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Original Research Article

A Comparison the Biomass of Productivity, Protein and Lipid Content of *Spirulina platensis* Cultured in the Pond and Photobioreactor*

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ABSTRACT

Dry matter, chlorophyll *a*, optical density, protein and lipid production of microalgae *Spirulina platensis* cultured in tubular and panel photobioreactors, and pond in June and September were compared. The highest dry matter of 4.951±0.03 gL^{-1} and 71.89±1% protein were determined from tubular photobioreactor, while the lowest dry matter of 2.710±0.02 gL^{-1} and 53.92±0.8% protein were recorded from panel photobioreactor. *S. platensis* cultured in the pond have 3.150±0.08 gL^{-1} dry matter and 70.29±0,8% protein. The water temperatures in June and September were similar, and it was observed that the culture in the panel was affected to the higher temperature (39.35±1.82 °C, 40.22±0.70 °C), negatively. The highest optical density (3.280±0.009) and lipid (7.74±0.5%) amounts were obtained from the culture produced in the tubular photobioreactors.

Key Words: *Spirulina platensis*, Outdoor culture, Photobioreactor, Dry matter, Protein

Fotobiyoreaktörlerde ve Havuzda Kültüre Alınan *Spirulina platensis*'in Ürün Verimliliği, Protein ve Lipid Miktarlarının Karşılaştırılması

ÖZET

Mikroalg *Spirulina platensis*, tubular ve panel fotobiyoreaktörlerde ve havuzda Haziran ve Eylül aylarında kültüre alınarak, kuru madde, klorofil *a*, hücre yoğunluğu, protein ve lipid miktarlarının karşılaştırması yapılmıştır. Tubular fotobiyoreaktörde 4.951±0.03 gL^{-1} kuru madde miktarı ve %71.89±1 protein oranı ile en yüksek değerler elde edilirken, 2.710±0.02 gL^{-1} kuru madde ve %53.92±08 protein miktarları ile en düşük değerler panel fotobiyoreaktörde saptanmıştır. *S. platensis*'in havuzdaki kültüründe ise 3.150±0.08 gL^{-1} kuru madde miktarı ve %70.29±0.8 protein oranı belirlenmiştir. Haziran ve Eylül denemelerinde sıcaklık ortalamalarının benzer olduğu ve panel fotobiyoreaktördeki kültürün yüksek sıcaklıktan (39.35±1.82 °C, 40.22±0.70 °C) olumsuz etkilendiği gözlenmiştir. Havuz, panel ve tubular fotobiyoreaktör arasında en yüksek optik yoğunluk (3.280±0.009) ve lipid (%7.74±0.5) ve miktarları tubular fotobiyoreaktörde üretilen kültürden elde edilmiştir.

Anahtar Kelimeler: *Spirulina platensis*, Dışarı kültür, Fotobiyoreaktör, Kuru madde, Protein

INTRODUCTION

Spirulina platensis, used as food for a long time is a filamentous microscopic cyanobacterium, takes it's name from the spiral structure of filaments. Highly alkaline medium is required for the growth of *S. platensis* (Zarrouk 1966). Optimum pH is from 8.5-11 for *S. platensis*. The high pH value in the medium is due to the CO_2 from in the bicarbonate and carbonate (Richmond 1988).

Optimum temperatures are 30-35 ºC for *S. platensis*. The species can live at the 18 °C minimum and 39 °C maximum (Fox 1996, Richmond 1992).

The biomass productivity of *S. platensis* cultured in the ponds or closed systems, photobioreactors is affected outdoor environmental factors considerably. In order to increase the biomass productivity for unit area and period different photobioreactor design. There are advantages and disadvantages of the ponds and *S. platensis*.

For the aim of to compare biomass productivity, lipid and protein content of *S. platensis* cultured in the ponds and panel and tubular photobioreactors, outdoor.

