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The Effects of Different Salinity Ratios on Reproductive Rates and Proximate Composition of *Spirulina platensis* in a Helical Photobioreactor

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Abstract: *Spirulina platensis*, cyanobacteria was cultivated in a helical photobioreactor and in a continuous system. Cultivation continued at different salinity ratios (10, 20, 30%) for 10 days. The total volume of the photobioreactor was 24.3 L comprising of 18 L nutrition medium and 6.3 L algae. Each day, the system yielded 10 L of algae and 10 L of nutrition medium was added into the system. At the end of 10 day experiment, the medium reached to1.042×10^7 filament L^{-1} from an initial filament density of 7.596×10^6 filament L^{-1} at 10% salinity, 7.409×10^6 filament L^{-1} from an initial filament density of 7.60×10^6 filament L^{-1} at 20% salinity and to 7.381×10^6 filament L^{-1} from an initial filament density of 7.62×10^6 filament L^{-1} at 30% salinity. Variations in proximate composition of *S. platensis* occurred due to the different rates of salinity. Carbohydrate, TMS (Total Mineral Substance) and lipid values increased; protein levels decreased in parallel with the increase in salinity. A reduction of approximately 13.05% occurred in the protein values, 26.08% increase in the lipid value, 31.85% increase in TMS and 9.86% increase in carbohydrate values were observed.

Key words: *Spirulina platensis*, helical photobioreactor, salinity ratios, reproductive productivity, proximate composition

INTRODUCTION

Sprulina platensis, a member of the blue-green algae class (Cyanophyceae) is a filamentous, spiral prokaryotic organism composed of microscopic cells. *Spirulina* sp. contains plantal protein with the richest biological value in nature. *S. platensis* is a highly important cyanobacteria species in that it contains high levels of protein, pigments, and gamma linoleic acid (Borowitzka, 1992; Cohen, 1997). Therefore, it is widely cultivated to be used commonly as a human and animal food source and in cosmetics, pharmaceuticals and other various industrial fields (Belay *et al*., 1996; Cirik, 1989; Chen *et al*., 1996; Glazer, 1999; Wikdors and Ohno, 2001). It is produced commercially as a food source in health foods and the pharmaceutical industry, especially in developing countries (Richmond, 1988; Zeng and Hu, 1992).

Commercial mass culture of *S. platensis* as a food supplement began at the end of 1970s (Ciferri, 1983). The production of *S. platensis* species from blue-green algae has become popular due to its easy reproduction and high productivity (Kilic *et al*., 2006). Turkey has highly appropriate climatic conditions for cultivation of Spirulina culture. *S. platensis* is resistant to high pH levels (9-10) and its optimum growing temperature is 35-37°C. These characteristics make *S. platensis* relatively easy to cultivate.

For the production of *Spirulina* sp. closed systems are suggested for effective control of growing parameters and preventing contamination. Photobioreactors utilized in the production of photosynthetic microorganisms are transparent plain panel reactors and helical tubular photobioreactors. The fundamental principle of the photobioreactor design is to achieve higher biomass densities by ensuring the cells in the cultivation area to benefit from light in the most efficient way.

The density of nutrient, lighting, temperature, beginning density, circulation flow rate, pH, water quality, macro and micro nutrients are principal environmental factors which affect the productivity of Spirulina (Habib *et al*., 2008).

To find out the changes in proximate compositions of *S. platensis* stemming from various stress sources (Salinity, temperature, etc.), a lot of studies have been carried out by Vonshak *et al.* (1996), Rafiqul *et al.* (2003), Koru and Cirik (2003). Rafiqul *et al.* (2003) determined variations in the levels of protein (38-61.5%), lipid (7.5-19.6%) and carbohydrate (20.1-41.4%) of *Spirulina*

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fusiformis cultivated in different salinities. The present study investigated the effects of different salinity ratios on the reproduction efficiency and proximate composition of *S. platensis*in a helical photobioreactor.

MATERIALS AND METHODS

The experiment was conducted in the Plankton Laboratory of Faculty of Fisheries in Mersin University. A helical tubular photobioreactor (Lu *et al*., 2001; Travieso *et al*., 2001; Soletto *et al*., 2008) was utilized in the experiment. The aim was to sustain the same instantaneous growth rate of the culture which has reached the maximum instantaneous growth rate through production in continuous system. The helical photobioreactor consisted of a transparent hose with inner diameter, length and volume of 1.8 cm, 80 m and 20.3 L, respectively. A stock tank which contained a constant volume of 4 L of alga was located on a platform above the helical part. The total volume of the system was 24.3 L. A peristaltic pump was used to ensure the circulation of the alga in the bioreactor. Four 40 W day light fluorescent lamps were used to provide constant lighting during the study (Fig. 1). CO_2 was continually added to the system for photosynthesis and pH stability.

