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Potential Biodiesel Production from Four Green Microalgae Cultures Collected off the Lebanese Coast

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***Abstract*— Seawater samples were collected from various locations along the Lebanese coast. Four unilagal isolates were acquired using streaking and serial dilution methods. The four isolates were cultured in 20 L flat photo-bioreactors containing seawater fertilized with Guillards F/2 medium. The growth dynamics, dry matter yield, lipid content, fatty acid profile and potential biodiesel yield were determined. The lipid content varied among growth phases and was greatest in the stationary phase, where it ranged between 14.5% DW in the Jod isolate and 16.5% DW in Jbd isolate. The best microalgal isolate tested gave a biodiesel production of around 50mg/ L. Optimization of culture conditions to improve biodiesel yield and the search for better performing local phytoplankton species are in progress.**

Index Terms*— *Microalgae, Renewable energy, Lipids, Fatty acids, Biofuel

I. INTRODUCTION

Fossil fuels account for 70% of the global energy market [1]. Accordingly, the price of fossil fuels is the main determinant of the amount of research and investment into developing novel sources of "green" energy such as solar and Aeolian power and biofuels. During the past decade or so, the price of a barrel of crude has risen from around US$ 20 to US$ 140 in 2008. This rise caused an increased interest in developing alternative fuels such as biodiesel, thus a search new fuel feedstock candidates (high organic biomass and lipid content, easy extraction and harvesting, and low cost) a must [2]. Higher plants like sugarcane, soybean, oil palm as well as microalgae are currently studied for biofuel production. Although energy crops have been commercially used as feedstocks for biofuel production, they are still not favored for long term fuel energy production mainly because they compete with food and feed production [1]. On the other hand, microalgae can provide various types of renewable biofuels including biodiesel, methane and biohydrogen [2]. Microalgae are considered as the highest yielding feedstock for biodiesel since they can produce 250 times more oil when compared to soybean, and 7 to 31 times more when compared to palm oil [2]. Statistics have shown that converting 1 to 3% of the US area for microalgal production would be enough to satisfy 50% of the country's fuel transport needs [3]. Microalgae, having the characteristics mentioned above are a renewable resource that can be sustainably supplied with no limited reserves, and thus will not be subjected to fluctuations in prices.

Microalgae are suitable for energy production because 1) They do not need productive arable land based sources, they can be grown in marginal areas such as arid lands and therefore reduce competition with food crops and fresh water [1], [4], [6]; 2) They can be produced in a variety of water sources such as fresh, brackish, sea water and waste water [1], [4]; 3) They perform efficient photosynthesis and thus they are efficient converters of sun energy into chemical energy [1], [4]; 4) They grow in high densities leading to high production if mass cultivation is well performed [1], [4]; and 5) Their cultivation does not require herbicides or pesticides; and their residuals after oil extraction can be used as feed or fertilizers [7], and/or fermented to produce alcohol or methane [3]. Moreover, energy from microalgae is carbon neutral and very environmentally friendly [1] [2]. Microalgal biodiesel contains negligible amounts of sulfur and performs as well as petroleum diesel, while reducing emissions of particulate matter, CO, hydrocarbons, and SOx [1]. In addition, microalgae have the capacity to convert 3-8% of solar energy to biomass as compared to terrestrial plants that are capable of 0.5% conversion only [8]. Unfortunately, present technology is not yet capable of producing biodiesel at prices that can compete with fossil fuels [4]. We need to reduce the cost of microalgal oil from $2.80/L to $0.48/ L in order to eliminate dependence on petroleum [9].

In the present work, we assessed the biodiesel producing potential of four promising microalgal species collected off the coast of Lebanon.

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Growth curves, oil production and biomass production were investigated.

II. MATERIALS AND METHODS

A. Microalgae culture

Water samples for microalgae isolations were collected at various locations along the Lebanese coast. Samples were immediately passed through 100 µm filters to remove macroalgae and zooplankton and then enriched with Guilliard f/2 medium. After isolating a pure monoculture, larger volumes were obtained by transferring 100 ml of culture inoculum from the exponential phase to 2 L culture flasks and incubating these under 450 lux fluorescent lamps for 16:8 hrs light and dark cycles. Temperature and salinity were maintained at an average of 21°C and 28g/L, respectively. Continuous aeration was also provided for cultures. For studies on growth kinetics, 20L flat-sided photobioreactors were used. Microscopic and molecular biology tools were used to identify the microalgae (details will be published in a separate article).

B. Estimation of algal density and biomass

Microalgal biomass was harvested using a static high volume centrifuge (cream separator) and the harvested microalgae were then frozen and lyophilized (Labconco, USA).

