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Optimization of Biomass and Oil Production of *Scenedesmus obliquus* Grown in Photobioreactor

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Abstract

The aim of this study was to grow *Scenedesmus obliquus* using vertical photobioreactor (PBR) and examine the oil production capacity of *Scenedesmus obliquus* grown under nitrogen repletion and starvation conditions. The volume of PBR was 4000 liters as a demo unit with the facility of computerized controlled system. Three dilution rates (0.125%, 0.18%, and 0.25%) were applied. The results showed that, the maximum biomass achieved with the highest dilution rate (0.25%) was 43.2 g /m^2.day. This was detected when the dry weight was 2.1 g/L with consuming about 62.5 ≈ g/m^2/day of CO_2. The maximum oil content reached to 26%±0.23 after 29 days under nitrogen repletion conditions. However, under nitrogen starvation, the oil content was dramatically increased and reached to 41.9%±0.6 after 8 days. Fatty acids profile showed that, both saturated and unsaturated were detected. The major saturated fatty acids were palmitic (C:16:0) and stearic (C:18:0). The unsaturated fractions were detected as palmetoleic (C:16:1), oleic (C:18:1), linoleic (C:18:2) and linolenic acids (C:18:3). The fatty acids with four or more double ponds were not detected. Total saturated fatty acids represented 60.47% and 67.43% under nitrogen repletion and nitrogen starvation respectively. Therefore, nitrogen content not only affects oil content but also affects oil fractions.

Keywords: Photobioreactor; *Scenedesmus obliquus*; Biomass; Dilution rate; Oil enhancement

1. Introduction

Algae's varied nature, striking photosynthetic efficiency and metabolic plasticity, and capability to adapt and bloom in a range of different environments have made them ubiquitous across the earth, though they are most common in aquatic environments. Because of their hasty rate of adaptation to potentially challenging environments, algae are considered excellent feedstocks for future bioenergy production; algae-based biofuels and bio-product applications and their associated promises and challenges have been the issue of a number of recent studies (Lieve, 2017). Microalgal biomass widely used as energy source, such as biodiesel, bioethanol, bio-hydrogen and photosynthetic microbial fuel cell (El Mekawy *et al.*, 2014). Biomass of microalgae also has potential benefits in cleaning the environment, owing to their CO_2 sequestration capability (Singh *et al.*, 2012). It is estimated that, 1 kg of dry algal biomass utilizes

about 1.83 kg of CO_2.

Nitrogen considered as the most important nutrient component that affects lipid metabolism in algae. Lipids accumulation, particularly triacylglycerol (TAG), due to nitrogen deficiency has been investigated in different microalgal strains (Yeh and Chang, 2011). Nutrient content has a significant effect on growth of microalgae, lipid, cell division and fatty acids composition of microalgae (Sato *et al*., 2000; Guschina and Harwood, 2006). Under nitrogen stress the responses of different green microalgae revealed a significant rise in lipid production (Hu *et al*., 2008).

Several types of algae cultivation systems were used for growing algae such as, bubble columns, open ponds, flat-plate photo-bioreactors (PBRs) or tubular PBRs (Ugwu *et al*., 2007). On large-scale, PBRs showed the most promising systems due to the low of contamination risk, no CO_2 loss, and high biomass concentration (Pulz, 2001; Richmond, 2004). Growing of microalgae in PBRs permits utilize of biomass for several applications such as foods production. In biodiesel production process, the high cetane number is directly attributed to good performance characteristics of biofuel, referring to good ignition quality in an engine (Van 2009; Chinnasamy *et al*., 2007). The first step to develop an algal process is selecting the most suitable species with high fatty acids contents (Pulz and Gross, 2004; Hempel *et al*., 2012).

In addition, commercial applications of microalgae have been used for a wide range of functions including pharmaceutical, food industries, andcosmetics (Gupta *et al*., 2015; Cardozo *et al*., 2007).

The aim of the current work is to investigate effect of nitrogen repletion and starvation on the biomass and oil production capacity of *S. obliquus* grown in outdoor PBR.

2. Materials and Methods

2.1 Isolation, Purification and Identification of Scenedesmus obliquus

S. obliquus was isolated and identified according to El-Baz *et al*. (2015).

2.2 Inoculum preparation

Scenedesmus obliquus was cultivated in Bold's Basal media indoor in conical flasks containing 15 L. about 1000 liter was used as inoculums for the vertical photobioreactor.

