ISSN: 2229- 6905

Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © 2009 PHYCOSPECTRUM - ISSN: 2229 – 6905

Growth Rate and Fatty Acids Profile of 19 Microalgal Strains Isolated from River Nile for Biodiesel Production

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Abstract:

Fatty acid composition and total lipids in 19 freshwater microalgal strains isolated from River Nile of Egypt were investigated. The species grown in BG11 media and harvested at stationary phase. Algal growth was monitored by measuring chlorophyll *a* content. The lipid content was determined with two methods of lipid extraction using co-solvent system for each species. First method used chloroform-methanol (1:1v/v) and the second one used a mixture of n-hexane and isopropanol (3:2v/v). The Gas chromatography analysis showed variation in fatty acids composition of the isolated algal strain. The highest total lipid content was detected in *Microcystis aeruginosa* (30%) followed by *Chlamydomonas variabills* (21%). Among the fatty acid detected palmitic acid C16:0 at relatively high concentrations in all strains. Also the unsaturated fatty acids oleic, linoleic and linolenic found in most of species. This means that the algae oil of most isolated species have the suitable fatty acid composition for biodiesel production.
Keywords: microalgae; lipid productivity; biodiesel; River Nile; fatty acids profile

1. Introduction

The last few decades have seen a growing interest in using microalgae, cyanobacteria and other photosynthetic bacteria as potential producers of renewable fuels, such as biodiesel, biohydrogen and biogas. Biodiesel production from microalgae is a relatively novel concept and these organisms offer the greatest photosynthetic efficiency, as a consequence of a minimum of internally competitive plant functions and limited nutrient requirements, besides exhibiting fast reproductive cycles. The yield of biodiesel from microalgae depends up on both the biomass concentration of the cultures and the oil content of individual cells (Momocha et al., 2012). Algal lipids have been suggested as a potential fuel substitute (Abdeshahian et al., 2010, Jegannathan et al., 2011) due to their accumulation inside the cells at the end of the growth stage (Mohammady and Fathy, 2007). Lipids produced by microalgae generally include neutral lipids, polar lipids, wax esters, sterols and hydrocarbons, as well as phenyl derivatives such as tocopherols, carotenoids, terpenes, quinines and pyrrole derivatives such as the chlorophylls (Kalpesh et al., 2012).

Factors such as temperature, irradiance and nutrient availability have been shown to affect both lipid composition and lipid content in several algae (Rao et al., 2007). In addition, to develop cost-effective algal oil production, researchers have experimented with photoheterotrophy / mixotrophy and heterotrophy for enhancing lipid productivity, especially with species of *Chlorella*. Furthermore, large-scale cultivation of microalgae may be 10–20 times more productive on a per hectare basis than other biofuel crops, are able to use a wide variety of water sources, and have a strong potential to produce biofuels without the competition for food production (Momocha et al., 2012).

It is very important to screen microalgal strains before suitable strains can be selected for their application. Ideally, primary screening should be rapid, inexpensive, predictive, specific, and effective for broad range lipids and applicable on a large scale. There are over 40000 species of algae already identified and many new species are yet to be identified (Hu et al., 2008). Among these very few were identified for their best lipid content in the cell and still more to be screened for their efficiency in lipid content. In any algal process, species selection is a key decision influencing choice of location, reactor design, culture conditions, harvesting method and product range (Pulz and Gross 2004). Qualities generally desirable for mass culture for lipid production include rapid growth rate, high lipid productivity, resistance to contamination (through high growth rate or growth in an extreme environment), tolerance of a range of

ISSN: 2229- 6905

environmental conditions (particularly those difficult to control in an outdoor environment such as temperature and changes in salinity due to evaporation or flooding), rapid CO_2 uptake and tolerance of shear force. Factors, such as temperature (Converti et al., 2009) light (Rodolfi et al., 2009) and nutrient availability (Shifrin, and Chisholm 1981**)** have been shown to influence lipid productivity. It is well known that nutrient limitation can increase lipid content, although this response is species-specific (Illman et al., 2000, Hsieh and Wu 2009)

The present study is aimed to identify high lipid producers from green, blue-green and diatoms algal strains which were isolated from freshwater River Nile. The growth rate, lipid accumulations as well as the lipid productivities were evaluated systematically. Results of this study will provide important information relating to screening technologies as well as the useful oil producers for future researches on algae-based biodiesel.