C. Determination of total lipids

Total lipid content was determined gravimetrically using the Folch method [10]. A methanol: dichlormethane (DCM) (2:1) mixture was added to a known mass of freeze dried algae. The mixture was then homogenized to a volume 20 times the volume of tissue sample (1 g in 20 ml of solvent mixture). Tubes were placed in an ultrasonic bath for an hour and a half (at 40ºC) then vortexed. Homogenates were filtered into clean screw cap tubes and 0.4 volumes of water were added (8 ml of water for 20 ml of solvent mixture). After vortexing for a few seconds, mixtures were centrifuged for 10 minutes at 4000 rpm to allow phase separation. Upper phases were discarded while the lower phases representing the DCM containing lipids were retained. Sodium sulfate was then added for moisture absorption. The mixture was decanted into a clean tube and the solvent evaporated under vacuum in a rotary evaporator. Dry weights of tubes were measured and lipid content estimated. Proportion of lipids in dry sample weight was calculated as follows :

%Lipid = (weight of lipid in g/ dry weight of sample in g) x 100.

D. Determination of fatty acid methyl esters and biodiesel production

After the lipid extraction processes, the resulting algal lipids were converted into biodiesel through transesterification. Fatty acid methyl esters (FAME) were prepared according to Freedman et al [15]. GC-MS was used for an efficient separation, identification and quantification of the particular fatty acid methyl ester present in the sample. The injector of the GC (Trace GC Ultra, USA) was set at a temperature of 260ºC. 1µL sample volume was injected into the split injector with a dilution split ratio of 100:1. Helium was used as a carrier and was injected in the DB 23 column at a rate of 30cm / sec (0.54 ml/ min). Initial column temperature was set at 50ºC for 2 minutes, then temperature was increased to 220ºC at a rate of 4ºC / min. Temperatures of the transfer line as well as of the MS were set at 260ºC.

The transformation or conversion of lipids into biodiesel was performed by base-catalyzed methylation. Fifty mg of the collected lipids were added to 15 mL of 1% MeONa in methanol and refluxed for about 2 hours. After complete conversion as monitored by thin layer chromatography (TLC), the mixture was extracted with hexane (2 x 15 mL), and the combined hexane layers were washed with water until pH was neutral, then the hexane extract was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure in a rotary evaporator to recover the fatty acid methyl ester fraction (biodiesel).

III. RESULTS

A. Growth kinetics

Experiments on growth kinetics were conducted for four isolates. The graphs representing readings of optical density taken at 560 nm are shown in the four figures: Jbd (Fig. 1), Had (Fig. 2), Jod (Fig. 3), and Ozd (Fig. 4).

After an initial lag phase of about two days, exponential growth started and lasted for a period of two to four days in three out of four isolates (Fig. 1, 2, 4). On the other hand, Jod isolate had a very short exponential phase (Fig. 3). The relatively high initial growth rate of Had, Jod, and Jbd was reflected by their specific exponential growth rate (µ max/d) 1.58, 1.50, 1.46, respectively as compared to µ max of 0.885 for Ozd. The highest optical density readings were obtained with the Had isolate with A560nm value of 0.8 corresponding to about 4x 10^6cells and a dry matter of 406 mg/ml in the stationary phase (TABLE I) while the lowest reading was recorded with Jbd isolate which reached an A_{560}

of 0.25 with $0.8x10^6$ cells/ml and a dry matter of 445mg /ml in the stationary phase. However, growth stopped at this O.D., and the number of cells declined to 0.45 x 10^6 cells/ml after a few days.

In the Ozd and Had isolates, at an A_{560} reading close to 0.4, the number of cells reached close to $3x10^6$ cells/ml. In the Jod isolate, the cell number recorded at the same absorbance was about $5x10^5$ cells/ml and reached later 2.5 x 10^6 cells/ml at an O.D. of 0.55.

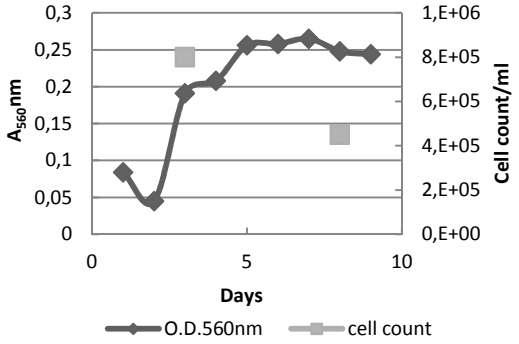

Fig.1. Growth kinetics of Jbd isolate as measured by optical density readings (A_{560nm}). The number of cells/ml is provided at specific dates and O.D. values.

