



## **Standardization of pH and Light Intensity for the Biomass Production of *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 the present investigation, the influence of light intensity and pH for *Spirulina platensis* growth, protein and chl a content were examined. In the present investigation the production of *Spirulina platensis* was optimized in terms of biomass and metabolites. The dry weight of *Spirulina platensis* was 0.91g/500ml and protein and Chlorophyll a content were 64.3% and 13.2mg/gm respectively at pH 9. At 5 Klux light intensity the dry weight of *Spirulina platensis* was 0.85g/500ml while protein content and Chlorophyll a were 64.3% and 9.8mg/gm respectively.

Keywords: *Spirulina platensis*, Biomass, Chlorophyll a, Light intensity, pH, Protein.

### **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; Zhang et al., 1999). Photosynthetic cyanobacterium *Spirulina platensis* has been cultivated for mass production in many countries in tropic,

subtropical and temperate regions for use in human health food, as an animal feed additive (Ciferri, 1983; Belay et al. 1993), making it one of the most commercialized microalgae. More recently, special attention has been given to *Spirulina platensis* as a potential source of pharmaceuticals, and other high value products such as chlorophyll (Borowitzka, 1995). The utilization of chlorophyll from *Spirulina platensis* is an attractive

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alternative that should be considered due to its high content of this pigment, and ease of cultivation. The cyanobacterium *Spirulina platensis* possesses a high tolerance to alkaline pH, for ease of cultivation; a large size for its cell aggregates for ease of harvest, and an easily digestible cell wall (Jensen and Knutsen, 1993).

To produce high quality biomass, much attention must be paid to culture status. Generally, in cultivation of cells, dry cell weight has been used to obtain information on cell growth with respect to biomass productivity or specific growth rate. Chlorophyll *a* concentration can also be used to determine cell activity and has even been used in remote estimation for determination of harvesting time and nutrient addition time in indoor cultivation of *Spirulina platensis* (Gitelson et al., 1995). The pH value of the culture medium combined with dry cell weight may be a simpler, indirect method for determining the degree of the cell growth of *Spirulina platensis*. This is because the pH gradually rises as bicarbonate added to the culture medium is dissolved to produce CO<sub>2</sub>, which releases OH<sup>-</sup> during cultivation of *Spirulina platensis* (Richmond and Grobbelaar., 1986). However, because the increased pH acts as an autoinhibitor of cell growth, it has been

suggested that controlling the pH of the culture medium is necessary (Richmond, 2000).

It has been shown that the composition of the cultivation medium, cellular age, and light intensity are the main factors influencing chlorophyll content in *Spirulina 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). Moreover, the use of high light intensity in *Spirulina platensis* cultivation can lead to two main effects: (i) photo inhibition, decreasing the cellular growth rate, and (ii) photooxidation, with severe cell damage and, in extreme cases, total loss of the cultivation (Jensen and Knutsen, 1993; Vonshak et al., 1994). Although photo inhibition 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.

The aim of this work was to study the effects of pH, temperature, and light intensity on the growth of *Spirulina*

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*platensis*. Additionally, protein and chlorophyll a production was also examined at different physical conditions.

## MATERIALS AND METHODS

### *Microorganism and culture medium*

The cyanobacterium *Spirulina platensis* was used in this study. The strain of *Spirulina platensis* was obtained from School of Studies in Biotechnology Jiwaji University Gwalior M.P., which is previously maintained in Zarrouk's agar media slants in 4°C. (Zarrouk., 1966). All the reagents used were of analytical grade, obtained from the Rankam Chemical Co.

### *Cultivation*

*Spirulina platensis* was axenically grown in Zarrouk's medium. Firstly we had transferred our culture to Zarrouk's broth from Zarrouk's agar slant. Cultures were incubated in a culture room at temperature of  $30 \pm 1^\circ\text{C}$  and illuminated with day- light fluorescent tubes having 4Klux at the surface of the vessels. During the process of growth the flask was shaken 3 to 4 time/day. The experiments were run in duplicates. All manipulations involving the transfer of cultures in the liquid media or on agar plates were carried out under aseptic conditions on a laminar flow chamber.

