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Growth Promotion of indigenous *Scenedesmus dimorphus* strain under different conditions using stirred tank photobioreactor

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Abstract: The effects of culture conditions at different temperature (15-25-35°C) and nitrate concentration (0.1-0.25-0.4g/L) on biomass production and growth rate of *scenedesmus dimorphus* were determined. The results shown that the dry biomass weight (0.153g/L) at 15°C was the lowest. No significant difference was observed between the dry biomasses at 25°C (0.289g/L) and 35°C (0.240g/L). While the lowest growth rate was at 15°C($0.157d^{-1}$), the highestone was at 25°C ($0.229d^{-1}$) and 35°C ($0.195d^{-1}$) with insignificant deference, and The nitrate concentration had no effect on both of biomass production and growth rate.
Key words: *Scenedesmus dimorphus*, biomass, growth rate, Photobioreactor.

Introduction

Microalgae are a group of fast growing unicellular or simple multicellular microorganisms[1] which have several advantages to consider as energy crop, including higher photosynthetic efficiency, higher growth rate and higher biomass production[2]. They are promising for economic industrial-scale production in the 21st century. Microalgae can be utilized in the production of nutritional supplements, antioxidants, cosmetics, natural dyes[3]. They can also be used in gum, beverages, candy and snack foods[4].

Among microalgae, *Scenedesmus* genus has the most desirable features for efficient and economic combination of CO_2 fixation, wastewater treatment, and lipid synthesis toward biodiesel production[5, 6].Recently, it has drawn attention as commercially a valuable source for a wide spectrum of compounds such as; high quality protein, B_{12}, C and E vitamins, pigments and other bioactive chemicals, long chain polyunsaturated fatty acids especially"omega-3" and "omega-6", glycolipids, sulfolipids and phenolic compounds[7,8,9]. *Scenedesmus dimorphus* seems to be the ideal species, due to its good ability to grow and tolerate different environmental conditions[10].

Recently, many studies reported various cultivation technologies to produce microalgae[11].Besidesopen ponds, the primary and effective methods for the large-scale production[3, 12], photobioreactors offered a better opportunity for controlling and optimizing all cell growth parameters to meet the specific demands[13, 14]. The most important and common design of this reactor is Stirred-tank reactors (STR)[15], for being simple in operation and structure, and ideal for cultivating different types of cells[16].

The main environmental factors influencing microalga growth and its chemical composition are light, nutrients, temperature and pH[17].

So, several strategies have been applied to improve the growth, biomass production and lipid content, by optimization of the medium composition (e.g., carbon source, nitrogen, phosphorus, vitamins and salts) [18],and physical parameters (e.g., pH, temperature and light intensity) [19].

Temperature strongly influences the growth rates for allalgae species[20], and for *Scenedesmus sp.*,the optimum temperature to grow is between 20 and 40°C[21], but the optimal one to produce biomass and lipids was 20°C[22]. Cassidy (2011) measured *Scenedesmus* and *Chlorella*'s growth rate at different temperatures (25, 30 or 35ºC), and found that 30ºC had the best growth rates for both algae.

Nitrogen was quantitatively the most important nutrient affecting the biomass and lipid productivity [23].Therefore, it is important to utilize an appropriate nitrogen source at a certainconcentration[24],so many studies investigated the effect of both nitrogen source and its concentration on biomass growth. According toArumugam*etal.*(2013)nitrate was found tobe a preferred form of nitrogen source, that potassium nitrate (0.32 g/L) and sodium nitrate (0.28 g/L) were the best for biomass growth of *Scenedesmus* sp. For *Scenedesmus dimorphus* species, the maximum growth rate in terms of biomass and lipid productivity was at 0.1g/L urea, but the biomass and cell numbers decreased gradually by increasing urea concentration beyond 0.1g/L[2].

The aim of this study was to evaluate the effect of temperature and nitrogen at various levels on *Scenedesmus dimorphus* growth using Photobioreactor to determine the best conditions for cell growth and biomass production.

Materials and methods

Microalgae and culture conditions

The experimental organism *scenedesmus dimorphus* was isolated from fresh water ponds located in Quneitra Province (south of Syria). The algae broth medium from sigma co., solidified with agar, was used to isolate and purify the algae in Petri-Dishes, then species was identified at the Center of Advanced Studies in Botany, University of Madras, Chennai, Egypt.