The strain of *S. platensis* that was used as the experiment material was supplied by Cukurova University, Faculty of Fisheries. Spirulina medium (Schlosser, 1982) was utilized as the nutritional medium.

Medium of schlosser: About 13.61 g $NaHCO_3$, 4.03 g Na_2CO_3, 0.50 g K_2HPO_4, 2.50 g $NaNO_3$, 1.00 g K_2SO_4, 1.00 g NaCl, 0.20 g $MgSO_4 \cdot 7\ H_2O$, 0.04 g $CaCl_2 \cdot 2\ H_2O$. While 6 mL of metal solution (97 mg $FeCl_3 \cdot 6\ H_2O$, 41 mg $MnCl_2 \cdot 4\ H_2O$, 5 mg $ZnCl_2$, 2 mg $CoCl_2 \cdot 6\ H_2O$, 4 mg $Na_2MoO_4 \cdot 2\ H_2O$). Almost 1 mL of micronutrient solution (50.0 mg Na_2EDTA, 618 mg H_3BO_3, 19.6 mg $CuSO_4 \cdot 5\ H_2O$, 44.0 mg $ZnSO_4 \cdot 7\ H_2O$, 20.0 mg $CoCl_2 \cdot 6\ H_2O$, 12.6 mg $MnCl_2 \cdot 4\ H_2O$, 12.6 mg $Na_2MoO_4 \cdot 2\ H_2O$).

S. platensis was taken to culture at three salinity levels (10, 20, 30%) and daily cell increase and instantaneous growth rate were analyzed. Three replicates were made at each of the three salinity levels. Average initial densities were determined as $7.596 \times 10^6 \pm 410.0$ filament L^{-1} in 10% nutritional medium; $7.60 \times 10^6 \pm 379.5$ filament L^{-1} in 20% nutritional medium and $7.62 \times 10^6 \pm 219.5$ filament L^{-1} in 30% nutritional medium.

About 6.3 L of algae which had been cultivated using a batch method was inoculated in 18 L culture liquid. The total volume of the culture suspension was 24.3 L. Nutrient was added to the system through a dosage pump at a flow rate of 7 mL min^{-1} and 10 L of algae was harvested daily from the stock tank at the same flow rate.

Fig. 1: Helical photobioreactor used in the experiment

Temperature and pH were measured daily. The temperature was kept within the range 24.60-27.80°C and pH was kept within the range 8.78-9.82. The instantaneous growth rate of the Spirulina in the experiment was calculated according to the following formula (Abu-Rezq *et al*., 1999).

$$K = \frac{\ln N_t - \ln N_0}{t}$$

Where:

K = Instantaneous growth rate
N_t = The number of cells at the end of experiment
N_0 = The number of initial cells
t = Time period (day)

The single drop tecnique was used to calculate the number of filaments (Semina, 1978; Venrick, 1978). The samples which were going to be counted were diluted according to the density of the culture. Each sample was counted for 3 times and then the average number of filament was calculated for a volume of 1 mL.

Proximate analysis: Proximate analyses of the obtained samples were performed using dry matter. Algae samples were dried for 4-5 h at 103°C (Ludorf and Meyer, 1973) for obtaining dry matter. Total Mineral Substance (TMS) were obtained by burning them in an oven at (550°C) until gray ash was produced (AOAC, 1984).

The Kjeldahl method was used to determine the level of crude protein (AOAC, 1984) and the Bligh and Dyer (1959) method was used for lipid analysis. Values of carbohydrates were calculated mathematically.

Statistical analysis: Prior to the analyses all data were checked for outliers and homogeneity of variance was also tested. Statistical analysis of data was carried out with the SPSS statistical program. ANOVA (Analysis of Variance) was used to evaluate the effect of different salinity on the reproductive rates and proximate composition.

RESULTS AND DISCUSSION

During the experiment a density of 1.042×10^4 filament mL^{-1} was acquired from an average initial filament density of 7.596×10^3 filament mL^{-1} in 10% medium. A density of 7.409×10^3 filament mL^{-1} was acquired from an average initial density of 7.60×10^3 filament mL^{-1} in 20% medium. A density of 7.381×10^3 was acquired from an average initial density of 7.62×10^3 filament mL^{-1} 30% medium (Table 1). The cells were seen to be in a logarithmic growth phase between the 1st and 4th day in each of the three medium (Fig. 2).

The highest cell density and instantaneous growth rate were recorded on the 5th and 7th days in 10% nutritional medium (Fig. 2). The highest cell density was found to be 1.240×10^4 instantaneous growth rate was observed to be 0.013 division day 1 was found on the 5th day. Cell density was 1.373×10^4 filament mL^{-1}; instantaneous growth rate was 0.157 division day 1 on the 7th day.

