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Cultivation of Local Fresh Water Microalgae in Closed Systems

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Abstract

Alternative renewable energy is considered the optimal solution to solve the global energy crisis. Biofuel is one of the promising alternatives; especially that are produced from microalgae. Microalgae have the potential to produce 5000 – 15000 gallons biodiesel/ (acre-year). However, there are challenges; these include high yield of biomass and high lipid content. In this paper the authors studied the concentration of inoculum and the light penetration as a part of the parameters affecting the kinetics of the cultivation process of local strains of microalgae (*Spirulina platensis, Scenedesmus obliquus,* and *Nannochloropsis sp.)* that isolated from the Nile to obtain the maximum amount of oil, applying the factors affecting algal growth as light intensity, mixing, temperature and aeration for optimal design of photo-bioreactor. The achieved productivity was 0.54 g/l of algae biomass in 13 days with doubling time 2.8 days and specific growth rate 0.25 d^{-1}. Optimizing the culture dense and the light penetration distance; the light was not a limiting parameter for inoculum chlorophyll concentration up to 1500 µg/l.

Key word:s *growth rate, light penetration, Photo-bioreactor, Scenedesmus obliquus***.**

1. Introduction

One of Egypt's challenges is to satisfy increasing domestic demand for energy in the midst of falling domestic production. Egypt produced 34.5 million tons of fossil fuel in 2014 representing only 0.8% of total world production whereas the local consumption was 35.7 million tons sharing the world consumption by 0.9% [1]. Compared to the situation in 2002, the local production was 37 million tons while the consumption was 25.2 million tons [2]. These statistics foretell the local energy crisis and emphasize the importance of alternative renewable energy not only to cover our demands but also to decrease the Greenhouse Gases (GHG) emissions.

The energy sector is the main source of GHG emissions. Egypt is 92% dependent on fossil fuel either oil or natural gas [3]. The local emissions in 2010 were in the range of 275 metric ton carbon dioxide equivalent contributing 0.6% of the world emissions [4]. This makes sense that renewable fuels are necessary for environmental and economic sustainability.

Biofuel appear to be an attractive alternative source of energy compared with other forms of renewable energy as wind energy, since biofuel allow energy to be chemically stored and can be used in existing engines and transportation infrastructures after blending to various degrees with petroleum diesel [5]. The United States Environmental Production Agency EPA [6] reported that emissions varied with the biofuel source either the first generation from edible crops as soybean or the second generation from non-edible crops as rape seed. Even microalgae which represent the third generation reduce the particulate emissions by 47%, carbon dioxide by 48%, and hydrocarbons by 67%. In spite of increasing of nitrogen oxides by 10%, the lack of sulfur in biofuel allows nitrogen oxides control technologies that cannot be used with conventional engines [7].

Microalgae may be considered as a promising source of biofuel in Egypt due to the nature of these organisms, the sunny climate that increase the availability of capturing light, the moderate temperature and no need to fertile land. Nevertheless microalgae species contain oil up to 75% of their weight, they can be cultivated in closed systems, open systems and natural lakes [8].

Successful algal biofuel production depends mainly on choosing the suitable species with relevant properties such as biomass and oil productivity [9], [10]. A high content of the desired product increases the process yield and reduces the cost of extraction and purification per unit product [11], [12].

This research paper is studying the concentration and the light penetration as a part of the parameters affecting the kinetics of the cultivation process of local strains of microalgae to obtain the maximum amount of oil, applying the factors affecting algal growth as light intensity, mixing, temperature and aeration.

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2. Materials and Methods

2.1 Algal source and cultivation methodology

In this research, three strains of fresh water microalgae were isolated from River Nile at Water Pollution Department, Environmental Research Division, NRC; Cairo. The strains *Spirulina platensis, Scenedesmus obliquus,* and *Nannochloropsis sp.* were cultivated in 100 ml Erlenmeyer flasks at room temperature for a week illuminated by tubular fluorescent with photo-period 24 hours and photosynthetic photon flux 33.8 µmole/µEinstein. These flasks were subjected on a shaker to insure proper mixing. This sub-cultivation is important to prepare healthy inoculum for scaling up cultivation avoiding any lag phase.

