RESEARCH PAPER
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Effect of different nitrogen concentrations on the biomass and biochemical consituents of *Spirulina platensis* [Geitler]

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Nitrogen is one of the primary requirements of growth media for any cell. Absence of nitrogen or starvation condition is considered as stress by the organisms. Increasing the nitrogen concentration upto 0.04 M significantly increased the biomass, protein, phycocyanin and lipid content of *Spirulina*, while total carotenoids and β-carotene content were reduced compared to control. In contrast, the biomass, protein, phycocyanin and lipid content were reduced in the control indicating that nitrogen requirement for synthesis of aminoacids, which make up protein and other cellular components such as phycocyanin. The accumulation of carotenoids under nitrogen starvation may be due to production of a large amount of acetyl-CoA, which serves as a precursor for synthesis of carotenoids.

Key words : *Spirulina platensis,* Nitrogen, Biomass, Pigment, Lipids

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INTRODUCTION

Spirulina platensis is a microscopic, filamentous cyanobacterium rich in protein, vitamins, essential amino acids, minerals, and essential fatty acids. *Spirulina* has been commercially exploited for the production of humanfood supplements, animal feed and pharmaceuticals because of its ability to produce large quantities of valuable products, such as phycocyanin (Estrada *et al.,* 2001).The large-scale production of *Spirulina* biomass depends on many factors, the most important ofwhich are nutrient availability, temperature andlight. These factors can influence the growth of *Spirulina* and the composition of the biomass produced by causing changes in metabolism, which considerably modify the time course of the accumulation of the main biomass components (Kim and Young, 2007). The objective of the present investigation was to evaluate the influence of nitrogen concentration on the growth and biochemical constituents of *Spirulina platensis* to optimize the culture condition for its large scale production.

RESEARCH METHODOLOGY

Spirulina platensis was isolated from the alkaline lake samples of Kodaikanal (Tamil Nadu, India) using Zarrouk's medium (Zarrouks, 1966). In order to evaluate the effect of different NaNO3 levels, *S. platensis* (10%) was added to 250 ml Zarrouks medium with different $NaNO_3$ concentrations *viz.*, 0.01 M (0.835 g), 0.02 M (1.045 g), 0.03 M (1.265 g),0.04 M (1.465 g) and control (0.625 g). The flasks were kept in shade net under diurnal natural light conditions. Cultures were analyzed for their growth and biochemical (nutrient and pigment) constituents on 30^{th}day after inoculation (DAI).

For biomass content, a known volume of *Spirulina* was filtered in a pre-weighed filter paper and oven dried at 75^0 C for 4 to 6 h, cooled and then again weighed. The difference between the initial and final weight were taken as dry weight. Protein content was determined as per Lowry *et al.* (1951), using bovine serum albumin as a standard. The total lipid content was determined from chloroform: methanol (1:2) (v/v) extract (Bligh and Dyer, 1959).

Chlorophyll-a was extracted in methanol as described by Vonshak (1997). For phycocyanin, the culture was precipitated using 50 per cent ammonium sulphate and the pigment was extracted completely by repeated freezing and thawing (Boussiba and Richmond, 1979). Total carotenoids and β-carotene were extracted as described by Rafiqul *et al.* (2003).

RESEARCH FINDINGS AND ANALYSIS

Nitrogen is the major form of nitrogen commonly used for assimilation by non-nitrogen fixing cyanobacterium under laboratory conditions. The assimilatory reduction of nitrate is a fundamental biological process in which a highly oxidised form of organic form of nitrogen is reduced to nitrite and then to ammonia (Bhattacharya and Shivaprakash, 2006).

In the present study, various $NaNO_3$ concentrations in Zarrouks medium were tried for the optimal growth and chemical composition of *S.platensis* and the results revealed that increase in the nitrogen concentration upto 0.04 M recorded relatively higher growth (Fig. 1) in terms of biomass (2.72 mg/ml) followed by 0.03 M (2.65 mg/ml). Filali *et al.* (1997) reported that *S.platensis* is able to use numerous mineral and organic nitrogen sources for growth. Among them nitrate is the more convenient because it may be provided to the culture at higher concentrations (up to 100 mM), that delay the appearance of nitrogen limitation and therefore, allow long term cultures.

With regard to carotenogenesis (Fig. 1), the total carotenoids (3.124 µg/mg) were found to be increased with decrease in the nitrogen concentration. El-Baz *et al.* (2002) stated that the mechanism suggested for the acclimation of carotenoids in *Chlorella* and *Dunaliella* to grow under stress conditions also applies to *Spirulina.* It seems that the division of algae cells grown under nitrogen starvation is blocked, while photosynthesis continues, leading to storage of carotenoids, carbohydrates and triglycerides as they do not require nitrogen for their synthesis. The accumulation of carotenoids grown under nitrogen starvation may be due to stimulation of lipolysis process which produced a large amount of acetyl-CoA, which serves as a precursor for synthesis of carotenoids. β-carotene has anti-oxidant properties that quench excessive free radicals, restoring the physiological balance. The increase in β-carotene (Fig. 1) content of control (1.520 µg/mg) in nitrogen starved cells may be attributed to excessive formation of free radicals under the stress. Additional β-carotene is produced in order to protect the cells and to continue their growth (Moller *et al.*, 2000). Hence, β-carotene production is markedly increased in nitrogen starvation.

