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Effect of different conditions on the production of chlorophyll by *Spirulina platensis*

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Abstract

The cyanobacterium *Spirulina platensis* is an attractive alternative source of the pigment chlorophyll, which is used as a natural color in food, cosmetic, and pharmaceutical products. In this work, the influence of the light intensity and temperature using Zarrouk media and RM-6 media through batch cultivation for *S. platensis* on growth and chlorophyll content was examined. Cultivation was carried out in 3 L culture vessel, with 2 klux, 3 klux, 4 klux, 5 klux light intensity at constant temperature 28±1^{0}C, and 26^{0}C, 28^{0}C, 30^{0}C, 32^{0}C. The best growth was observed with 5 klux and 28^{0}C, whereas the highest chlorophyll in the biomass was observed with 2 klux and 28^{0}C. Overall, the best chlorophyll productivity was observed with 3±1 klux light intensity and 28^{0}C temperature.

Keywords: *Spirulina platensis*; Culture media; Chlorophyll content; Light intensity; Temperature

Introduction

Spirulina platensis is a cyanobacterium that has been largely studied due to its commercial importance as a source of protein, vitamins, essential amino acids, and fatty acids (Ciferri and Tiboni, 1985). Photosynthesizing microorganisms, like *Spirulina platensis*, can be an alternative source of proteins for food and feed purposes. Also, there is the possibility of obtaining pigments such as carotenoids, phycocyanin and chlorophyll (Henrikson, 1989). Chlorophyll a, due to its stability properties, has been widely used as a coloring substance. This substance is conventionally obtained from higher plants, in which occurs the synthesis of other kinds of chlorophyll. Therefore, adequate extraction and separation processes are required for the utilization of chlorophyll from plants. The *S. platensis*, on the contrary, presents only chlorophyll a on its composition. In addition, this micro alga presents one of the highest chlorophyll contents found in

nature, corresponding to 1.15% of its biomass (Henrikson, 1989). The use of the *Spirulina* sp for pigments as colorant has already been explored by the cosmetic, pharmaceutical and food industries. Phycocyanin, a blue pigment, is used as colorant for food and drinks in Japan. The world trend for colorants is to substitute artificial products for natural ones, which suggests the possibility of exploring Spirulina sp for this purpose, because this micro alga is one of the largest sources of chlorophyll in nature. In Brazil, the chlorophyll used as natural green colorant is obtained from spinach, which contains approximately 0.06 mg g^{-1} (Gross, 1991), whereas the *Spirulina* sp biomass contains 1:15 mg g^{-1} of this pigment (Henrikson, 1989). The use of fermentation processes possesses a number of advantages when compared to vegetable sources, including the possibility of continuous cultivation, and the rapid multiplication of microorganisms (Taylor, 1984). It has been shown that the composition of the cultivation medium, cellular age, and light intensity are the main factors influencing chlorophyll content in *S. platensis* biomass. Cultivations carried out under poor illumination conditions present higher biomass chlorophyll content than cultivations carried out under high illumination conditions, suggesting an inverse proportional relationship between light intensity and chlorophyll content (Bogorad, 1962; Eloranta, 1986). Moreover, the use of high light intensity in *S. platensis* cultivation can lead to two main effects: (i) photoinhibition, decreasing the cellular growth rate, and (ii) photo oxidation, with severe cell damage and, in extreme cases, total loss of the cultivation (Jensen and Knutsen, 1993; Vonshak *et al*., 1994). Although photoinhibition usually occurs at light intensities above the saturation of the photosynthetic rate, this phenomenon can be observed at light intensities below the saturation of the photosynthetic rate in cultivations under stress conditions, such as low temperatures (Samuelson *et al*., 1985). According to Piorreck *et al*., (1984), the concentration of chlorophyll in *S. platensis* biomass increases with an increase of nitrogen concentration in the cultivation medium. Based on these facts, the utilization of *S. platensis* for chlorophyll production can be considered an interesting alternative to be studied. In this work, the influence of

light intensity and temperature on *S. platensis* growth and its chlorophyll content was investigated.