The medium of nutrients and vitamins used for cultivation were modified of BG11 with composition illustrated in table (1) [13]. The three species of microalgae were scaled up in 250 ml flasks for two weeks at 33.8 µmole/µEinstein and photoperiod 24 hours with same initial biomass content. After two weeks microalgae were harvested by settling and decantation, and then dried over nigh at 80°C, weighed and grinded to be prepared for extraction process.

Table 1: Composition of used medium

Nutrient Solution	Stock Weight (g)/500 ml	Amount of stock added (ml_{stock}/L)
$K_2HPO_4.H_2O$	9.7	0.64
$NaNO_3$	150	1.67
$MgSO_4.7H_2O$	37.5	0.66
$CaCl_2.6H_2O$	27	0.36
$NaHCO_3$	2.5	20
NaFeEDTA	3.3	1

2.2 Oil Extraction

A method modified of Floch and Blight [14] stated that homogenizing one gram of dry algal cells with 20 ml of chloroform/methanol solvent (1:1 V/V) at 800 rpm for 5 minutes rupture the cell wall and rapid extraction. The mixture is subjected to a magnetic stirring at 40°C for two hours before filtration. The filtrate is washed by distillate water and oil is separated by separating funnel followed by Rota-vapour. The combination of polar and non-polar solvents enhances the extraction of both polar and non-polar lipids [15].

2.3 Selecting the most promising strain

Comparing the oil and biomass content of the three strains, the most suitable strain for oil production was selected.

2.4 Growth conditions

The selected strain was cultivated in a Fermenter Type photo-bioreactor made of Plexiglas at ambient temperature (25-30°C) and pH (8-10). Air stream of flow rate 0.5 v/v was applied from the bottom of the photo-bioreactor while the culture was illuminated by fluorescent lamps with photo-period 24 hours and photosynthetic photon flux 33.8 µmole/µEinstein. Applying these conditions on two photo-bioreactors with active volume 11 L and 17 L but with same volume to surface ratio; the growth cultivation was followed-up by measuring the chlorophyll content and the cellular dry weight. The growth culture was also monitored by microscopic investigation using Olympus microscope, and by measuring the optical density at 680 nm using Jenway model 6310 spectrophotometer as a measure of the culture turbidity. The pH of culture was measure using Jenway model 350 pH meter, since the strains followed photoautotrophic and mixotrophic mechanism release OH- [16] according to the following reactions:

Reaction 1: Photoautotrophic mechanism

$$H_2O + HCO_3^- \rightarrow C\ (biomass) + \frac{1}{2}\ O_2 + 3\ OH^-$$

Reaction 2: Mixotrophic mechanism

$$a\ HCO_3^- + b\ CH_2O \rightarrow c\ C\ (biomass) + d\ CO_2 + 3\ OH^-$$

2.5 Chlorophyll measurement

Chlorophyll concentration was measured periodically using methanol extraction method [17] where 1ml MgCO3 was added to 10 ml of aquatic algal sample, and then centrifugation takes place for 10 minutes at 2000 rpm. After that the chlorophyll in the sediments are extracted in water bath using methanol then centrifuged for 10 minutes at 2000 rpm and the chlorophyll is measured at wave length 664 nm, 647 nm, and 630 nm. The chlorophyll concentration [18] was calculated as:

$$\text{Chlorophyll concentration } (\mu g/l) = (11.85\ R_{664} - 1.54\ R_{647} - 0.08\ R_{630}) \times \frac{methanol volume(ml)}{sample volume(ml)} \times 1000. \quad (1)$$

2.6 Cellular dry weight

Cellular dry weight (CDW) was measured after overnight drying at 80°C, and the biomass productivity was calculated as:

$$\text{Biomass productivity } (g\ CDW\ L^{-1}\ d^{-1}) = (CDW_L - CDW_E) \times (t_L - t_E)^{-1}. \quad (2)$$

Where CDWE represents the CDW (g L-1) at days of early exponential phase (tE) and CDWL represents the CDW at days of late exponential phase (tL) [19].

