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THE INFLUENCE OF LIGHT INTENSITY ON GROWTH AND BIOCHEMICAL CONSTITUENTS OF *SPIRULINA*

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Abstract: The influence of light intensity on growth and biochemical constituents of two species of *Spirulina; Spirulina platensis* and *Spirulina fusiformis* was studied. Various light intensities of 1000, 1500, 2000 and 2500 lux were investigated. The best growth for both *S. platensis* and *S. fusiformis* were found at the intensity of 2500 lux and at 1000 lux both species showed slower growth. The results indicated that at highest light intensity the protein content increased while the carbohydrate and lipid content decreased. Maximum protein content of 59.2 % and 60.6% were found at 2500 lux for *S. platensis* and *S.fusiformis* respectively. Maximum biomass productions were also achieved at 2500 lux for both the species and they were 2.1 and 2.2 g/l for *S. platensis* and *S. fusiformis* respectively. Carbohydrate and lipid content was high at 1000 lux for both the species. Phycocyanin and carotenoid content was found higher at optimal light intensity of 2500 lux.

Key words: *Spirulina,* light intensity, Growth, Biochemical composition

Introduction

Spirulina, like many other algae grown photoautotrophically depends on light as its main energy source. In fact, light is the most important factor affecting photosynthetic organisms. When nutrient and temperature are not limiting the growth of S*pirulina*, light availability to the average cell becomes the dominant limiting factor (Richmond, 1992). Photosynthesis in most algae occurs in the presence of light with wavelength of 300-700 nm. Light stimulates inorganic nitrogen uptake and assimilation by algae. Light affects mainly the nitrite-reducing step, which requires ferredoxin. Due to prokaryotic nature of *Spirulina* light does not affect the differentiation of development processes. In the dark the rate of oxygen evolution or carbon fixation will be negative because of respiration. As irradiance increased a point is reached when the photosynthetic rate is just balanced by respiration. This is known as compensation point. Different strain of *Spirulina* may differ in their sensitivity to the light stress (Vonshak and Richmond, 1988). If no nutrient limitation occurs light becomes the growth-regulating factor. The study aims to identify in detail, the effect of different light intensity on the chemical composition of *Spirulina*, cultivated in this experiment.

Materials and Methods

Two species of *Spirulina* were examined in different light intensities as 1000, 1500, 2000 and 2500 lux. Illumination will be provided by fluorescence tube of 36 watt. Temperature of the room was 31±1^{0}C and the pH was 9.5±2. Erlenmeyer flasks of 500 ml capacity containing 250 ml sterilized media and 10% (v/v) of the prepared inoculums (0.07 O.D.) were used for the experiment. Three replicates of each treatment were used.

Analytical procedure

Chlorophyll-a was determined spectrophotometrically after absolute methanol extraction utilizing the absorption coefficient factor reported by Vonshak, (1997). Dry weight determination was done by 20 ml algal sample of suspension that was filtered through a Whatman GF/C filter of 47 mm diameter. The filter was dried in an oven for overnight at 70^{o}C, put in desiccators for 20 min for cooling and weighed. The specific growth rate was measured by using the formula:

$$SGR = \frac{\ln x_2 - \ln x_1}{t_2 - t_1}$$

Where, x_1 and x_2 are biomass concentration at time interval t_1 and t_2.

For protein determination, 6 ml 0.5 N NaOH was added to filtered algal samples then sonicated for two minutes and 80^{o}C water bath for 20 minutes. Then the sample was centrifuged in 3000 rpm for 10 minute. The protein content was then measured by Bradford method (1976) using BSA as protein standard. Carbohydrate was determined based on method of Kochart, (1978) using glucose as a standard. Total Lipid determination was done based on the method of Bligh and Dyer (1959) as modified by Kates and Volcani (1966). The method used for estimating total carotenoids was spectrophotometry after extracting the cells in 90% acetone. The phycobilliproteins content was estimated after extraction in a phosphate buffer (pH 7) following the procedure of MacColl and Guard- Friar (1987). Three replicates were used for each species and for each temperature and the average was calculated.

Results and Discussion

The specific growth rates of *S. platensis* and *S. fusiformis* at different light intensities are presented in Fig 1. The highest specific growth rate of 0.120 for *S. platensis* and specific growth rate of 0.121 for *S. fusiformis*

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were found at the light intensity of 2500 lux. The specific growth rate of both the species under the continuous light of 2000 lux was the second (0.115 for *S. platensis* and 0.109 for *S. fusiformis*). The lowest specific growth rate of both the species was found under the continuous light of 1000 lux (0.092 for *S. platensis* and 0.090 for *S. fusiformis*). Maximum biomass production was also achieved at 2500 lux for both species; 2.1 g/l for *S. platensis* and 2.2 g/l for *S. fusiformis* (Table 1). The lowest biomass of 1.4 g/l was achieved for *S. platensis* and 1.2 g/l was attained for *S. fusiformis* under the continuous light of 1000 lux. Becker and Venkataraman (1984) stated that among the climatic factors affecting the cultivation of *Spirulina* light intensity plays the most important role on its growth and in outdoor culture high light intensity limits the growth and should be avoided by shading the culture.