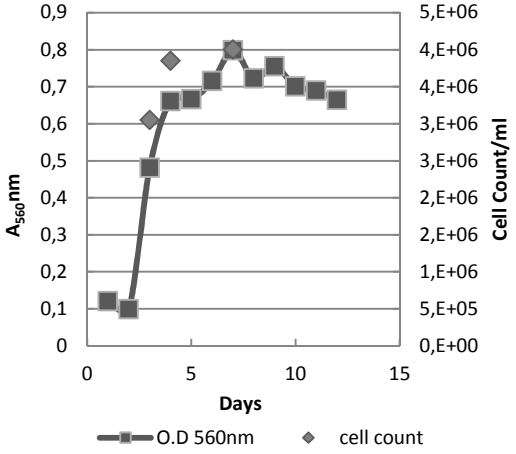

Fig. 2. Growth kinetics of Had isolate as measured by optical density readings (A_{560nm}). The number of cells/ml is provided at specific dates and O.D. values.

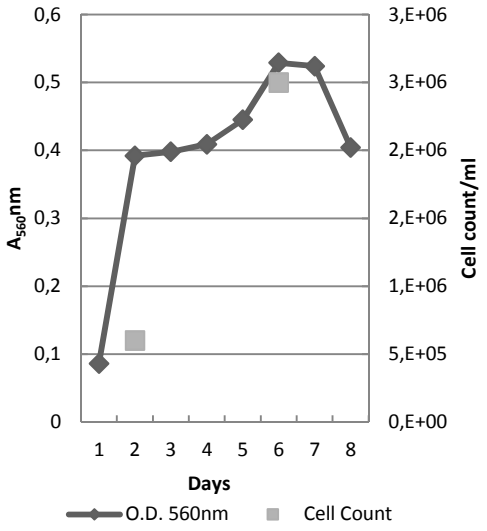

Fig. 3. Growth kinetics of Jod isolate as measured by optical density readings (A_{560nm}). The number of cells/ml is provided at specific dates and O.D. values.

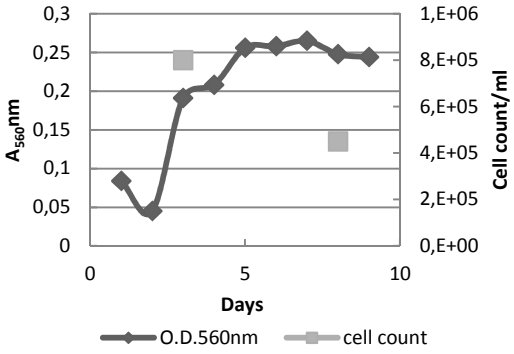

Fig. 4. Growth kinetics of Ozd isolate as measured by optical density readings (A_{560nm}). The number of cells/ml is provided at specific dates and O.D. values.

TABLE I.
Dry weights of four microalgal isolates at corresponding optical densities during exponential and stationary growth phases

Exponential phase			Stationary phase	
Isolate	**A_{560}**	**(mg / L)**	**A_{560}**	**(mg / L)**
Jbd	0.191	42.8	0.244	445
Jod	0.392	62	0.404	443
Had	0.481	129.8	0.664	406
Ozd	0.247	104.4	0.336	495

B. Lipids and Fatty Acid content

The total lipid content and the relative percentage of saturated and unsaturated fatty acids for each isolate are presented in TABLE II. The highest total lipid content was recorded during the stationary phase. It reached about 16% DW in Jbd and Had isolates and about 14.5% in Jod and Ozd isolates. In the exponential phase, the lipid content was 1-2% less that in the

stationary phase (TABLE II, Fig. 5,Fig. 6). The proportions of saturated and monounsaturated FAs were greater in the stationary phase of the growth period whilst polyunsaturated FA were considerably more in microalgae during the exponential phase of growth.

Among the four tested isolates, Jbd, Jod and Had had a biodiesel production of 49.84, 42.8 and 48.72 mg/liter of media culture respectively, whilst Ozd had a biodiesel production of 52.6mg/liter of media culture (Fig. 6).

TABLE II. Total lipid content (%) and percentage saturated and unsaturated FA in four local microalgal isolates measured at two growth stages

Phase	Isolate	O.D./ no. of days	Total Lipid* Content (%DW)	%Sat-urated FA	%Unsaturated fatty acid			
					Total	Mono	Di	Poly
Exponential	Jbd	0.191/3	14*	27.98	70.61	36.28	7.95	26.38
	Jod	0.398/3	12.17*	33.14	66.85	35.87	6.67	24.31
	Had	0.661/4	15*	31.24	67.49	37.68	4.81	25
	Ozd	0.247/5	13.33*	30.71	68.19	29.68	14.39	24.12
Stationary	Jbd	0.244/9	16.5*	34.8	65.19	48.25	6.59	10.35
	Jod	0. 404/8	14.63*	31.92	68.07	38.32	8.87	20.88
	Had	0.710/10	16.03*	36.25	62.84	45.25	5.72	11.87
	Ozd	0.336/11	14.53*	35.89	64.13	47.16	7.5	9.47

* Average of three replicates.

