Effects of Carbon Source on Growth Characteristics and Lipid Accumulation by Microalga *Dictyosphaerium* sp. with Potential for Biodiesel Production

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Abstract

Sustainability and eco-friendliness have both engendered research on alternative replacement of fossil fuel. This study was aimed at determining the effects of varying levels of glucose (10 ~ 40 g/L), and glycerol (0.25 ~ 1.0 mL/L) on the heterotrophic and mixotrophic growth and lipid production by *Dictyosphaerium* sp. The microalga was cultivated in 2000 mL amber-coloured bottles each containing 1000 mL of a sterile modified BG-11 medium at pH of 7.3. Each bottle was inoculated with a one-week-old pure culture of the isolate (inoculum ratio = 15%) and incubated in the dark at room temperature (30˚C ± 2˚C) for 10 d. *Dictyosphaerium* sp. showed the ability to grow heterotrophically and mixotrophically on glucose and on glycerol as a sole carbon substrates. Biomass productivity and specific growth rates did not vary when the initial medium glucose was varied. Lipid accumulation was not dependent on the initial medium glycerol contents. The mean lipid content and productivity of the organism in the present study were high enough to be utilised for industrial processes. Growth and lipid accumulations were better in mixotrophic cultures than both heterotrophic and autotrophic. However, both were better than autotrophic. The percentage compositions of the major fatty acids from *Dictyosphaerium* sp. grown under different culture conditions show at least five components each. The carbon skeletons eluted ranged from C14 to C22. Oleic acid was a major component of all the fatty acids, which confirm the suitability of the use of the oil for biodiesel production.

Keywords

Algal Growth, Biodiesel Production, Carbon Substrates, *Dictyosphaerium* sp.,

Lipid Accumulation

1. Introduction

The demand for alternative fuel for automobiles has been, and will continue to be on the increase throughout the world. In most developing countries, the numbers of automobiles are on the increase as a result of a pronounced increase in population and crave for industrialisation. At the moment, the energy needed by most countries is supplied by the fossilised resources. This fossilised fuel consumption is linked with some bottlenecks including ecological menaces and finite nature. Hence, the increased quest for bioenergy correlates with the considerations for environment and energy sustainability. Bioenergy production is eco-friendly and is perceived to be sustainable.

Chisti [1] reviewing the important biological feedstock for biodiesel production arrived at oil-rich microalgae as the best candidate for lipid accumulation which subsequently is converted into biodiesel. Some of the advantages of the use of microalgae as biofuel feedstock including rapid growth rates and high lipid accumulation are important because of volumetric productivities of both biomasses and lipids. Biodiesel production is dependent on lipid quantity [2]. Therefore, insight into what microalga to use, and the cultivation conditions are very critical. Consequently, the search for novel isolates that could be utilized in the production process is essential.

Conventionally, most species of microalgae are cultivated photoautotrophically, using mineral salt media. This is because all microalgae are sunlight energy converters [3]. However, autotrophic cultivation has not solved the present need considering volumetric productivities of both biomasses and lipids. Therefore, it is not enough to say that all microalgae can grow autotrophically. Considering side by side the merits and the demerits of the different growth modes, in autotrophic cultivation, light supply limitation and other technical photobioreactor issues would make autotrophic cultivation costly.

Cost is an integral component of the pursuit of sustainable bioenergy production. Thus, as a replacement for autotrophic growth, heterotrophic or mixotrophic cultivation has been suggested for growing microalgae as a way of circumventing the limitations of photobioreactor. Not all microalgae can grow in absence of light energy. Many strains, however, are either known to grow heterotrophically or mixotrophically [4] [5] [6] [7] [8]. If the issue of cost is solved by getting such species, another key problem is that only a few of the strains are oil producing. An oil-producing strain can produce up to 20% lipid under unoptimized conditions. Even if a strain is an oil producing strain, other bottlenecks are the quality of the oil and the productivity.

Not all oils are suitable for biodiesel production *de novo*. Ogbonna and McHenry [9] and Ogbonna and Moheimani [10] reported that oil quality of heterotrophically cultivated algal cells is better than those under photoautotrophic

cultivations. Therefore, a central challenge faced by researchers utilising microalgae as feedstock for biofuel production is how to get species capable of combining the above characteristics. The present study investigated the effects of carbon source on growth characteristics and lipid accumulation by a novel isolate of microalga *Dictyosphaerium* sp.

