodiesel, lipid productivity, photoperiods, microalgae

Introduction

The last few decades have witnessed an increased interest in microalgal research especially with regards to their application as an important bioresource in the production of greener and renewable fuels. So, the idea of developing microalgae as a source of biofuels is not a new concept (McGuin *et al.*, 2011). With the controversies and complexities surrounding 1st and 2nd generation biofuels, in addition to the finite nature of fossil fuels and the attendant environmental issues arising from their very usage, microalgae have been viewed and researched as viable and sustainable alternative. The reduction of the emission of greenhouse gasses and consequently contributing significantly to mitigating global warming and the concomitant climate change is one of the key advantage of microalgae (Darzins *et al.*, 2010). Compared to the terrestrial plants, these microorganisms have more CO_2 use efficiency, sequestering CO_2 to make biomass with better efficiency than terrestrial plants and some have 50% of

their dry cell weight as lipid (Darzins *et al*., 2010).

The selection of oleaginous, high lipid-containing microalgal strains for biodiesel production is focused not only on lipid content but also on growth conditions (Han *et al*., 2015). A wide range of factors including temperature, CO_2, available nutrients, light and salinity influence the growth and by extension microalgal lipid accumulation. However, to a very large extent, light is considered the strongest factor affecting growth and storage products of microalgae (Han *et al*., 2015) and hence, has been studied as it affects the growth of various microalgae. For example, Scott *et al*. (2010) obtained low biomass with low light intensity (LI) and photoinhibition with high LI. This situation was previously described by Campbell *et al*. (1995) and alluded to by McGuin *et al*. (2011). However, Han *et al*. (2015) inferred that under most incubation conditions, especially with relatively high cell concentration, the light is limited without photoinhibition. Ruangsomboon (2012) observed that the microalga *Botryococcus braunii* had the highest lipid yield of 0.45 g L^{-1} as the intensity of the tested light was increased from 0.3 to its highest 538 µE $m^{-2}s^{-1}$. The findings further suggested that a relatively high light intensity limited algal growth, but favored an increase in the content and yield of the lipids (Han *et al*. 2015).

Light intensity affects lipid yields in microalgae which in turn is of particular interest in algal biodiesel production (Nzayisenga *et al*., 2020). Increasing light intensity has been reported to reduce lipid production in some microalgae (Cheirsilp and Torpee 2012), but enhancing lipid biosynthesis in some other species and additionally may have no effect on some other species (Pancha *et al*., 2015; Seo *et al*., 2017). Therefore, Nzayisenga *et al*. (2020) recommended that studying the effect of light intensity on lipid production should be done on a species-by-species basis.

The present study investigated the influence of the light intensity and photoperiod on the growth and lipid accumulation of *Desmodesmus subspicatus* LC172266. The microalga was isolated locally and characterized morphologically and by molecular approaches as reported below. Effects of light intensity and light: dark cycles on biomass production and lipid accumulation were investigated. Fatty acid composition of the algal lipid extracted from growth under optimal and un-optimized light conditions were determined by gas chromatography-mass spectrometry. Biodiesel property analysis was deduced using a BiodieselAnalyzer software and compared to ASTM and EN standards.

Materials and Methods

The Microalga

Desmodesmus subspicatus LC172266 was isolated from wastewater in Maiduguri, North-East Nigeria (Ogbonna, 2014) and was identified using both morphological and molecular approaches (Ogbonna and Ogbonna, 2015; Eze *et al*., 2017).

Effects of light intensity on biomass production and lipid accumulation

The influence of light intensity on the growth and lipid accumulation by the alga was determined by cultivating the microalga at different light intensities: 0, 1000, 2000, 3500 and 5000 lx, and measuring the growth and lipid biosynthesis. The alga was cultivated in 2000 mL transparent Teflon bottles containing 1000 mL of a sterile BG-11 medium (Ogbonna and Ogbonna, 2015). Dilute hydrochloric acid and sodium hydroxide solutions were used to equilibrate the initial medium pH to 7.3 before the autoclave-sterilization. The inoculation of each bottle was done by adding 15% (v/v) of a week's old seed culture of *Desmodesmus subspicatus* LC172266. The culture vessels capped with quality urethane foam were incubated at room temperature of 30 ± 2 ºC for 12 days (Ogbonna and Ogbonna, 2015) and the carbon source was atmospheric carbon IV oxide (CO_2) allowed to diffuse into the culture through quality foam plugging. The cultures were illuminated using four pieces of 2-feet white fluorescent tubes arranged in parallel and light intensity variation (with intensities 0 ~ 5000 lx) were achieved by distance adjustment of the light and incubation in the dark. The duration of light

