Microalgal culture in photo-bioreactor for biodiesel production: case studies from Egypt

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Ejemplos en Egipto, de cultivo de microalgas en reactor foto-biológico para la producción de biodiesel

Exemples a Egipte, de cultiu de microalgues en reactor foto-biològic per a la producció de biodièsel

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RESUMEN

Se ha investigado la producción de biodiesel a partir de tres microalgas autóctonas de Egipto. Estas especies de microalgas difieren en su patrón de crecimiento, ya que mientras una de las cepas de cianobacterias es *Phormidium sp.*, filamentosa y forma alfombras, la otra cepa *Microcystis sp*. es cocoide y forma colonias, y la tercera, *Botrydiopsis sp.*, es un xantófito cocoide. El orden de producción de biomasa en un reactor foto-biológico utilizando un cultivo semi-continuo, según las cepas, fue: *Microcystis sp.* > *Botrydiopsis sp.* > *Phormidium sp.* La producción de biomasa puede ser aumentada, incrementando el periodo de iluminación en el caso de *Botrydiopsis sp.* y *Microcystis sp.* El contenido de lípidos se determinó utilizando distintos disolventes para extracción de lípidos. La *Botrydiopsis sp.* proporcionó el contenido de lípido más alto (48%) en el caso de *Botrydiopsis sp.* en medio de crecimiento *Oscillatoria*. *Microcystis sp.* presentó un 28% de contenido en lípido mientras que *Phormidium sp.* presentó el menor contenido en lípidos (15%). Los principales componentes del conjunto de ácidos grasos en las distintas especies de algas estudiadas fueron los ácidos linoléico, palmítico, oléico y esteárico. En conclusión, el cultivo de microalgas en reactor foto-biológico da como resultado una elevada producción de biomasa cuando se aplica una técnica semiautomática de alimentación. La producción de biomasa más elevada no implica un contenido de lípido más alto. El análisis por cromatografía de gases mostró que el aceite de algas tiene la composición adecuada de ácidos grasos para la producción de biodiesel.

Palabras clave: Biodiesel, *Botrydiopsis sp., Microcystis*, *Phormidium,* reactor foto-biológico.

SUMMARY

Biodiesel production from three local microalgae from Egypt was investigated. These microalgae strains differ in their growth pattern as one of the cyanobacterial strains is filamentous mat-forming *Phormidium* sp. whereas the other strain is coccoid colony-forming *Microcystis* sp. The third is coccoid yellow-green *Botrydiopsis* sp. The mass productivity for the strains in a photobioreactor using semi-continuous culture was arranged as: *Microcystis* sp.> *Botrydiopsis* sp.> *Phormidium* sp. The mass productivity can be increased by increasing the illumination period in case of *Botrydiopsis* sp. and *Microcystis* sp. The lipid content was determined by using different solvents for lipid extraction. The *Botrydiopsis* sp. gave the highest lipid content (48%) for *Botrydiopsis* sp. cultured in *Oscillatoria* medium. *Microcystis* sp. had (28%) lipid content while the *Phormidium* sp. had the lowest lipid content (15%). The major components of the fatty acid compositions in different algal species studied were linoleic, palmitic, oleic and stearic. In conclusion, the cultivation of microalgae in photo-bioreactor has given high biomass productivity by applying semi-continuous feeding technique. The highest mass productivity doesn't mean the highest lipid content. The Gas chromatography analysis showed that the algae oils have the suitable fatty acid composition for biodiesel production.

Keywords: Biodiesel, *Botrydiopsis* sp., *Microcystis*, Photobioreactor, *Phormidium*

RESUM

S'ha investigat la producció de bio-dièsel a partir de tres microalgues autòctones d'Egipte. Aquestes espècies de microalgas difereixen en el seu patró de creixement, ja que mentre una de les soques de cianobacteris és Phormidium sp., filamentosos i que formen catifes, l'altre soca Microcystis sp. Son cocs i formen colònies, i la tercera, Botrydiopsis sp., son xantòfits i cocs. L'ordre de producció de biomassa en un reactor foto-biològic utilitzant un cultiu semi-continu, segons les cepes, va ser: Microcystis sp. > Botrydiopsis sp. > Phormidium sp. La producció de biomassa pot ser augmentada, incrementant el període d'il-