2. Materials and Methods

2.1. The Microalga and the Culture Conditions

Dictyosphaerium sp*.* obtained from a water body in North East Nigeria [11] was used for this study. The alga was cultivated autotrophically in BG-11 medium (pH = 7.3) under a light intensity of 1000 lux at 30˚C ± 2˚C. To test the ability of the alga to grow heterotrophically, glucose was added to a BG-11 medium to obtain a modified medium with initial glucose levels of 10, 20, 30 and 40 g·L^{-1}. Furthermore, the ability of the alga to grow on glycerol was tested using a BG-11 medium containing different levels of glycerol (0.25; 0.5; 0.75 and 1.0 mL·L^{-1}). Prior to the addition of glucose or glycerol, the initial medium pH was adjusted to 7.3. Each microalga was cultured in 2000 mL amber-coloured polytetrafluoroethylene plastic bottle which contained 1000 mL of sterile BG-11 medium with the organic carbon substrate. All the flasks were inoculated with 15% (v/v) inoculum of a one-week-old pre-culture of the microalgae (obtained by inoculating a BG-11 medium supplemented with 1% glucose or glycerol respectively and incubating in the dark). Cultures were incubated at room temperature (30˚C ± 2˚C) in the dark for 10 d. For the mixotrophic cultures, all the other conditions in heterotrophic growth were the same except that transparent bottles were used, and the mixotrophic cultures were incubated under the sun for 4 h daily between 8:00 and 12:00 noon. This was followed by incubation in the dark. The cultures were agitated at 120 rpm for 3 min twice every day in a shaker in Gallenkamp orbital shaker [Gallenkamp Ltd., UK] and about 10 mL sample was collected every two days for analyses of biomass, chlorophyll and lipid contents. All the experiments were performed in triplicates and the results presented as the mean ± standard error of means.

2.2. Analytical Protocol

2.2.1. Biomass

The biomass was determined as cell dry weight. Measurements were carried out 48 hourly until a stationary phase was reached. The specific growth rate (μ) was calculated according to Equation (1).

$$\mu = \left(\ln N_t - \ln N_0 \right)/\left(t - t_0 \right) \tag{1}$$

where N_t = cell density at the time (t) and N_0 = cell density at the start of the exponential phase (t_0). Algal productivity was expressed in gL^{-1}·day^{-1}.

2.2.2. Cell Dry Weight

Samples were harvested by centrifugation (at 3000 rpm) in a bench-top centri-

fuge for 15 minutes. This was thereafter washed three times with distilled water, transferred to a pre-weighed filter paper (w_1) and dried to a constant weight in a hot air oven at 70°C overnight. They were left in desiccators for 5 h before re-weighing (w_2) and the cell dry weight was calculated as in Equation (2).

$$\text{Cell Dry Weight}(CDW) = w_2 - w_1$$

$$\text{Converting to g·L}^{-1}, \quad CDW = \frac{w_2 - w_1}{v} \times \frac{1000}{1} \quad (2)$$

where: w_2 = weight of filter paper and dried cells (g);

w_1 = weight of filter paper (g);

v = volume of culture (mL).

2.2.3. Chlorophyll Estimation

The chlorophyll contents were determined by the method of Becker [12] using methanol and water. To determine the chlorophyll a (chl a) content, 10 mL of algal suspension was centrifuged at 3000 rpm for 30 min and the supernatant was discarded. The algae were suspended in 3 mL of methanol and boiled for 5 min in a water bath. The samples were cooled to room temperature and then the volume was made up to 5 mL by adding methanol. The *chl* concentration in the extract was calculated by reading the absorption (A) of the pigment extract in a spectrophotometer (SpectrumLab 22) at a given wavelength against a solvent blank using the equation [12]:

Chlorophyll a (mg·L^{-1}) = $(16.5 \times A_{665}) - (8.3 \times A_{650})$.

Chlorophyll b (mg·L^{-1}) = $(33.8 \times A_{650}) - (12.5 \times A_{665})$.

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

2.2.4. Lipids Extraction

Lipids were extracted in a chloroform-methanol-water solvent system as described by Bligh and Dyer [13]. 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. The upper phase was discarded and the lower phase collected through the protein disk with a Pasteur pipette. After evaporation, the lipid extract (lower phase) was re-dissolved in a small volume of chloroform/methanol (2/1). Lipid productivity was calculated as the product of average lipid content and biomass productivity (Griffiths and Harrison, 2009) as seen in Equation (3).