exposure was 12 h followed by 12 h darkness every day. Prior to choosing the range of light intensities, an initial measurement of the LI of the natural environment of the growing alga was made. Light intensity measurement (using light meter, LX-1000, Japan) of the culture medium was done at centre of the culture bottle. Each day, the cultivated algal culture was agitated two times by shaking at 120 revolutions per minute (rpm) for 10 min in an orbital shaker (Gallenkamp Ltd, United Kingdom). Biomass, chlorophyll and oil contents were analyzed every two days using 10 mL of the algal culture broth for each determination. For each set-up, there were 3-replicates and the mean and standard error of means were deduced.

Influence of light: dark cycles on biomass yield and lipid accumulation

Optimal light: dark cycle for biomass yield and biosynthesis of lipids was assessed by culturing *Desmodesmus subspicatus* LC172266 at light: dark timings of 12:12, 14:10, 16:08 and 18:06 h and testing for biomass, lipid and chlorophyll contents. The cultivation conditions were as reported for LI above. However, cultures were illuminated using 2-feet white fluorescent tubes arranged in parallel with an LI of 5000 lx only. Other culture variables not mentioned, sample collection and analyses were as described above for light intensity.

Effects of optimal and un-optimal light intensity and light: dark cycles on lipid composition

Effects of optimal and un-optimal light intensity and light: dark cycles on lipid composition were demonstrated. For optimal cultivation, *Desmodesmus subspicatus* LC172266 was grown at light: dark timings of 18:06 h and a light intensity of 3500 lx for eight days. The cultivation at un-optimal light regime was done at a photoperiod of 12:12 h per day and light intensity of 1000 lx for eight days. Other cultivation conditions remained constant as reported above. Cultures were harvested on the eightieth day of incubation, followed by the extraction of lipid by a protocol reported below. **L**ipid composition was measured using the GC-MS protocol described below.

Determination of biomass concentrations

Biomass concentration was determined as cell dry weight every 48 h up to stationary phase.

Algal biomass productivity was measured as gL^{-1} day^{-1} as in Okpozu *et al*. (2019) (Equation 1).

$$Biomass\ productivity, P_{B,}(gL^{-1}day^{-1}) = \frac{\frac{C_B - C_A}{t}}{t} \quad \text{Equation 1.}$$

where, $C_A\,(gL^{-1})$ is the initial biomass concentration, $C_B\,(gL^{-1})$, cell dry weight at a time (t) of the batch culture.

The dry cell weight of the *Desmodesmus subspicatus* LC172266 was obtained by centrifuging ten milliliters of the broth culture at 3000 x g for 15 min. The cell pellets were washed consecutively for three times using sterile distilled water. This was thereafter transferred into a Whatman No. 1 filter paper of known weight and tagged w_1. The biomass extract was dried in a hot air oven at 70 ºC for 7 h until a constant weight was achieved. This was desiccated for 5 h before getting the weight 2 (w_2). To obtain cell dry weight, Liang *et al*. (2005) equation was adapted as in equation 2.

$$Cell\ Dry\ Weight\ (CDW) = W_2 - W_1,$$

$$CDW\ (gL^{-1}) = \frac{W_2 - W_1}{V} \times \frac{1000}{1}$$

(Equation 2)

where, w_2 = weight of filter paper and dried algal mass (g), w_1 = weight of the filter paper (g) and v = volume of broth (mL).

Chlorophyll contents estimation

To determine the chlorophyll quantity in the culture, we used water and absolute methanol as extractants and Becker (1994)'s documented protocol was adapted. Ten-milliliter quantities of the algal broth were harvested with the washing as reported above for cell dry weight. Chlorophyll was extracted from the cell pellets by adding 3 mL methanol and boiling in a water bath for 5 min. The contents of the boiled chlorophyll extract were cooled and made up to 5 mL volumes using methanol and measured in a spectrophotometer (Spectrumlab 22) at different wavelengths against the solvent blank. Chlorophyll contents calculations

were as shown in Equations 3 (Becker, 1994).

$$\text{Chlorophyll } a + b\ (\text{mg L}^{-1}) = (4.0 \text{ x } A_{665}) + (225.5 \text{ x } A_{650})$$

(Equation 3)

The chlorophyll contents in the algal biomasses (mg g^{-1} biomass) were calculated by dividing the concentration of the chlorophylls (mg L^{-1}) by the cell dry weight (Cheirsilp and Torpee 2012).