Material and methods

Micro alga, Culture media and growth condition

Spirulina platensis was grown in Zarrouk's media (Zarrouk, 1996) and another media was Revised medium 6 (Raoof. *et al*.). Experiment were carried out in 3 L culture vessel and agitation was provided by aeration pump. The standard cultivation corresponds to ones curried out utilizing the both standard culture media. In the first standard cultivation four different light intensity was arranged; 2 klux, 3 klux, 4 klux, 5 klux at a temperature of 28±1 ^{0}C. The light intensity varied through the adjustment of fluorescent lights in the separate culture shelf. Forty watt fluorescent lamps (Phillips India) were employed, and the light intensity was measured using a Lutron (Taiwan) luxmeter. The total volume of the cultivations was kept constant through daily replacement of the water lost by evaporation. The initial pH of culture media was 9.0± 0.2. Harvesting time of culture was 16 days from the inoculation day.

In second standard cultivation four different temperatures were arranged 26^{0}C, 28^{0}C, 30^{0}C, 32^{0}C in separate culture shelf at fixed light intensity 3 klux in all culture shelves. The temperatures were maintained by thermostats. Harvesting time after 20 days from the inoculation day and initial pH of both media was 9.0±0.2. One control was kept in all experiment at the temperature 28±1^{0}C and 3 klux light intensity and the pH of media was 9.0±0.2 for comparison with the experimental culture flasks.

Preparation of inoculums

Algal biomass was collected by filtration using filter paper 8 μm pore size (Screen printing paper) than cells were washed with buffer solution (pH 7), diluted to known volume and processed for further inoculation. Diluted inoculums shake in cyclomixture for making homogenized mixture at a temperature of 28±1^{0}C and light intensity of 3 klux. For all experiments, the starting cellular concentration was 50 mg/l.

Biomass analysis

The cellular concentration was determined by measurements of optical density at 560 nm. The chlorophyll biomass content (mg /g) was determined spectrophotometrically at 661 nm from a fresh biomass on a D5 spectrophotmeter (Electronics India) following the methods of Mackinney (1941). The protein content was (% of dry weight) determined by Lowery *et al*., (1951) methods. *Spirulina platensis* dry weight was determined by filtration through screen printing paper (pore size of 8 μm) and oven dried at low temperature for 4 to 6 hours. The dry weight was expressed as g/l.

Kinetic parameters calculation

The total chlorophyll was calculated as the product of the chlorophyll biomass content by the corresponding cellular concentration and the total cultivation volume. The chlorophyll productivity (P_C) was calculated dividing the total chlorophyll by the total cultivation volume and the cultivation time T.

Results and discussion

The results presented in Fig. 1 show that the best cellular growth was observed at 5 klux and the lowest at 2 klux. From the results in Fig. 1 and Tables 1-2, it can be inferred that, even at the highest light intensity used, photoinhibition and/or photooxidation may not have occurred. Although the best growth was observed at 5 klux, the biomass from cultivations at 2 klux presented higher contents of chlorophyll than that grown at 5 klux.

It should be pointed out that *S. platensis* requires more light for photosynthesis and cellular growth than other cyanobacteria, since it grows under high salinity and pH conditions (Kebede, *et al*.,1996).