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luminació en el cas de Botrydiopsis sp. i Microcystis sp. El contingut de lípids es va determinar utilitzant diferents dissolvents per a l'extracció de lípids. La Botrydiopsis sp. va proporcionar el contingut de lípid més alt (48%) en el cas de Botrydiopsis sp. enmig de creixement Oscillatoria. Microcystis sp. va presentar un 28% de contingut de lípids mentre que Phormidium sp. va presentar el contingut en lípids més petit (15%). Els principals components del conjunt d'àcids grassos en les diferents espècies d'algues estudiades van ser els àcids linolèic, palmític, olèico i esteàric. En conclusió, el cultiu de microalgas en reactor foto-biològic dóna com a resultat una elevada producció de biomassa, quan s'aplica una tècnica semiautomàtica d'alimentació. La producció de biomassa més elevada no implica un contingut de lípid més alt. L'anàlisi per cromatografia de gasos va mostrar que l'oli d'algues té la composició adequada d'àcids grassos per a la producció de bio-dièsel.

Paraules clau: Biodiesel, Botrydiopsis sp., Microcystis, Phormidium, reactor foto-biològic.

1. INTRODUCTION

The world is confronting an energy crisis due to the consumption of unrenewable resources of fossil fuel (Guan Hua Huang *et al*., 2010). Moreover, the continued use of petroleum-based fuels represents an extremely dangerous environmental hazard (Goldenberg, 2000). This is due to their contribution to air pollution through accumulation of carbon dioxide and some of the volatile toxic organic compounds (Goldenberg, 2000). Biodiesel production from biomass is one plausible alternative to fossil fuel (Guan Hua Huang *et al*., 2010). Biodiesel (Monoalkyl esters) is nontoxic and biodegradable fuel obtainable from soybean, sunflower oil, algal oil and can be prepared by the trans-esterification of triglyceride oil with monohydric alcohols (Rurkenburg, 2000 and Lang *et al*., 2002). Biodiesel production from microalgal biomass is particularly one of the most promising clean carbon-neutral sources of energy (Spolanore *et al*., 2006). The microalgae are microscopic photosynthetic microorganisms that use light energy and carbon dioxide, with a higher photosynthetic efficiency than plants for the production of biomass (Spolanore *et al*., 2006). Oil levels 20 –50 % are common in some microalgae and the cultivation of microalgae for the purpose of oil production does not require vast land plots or extensive labour on contrary to plants used for the same purpose (Chisti, 2007). Microalgae can transform carbon dioxide from the air and light energy through photosynthesis to organic matter as they are mainly photoautotrophic in their nutrition (Spolanore *et al*., 2006). This recycling of carbon dioxide and the release of oxygen through photosynthesis is of major importance to combat the increased levels of carbon dioxide that are linked to harmful global warming and green house effect. Microalgae can be grown in two systems such as open ponds and enclosed photobioreactors (Chen *et al*., 2009). Because of better environmental control, enclosed photo-bioreactor system has been favoured for culturing microalgae as growth conditions can be optimized without threats of contamination. In addition, photobioreactors have high rates of productivity (Guan Hua Huang *et al*., 2010). The quantity and quality of algae oil within the cell can vary as a result of changes in growth conditions (temperature and light intensity) or nutrient media characteristics (Lin *et al*., 2004). As the right choice of cultivation system and the optimisation of growth conditions are important for mass production of microalgae, the extraction methods for lipids from those algae for the purpose of biodiesel production are equally important (Porphy & Farid, 2011). An ideal lipid extraction process for microalgae biodiesel production needs to not only be lipid-specific in order to minimize the co-extraction of non-lipid contaminants but also selective towards desirable lipid fractions (neutral lipids containing mono-, di-, and tri-enoic fatty acid chains (Miao & Wu, 2006). Even though the classic Folch chloroform-based lipid extraction protocol is effective for the majority of microalgae lipid analyses (Xu et al., 2006) an alternative organic solvent method that is more user-friendly is needed for scale-up. Hexane, despite being reported to be less efficient than chloroform when extracting from microalgae, is less toxic, has minimal affinity towards non-lipid contaminants, and apparent higher selectivity towards neutral lipid fractions (Chen *et al*., 1996). Hexane, iso-propanol, butanol and various combinations have been investigated as less toxic substitutes for lipid extraction (Miao & Wu, 2006). In this study, a medium-sized closed photo-bioreactor has been used for producing microalgae biomass. The factors optimized here in an attempt to increase the lipid content in microalgae were illumination period and growth media for the microalgae tested. The lipid extracted from the three microalgae using different extraction methods and the lipid quantity and quality were estimated by gravimetric and gas chromatographic (GC) methods, respectively. The study investigates the potential of using photobioreactor associated with semi-continuous feeding system in obtaining the best growth and lipid productivity for the strains studied taken into consideration their difference in growth habits and illumination needs.

