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Effect of temperature on growth and fatty acid profile of the biodiesel promising microalga *Scenedesmus obliquus*

Abd El-Fatah Abomohra[1*], Mostafa El-Sheekh[1], Metwally Abd El-Azim[1] and Reda Abou-Shanab[2]

1- Botany Department, Faculty of Science, Tanta University, 31527 Tanta, Egypt
2- Genetic Engineering & Biotechnology Research Institute (GEBRI), City of Scientific Res. and Technology Applications, New Borg El-Arab City, 21934 Alexandria, Egypt
***Corresponding author:** e-mail: abomohra@yahoo.com

ABSTRACT

The present study examined the effect of incubation temperature (15, 20, 25, 30, 35 and 40 °C) on biomass, esterified fatty acids content and fatty acid productivity of the promising biodiesel producing microalga *Scenedesmus obliquus*. Incubation of this species at 30 °C showed the maximum growth, however no significant difference was recorded in biomass productivity at 25 and 30 °C (0.411 and 0.423 g /L/ d , respectively). The highest fatty acids content (104.1 mg/ g CDW) was recorded at 15 °C which decreased by increasing of temperature. As a result of high biomass production, fatty acids productivity showed the highest values at 25 and 30°C (41.27 and 42.10 mg/ L / d, respectively). With respect to fatty acids profile, the proportion of saturated and mono-unsaturated fatty acids increased, while the poly-unsaturated fatty acids decreased with increasing the incubation temperature. The proportion of saturated and mono-unsaturated fatty acids was increased from 13.72 to 23.79 % and from 11.13 to 33.10 % of total fatty acids, respectively, when the incubation temperature increased from 15°C to 40°C. While, poly-unsaturated fatty acids decreased from 75.15 % to 43.10 % of total fatty acids at incubation temperatures 15°C and 40°C, respectively. The present study concluded that incubation temperature is a critical parameter for quantitative and qualitative fatty acids compositions of *Scenedesmus obliquus*. In addition, the type and proportion of individual fatty acids, which controls the biodiesel quality, can be modified using different incubation temperatures to meet the biodiesel international standards.

Key words: Biomass, *Scenedesmus obliquus*, biodiesel, fatty acids, temperature.

INTRODUCTION

Nowadays, the global energy usage is based on utilization of fossil fuels including natural gas, coal and oil. Barbir (2009) concluded that using of fossil fuels has several problems, such as: 1) it results in pollution on local, regional and global scales, 2) due to the quick development of human activities and overconsumption of fossil fuels, the energy crisis is representing the largest challenge of the 21st century, and 3) due to continuous and increasing combustion of fossil carbon, the amounts of greenhouse emissions (i.e. NOx, CO_2 and SOx) increase and cause global warming and climate change problems. Biofuel (fuel derived from biomass) has received considerable attention because it is a renewable, biodegradable and non-toxic fuel (Mutanda *et al.*, 2010). Biodiesel from microalgae is a promising renewable biofuel that has the potential to completely displace petroleum-derived transport fuel without adversely affecting the supply of food and other crop products (Chisti, 2008). In fact, algae are the highest yielding feed stock for biodiesel comparing to crop plants (Abomohra *et al.*, 2014). Oil yield of algae is 7 to 31 times greater than palm oil due to their ability to accumulate lipid and their very high actual photosynthetic yield (Li *et al.*, 2008; Abomohra *et al.*, 2014). Biodiesel yield depends strongly upon the lipid content of the algal strain and its growth rate. For biodiesel production, the economic biomass production of microalgal has to be taken into consideration, using of microalgal species with high lipid content and high cell growth (Lv *et al.*, 2010). Abomohra *et al.* (2013) reported that there are two categories of microalgae that used for lipid production, 1) high lipid content (43%) with low growth rate (30 mg L^{-1} d^{-1}) , such as *Botryococcus braunii* and 2) low lipid content (15%) with high growth rate (250 mg L^{-1} day) such as *Scenedesmus obliquus.*

Several studies have shown that the quantity and quality of lipids within the cell, and biomass production can vary as a result of changes in growth conditions; light intensity, temperature, CO_2 concentrations, nitrogen deficiency, phosphate limitation, silicon deficiency and iron supplementation (Tzovenis *et al.*, 2003; Ho *et al.*, 2010; El-Sheekh *et al.*, 2013; Liu *et al.*, 2008; Griffiths and Harrison, 2009; Kumar *et al*., 2014). Among the factors mentioned above, temperature is a sensitive limiting factor for microalgal growth and metabolic activities. Moreover, it is an easy-control factor in the practical operation of microalgae cultivation. Most of the previous studies have been investigated the effect of those factors on cell growth and/or lipid production of various microalgal species separately. Therefore, to get better understanding of the relationship between cell growth and lipid accumulation, they have to be measured as biomass and lipid productivities.