2.3 Bold's Nutrient Composition

Macroelements, g/L:

Urea 0.3, K_2HPO_4 0.075, KH_2PO_4 0.175, $MgSO_4(7H_2O)$ 0.075, NaCl 0.025, $CaCl_2(2H_2O)$ 0.025

Microelements mg/L:

$ZnSO_4(7H_2O)$ 8.8, $MnCl_2(4H_2O)$ 0.44, MoO_3 0.071, $CuSO_4(5H_2O)$ 1.57, H_3BO_3 11.42, EDTA 50, KOH 31, $FeSO_4(7H_2O)$ 4.98, $Co(NO_3)_2.6H_2O$ 0.49, H_2SO_4 1 µl/ L.

2.4 Vertical photobioreactor

Cultivation and optimization of microalgae strains started at NRC using the (indoor outdoor) facilities. Two PBRs were established on lab scale with the total capacity of approximately 600L. The optimization of growth under outdoor conditions started using the demo PBR unit with the capacity of 4000 liters (Fig. 1) and Table 1.

This class of models is based on the assumption that, the micro-organisms growth

Table 1. Physical considerations of the vertical photobioreactor

Volume	4 m^3
Areal footprint	56 m^2
Height	3 m
Shape	Cylindrical
Mixing	Aeration
Electricals	aerating, control unit

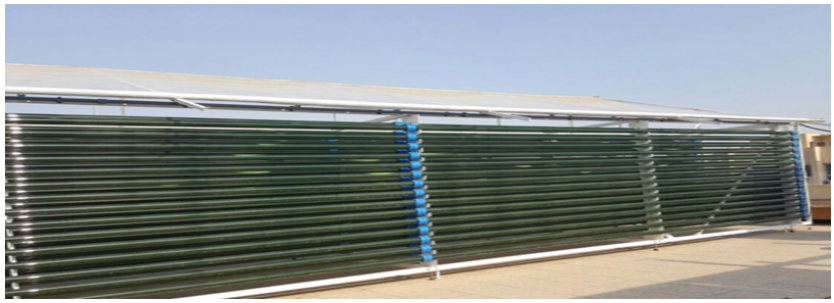

Figure 1. Vertical Photobioreactor 4000L

rate is proportional to their consumption rate of some extracellular limiting nutrient. Thus, these models are often referred to as constant yield models (Smith and Waltman, 1995).

The systems was run continuously as chemostat (dilution rate is kept constant). The photobioreactor was diluted with a daily fixed dilution rates. After four days, dilution rate was changed to the next dilution rate. The range of dilution rates was according to growth rates.

When the system was harvested, the level drops inside the stripper. This generates a "low level" signal by level sensors, water and concentrated stock solutions were then added.

2.5 Ground areal biomass productivity (Bosma et al., 2014)

$$P_{x.ground} = \frac{\{(Vharvest.Cxharvest) + VR\square\, Cx_{(t)} \square\, Cx_{(t\square 1)}\}}{A_{ground}}$$

Where: Px, ground: ground areal biomass productivity (g/m$^{2/}$d); V harvest: harvested volume (L); Cx, harvest: dry weight algal concentration in the harvest (g/L); VR: photobioreactor volume (L); A ground: occupied ground area photobioreactor (m^2); $Cx_{(t)}$, $Cx_{(0)}$: dry weight algal concentration in photobioreactor (g/L), on consecutive sampling points; t: time between consecutive sampling points; ±24 hours (Bosma *et al.*, 2014).

To insure the purity of the culture, samples were taken regularly and examined microscopically. The culture was left to grow until the biomass reached 1.5 g/L. Then a representative samples were taken to determine oil content at zero time and the other biomass was recultivated in the photobioreactor under N- stress condition (0.025 g/L urea).

2.6 Growth measurements

Algal biomass and chlorophyll a content

Cell growth was monitored by gravimetric determination of algal biomass dry weight (dw) with each determination made in duplicate every day. Aliquots of 100 mL algal suspension were filtered through 0.5 µ filter paper, dried at 105°C to a constant weight, cooled in a desiccator and weighed. The growth curves were determined from parallel cultures starting from the same inocula and were calculated from average algal biomass as function of time. Chlorophyll a content was monitored to evaluate the growth of the culture (Rice *et al.*, 2012).

