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The Proximate Composition and Growth of *Spirulina platensis* Biomass (*Arthrospira platensis*) at Different Temperatures

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Abstract: *Spirulina platensis*, Cyanobacteria was cultivated in a batch culture system at different temperature (30±1, 35±1 and 40±1°C) for 10 days. The total volume of a glass container in batch culture was 2 L comprising of 1.9 L Spirulina medium and 0.1 L algae. It was determined to changes of cellular concentration, specific growth rate and proximate compositions (protein, lipid and total mineral substance) of *Spirulina platensis* which was produced in different temperature. Maximum cellular concentration of 30 and 40°C in batch cultures was found at 3rd and 6th days, 6.783 and 6.333 g L^{-1}, respectively. The highest cellular concentration was found out of 35°C at 6th day as 7.967 g L^{-1}. Maximum specific growth rate at 30°C was found out at 4th day as 0.34 division day^{-1}. The highest specific growth rates at 35 and 40°C was observed at 2nd day as 0.58 and 0.63 division day^{-1}, respectively. The most appropriate growth rate for *S. platensis* was detected at 35°C. The results of proximate analysis showed that carbohydrate, TMS (Total Mineral Substance) and lipid levels increased protein levels decreased in parallel with the increase temperature of culture medium. A reduction of approximately 8.63% occurred in the protein levels, 11.44% increase in the lipid levels, 10.40% increase in the TMS levels and 19.95% increase in carbohydrate levels were observed.

Key words: *Spirulina platensis*, temperature, proximate composition, cellular concentration, specific growth rate, Turkey

INTRODUCTION

Sprulina platensis, Cyanophyceae is a filamentous blue-green algae. This spiral prokaryotic blue-green algae species is composed of microscopic cells. Spirulina contains high levels of protein, pigments and gamma linoleic acid (Borowitzka, 1992; Cohen, 1997). Thus, it is extensive cultivated to be used which in human and animal food industry in cosmetic in pharmaceutical, etc. (Belay *et al*., 1996; Chen *et al*., 1996; Glazer, 1999; Wikfors and Ohno, 2001; Simpore *et al*., 2005; Sixabela *et al*., 2011).

Commercial mass culture of *S. platensis* as a food supplement began at the end of 1970s (Ciferri, 1983). The production of *S. platensis* has become popular because of this it is easy reproduction and high productivity (Kilic *et al*., 2006). Turkey has highly suitable in term of climatic conditions for cultivation of Spirulina. The range of optimum growing temperature of *Spirulina platensis* is 35-37°C. This species is also resistant to high pH levels (9-10). Temperature represents one of the most important biological limitations for the production of Spirulina biomass. It is an important environmental factor affecting it's all metabolic activities. Furthermore, temperature changes affect the growth and lipid contents of *S. platensis* (Tedesco and Duerr, 1989). The optimum growth temperatures for Spirulina species indicate differences (Vonshak, 1997). According to Tomaselli *et al.* (1987) laboratory experiments, the maximum biomass productivity is obtained when Spirulina grows up at 35°C optimal temperature. *S. platensis* grew up the most at 32 and 30-35°C. The most proper growth rate was detected as 35°C for *S. platensis* (Rafiqul *et al*., 2003). According to Richmond (1992), optimum temperature ranges for the growth rate of different *Spirulina* types were 35-37°C. He reported that it would definitely be damaged at 40°C.

To find out the changes in proximate compositions of *S. platensis*, a lot of studies have been carried out by Vonshak *et al.* (1996), Rafiqul *et al.* (2003), Yilmaz (2010) and Yilmaz *et al.* (2010).

The present study investigated the effects of different temperatures on the reproduction efficiency and proximate composition (protein, lipid, total mineral substance) of *S. platensis*in a batch cultured.

MATERIALS AND METHODS

Experiment: The experiment was conducted in the Plankton Laboratory of Faculty of Fisheries in Mersin University. Batch cultures were utilized in the experiment.

Spirulina platensis, Cyanobacteria was cultivated at different temperature (30±1, 35±1 and 40±1°C) for 10 days. The total volume of a glass container in batch culture was 2 L comprising of 1.9 L Spirulina medium and 0.1 L algae.