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3. Results and Discussion

3.1 Strain selection

The selection of the most promising strain for biofuel production is based on the extracted oil content. Strain cultivation and oil extraction methods were discussed previously. The results revealed that *Scenedismus obliquus* has the highest oil content of the three strains fig (1).

This result is consent with the fatty acid content of each strain. The most valuable carbon chains in biofuel production vary between C16 to C20, *Scenedismus obliquus* contain 38.78% of palmilic acid C16:0, 28.6% of oleic acid C18:1, and 13.2% of alpha-linolenic acid C18:3n-3 [20]. While *Nannochloropsis* sp. contains 14.39% of C16:0, 5.59% of C18:1, 1.88% of C18:3n-3 [21] and *Spirulina platensis* contains 30.6%of C16:0, 1.7% of C18:1[22]. Therfore; *Scenedismus obliquus* is the most suitable strain for biofuel production containing the highest oil content of saturated and mono saturated fatty acid.

3.2 Effect of seeding concentration

The selected strain *Scenedesmus obliquus* was cultivated in 11L and 17L photo-bioreactor at same surface to volume ratio and same conditions of temperature, pH and luminance. The growth curves are plotted in fig (2), the chlorophyll content of broth solution is plotted versus time as semi-log graph. It shows that the chlorophyll content increases exponentially.

This strain that cultivated in both 11L and 17L was studied where initial seeding was 360 µg/l (inoculums concentration 0.024 g/l) and the cultivated microalgae in 11L when initial seeding was 1500 µg/l, also the cultivated strain in 17L when initial seeding was 4000 µg/l. Based on these measurements and assuming that one nutrient is limiting the growth, it was possible to determine the parameters of the Monod Equation [23]:

$$\mu = \mu_{\max} * \frac{S}{K + S} \qquad (3)$$

Where µ is the specific growth rate of cells, µmax is the maximum specific growth rate for the algae, S is the concentration of the limiting nutrient, and K is the half saturation coefficient. No lag phase is evident since the strain was sub-cultivated in 250 ml flasks at the same growth conditions and the cells use the lag phase to adapt to their new environment. The Monod equation was applied during the exponential growth phase. Cell growth rates are often expressed in terms of the doubling time (Td) or the time of generation which can be calculated [23] from the specific growth rate.

$$T_d = \frac{\ln 2}{\mu_{\max}} \qquad (4)$$

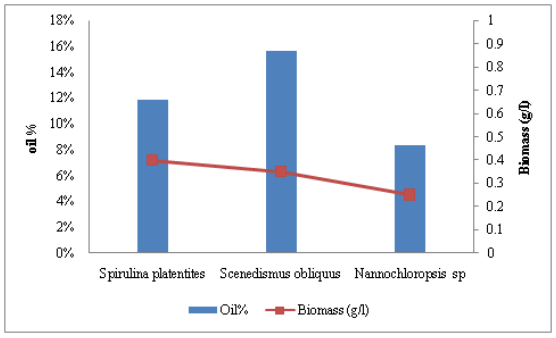

Fig (1) Lipid content of microalgae strains

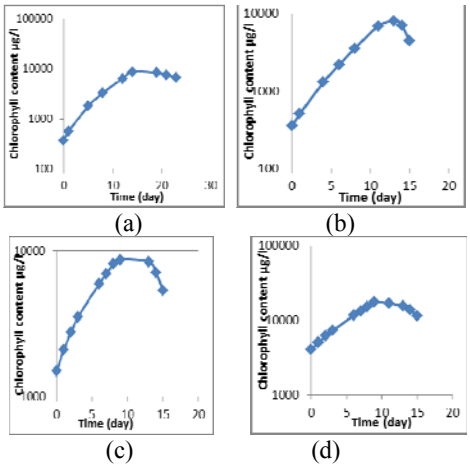

Fig (2) Growth curve of scenedesmus obliquus (a)cultivated in 11L with initial seeding 360 µg/l, (b) cultivated in 17L with initial seeding 360 µg/l, (c) cultivated in 11L with initial seeding 1500 µg/l, (d) cultivated in 17L with initial seeding 4000 µg/l.

The maximum specific growth rate of algae, the doubling time, the duration of the exponential phase, and the cell dry weight are summarized in table (2).