2.7 Oil content (Bligh and Dyer, 1959).

The biomass of microalgae was dried and ground into homogenous fine powder. The dry cells were mixed with methanol-chloroform (1:1, v/v) as a co-solvent using homogenizer for 5 minutes at 800 rpm in a proportion of 1 gm in 75 ml of solvent mixture. The homogenate mixture was subjected to a magnetic stirrer at 30°C for 2 hrs. Cell residue was removed by filtering. The filtrate was transferred into a separating funnel and sufficient water was added to induce biphasic layering. After settling the solvent mixture was partitioned nto two distinct phases, top light green aqueous methanol layer containing most of the co-extracted non- lipids and bottom dark green chloroform layer containing most of the extracted lipids. The chloroform layer was collected in a pre-weighted flask and evaporated using a rotary evaporator.

2.8 Oil content was calculated as follows:

$$Oil\ content = \frac{Oil\ weight}{Dry\ cell\ weight} \times 100$$

2.9 Harvesting and drying

Algal biomass was harvested using basket centrifuge at 2000 rpm and dried at sun dryer where the temperature reached 45°C.

2.10 Fatty Acids profile Analysis

The fatty acid profile of the extracted oil sample of *S. obliquus* was determined by converting the fatty acids in the oil to Fatty acid methyl esters (FAMEs) according to the method described by El-Baz *et al.* (2015).

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.) . Helium was used as carrier gas at a flow rate of 1 ml/min. The column was held at 150 °C for 1 min and ramped to 240 °C at rate 30 °C/min, and it was then held at 240 °C for 30 min. Standards were used to give rise to well-individualized peaks that allow the identification of the fatty acid composition.

3 Results and discussion

3.1 Growth of the culture

The growth was monitored by measuring chlorophyll a content and dry weight (Fig. 2). The maximum chlorophyll was detected after 14 days where it reached 67,964 µg/L with a dry weight 2 g/L±0.1. The results presented in figure 2 showed that *S. obliquus* was an appropriate strain to be cultivated in PBR due to its fast growth. Results also indicated that, the nutrient media of Bold's basal is suitable for growing *S. obliquus* for mass production. These results were in harmony with those found by Al-Shatry *et al.* (2014) who stated that the Bolds basal medium (BBM) is the optimal suitable medium for culturing *Scenedismus* sp. due to the high productivity of biomass.

Referring to CO_2 consumption, the pH-control system attempted to reduce the pH by more frequently injecting the gas, the maximum injection rate was deliberately set low to prevent too much loss of CO_2. 62.5 ≈g/m^2/day of CO_2 was consumed. Asmare *et al.* (2013) stated that CO_2 may be consumed at a rate as high as 26 g CO_2/m^2 h which equal to 624 g/m^2/day.

3.2 Effect of dilution rate on biomass production

The cultivation systems can be operated batch wise as chemostat (constant dilution rate) and turbidostat (constant biomass concentration) (Bosma *et al.*, 2014). In the current study, chemostate was used at first to insure the biomass production then turbidosate was appliedto study the effect of different dilution rates on biomass production.

The dilution rate is an important factor which affects the biomass productivity rate, and ultimately what needs to be maximized. Thus, it is necessary to maximize the biomass concentration while maintaining a high rate of dilution. In Table 2, the highest recorded biomass production 43.2 g/m^2/d was achieved. This was detected when the dry weight was 2 g/L. Thus,

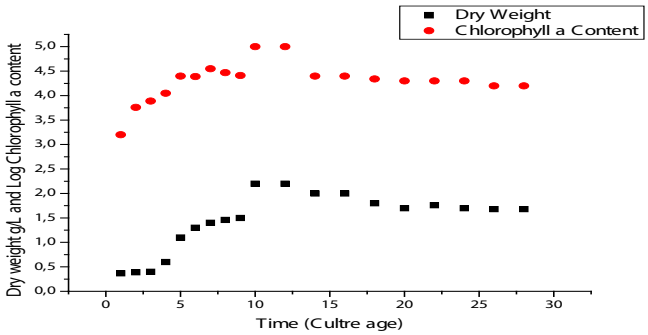

Figure 2. Chlorophyll a content and dry weight of *S. obliquus* grown in photobioreactor

Table 2. Dilution rates and ground areal biomass productivity of *S. obliquus* grown in PBR

Parameters	Biomass average
Dilution rate d^{-1}	0.125 (500 L)
Px.ground (g/m^2d)	10.2
Dilution rate d^{-1}	0.18 (750 L)
Px.ground ($g/m^{2/}d$)	39.6
Dilution rate d^{-1}	0.25 (1000 L)
Px.ground ($g/m^2/d^1$)	43.2

*Px, ground: ground areal biomass productivity

the higher the dilution rate the higher the light availability and the lower the photolimitation of the cultures.

