Plant Archives Vol. 20, Supplement 2, 2020 pp. 4259-4263 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF ABIOTIC FACTORS ON THE GROWTH OF *SPIRULINA PLATENSIS* STRAINS

Kumaresan G., K. Sivakumar* and Rajan L Fradlin Singh
Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar-608002, Chidambaram, Tamilnadu, India
Email - microsen1974@gmail.com
*corresponding author Email – ksivamicro@gmail.com

Abstract

Spirulina is microscopic cyanobacterium. This microalga is present in fresh water, springs, marine water etc. *Spirulina* is cultivated in major parts of the country due to the presence of crude protein, pigments and other food elements and it is commercially used as food supplementary for human beings and feed for animals. The present research works were studied that, the effects of temperature, pH, salinity and light intensity on the growth of *Spirulina platensis* strains (Sp1, Sp2, Sp3, Sp4 and reference strain (CAS10) cultivation in Zarrouk's medium. In vitro studies were performed at temperatures from 30 to 45ºC, pH from 8.0 to 11.0, salinity (sodium chloride) concentration ranges from 0.10 to 0.35 M and light intensities from 2.0 to 5 K lux, in Zarrouk's medium and incubated for 30 days in light chamber. The highest cell dry biomass was obtained at temperature of 35ºC (2.75 g L^{-1}), pH of 9.5 (2.56 g L^{-1}), salinity of 2.0 M (3.88 g L^{-1}) and light intensity of 5.0 K lux (2.95 g L^{-1}) in Sp2 strain of *S. platensis* at 30th days of incubation. The present results revealed that, the optimum abiotic conditions for better growth were optimized and efficient strain was found for *Spirulina* production.
Keywords: Dry biomass, temperature, salinity, pH, light intensity, *Spirulina platensis*

Introduction

Spirulina platensis is a cyanobacterium rich in proteins (60-70%), essential fatty acids, beta-carotene, vitamins and mineral elements (Charpy *et al*., 2008). This alga (200-400 µm) lives in shallow water with a salinity ranging from 7-8% approximately and high alkalinity. Recent studies show that the bioavailability of nutrients in this alga was higher than that of dietary fiber (Cruchot, 2008 and Falquet and Hurni, 2006). It may therefore be essential to use *S. platensis* as a supplement to protein, energy and as a means of avoiding malnutrition. Mineral elements, sunlight and CO_2 are directly used by alga cells for their growth. Environmental stresses affect growth and biopigment accumulation of microalgae, including nutrients availability, high pH, light, salinity and temperature (Pandey *et al*., 2011) The pH plays important role in the metabolic activities of microalgae. It strongly affects biomass production, chemicals dissociation and cell physiology. Therefore, the effect of different pH levels on the growth of microalgae was continuously evaluated under different environmental condition (Ogbonda, 2007 and Celekli, 2009) To obtain biomass is a complicated issue during the large-scale production of S. platensis since the process involves many optimum conditions for the successful growth of the alga (Pandey *et al.,* 2010). The objective of the present work was to assess the influence of stress conditions including temperature, pH, salinity and light intensity on the biomass production of *S. platensis* to optimize the best culture condition for improvement of dry biomass production.

Materials and Methods

Organism used:

The strains of *Spirulina platensis viz.* Sp1, Sp2, Sp3, Sp4 were isolated from different temple pond and CAS10 strain was obtained from Department of Botany, University of Madras, Chennai. Which were maintained in Zarrouk's agar medium slant and broth.

Multiplication of Culture

The isolated strains of *S. platensis* Sp1, Sp2, Sp3, Sp4 and reference strain CAS10 were grown in Zarrouk's medium under laboratory condition at 30°C in the light chamber for 30 days and estimated the growth parameters like cell population, specific growth rate and doubling time at 10 days interval on 10th, 20th and 30th days.

Specific growth rate (Abu-Razq *et al.,* 1999)

The specific growth rate of the *S. platensis* strains was calculated according to the following equation.