It was observed that the cultivation of microalgae using initial chlorophyll content 360µg/l in 11L photobioreactor decreases the doubling time which represents the growth rate to be 3.1 days, producing high final biomass (0.578 g/l) with proper cell shape. While increasing the initial seeding to 1500 µg/l increases the doubling time to be 3.5 days with approximately same final biomass; therefor it is preferred to cultivate microalgae with low initial seeding. However scaling-up the active volume with same surface to volume ratio, and same initial seeding; decreases the doubling time to 2.8 days with 6.9% final biomass decreasing.

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It is believed that the doubling time decreasing is a good indication to the proper growth rate, while the decrease of final biomass may be referred to the loss in harvesting steps as filtration and drying. In spite of increasing the initial seeding to more than 1500 µg/l increases the final biomass during low cultivation period; this have high doubling time which means that improper growing.

Table 2: some of the kinetic parameters of Scenedismus obliquus

volume (L)	Initial chlorophyll content (µg/l)	Initial cell concentration (g/l)	Growth phase duration (day)	$\mu_{max}(d^{-1})$	T_d(day)	CDW(g/l d^{-1})	Final cell concentration (g/l)
11 L	360	0.024	14	0.22	3.1	0.04	0.58
11L	1500	0.101	9	0.2	3.5	0.06	0.58
17L	360	0.024	13	0.25	2.8	0.04	0.54
17 L	4000	0.268	9	0.16	4.4	0.1	1.16

3.3 Light effect on algal growth in photobioreactor

The light system was arranged around the photobioreactor to insure proper light distribution. The penetration distance was evaluated using the following empirical formula [24].

$$d_p = \frac{6000}{C} \quad (5)$$

where dp is the light penetration distance (in cm) and C is the algal biomass concentration (in mg/l). The light penetration for photobioreactor with an algal biomass concentration 0.58 g/l or 580 mg/l is 10.34 cm, while for biomass concentration 0.54 g/l the penetration distance is 11 cm. The radius of the photobioreactor was 10 cm; therefore, light was not a limited factor for initial seeding lower 1500 µg/l. Nevertheless the suitable dimensions, the mixing system insure adequate cell share of light. However for biomass concentration 1.16 g/l, the penetration distance is 5.17 cm; therefore, light was a limited factor for the high initial seeding and the design should be modified according to light intensity and light penetration.

The cultivation system itself affects the biomass productivity. It was noticeable that *Scenedesmus obliquus* grown in PBR have higher cell concentration than that grown in flasks with same conditions of light intensity and light duration. In general the aeration and agitation are important factors to gain healthy cells with high biomass concentration.

4. Conclusion

Microalgae, the new trend of biofuel production, have a wide taxonomy depends on the environment that reflect the structure of cells. However selecting certain strain depends on the purpose of production and the bio-refinery products if exist.

The selected strain in this paper exhibited the typical growth curve of other micro-organisms, the exponential, the stationary, and the lysis phases. While there was no lag phase since the inoculum was taken from the healthy exponentially growing culture under similar growth conditions. The duration of exponential phase depends upon the size of the inoculum and the growth rate. It was more effective to use low inoculum chlorophyll concentration about 360µg/l was used in this paper with agitation and enough aeration to promote gas exchange without cell rupture. Optimizing the culture dense and the light penetration distance is an essential factor in designing the photo-bioreactor dimensions. While the smaller surface to volume ratio, the high culture denes.

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References

[1] BP Statistical Review of World Energy. 63rd edition [Internet]. June 2014. Available from: http://www.bp.com/statisticalreview.
[2] BP Statistical Review of World Energy [Internet]. June 2013. Available from: http://www.bp.com/statisticalreview.
[3] Egypt Climate Change. Egyptian Environmental Affair Agency. Ministry of State for Environmental Affairs [Internet]. Available from: http://www.eeaa.gov.eg/ecc/climate
[4] Egypt GHG emissions, reduction strategy. Mckinsey and company modernization program. Industrial Modernization Programme [Internet]. Jan 2012. Available from: http://www.imc-egypt.org
[5] Biodiesel Emissions Overview. In: The Impact on California Air Quality [Internet]. 2004[cited 2012 Apr 16]. Available from: http://www.crimsonrenweable.com
[6] A comprehensive Analysis of Biodiesel Impacts on Exhaust Emissions-draft Technical Report. US EPA. October 2002; EPA420-P-02-011.