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MATERIALS AND METHODS

Spirulina platensis, cyanobacteria, multicellular and filamentous blue-green microalgae was used in this study. Dry matter, chlorophyll *a*, optical density, protein and lipid contend of the biomass obtained from tubular and panel photobioreactors and the pond in June and September were compared. The experiments were carried out in fiberglass ponds, 1x5x0.2 m in size $1m^3$ capacity, in tubular photobioreactor 300 L and panel photobioreactor 81 L. The cultures were grown in *Spirulina* medium. The content of the medium consists of the following composition (gl^{-1}): 18.6 $NaHCO_3$, 8.06 Na_2CO_3, 1.00 K_2HPO_4 5.00 $NaNO_3$, 2.00 K_2SO_4, 2.00 NaCI, 0.40 $MgSO_4.7H_2O$, 0.02 $CaCI_2.2H_2O$, 0.02 $FeSO_4.7H_2O$, 0.16 $EDTANa_2$ and micronutrient elements (0.001 $ZnSO_4.7H_2O$, 0.002 $MnSO_4.7H_2O$, 0.01 H_3BO_3, 0.001 $Na_2MoO_4.2H_2O$, 0.001 $Co(NO_3)_2.6H_2O$, 0.00005 $CuSO_4.5H_2O$, 0.7 $FeSO_4.7H_2O$, 0.8 $EDTANa_2$) were added 10 mL to 1 L, (Zarrouk 1966).

S. platensis stock cultures were maintained at 30±1 ^oC and continuous illumination with flourescent (Tekfen, TLD36 watt) lights in laboratory conditions. The light intensity was as measured by a Radiation Sensor LI-COR (LI-250) 80 µmol m^{-2} s^{-1}. Initial cultures in which erlenmayers (250 mL, 500 mL, 2 L) and carboys (5 L and 10 L) in laboratory conditions were used. The stock cultures in 10 L volume were adapted to outdoor climate conditions for one day aproximately before inoculation to the ponds and photobioreactors. The culture period continued to 14 days in the study started in the pond, tubular and panel photobioreactors in June. Pond, tubular and panel photobioreactors continued to 17, 10, 13 days for the culture period in September lasted, respectively. Tubular photobioreactor, diameter of the transparent acrylic tubes was 2.6 cm and tubes were set up horizontally. The CO_2 gas inlet was provided with flow meter. The pH and the flow rate of the cultures were adjusted to 9,5 and 0.3 m sec^{-1} respectively. The light intensity, pH, temperature, dry matter and chlorophyll *a*, optical density were measured daily. For lipid and protein analyses, samples of microalgae were collected in the stationary phase.

Dry matter was determined according to the method developed by Boussiba et al. (1992). Chlorophyll *a* was measured with 90% acetone of the solvent liquid (Parsons and Strickland 1963). Optical density was measured at 680 nm (Costa et al. 2003) a UV-VIS. spectrophotometer (Shimadzu, UV mini 1240 model). The amount of total protein was determined by Kjeldahl method (AOAC 1998). Lipids were extracted from the algae biomass according to the Bligh and Dyer (1959) method. All the applications were repeated three replicates.

Statistical analysis One-way analysis of variance (One-Way ANOVA) and analysis of the results differ depending on the case of differences in order to determine the purpose of treatment between the Duncan multiple comparison test and months t-test SPSSX 20.0 package program was used.

RESULTS AND DISCUSSION

S. platensis was cultured in open system, the pond and the closed system tubular and panel photobioreactor, outdoor in order to determine how the biomass was affected temperature and light. For this aim temperature (°C), pH, light intensity (µmol m^{-2} s^{-1}), chlorophyll *a* (μgl^{-1}), dry matter (gl^{-1}) and optical density (OD_{680}) were measured daily. Protein and lipid content of was determined from harvested biomass.

Table 1. Temperature and light intensites of *S. platensis* cultures.