After the inoculum was prepared, the initial cell concentration was set to be its OD_{680}= 0.150 (optical density at 680nm). All experiments were carried out in the Stirred Tank (STR)with a maximum capacity of 14L and 10L as working volume figure (1).

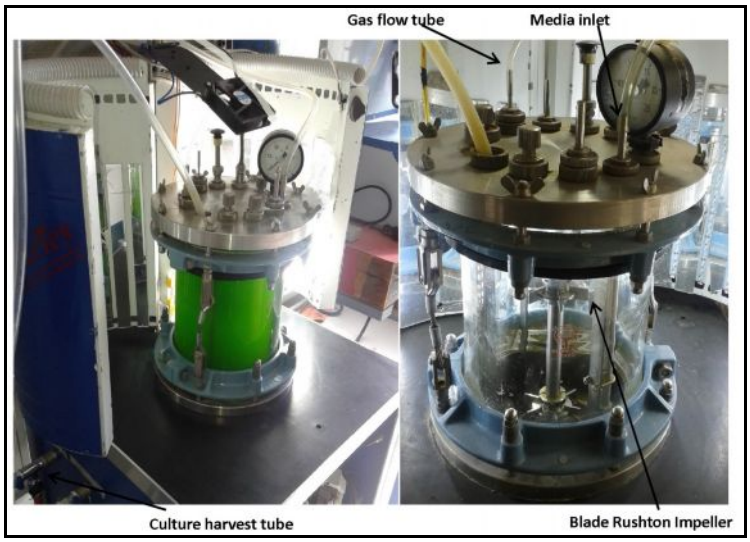

Fig. 1: The modified Stirred Tank Reactor

STR was equipped with PLC (Programmable Logic Controller), controlling and monitoring all parameters (Temperature, light, pH, gas flow; CO_2 and air, mixing speed).

All Growth experiments were done at three different temperatures(15, 25, 35◦C), then three different nitrate concentrations(0.1, 0.25, 0.4g/l) in 10 L autoclaved Bissch off & Bold medium (BBM).Each batch cultivation was carried out three times for 15 days at fixed parameters(6000Lux continuous illumination ,250 rpm mixing speed, PH= 7, air mixed withCO2 500ppm with flow rate 2L/min, temperature 25◦C with variable nitrate concentration, and nitrate concentration 0.25g/L at variable temperature) all parameters were established on the basis of the last studies[26,27].The central values of temperature, 25◦C were chosen depending on Hernande *et al*. (2009) and Brown *et al*. (1998). But the additional experiments were done either increasing or reducing the growth temperature by 5◦C. The central concentration of nitrogen in medium (0.25 g/ L)was selected according to Guillard(1975), and the additional cultivations were run at 0.1 and 0.4g/L.

Estimation of Algal Growth

The growth rate (GR) was calculated by fitting $OD_{680}$of the daily samples using spectrophotometer (HITACHI U-2900) in the following formula[29]:

$GR = (\ln OD_t - \ln OD_0)/t$.
OD_0: the optical density at inoculation day. OD_t: the optical density measured on day t.

Determination of Biomass Dry Weight (DW)

Dry Biomass content was determined according to the modified method of Yadavalli *et al* (2012),that used a standard curve of OD_{680} plotting versus serial dilutions of DW figure (2).

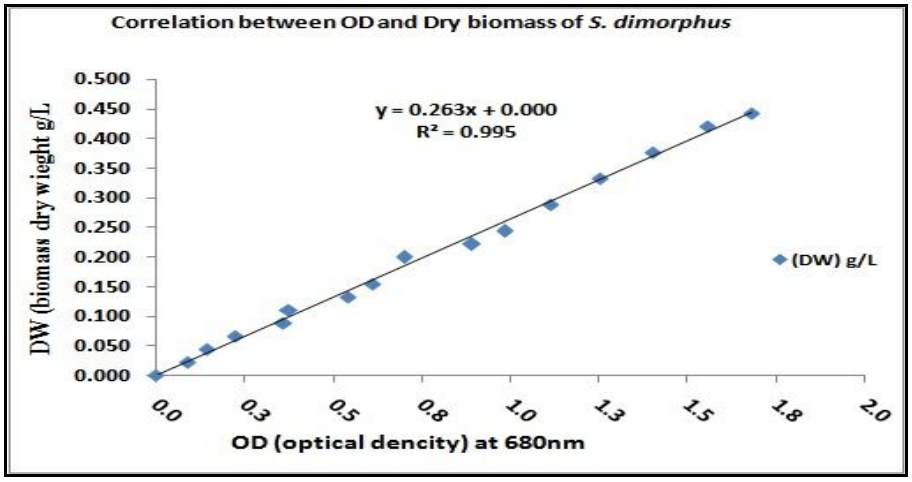

Fig. 2 Linear regression equation of relationship between OD and dry biomass

The algal cells were collected by centrifugation (5,000×g, 10 min), and washed with water, then dried for determining its DW gravimetrically.