Determination of lipid content

Lipid content was determined using a protocol adapted from two-step Bligh and Dyer (1959). The solvent system was water, methanol and chloroform. To a sample containing 1 ml water, 3.75 ml of a mixture chloroform/methanol (1/2) was added and vortex mixed for 10-15 min. Then 1.25 ml chloroform was added with mixing for 1 min and 1.25 ml water with mixing another minute before centrifugation. Using this means, lipid was extracted and transferred to a vial of known weight. The chloroform was evaporated in a water bath set at 55 °C with a steady flow of N_2 gas. The sample was thereafter dried in an oven at 105 ºC for 1 h and the vial weighed again and the extracted lipid content expressed as percentage lipid. The LP was expressed in $gL^{-1}day^{-1}$ as in Equation 4 (Griffiths and Harrison, 2009).

$$Lipid\ productivity, LP, (gL^{-1}day^{-1}) = \frac{Total\ microalgae\ biomass\ production\ (g) \times lipid\ content\ (\%)}{working\ volume\ (l) \times cultivation\ time}$$

(Equation 4)

Determination of fatty acid composition

The fatty acid composition of *Desmodesmus subspicatus* LC172266 lipid was determined by gas chromatography-mass spectrometry (GC-MS-QP2010 PLUS Shimadzu, Japan) using a micro-bore capillary column (Db 30.0). Helium was the gas carrier (flow rate was 1.8 mL min^{-1}). The oven temperature was 70 ºC, injection temperature, 250 ºC with split injection mode. A total flow of 40.8 mL min^{-1} was recorded and MS table start time of 3.00 min, and retention time of 24.00 min were also recorded. The fatty acids were identified from the chromatograms by comparing the spectrum of the unknown component with the spectrum of the known components stored in the National Institute of Standards and Technology (NIST) library. The retention time, the spectra and the molecular name, molecular weight and formula were used as identification parameters. The properties of the fatty acid methyl ester (biodiesel) was measured by means of a BiodieselAnalyzer software (Talebi *et al*., 2014).

Statistical analyses of the data

Data were analyzed by One-way ANOVA using STATGRAPHICS Centurion XVI Version 16.1.05 ($P < 0.05$).

Results and Discussion

Influence of light intensity on the growth and lipid accumulation by Desmodesmus subspicatus LC172266

Light intensity significantly influences algal growth and accumulation of lipids to such an extent that when the nutrients are not limited, the growth of microalgae depend on the light conditions (Han *et al*., 2015). Growth of *Desmodesmus subspicatus* LC172266 was promoted by light intensity (LI) in an increasing order of 1000, 2000, 3500 and 5000 lx (Fig. 1). Light intensity tolerance of up to 300 $\mu Em^{-2}s^{-1}$ has also been documented for another *Desmodesmus* sp (Nzayisenga *et al*., 2020). However, in the present study, increasing LI beyond 5000 lx to 10000 lx and above (for between 24 ~ 48 h) led to the bleaching of the culture broth and consequent destruction of the microorganism (result not shown since it was clear the cells were dead). This could imply that there was a photo-inhibition at high irradiance. The apparent photo-inhibition phenomenon observed in this study by very high light irradiation had previously been described by Subramaniam *et al*. (2010) for a related species of microalga.

At the other end of the scale (in the present study), incubation of the alga in the dark (0 lx) resulted in some instances of very low biomass but in most cases a total cessation of growth. This is not surprising since *D. subspicatus* is a freshwater green microalga requiring light for its food

manufacture implying that incubation in the dark may have affected a key process in the metabolism of the alga. Similarly, in this culture incubated in the dark (0 lx), LP was not determined due to insufficient biomass to run a lipid content assay (Table 1). Here, the yield was very low and values almost stationary from the beginning of the study to the end, resulting in the inability to run the lipid content or productivity test.