The *S.platensis* grown with high nitrogen levels reported maximum protein (732.60 µg/mg) and total phycocyanin(173.02 µg/mg) contents(Fig.2) at 0.04 M $NaNO_3$. This might be due to the reason that nitrogen is required for synthesis of the aminoacids, which make up protein and other cellular components such as phycocyanin. When the cultures were completely deprived of nitrogen, a corresponding specific decrease in the cell content of phycocyanin was observed. Hanaa and El-Baky (2003) reported that increase in nitrogen concentration led to increase in phycocyanin pigments and soluble protein content, whereas, decrease in nitrogen levels led to decrease in phycocyanin, chlorophyll and protein content due to breakdown of the whole chloroplasts.Similarly to these results, in the present study phycocyanin(121.80µg/mg), chlorophyll (8.356 µg/mg) and protein (493.70 µg/mg) contents were observed to be lower in the control. The significant decrease in chlorophyll during nitrogen starvation may be because chlorophyll molecule contains four nitrogen

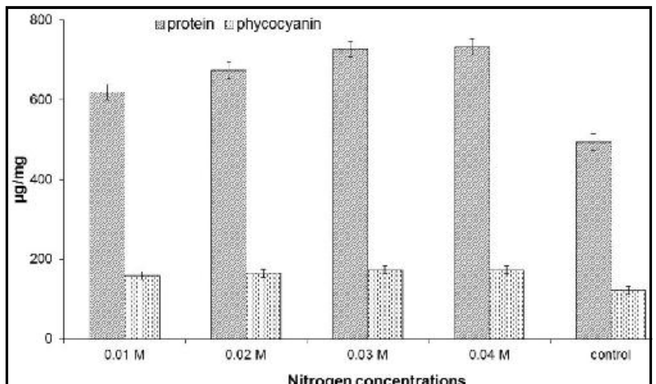

Fig. 2 : Effect of different nitrogen concentrations on protein and phycocyanin contents of *Spirulinaplatensis*

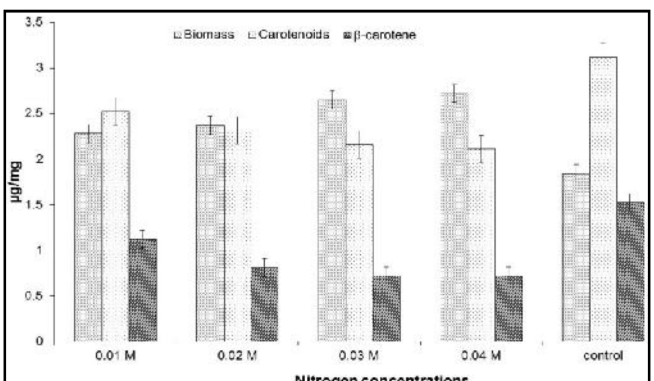

Fig. 1 : Effect of different nitrogen concentrations on growth and carotenoid contents of *Spirulinaplatensis*

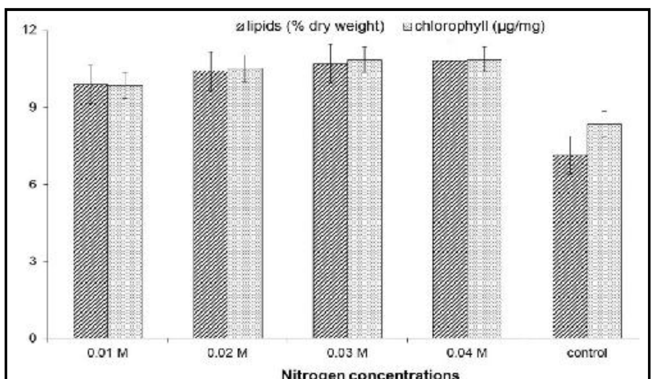

Fig. 3 : Effect of different nitrogen concentrations on lipid and chlorophyll contents of *Spirulinaplatensis*

atoms in its structure and, therefore, it becomes very difficult for the cell organelles to synthesize chlorophyll in the absence of nitrogen (Pisal and Lele, 2005).

With regard to lipids (Fig. 3), higher concentration of sodium nitrate resulted in an increase in lipids (10.82 % dry weight) at 0.04 M, similar to the results Olguin *et al*. (2001) observed a higher content of total lipids in *Spirulina* growing in Zarrouks medium is compared to cultivate under nitrogen starvation.

To summarize enhanced production of biomass, chlorophyll, lipids and phycocyanin are enhanced by increased nitrogen concentration, while carotenoids and β-carotene content could be enhanced under nitrogen starvation.

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