2. Materials and Methods

2.1. Isolation and Purification of Algal Strains

Algal species were isolated from River Nile water which concentrated via phytoplankton net (80µm mesh size) using BG11 media (Carmichael, 1986**)** and Diatom Medium, Modified **(**Cohn et al., 2003) Some modifications in the nitrate concentration of the BG11 media (1.5mg/L $NaNO_3$) were made according to different algal species requirements. *Oscillatoria limnetica* and *Spirulina platensis* were successfully isolated after the $NaNO_3$ was diluted to 1/5 the original concentration. The source of nitrogen was completely omitted from the media to obtain the isolates of blue-green heterocystis algal form. In addition, species of green algae were isolated and purified in nitrate modification while *Nitzschia linearis* and *Navicula cuspidate* were flourished by using Diatom Medium.

Algal strains were isolated by spreading 0.1ml of water samples into petri dishes containing BG11 and Diatom Medium plus 1.5% agar (for solidification). Single colonies of algae were then recultivated in specified liquid media for each strian as non-axenic batch cultures (50ml) at 25±2°C and a photoperiod 24hr with white fluorescent light intensity ≈2500Lux.

Nineteen algal species belonging to blue-green algae group (Cyanophyta), green algae group (Chlorophyta) and diatoms group (Bacillariophyta) were successfully isolated. Algal identification has been done according to the keys of identification (Streble and Krauter 1978 , Komárek, 1989).

2.2. Cultivation of the Isolated Strains

Cultivation was carried out in sterilized 1 liter conical shoulder flasks containing 600ml of the corresponding culture medium under continuous illumination. The cultivation time differed from one strain to another depending on the optimum growth rate till reaching stationary phase which always ranged between (15-20) days.

2.3. Chlorophyll a Content Measurement

The fresh Sample (25ml) of each strain was taken every 48 h and filtered through 0.45µm membrane filter and extracted with hot methanol (Fitzgerald et al., 1971) after the addition of 0.5ml magnesium carbonate solution (1%) in order to prevent chlorophyll degradation. The concentration 0f chlorophyll *a* was calculated according to the sited equation (Standard Methods 1998).

$$\mathbf{C}_{\mathbf{a}} = 11.85(OD664) - 1.54(OD647) - 0.08(OD630)$$

Chlorophyll *a* µg/L = C_a X extract volume, L/ volume of sample, L

Where: OD664, 647 and 630 are the absorbance at 664,647 and 630.

2.4. Algal Biomass Harvesting

In order to harvest algal biomass, a suitable harvesting method may involve one or more steps and be achieved in several physical, chemical, or biological ways. Filter a press operating under pressure was used with *Microcystis aeruginosa* and *Chlamydomonus variabills*, through membrane filter 0.8µm, while Filter presses operating under vacuum was used with *Scenedesmus obliquus* and *Scenedesmus quadricauda* by using phytoplankton net 5µm mesh size. Other microalgal strains were harvested through settling then centrifugation, these include, *Chlorella vulgaris*, *Haematococcus pluvialis*, *Nitzschia linearis*, *Navicula cuspidate* and *Anabaena constricta*. In contrast, other *Anabaena species* which include, *Anabaena spiroides* and *Anabaena sphaerica*, in addition to *Lyngbya limnetica, Nostocoposis wichmannii, Oscillatoria agardhii, Oscillatoria limosa, Phormidium rimosum and Spirulina platensis* were harvested using simple method using a handmade thieve consists of a phytoplankton net with a pore size of 30µm mesh size. Centrifugation also was used with only two strains, *Chroococcus turgidus* and *Oscillatoria limentica.*

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2.5. Lipid Extraction

All species after harvesting were subjected to two methods for oil extraction:

2.5.1. Modified method of Bligh and Dyer (Bligh and Dyer, 1959)

Mixing chloroform-methanol (1: 1, v/v) with the dry cells using homogenizer for 5-minutes at 800 rpm in a proportion of 1g in 20 ml of solvent mixture. The homogenate mixture subjected to a magnetic stirring at room temperature for 4-8 h. The mixture was washed with distilled water. After vortexing, the mixture was transferred into a separating funnel and the solvent evaporated using a rotary evaporator. The weight of the crude lipid obtained from each sample was measured.