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

$$(3)$$

2.2.5. Fatty Acids Analyses

The fatty acids profiles were determined in a Shimadzu Gas Chromatograph (Shimadzu, Japan), Model GCMS-QP2010 Plus. The GC-2010 column oven

temperature was 70˚C, injection temperature was 250˚C, injection mode was split, and the flow control mode was linear velocity while the pressure was 116.9 kPa. Total flow was 40.8 mL/min, column flow was 1.80 mL/min, linear velocity was 49.2 cm/s and purge flow was 3.0 mL/min while the split ratio was 20.0. High-pressure injection, carrier gas saver and splitter hold were all off. The GC program of the GCMS-QP2010 Plus ion source temperature was 200˚C, interface temperature was 250˚C, solvent cut time was 2.50 min, detector gain mode was relative and detector gain was 0.00 kV while threshold was 2000. The MS table start time was 3.00 min, and the end time was 24.00 min, ACQ mode was scan, event time was 0.50 s, scan speed was 666 and start m/z was 30.00 while end m/z was 350.00.

2.3. Statistical Analysis

Data were analysed by Multiple-Sample Comparison using STATGRAPHICS Centurion XVI Version 16.1.05 (32-bit) statistical software. All the experiments were done in triplicates and the results were presented as the mean ± standard deviation and descriptive statistics.

3. Results

3.1 Effects of Glucose Concentration on the Heterotrophic Growth and Lipid Accumulation by *Dictyosphaerium* sp.

The effects of different concentrations of medium glucose on the heterotrophic growth and lipid content of *Dictyosphaerium* sp. is shown in Figure 1.

There was a short lag phase within the first two days of the cultivation which was followed by a logarithmic growth phase. The algal growth was not proportional to the initial glucose concentration in the medium. In addition, there was no significant effect of initial glucose concentration on biomass concentrations ($p > 0.05$). The highest biomass concentrations obtained were 2.26 ± 0.15, 2.43 ± 0.11, 2.89 ± 0.03 and 2.75 ± 0.11 from media with initial glucose concentrations of 10, 20, 30 and 40 g·L^{-1} respectively (Table 1).

The BG-11 medium augmented with 20 g·L^{-1} of glucose gave the maximum lipid content of 42.3% ± 1.33%. Although the lipid contents of the cells varied with the medium glucose concentration, there was no significant different between the lipid contents obtained from media with glucose concentrations of 30 and 40 g·L^{-1}. Furthermore, the growth phase did not significantly affect the lipid contents as the values obtained at the stationary growth phase did not vary significantly with those obtained at the exponential growth phase. Irrespective of the initial medium glucose concentration, the maximum chlorophyll content, specific growth rate, maximum biomass concentration and biomass productivity were not significantly different (Table 1). The table shows that lipid productivity was highest (96.6 ± 2.55 mg·L^{-1}·day^{-1}) when the medium glucose concentration was 20 g·L^{-1}.

(a)

(b)
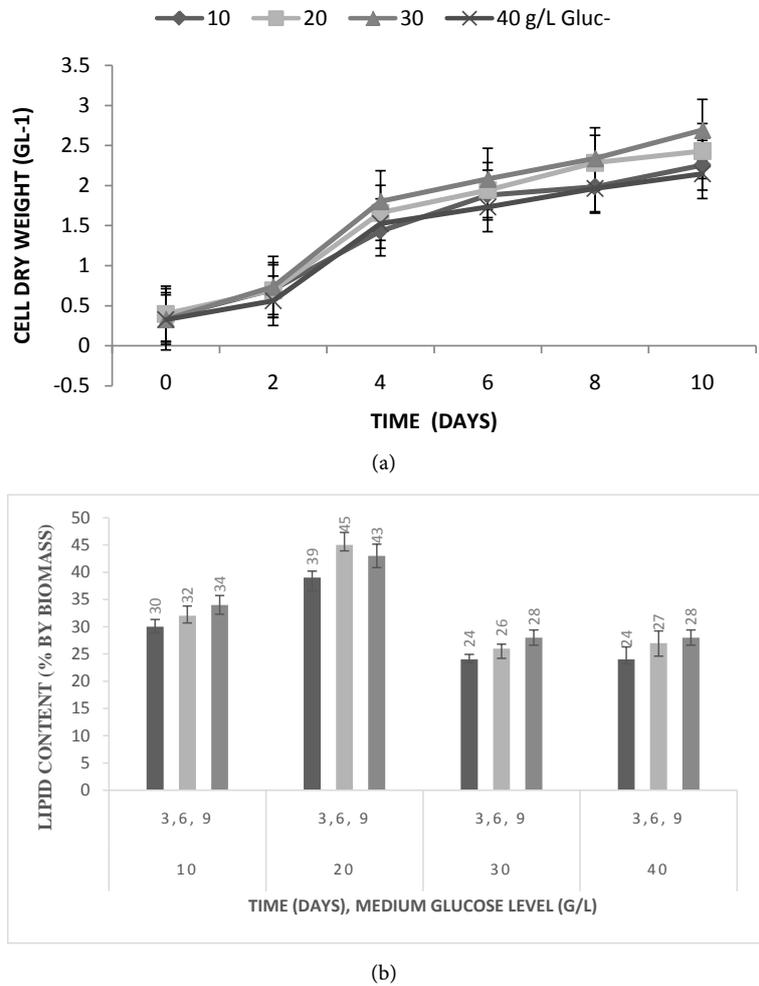
Figure 1. Effects of different levels of glucose on the heterotrophic growth and lipid accumulation by *Dictyosphaerium* sp. [NB: 3, 6, 9 are days 3, 6 and 9 of incubation respectively].