ISSN 2348 – 7968

[7] Green Naval Ships. Center of Electromechanics. The University of Texas at Austin [Internet]. [cited 2014 May 20]. Available from: http://www.utexas.edu.
[8] Chisti Y. Biodiesel from microalgae: research review paper. Biotechnol Adv. 2007; 25: 294-306.
[9] Menetrez M Y. An Overview of Algae Biofuel Production and Potential Environmental Impact. Environ Sci Technol. 2012; 46: 7073-7085.
[10] Demirbas A, Demirbas M F. Importance of algae oil as a source of biodiesel. Energ Convers Manage. 2011; 52: 163-170.
[11] Huesemann M H, Van Wagenen J, Miller T, Chavis A, Hobbs S, Crowe B. A Screening Model to Predict Microalgae Biomass Growth in Photobioreactors and Raceway Ponds. Biotechol Bioeng. 2013; 110(6): 1583-1594.
[12] Ahmed F, Li Y, Schenk P M. Algal Biorefinery: Sustaiable Production of Biofuels and Aquaculture Feed. Chapter 3 In: Gordon R, Seckbach J editors. The Science of Algal Fuels: Phycology, Geology, Biophotonics, Genomics and Nanotechnology. Dordrecht; New York: Springer; 2012.
[13] El-Ardy O, Abo El-Enin S, El-Semary N A, El-Diwani G. Microalgal Culture in Photobioreactor for Biodiesel Production: Case Studies from Egypt. Afinidad Lxix. 2021; 558: 137-143.
[14] Lee J Y, Yoo C, Jun S Y, Ahn C Y, Oh H M. Comparison of Several Methods for Effective Lipid Extraction from Microalgae. Bioresource Technol. 2010; 101: 575-577.
[15] Young G, Nippgen F, Titterbrandt S, Cooney M J. Lipid Extraction from Biomass Using Co-Solvent Mixtures of Ionic Liquids and Polar Covalent Molecules. Sep Purif Technol. 2010; 72(1): 118- 121.
[16] Chojnaka K, Marques-Rocha F J. Kinetic and Stoichiometric Relationships of The Energy and Carbon Metabolism in the culture of Microalgae. J Biotechnol. 2014; 3(1): 21-34.
[17] Fitzgerald J W, Thomas P, Tudball N, Witerburn P J. Food Ingredients from Algae. Trends Food Sci Technol. 1971; 19: 31- 39.
[18] Standard Methods for the Examination of Water and Waste Water. 20th edition. .APHA; 1998.
[19] Abomohra A, Wagner M, El-Sheekh M, Hanelt D. Lipid and total fatty acid productivity in photoautotrophic fresh water microalgae: Screening studies towards biodiesel production. J Appl Phychol. 2012; DOI 10.1007/s10811-012-9917-y.
[20] Makulla A. Fatty Acid Composition of *Scenedesmus obliquus*: Correlation to Dilution Rates. Limnologiga. 2000; 30: 162- 168.
[21] Roncarati A, Meluzzi A, Acciarri S, Tallarico N, Melotti P. Fatty Acid Composition of Different Microalgae Strains (*Nannochloropsis sp., Nannochloropsis oculata* (Droop) Hibberd, *Nannochloris atomus* Butcher and *Isochrysis sp.*) According to the Culture Phase and the Carbon Dioxide Concentration. 2004; 35(3): 401- 411.
[22] Palmegiano G P, Agradi E, Forneris G, Gai F, Gasco L, Rigamonti E, Sicuro B, Zoccarato I. Spirulina as a nutrient source in diets for growing sturgeon (*Acipenser baeri*). Aquaculture Research. 2005; 36:188- 195.
[23] Doran P M. Bioprocess Engineering Principles: Elsevier Science and Technology Books; 1995.
[24] Andersen A R. Algal Culturing Techniques: Elsevier Academic Press, Burlington, Massachusetts; 2005.

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