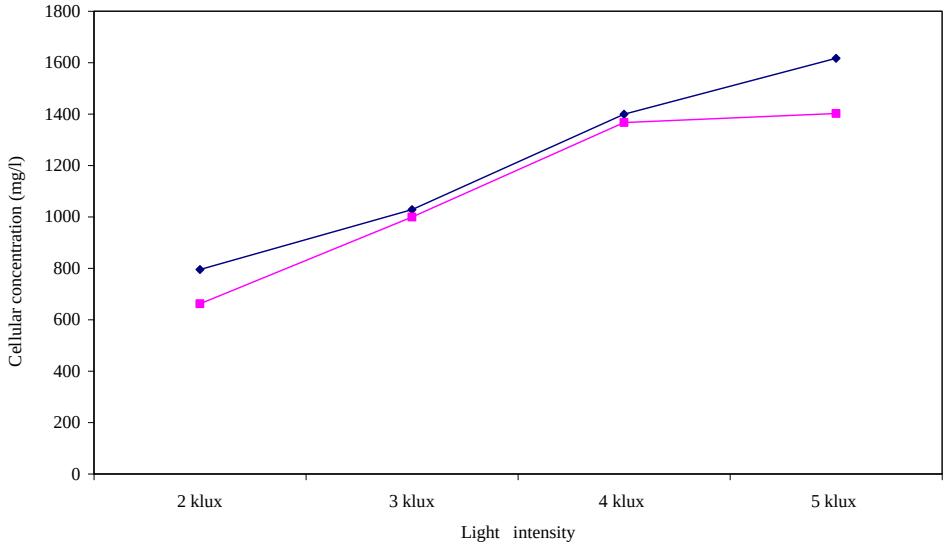

Fig-1 Growth curves of S. pletensis cultivations under different light intensity using two media: (♦) Zarrouk media; (■) RM-6 media

Table 1- Effect of light intensity on the growth and chlorophyll content in *S. platensis*.

Final cellular concentration (C_f), temperature 28±1^{0}C,

Parameters	Control	Experimental protocol							
		1[a]	2[a]	3[b]	4[b]	5[c]	6[c]	7[d]	8[d]
C_f (mg/l)	1362	786	804	642	682	1044	1012	996	1002
Chlorophyll biomass content (mg/g)	13.3	15.9	15.1	14.7	14.9	13.8	13.6	13.1	12.8
Protein biomass content (%)	61.1	59.2	60.2	61.1	58.1	61.5	62.0	61.7	61.2
Total chlorophyll per cultivation *	54.3	37.5	36.4	28.3	30.5	43.2	41.3	39.1	38.5
Chlorophyll productivity (P_c)(mg/l/day)	1.1	0.8	0.7	0.6	0.6	0.9	0.8	0.8	0.8

[a] Experiment carried out with Zarrouk media at 2 klux. [b] Experiment carried out with RM-6 media at 2 klux.

[c] Experiment carried out with Zarrouk media at 3 klux. [d] Experiment carried out with RM-6 media at 3 klux.

*Considering 3 l of cultivation Cultivation time was 16 days.

Table 2: Effect of light intensity on the growth and chlorophyll content in *S. platensis*.

Final cellular concentration (C_f), temperature 28±1^{0}C,

Parameters	Experimental protocol							
	9[e]	10[e]	11[f]	12[f]	13[g]	14[g]	15[h]	16[h]
C_f (mg/l)	1396	1402	1356	1378	1592	1642	1408	1396
Chlorophyll biomass content (mg/g)	11.6	12.3	10.2	10.8	5.9	6.1	6.3	7.0
Protein biomass content (%)	63.1	66.1	65.5	62.4	65.2	67.1	64.3	63.3
Total chlorophyll per cultivation *	48.6	51.7	41.5	44.6	28.2	30.0	26.6	29.3
Chlorophyll productivity (P_c)(mg/l/day)	1.0	1.0	0.8	0.9	0.6	0.6	0.5	0.6

[e] Experiment carried out with Zarrouk media at 4 klux. [f] Experiment carried out with RM-6 media at 4 klux.
[g] Experiment carried out with Zarrouk media at 5 klux. [h] Experiment carried out with RM-6 media at 5 klux.
*Considering 3 l of cultivation. Cultivation time was 16 days.

This light effect was observed in this work for both culture media in batch cultivation. Moreover, it also has been reported a possible ability of *S. platensis* cells to regulate its photosynthetic efficiency by varying the pigments content. Concerning to the culture media studied, its clear that the use of Zarrouk media is better then the use of RM-6 media.