Table 1. Chlorophyll content, biomass and lipid production by *Dictyosphaerium* sp. grown in BG-11 medium with different initial glucose concentrations under heterotrophic condition.

Glucose concentration (g·L⁻¹)	Max. Chlorophyll a + b (mg/g cell)	Specific growth rate, μ (d⁻¹)	Max. Biomass concentration (g·L⁻¹)	Biomass productivity (g·L⁻¹·day⁻¹)	Average lipid content (%)	Lipid productivity (mg·L⁻¹·day⁻¹)
10.0	3.10 ± 0.12	0.15 ± 0.01	2.26 ± 0.15	0.23 ± 0.01	32.0 ± 1.22	73.6 ± 3.02
20.0	3.38 ± 0.67	0.16 ± 0.04	2.43 ± 0.11	0.23 ± 0.02	42.3 ± 1.33	96.6 ± 2.55
30.0	3.92 ± 0.23	0.16 ± 0.01	2.89 ± 0.03	0.24 ± 0.02	26.0 ± 0.80	62.4 ± 2.04
40.0	2.36 ± 0.47	0.17 ± 0.02	2.75 ± 0.11	0.21 ± 0.02	26.3 ± 1.08	55.3 ± 1.56

a: Results are means of triplicate tests.

3.2. Effects of Glucose Concentration on the Mixotrophic Growth and Lipid Accumulation by *Dictyosphaerium* sp.

The effects of different initial glucose concentrations on the mixotrophic growth of *Dictyosphaerium* sp. are shown in **Figure 2**. For all the initial medium glucose
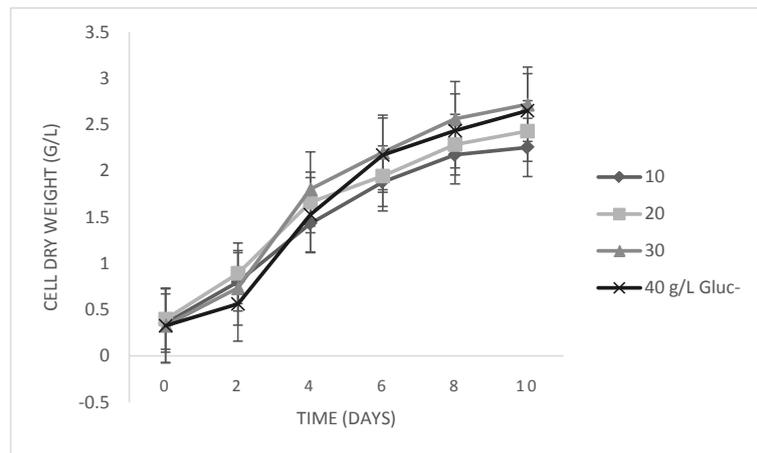
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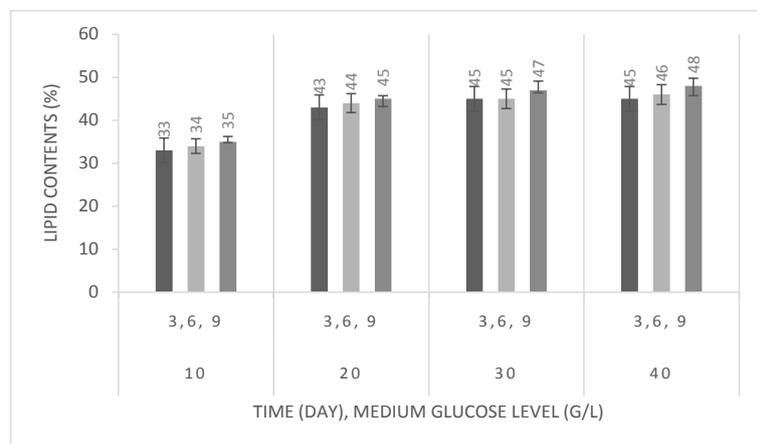
(b)

Figure 2. Effects of glycerol concentration on the heterotrophic growth and lipid accumulation by *Dictyosphaerium* sp.