**Filtration:** - Cells were collected by filtration using filter paper 8 mm pore size (Screen printing paper).

**Washing:** - Cells were washed with buffer solution (pH 7), diluted to known volume and processed for further inoculation.

**Shaking in cyaclomixture:-** Diluted inoculum shaken in cyaclomixture for making homogenized mixture. Analysis of variance (ANOVA) was used to compare the data during experiments.

### *Analytical methods*

Biomass concentration ( $\text{gl}^{-1}$ ) was calculated by measuring dry weight. For dry weight measurement homogenous suspensions of known quantity of *Spirulina* sample were filtered through screen-printing paper and oven dried at  $75^\circ\text{C}$  for 4 to 6 hours. The dried filter paper containing *Spirulina* biomass were cooled and weighed. The difference between the initial and final weight were taken as the dry weight of *Spirulina* biomass. The dry weights were expressed in terms of g/l. Chlorophyll a was estimated by the Mackinney method (Mackinney, 1941). Protein was determined by the Lowry method (Lowry, *et. al.* 1991).

As pH is important for the growth of *Spirulina* for biomass, different pH

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levels viz. 7, 8, 9, 10, 11, 12 were set for the experiment. The pH was adjusted with the help of 8 M NaOH and 1N HCl solution. Flasks were prepared as described above and inoculated with equal biomass concentration of *Spirulina platensis* in 500ml-modified Zarrouk's media. Subsequent harvesting for biomass estimation was done after 20 days of growth.

As light is important for the photosynthesis of *Spirulina platensis*, different light intensities such as 3 Klux, 3.5 Klux, 4 Klux, 4.5 Klux and 5 Klux light were set for the light intensity test (The culture was prepared in flasks as explained before). The flasks were taken in triplet for each light intensity.

## RESULTS AND DISCUSSION

Culturing *Spirulina platensis* in conical flask has its limitation in providing complete information related to growth, development and production of value added chemicals viz. vitamins, amino acids, fatty acids, protein and polysaccharides both in quantity and quality and disposing of carbon dioxide one of the major causes of global warming. (Capone, *et al.*, 1997). Extensive research has been conducted on production of *Spirulina platensis* living at salt lakes in

the tropical regions (Sassano, *et al.*, 2004; Costa, *et al.*, 2003).

Physico-chemical profile of *Spirulina platensis* is describing the relationship between growth and environmental factors especially irradiance flux, density and temperature (Vonshak, *et al.*, 2000), which are important in the evolution of micro algae and cyanobacteria for biomass production, as well as their general characterization. High alkalinity is mandatory for the growth of *Spirulina platensis* and bicarbonate is used to maintain high pH (Belkin, *et al.*, 1971; Grant, *et al.*, 1990). Sources of nutrition also affect the growth rate of cyanobacteria (Faintuch, *et al.*, 1991). The growth of *Spirulina platensis* was maximum at 30-35 °C. Because the *Spirulina platensis* cells had previously been adapted to the medium there was no lag phase. It has been shown by previous workers (Danesi *et al.*, 2001) that the optimal growth temperature for *Spirulina platensis* is between 30 and 35 °C.

The pH value of the culture medium combined with dry cell weight may be an indirect method for determining the degree of cell growth of *Spirulina platensis*. This is because the pH gradually rises as bicarbonate added to the culture medium is dissolved to produce CO<sub>2</sub>, which releases OH<sup>-</sup> during cultivation of

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*Spirulina platensis* (Richmond, *et al.*, 1986). *Spirulina platensis* was grown at different pH (7, 8, 9, 10, 11, and 12) in flask culture and monitored and expressed in term of dry weight (Table 1). The maximum bulk density about 0.91 g/500ml was noticed when the pH of culture medium was, maintained at 9.0 with medium volume 500 ml in a 1000 ml flask. The maximum bulk density was attained on 25<sup>th</sup> day after the inoculation of culture in medium. The increase in the production of *Spirulina platensis* could have been due to the availability of mire space, oxygen and light to the culture flask. Earlier

results also demonstrated that optimum pH for maximum growth of *Spirulina platensis* was 9 to 9.5 ranges (Belkin, *et al.*, 1971). *Spirulina platensis* is considered to be an alkalophilic organism by nature (Grant, *et al.*, 1990). Chlorophyll *a* content and protein content is also maximum in pH 9. The Chlorophyll *a* content is 13.2 mg/g and protein content is 64.3 % of dry weight. Similar studies have also been done by various workers of cyanobacteria. (Carvalho, *et al.*, 29 November 2002; Kim, *et al.*, April 2007).

