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EFFECT OF DIFFERENTPH AND LIGHT QUALITY ON THE BIOMASS PRODUCTION AND CHLOROPHYLL CONTENT IN *SPIRULINA PLATENSIS*

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The cyanobacterium *Spirulina platensis* has been used by humans because of its nutritional and medicinal effects. It was cultivated in laboratory condition with the objective of determining the effect of different pH and different wavelengths of light on the biomass production and chlorophyll-a content. In the present study, the production was optimized in terms of biomass and metabolites. The cultivations were carried out in pure CFTRI medium, with constant agitation (100 rpm) twice a day and 16/8 hours photoperiod for 30 days. The dry weight of S1 strain was 17.85mg ml^{-1} and chlorophyll -a content was 10.421µg mg^{-1} and dry weight of S2 strain was 14.62 mg ml^{-1} and chlorophyll-a content was 9.75µg mg^{-1}at pH 10. Similarly, under red light, the dry weight of S1 strain was 19.29 mg ml^{-1} and chlorophyll -a content was 8.74µg mg^{-1} where as the dry weight of S2 strain was 17.98 mg ml^{-1} and chlorophyll a content was 7.89µg mg^{-1}.

Key words: Biomass, Chlorophyll-a, pH, light wavelength and *Spirulina platensis.*

Spirulina platensis is a Cyanobacterium which has been studied extensively with respect to the biomass production. *Spirulina platensis*, renamed as *Arthrospira platensis* is a planktonic blue green alga, dominating the flora of alkaline saline water having pH upto 11. It is widespread and the main species can exist in various types of habitats, namely ground, brackish water, freshwater, sea water and water of industrial and domestic use (Padhi *et al*. 1992, Sankaran and Radharukman 1992, Santra 1992, Laliberte *et al*. 1997). *Spirulina* has been used as an alternative source of protein and nutrients for human food and animal feed (Pelizer *et al*. 2003, Andrade & Costa 2007). *Arthrospira platensis* is naturally found in tropical regions inhabiting alkaline lakes (pH 11) with high concentration of NaCl and bicarbonates (Cozza and costa 2000, Richmond 1990, Richmond 2004, Vonshak 1996). The absorption of light energy by *Spirulina* is due to the occurrence of photosynthetic pigments including chlorophyll-a, carotenoid and c- phycocyanin. Carotenoids are accessory pigments in photosynthesis and are normally found in the chloroplasts of algae and cytoplasm of cyanobacteria. *Spirulina* synthesize phycobilins (c-phycocyanin and allophycocyanin) which are the major accessory light harvesting pigments (Babu *et al*. 1991). The objective of the work presented in this paper was to evaluate the influence of different pH and light quality on the biomass production and chlorophyll-a content of *S. platensis*.

MATERIALS AND METHODS

Microorganism- Two strains of namely S1 and S2 of *S. platensis* were used in this study. Strain S1 of *S. platensis* was procured from National Facility for Blue Green Algal collection, IARI, New Delhi. Strain S2 of *S. platensis* was procured from Botany Department., University of Rajasthan, Jaipur. Algal stocks were inoculated in the pure CFTRI liquid medium.

Culture Conditions- Both the strains were cultivated separately in 500ml flask containing

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Table-1 Effect of pH on biomass production and chlorophyll-a content on S1 and S2 strains of *S. platensis*

S.No.	pH of CFTRI media	Biomass (mg ml^{-1} dry wt.)		Chl-a content(µg mg^{-1} dry wt.)	
		S1 strain	S2 strain	S1 strain	S2 strain
1	8	9.58a	6.25d	2.87c	1.52b
2	9	13.12b	10.41a	6.45d	5.56c
3	10	17.85c	14.62b	10.421a	9.75d
4	11	12.22d	10.11c	5.62b	5.26a

Different letters in each column indicate statistically significant difference in mean values (P =0.001)

200ml pure CFTRI medium, and an initial biomass concentration was 50ml. Cultures were incubated at temperature of 30±2ºC and illuminated with fluorescent tubes (4K lux). Constant agitation (100 rpm) was applied twice a day with 16/8 hours of photoperiod. The experiments were carried out in triplicates.