Culture medium (Schlosser): Spirulina medium was utilized as the nutritional medium. Medium of Schlosser: 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 $MgSO_4.7\ H_2O$, 0.04 g $CaCl_2.2\ H_2O$. About 6 mL of metal solution (97 mg $FeCl_3.6\ H_2O$, 41 mg $MnCl_2.4\ H_2O$, 5 mg $ZnCl_2$, 2 mg $CoCl_2.6\ H_2O$, 4 mg $Na_2MoO_4.2\ H_2O$). About 1 mL of micronutrient solution (50.0 mg Na_2EDTA, 618 mg H_3BO_3, 19.6 mg $CuSO_4.5\ H_2O$, 44.0 mg $ZnSO_4.7\ H_2O$, 20.0 mg $CoCl_2.6\ H_2O$, 12.6 mg $MnCl_2.4\ H_2O$, 12.6 mg $Na_2MoO_4.2\ H_2O$).

Daily cell concentration: The *S. platensis* samples and filter papers (Whatman ME 25 membrane filter 10 401 612) for the daily cell concentration were dried 105°C to reach constant weight (12 h). After, they were filtered (2 mL) and were calculated.

The daily cell concentration and specific growth rate of *S. platensis* were determined. Three replicates were made at each of the three temperatures. Average initial culture densities were find out as 4.58±1.06 g L^{-1} at 30°C; 4.78±0.97 g L^{-1} at 35°C; 4.57±0.11 g L^{-1} at 40°C.

DCC (Daily Cell Concentration, g L^{-1}) =
[Sample (2 mL)+Filter paper-Filter paper] × 500

Specific growth rate: The specific growth rate of the Spirulina in the experiment was calculated according to the following equation (Abu-Rezq *et al*., 1999):

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

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

Proximate analysis: Proximate analyses of the obtained samples were performed using dry matter. Algae samples were dried for 4-5 h at 103°C 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 temperature on the reproductive rates and proximate composition.

RESULTS AND DISCUSSION

Cellular concentrations: Maximum cellular concentration at 30 and 40°C batch cultures was found out at 6th and 3rd days, as 6.783 and 6.333 g L^{-1}, respectively. The highest cellular concentration was determined of 35°C at 6th days 7.967 g L^{-1} (Table 1).

Specific growth rates: Maximum specific growth rate at 30°C was found out at 4th day, as 0.34 division day^{-1}. The highest specific growth rates at 35 and 40°C was observed at 2nd day, as 0.58 and 0.63 division day^{-1}, respectively (Fig. 1).

Proximate compositions: The results of proximate analysis showed that carbohydrate, TMS and lipid levels increased protein levels decreased in parallel with the

Table 1: The cell concentrations of *Spirulina platensis* at different temperatures (g L^{-1})

Days	30°C	35°C	40°C
1	2.057±0.072^a	2.033±0.148^a	2.083±0.164^a
2	2.783±0.205^a	3.617±0.073^b	3.883±0.093^b
3	3.133±0.148^a	3.900±0.304^a	6.333±0.186^b
4	4.383±0.311^a	5.317±0.130^b	3.700±0.161^a
5	5.450±0.312^b	6.287±0.236^c	3.217±0.148^a
6	6.783±0.377^b	7.967±0.220^c	3.183±0.169^a
7	6.000±0.076^b	5.667±0.209^b	3.917±0.083^a
8	4.783±0.349^b	5.633±0.101^b	3.717±0.235^a
9	4.250±0.161^b	4.217±0.073^b	3.067±0.109^a
10	4.200±0.132^c	2.750±0.126^a	3.283±0.060^b

$\bar{X}\pm S_{\bar{x}}$: Mean±Standart Eror *There is a significant difference at the level of $p<0.05$ among those which are shown by different letters in the same line