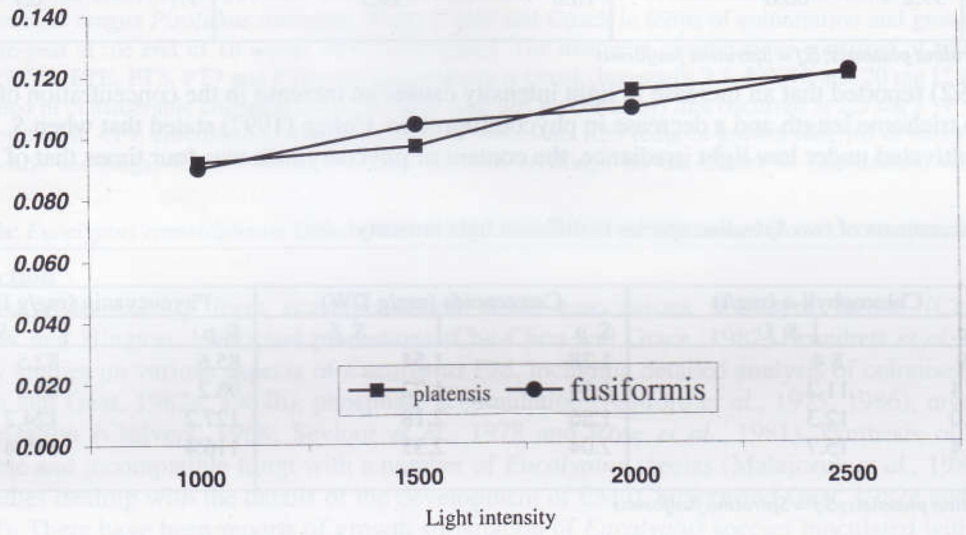

Fig. –1. Specific growth rate of *S. platensis* and *S. fusiformis* grown in different light intensity

Table 1. Biomass of two *Spirulina* species grown in different light intensity

Light (lux)	*S. platensis*	*S. fusiformis*
1000	1.4	1.2
1500	1.7	1.8
2000	2.0	1.8
2500	2.1	2.2

Results presented in Table 2 show the biochemical composition of two species grown in different light intensity. Protein content of *S. fusiformis* varied from 54.2 to 60.6 %. Protein content of *S. platensis* varied from 53.5 to 59.2 %. The result indicates that protein content increased with increasing light intensity. The lowest protein content of 54.2 % was achieved at 1000 lux and protein content was high at 2500-lux intensity and found 60.6 % for *S. fusiformis*. The lowest protein content of 53.5 % was achieved at 1000 lux and protein content was highest at 2500-lux intensity and found 59.2 % for *S. platensis*.

Carbohydrate content found higher at low light (1000-lux) intensity and found 23.7 % for *S. fusiformis*. Lowest carbohydrate content of 19.2 % achieved at 2000 lux. Carbohydrate content of 21.1 and 19.3 % attained at 1500 and 2500 lux, respectively. Carbohydrate content was high at 1000-lux intensity and found 28.4 % for *S.platensis*. Lowest carbohydrate content of 18.0 % achieved at 2500 lux. Carbohydrate content of 27.1 and 21.6 % attained at 1500and 2000 lux, respectively.

Lipid content of *S. fusiformis* varied from 6.7 to 8.7%. Lipid content was higher at low light (1000-lux) intensity and found 8.7 % for *S. fusiformis*. Lowest lipid content of 6.7% achieved at 2500 lux. Lipid content of 8.3 and 7.5% attained at 1500 and 2000 lux, respectively. Lipid content of *S. platensis* varied from 7.7 to 9.2%. Lipid content was high at 1000-lux intensity and found 9.2% for *S. platensis*. Lowest lipid content of 7.7% achieved at 2500 lux. Lipid content of 8.5 and 8.1 % attained at 1500and 2000 lux, respectively.

Olguin. (1996) studied *S. Maxima* under two light intensities and observed that the strain was more productive under the higher light intensity with more protein at outdoor cultivation.