2. MATERIALS AND METHODS

2.1. Algal source and culture technique

Sampling and isolation procedures for the three microalgae strains are listed below but the characteristics and culture conditions are listed in Table 1.

(1) ***Phormidium* sp.** Water samples were collected from the benthos of a small alkaline (pH 9.5) water body called Lake El Baida, Wade El Natroun, Egypt. Single filaments

Table (1). *Microalgal strains, their characteristics and culture conditions.*

Strain	Characteristics	Optimum growth conditions
Phormidium sp.	Filamentous mat-forming blue-green alga	High temperatures (30 – 40 ºC) and an incident irradiance of 5-15 µmol photon m^{-2} s^{-1}
Microcystis sp.	Coccid colony-forming blue-green alga	Moderate temperature range of (20–30 °C) and an incident irradiance of 20–40 µmol photon m^{-2} s^{-1}
Botrydiopsis sp.	Green yellow coccoid alga	High temperature range (30 – 40 ºC) and an incident irradiance of 30–60 µmol photon m^{-2} s^{-1}

Table (2). *Fatty acid profile for Microcystis sp. and Botrydiopsis sp.*

Fatty Acid	*Microcystis* sp.	*Botrydiopsis* sp.
Caprice (C10)	0	0.45
Undecanoic(C11)	3.9	2.51
Lauric(C12)	1.7	5.47
Tridecanoic(C13)	3.3	0.65
Myristic(C14)	0	0.7
Palmitic(C16)	9.80	8.82
Palmitoleic(C16-1)	0	1.09
Heptadecanoic(C17)	0	0 .021
Stearic(C18)	0 .55	0.72
Oleic(C18-1)	7.99	9.83
Linoleic(C18-2)	34.66	23.31
Linolenic(C18-3)	0	1.45
Eicosadienoic(C20-2)	6.4	0
Archidonic(C20-4)	13.9	1.29
Erucic(C22-1)	17.8	0.37
Docosahexaenoic(C22-6)	0	1.01
Methyl Hexadecanoic(C26)	0	42.33

were picked under the microscope, washed through drops of sterile water and used to establish monospecific axenic cultures in *Oscillatoria* medium (Feuillade, 1994).

(2) *Microcystis* sp. Water samples (M1) were collected in triplicates, from the banks of water canal "Treat El Khashaab", Helwan area, in sterile containers. Samples were spun down, and the pellets were spread over solidified and liquid *Oscillatoria* medium (Feuillade, 1994).

***(3) Botrydiopsis* sp.** Water samples were collected in sterile containers from banks of The Nile, Maadi area. Samples were spun down, and the pellets were spread over solidified medium (1.5 % agarose, w/v of growth medium). Colonies were picked up, examined under microscope and used to establish axenic cultures. After the establishment of axenic cultures, the microalgae strains were subcultured in growth medium (200 mL), allowed to obtain equilibrium with their new environment at ambient temperature and then inoculated into photo-bioreactor vessel in *Oscillatoria* growth medium 1. Another growth medium; modified Bold's Basal medium (Nicholas, 1973) commonly-used for eukaryotic freshwater (M2) algal culture, was applied in the culture system of the *Botrydiopsis* strain.

2.2 Experimental culturing systems

The culturing systems involved two types (M2); batch and semi-continuous systems. In batch system, the growth medium was inoculated with the microalgal strain followed by daily determination of O.D., and continued until fixed O.D. was obtained. The semi-continuous photobioreactor used cylindrical Plexiglas photo-bioreactor with a working volume of 5 L provided with mechanical steering, pH-controller unit, aeration unit and mass flow meter. The semi-continuous process involved the withdrawal of a certain volume of the medium at a definite time, and feeding with the same volume fresh medium. This was repeated several times. The culture temperature was the ambient temperature (30 ± 5 ºC). Fluorescent lamps were arranged around the photo-bioreactor to supply illumination. The light intensity was measured with a digital luxmeter (7500D, HANNA Instrument, Romania, Europe) with the scale range of (0 -1999.9 lux).

2.3 Growth conditions

Algae cultures were maintained at ambient temperature (30 ± 5 ºC), pH media (6.5-7.5), air flow 0.5 volume air / volume culture and illuminated with 2400 lux in the photobioreactor. All cultures were kept at illumination period of 8 h/ 16 h, light/dark cycle. In addition, Each culture, other than *Phormidium* which was very sensitive to long illumination period, was kept under its own optimized illumination period(s) and growth medium to obtain the best growth possible,. The second strain (*Microcystis* sp.) was subjected to different illumination periods (8, 16 & 24 h), whereas *Botrydiopsis* sp. was kept under 8 hours light period in culture medium 2 and 24 h light period in culture medium 1.