2.5.2. Hexane-Isopropanol Extraction Method (Halim et al., 2011)

A mixture of n-hexane and isopropanol (3: 1ml) was added to 1g of microalgae powder. The conical flasks were sealed with aluminum foil to reduce solvent evaporation and all extraction mixtures were agitated at 800 rpm at ambient conditions for 7.5 h. Cell residue was removed by filtering through Whatman GF/C paper. The filtrate was transferred into a separating funnel and sufficient hexane and water (approximately 40 ml each) were added to induce biphasic layering. After settling, the solvent mixture was partitioned into two distinct phases: a top dark-green hexane layer containing most of the extracted lipids and a bottom light green aqueous-isopropanol layer containing most of the co-extracted non-lipid contaminants. The hexane phase was collected in a pre-weighed flask before it was heated to dryness in the oven (60 °c) to enable gravimetric quantification of the lipid extract. The crude lipid was re-dissolved in hexane (approximately 20 ml) and transferred into a sealed glass vial for storage.

2.6. Fatty Acid Analysis

The fatty acid profile of the extracted oil sample of all species were determined by converting the fatty acids in the oil to fatty acid methyl esters (FAMEs).The FAME composition was determined using a Gas-Chromatography (GC) with a split automatic injector and silica capillary column DB-5 (length: 60 m; ID: 0.32 mm.) .Details of the procedure have been described elsewhere. Helium was used as carrier gas at a flow rate of 1 ml/min. The column was held at 150 ºC for 1 min and ramped to 240 ºC at rate 30 ºC/min, and it was then held at 240 ºC for 30 min. Standards were used to give rise to well-individualized peaks that allow the identification of the fatty acid composition.

2. Results and Discussion

3.1. Growth Characteristic and Chlorophyll a Content

Chlorophyll *a* content as a major growth indicator of algae was measured to evaluate the specific growth rate (μ) and biomass production of each algal species under the investigation. The growth rate of algal strains till reached stationary phase ranged between 14 to 20 days according to the behavior of each strain. The stationary phase of strains differed from one strain to another. The maximum standing biomass of each isolated strain as chlorophyll a content and maximum specific growth rate (μ) are presented in Figure 1.

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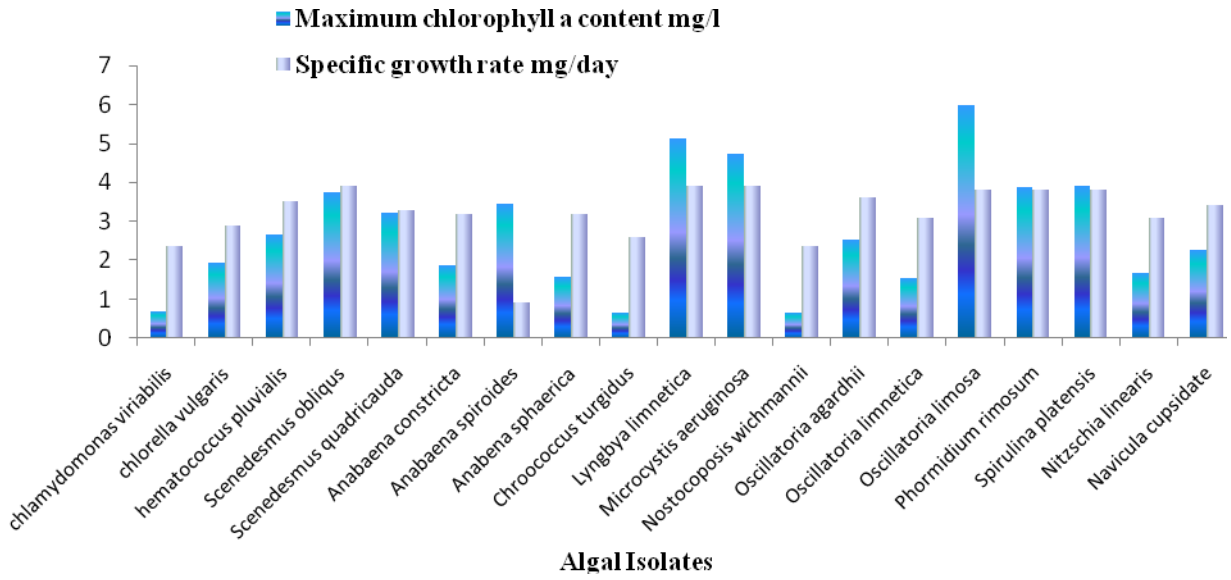
Figure 1. The maximum chlorophyll a content and specific growth rate of selected algal isolates