Parameter Values		June	September
The average temperature (°C) of day (morning and midday)	**Tubular photobioreactor**	34.90±2.96^b	34.30±0.07^b
	Panel photobioreactor	37.78±2.80^a	36.80±0.5^a
	Pond	32.37±3.48^b	28.19±0.2^c
The average midday temperature (°C)	**Tubular photobioreactor**	37.63±0.7^b	38.03±0.29^b
	Panel photobioreactor	39.35±1.82^a	40.20±0.65^a
	Pond	34.96±1.78^c	31.14±0.25^c
		June	**September**
The average light intensity	**Tubular photobioreactor**	725.54 ±48^b	566.46 ±96^c

(µmol m^{-2} s^{-1}) of day (morning and midday)	**Panel photobioreactor**	781.26±94[b]	699.74±64[c]
	Pond	992.56±76[a]	538.86±46[c]
The average midday light intensity (µmol m^{-2} s^{-1})	**Tubular photobioreactor**	769.4±126[c]	653.4±18[c]
	Panel photobioreactor	828.43±94[b]	830.60±65[b]
	Pond	1053.10±113[a]	637.52±52 [c]

[a, b, c,] ($p<0.05$), Means values, n=5; *Different letters between the lines and columns indicate significant differences

Table 2. *S. platensis* of cultured in the pond and photobioreactors optical density, dry matter, chlorophyll *a* protein and lipid values.

Parameter Values (Last Day)		**June**	**September**
Optical Density (OD_{680})	**Tubular photobioreactor**	3.152±0.006[a]	3.280±0.009[a]
	Panel photobioreactor	2.726±0.001[b]	2.457±0.007[c]
	Pond	2.630±0.003[b]	2.319±0.005[c]
Chlorophyll *a* (µgL^{-1})	**Tubular photobioreactor**	502.2±3[a]	510.1±6 [a]
	Panel photobioreactor	302.1±3[c]	381.25±6[b]
		422.74±4[b]	517.2±5[a]
Dry matter (gL^{-1})	**Tubular photobioreactor**	4.951±0.008[a]	3.500±0.008[b]
	Panel photobioreactor	2.710±0.02[c]	2.766±0.004[c]
	Pond	2.900±0.008[c]	3.150±0.08[b]
Protein (%)	**Tubular photobioreactor**	71.89±1[a]	71,48±0.71[a]
	Panel photobioreactor	55.20±0.2[c]	53,92±0,8[c]
	Pond	69.15±0.2[b]	70,29±0,8[b]
Lipid (%)	**Tubular photobioreactor**	7.66±0.1[a]	7.74±0.5[a]
	Panel photobioreactor	5.48±0.2[c]	5.21±0.1[c]
	Pond	6.23±0.3[b]	6.35±0.2[b]

[a, b, c,] ($p<0.05$), Means values, n=5; *Different letters between the lines and columns indicate significant differences.

It was aim that different culture systems were compared in order to observe the biomassproductivity and protein and lipid content outdoor closed and open systems.

During the experiment dry matter, chlorophyll *a*, optical density protein and lipid amounts were determined (Table 2).

Microalgae *S. platensis* was made culured in the months of June and September. On June, the average temperature of the cultures (midday and morning) for tubular photobioreactor was 34.90±2.96 °C, for panel photobioreactor was 37.78±2.80 °C and for the pond was 32.37±3.48°C while the average temperature was 34.30±0.07 °C, 36.80±0.5 °C and 28.19±0.2 °C on September, respectively. Even though the two experiment were conducted on the different seasons, the temperatures of June and September were similar in Cukurova climate conditions (Table 1). The temperatures average at midday were determined as similar in these two months. The average temperatures at the midday and mornings are found similar except for the pond amounts. For the growth of *S. platensis*, the most appropriate temperature is known as 35-38 °C (Richmond 1986). Generally, all the temperatures measured were optimum for *S. platensis* culture.