Filtration and Washing- Trichomes were collected by filtration using filter paper 8µm pore size (Screen printing paper) and washed with sterilized distilled water to remove the impurities, and finally washed with buffer solution (pH 7), diluted to known volume for further inoculation.

Centrifugation- Diluted inoculum centrifuged at 15,000 rpm.

Analytical methods- Biomass concentration (mg ml^{-1}) was calculated by measuring dry weight. For dry weight measurement the known quantity of *Spirulina* samples were filtered through screen printing paper and oven dried at 75ºC for 5 to 7 hours. The dry weight was expressed in terms of mg ml^{-1}. Hot method (Mackinney 1941) was employed for chlorophyll- a estimation.

As pH is important for the growth of *Spirulina* biomass, different pH levels viz 8, 9, 10, 11 were set for the experiments for both the strains. Subsequent harvest for biomass estimation of *Spirulina platensis* was done after 30 days. The different light sources including red (640- 740 nm), blue (450-520 nm), green (520-570nm) and white light (350-740 nm) were set for the experiments for both the strains.

Statistical analysis: Two-way ANOVA (analysis of variance) was applied to test variance among the mean values of chlorophyll-a and biomass in *Spirulina*. Fisher's LSD test was employed to further isolate the group(s) that differed significantly.

RESULTS AND DISCUSSION

The biomass produced by the S1 and S2 strains of *Spirulina platensis* at different pH levels ranging from 8.0-11.0 at intervals of 30 days after inoculation and the results are presented in In the present study, the biomass production and chlorophyll-a content of both the strains of *Spirulina platensis* has been evaluated under different pH levels. Maximum growth of both the strains was observed at pH 10.0. The reason could be attributed to optimal activity of all the enzymes needed for photosynthesis and respiration, at this pH, however, at high acidic and alkaline pH, there was a decreased activity of the photosynthetic

S.No.	Light wavelength (λ max)	Biomass (mg ml^{-1} dry wt.)		Chl-a content(µg mg^{-1} dry wt.)	
		S1 strain	S2 strain	S1 strain	S2 strain
1	Blue (450-520)	14.47a	12.22d	5.25c	4.58b
2	Red (640-740)	19.29b	17.98a	8.74d	7.89c
3	Green (520-570)	6.65c	8.74b	2.02a	3.57d
4	White (350-740)	19.25d	17.62c	8.521b	7.51a

Different letters in each column indicate statistically significant difference in mean values (P =0.001)

enzyme RUBP- carboxylase and an increased activity of respiratory enzymes glucose 6-phosphate dehydrogenase (Kaushik and Sharma 1997), leading to the reduction in the biomass production. Analysis of variance (ANOVA) indicated significant statistical difference among these values ($P \leq 0.001$). According to Fisher's LSD test (alpha = 0.050), all the factors differ significantly amongst themselves.

The biomass produced by the S1 and S2 strains of *Spirulina platensis* under different wavelengths of lights at intervals of 30 days after inoculation and the results are presented in In the present study, the biomass production and chlorophyll- a content of both strains of *Spirulina platensis* has been evaluated under different wave lengths of lights. Maximum growth of both the strains of *S. platensis* was observed under red light. It was found that the biomass production under red and white lights was significant initially, but later it was non-significant. The reason could be attributed to the maximum absorption of chlorophyll initially under red light, but after some time the red light is converted into white light, which has inhibitory effect on photosynthesis resulting in reduced biomass. Similar results were obtained by Vonshak (1996) who stated that the growth of *S. platensis* became saturated at a range of 150-200$\mu^{-2}sec^{-1}$ and this was about 10-15% of the total solar radiance at 400-700 nm. Analysis of variance (ANOVA) also revealed highly significant statistical difference ($P<0.001$).

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