2.4. Biomass determination

The *Microcystis* sp. and *Botrydiopsis* sp. strains were determined daily by optical density measurements as a function of biomass (illustrated in Tables 6 & 7) at 680 nm as by a spectrophotometer model Jenwey 6310. Each sample was shaken for 10 s then measured in triplicates and the average was computed. For *Phormidium* sp. and due to its mat-forming growth pattern, the dry weight as a function of biomass was determined gravimetrically.

2.5 Lipid extraction

The total lipid was extracted by one of the following methods:

Soxhlet extraction (Converti et al., 2009) using hexane as a solvent. In this method, the sample is dried, ground into small particles and placed in a porous cellulose thimble. The thimble is placed in an extraction chamber which is suspended above a flask containing the solvent and below a condenser. The flask is heated in a water bath at the evaporation temperature of the solvent and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber. At the end of the extraction process, which lasts a few hours, (4-6 h), the solvent in the flask is then evap-

Table (3). *Fatty acid profile for various plants-producing oil & microalgae.*

Fatty Acid	Canola	Rapeseed	Soybean	Jatropha	*Microcystis sp.*	*Botrydiopsis sp.*
Myristic C14	0	0	0	0	0	0.7
Palmitic C16	4	2.2	9.9	18.22	9.8	8.82
Stearic C18	2.4	0.9	3.6	5.14	0.55	0.72
Oleic C18-1	65.0	12.6	19.1	28.46	7.99	9.83
Linoleic C18-2	17.3	12.1	55.6	48.18	34.66	23.31
Linolenic C18-3	7.8	8	10.2	0	0	1.45
Eicosenoic C20- 1	1.3	7.4	0.2	0	0	0
Behenic C22-0	0.4	0.7	0.3	0	0	0
Erucic C 22-1	0.1	49.9	0	0	17.8	0.37

Table (4). *The effect of semi-continuous feeding system on the growth, expressed in terms of optical density, of Botrydiopsis sp. (30 °C, 100rpm, 1/2 v/v aeration & 24 h illumination).*

O.D. (batch)	O.D. (semicon.)
0.200	1.480
0.544	2.420
1.367	2.480
1.240	2.950
1.260	2.793
1.340	3.488
1.628	3.250
1.488	3.476
1.824	4.070
2.148	4.250
2.208	3.880
2.530	3.400

Table (5). *The growth of the three strains of microalgae at the same incubation conditions (30°c, 100 rpm, 1/2 v/v aeration & 8 h illumination).*

O.D. of *Microcystis* sp. at 680 nm	O.D. of *Botrydiopsis* sp. at 680nm	O.D. of *Phormidium* sp., at 680 nm	Time, days
0.083	0.145	0.261	0
0.084		0.304	1
0.113			2
0.142	0.294		3
0.370		0.363	4
0.575			5
0.782		0.451	6
0.974	0.775		7
1.104		0.845	8
1.235			9
1.365	1.294		10
1.621		1.328	11
2.115			12
2.241			14
2.347		1.386	15
2.449	2.150		17
2.551			18
2.608		1.401	19
2.783			20
3.045	2.020		21
2.989			22
3.076			23
3.206			24
3.337	2.916	2.553	25
3.800			26
			27
		2.643	28
	2.859		29
		3.008	30

[1]N.D. Not determined due to end of incubation period.

orated and the mass of the remaining lipid is measured. The percentage of lipid in the initial sample can then be calculated.

Modified method of Folch and Bligh & Dyer's (Bligh & Dyer, 1959) (Lee *et al.*, 2010). By mixing chloroform-methanol (1: 1, v/v) with the dry cells using homogenizer Model WiseTis HG-150 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 0.2 volume (4 mL for 20 mL distilled water). After vortexing, some seconds, 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.

Hexane-isopropanol extraction method (Halim et al., 2011)

A mixture of n-hexane and isopropanol (3: 2, 300 mL.) was added to 4 g 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 *Microcystis* sp. & *Botrydiopsis* sp. was 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 (Tang *et al*., 2008). 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.