Bosma *et al*. (2014) stated that high dilution rates will result in low biomass concentration and photo saturation of the culture will take place, on the other hand too high dilution rates can result in a washout of the culture. At decreasing dilution rates the biomass concentration will increase. While at too low dilution rates, biomass concentrations will become too high and this will lead to photo limitation of the culture. All these phenomena can result in a suboptimal operation of outdoor PBR.

On average of biomass produced in different countries, Sudhakar *et al*. (2012) estimated the annual average productivity of 75 g/m^2/day for India condition.

It was noted that the dilution rate should not be continued for a long time hence, the biomass decreased and the desired biomass not reached. So, it is advisable to monitor the growth daily in order to determine the appropriate dilution rate.

3.3 Oil content of S. obliquus grown under N-repletion conditions

The results given in Figure 3 showed that, oil content of *S. obliquus* at the beginning of growth was 15.2%±0.5. Growing *S. obliquus* for longer period oil content reached 26±0.2 (when the culture was 29^{th} days old) reached more than 71% increase in oil content. The oil content was monitored during the cultivation period and the results showed that, there were variations in the oil percentage; this may be due to the dilution rate and the increase in biomass concentration.

Table 3 showed GC analysis of the oil extracted from *S. obliquus* grown under N-repletion at zero point (15.2%) and at the maximum oil concentration (26%). The major saturated fatty acids was palmitic acid (C:16:0), which is one of the most important fractions in biodiesel production (17.35 and 31%) for both samples at zero point and maximum oil content, respectively. Stearic acid (C18:0) was also detected in a higher percentage (25.32%) in sample having the highest oil percentage, while it was 19.37% at the zero point sample. Unsaturated fatty acids were also found in varying concentrations. Palmetolic acid (C16:1) was detected in both samples with a percentage 7.75 and 8.02%. Linolenic acid was found in high concentration at zero point sample and reached to 40.28% compared to 2.8% in sample contained high oil content. Linoleic acid was found in high concentration 22.54% in sample containing maximum oil content. The results of the presented study indicated that fatty acids with four or more doublé ponds were not detected which cause theoxidation and reduce their acceptability for utilization in biodiesel (Yusuf, 2007).

Total saturated fatty acids reached 60.47% in simple containing máximum oil content which indicated that the oil properties fit to be used as a source for biofuel since the percentage of saturated fatty acids is higher tan unsaturated fatty acids.

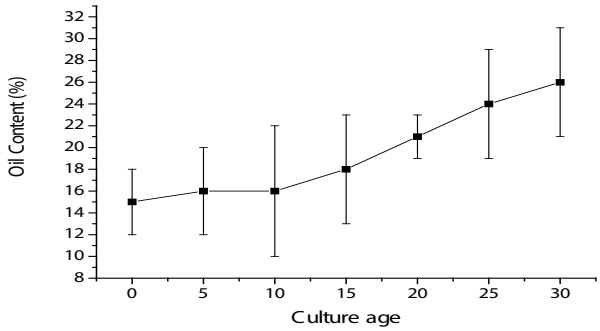

Figure 3. Oil content during cultivation period of *S. obliquus* grown under N-repletion condition

3.4 Oil enhancement under N starvation conditions

The results presented in figure 4 showed that oil percentage was 20.5% ±0.7 after inoculation representing zero point. After 8 days oil percentage increased to 41.9%±0.6. This increase may be due to n- depletion condition where the concentration of urea was 0.025 g/L. After that the oil percentage decreased and the biomass increased slightly, where at the beginning the dry weight was 1.2±0.13 g/L and reached to 2.1±0.21 g/L and chlorophyll content reached to 511,175 µg/L.

These results were confirmed with the findings of (Widjaja *et al*., 2009) they found that high oil production because of nitrogen stress may take 2–5 days and it is also complemented with slow growth rates and consequently low cell counts that finally affect the total biomass and oil productivity.

Rodolf *et al*. (2009), Gouvela and Olivera (2009) suggested that the reasons for the increase of oil content under nutrient stress conditions due to the rate of production of all cell compounds is lower but oil production remains high leading to accumulate the oil in cells. Additionally, they added that, enough biomass as inoculum should be produced under N-sufficient, before N-starvation of the culture for oil production followed by a second stage for efficient lipid accumulation under N-deprivation.