Travieso (2003) et al. reported helical tubular photobioreactor was operated, under steady-state conditions, for *Spirulina sp.* production as a semi continuous reactor using different dilutions. a maximum value of 5.82 dry weight was obtained. In the study, the highest optical density values were measured in tubular photobioreactor during June and September. The optical density values determined for the panel photobioreactor and the pond were lower than tubular photobioreactor (Table 2). The highest dry matter of 4.951±0.03 gL^{-1} was determined from tubular photobioreactor, while the lowest dry matter of 2.710±0.02 gL^{-1} was recorded from panel photobioreactor. *S. platensis* cultured in the pond determined as 3.150±0.08 gL^{-1} dry matter. The results about biomass productivity can be explained that the water temperatures in June and September were similar, and it was observed that the culture in the panel was affected negative to the higher temperature (39.35±1.82 °C,

40.22±0.70 °C). The highest optical density (3.280±0.009) and dry matter (4.951±0.008) amounts were obtained from the culture produced in the tubular photobioreactors (Table 1, 2).

During the experiments, the highest chlorophyll *a* amounts of 517.2±5 μgL^{-1} and 510.1±6 μgL^{-1} were obtained from the pond culture and tubular photobioreactor during September. These amounts was followed by the pigment amounts of tubular photobioreactor and the pond on June (Table2). In both June and September, the least pigment amounts was belonged to the panel photobioreactor (302.1±3 μgL^{-1} and 381.25±6 μgL^{-1}).

When we consider that the the midday temperature of June and September in tubular photobioreactor 37.63±0.7 °C, and 38.03±0.29 °C respectively, in pond 34.96±1.78 °C and 31.14±0.25 °C, in panel 39.35±1.82 °C, and 40.22±0.70 °C, the green pigment chlorophyll *a* was considered to be effected negatively by the high temperature.

In the study, the highest protein contents of 71.89±1% and 71,48±0.71% respectively were obtained in tubular photobioreactor during June and September. The protein rates 69.15% and 70.29% of the biomass were obtained from the pond the protein rates of 55.20% and 53.92% were obtained from panel photobioreactor for June and September, respectively (Table 2).

Göksan (2007) et al., were cultured *S. platensis* in transparent jars, polyethylene bags and in the ponds and determined growth characteristics of the microalgae. At the end of the experiments, the calculated protein amounts for transparent jars, bags and pond cultures were determined as 33.4%, 54.5% and 58.3%, respectively. The protein amounts in the transparent jars were lower than other. It was thought that the reason for this, temperature of the culture is higher than others. In the our study in the panel photobioreactor on June and September the midday temperature of 39.35±1.82 °C and 40.22±0.70 °C affected the protein content negatively.

In this study, tubular and panel photobioreactor and in the pond, *S. platensis* lipid amount, it was no observed differences in *S. platensis* cultured in different systems, there were differences in the amount of the lipid amount (Table 2). In this study, the highest lipid amounts were obtained as 7.66% and 7,44% in the tubular photobioreactor. The lipid amounts were determined as 6.23% and 6.35% in the pond. The lowest lipid amounts were determined as 5.48% and 5.21% in the panel photobioreactor. Koru and Cirik (2003) reported, the temperatures of 28 °C to 45 °C, *S. platensis* culture and the affected metabolism was researched and the lipid rates were determined as relatively reported 7.4% and 11.3%. They reported that when the temperature increased, the lipid rate increased, too. In this study, unlike others, in the high temperature, less lipid amounts was determined. In this study in the high temperature. There is a decline in lipid and protein amounts.

CONCLUSION

As a result of this study, in the climate conditions of the conducted study, much higher biomass, protein and lipid amounts are obtained from photobioreactor in comparison with the pond and panel photobioreactor. On June and September, on the experiments, panel photobioreactors are much more affected by sunlight and its culture temperature is much higher.

Koru and Cirik (2003), determined that the lipid content of 7.4% and 11.3% in *S. platensis* culture in temperature and the microalgae metabolism affected. When tubes of tubular photobioreactor are kept in the pond, overheating was prevented. Accordingly Cukurova conditions on June and September without cooling take measures on the culture in the panel was negatively affected to the higher temperature.

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