levels tested, there was a short lag phase. This was followed by a patterned growth that did not show a clear-cut variation among the different initial glucose concentrations tested. The maximum biomass densities were 2.28 ± 0.05, 2.44 ± 0.15, 2.91 ± 0.22 and 2.90 ± 0.06 for media with initial glucose levels of 10, 20, 30 and 40 g·L^{-1} respectively (Table 2). There were no significant variations in chlorophyll a + b (mg·g^{-1} cell), specific growth rate, μ (d^{-1}), maximum biomass concentration (g·L^{-1}) and biomass productivity (g·L^{-1}·day^{-1}). Lipid accumulation under mixotrophic cultivation seemed to be positively influenced by organic substrate addition (Figure 2). However, there were no significant differences between the lipid accumulated when the initial media glucose were 20, 30 and 40 g·L^{-1}. The mean lipid contents of 34.0 ± 3.20, 44.0 ± 0.60, 45.7 ± 1.80 and 46.3 ± 2.10 were obtained when the initial media glucose was 10, 20, 30 and 40 g·L^{-1} respectively. Lipid productivity was highest (115.8 ± 2.30 mg·L^{-1}·day^{-1}) when the medium glucose was 40 g·L^{-1}.

There were no significant effects of varying concentrations of glycerol on the heterotrophic growth and lipid accumulation by *Dictyosphaerium* sp. from the

Table 2. Chlorophyll content, biomass and lipid production by *Dictyosphaerium* sp. grown in BG-11 medium with different initial glucose concentrations under mixotrophic condition.

Glucose concentration(g·L^{-1})	Max. Chlorophyll a + b (mg/g cell)	Specific growth rate, μ (d^{-1})	Max. Biomass concentration (g·L^{-1})	Biomass productivity (g·L^{-1}·day^{-1})	Average lipid content (%)	Lipid productivity (mg·L^{-1}·day^{-1})
10.0	10.70 ± 1.22	0.17 ± 0.01	2.28 ± 0.05	0.24 ± 0.05	34.0 ± 3.20	81.6 ± 1.4
20.0	10.84 ± 1.07	0.17 ± 0.01	2.44 ± 0.15	0.25 ± 0.01	44.0 ± 0.60	110.0 ± 3.20
30.0	11.49 ± 1.06	0.18 ± 0.02	2.91 ± 0.22	0.25 ± 0.02	45.7 ± 1.80	114.3 ± 2.10
40.0	10.07 ± 0.60	0.18 ± 0.01	2.90 ± 0.06	0.25 ± 0.01	46.3 ± 2.10	115.8 ± 2.30

b: Results are means of triplicate tests.

first to the sixth day of cultivation (Figure 3). However, after the sixth day of incubation, higher medium glycerol (0.75 and 1 mL·L^{-1}) gave higher biomass concentrations than at lower medium glycerol level. The maximum biomass contents obtained were 1.39 ± 0.20, 1.42 ± 0.03, 1.88 ± 0.18 and 1.84 ± 0.08 g·L^{-1} for 0.25, 0.5 0.75 and 1.0 mL·L^{-1} (v/v) of glycerol concentration respectively (Table 3). The range of specific growth rates and biomass productivity also followed the same pattern.

Lipid accumulation did not also depend on the initial medium glycerol level. There was no significant variation ($p > 0.05$) in the lipid contents among the initial medium glycerol concentrations tested. Similarly, there was no statistically significant difference ($p > 0.05$) between the lipid contents at the exponential growth phase and the stationary growth phase. Overall, the lipid contents ranged from 23.0% ± 0.24% to 27.7% ± 0.6%. Lipid productivity did not follow a general pattern. The highest lipid productivity (60.9 ± 1.05 mg·L^{-1}·day^{-1}) was obtained when the initial medium glycerol content was 0.75 mL·L^{-1} and lowest (39.1 ± 2.82 mg·L^{-1}·day^{-1}) at the medium glycerol concentration of 0.25 mL·L^{-1} (Table 3). The chlorophyll contents, specific growth rates and biomass productivities were higher at lower concentrations of glycerol.

3.3. Effects of Glycerol Concentration on the Mixotrophic Growth and Lipid Accumulation by *Dictyosphaerium* sp.

The initial medium glycerol did not significantly affect the growth pattern of *Dictyosphaerium* sp. (Figure 4). Maximum cell densities of 1.48 ± 0.50, 1.52 ± 0.20, 1.80 ± 0.05 and 1.85 ± 0.06 were obtained with initial medium glycerol concentrations of 0.25, 0.50, 0.75 and 1.00 mL·L^{-1} respectively (Table 4). The chlorophyll contents and the specific growth rates were higher than the values obtained in heterotrophic cultivation (Table 4).