The maximum standing biomass of *Chlamydomonas variabills* was reached after 8 days with maximum chlorophyll *a* content reached 0.6 g/L and start the stationary phase up to 12 day, while *Chlorella vulgaris* growth rate showed maximum value at 10th day of culture and reached 1.9 g/L. The growth rate start in stationary phase with no decline phase up to 14th day. *Haematococcus pluvialis* chlorophyll *a* content attains its maximum value 2.6 g/L at 10th day and the stationary phase was continued up to 14th day. In addition, *Scenedesmus obliquus* and *Scenedesmus quadricauda* both of strains continue to grow up to 16 days with maximum chlorophyll *a* 3.7 g/L and 3.2 g/L, respectively. Maximum chlorophyll *a* content for the heterocystus strains (blue-green algae) including *Anabaena constricta, Anabaena spiroides, Anabaena sphaerica* and *Nostocoposis wichmannii* was detected at 10th day and the stationary phase was continued up to 14th day and the maximum chlorophyll *a* production are 1.2 g/L, 3.4 g/L, 1.5 g/L and 0.66 g/L, respectively. *Chroococcus turgidus* reached maximum growth at 12th with chlorophyll content 0.65 g/L. Growth rate of *Lyngbya limnetica, Oscillatoria agardhii, Oscillatoria limnetica, Oscillatoria limosa, Phormidium rimosum* and *Spiulina platensis* is the same where maximum chlorophyll at 10th day and continue in stationary phase up to 14th. *Microcystis aeruginosa* showed maximum growth at 16th and continue in stationary phase up to 20 days. In addition, diatoms strain *Nizschia linearis* and *Navicula cuspidate* reached maximum growth at 10th and 14th with chlorophyll content 1.6 g/L and 2.2 g/L, respectively. 10th and 14th with chlorophyll content 1667.4 µg/L and 2262.7 µg/L, respectively.

3.2. Total Lipid Content

Total lipid content and Lipid productivity of all isolated algal strain were shown in Figure 2. The results revealed that the highest total lipid content was detected in *Microcystis aeruginosa* and *Chlamydomonas variabills* 30% and 21% respectively, these results matches that of Kodandoor and Madaiah (2011**)** who found that *Microcystis aeruginosa* has total lipid of 28.15%, and the results of Giuliano et al., (2010) showed that *Chlamydomonas variabills* has a total lipid of 21%. Green alga *Scenedesmus quadricauda* and blue-green alga *Oscillatoria limosa* both of them have the same percentage of total lipid content 16% despite they are belong to differed algal group, these results comes in harmony with Mohparta (2006) who found that *Scenedesmus quadricauda* has a total lipid of 19.9, while Kodandoor and Madaiah (2011) stated that *Oscillatoria chlorina* has a total lipid of 16.6% near to the percentage of *Oscillatoria limosa*. Moreover, *Nitzschia linearis* showed the lowest total lipid percentage 6%. Other strains varied in their lipid content where, *Spirulina platensis* and *Chlorella vulgaris* showed 15.8% and 14.8% respectively. *Haematococcus pluvialis, Scenedesmus obliquus* and