All statistical analyses were done using SPSS (version 17), according to ANOVA followed by Tukey test.

Results and discussion

Effect of temperature:

Biomass production

The daily increase in dry biomass at the three temperatures showed that the actual increase in dry biomass began after the second day of inoculation at all temperatures, figure (3). This

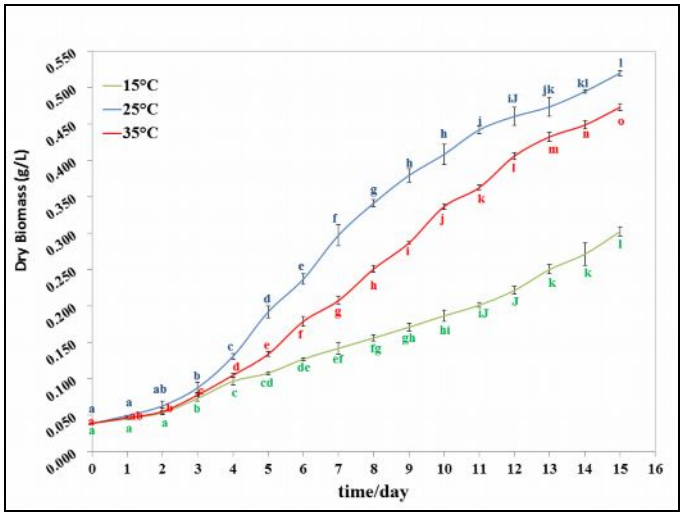

Fig. 3: dry biomass curve grown in different temperatures. Different letters above the lines indicate significant differences (p<0.001) among means within each temperature. (ANOVA followed by Tukey Test).

Suggests that the studied temperature had no effect during the lag phase. As that the Exponential phase started in the third day of inoculation, and continued with a significant increase (P>0.001), in most days, till day 15; the harvest day, but at 25°C, the difference between the fourteenth and fifteenth days wasn't significant, this may make the fourteenth day more economical to harvest. This could refer to the acceleration of growth at 25, that led to shortcut the exponential phase. This result was lower than that of Velichkova *et al* (2013),who clarified that the sixteen day was the harvest day. On the other hand, the effect of temperature in table (1) shown that the difference between the means of biomass at25°C and

Growth rate

The daily growth rate for the three temperatures is shown in Figure (4), where the growth rate

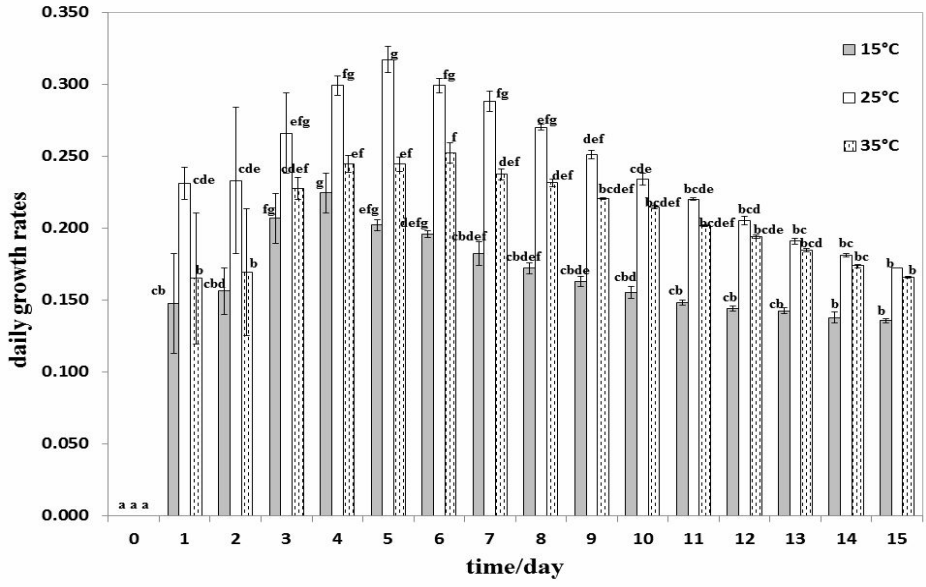

Fig. 4: Comparison of daily growth rate grown in different temperatures. Different letters above the bars indicate significant differences (p<0.001) among means within each temperature. (ANOVA followed by Tukey Test).