µ= Specific growth rate

In Xl = Initial weight of *S. platensis* of biomass

In X2 = Final weight of *S. platensis* of biomass

t = Time period (day)

Doubling time (Vonshak, 1997)

The doubling time of the *S. platensis* strains was calculated according to the following equation.

µ = Specific growth rate, D.T = Doubling time

Estimation of dry weight of *S. platensis.* (Pandey *et al.,* 2010).

Dry biomass concentration (gL^{-1}) was calculated by measuring dry weight. For dry weight measurement, homogeneous suspensions of known quantity of *S. platensis* samples were filtered through filter paper and oven dried at 75°C for 4 to 6 hours. The dried filter paper containing *S. platensis* biomass were cooled and weighed. The difference between the initial and final weight were taken as the dry weight of *S. platensis* biomass. The dry weights were expressed in terms of g/l.

Effect of various temperature on biomass productivity of *S. platensis* strains

The effect of the temperature on growth of Sp1, Sp2, Sp3, Sp4 and CAS10 of *S. platensis* strains was determined by separately inoculating the strains in 1000 ml of Zarrouk's medium in two litre Erlenmeyer conical flasks. Each flask was inoculated with 50 ml (0.25 mg wet weight) of the 30 days old pure culture of *S. platensis*. Each temperature regime was in triplicates. The culture flasks were incubated in BOD incubator with fluorescent light at the various temperatures *viz.,* 30, 35, 40 and 45°C and periodically replenished with the growth medium to prevent drying up of the cultures. After 30 days incubation the specific growth rate, doubling time and dry biomass were determined.

Effect of various pH on biomass productivity of *S. platensis* strains

The effect of the pH on the growth of Sp1, Sp2, Sp3, Sp4 and CAS10 *S. platensis* strains was determined by separately inoculating the strains in 1000 ml of Zarrouk's medium in two litre Erlenmeyer conical flasks. Each flask was inoculated with 50 ml (0.25 mg wet weight) of the 30 days old pure culture of *Spirulina platensis.* Each pH level (8.0, 8.5, 9.0, 9.5, 10.0, 10.5 and 11.0) was adjusted with 0.1 N NaOH and incubated for 30 days in light chamber at room temperature. After incubation the cell dry biomass was determined as described earlier (2.5).

Effect of salinity on the growth of *Spirulina platensis* strains

The effect of salinity on growth of Sp1, Sp2, Sp3, Sp4 and CAS10 *S. platensis* strains was determined by separately inoculating the strains in 1000 ml of Zarrouk's medium in two litre Erlenmeyer conical flasks with different concentrations of NaCl (0.10, 0.15, 0.20, 0.25, 0.30, and 0.35 M). The controls were grown in Zarrouk's medium without the addition of salt supplement. Each flask was inoculated with 50 ml (0.25 mg wet weight) of the 30 days old pure culture of *S. platensis* and incubated for 30 days in light chamber at room temperature. After incubation the cell dry biomass was determined as described earlier 2.5).

Effect of light intensity on the growth of *S. platensis* strains (Pandey *et al.,* 2010)

Light is important for the photosynthesis of *S. platensis,* different light intensities such as 2.0. 2.5, 3.0, 3.5, 4.0, 4.5 and 5 Klux light were set with the help of Luxi meter. The effect light intensity on growth of Spl, Sp2, Sp3, Sp4 and CAS10 strains was determined by separately inoculating the strains 1000 ml of the Zarrouk's medium was taken in two litre Erlenmeyer conical flasks. The seven sets of flasks were prepared in triplet for each light intensity incubated in a room temperature (30±2.0°C) under artificial lighting of 20 W fluorescent lamps with light intensity of 1.00 Klux were used in light chamber.

Statistical analysis

The experimental results were statistically analyzed as suggested by Gomez and Gomez (1984). For the significant results, critical difference was worked out at 5 per cent probability level.