**Table 1- Effect of different medium pH on biomass production of *Spirulina platensis***

S. No.	Initial pH of ZM media	Dry weight in g/500ml (Mean±sem)	Final pH of culture (Mean±sem)	Chl a content in mg/g (Mean±Sem)	Protein content in % of dry wt (Mean±Sem)
1	7	0.63±0.046	9.34±0.16	11.01±0.63	60.1±0.16
2	8	0.82±0.038	9.53±0.13	11.2±0.18	59±0.16
3	9	0.91±0.061	10.02±0.18	13.2±0.42	64.3±0.11
4	10	0.29±0.015	10.16±0.09	6.5±0.23	50±0.18
5	11	0.22±0.025	10.20±0.04	6.8±0.16	48±0.025
6	12	0	11.95±0.02	0	0

Growth Condition – Light Intensity - 5 Klux, Inoculum (in fresh weight) - 1 g/500ml  
Relative Humidity - 75%, Room Temperature- 30 ±2 °C  
Incubation Time- 25 days

**Table 2- Effect of different light intensity on biomass production of *Spirulina platensis***

S.No.	Light intensity	Dry weight in g/500ml (Mean±sem)	Chl a content in mg/g (Mean±Sem)	Protein content in % of dry wt (Mean±Sem)
1	3Klux	0.60±0.020	14.2±0.076	59.2±0.065
2	3.5Klux	0.68±0.035	13.3±0.045	61.2±0.086
3	4Klux	0.73±0.076	11.4±0.038	63.4±0.076
4	4.5Klux	0.75±0.089	11.6±0.075	60.5±0.039
5	5Klux	0.85±0.030	9.8±0.074	64.3±0.035

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Growth Condition – Initial pH - 8.25, Inoculum (in fresh weight) - 1 g/500ml  
 Relative Humidity - 75%, Room Temperature – 30 ±2 °C  
 Incubation Time - 25 day

Figure 1. Effect of different medium pH on biomass production of *Spirulina platensis*