Maximum chlorophyll-a content of 15.4 mg l^{-1} was found for *S. platensis* at 2500 lux and highest 15.7 mg l^{-1} for *S. fusiformis* at 2500 lux. Carotenoids were high at 2500 lux for both species; 2.04 mg g^{-1} dry weight for *S. platensis* and 2.37 mg g^{-1} dry weight for *S. fusiformis* (Table 3). Maximum phycocyanin occurred at 2000 lux in both the species, which was 127 mg g^{-1} dry weight for *S. platensis* and124 mg g^{-1} dry weight for *S. fusiformis* (Table 3). Allophycocyanin was found also high at 2000 lux for both the species (55 mg g^{-1} dry weight for *S. platensis* and 53 mg g^{-1} dry weight for *S. fusiformis*). Phycoerythrin content of 10.4 mg g^{-1} dry weight was found at 1000 lux for *S. platensis* and for *S. fusiformis* phycoerythrin content of 9.4 mg g^{-1} dry weight was also found at 1000 lux.

Table 2. Biochemical compositions of two *Spirulina* species in different light intensity

Light lux	Protein (%)		Carbohydrate (%)		Lipid (%)	
	S. p.	*S. f.*	*S. p.*	*S. f.*	*S. p.*	*S. f.*
1000	53.5	54.2	28.4	23.7	9.2	8.7
1500	55.0	55.5	27.1	21.1	8.5	8.3
2000	57.7	59.5	21.6	19.2	8.1	7.5
2500	59.2	60.6	18.0	19.3	7.7	6.7

Note~ *S.p.= Spirulina platensis; S.f = Spirulina fusiformis*

Richmond (1992) reported that an increase in light intensity causes an increase in the concentration of gas vesicles and in trichome length and a decrease in phycobilliprotein. Cohen (1997) stated that when *S. subsala* was cultivated under low light irradiance, the content of phycoerythrin was four times that of phycocyanins.

Table 3. Pigment contents of two *Spirulina* species in different light intensity

Light lux	Chlorophyll-a (mg/l)		Carotenoids (mg/g DW)		Phycocyanin (mg/g DW)	
	S. p.	*S. f.*	*S. p.*	*S. f.*	*S. p.*	*S. f.*
1000	8.9	8.4	1.28	1.54	85.6	82.5
1500	10.4	11.2	1.34	1.72	98.2	85.3
2000	14.0	12.3	1.86	2.18	127.2	124.2
2500	15.4	15.7	2.04	2.37	116.4	118.4

Note~ *S.p.= Spirulina platensis; S.f = Spirulina fusiformis*

References

Bligh, E.G. and W.J, Dyer, 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology.*, 37: 911-917

Bradford, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-Dye binding. *Analytical Biochemistry,* 72: 248-254.

Becker, E.W. and L.V., Venkataraman, 1984. Production and utilization of the blue green algae *Spirulina* in India. *Biomass,* 4(2): 105-125.

Cohen, Z. 1997, Chemistry of Spirullina. In: Vonshak, A.(ed). *Spirulina Platensis (Arthospira) Physiology, Cell biology and Biotechnology.* London: Taylor and Francis. P.175-204

Kates, M. and B.E.,Volcani, 1966. Lipid composition of diatoms. *Biochemical and Biopysical Acta,* 116: 264-278.

Kochart, A.G. 1978. Carbohydrate determination by the phenol-sulphuric acid method. In: Hellebust, J.A. and Craigie, J.S. (eds). *Handbook of Phycological and Biochemical Methods.* Cambridge Univ. press publ. P.95-97.

MacColl, R. and D., Guard-Friar, 1987. Phycobilliproteins. CRC press, Boca. Raton.Richmond, A. (1992). Open system for the mass production of photoautotrophic microalgae outdoor: physilogocal principles. *Journal of Applied Phycology,* 4: 281.

Olguin, E. J. 1986. Appropriate biotechnological system in the arid environment. In: *Applied Microbiology,* Doelle, H. W. and Heden, C. G. (Eds.). Dorderrcht, D. Reidel Publishing Company; Paris UNESCO, Trends in Scientific research. 2: p 111-134.

Richmond, A. 1992. Open system for the mass production of photoautotrophic microalgae outdoor: physilogocal principles. *Journal of Applied Physiology,* 4: 281.

Vonshak, A. and A., Richmond, 1988. Mass production of the Blue-green Algae Spirulina: An Overview. *Biomass,* 15: 233-247

Vonshak, A., 1997, Use of Spirulina biomass. In: Vonshak, A.(ed). *Spirulina Platensis (Arthospira) Physiology, Cell biology and Biotechnology.* London: Taylor and Francis. P. 205-212.