Results and Discussion

Effect of temperature on biomass productivity of *S. platensis* strains

The result showed that the effect of various temperature (30, 35*,* 40 and 45°C) on biomass productivity of *S. platensis* strains (Sp1, Sp2, Sp3, Sp4 and reference strain (CAS10) was studied and results are summarized in Table (1).The optimum temperature for the growth of all *S. platensis* strains was found to be 35°C and the highest biomass was obtained at 35°C (2.75 g L^{-1}) in Sp2 strain followed by Sp4 (2.37 g L^{-1}), CAS10 (2.29 g L^{-1}), Sp3 (1.96g L^{-1}) and Sp1(1.56 g L^{-1}) strains in 30 days.

The most elementary factor of all living organisms is temperature as it affects metabolic processes and biochemical composition of cells. In addition, optimal growth temperature and tolerance to the extreme values generally differ for different microalgae strains. Temperature is one of the most important and well-studied factors influencing the chemical composition of algae, particularly lipids and fatty acids. *Spirulina* is a thermophilic alga, the optimum temperature for its growth falls between 35 to 37°C (Ciferri, 1983). Tomaselli *et al.* (1987) have shown in their laboratory experiment that the maximum biomass yield was obtained when *Spirulina* grown at the optimal temperature of 35°C.

Manoj kumar *et al.* (2011) studied the effect of temperature on growth rate, biomass composition and pigment production of *S. platensis.* Growth kinetics of cultures showed a wide range of temperature tolerance from 20°C to 40°C. Maximum growth rate, cell production with maximum accumulation of chlorophyll and phycobiliproteins were found at temperature 35°C. Similarly, the present investigation revealed that, all the five *S. platensis* strains recorded the highest rate of growth at 35°C. The highest biomass (2.75 g L^{-1}), specific growth rate (0.047 h^{-1}) and doubling time (36.48 h) were recorded in *S. platensis*-Sp2 at 35°C followed by Sp4, CAS10, Sp3 and Sp1.

Effect of pH on biomass productivity of *S. platensis* strains

The effect of pH on growth of four *S. platensis* strains and reference strain (CAS) was studied. The present investigation revealed that, the effect of pH (8.0, 8.5, 9.0, 9.5, 10.0, 10.5 and11.0) on growth of *S. platensis* strains studied in Fig. (1). The *S. platensis* Sp2 strain augmented highest biomass (2.56 g L^{-1}) at pH 9.5 followed by Sp4 (2.44 g L^{-1}), CAS10 (2.17 g L^{-1}), Sp3 (1.83g L^{-1}) and Sp1 (1.80 g L^{-1}) strains in 30 days.

In *Spirulina* cultivation, pH was one of the most important factors and should be maintained above 9.5 to avoid contamination by microalgae (Belay, 1997). The optimum pH 9.5 to 10.5 was considered ideal for *Spirulina* cultivation in Zarrouk's medium, the best biomass as observed at pH 9.5. (Richmond,1992). Pandey *et al.,* 2010 were found that, the optimum pH for maximum growth of *S. maxima* was 9 to 9.5 ranges and biomass was obtained (0.73g/500 ml) at 25th day. Similarly, in present investigation, the optimum pH for the growth of *S. platensis* was 9.5 and highest biomass was obtained (2.56 g L^{-1}) in Sp2 strain at pH 9.5.

Effect of salinity on biomass productivity of *S. platensis* strains

The effect of sodium chloride concentration (0.0, 0.10, 0.15, 0.20, 0.25 and 0.30 M) on growth of *S. platensis* strains was studied and the data are presented in Table (8). The *S. platensis* recorded highest biomass in 0.20 M NaCl conc. and the highest biomass was obtained (3.88 g L^{-1}) in Sp2 strain followed by Sp4 (3.32 g L^{-1}), CAS10 (2.66 g L^{-1}), Sp3 (1.97 g L^{-1}) and Sp1 (1.90 g L^{-1}) strains in 30 days.

