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BOTANY

Biodiesel Production from *Scenedesmus obliquus* Cultivated in Outdoor Conditions at Large Scale

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Abstract *Scenedesmus obliquus* was discussed by phycologists as a promising microalga for bio- diesel production based on its biomass and fatty acid productivity. In the present study, *S. obliquus* was pilot cultivated for large scale production in a semi continuous culture for three months using bubbled glass boxes photo- bioreactor. Cultivation of *S. obliquus* resulted in a maximum biomass productivity of 0. 59 g L^{-1} d^{-1} and maximum esterified fatty acid productivity of 20.37 mg l^{-1} d^{-1} in (March, April and May), different flocculants for biomass harvest showed maximum flocculation efficiency of 82% using 250 mg l^{-1} of NaOH for 2 h. Solar drying of the harvested biomass showed significant increase of esterified fatty acid content by 50% with respect to control. In addition, fatty acid profile and iodine number of *S. obliquus* oil meet biodiesel standard specifications which make the fatty acid of *S. obliquus* eligible for further research to be used as a feed stock for biodiesel production.

1.Introduction

Microalgae are the largest autotrophic microorganisms of plant life taxa in the world that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure. Microalgae are present in all existing earth ecosystems, not just aquatic but also terrestrial, representing a big variety of species living in a wide range of environmental conditions. It is estimated that more than 50,000 species exist, but only a limited number, of around 30,000, have been studied and analyzed (1).

The biomass produces three major biochemical components by denovo synthesis consisting of carbohydrates, proteins and lipids (natural oils)(2). Microalgae are known to synthesize and can rapidly accumulate substantially higher amounts of lipids than terrestrial plants due to their high growth rates (3), concomitantly by alteration of the lipid biosynthetic pathways for storage as neutral lipids. The lipid yields and growth rates vary significantly among different species(4, 5). Micro algal lipids contain twice the energy stored per carbon atoms than carbohydrates, which translates directly into a twofold increase in fuel energy content thereby outcompeting terrestrial plants for biodiesel production (1).

Microalgae cultivation can be done in open-culture systems such as ponds or in highly controlled closed-culture systems called photo bioreactors (6, 7).

There are many methods for harvesting of microalgae like centrifugation, filtration and gravity sedimentation which may be preceded by a flocculation step(8). Choosing of the suitable method depends on algae species, growth medium, algae production , end product and production cost benefit (9). Various methods of flocculation can be used to aggregate the microalgae cells to increase the effective 'particle' size and hence ease sedimentation (10). Flocculation of algal biomass is preferred in large scale due to its low costs compared to other methods (11).

The present work was intended to throw some light on using of *Scenedesmus obliquus* as a feedstock for biodiesel production. This species was manipulated to stimulate fatty acid productivity and cultivated in large scale cultures. The quality of extracted oil as a source for biodiesel and recycling of byproducts were measured.

2. Materials and methods

2.1. Cultivation of *S. obliquus* in large scale

S. obliquus was cultivated in a bubbled glass boxes photo bioreactor. The system was built of 4 mm thick glass and had an internallength of 60 cm, width of 30 cm, a height of 35 cm and a nominal working volume of 40 L. The dispersion system for the reactor consisted of an air pump located in the center of the system. The

reactor was illuminated with (daylight-type). The duration of the light cycle was controlled by a timer. Airflow into the photo bioreactor was provided via filtered air through Teflontubing (fig .1)

S. obliquus was cultivated in 10 glass boxes each one was filled with 40 L of stock moh202 medium (see table 1) and 2L of 2 weeks-old inoculums previously grown in plastic bags in sun light. Temperature range between 16-30 ºC. The light intensity was 1185 µmol m^{-2} s^{-1} with a photoperiod 14:10 h light: dark. The pH was adjusted at 7 ± 0.2 by pH controller using air babbles.

2.2. Biomass assay

Algal growth was monitored using the optical density of the culture at 680 nm (OD_{680}) using a Shimadzu UV 2401 PC spectrophotometer and by determination of algal cellular dry weight (CDW). Biomass productivity was calculated according to (12).Biomass productivity (g $CDWL^{-1}d^{-1}$) = $(CDWL-CDW_E)\cdot(t_L - t_E)^{-1}$ Where CDWE and CDWL representing the CDW (g L^{-1}) at days of early exponential phase (t_E) and late exponential phase (t_L), respectively.