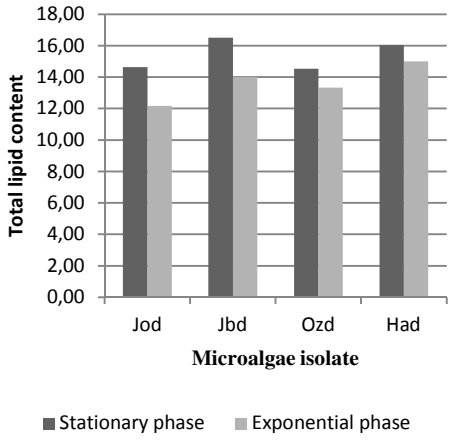

Fig. 5. Total lipid content of microalgal isolates in both exponential and stationary phases.

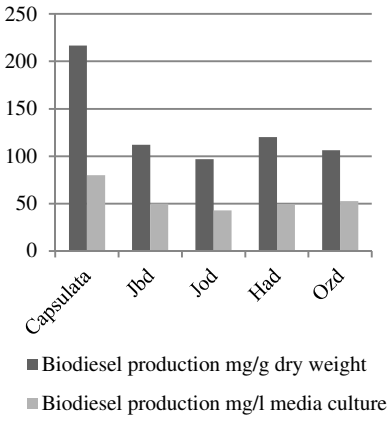

Fig. 6. Biodiesel production of four local microalgal isolates measured at the stationary stage [1].

[1] Capsulata is a reference "Chlorella" microalgae.

IV. DISCUSSION

With the increasing demand on fossil fuels and the expected shortage in world reserves over the coming decades, the search for renewable sustainable sources of clean energy has become a priority. In addition the pollution generated by burning fossil fuels and their contribution to global warming has led many developed countries to issue legislations pertaining to reduction of CO_2 emissions [1]. Microalgae have been for a long time considered as potential feedstocks for biodiesel production. However further investigations and developments are required for biodiesel production to be become economically sustainable. In this preliminary study, four local isolates were evaluated for their potential use for biodiesel production. Since no optimization of any growth conditions was attempted, these results may be considered as preliminary. Under the experimental conditions, the maximum growth rate (μ) in three isolates reached 1.5, suggesting that doubling of the cells occurred every 11 hours. However, this rapid growth rate lasted only for about two days and then decreased. This was reflected in the level of biomass produced which ranged from 406 to 495 mg dry weight (DW)/L of culture medium/harvest every 8-10 days. This level of production is of the same order of magnitude reported in many recent publications [14, 16, 17] following optimization of growth conditions. However, the biomass yield of the Lebanese isolates is considerably less than those reported by [3], where the maximum dry matter yield reached about 300 mg/L/day. This suggests that under our experimental conditions, there is one

or more limiting factor(s) since the growth rate of microalgae depends on various parameters including species and several environmental factors such as temperature, light duration and intensity, CO_2, salinity, pH, nutrient availability and toxic materials excreted into the medium. The total lipid content during the stationary phase varied between 14.5 and 16.5%, with the Had isolate (*Tetraselmis marina*) and Jbd isolate (*Micratinium* sp.) containing 16.5 and 16.0 % total lipids, respectively. This lipid content is similar to that reported for most green algae [18]. However it is less than that reported for *Scenedesmus dimorphus* (16-40%) [18], *Botryococcus braun*ii (35-63%) [19], or for *Nannochloropsis* sp., where lipid content supposedly attained 60% (DW) after nitrogen starvation [3]. Significant differences in total lipids and the FA profiles were observed depending on the growth phase. Greater content of total lipid was observed at the stationary phase. However, the greatest proportions of PUFA and omega-3 FAs were observed during the exponential phase. For biodiesel production a high content of saturated and mono unsaturated fatty acid is preferred to get a good quality biodiesel, three out of the four isolates tested may fit this specification especially in the stationary phase where biomass production is highest. The lipid content of microalgae and the composition of their saturated and unsaturated fatty acids may be affected by several factors including the light conditions, nutrient composition of the medium, pH, salinity and mainly nitrogen starvation [14]. Therefore, optimization of culturing conditions may improve the total biomass production and/or the total lipid content.

The four isolates were also evaluated for their biodiesel potential and showed biodiesel productivity around 5 mg/L/day of culture media in three isolates. This represents 9 to 11.2% of the microalgal dry weights. According to the literature "[13]", microalgal oil contents are usually between 20% and 50% of dry algal biomass weight which makes our isolates non-competitive and non-economical for current biodiesel production.

Optimization of culturing conditions might allow at least doubling the biodiesel production potential of our isolates. The four tested isolates may also be considered for other potential uses; preliminary data show that one isolate has a potential to produce Omega-3 FA and another isolate can be used to produce protein as food or feed supplement.

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