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Enrichment of chlorophyll and phycobiliproteins in *Spirulina platensis* by the use of reflector light and nitrogen sources: An *in-vitro* study

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ABSTRACT

The use of reflector film in a cyanobacteria culture rack provides a good light intensity. The effect of light intensity on *Spirulina platensis* biomass, phycobiliprotein and amino acid content using KNO_3 and urea as a nitrogen sources were studied. Maximum biomass productivity 6.9 mg $ml^{-1}$and 6.1 mg ml^{-1} with light reflector (LR) + urea and LR + KNO_3 respectively, were noticed. The C-phycocyanin (148.1 ± 2.2 mg g^{-1}) and allophycocyanin (45.2 ± 0.94 mg g^{-1}) contents were reached maximum at 4 klx. The rack system with reflector gave good effect of light intensity (4.8–6.0 klx) than the normal system (2–3 klx). Results showed that reflector used cultivation improved biomass, proteinic pigments and protein biosynthesis in *S. platensis*. This method of cultivation is simple, inexpensive and requires low energy.

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1. Introduction

Cyanobacteria are photosynthetic organism that can efficiently capture light energy and this energy to convert CO_2 into carbohydrates and proteins, with higher areal efficiency than the land plants [1]. The *Spirulina platensis* is a rich source of protein, which is used as a protein supplement for humans, chicks and also in aquaculture [2]. In addition to the high contents of proteins, other substances such as vitamins, polyunsaturated fatty acids, phycocyanin, carotene and chlorophyll pigments that have been used as food and drink, cosmetic and pharmaceutical colorants are emphasized [3]. The photosynthetic pigment, chlorophyll *a*, is the principal photochemically active compound, which functions as a receiver of light for driving photosynthesis [4]. Therefore, efficient use of this energy source is important. Theoretically, a maximum of 9% of total sunlight energy can be converted to algal biomass, i.e. the maximal photosynthetic efficiency is 9%. The widely used systems for *Spirulina* large-scale productions are open ponds. Conversely such systems do not lead to high biomass concentration [5]. The main reasons for reducing biomass are variability of temperature, susceptibility to contamination and low efficiency of light penetration due to the low surface/volume ratio. The use of a closed system could reduce these disadvantages and increase productivity and cell concentration. Thus production of *Spirulina* in a closed system appears to be a powerful and competitive tool, already implemented in developed countries [6].

Phycobiliproteins are assembled into particles named phycobilisomes which are attached in regular arrays to the

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external surface of the thylakoid membrane and act as major light harvesting pigments in cyanobacteria. Phycobilisomes consist of allophycocyanin cores surrounded by phycocyanin on the periphery. Phycocyanin is the major constitute while allophycocyanin functions as the bridging pigment between phycobilisomes and the photosynthetic lamella [7]. Phycocyanin is used as a colorant in food and cosmetic products and also has a therapeutic value [8].

This organism consists of one of the highest chlorophyll contents found in nature, corresponding to 1.15% of its biomass [9], and it depends on good cultural practices and energy. Today's, life higher amount of electric energy used for different sector. On average, the commercial sector consumes about 38% of its electricity for lighting. Plant tissue culture, microalgal and cyanobacterial culture laboratories expend a lot of power to provide the necessary light energy up to 12 klx in a 16 h/8 h light dark cycle. Besides the depletion of the natural coal resources, it involves the release of substantial amounts of carbon dioxide, which is one of the chief green house gases responsible for global warming and climate-change [10,11].

Light intensity plays an important role in algal culture, but its requirements vary greatly with the culture depth and the density of the algal culture. At higher depths and cell concentrations, the light intensity must be increased to penetrate through the culture. One of the major factors influencing chlorophyll biosynthesis is the light intensity, and it changes the biochemical content and growth of an organism under both limited and high illumination conditions [12]. From the laboratory studies, it was observed that not only the light intensity on the growth of cyanobacteria but also culture media composition can improve the protein biosynthesis in Spirulina. Nitrates, such as KNO_3 and $NaNO_3$, are commonly used as a nitrogen source for Spirulina cultivation. The objective of the present work is to study the effect of reflector light and nitrogen sources for the chlorophyll, biomass and phycobiliproteins production of *S. platensis* through saving the amount of electric energy consumption in the culture rack.