Salinity is a primary factor influencing the growth of marine microalgae as algae often have a negative response in morphology and physiology with fluctuating salinity. The present study revealed that, the growth of all the five *S. platensis* strains recorded the higher growth at 0.2 M NaCl. The highest biomass (3.88 mg ml^{-1}) was recorded in *S. platensis*-Sp2 in salinity (NaCl) concentration at 0.20 M. These results agree with Cassan *et al.* (2011) have reported that, the optimum microalgal growth was obtained (13g L^{-1}) with salinity of 0.22M.

Effect of light intensity on the growth of *S. platensis* strains

The effect of light intensity (2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 Klux) on growth of *S. platensis* strains was studied in Fig. (2). The *S. platensis* recorded highest biomass in 5.0 Klux and the highest biomass was obtained (2.95 g L^{-1}) in Sp2 strain followed by Sp4 (2.25 g L^{-1}), CAS10 (2.14 g L^{-1}), Sp3 (2.11 g L^{-1}) and Sp1 (1.60 g L^{-1}) strains in 30 days.

Light is major physical factors that affect overall biomass productivity in photoautotropic algal cultures (Carvalho and Malcata, 2003). Danesi *et al.* (2004) have reported that, the light intensity is one of the major factors influencing chlorophyll biosynthesis, in a way that under limited illumination conditions high pigment contents was observed. The best cellular growth recorded at higher light intensity values. Efficient utilization of light is one of the major challenges in microalgal biotechnology, especially when an increase in the biomass yield is desired (Barbosa *et al.,* 2001). Pandey *et al.* (2010) have reported that, the higher dry weight (0.85g/500ml), protein (64.3%) and chl -a (9.8mg/gm) content of *S. platensis* were obtained at 5 Klux light intensity. Similarly, the present study revealed the effect of different light intensity (3.5, 4.0, 4.5 and 5.0 K lux) on growth of *S. platensis* strains were studied. The highest biomass (2.95 mg ml^{-1}) was recorded in *S. platensis*- Sp2 with light intensity level at 5.0 (K lux).

Conclusion

The present study clearly indicated that, the optimum environmental conditions required for better growth of *Spirulina platensis are* temperature (35°C), pH (9.5), salinity (0.2 M NaCl conc.) and light intensity (5 Klux). The Sp2 strain was showed better growth in all abiotic factors compared to other strains (Sp1, Sp3, Sp4 and CAS10) of *S. platensis.* Hence, we concluded that the Sp2 strain was selected for further mass production.

Table 1 : Effect of temperature on the growth of *S. platensis* strains

S. platensis strains	***S. platensis* biomass (gL^{-1}) at 30th Day**											
	Temperature (°C)											
	30			**35**			**40**			**45**		
	µmax (h^{-1})	**Doubling time (h)**	**Dry weight (gL^{-1})**	**µmax (h^{-1})**	**Doubling time (h)**	**Dry weight (gL^{-1})**	**µmax (h^{-1})**	**Doubling time (h)**	**Dry weight (gL^{-1})**	**µmax (h^{-1})**	**Doubling time (h)**	**Dry weight (gL^{-1})**
Sp1	0.026	14.75	1.23	0.016	17.90	1.56	0.021	16.70	1.15	0.012	21.74	1.15
Sp2	0.047	29.85	2.46	0.045	36.48	2.75	0.049	38.45	2.20	0.048	75.00	1.74
Sp3	0.030	16.90	1.61	0.025	19.67	1.96	0.024	20.65	1.30	0.015	23.29	1.28
Sp4	0.042	23.89	2.05	0.039	28.75	2.37	0.038	28.21	1.90	0.025	56.66	1.67
CAS10	0.035	19.24	1.65	0.030	20.00	2.29	0.026	22.33	1.55	0.018	34.66	1.32
Mean	0.03	17.5	1.79	0.03	23.8	2.33	0.03	24.3	1.58	0.02	42.6	1.3
SEd	**0.0050**	**-**	**0.0041**	**0.0060**	**-**	**0.038**	**0.011**	**-**	**0.0170**	**0.023**	**-**	**0.070**
CD (p=0.05)	**0.0025**	**-**	**0.0205**	**0.0030**	**-**	**0.0190**	**0.0650**	**-**	**0.0070**	**0.0125**	**-**	**0.0035**