There was no statistically significant difference between the lipid contents obtained when the initial medium glycerol was 0.25 and 0.50 or when it was 0.75 and 1.00 mL·L^{-1} (Figure 4). The average percentage lipid contents were 24.0 ± 0.24, 23.7 ± 1.20, 28.7 ± 0.60 and 30.1 ± 1.40 for media with initial medium glycerol of 0.25, 0.50, 0.75 and 1.00 mL·L^{-1} respectively (Table 4). Lipid productivity ranged from 45.6 ± 1.20 to 69.0 ± 1.40 with the highest being achieved when
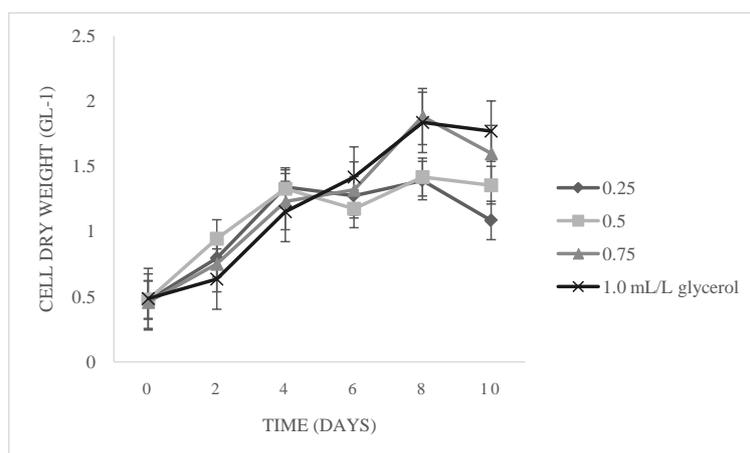
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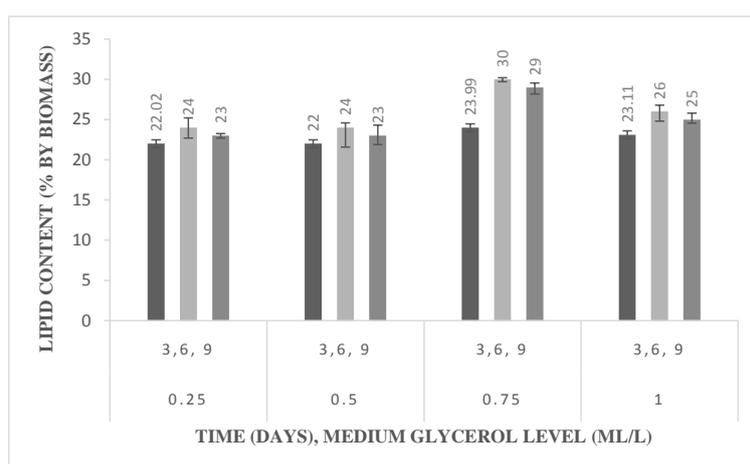
(b)

Figure 3. Effects of different levels of glycerol on the growth and lipid accumulation by *Dictyosphaerium* sp. under heterotrophic condition. [NB: 3, 6, 9 are days 3, 6 and 9 of incubation respectively].

Table 3. Chlorophyll content, biomass and lipid production by *Dictyosphaerium* sp. grown in BG-11 medium with different initial glycerol concentrations under heterotrophic condition.

Glycerol concentration (mL·L⁻¹)	Max. Chlorophyll a + b (mg/g cell)	Specific growth rate, μ (d⁻¹)	Max. Biomass concentration (g·L⁻¹)	Biomass productivity (g·L⁻¹·day⁻¹)	Average lipid content (%)	Lipid productivity (mg·L⁻¹·day⁻¹)
0.25	4.85 ± 0.11	0.14 ± 0.03	1.39 ± 0.20	0.17 ± 0.02	23.0 ± 0.24	39.1 ± 2.82
0.50	4.30 ± 2.04	0.15 ± 0.01	1.42 ± 0.03	0.18 ± 0.02	23.3 ± 1.20	41.4 ± 2.25
0.75	3.90 ± 0.53	0.17 ± 0.03	1.88 ± 0.18	0.22 ± 0.01	27.7 ± 0.60	60.9 ± 1.05
1.00	3.40 ± 0.60	0.17 ± 0.04	1.84 ± 0.08	0.22 ± 0.01	24.7 ± 1.40	54.3 ± 1.44

c: Results are means of triplicate tests.