Based on the results presented in Tables 1-2 and Fig-1, in the cultivation the higher light intensity applied to the reactor would be favorable to the cellular growth, aiming to achieve high cellular concentration. Then the light would be lowered to favor the chlorophyll accumulation (Kebede *et al.*,1996).

Cultivation carried out with 5 klux in Zarrouk media, a chlorophyll biomass content of 6.1±1.2 mg/g was obtained, similarly, cultivation carried out with RM-6 media 6.2±1.0 mg/g chlorophyll content obtained. But as the light intensity was decreased the chlorophyll biomass content increased and concentration of biomass was decreased. In the Table 1-2 results was shown that chlorophyll content in 4 klux, 3 klux, 2 klux was 11.8±0.5 mg/g, 12.9±1mg/g, 14.6±1.1 mg/g respectively; and biomass concentration was 1399±10 mg/l, 998±11 mg/l, 795±15 mg/l respectively.

Table 2 shows that highest chlorophyll per cultivation was obtained in 4 klux light intensity with both Zarrouk and RM-6 media and here the higher cellular concentration was also observed.

In the second experiment using different temperatures, the best results (Table 3-4) of cellular concentration were obtained in the cultivation at 28 ± 1^0C with both culture media (Fig-2). This result is in line with observations made by Jensen and Knutsen (1993), and Vonshak, *et al.*, (1996).

Table 3: Effect of temperature on the growth and chlorophyll content in *S. platensis*.

Final cellular concentration (C_f), light intensity 3 klux, temperature (T)

Parameters	Control	Experimental protocol							
		1[a]	2[a]	3[b]	4[b]	5[c]	6[c]	7[d]	8[d]
C_f (mg/l)	1306	112	120	110	108	159	149	112	106
Chlorophyll biomass content (mg/g)	11.6	2	1	0	6	1	0	9	8
Protein biomass content (%)	63.6	12.3	10.3	10.7	11.2	11.6	10.9	10.3	11.2
Total chlorophyll per cultivation *	45.4	60.4	65.4	60.1	59.8	65.6	61.1	60.1	61.6
Chlorophyll productivity (P_c)(mg/l/day)	0.7	41.4	37.1	35.3	36.5	55.4	34.9	34.9	35.9
		0.7	0.6	0.6	0.6	0.9	0.6	0.6	0.6

[a] Experiment carried out with Zarrouk media at T- 26^{0}C. [b] Experiment carried out with RM-6 media at T -26^{0}C.

[c] Experiment carried out with Zarrouk media at T- 28^{0}C. [d] Experiment carried out with RM-6 media at T -28^{0}C.

*Considering 3 l of cultivation. Cultivation time was 20 days.

Table 4: Effect of temperature on the growth and chlorophyll content in *S. platensis*.

Final cellular concentration (C_f), light intensity 3 klux, temperature (T)

Parameters	Experimental protocol							
	9[e]	10[e]	11[f]	12[f]	13[g]	14[g]	15[h]	16[h]
C_f (mg/l)	1396	1405	1262	1213	1191	1091	1051	942
Chlorophyll biomass content (mg/g)	12.3	11.9	10.7	10.5	9.9	8.7	8.6	9.1
Protein biomass content (%)	64.3	67.1	63.1	60.9	62.3	61.5	60.2	59.1
Total chlorophyll per cultivation *	51.5	50.1	40.5	38.2	35.4	28.5	27.1	25.7
Chlorophyll productivity (P_c)(mg/l/day)	0.8	0.8	0.6	0.6	0.6	0.5	0.4	0.4

[e] Experiment carried out with Zarrouk media at T-30^{0}C. [f] Experiment carried out with RM-6 media at T-30^{0}C.

[g] Experiment carried out with Zarrouk media at T-32^{0}C. [h] Experiment carried out with RM-6 media at T-32^{0}C.

*Considering 3 l of cultivation. Cultivation time was 20 days.