Fig.1.Cultivation system of *S. obliquus* at large scale showing the glass boxes. Each one contains 40L of moh202 media in the sun light with a photoperiod 14:10 h light: dark and aerated using air pump.

Table1. Composition of Moh202 media pH 7.5 (12)

Elements	Nutrient Solution[g/L]
KNO_3	0.80
K_2HPO_4	0.15
KH_2PO_4	0.20
$CaCl_2$	0.03
$MgSO_4$	0.10
NaCl	0.03
Hutner's trace elements*	1.00 ml l^{-1}
$NaHCO_3$	1.25
Vitamin B_1	0.12 mg l^{-1}
Vitamin B_{12}	0.01 mg l^{-1}

Hunter's trace elements (1950) *

For 1-liter final mix, dissolve each compound in the volume of water indicated. The EDTA should be dissolved in boiling water, and the $FeSO_4$ should be prepared last to avoid oxidation.

Table2. Hunter's trace elements (1950) *

compound	amount	water
EDTA disodium salt	50 g	250 ml
$ZnSO_4$. 7 H_2O	22 g	100 ml
H_3BO_3	11.4 g	200 ml
$MnCl_2$. 4 H_2O	5.06 g	50 ml
$CoCl_2$. 6 H_2O	1.61 g	50 ml
$CuSO_4$. 5 H_2O	1.57 g	50 ml
$(NH_4)_6Mo_7O_{24}.4H_2O$	1.10 g	50 ml
$FeSO_4$. 7 H_2O	4.99 g	50 ml

2.3. Biomass harvesting and drying

In this study, different flocculants were applied including NaOH and Al_2 $(SO_4)_3$ to reduce the cost of harvest (12). Different concentrations (0, 50, 150 and 250 ppm) of NaOH and Al_2 $(SO_4)_3$ were tested to harvest the cells with initial OD_{680} of 2.7 (A_1) by flocculation. The flocculation efficiency was determined by measuring the OD_{680} at 2 cm from the bottom of the tube (A_2) according to Tao and Salihon (2011) Flocculation efficiency = $(A_1-A_2)/A_1\times100\%$. After choosing the suitable flocculent, the culture was collected in 50 L container and mixed with suitable concentration of flocculent (270 ppm corresponding to OD_{680} 2.7). The precipitated cells were collected after 2 hours of flocculation and dried(13).

The drying temperature before lipid extraction from algal biomass was found to affect the fatty acid content. It has been found that, the solar drying resulted in increase of fatty acid content by 50 %, with respect to control; in this test, the harvested cells were dried through -out the hours of the day.

2.4. Extraction of lipid by Bligh and Dyer (1959):

In detail, a 15-ml glass vial containing 20 g algal biomass, 100 ml methanol, and 50 ml chloroform were added and kept for 24 h at 25°C. The mixture was agitated in a vortex for 2 min. 50 ml of chloroform was again added and the mixture was shaken vigorously for 1 min. (fig.2). After, 50 ml of distilled water was added and the mixture was mixed in a vortex again for 2 min. fig.3. The layers were separated by separating funnel. The lower layer was collected into a previously weighed clean vial (W_1). Evaporation was carried on in a water bath and the residue was further dried at 104°C for 30 min. The weight of the vial was again recorded (W_2). Lipid content was calculated by subtracting W_1 from W_2, and was expressed as % DCW(14).