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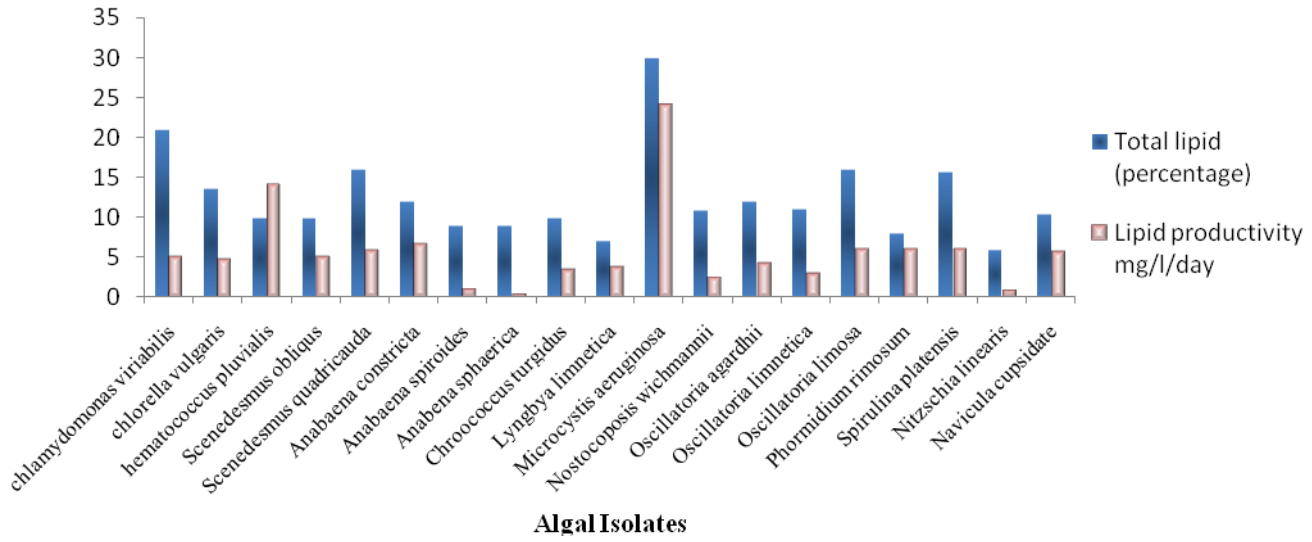
Chroococcus turgidus have the same percentage of total lipid which is 10% although they are not belonging to the same algal group. Becker (1994) results showed that *Scenedesmus obliquus* total lipid range from 12-14%, and Cecilia et al. (2010) stated that *Haematococcus pluvialis* total lipid percent is 15.6%.

Figure 2. Total lipid content and lipid productivity of selected algal Isolate*3.3. Lipid Productivity*



The results of lipid productivity presented in Figure 2 of all algal strains varied from one strain to another even in the same group. The highest lipid productivity was found at *Microcystis aeruginosa* 24 (mg/L/day), this result differed from that of Patrícia et al. (2012) where the lipid productivity of *Microcystis aeruginosa* is 46.92 and total lipid of 28%, this may be due to the use of different media (ASM-1 medium). In addition, *Hematococcus pluvialis* revealed lipid productivity 14 (mg/L/day) which has a total lipid percentage of 10%, while lipid productivity of *Chlamydomonas variabills* is 5 (mg/L/day). The lowest lipid productivity was detected in *Nitzschia linearis* 0.9 (mg/L/day).

3.4. Fatty Acids Profile in Different Algal Strains

Saturated and unsaturated fatty acids of investigated algal strains are represented in Table 1. The major saturated fatty acids present were palmitic acid (C16:0) present in all species in varied concentrations. *Anabaena spiroides* has the highest concentration of palmitic acid 48% followed by *Anabaena sphaerica* and *Microcystis aeruginosa* 37.8%, 36.4% respectively. Stearic acid (18:0) was also present in most species. *Microcystis aeruginosa* showed the highest concentration of stearic acid, although it was not found in *Hematococcus pluvialis*, *Spirulina platensis* and *Navicula cuspidate.* The most important unsaturated fatty acids present in algal strains are, palmitoleic acid (C16:1), oleic acid (C18:1), lenoleic acid (C18:2) and linolenic (C18:3). Palmitoleic acid was found in high concentrations reached 36% and 29.9% for *Nostocoposis wichmannii* and *Scenedesmus quadricauda* respectively, and oleic acid was high in *Chroococcus turgidus* and *Oscillatoria limosa* 25.2%, 20.9% respectively, while it was not found in *Lyngbya limnetica* . Lenoleic acid was found in most of algal species more than linolenic acid.