Although the cultures irradiated with LI 5000 lx had numerically more cell mass, the 3500-lx showed higher lipid productivity. This could be because lipid productivity is a dual function of the biomass productivity and the lipid content. Therefore, even though the cell biomass may numerically be higher, the lipid productivity may not since the biomass content, the lipid content and the working volume are all taken into account in deducing lipid productivity (Griffith and Harrison, 2009). Ruangsomboon (2012) had previously obtained an equivalence of 5000 lx for optimal growth of *Botrycoccus braunii* KMITL 2 whereas Cheirsilp and Torpee (2012) reported the range of 2000 ~ 8000 lx for optimal growth and oil accumulation by two related species of microalgae. In all cases of the present investigation, the peak biomass concentrations were obtained on the day eight of the cultivation (Table 1) implying that maximal biomass harvesting needs to be targeted towards the eightieth day of incubation for this particular microalga under the studied cultivation condition.

In this study of the influence of light intensity, although the highest lipid content was achieved at 1000 lx (Fig. 2), lipid productivity (LP) was maximum (77.50 ± 1.23 mg $L^{-1}day^{-1}$) when the incubation LI was 3500 lx. This could have resulted because the low light intensity of 1000 lx that supported the highest lipid content did not support maximal algal biomass accumulation which is an integral component of lipid productivity. Therefore, 3500 lx could be regarded as the optimal condition for better lipid accumulation of *Desmodesmus subspicatus.* This deduction was made because the overall attribute of a biological feedstock needed for biodiesel production centres around lipid productivity and lipid class. It was not entirely clear why highest lipid content and lipid productivity (LP) were not obtained at the same light intensity as previous in findings (Ruangsomboon, 2012) showed that algae grown at high light intensities often accumulated more lipids. Even with a *Desmodesmus* sp, Nzayisenga *et al*. (2020), had noted that a high light intensity of 300 $\mu Em^{-2}s^{-1}$ supported higher fatty acid content. Krzeminska *et al*. (2015) suggested that increases in lipid content during condition of high light intensity could partly be caused by starvation. That could be true for the present study because at day 8, lipid production was maximal when nutrient was presumably minimal. Nevertheless, in some other studies (Cheirsilp and Torpee, 2012; George *et al*., 2014), high light intensities led to reduction of the lipid in the cultures of *Chlorella* and *Nannochloropsis* spp., even though their biomass contents increased. Similarly, Nzayisenga *et al*. (2014) obtained a reduction in lipid contents of *Chlorella vulgaris* and *Ettlia pseudoalveolaris* cultivated at LI 300 $\mu E\ m^{-2}s^{-1}$ and higher biomass than in cultures with lower LI.

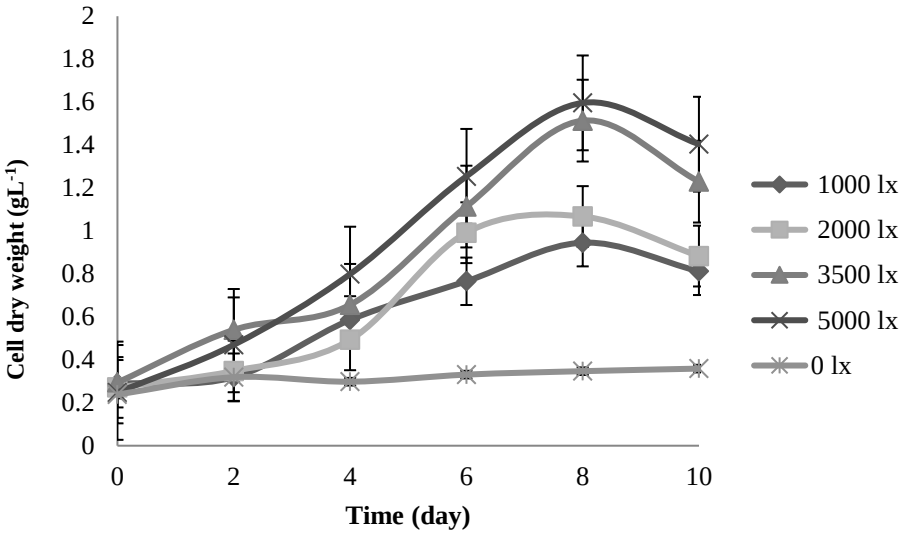

Fig. 1: Growth of *Desmodesmus subspicatus* under different Light intensity**.**

Table 1: Chlorophyll content, biomass and lipid production of *Desmodesmus subspicatus* under different light intensity**.**