Table 1: Means ±SD of dry biomasses and growth rate during the entire growth period within three temperatures

Temperature	dry biomass ±SD		Growth rate/day ±SD	
15°C	0.227±0.150	[a]0.153±0.080	[a]0.157±0.050	0.079±0.041
25°C		[b]0.289±0.171	[b]0.229±0.075	
35°C		[b]0.240±0.152	[b]0.195±0.060	

Different letter in each Column for each species indicate significant differences ($p < 0.001$) between means (ANOVA followed by Tukey Test) 35°C was not significant. X in *et al*. (2011) showed that the temperature 20°C gave the highest biomass in *Scenedesmus sp*., so it is possible that the range 20°C to 35°C is the best to get the highest dry biomass.

Increased up to the highest values 0.224, 0.317 and 0.252 d^{-1} in days fourth, fifth and sixth at 15°C, 25°C and 35°C respectively, that indicated to a positive relation between temperature (15 to 35°C) and time elapsed to achieve the highest growth rate. This seemed to belogical, as the lower temperature reduces the length of the exponential Phase.

According to the table (1), the lowest growth rate was at 15°C 0.157±0.050d^{-1}, while the difference between the growth ratesat 25°C and 35°C, respectively, was not significant.

This agreed with other studies, as Westerhoff *et al.* (2010) found that growth rates didnot vary by temperatures ranging between 27 and 39°C.Also, Xin *et al*(2011) showed that the highest growth rate was obtained at 25°C. In addition, Sanchez *et al* (2008) explained that the ideal growth of *Scenedesmus sp.* was within the range of temperature20 to 40°C.

Effect of nitrate concentrations:

Biomass production

The actual increase of biomass, at concentrations 0.4 and 0.25g/L, began after two days of inoculation, while it delayed till the third day for the concentration 0.1g/L Figure (5). That

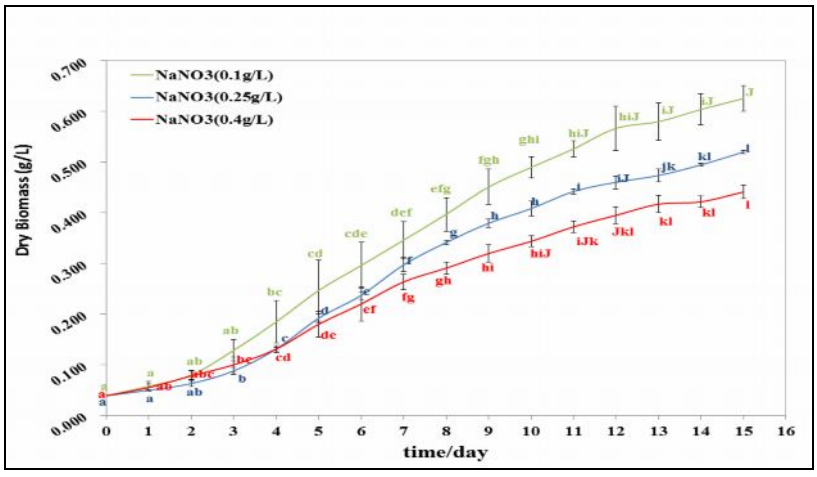

Fig. 5: The change of dry biomass by the time,in different concentrations of sodium nitrate. Different letters above the lines indicate to significant differences (p<0.001) among means within each concentration. (ANOVA followed by Tukey Test). might be explained by elongation of the Lag phase,that algae need to adapt with the growth medium according to Spencer (1954), at the low concentration of nitrate.