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ISSN: 2229- 6905

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Many microalgae species can be induced to accumulate substantial quantities of lipids, thus contributing to a high oil yield. The average lipid content varies between 1 and 70% but Chisti (2007) and Li et al., (2008) stated that under certain conditions some species can reach 90% of dry weight [28, 29]. In this study the lipid content varied from 6% to 30%. *Microcystis aeruginosa* seems to be a good strain for biodiesel production. Yet, as other species are so efficient and productive as this one, the selection of the most adequate species needs to take into account other factors, such as for example the ability of microalgae to develop using the nutrients available or under specific environmental conditions. All these parameters should be considered simultaneously in the selection of the most adequate species or strains for biodiesel production. Also, the composition of fatty acids of the different microalgae species, as they can have a significant effect on the characteristics of biodiesel produced. These are composed of saturated and unsaturated fatty acids with 12–22 carbon atoms, some of them of v3 and v6 families. Thomas et al. (1984) analyzed the fatty acid compositions of seven fresh water microalgae species showing that all of them synthesized C14:0, C16:0, C18:1, C18:2, and C18:3 fatty acids.

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Table 1. Fatty acid profile (saturated and unsaturated fatty acids) of 19 isolated microalgal species

Algal Isolates	Fatty Acids Composition												
	C:16-0	C:17-0	C:18-0	C:20-0	C:22-0	C:24-0	C:16-1	C:16-2	C:18-1	C:18-2	C:18-3	C:20-1	C:20-2
Chlorophyta													
Chlamydomonus variabills	24.7	3.3	10.7	-	6.7	11.9	-	-	5.3	6.5	5.7	-	-
Chlorella vulgaris	11.7	-	10.4	-	0.3	0.5	6.5	-	16.3	19.1	1	0.2	1.3
Haematococcus pluvialis	2.6	14.8	-	-	5.5	5.5	6.8	-	2.6	1.4	-	4.4	7.4
Scenedesmus obliquus	11.4	2.3	14.8	1.3	-	-	0.8	-	4.9	5	3.4	2.1	7.9
Scenedesmus quadricauda	11.5	1.5	4.8	-	-	-	29.9	-	8	5.3	-	-	-
Cyanophyta													
Anabaena constricta	34.6	1.1	3.7	1	-	1.4	2.7	-	16.7	15.7	0.4	0.9	3
Anabaena spiroides	48	1.2	9.2	-	-	-	2.2	-	10.6	12.8	-	-	2
Anabaena sphaerica	37.8	1	4.5	-	-	0.39	2.6	-	18.52	16.5	-	0.2	1.72
Chroococcus turgidus	26.9	2.4	1.7	-	-	-	10	2.6	25.2	4.6	2.5	-	-
Lyngbya limnetica	0.5	7.9	3.7	-	-	-	10.2	-	-	0.9	-	16.3	1.8
Microcystis aeruginosa	36.4	3	17.7	-	-	-	5.3	-	17.7	5.7	1.4	-	-
Nostocoposis wichmannii	17.6	0.45	2.9	-	-	0.4	36	2.4	8.2	3.2	2.8	0.15	4.9
Oscillatoria agardhii	31.6	0.5	5.7	-	0.64	0.58	-	-	5.8	14.5	-	-	5.3
Oscillatoria limnetica	5.3	15	4	-	11.6	-	-	-	1	7.8	-	3.5	4.5
Oscillatoria limosa	23.3	0.7	4.8	-	0.56	0.4	5.7	-	20.9	3.1	0.4	0.3	2.9
Phormidium rimosum	25.4	3.8	5.7	7.1	5.2	0.8	0.1	-	2.5	2.3	1.4	-	0.8
Spiulina platensis	17.6	3.9	-	2.3	1.4	-	-	-	3.4	-	2.1	-	-
Bacillariophyta													
Nizschia linearis	17.5	4.4	-	-	-	-	8.5	-	2.1	0.9	1.5	2	6.3
Navicula cuspidate	37	4	3.8	-	-	-	28	-	2.1	1.5	-	-	-

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4. **Conclusion**

The total lipid content in microalgae varies greatly from one species to another although those belong to the same algal group. So, it is very important to screen microalgal strains before suitable strain can be selected for their application. Two species from 19 microalgal isolated species revealed high lipid content, these are *Microcystis aeruginosa* and *Chlamydomonus variabills*. Albeit, *Anabaena spiroides* revealed low lipid productivity it gives high palmitic acid (C: 16-0) conten.

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