The highest cell density was recorded on the 5th day in 20% nutritional medium. Besides, the highest instantaneous growth rate was recorded on the 4th day in 20% nutritional medium. At 5th day cell density was 8.563×10^3 filament mL^{-1}; instantaneous growth rate was 0.037 division day 1 in 20% nutritional medium. At 4th day cell density was 8.337×10^3 filament mL^{-1}; instantaneous growth rate was 0.270 division day 1 in 20% nutritional medium.

The highest cell density was recorded on the 11th day in 30% nutritional medium The highest instantaneous growth rate (0.503 division day 1) were recorded on the 2nd day in the 30% nutritional media. The average instantaneous growth rates were found to be 0,094 division day 1 in 10% medium; 0.085 division day 1 in 20% medium and 0.074 the division day 1 in 30% medium.

In terms of filament density, the 10% salinity group showed a statistically significantly variation on every day except for the 1st day when compared with the 20 and 30% groups ($p<0.05$). The 10% group showed a statistically significant variation on the 2nd and 7th days in terms of instantaneous growth rate when compared with the two other groups ($p<0.05$). In addition, a significance difference was found in 10% group in comparison to 30% group on 2, 3, 4 and 7 days ($p<0.05$). Variations were determined in proximate composition of *S. platensis* in different salinity ratios (Table 2, Fig. 3).

Table 1: Average density of filament of *Spirulina platensis* in 10, 20 and 30% nutritional medium

	Salinity		
Days	10% filament mL^{-1} ($X_{1,2,3}$)	20% filament mL^{-1} ($X_{1,2,3}$)	30% filament mL^{-1} ($X_{1,2,3}$)
1	$3.457\times10^3\pm230.0^a$	$3.350\times10^3\pm280.6^a$	$3.757\times10^3\pm171.5^a$
2	$6.850\times10^3\pm135.0^b$	$5.507\times10^3\pm449.3^a$	$6.247\times10^3\pm96.7^{ab}$
3	$8.270\times10^3\pm375.4^b$	$6.333\times10^3\pm303.4^a$	$6.980\times10^3\pm103.9^a$
4	$1.222\times10^4\pm264.6^b$	$8.337\times10^3\pm757.7^a$	$8.217\times10^3\pm603.8^a$
5	$1.240\times10^4\pm360.6^c$	$8.563\times10^3\pm127.8^b$	$7.353\times10^3\pm171.7^a$
6	$1.118\times10^4\pm953.9^b$	$8.040\times10^3\pm378.5^a$	$7.823\times10^3\pm428.5^a$
7	$1.373\times10^4\pm120.2^b$	$8.397\times10^3\pm236.7^a$	$8.077\times10^3\pm267.4^a$
8	$1.093\times10^4\pm425.6^b$	$8.090\times10^3\pm404.5^a$	$8.050\times10^3\pm270.6^a$
9	$1.180\times10^4\pm57.7^b$	$8.313\times10^3\pm459.6^a$	$8.123\times10^3\pm58.4^a$
10	$1.167\times10^4\pm33.3^b$	$8.133\times10^3\pm422.6^a$	$8.107\times10^3\pm399.4^a$
11	$1.153\times10^4\pm284.8^b$	$8.437\times10^3\pm176.8^a$	$8.460\times10^3\pm242.1^a$
$\sum_{ort}$	$1.042\times10^4\pm516.7$	$7.409\times10^3\pm297.7$	$7.381\times10^3\pm242.1$

*There is a statistical difference at the level of $p<0.05$ among the data which are shown by different letters in the same line in terms of density of filament

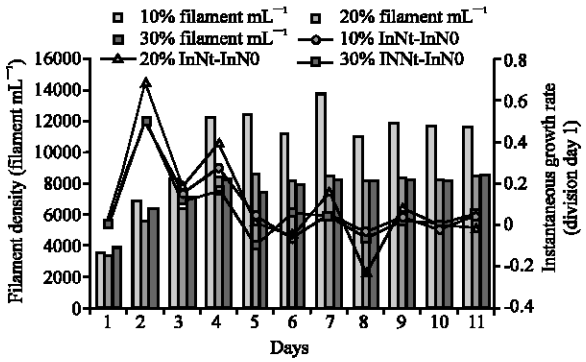

Fig. 2: Average density of filament and instantaneous growth rate of *Spirulina platensis* in 10, 20, 30% nutritional media

In terms of protein and TMS, algae cultivated in 10% salinity differed significantly from those cultivated in 20 and 30% salinity ($p<0.05$). In terms of lipid and carbohydrate levels, statistically significant variations were observed between all three nutritional medium ($p<0.05$). The level of carbohydrates and lipid values were observed to increase with increasing salinity.