In this study, the effect of different incubation temperatures on the batch culture of the promising biodiesel microalga *Scenedesmus obliquus* was carried out. The variation methods of fatty acids measurement were applied, including fatty acid content and fatty acid productivity. The influence of incubation temperature on cell growth, biomass production, fatty acids content and fatty acid productivity were also assessed.

MATERIALS AND METHODS

Alga Strain and Growth Conditions

Scenedesmus obliquus was isolated from waste water at Tanta city, Egypt, and was cultivated axenically in 1 L Erlenmeyer flasks with 700 ml KC medium (Kessler and Czygan, 1970) at an initial OD_{680} of 0.05. Sterile filtered air enriched with 3% CO_2 (v/v) was continuously applied to the cultures. Cultures were illuminated by tubular fluorescent lamps (PHILIPS Master TL-D 85 W / 840). The light intensity at the surface of the culturing vessels was 130±10 µmol photons m^{-2} s^{-1} with a photoperiod of 14:10 h light: dark at different incubation temperatures (15, 20, 25, 30, 35 and 40 ˚C). OD_{680} was measured every other day; dry weight and esterified fatty acids (EFA) were measured after 22 days of incubation. Biomass productivity and fatty acids productivity were calculated.

Biomass Assay

Algal growth was monitored using the optical density of the culture at 680 nm (OD_{680}) and by determination of algal cellular dry weight (CDW). Biomass productivity was calculated as according to Abomohra *et al.* (2013)

$$\text{Biomass productivity}\left(\text{g CDW L}^{-1}\text{d}^{-1}\right) = (\text{CDW}_L - \text{CDW}_E)\cdot(t_L - t_E)^{-1}$$

Where, CDW_E representing the CDW ($g\ L^{-1}$) at days of early exponential phase (t_E) and CDW_L at days of late exponential phase (t_L).

Lipid Extraction

To analyse the cellular fatty acids composition, 5 ml aliquots of each culture was collected at times specified. Lipids were extracted following the method of Bligh and Dyer (1959). Prior to extraction, trinonadecanoylglycerol was added to the samples as internal standard esterified fatty acids.

Fatty acids profiles

Esterified fatty acids (EFA) from the extract of intracellular lipids were subjected to transmethylation for GC analysis as described previously (Kaczmarzyk and Fulda 2010; Scharnewski *et al.* 2008). The fatty acids methyl esters were subjected to analysis by GC/FID. The GC analysis was performed with a Varian 3900 GC-system equipped with a capillary column (Select Fame, 50 m x 0.25 mm; Varian). Fatty acids content (mg g^{-1} CDW) was calculated; in addition, fatty acids productivity was calculated according to Abomohra *et al.* (2013).

$$\text{Fatty acid productivity } (mg\ L^{-1} d^{-1}) = (FA_L - FA_E) \cdot (t_L - t_E)^{-1}$$

Where, FA_E and FA_L representing the total fatty acids content ($mg\ L^{-1}$) at days of early exponential phase (t_E) and late exponential phase (t_L).

Statistical analysis

Results are presented as mean ± standard deviation (SD) from four replicates. The statistical analyses were carried out using SAS (v 6.12). Data obtained were analyzed statistically to determine the degree of significance using one way analysis of variance (ANOVA) and paired-samples t-test at probability level $p \leq 0.05$.

RESULTS

Effect of incubation temperature on the growth rate of *Scenedesmus* sp. (Photo 1) was examined based on cell density (measured as optical density) and cellular dry weight (biomass productivity). Figure (1) shows the effect of different temperatures on the growth of *Scenedesmus obliquus* for 28 day of incubation. At late exponential phase (after 22 day), incubation at 30 ˚C showed the maximum growth, while the lowest growth was recorded at 15˚C. The reduction of growth was less pronounced at 40 ˚C which showed 24 % lower optical density than that obtained at 30˚C. Biomass productivity showed the same pattern, the lowest significant (one way ANOVA, $p \leq 0.05$) biomass productivity (0.282 g L^{-1} d^{-1}) was recorded at 15˚C (Fig. 2), while the highest biomass productivity (0.423 g L^{-1} d^{-1}) was recorded at 30˚C which showed insignificant difference with that at 25˚C. Incubation at 40˚C showed significant reduction in biomass productivity by 18 % than 30˚C (Fig. 2).