During the Exponential phase, the dry biomass increased significantly by the time in all concentrations, in most days. This is consistent with Becker (1994),who explained the systematic duplication of the cells in this

phase. In the table (2), the insignificant differences (P> 0.001)in dry biomass values might be referred to that the concentration of nitrates

Table 2: Means ±SD of dry biomasses and growth rate during the entire growth period within three concentrations of sodium nitrate

NaNO3(g/L)	dry weight±SD		Growth rate ±SD	
0.1	[a]0.298±0.177	[a]0.351±0.205	[a]0.272±0.106	[a]0.113±0.047
0.25		[a]0.289±0.171	[a]0.229±0.075	
0.4		[a]0.254±0.139	[a]0.233±0.090	

Different letter in each Column for each species indicate significant differences ($p < 0.001$) between means (ANOVA followed by Tukey Test) within the range (0.1g/L to 0.4g/L)did not have any effect on the produced dry biomass. While in the study carried out by Arumugam *et al*. (2013) that tested four different concentrations of sodium nitrates 05, 10, 15 and 20 mM, the best one was (10Mm=0.28g/L). This could be attributed to the effects of other condition on growth.

Growth rate:

The daily growth rate at different concentrations of sodium nitrate shown in Figure (6), where the highest growth rates for concentrations 0.1, 0.25, 0,4g/L were at days third, fifth and second respectively.

Also, according to table (2) the increase of sodium nitrate concentration from 0.1g/L to 0.4g/L had no effect on growth rate of algae. This result correspond with Goswami and Kalita (2011) who used urea as a nitrate source, since the highest growth rate achieved at 0.1g/L, then the more increase in the urea concentration led to a gradual decline in growth.

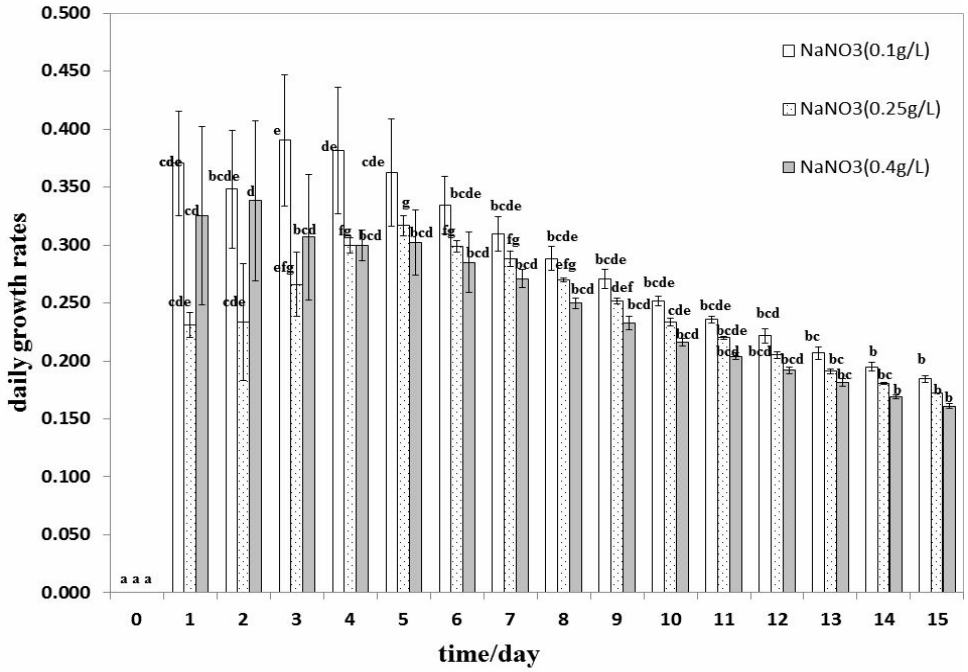

Fig. 6: Comparison of daily growth rate grown in different concentrations of sodium nitrate. Different letters above the bars indicate to significant differences (p<0.001) among means within each concentration. (ANOVA followed by Tukey Test).

Conclusion

Because of *Scenedesmus dimorphus* is used in food and pharmaceutical industries, the objective of this research was to optimize the best culture conditions for the highest biomass production. It has been mentioned above that 25 and 35°Cas a growth temperature, achieved the optimal biomass yield and the highest growth rate, too. In addition, biomass and growth rateof *S. dimorphus* was not significantly influenced by nitrate concentration, so any increase in nitrate concentration above 0,1g/L was useless and not economic. Therefore, we recommended culturing *S. dimorphus* at 25-35°C and 0.1g/L NaNO3to get the optimal production of its biomass.

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