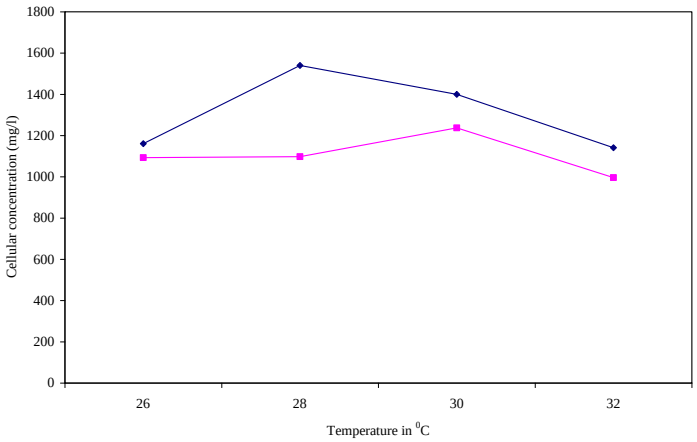

Fig-2 Growth curves of S. pletensis cultivations under different tenperature using two media: (♦) Zarrouk media; (■) RM-6 media

Fig 4 has shows the higher chlorophyll obtained at the temperature 28^{0}C and the productivity of chlorophyll was highest in 28^{0}C with Zarrouk media. The results at 32^{0}C showed a decrease in the cellular growth in all of the cultivations, in second experiment. Also at high temperature, the water loss could have provoked changes in the osmotic pressure in culture medium causing damage to the cells.

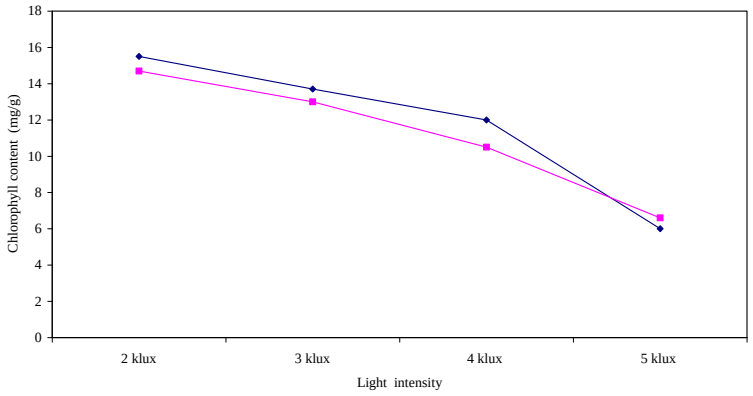

Fig-3 Chlorophyll biomass content of S. pletensis cultivations under different light intensity using two media: (♦) Zarrouk media; (■) RM-6 media

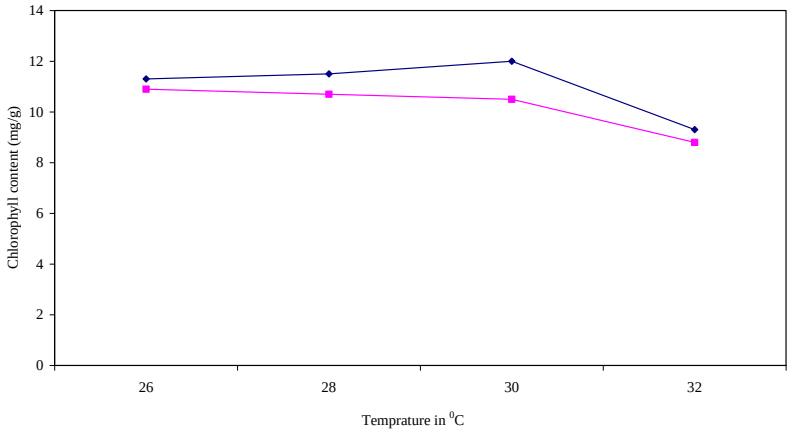

Fig-4 Chlorophyll biomass content of S. pletensis cultivations under different tenperature using two media: (♦) Zarrouk media; (■) RM-6 media

The pH of the order of 11 at the end of the cultivation hindered the absorption of the inorganic carbon source, because in this pH the carbonate form is predominant and the bicarbonate form is the one utilized by the microorganism and the cultivation with higher saline concentration had lower chlorophyll and protein contents (Vonshak, *et al*., 1996). The contents of proteins and lipids were not influenced significantly by the experimental conditions and their high values prove the importance of this biomass for nutritional value (Richmond, 1988).