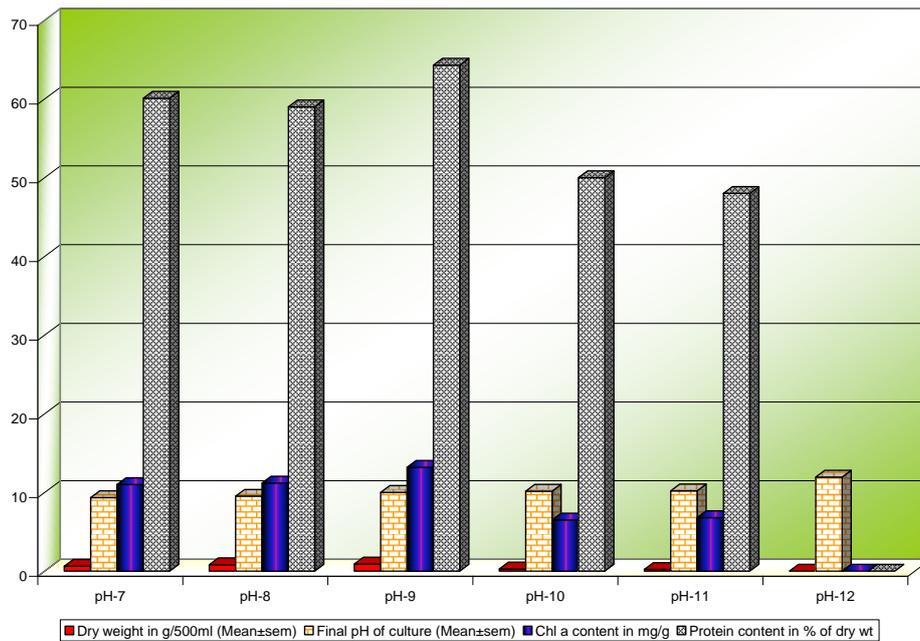
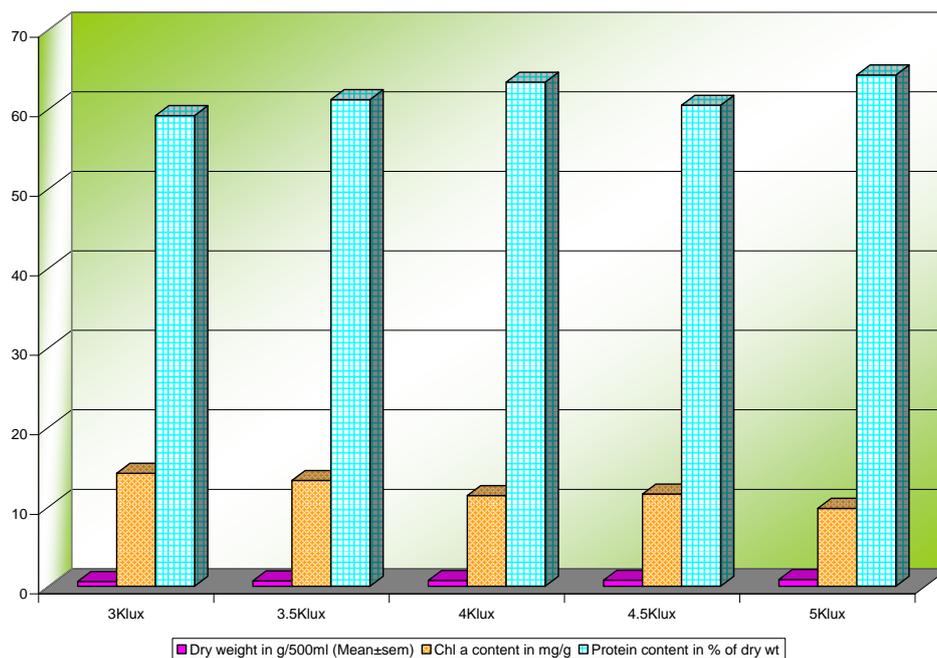


Figure 2. Effect of different light intensity on biomass production of *Spirulina platensis*



The duration, intensity and quality of light are the most important factors in the success of photosynthetic organism. The synthesis of various cell components is known to be influenced by light intensity. Sorokin, *et al.*, (1965) had reported that an increase in light intensity first favors cell division then, after the optimal light intensity was attained, a further increase in light intensity inhibited cell division. Dubey (2006) Reported moderate light intensity in the cultivation of *Spirulina*, suggesting low light intensity at the beginning to avoid photolysis. He also noted that exposing *Spirulina* to high light intensity photolysis them. Result suggests 5 Klux is optimum light for the growth of *Spirulina platensis* in subtropical region of Madhya Pradesh (Table 2). The Chlorophyll *a* and protein content is 9.8 mg/g and 64.3 % at 5 Klux light intensity. Chlorophyll *a* content is maximum at 3 Klux light intensity, which is 14.2 mg/g. The similar studies were done by Danesi, *et al.*, (2004) and Richmond, *et al.*, (1986).

## CONCLUSIONS

This paper has demonstrated that temperature has an important influence on the production of biomass, proteins and chlorophyll *a* by *Spirulina platensis*. In present study, we set out to investigate how

a combination of a set of parameters namely pH and light intensity affect maximum production of biomass and protein. On the basis of utility, *Spirulina platensis* can be cultured under variable natural, artificial and laboratory conditions. Nutrients content of *Spirulina platensis* depends on the location and environment in which the cyanobacterium grows. Percentage of specific components of *Spirulina platensis* can increase or decrease according to need by growing under regulated growth conditions.

## REFERENCES

- Belay, A., Y. Ota, K. Miyakawa, and H. Shimamatsu. 1993. Current knowledge on potential health benefits of *Spirulina*. *J. Appl. Phycol.* 5: 235-241.
- Belkin, S. and Boussiba, S. 1971. Resistance of *Spirulina platensis* (Cyanophyta) to high pH values. *Plant cell Physiol.* 32:953-9589.
- Bogorad, L. 1962. Chlorophylls. In: Lewin, R.A. (Ed.), *Physiology and Biochemistry of Algae*. Academic Press Inc, New York.
- Borowitzka, M.A. 1995. Microalgae as sources of pharmaceuticals and other

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biologically active compounds. *J. Appl. Phycol.* 7: 3–15.