Fig.2. Extraction of lipid by Bligh and Dyer

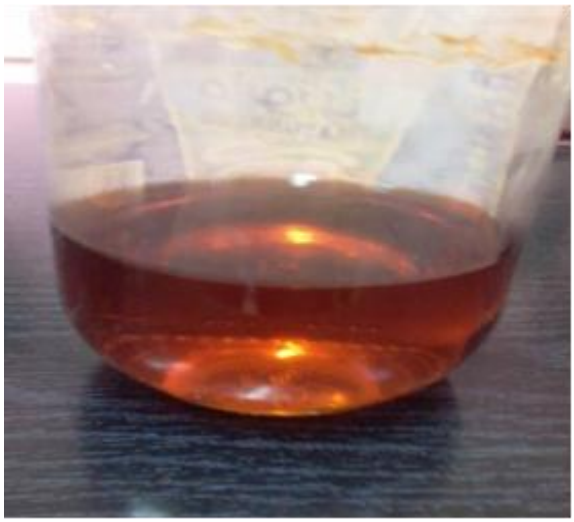

Fig.3. Lipid after washing and drying

2.5. Transesterification process

In which, 0.5g of NaOH and or KOH pettets were crushed with methanol. Then Stirring for 5-10 min until the NaOH and or KOH pettets were dissolved. Measure out 60 ml of the algal oil. Add all the contents to the reaction flask. Heat the flask contents to 50c^{o} for 20 min. with continuous stirring. While still warm, pour the mixture into a separating funnel and allow cooling until the mixture separates into two layers. The upper layer is biodiesel while the lower is glycerol. Don't let it stand for too long as the lower layer may solidify(15).

2.6. Biodiesel quality

Fatty acid composition was estimated by GC analysis and degree of unsaturation by determination iodine. The obtained values could be used as indicators for the quality of *S. obliquus oil* as a feedstock for biodiesel. The iodine value was determined according to the modified method of (16). 0.2 g of the oil was introduced in a dry glass stoppered Erlenmeyer flask. 25 ml of the reagent A were added. A previously moistened stopper with KI solution was inserted for 1 min, and then 10 ml of 20% KI solution were added. The stopper and the neck of the flask were washed with water and the contents were diluted to 200 ml. The librated iodine was titrated with 0.1 N sodium thiosulphate solution using starch as indicator. A blank was carried out and the iodine value was calculated according to the following equation: IV= (B-E) *1.27/W, where: IV= iodine value B= weight of 0.1 N sodium thiosulphate of the blank (g) E= weight of 0.1 N sodium thiosulphate of the sample (g) W= weight of the oil (g) *Reagent A:* 45 ml of dioxin and 6 ml of bromine solution were added to 500 ml of chloroform and mixed thoroughly. The volume was completed to 1 liter with chloroform then stored in a dark bottle.

3. Results

In order to keep costs down in large scale cultivation of microalgae, it is important to develop massive cultivation systems character- ized by low cost, high biomass production and ease of handling. The growth pattern of *S. obliquus* cultivated in experimental photobio- reactor for 2 weeks at different time periods of year. As a general trend, the increase in light intensity accelerated the growth and EFA content. Biomass productivity increased from 0.24 g L^{-1} d^{-1} in (October, November and December) to 0.59 g L^{-1} d^{-1} in (March, April and May) (fig.4). Also, EFA productivity of *S. obliquus* increased from 10.5 mg L^{-1} d^{-1} at (October, November and December) to 20.37 mg L^{-1} d^{-1} at (March, April and May) (fig.5).

It has been found that, the solar drying resulted in increase of fatty acid content by 50 %, with respect to control (fig.6). In this test, the harvested cells were dried throughout the hours of the day.

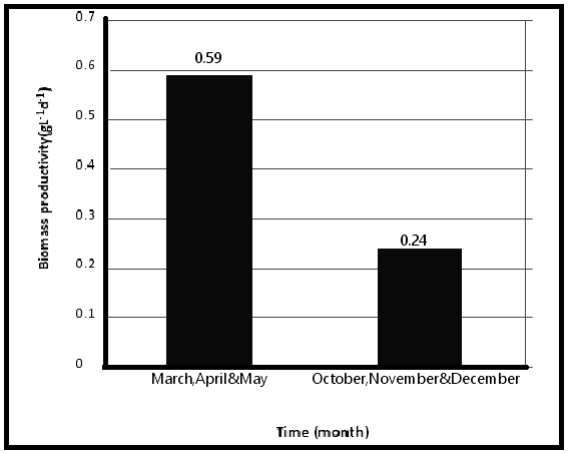

Fig.4. Biomass productivity of S.obliquus cultivated at different time periods of year.