Conclusion

Spirulina platensis was cultivated at different light intensity and different temperature, utilizing two different media. The results show that better biomass productivity and chlorophyll production values are observed with Zarrouk media at 28^0C temperature and 3.5±0.5 klux light intensity, when compared to RM-6 media.

References

Bogorad, L., 1962. Chlorophylls. In: Lewin, R.A. (Ed.), Physiology *and Biochemistry of Algae*. Academic Press Inc, New York.

Ciferri, O., Tiboni, O., 1985. The biochemistry and industrial potential of Spirulina.*Ann. Rev. Microb*. **39**: 503–526.

Eloranta, P., 1986. Paper chromatography as a method of phytoplankton community analysis. *Ann. Bot. Fennici* **23**: 53–159

Gross J. 1991. Chlorophylls. In: Reinhold VN, editor. *Pigments in*

vegetables—chlorophylls and carotenoids. New York: AVI, 1991. p. 3–74.

Hendry, G.A.F., Houghton, J.D. (Eds.), (1996) *Chlorophylls and chlorophyll derivates natural Food Colorants.* Blackil Academic Professional, London, pp. 131–155.

Henrikson R. 1989. Earth food *Spirulina*. California, Ronore Enterprises Inc. 180p.

Jensen, S., Knutsen, G., 1993. Influence of light and temperature on photoinibition of photosynthesis in *Spirulina platensis*. *J. Appl. Phycol*. **5**: 495–504.

Kebede, E., Ahlgren, G., 1996. Optimum growth conditions and light utilization efficiency of *Spirulina platensis* (Arthrospira fusiformis)Cyanophyta. *Hydrobiologya* **332**: 99–109.

Lowry, O.H., Rosebrough, N.L., Farr, A.L. and Radall, R. J. (1951). Protein measurement with the folin-phenol reagent. *J. Bio. Chem*. **193**: 265-275.

Mackinney, G. (1941). Absorption of light by chlorophyll solution. J. *Biol. Chem*. 140: 466-469.

Piorreck, M., Baasch, K.-H., Pohl, P., 1984. Biomass production total protein, chlorophyll, lipids and fatty acids of freshwater green and blue–green algae under different nitrogen regimes. *Phytochemistry* **23**: 207–216.

Raoof, B., Kaushik, B.D., Prasanna, R. 2006. Formulation of a low-cost medium for mass production of *Spirulina*. *Biomass and Bioenergy*. **30**(6), 537-542

Richmond A. Spirulina. 1988. In: Borowitzka MA, Borowitzka LJ, editors. *Microalgal biotechnology.* Cambridge: Cambridge University Press, p. 85–119

Samuelson, G., L€onneborg, A., Rosenqvist, E., Gustafsson, P., €OOquist, G., 1985. Photoinhibition and reactivation of photosynthesis in the cyanobacteria *Anacystis nidulans*. *Plant Physiol*. **79**: 992–995.

Taylor, A.J., 1984. Natural colours in food. In: Walford, J. (Ed.), *Developments in Food Colours.* Academic Press Inc, New York.

Vonshak, A., Kancharaksa, N., Bunnag, B., Tanticharoen, M.,1996. Role of light and photosynthesis on the acclimation process of the cyanobacteria *Spirulina platensis* to salinity stress. *J. Appl. Phycol*. **8**:119–124

Vonshak, A., Torzillo, G., Tomaseli, L., 1994. Use of chlorophyll fluorescence to

estimate the effect of photoinhibition in outdoor cultures of *Spirulina platensis*. *J. Appl. Phycol.* **6**: 31–34.

Zarrouk, C. 1966. Contribution à l´étude d´une cyanophycée influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima* (Setch. et Gardner) Geitler (Ph. D. thèse). Université de Paris.