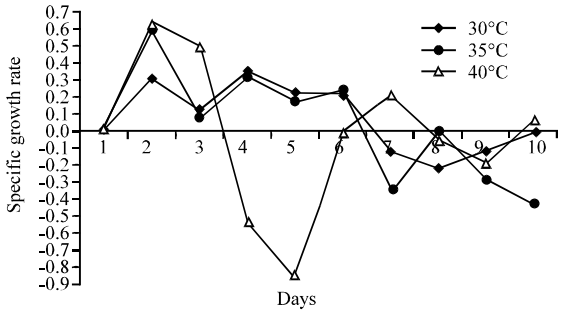

Fig. 1: The specific growth rates of *Spirulina platensis* at different temperatures

Table 2: The proximate compositions of Spirulina platensis at different temperatures (%)

Composition	30°C $\bar{X}\pm S_{\bar{x}}$	Min.-Max.	35°C $\bar{X}\pm S_{\bar{x}}$	Min.-Max.	40°C $\bar{X}\pm S_{\bar{x}}$	Min.-Max.
Protein	64.79±0.37^{c}	64.38-65.11	60.59±0.57^{b}	59.99-61.13	59.20±0.21^{a}	59.00-59.42
Lipid	6.82±0.08^{a}	6.77-6.910	7.22±0.08^{b}	7.15-7.300	7.60±0.18^{c}	7.45-7.800
TMS	8.94±0.06^{a}	8.88-9.000	9.35±0.11^{a}	9.22-9.430	9.87±0.38^{b}	9.60-10.30
Carbohydrates	19.45±0.37^{a}	19.10-19.83	22.88±0.49^{b}	22.50-23.43	23.33±0.57^{b}	22.73-23.85

*Different letters in the same row indicate significant differences ($p<0.05$); $\bar{X}\pm S_{\bar{x}}$: Average±Standart deviation

increase temperature of culture medium (Table 2). Protein and lipid levels of all groups were significantly ($p<0.05$) different.

The results indicate that, in comparison to the 40°C, the reproductive productivity of the other two different temperatures (30 and 35°C) was higher ($p<0.05$) (Table 1). These groups showed some similarities in terms of reproductive productivity.

Specific growth rates of *S. platensis* were reported to be higher in increased temperature of medium. In parallel with the increase in temperature, photosynthesis and protein synthesis capacities of the cells increase and higher temperature was found to lead to increase growing rate. According to the data obtained in the study, the highest cell concentrations at 35°C were detected as 7.967 g L^{-1}. Richmond (1988) and Ogawa *et al.* (1970) reported that the optimum temperature range for the growth of *S. platensis* was 35 and 37°C. Chanawongse (1992) found out that the optimum temperature for *S. platensis* was 35°C. They reported that the speed of growth rate of Spirulina cells decreased at 40°C. These results are similar to those obtained in this study. Oliveira *et al.* (1999) observed an increase in both of the species until they reached the optimum temperature. *S. maksima* productivity value dropped by 50% at 40°C and a lower percentage was reported for *S. platensis*. In the study, the growth rate for *S. platensis* at 40°C dropped by 50% as well. Danesi (2001) and Vonshak (1997) reported that the most appropriate growth rate for *S. platensis* was between 30 and 35°C. Besides, they found out thatthe heat of 40°C was certainly harmful for Cyanobacteria. Specific growth rate at 40°C decreased as -0.86 division day^{-1} after the 3rd day.

According to Chaiklahan *et al.* (2007) whereas when it was grown at the optimum temperature (35°C), *S. platensis* alg cells specific growth rate was detected as 0.0247 h^{-1}, it dropped down to 0.008 h^{-1} at 43°C. According to Richmond (1988), optimum temperature for different Spirulina types were between 35 and 37°C whereas at 40°C this species was negatively affected. These results are similar to those obtained in the study. The present study found that carbohydrate, TMS and lipid values increased; those of protein decrease in parallel with the increase in temperature (Table 2). A reduction of approximately 8.63% occurred in the protein levels, 11.44% increase in the lipid levels, 10.40% increase in the TMS levels and 19.95% increase in carbohydrate levels were observed. In this study, the protein metabolism slowed down because of stress from temperature but the speed of lipid metabolism increased.

CONCLUSION

While protein metabolism slows down in *S. platensis* species in accordance with temperature increase, an increase is observed in lipid and carbonhydrate metabolism. The detection of the metabolic characteristics of this species consumed by people will be fruitful for the production of it for industrial purposes.

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