2. Material and method

2.1. Microorganism and culture media

S. platensis was obtained from the National Centre for Conservation and Utilization of Blue-green Algae, IARI, New Delhi. The culture media suggested by Kaushik [13] was used, having $NaNO_3$ as the nitrogen source. 16.8 g l^{-1} $NaHCO_3$, 0.5 g l^{-1} K_2HPO_4, 2.5 g l^{-1} $NaNO_3$, 1.0 g l^{-1} K_2SO_4, 1.0 g l^{-1} NaCl, 0.2 g l^{-1} $MgSO_4 \cdot 7H_2O$, 0.04 g l^{-1} $CaCl_2$, 0.01 g l^{-1} $FeSO_4 \cdot 7H_2O$, 0.08 g l^{-1} EDTA. A culture media similar to the one suggested by this author, having urea and KNO_3 (2.5 g l^{-1}) as the nitrogen source in the place of $NaNO_3$ was used, as well.

2.2. Culture condition and experiments

The Spirulina was cultivated in the 500 ml culture bottle containing 250 ml of sterilized medium. The pH was set at 9.5 ± 0.1, and the temperature was maintained at 28–30 °C. The rack shelves have a dimension of 120 cm × 37.5 cm × 45 cm ($l \times b \times h$) (Sigma Culture Rack). Each shelf was fitted with four fluorescent tube lights (36 W Supersaver $_{xLR}$ fluorescent lamp, India). A silver-tinted polyester film was fixed to the shelves on all sides to serve as a light reflector. In order to increase photosynthetic efficiency, the rack culture design can be adapted. The light intensity was measured at different points in the rack with and without reflector by luximeter. The light intensities recorded at different points were ranged from 2.5 to 3.0 klx without the reflector, and from 4.8 to 6.0 klx with reflector film. The experiments were carried out in two types of shelves, (a) Shelves without reflector and (b) Shelves with reflector one [14]. The nitrogen sources, KNO_3 and urea were added continuously in the form of aqueous solution in both experiments.

2.3. Biomass yield

At 2-days intervals, 10 ml of homogenized algal suspension were filtered through a Whatman GF/C filter paper that had been dried in an oven for 24 h at a constant temperature of 60 °C. The biomass yield was determined by the method of Vonshak [15].

2.4. Biochemical analysis

Chlorophyll and carotenoid content was estimated by Mackinney [16]. Protein content was determined by the Bradford method [17] using bovine serum albumin as a standard. Amino acids were estimated by paper chromatography by the method of Allen et al. [18], and the concentration is expressed as g/16 N (approximately 100 g protein). The algal biomass were harvested through centrifugation and then washed and dried overnight (50 °C) for phycobiliproteins extraction. It was extracted in the potassium phosphate buffer (pH − 7) until the colored supernatant obtained from the pellet through repeated freezing and thawing method. The absorbencies of phycobiliproteins containing supernatant were measured at 562, 615 and 652 nm using phosphate buffer as a blank. For estimation, the following equations were used and expressed in mg g^{-1} per dry weight [19].

$$\text{Phycocyanin (C-PC)} = \{A_{615} - (0.474 \times A_{652})\}/5.34 \quad (1)$$

$$\text{Allophycocyanin (APC)} = \{A_{652} - (0.208 \times A_{615})\}/5.09 \quad (2)$$

$$\text{Phycoerythrin (C-PE)} = \{A_{562} - (2.41 \times \text{PC}) - (0.849 \times \text{APC})\}/9.62 \quad (3)$$

2.5. Statistical analysis

The data were presented as means ± standard deviation of three determinations. Analysis of variance (ANOVA) method was used to find out if the significant difference existed among the treatment at $p < 0.05$ level. (SPSS version 11.5, 2001).

3. Results and discussion

Different light intensities and nitrogen sources were tested in order to specify the most appropriate culture condition for intensive accumulation of biomass and phycobiliproteins of S.

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