Table 2 : Effect of salinity on the growth of *S. platensis* strains

***S. platensis* strains**	***S. platensis* biomass (gL^{-1}) at 30th Day**					
	NaCl concentration (M)					
	0	**0.10 (5.84 g)**	**0.15 (8.76 g)**	**0.20 (11.68 g)**	**0.25 (14.61 g)**	**0.30 (17.53 g)**
Sp1	1.66	1.54	1.90	1.48	0.45	1.09
Sp2	2.46	2.90	2.96	3.88	1.87	1.34
Sp3	1.79	1.84	1.97	2.38	1.02	1.16
Sp4	2.35	2.81	2.83	3.32	1.29	1.21
CAS10	2.00	2.08	2.31	2.66	1.13	1.20
Mean	2.162	2.19	2.242	2.66	1.15	1.256
Sed	**0.011**	**0.090**	**0.013**	**0.060**	**0.058**	**0.013**
CD (p=0.05)	**0.0055**	**0.0450**	**0.0650**	**0.0030**	**0.0290**	**0.0650**

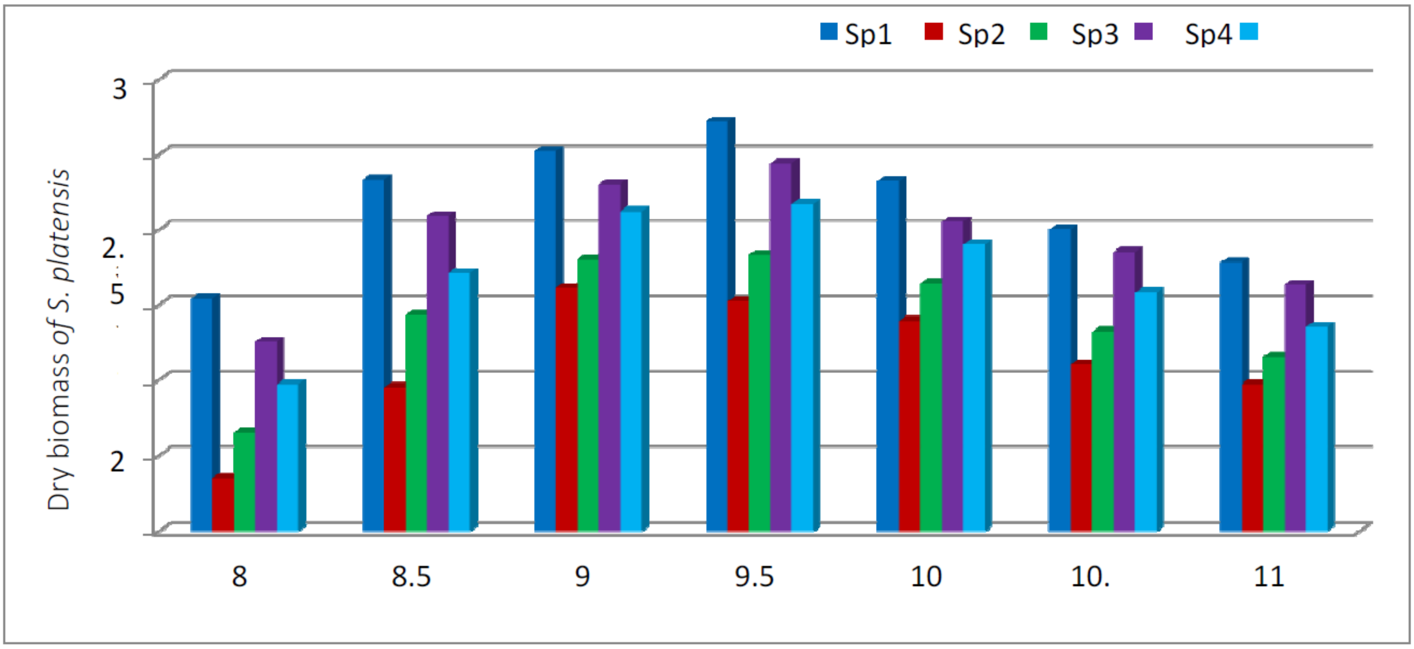

Fig. 1 : Effect of various pH on biomass productivity of *S. platensis*

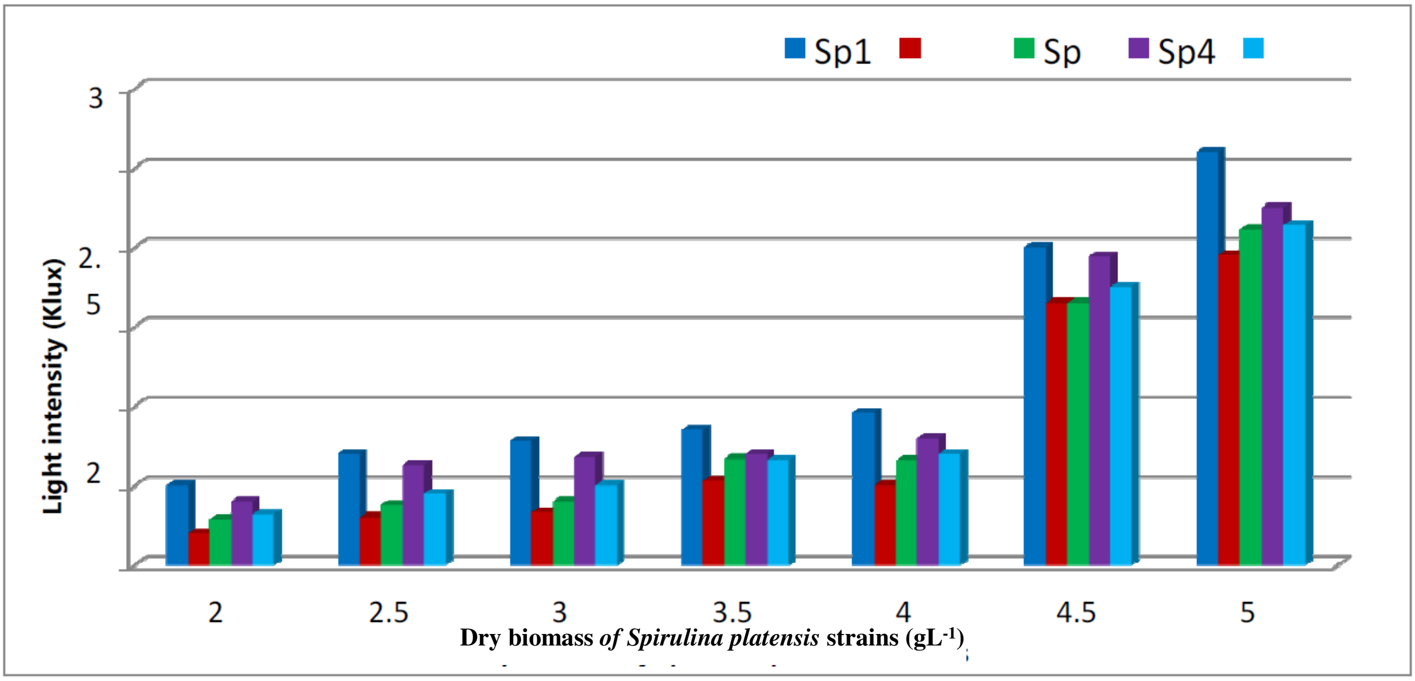

Fig. 2: Effect of light intensity on the growth of *Spirulina platensis* strains in Zarrouk′s medium

References

Abu-Razq, T.S.; Musallam, T.S.; Shimmari, J.A.L. and Dias, P. (1999). Optimum production-condition for different high-quality marine algae. Hydrobiol., 103; 97-107.