3. RESULTS AND DISCUSSION

3. 1 Cell biomass

The three micro algal species were incubated until a high algal biomass was produced. All strains were cultured under the same ambient temperature 30 ºC and aeration rate 1/2 v/v at 100 rpm. *Phormidium* sp. was cultured with 8 hours illumination period. The growth rate reached 1.7 g/L dry weight after 17 days in batch culture system and still constant for 3 days, then by using semi-continuous feeding system (withdrawing 2- 2.5 L old medium and feeding with equal volume fresh medium), the cell biomass reached 4 g/ L on the eleventh day, "Figure 1". In comparison, *Microcystis* sp. growth rate reached 3 O.D. after 13-days

Table (6). *Standard curve between O.D. and the biomass (g/L) of Microcystis sp.*

Dry weight (g/L)	O.D.
2.98	2.42
4.20	2.95
4.70	3.25
5.39	4.32
3.60	2.80
3.80	2.80

Table (7). *Standard curve between O.D. and the biomass (g/L) of Botrydiopsis sp.*

Dry weight(g/L)	O.D.
2.00	2.50
2.60	2.97
3.20	3.56
4.90	4.98

in batch culture system under the same conditions with 8 h illumination period. The use of semi-continuous feeding system to starting always by 1.5 culture optical density (O.D.), as shown in "Figure 2", the effect of illumination period on the growth rate was studied with semi continuous feeding system and found that; the growing rate reached 3 O.D. after 13-days with 8 hr illumination period, while after7-days with 16 h illumination reached to the same rate (3 O.D.) and with 24 h. Illumination period reached to the same value (3 O.D.) after 5-days only. So, the illumination period 16 hr is the best one economically. "Figure 3" illustrates that 16 h illumination period given the maximum biomass of *Microcystis* sp. after 14-days (5.8 O.D.) by using semi-continuous system. The biomass of *Botrydiopsis* sp. reached its maximum after about 26 days (2.5 O.D.) under 24 h illumination period in batch cultures, but by semi-continuous feeding system increased the growing to reach 4.3 O.D. after 21-days as shown in "Table 4".

3.2 Comparison of growth of the tested strains

The difference in the growth between the three micro-algal strains was studied under the same culture conditions of 8 h illumination/day, at temperature 30 °C with stirring rate 100 rpm and aeration rate 1/2 v/v. As shown in "Table 5", the *Microcystis* sp. gave the highest growth followed by *Botrydiopsis* sp. and *Phormidium* sp. which gave the lowest growth.

3.3 Effect of media type on algal growth

The *Botrydiopsis* sp. was subjected to growth in two different media (media 1& 2) under the same conditions (30 °C, aeration 1/2 v/v at 100 rpm). Figure 4 clearly shows that the biomass productivity of that strain at first 5-days in medium1 was higher than that in medium 2. From sixth.-day, the algae growing rate in medium 2 was increasing rapidly than medium1. The biomass productivity of *Botrydiopsis* sp. in medium 2 reached twice that in medium1. This may be attributed to the difference in composition of two media especially due to the presence of vitamin supplements in the second medium as yellow green algae can use these vitamins as enzymatic cofactors.

3.4 The optical density

The relation between optical density (O.D.) and dry weight for *Microcystis* sp. and *Botrydiopsis* sp. through different growth stages are shown in Table 6 and Table 7.

3.5 lipid Content Determination

This study dealt with the extraction of lipid from the cells in order to determine the percentage of this component in the microalgae dry mass. Hexane soxhlet extraction, chloroform-methanol (1: 1, v/v) solvent mixture and hexane-isopropanol extraction methods were used for lipid content determination. Hexane soxhlet extraction method has given the highest lipid percentage for *Phormidium* sp. (15 % of the dry weight), chloroform-methanol (1: 1, v/v) solvent mixture method recorded the highest lipid content for *Microcystis* sp. (28 %) and the best extraction method for *Botrydiopsis* sp. was hexane-isopropanol extraction method which has given 48% lipid content of the biomass for *Botrydiopsis* sp. cultured in medium 1 and that cultured in medium 2 has given 40 % lipid of the biomass.

3.6 Analysis of algal oil as fatty acid methyl esters (FAME)

The analysis of fatty acid methyl esters derived from *Microcystis* sp. (El-Diwani et al., 2011) and *Botrydiopsis* sp. algal oil were determined by gas chromatograph (GC) shown in Table (2). The most abundant saturated fatty acid was Palmitic acid (C16:0), and Stearic acid (C18:0). The most abundant unsaturated fatty acid was Linoleic acid (C18:2) and Oleic acid (C18:1).