But the level of protein values was observed to decrease with increasing salinity (Table 2, Fig. 3). The level of TMS was observed to be lower in 10% medium than those which had been cultivated in 20 and 30% nutritional medium. However, no statistically significant difference was observed between those which had been cultivated in 20 and 30% nutritional medium ($p>0.05$). The highest level of TMS was observed in algae cultivated in a 20% nutritional medium (Table 2, Fig. 3).

Table 2: Variations in proximate composition of *S. platensis* in different nutritional medium

	Salinity		
Parameters	10%	20%	30%
Protein	57.47±1.61[b]	51.87±0.74[a]	49.97±0.46[a]
Lipid	7.44±0.05[a]	8.41±0.05[b]	9.38±0.06[c]
TMS	10.14±0.09[a]	13.37±0.09[b]	13.24±0.04[b]
Carbohydrate	24.95±0.15[a]	26.35±0.21[b]	27.41±0.20[c]

*There is a significant difference at the level of $p<0.05$ among those which are shown by different letters in the same line

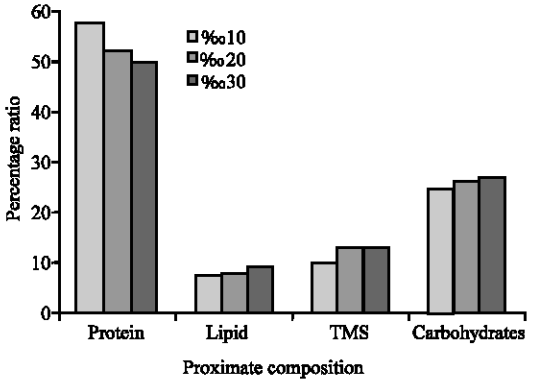

Fig. 3: Variations in proximate composition of *S. platensis* in different nutritional medium

The results indicate that in comparison to the 10% salinity media, the reproductive productivity of the other two nutritional media was lower ($p<0.05$). The 20 and 30% salinity media showed some similarities in terms of reproductive productivity. Specific growth rates of *S. platensis* were reported to be lower in increased salinity concentrations (Zeng and Vonshak, 1998). Kebede (1997) stated that the highest growth rate was achieved at the lowest salinity ratio for studies which were performed with various concentrations of $NaHCO_3$, NaCl and Na_2SO_4 salts (13-88%). The results presented by the researchers were similar to those obtained in the present study. Gokpinar (1983) indicated that salinity concentrations are one of the principal factors which affect phytoplankton cultures. In parallel with the increase in salinity, photosynthesis and protein synthesis capacities of the cells decrease and cells show reduced adaptation to higher salinity environments; higher salinity was found to lead to reduced growing rate.

Rafiqul *et al.* (2003) indicated that the growth rate of *S. fusiformis* showed an inverse relationship to salinity concentration and the study found that higher salinity led to lower growth rates. They also detected that biomass and growth rate decreased at higher salinity. The results presented by the researchers were similar to those obtained in the present study. Rafiqul *et al.* (2003) determined variations in the levels of protein (38-61.5%), lipid (7.5-19.6%) and carbohydrate (20.1-41.4%) of *Spirulina fusiformis* cultivated in different salinities. The ranges of nutritional components found by Rafiqul *et al.* (2003) support the findings of the present study (Table 2). Koru and Cirik (2003) determined variations in the protein levels (45.7-59.0%), lipid (7.4-11.3%) and carbohydrate (28.9-37.6%) of *S. platensis* cultivated at different temperatures. The ranges presented by the researchers were similar to those obtained in the present study (Table 2).

The present study found that carbohydrate, TMS and lipid values increased; those of protein decrease in parallel with the increase in salinity (Fig. 3). A reduction of approximately 13.05% occurred in the protein value, 26.08% increase in the lipid value, 31.85% increase in TMS and 9.86% increase in carbohydrate values were observed. It was found that the increase in TMS level occurred because the species accumulated much more inorganic matter in highly saline medium. Rafiqul *et al.* (2003) pointed out that the protein level of *S. fusiformis* cultivated in nutritional medium of different salinity decrease but lipid and carbohydrate levels increase with increasing salinity. Kebede (1997) found that the Nitrogen/Carbon rate (N/C,%) decreased as the salinity increased at the sodium bicarbonate ($NaHCO_3$) series. The results presented by the researchers were similar to those obtained in the present study (Table 2). Vonshak *et al.* (1996) stated that higher salinity leads to lower chlorophyll and protein content in *S. platensis* species.

CONCLUSION

In the present study, the protein metabolism slowed down because of stress from salinity but the speed of lipid and carbohydrate metabolisms increased.

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