Capone, D.J., Zehr, J.P., Paerl, H.W., Bergman, B. and Carpenter, E.J. 1997. *Trichodesmium*, a globally significant marine cyanobacterium. *Sci.* 276:1221-1229.

Carvalho, J.C.M., Sato, S., Moraes, I. DE O. and Pelizer, L.H. 2002. *Spirulina platensis* growth estimation by pH determination at different cultivation conditions. *Electronic Journal of Biotechnology.* 5(3):251-257.

Ciferri, O. 1983. *Spirulina*, the edible micro-organism. *Microbiol. Rev.* 47: 551-578.

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

Costa, J.A., Colla, L.M. and Duarte, F.P.E. 2003. *S. platensis* growth in open raceway pond using fresh water supplemented with carbon, nitrogen and metal ions. *Z. Naturforsch. (C).* 58 (1-2):76-80.

Danesi, E.D.G., Rangel Y. C.O., Carvalho, J.C.M. and Sato, S. 2004. Effect of reducing

the light intensity on the growth and production of chlorophyll by *Spirulina platensis*. *Biomass and Bioenergy.*26: 329-335.

Danesi, E.D.G., Rangel, C.O., Pelizer, L.H., Carvalho, J.C.M., Sato, S. and Moraes, I.O. 2001. Production of *Spirulina platensis* under different temperatures and urea feeding regimes for chlorophyll attainment. In Proceedings of the Eighth International Congress on Engineering and Food, vol. 2: 1978–1982.

Dubey, R.C. 2006. A textbook of Biotechnology. Fourth revised and enlarge edition, S. Chand and company limited.419-421.

Faintuch, B. L., Sato, S. and Aquarone, E. 1991. Influence of the nutritional sources on the growth rate of cyanobacteria. *Arch. Biol. Technol.* 34:13-30.

Gitelson, A. S., Laorawat, G.P.P., Keydan, and A. Vonshak. 1995. Optical properties of dense algal cultures outdoors and its application to remote estimation of biomass and pigment concentration in *Spirulina platensis*. *J. Phycol.* 31: 828-834.

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Grant, W.D., Mwatha, W.E. and Jones, B.E. 1990. Alkaliphiles: ecology, diversity and application. *FEMS Microbiol rev.* 75:225-270.

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

Kim C.J., Jung, Y.H. and OH, H.M. 2007. Factors indicating culture status during cultivation of *Spirulina (Arthrospira) platensis*. *The Journal of Microbiology.* 45 (2):122-127.

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.

Richmond, A. 2000. Microalgal biotechnology at the turn of the millennium: A personal view. *J. Appl. Phycol.* 12: 441-451.

Richmond, A. and J.U. Grobbelaar. 1986. Factors affecting the output rate of *Spirulina platensis* with reference to mass cultivation. *Biomass* 10: 253-264.

Sassano, C. E., Carvalho, J. C., Gioielli, L. A., Sato, S., Torre, P. and Converti, A. 2004. Kinetics and bioenergetics of *Spirulina platensis* cultivation by fed-batch addition of urea as nitrogen source. *Appl. Biochem. Biotechnol.* 112(3):143-150.

Sorokin, C. and Krauss, R.W. 1965. The dependence of the cell division in chlorella on temperature and light intensity. *Am. J. Bot.* 52:331-339.

Vonshak, A. and Tomaselli, L. 2000. *Arthrospira (Spirulina): Systematics and ecophysiology*. In: Whitton, A., Potts, M., Eds. *The Ecology of Cyanobacteria*. Kluwer Academic Publishers. The Netherlands: 505-522.

Vonshak, A., Torzillo, G. and 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.

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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*. Ph.D. Thesis, Université de Paris, Paris.

Zhang, X.-W., Zhang, Y. M. and Chen, F. 1999. Application of mathematical models to the determination optimal glucose concentration and light intensity for mixotrophic culture of *Spirulina platensis*. *Process Biochem.* 34: 477–481.