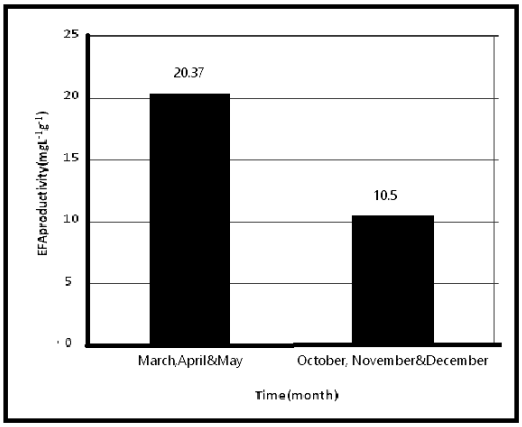

Fig.5. EFA productivity of S.obliquus cultivated at different time periods of year.

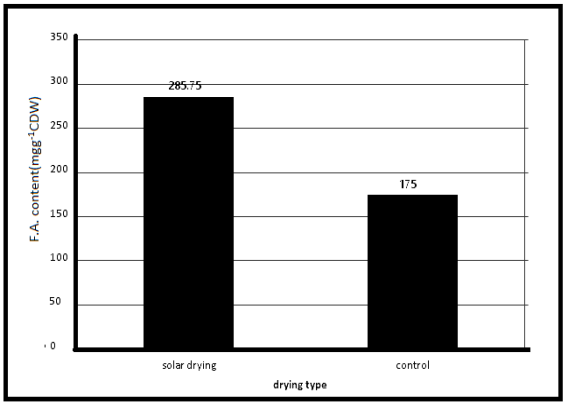

Fig.6. The effect of solar drying on fatty acid content of *S. obliquus*. Control means the fresh algal cells without drying, while solar drying was conducted using the sun throughout the hours of the day until constant weight.

As a general trend, the flocculation efficiency increased by increasing the flocculant concen- tration. The maximum efficiency for $Al_2(SO_4)_3$ was 75% using 270 ppm after 2 h(fig.7), while the maximum flocculation efficiency for NaOH was 82% using 270 ppm for 2 h (fig.8). Therefore, the harvest took place using 270 ppm of NaOH corresponding to OD_{680} 2.7 for 2 h.

Coagulation of cells resulting in precipitation or floating to the surface may be induced by the addition of multivalent metal salts such as aluminium sulphate and ferric chloride. Recovery of the microalgal biomass is then accomplished by siphoning off the supernatant or skimming cells off the surface respectively. These multivalent metal salts e.g. aluminium sulphate are effective coagulants for *Scenedesmus*(17)

The acceptability of biodiesel from microalgae as a substitute of fossil diesel fuel is strongly dependent on compliance with existing standards. The benchmark standard currently is the European Union (EN 590:1999) (18). Table1 show the fuel properties of biodiesel from micro algal oil such as viscosity, heating value, density, viscosity, flash point and solidifying point.

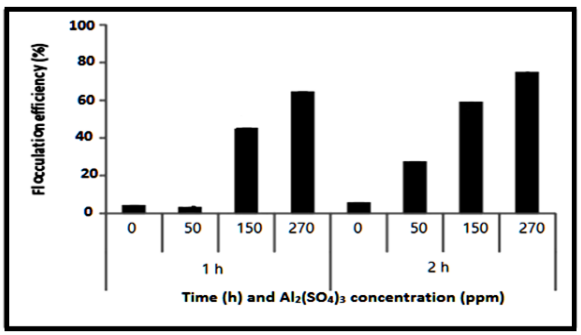

Fig.7.Flocculation of algal culture of *Scenedesmus obliquus* using different concentrations of $Al_2(SO_4)_3$ after 1 and 2 hours after mixing.