Light intensity (LI) (lx)	**Max. Chlorophyll a + b ($mg^{-1}g$ cell)**	**Max. Biomass concentration ($g\ L^{-1}$)**	**Biomass productivity ($g\ L^{-1}day^{-1}$)**	**Lipid productivity ($mg\ L^{-1}day^{-1}$)**
1000	11.20 ± 1.02^{d}	0.95 ± 0.07	0.16 ± 0.01^{b}	52.50 ± 2.01^{d}
2000	15.78 ± 1.99^{b}	1.08 ± 0.03	0.18 ± 0.02^{b}	57.50 ± 0.80^{c}
3500	14.42 ± 1.46^{c}	1.51 ± 0.07	0.25 ± 0.01^{a}	77.50 ± 2.20^{a}
5000	20.40 ± 0.50^{a}	1.60 ± 0.24	0.27 ± 0.01^{a}	67.50 ± 2.03^{b}
0	7.05 ± 0.25^{e}	0.36 ± 0.05	0.06 ± 0.00^{c}	ND

Results = means ± SEM. ND = Not determined. Means that share different letters along the column are significantly different at 95% confidence limit. F (Chlorophyll content) =121.30, P=0.000 (P<0.05) (Significant differences exist in chlorophyll content under different light intensity). F (Biomass productivity) =126.35, P=0.000 (P<0.05) (Significant differences exist in biomass productivity under different light intensity). F (Lipid productivity) =3239.06, P=0.000 (P<0.05) (Significant differences exist in lipid productivity under different light intensity).

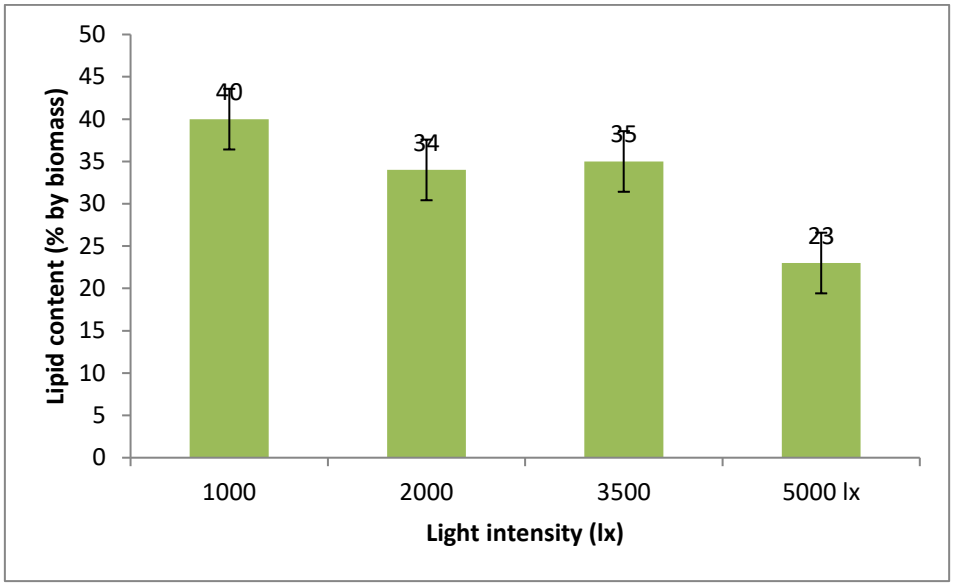

Fig. 2: Lipid accumulation by *Desmodesmus subspicatus* LC172266 under different Light intensity**.**

No statistical differences (P> 0.05) were observed with LI of 3500 and 5000 lx in terms of the biomass concentration. Chlorophyll content increased with LI with the highest (20.40 ± 0.50 mg g^{-1} cell) and lowest (11.20 ± 1.02 mg g^{-1} cell) at 5000 lx and 1000 lx respectively (Table 1). Although, the opposite had previously been reported by Neidhardt *et al*. (1998) when *Dunaliella salina* cells grown under high-light exhibited lower chlorophyll levels than those grown under low-light, with the lower chlorophyll content attributed to irradiance stress (Srirangan *et al*., 2015). Therefore, it is not certain whether the current observation is species dependent or other unforeseen intrinsic or extrinsic factor.