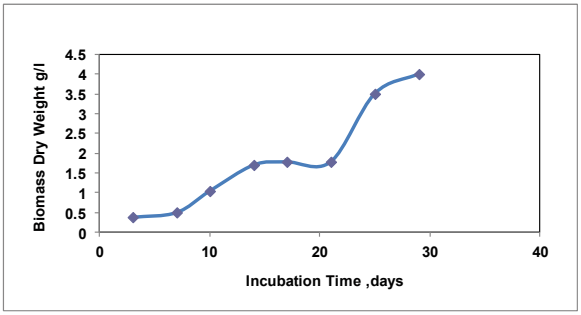

Figure (1). *The effect of semi-continuous feeding system on the growth rate of Phormidium sp. (30 °C, 100 rpm, 1/2 v/v aeration and 8 h illumination)*

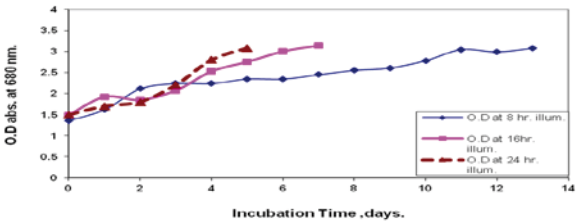

Figure (2). *The effect of illumination time on the growth rate of Microcystis sp. (30 °C, 100 rpm, 1/2 v/v aeration)*

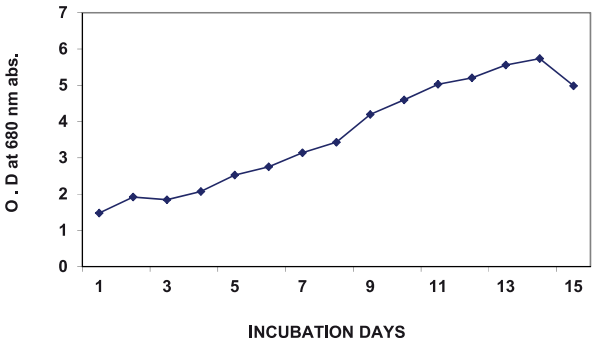

Figure (3). *The effect of semi-continuous feeding system on the growth of Microcystis sp. (30 °C, 100 rpm, 1/2 v/v aeration and 16 h Iliumination)*

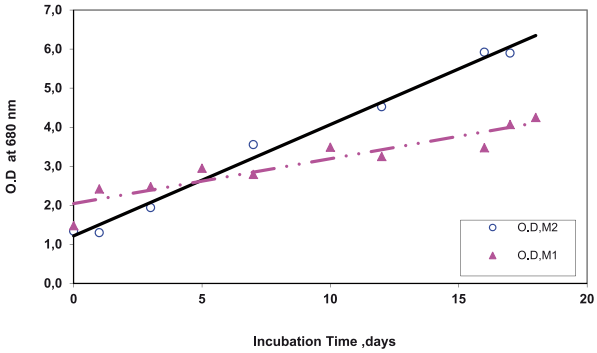

Figure (4). *The effect of media type on growth of Botrydiopsis sp. (30 °C, 100 rpm, 1/2 v/v aeration and 24 h illumination)*

Overall, the compositions were very similar, with 10-20% saturated fatty acids and 80-90% unsaturated fatty acids of various oils as shown in Table (3) **(**El Diwani *et al*., 2008**)**. In a previous research (Knothe, 2005), palmatic, stearic, oleic and linoleic acids were recognized as the most common fatty acids contained in biodiesel. In particular, oils with oleic acid content have been reported to have a reasonable balance of fuel properties (Rashid et al., 2008).

4. CONCLUSIONS

The cultivation of microalgae in closed photo-bioreactor has given high biomass productivity by applying semi-continuous feeding technique. The growth rate of microalgae can be accelerated by increasing the illumination period. The *Microcystis* sp. had the highest growing rate at 8 h. Illumination compared to other two strains. The *Botrydiopsis* sp. had the highest lipid content (48 %) for species cultured in medium1 and (40 %) lipid of the biomass for species cultured in medium 2. The highest mass productivity doesn't mean the highest lipid content, where the mass productivity for three tested strains was arranged as: *Microcystis* sp.> *Botrydiopsis* sp.> *Phormidium* sp. On the other hand, the lipid content for these strains arranged as: *Botrydiopsis* sp.> *Microcystis* sp.> *Phormidium* sp. Finally, the gas chromatography analysis showed that the algae oil have the suitable fatty acid composition which are required for biodiesel production.

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