the medium glycerol was 1.00 mL·L⁻¹. Overall, both the mixotrophic and heterotrophic cultivations had better biomasses and lipid contents for glucose and glycerol than their autotrophic counterpart (data not shown).
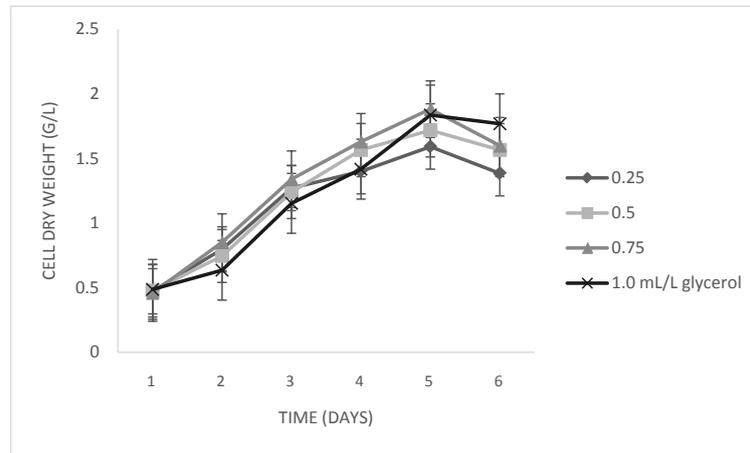
(a)
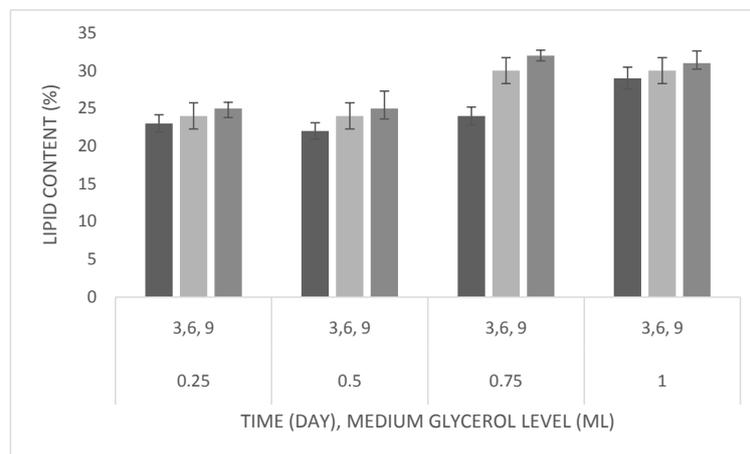
(b)

Figure 4. Effects of different levels of glycerol on the growth and lipid accumulation by *Dictyosphaerium* sp. under mixotrophic condition. [NB: 3, 6, 9 are days 3, 6 and 9 of incubation respectively].

Table 4. Chlorophyll content, biomass and lipid production by *Dictyosphaerium* sp. grown in BG-11 medium with different initial glycerol concentrations under mixotrophic condition.

Glycerol concentration (mL·L^{-1})	Max. Chlorophyll a + b (mg/g cell)	Specific growth rate, μ (d^{-1})	Max. Biomass con-centration (g·L^{-1})	Biomass productivity (g·L^{-1}·day^{-1})	Average lipid content (%)	Lipid productivity (mg·L^{-1}·day^{-1})
0.25	8.86 ± 1.06	0.16 ± 0.01	1.48 ± 0.50	0.19 ± 0.01	24.0 ± 0.24	45.6 ± 1.20
0.50	9.58 ± 2.01	0.16 ± 0.01	1.52 ± 0.20	0.20 ± 0.02	23.7 ± 1.20	47.4 ± 2.10
0.75	10.96 ± 1.22	0.18 ± 0.02	1.80 ± 0.05	0.23 ± 0.01	28.7 ± 0.60	66.0 ± 0.48
1.00	10.85 ± 0.05	0.19 ± 0.03	1.85 ± 0.06	0.23 ± 0.02	30.1 ± 1.40	69.0 ± 1.40

d: Results are means of triplicate tests.

3.4. Fatty Acids from *Dictyosphaerium* sp.

The percentage compositions of the major fatty acids from *Dictyosphaerium* sp. grown under different culture conditions show at least five components each

(Table 5). Oleic acid was present under all the culture conditions with autotrophic having the highest (63.60%). This was followed by the mixotrophic (glycerol) and heterotrophic (glucose) was the least. Generally, the carbon skeletons eluted ranged from C14 to C22.

4. Discussions

Dictyosphaerium sp. showed the ability to grow on glucose as the sole organic carbon source. This attribute is vital since the heterotrophic cultivation of algae has been recommended for biofuel production in terms of the cost of production. It has been pointed out previously that one major challenge of the use of algae for bioenergy is that many species cannot grow heterotrophically [9] [10].