Effects of light: dark cycles (LDC) on the growth and lipid accumulations (LA) by Desmodesmus subspicatus LC172266

The extent of exposure of the alga to light/dark regimes investigated showed that the growth and lipid elaboration by the alga were affected by the extent of the photoperiods. The optimal LDC for *Desmodesmus subspicatus* LC172266 growth and LA were 18:06 h (Fig. 3). This was followed by 16:08 h LDC and so on with decreasing influence, and with LDC of 12:12 h supporting the least algal growth irrespective of the LI. This is consistent with the findings of Ruangsomboon (2012) that light: dark cycle of 12:12 h produced the least biomass of a green microalga *Botryococcus braunii* KMITL 2. The peak algal cell concentrations were reached on day 8 of cultivation by all the light treatment cycles and the lag phases were very short and almost un-detectable in all cases. Light exposure time is a direct factor for lipid production (Kato *et al*., 2019), hence, lipid accumulation correlated directly with the lengths of the light phases. Therefore, the highest lipid content (53%) was obtained at 18: 06 LDC followed by 42% obtained in 16:08 h cycle (Fig. 4).

The lipid contents at 14:10 h, 16:08 h and 18:06 h photoperiods were statistically different ($p < 0.05$) and the ones at photoperiod 12:12 h were significantly lower ($P < 0.05$) compared to the rest. This result contrasted with the findings of Anjala *et al*. (2015) when they reported a significantly higher percentage lipid content of 12.96% when *Nannochloropsis* sp. was grown at photoperiod 12: 12 h compared to 12.36% increase when grown at 16:8 LDC. The lipid content results did not support the results obtained by Ruangsomboon (2012) and a more recent report by Ma *et al*. (2017) for light: dark cycles. This could be because whereas, Ruangsomboon worked on a green

microalga *Botryococcus braunii* KMITL 2, the present study investigated *D. subspicatus* LC172266.

Chlorophyll content was highest (20.40 ± 0.50 mg g^{-1} cell) and lowest (14.91 ± 1.32 mg g^{-1} cell) at LDC of 12:12 h and 18:06 h respectively using the 5000 lx (Table 2). This could imply that the longer the exposure of the organism to light, the smaller the chlorophyll yields. This could also be attributed to irradiance stress previously reported by Srirangan *et al*. (2015). The highest LP (118.80 ± 2.04 mg $L^{-1}day^{-1}$) was achieved at photoperiod 18 h per day. Biomass productivity (BP) was directly proportional to the lengths of light phases with the highest BP (0.32 ± 0.06 $gL^{-1}day^{-1}$) obtained at 18:06 h LDC cultures as observed by Anjala *et al*. (2015).