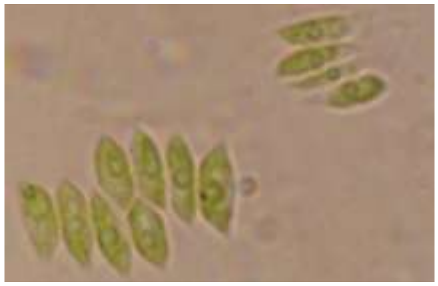

Photo 1. *Scenedesmus obliquus* isolated from wastewater.

The fatty acids content per microalgal biomass (mg g^{-1} CDW) of *Scenedesmus obliquus* after 22 day of cultivation at different incubation temperatures are shown in Figure (3). At 30 ˚C, which showed the maximum growth, the fatty acids content was 94.90 mg g^{-1} CDW. At lower temperature (15˚C), the fatty acids content was 104.12 mg g^{-1} CDW, significantly (paired-samples t-test, $p \leq 0.05$) higher than the one at 30˚C by 10 %, respectively. At higher temperature of 40 ˚C, the fatty acids content significantly decreased to 86.34 mg g^{-1} CDW.

Esterified fatty acids (EFA) productivity of *Scenedesmus* sp. at different cultivation temperatures is shown in Figure (4). Due to the high microalgal biomass, the highest EFA productivity was recorded at 30 ˚C (42.10 mg L^{-1} d^{-1}) which showed insignificant difference with that at 25˚C. EFA productivity (~ 30 mg L^{-1} d^{-1}) was almost the same at 15 and 40˚C , while at 20 and 35 ˚C (~ 35 mg L^{-1} d^{-1}) which were about 29 and 17 %, respectively, lower than the EFA productivity at 30˚C. Therefore, the optimal cultivation temperature to produce microalgal biomass (*Scenedesmus* sp.*)* and fatty acids for biodiesel production was ranged from 25 to 30 ˚C.

Fatty acid composition of *Scenedesmus* sp. at different cultivation temperatures is shown in Table 1. The ratio of the sum of saturated and monounsaturated fatty acids increased with the increase of cultivation temperature; while at the same conditions, polyunsaturated fatty acids decreased. At relatively low temperature (15°C), 35% of total fatty acids of *Scenedesmus* sp. was almost composed of polyunsaturated fatty acid (C18:3n-3). While at high temperature (40°C), the fatty acids were mainly composed of the monounsaturated fatty acids (C18:1n-9c) and saturated fatty acid (C16:0) with 28 and 21 %, respectively.

DISCUSSION

Microalgae are one of potential source for biodiesel production due to high efficiency of solar energy conversion to chemical energy. The impact of nutrient depletion and physical stress was examined on different algae for enhancement of lipid production. However, Francisco *et al.* (2010) and Abomohra *et al.* (2013) reported that biomass productivity and lipid content are inversely related. Therefore, for biodiesel production, the economic feasibility of microalgal mass culture has to be taken into consideration. The selection of microalgal species with high lipid contents and high cell growth is a great importance. In the present study, the cell growth and consequently biomass productivity of *Scenedesmus obliquus* were closely related with cultivation temperature. The highest biomass productivity was achieved at 25-30 °C, while the biomass productivity was decreased constantly by increasing temperature over 30 °C. Previous studies by Westerhoff *et al.* (2010) reported that the optimal cultivation temperature for the *Nannochloropsis oculata* was 20 °C. However, they concluded that the exponential growth rate constant did not vary between 27 and 39°C of *Scenedesmus* and *Chlorella*, but at 42°C the microalgae could not grow.

Temperature is a sensitive parameter to microalgal growth and their metabolic activities including lipid biosynthesis. In the coupled system of wastewater treatment and biodiesel production by microalgae, increasing of lipid productivity via temperature adjustment has an advantage that the composition of wastewater does not need to be changed or primarily chemically treated, making it suitable for processing and ecologically safe (Xin *et al*., 2011). Chen *et al.* (2008) reported that the cultivation temperature had little effect on the lipid content of microalgal biomass, but the triglycerides (TAGs) contents decreased with the decrease of temperature. However, Converti *et al.* (2009) reported that high temperature enhanced the lipid accumulation in *Nostoc oculata*. In our study, the effect of temperature on the lipid content of *Scenedesmus* sp. was in agreement with the findings of Chen *et al.* (2008). In the present study, low temperature would induce the fatty acid accumulation in *Scenedesmus obliquus* cells and results in high lipid content. From the economic point of view, enhancement of lipid productivity, which is related to the growth, has more feasibility than lipid content. Li *et al.* (2010) results showed high lipid contents of *Scenedesmus* sp. under stress conditions; however the lipid productivity was not high due to low growth rate and consequently low microalgal biomass productivity.