There was no statistical variation in the heterotrophic growth pattern of *Dictyosphaerium* sp. when glucose was the organic carbon source. In the same way, biomass productivity and specific growth rates did not vary as a result of the variation in the initial medium glucose. This implies that utilising large quantity of glucose in the medium might be unnecessary. The effects of different levels of glucose on the lipid accumulation by *Dictyosphaerium* sp. seemed to be dosage dependent and it is consistent with the dose-dependent results obtained previously [14] [15] in *Chlorella*.

There were no significant effects of initial glucose concentrations on the mixotrophic growth of *Dictyosphaerium* sp. In addition, there was no significant variation in the chlorophyll a + b (mg/g cell), specific growth rate, μ (d^{-1}), maximum biomass concentration ($g \cdot L^{-1}$) and biomass productivity ($g \cdot L^{-1} \cdot day^{-1}$) among all the levels of glucose tested. The mean lipid content and productivity obtained in the present study were lower than those reported by Chen and Walker [15] in *Chlorella* fed-batch growth mode.

Table 5. Percentage compositions of the major fatty acids from *Dictyosphaerium* sp. grown under different culture conditions.

Fatty acids	Autotrophic	Mixotrophic (glucose)	Heterotrophic (glucose)	Mixotrophic (glycerol)	Heterotrophic (glycerol)
Saturated					
C14:0	3.24	-	-	5.2	3.6
C16:0	7.45	5.6	15.0	11.4	8.7
C17:0	-	14.0	-	-	-
C18:0	10.4	6.4	7.32	15.6	11.2
C19:0	-	9.8	7.2	2.3	-
Unsaturated					
C18:1	63.60	23.5	10.98	45.5	52.4
C18:2	-	-	-	-	-
C18:3	-	-	-	-	1.4
C19:1	1.22	33.2	49.2	13.2	9.6
C22:1	1.85	-	-	-	-

- = not eluted.

Using a related microalga under mixotrophic culture condition, Liang *et al.* (2009) obtained biomass concentrations that were medium glucose dosage dependent. The final biomass densities (2.89 ± 0.03 g/L) obtained in the present study for glucose supplemented medium was higher than the 2 g/L obtained by Liang *et al.* [14]. Conversely, Zhao *et al.* [16] working with *Scenedesmus quasdricauda* obtained higher biomass (3.39 g·L^{-1}) and specific growth rate of 0.572 ± 0.024 d^{-1} which is also higher than the specific growth rates obtained in the present study.

The present study has also shown that *Dictyosphaerium* sp. can grow with glycerol as the only organic carbon source. The maximal algal growth of 1.88 ± 0.18 g/L obtained for glycerol supplemented medium was lower than that reported by Liang *et al.* [14] and Chen and Walker [15]. An interesting attribute of the use of glycerol as a feedstock for biodiesel production is that glycerol is itself a by-product of biodiesel production.

Lipid accumulation was not dependent on the initial medium glycerol contents in the present study. In the heterotrophic cultivation of algae for lipids, it has been reported that increasing the carbon substrates concentrations leads to increase in lipid accumulation [9] [10]. In the present study, lipid accumulation seemed to be uniform irrespective of the initial medium glycerol level.

Comparing heterotrophic and mixotrophic growths of this organism under the mixotrophic condition with glucose as the carbon source, there was a slight increase in biomass accumulation and lipid contents. Even though the highest lipid content did not seem to differ significantly with that of heterotrophic culture, lipid accumulation seemed to have a gradual increase with the increase in the concentration of organic carbon in the mixotrophic cultivation. In mixotrophic cultures, the % lipid content was somewhat directly proportional to the quantity of the medium glucose concentration. Another important distinction is that the lipid content seemed to be higher at stationary growth phase than at exponential phase under mixotrophic cultivation. This was not clear-cut in heterotrophic cultivation. Both the mixotrophic and heterotrophic cultivations had better biomasses and lipids than their autotrophic counterpart. If the proportion of oleic acid is used as an index of quality of biodiesel, then, autotrophic cultivation could offer the best quality biodiesel followed by mixotrophic cultivation in the present study. However, other assessment parameters as indicated by [17] should also help in giving final judgement.

5. Conclusion

The new isolate, *Dictyosphaerium* sp. can grow either with glucose or glycerol as sole carbon substrate and produced appreciable biomasses and lipids. For the autotrophic, mixotrophic and heterotrophic cultivation, at least five major fatty acids were eluted. These fatty acids are those that are important components of a good quality biodiesel. Furthermore, the presence of oleic acid in remarkably high percentage in all the test conditions further confirms the suitability of the use of the oil for biodiesel production.

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