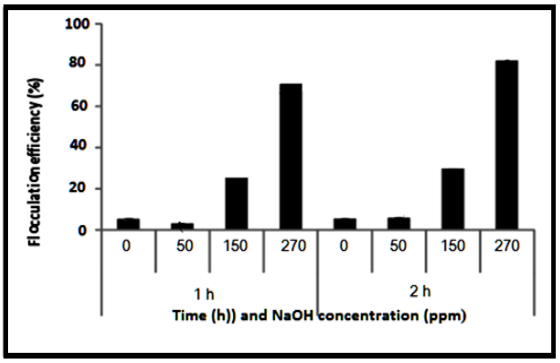

Fig.8. Flocculation of algal culture of *Scenedesmus obliquus* using different concentrations of NaOH after 1 and 2 hours from mixing

Table3. Comparison of properties of biodiesel from micro algal oil and diesel fuel

Properties	Biodiesel from micro algal oil	Diesel fuel (EN 590:1999)
Density (kg/l)	0.859	0.838
Viscosity(mm^2/ s, cSt at 40 ºC	4.9	2- 4.5
Flash point (ºC)	111	>55
Acid value (mg KOH/g)	0.371	max 0.5
Heating value (MJ/kg)	39	40- 45
H/C ratio	1.81	1.81
Solidifying point (ºC)	-12	-50 to 10

From fatty acid profile of *S. obliquus* revealed that, Lipids of *S. obliquus* were composed of 51% saturated and mono unsaturated fatty acids in the stationary phase. The predominant fatty acid at this growth phase was palmitic acid (16:0). The iodine value of *S. obliquus* was 70 g iodine/ 100 g oil. These results make the fatty acid composition of *S. obliquus* meet standard specifications of EN 590:1999(18).

4-conclusion

Microalgae have emerged as one the most promising feedstocks for biodiesel production(19). They have several key traits that make them a desirable energy source. They can be grown away from farmlands and forests and their yields of oil are higher than those from traditional oilseeds. In addition, they can provide several different types of high value derivatives such as nutritional supple- ments, antioxidants, cosmetics, natural dyes, and polyunsaturated fatty acids and renewable biofuels.

As a general trend, the increase in light intensity accelerated the growth and EFA content of *S.*

obliquus(20-23). It has been founded that, the sun light intensity increase biomass productivity from 0.24 g L^{-1} d^{-1} at (October, November and December) to 0.59 g L^{-1} d^{-1} at (March, April and May). Also, EFA productivity of *S. obliquus* increased from 10.5 mg L^{-1} d^{-1} at (October, November and December) to 20.37 mg L^{-1} d^{-1} at (March, April and May).

Harvest of *S. obliquus* cells has been done by chemical flocculation. In this study, different flocculants were tested and NaOH was selected as the best one.

The iodine value of *S. obliquus* was 70 g iodine/ 100 g oil. These results make the fatty acid composition of *S. obliquus* meet standard specifications of EN 590:1999(18). Measurements of other parameters including density, viscosity, flash point, acid value heating value, H/C ratio and solidifying are important to detect the suitability of *S. obliquus* oil for biodiesel.

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الملخص

اظهرت الدراسات السابقة ان السندزمس اوبلكس واحده من المواد الاولية الواعدة في مجال انتاج وقود الديزل الحيوي على اساس المحتوى الدهني ومعدلات النموالعالية .وفي هذه الدراسة تم اختيار السندزمس اوبلكس للزراعة لمدة ثلاثة اشهر على نطاق واسع باستخدام صناديق زجاجية شفافة كمفاعل ضوئي تجريبي. اظهرت النتائج ان زراعة السندزمس اوبلكس في اشهر(مارس ابريل مايو) يعطي انتاجية اعلى في معدلات النمو وانتاجية الاحماض الدهنية عنها في اشهر (اكتوبر نوفمبر ديسمبر) بمعدل يصل الى ٦٠% . في هذه الدراسة استخدمت طرقة الترسب باستخدام مواد كيمائية فى حصاد خلايا السندزمس اوبلكس وباختبار مرسبات مختلفة وجد ان استخدام ٢٥٠ مل غرام من هيدروكسيد الصوديوم لمدة ساعتان يزيد من معدل الترسيب بنسبة تصل الى ٨٢% عنها في المعدل الطبيعي . وباستخدام التجفيف الشمسي وجد ان هناك زياد ملحوظة في محتوى الاحماض الدهنية تصل الى ٥٠%عن تلك التي تجفف باستخدام الافران الكهربية .واعتمادا على أنواع الاحماض الدهنية والرقم اليودى للمواد الدهنية فى السندزمس اوبلكس تضح أن الزيت المستخرج مذا مطابق للمعايير الاوروبية لاستخدامه كوقود ديزل حيوى.