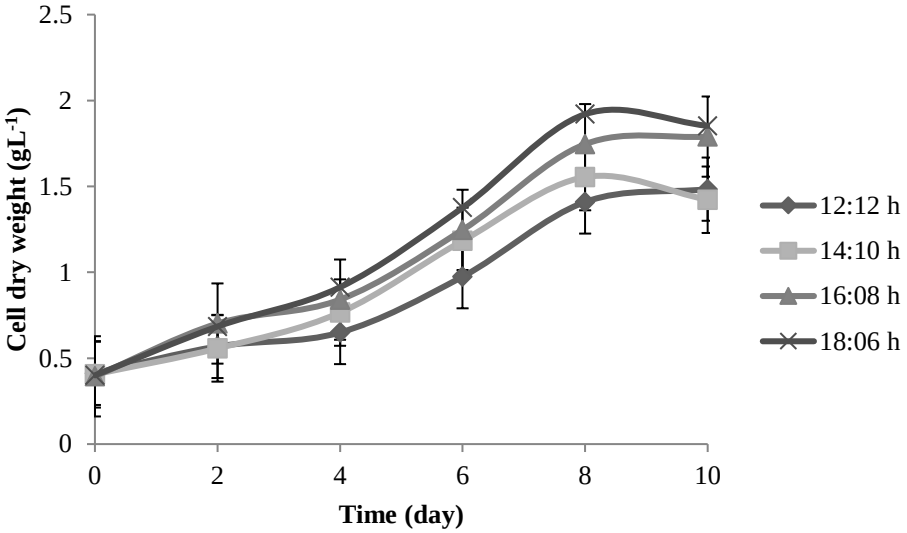

Fig. 3: Growth of *Desmodesmus subspicatus* under different light: dark cycles

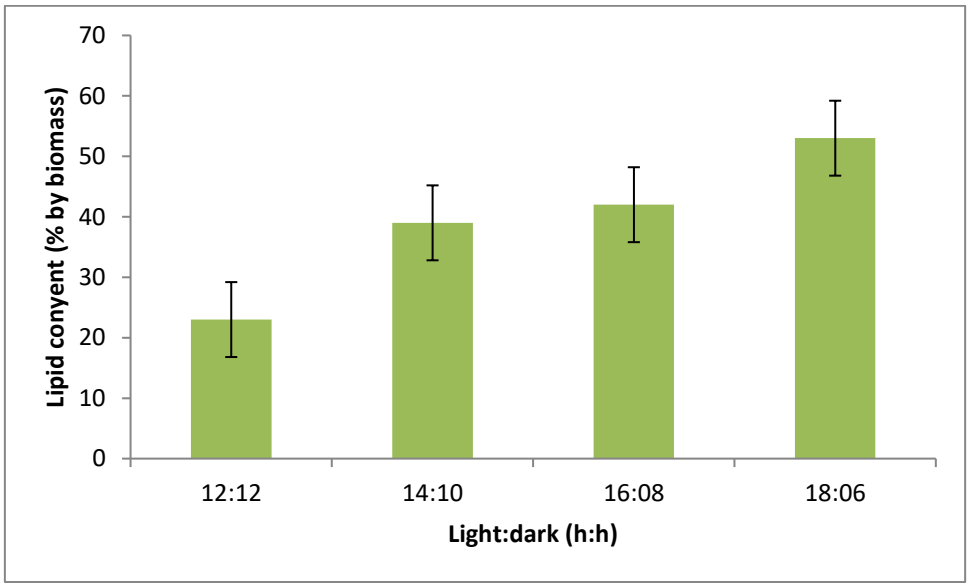

Fig. 4: Lipid accumulation by *Desmodesmus subspicatus* LC172266 under different light: dark cycles**.**

Fatty acid composition

The major fatty acid compositions of *Desmodesmus subspicatus* LC172266 lipid under un-optimized and optimized light intensities and photoperiods are presented in Table 3. The major fatty acids eluted (shown by % area of the peak of the fatty acid) under un-optimized conditions of light intensity and photoperiods were oleic acid (62.76), stearic acid methyl ester (7.96) and methyl 14-methyl-pentadecanoate (10.94) (Table 3). There were however, other minor fatty acids.

Similarly, under optimal conditions, the major fatty acids eluted were oleic acid (61.43), stearic acid methyl ester (9.69) and methyl 14-methyl-pentadecanoate (12.05) (Table 3).

There was no statistical difference between the fatty acid composition of the alga irrespective of the light intensity / photoperiod manipulation in culture conditions named optimized and un-optimized culture conditions. Solovchenko *et al*. (2008) noted that the higher the light intensity applied (from 35 to 400 μE $m^{-2}s^{-1}$), the higher the fatty acid and arachidonic acid content in the cells of a related microalga *Parietochloris incisa*, implying that it was in contrast with the results of the present study. The difference could be attributed to the differences in the algal species used.

Table 2: Chlorophyll content, biomass and lipid production of microalga *Desmodesmus subspicatus* under different light: dark regimes.

Light dark cycle (h)	Max. Chlorophyll a + b (mg g^{-1}cell)	Max. Biomass concentration (g L^{-1})	Biomass productivity (g $L^{-1}day^{-1}$)	Lipid productivity (mg $L^{-1}day^{-1}$)
12:12	20.40 ± 0.50^{a}	1.60 ± 0.24	0.27 ± 0.01^{c}	67.50 ± 2.03^{d}
14:10	15.65 ± 1.26^{b}	1.55 ± 0.09	0.26 ± 0.02^{c}	82.50 ± 1.45^{c}
16:08	20.02 ± 0.94^{a}	1.79 ± 0.01	0.29 ± 0.03^{b}	95.00 ± 2.17^{b}
18:06	14.91 ± 1.32^{c}	1.92 ± 0.03	0.32 ± 0.06^{a}	118.80 ± 2.04^{a}

Results = means ± SEM. Means that share different letters along the column are significantly different at 95% confidence limit. F (Chlorophyll content) = 804.61, P=0.000 (P<0.05) (Significant differences exist in chlorophyll content under different light dark cycle). F (Biomass productivity) = 31.26, P=0.003 (P<0.05) (Significant differences exist in biomass productivity under different light dark cycle). F (Lipid productivity) = 1198.89, P=0.000 (P<0.05) (Significant differences exist in lipid productivity under different light dark cycle).