Barbosa, M.; Jansen, M.; Ham, N.; Tramper, J. and Wijffels, R.H. (2001). Microalgae Cultivation in air-lift reactors. Biotech. Bioengg., 82(2): 170-179.

Belay, A. (1997). Mass culture of Spintlina outdoors—the earthrise farms experience. In: Vonhask; A. (Ed.), *S. platensis* (Arthrospira): Physiology, Cell-Biology and Biotechnology. Taylor and Francis, London, 13: 1-158.

Carvalho, A.P. and Malcata, F.X. (2003). Kinetic modeling of the autotrophic growth of *Pavlova lutheri*: study of the combined influence of light and temperature. Biotechnol. Prog., 19: 1128-1135.

Cassan, C.J.; Ravelonandro, P.H.; Arsene Isambert, D.H.R. and Raherimandimby, M. (2011). Improvement of the growth of Arthrospira *(Spirulina) platensis* from Toliara (Madagascar): Effect of agitation, salinity and CO_2 addition. Food and Bioproducts Proc., 89: 209-216.

Celekli, A.; Yavuzatmaca, M.; Bozkurt, H. (2009). Modeling of biomass production by Spirulinaplatensis as function of phosphate concentrations and pH regimes. Bioresour Technol., 100: 3625-3629.

Charpy, L.; Langlade, M.J. and Alliod, R. (2008). Spirulina can be an Asset to the Health and Development in Africa? Research Institute for Development (IRD), Marseilles, Pages: 43.

Ciferri, O. (1983). *Spirulina,* the Edible Microorganism." Microbiol. Rev., 47(4): 551-578.

Cruchot, H. (2008). Spirulina, results and prospects. M.Sc. Thesis, University of Franche Comte, Besancon, France.

Danesi, E.D.G.; Rangel-Yagui, 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. Bioengg., 26: 329-335.

Falquet, J. and Hurni, J.P. (2006). Spirulina, Nutritional Aspects. Antenna Technologies, Geneva, Switzerland, Pages: 26.

Manojkumar, J.K. and Singh, G.P. (2011). Growth and biopigment accumulation of cyanobacterium *Spirulina platensis* at different light intensities and temperature. Brazilian J. Microbiol., 42: 1128-1135.

Ogbonda, K.H.; Aminigo, R.E. and Abu, G.O. (2007). Influence of temperature and pH on biomass production and protein biosynthesis in a putative Spirulina sp. Bioresour Technol 98: 2207-2211.

Pandey, J.P.; Tiwari, A.; Singh, S. and Tiwari, D. (2011). Potential of different light intensities on the productivity of *Spirulina maxima*. J of Algal Biomass Utilization 2: 9-14.

Pandey, J.P.; Tiwari, A. and Mishra, R.M. (2010). Evaluation of biomass production of *Spirulina maxima* on different reported media. J. Algal Biomass Utilization, 1: 70-81.

Pandey, J.P.; Neeraj, P. and Tiwari, A. (2010). Standardization of pH and light intensity for the biomass production of *Spirulina platensis.* J. Algal. Biomass. Utln., 1(2): 93-102.

Richmond, A. (1992). Mass culture of cyanobacteria. In: Mann, N., Carr, N., Eds. *Photosynthetic prokaryotes.* 2nd ed. Plenum Press, New York and London, pp. 181-210.

Tomaselli, L. (1997). Morphology, ultrastructure and taxonomy of *Arthrospira (Spirulina) maxima* and *Arthrospira (Spirulina) platensis.* In: Vosnshak, A. (Ed.), *Spirulina platensis (Arthrospira).* Physiology, Cell-Biology and Biotechnology. Taylor and Francis, London, pp. 1-17.

Vonshak, A. (1997). Appendices. In: Vonshak, A., Ed. *Spirulina platensis (Arthrospira):* Physiology, Cell biology and Biotechnology. Taylor and Francis, London, Great Britain; 213- 226.