The saturation and chain length of fatty acids would affect the properties of biodiesel. Hu *et al.* (2008) mentioned that saturated fats produce a biodiesel with higher oxidative stability and acetane number, but rather poor low-temperature properties. In contrast, biodiesel feedstock rich in polyunsaturated fatty acids produce a biodiesel with good cold-flow properties, but susceptible to oxidation. In our study, at relatively low cultivation temperature the fatty acids of *Scenedesmus obliquus* were mainly composed of polyunsaturated fatty acids, while at high cultivation temperature the fatty acids are mainly composed of saturated and monounsaturated fatty acids. The fatty acid profile of *Scenedesmus obliquus* at different incubation temperature makes the microalgal cells adaptable to the ambient temperature. In addition, it makes the biodiesel from microalgae suitable to be used in cold or warm area depending on the cultivation temperature under which the feedstock are obtained.

In conclusion, the variation of temperature showed minor effect on lipid content of *Scenedesmus* sp., while strongly influenced the lipid productivity due to the synchronous effect on algal biomass production. The optimum temperature for maximum lipid production was (25-30 °C). The degree of saturation of fatty acids increased by increasing temperature, which gives advantage for biodiesel properties from *Scenedesmus obliquus* to be used at different climates.

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تأثير درجات الحرارة على النمو وبروفيل الاحماض الدهنية للطحلب سينيديزمس اوبليكس الواعد فى انتاج وقود الديزل الحيوى

عبد الفتاح ابومهره[1] ، مصطفى الشيخ[1] ، متولى عبد العظيم[1] ، رضا ابوشنب[2]
1 - قسم النبات-كلية العلوم- جامعة طنطا
2 - معهد الهندسة الوراثسة وبحوث البيوتكنولوجى – مدينة البحوث العلمية وتطبيقاتها التكنولوجية-مدينة برج العرب- الاسكندرية

المستخلص

تناولت هذه الدراسة تأثير درجات الحرارة المختلفه (15، 20، 25، 30، 35، 40 °م) على الكتلة الحية، محتوى وانتاجية الأحماض الدهنية لطحلب سينيديزمس الواعد فى انتاج وقود الديزل الحيوى. أوضحت النتائج ان زراعة الطحلب في درجة حراره 30 °م يعطى أعلى معدل للنمو، بينما لم يلاحظ اختلاف فعال في إنتاجية الكتلة الحية عند درجات الحراره 25 ، 30 °م (0.411 و 0.423 جم/لتر، على الترتيب). تم تسجيل أعلى محتوى للأحماض الدهنية (104.1 ملجم/جم من الكتله الحيه) عند 15 °م، والتي انخفضت بزيادة درجة الحرارة. كما لوحظ أنه نتيجة لزيادة إنتاج الكتلة الحية زادت إنتاجية الأحماض الدهنية لأعلى مستوياتها عند درجات الحرارة 25 و 30 °م (41.27 و 42.10 ملجم/لتر.يوم على الترتيب). وفيما يتعلق بأنواع الأحماض الدهنية، تم تسجيل زيادة الأحماض الدهنية المشبعه والغير مشبعة الأحادية، في حين انخفضت الأحماض الدهنية غير المشبعة العديده مع زيادة درجة الحراره. حيث كانت زيادة نسبة الأحماض الدهنية المشبعة وغير المشبعة الأحادية من 13.72 ٪ الى 23.79 ٪ ومن 11.13 ٪ الى 33.10 ٪ من مجموع الأحماض الدهنية بزيادة درجة الحرارة من 15 °م إلى 40 ° م على الترتيب. في حين انخفضت الأحماض الدهنية غير المشبعة العديده من 75.15 ٪ إلى 43.10 ٪ من مجموع الأحماض الدهنية عند درجة حرارة 15 و 40 °م على الترتيب. خلصت الدراسة الحالية إلى أن درجة حرارة النمو عامل مؤثر فى انتاجية ونوعية الأحماض الدهنية لطحلب السينيديزمس. بالإضافة إلى ذلك، نوع ونسبة الأحماض الدهنية ، التي تؤثر فى جودة وقود الديزل الحيوي، يمكن تغيير نسبتها فى نفس الطحلب بتغيير درجة حرارة النمو لانتاج وقود ديزل حيوى مطابق للمواصفات العالميه.