Table 3: Major fatty acid composition of *Desmodesmus subspicatus* LC172266 under optimal and un-optimized light intensity and photoperiod

Retention Time (min.)	Name of compound	Molecular Weight	Molecular Formula	Abundance (%) optimal	Abundance (%) un-optimal
15.497	Methyl 14-methyl-pentadecanoate	270	$C_{17}H_{34}O_2$	12.05	10.94
16.067	Hexadecenoic acid	256	$C_{16}H_{32}O_2$	5.46	6.32
17.435	Stearic acid methyl ester	298	$C_{19}H_{38}O_2$	9.69	7.96
17.780	Oleic acid	282	$C_{18}H_{34}O_2$	61.43	62.76
17.953	Octadecanoic acid	284	$C_{18}H_{36}O_2$	3.98	5.91

T (Optimization types) = 0.02, P = 0.987 (% abundance was statistically the same under the optimal and un-optimized light intensity

Biodiesel property of fatty acids obtained from Desmodesmus sp grown at optimal and un-optimal light conditions

Biodiesel quality of the fatty acids obtained from *Desmodesmus* sp grown at optimal and un-optimal light is presented in Table 4. Monosaturated fatty acid (MUFA) (61.43 and 62.76 % respectively) was the most abundant followed by saturated fatty acid (32.39 and 32.18 % respectively). In a previous study by Solovchenok *et al*. (2015) *Desmodesmus* sp acclimated to grow at high CO_2 level produced palmitic acid, oleic acid and α – linoleic acid as the predominant fatty acids. El Semary (2011) had earlier obtained palmitic and palmitoleic acid as the major fatty acids in the polyphasic description of *Desmodesmus* sp isolate with the potential of bioactive compounds production. More recently, Ogbonna *et al*. (2018), Okpozu *et al*. (2019), obtained Octadecenoic acid, methyl ester and oleic acids from *Desmodesmus* sp grown in cassava wastewater as the major fatty acids. Knothe (2008) had previously recommended these class of fatty acids as suitable of use in biodiesel production.

In the present study, there was no significant difference between each of the test parameters of optimal and un-optimal conditions. This implies that changes in the light intensity and photoperiods did not change the fatty acid class and the biodiesel properties. Iodine value ($gI_2/100g$) and cetane value were within the ASTM and EN recommended limits for biodiesel as were kinematic viscosity (mm^2/s), and density (g/cm^2). This implies that the oil produced using *Desmodesmus* sp under the test condition as feedstock leads to a good quality biodiesel. Previous report had also shown that *Desmodesmus* sp is an ideal feed stock for biodiesel production. For instance, Ogbonna *et al*. (2018), Okpozu *et al*. (2019), obtained values comparable with the present for other *Desmodesmus* sp grown in cassava wastewater.

Table 4: Comparison of biodiesel quality of *Desmodesmus subspicatus* under optimal and un-optimized light intensity and photoperiod

Biodiesel properties	Optimal light	un-optimal	ASTM D6751	EN 14214	DSME	DAME	PME
SFA	32.39	32.18	N	N			
MUFA	61.43	62.76	N	N			
PUFA	6.20	5.35	N	N			
Iodine Value (g I_2/100g)	68.23	67.84	N	120.00 (max)	7.20	57.00	49.56
Cetane number	58.15	58.17	47.00 (min)	51.00 (min)	91.10	75.00	61.00
Long Chain Saturated Factor	7.96	8.11	N	N	8.85	15.47	-
Cold Filter Plugging Point (°C)	8.53	8.99	N	≤5/≤ -20.00	-8.30	-12.50	13.00
Kinematic Viscosity (mm^2/s)	4.14	4.16	1.90–6.00	3.50-5.00	2.28	3.80	4.53
Density (g/cm^2)	0.87	0.88	N	0.86-0.90	0.49	0.65	0.87

SFA = Saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, max = maximum, min = minimum, n = no limit assigned, - = not reported, DSME = *Desmodesmus subspitcatus* oil methyl ester, DAME = *Desmodesmus armatus* oil methyl ester, PME = plant oil methyl ester, ASTM D6751 and EN 14214 vehicular biodiesel standards (DSME, DAME and PME were adapted from Ogbonna *et al*. (2018), Okpozu *et al*. (2019) and Arora *et al*.(2016) respectively)

Conclusion

The biomass yield, lipid content and lipid productivity of *Desmodesmus subspicatus* were high, 1.92 ± 0.03 g/L, 53% and 118.80 ± 2.04 $mgL^{-1}day^{-1}$ respectively, showing its potential applicability in biofuel biotechnology *vis-à-vis* the fatty acid methyl ester properties. Light utilization optimization did not change the major fatty acid which was composed mainly of MUFA and SFA, in addition, the biodiesel was of excellent quality.

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