3.5 Fatty acids composition of S. obliquus grown under N- starvation

The fatty acids analysis of the oil produced from algal biomass under N- starvation was presented in table 4. algal oil samples were analyzed for their fatty acids compositions, the first one at zero point (20%±0.3 total oil content) and the second one which contained maximum oil production (41.9%±0.6 total oil content). From the results presented in table 4. The saturated fatty acids were identified as Myristic, Palmitic and stearic acids. On the other hand, the unsaturated fatty acids were also detected as Palmetoleic, Oleic, Linoleic and Linolenic acid. The chain length of all fatty acids was ranged from C14 to C18. In addition, palmitic acid (C:16:0), which is one of the most important fractions in biofuel production was detected in relatively high concentration (35.6%) in the sample contained maximum oil content (8 days old). Stearic acid (C:18:0) was also detected in high concentration 23.33%. These results were in harmony with that of (Sharma *et al*., 2012) who stated that, under stress conditions many species of microalgae modify their lipid biosynthetic pathways to the trend of formation and accumulation of neutral lipids (20–50% DCW), mainly in TAG form leading to make microalgae endure these stress conditions.

Comparing oil production potentials of *S. obliquus* under N- starvation and sufficient conditions, it is clear that approximately 42% oil was achieved after 8 days culture age under starvation conditions, compared to 26% oil content after 29 days old under N- repletion conditions. The same trend was found concerning fatty acids, Palmitic, Stearic and total saturated fatty acids.

Table 3. Fatty acids profile of green microalgae *S. obliquus* grown under N-repletion

Fatty acids	Common name	(0.0 time) 15.20%	(max.) 26%
C:14:0	Myristic	8.7	2.47
C:16:0	Palmitic	17.35	31
C:16:1	Palmetolic	7.75	8
Total C:16		25.1	39
C:17:0	Margaric acid	1.55	1.68
C:18:0	stearic acid	19.37	25.32
C:18:1	Oleic	2.5	6.17
C:18:2	Linoleic	2.5	22.54
C:18:3	Linolenic	40.28	2.8
Total C:18		64.65	56.83
Lipidprofile			
Total identifiedfattyacids (%)		100	100
Total saturated (T.S.)		46.97	60.47
Total unsaturated (T.U.)		53.03	39.53

4. Conclusion

Dilution rate is an important factor in growing micro algae in photobioreactor and affects positively the biomass production to 2 g/L. Under nitrogen repletion oil content reached 26%±0.23. Under nitrogen stress dry weight reached 2.1±0.21 g/L and total oil content of *S. obliquus* was increased from 26% to 41.9% within 8 days. Nitrogen depletion is preferred in growing *S. obliquus* since it enhances oil production.

Oil extracted from algae has high concentration of saturated fatty acids (67.43%), in addition fatty acids with four or more double ponds was not detected. This indicated that the algal oil possesses a favorable fatty acids profile and can be used for biodiesel production.

Under nitrogen repletion, Linolenic acid was found in high concentration at zero point sample reached to 40.28% compared to 2.8% in sample contained high oil content.

Under nitrogen starvation, Linoleicacid-percentage was decreased from 34.1% to 17.8%.

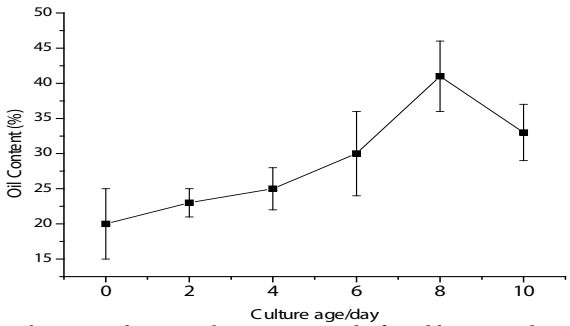

Figure 4. Oil content during cultivation period of *S. obliquus* under N-starvation

Table 4. Fatty acids profile of green microalgae *S. obliquus* grown under nitrogen starvation at different ages

Fatty acids	Common name	(0.0 time) 20%	8 days old 41.90%
C:14:0	Myristic	1.56	3.5
C:16:0	Palmitic	18.4	35.6
C:16:1	Palmetolic	7.3	8.2
C:17:0	Margaric acid	1	5
C:18:0	stearic acid	26.17	23.33
C:18:1	Oleic	10	6.5
C:18:2	Linoleic	34.1	17.8
C:18:3	Linolenic	1.4	-
Total Saturated (T.S.)		47.13	67.43
Total Unsaturated (T.U.)		52.87	32.57

This means the improvement of oil quality by decreasing the percentage of double ponds in FAME.

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