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Assessment for the influence of nitrate and phosphate on extracellular ammonium excretion by cyanobacterial strains of Loktak Lake

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ABSTRACT

In the present study, ten (10) cyanobacterial strains isolated from Loktak Lake were examined for their possibility of increasing the content of extracellular ammonium excretion subjecting to various concentrations of nitrate and phosphate. The concentrations of nitrate and phosphate required for maximum growth varied with species.In the influence of nitrate, among heterocystous forms (Nostoc spp. BTA60, BTA61, BTA80,BTA67 and BTA950; Calothrix sp. BTA73; Anabaena sp. BTA964), maximum extracellular ammonium was excreted by Nostocmuscorum BTA950 (238.07 µgml^{-1}) in 0N and minimum by Anabaena sp. BTA964 (25.95 µgml^{-1})in 2N. Among non-heterocystous forms (Phormidium spp. BTA52, BTA75 and BTA1048), maximum extracellular ammonium was excreted by Phormidium sp. BTA52 (134.09 µgml^{-1}) in 1N and minimumby Phormidium sp. BTA1048 (55.91 µgml^{-1})in 0N. On the contrary, experiments on influence of phosphate, Nostocmuscorum BTA950 showed the maximum excretion of extracellular ammonium (233.79 µgml^{-1}) in 1N and minimumby Anabaena sp. BTA964 (11.09 µgml^{-1}) in 0N concentrations of phosphate. The findings indicate that these strains mayproved as promising microorganism for the extracellular ammonium excretion which could be exploited biotechnologically for the benefit of biofertilizer applications.

Keywords: Biofertilizer, Cyanobacteria, Extracellular ammonium excretion, Loktak Lake, Nutrients

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INTRODUCTION

Cyanobacteria have the potential to produce a wide range of fine chemicals including polyunsaturated fatty acids, carotenoids, biliproteins, antibiotics, vitamins, polysaccharides, bioflocculants, biosurfactants, growth promoters etc. and thus can be utilized on a commercial scale. The biochemical constituents of cyanobacteria depends on the nature of strains, physiological state of the culture and the environment [1, 2, 3, 4].

Cyanobacteria can both photosynthesize and fix nitrogen and these abilities, together with great adaptability to various soil types, make them ubiquitous. Cyanobacteria also have a unique potential to contribute to productivity in a variety of agricultural andecological situations. They play an important role in maintenance and build-up of soil fertility, consequently increasing rice growth and yield as a natural biofertilizer [5].The utilizing cyanobacteria as an efficient source of biofertilizer for rice field has been practised and adopted in India [6]. Use of biofertilizers is cost-effective, cheap and renewable source to supplement the chemical fertilizers.

Culture experiments have been successful where ecophysiological requirements of particular species are known. Nutrients plays a very important role in the metabolic and physiological activities of cyanobacteria. It strongly

affects biomass production, chemicals dissociation and cell physiology. Nitrate, nitrite and ammonium ions and many dissolved organic nitrogenous compounds (urea, free amino acids and peptides) are the main sources of nitrogen for the algae used mainly for the synthesis of amino acids and proteins [7].

The work presented here was proposed to determine how different concentrations of nitrate and phosphate play a role in the excretion of extracellular ammonium by the cyanobacterial strains isolated from freshwater habitats of Loktak Lake.Data generated during the present investigation could be useful in understanding of a commercial or biotechnological potential of these strains.

MATERIALS AND METHODS

Cyanobacterial strains

Ten (10) cyanobacterial strains used in the present study were obtained fromNational Repository for Cyanobacteria and Microgreen algae (Freshwater)at DBT-IBSD, Imphal, Manipur, Indiawhich were isolated from Loktak Lake, the only largest freshwater lake in the North-Eastern region of India.

Growth conditions

Cyanobacterial strains were optimized for its growth and high yield extracellular ammonium excretion from it. In order to assess the influence of nutrients on the extracellular ammonium excretion, different concentrations of nitrate and phosphate of the BG-11 medium [8] was adjusted. A log phase culture was homogenized and 1 ml of the culture was inoculated into 250 ml cotton-plugged Erlenmeyer flask containing 100 ml of BG-11 medium (different concentrations of $NaNO_3$ and K_2HPO_4) broth medium in 150 ml cotton-plugged Erlenmeyer flask. Concerned nutritional treatments were 0=0.00 gl^{-1}, 0.5=0.75 gl^{-1}, 1=1.50 gl^{-1} and 2=3.00 $gl^{-1}$of $NaNO_3$ as concentrations for nitrate source; 0=0.00 gl^{-1}, 0.5=0.02 gl^{-1}, 1=0.04 $gl^{-1}$and 2=0.08 gl^{-1} of K_2HPO_4 for phosphate source. 1.50 $gl^{-1}$and 0.04 $gl^{-1}$for $NaNO_3$ and K_2HPO_4 respectively denoted the control. Cultures were allowed to grow in culture room with light intensity of 54-67 µmol photons $m^{-2}s^{-1}$provided by cool white fluorescent tubes following 14:10h light:dark conditions maintained at 28±2ºC. The flasks were stirred twice daily to allow circulation of air and uniform mixing of nutrients.

The amount of ammonium concentration excreted in the medium was measured by Solorzano's phenol-hypochlorite method [9]. All the experiments were carried out in triplicates and the data were presented as mean values ± SD of three replicates.

RESULTS AND DISCUSSION

Quantitative analysis on extracellular ammonium excretion against different concentrations of nitrate was presented (Fig. 1a-1j). Maximum amount was excreted in almost all heterocystous forms during their growth in nitrogen free medium.

In the present investigation, effect of nitrate showed that among heterocystous forms (*Nostoc* spp. BTA60, BTA61, BTA80,BTA67 and BTA950; *Calothrix* sp. BTA73; *Anabaena* sp. BTA964), maximum extracellular ammonium was excreted by *Nostocmuscorum* BTA950 (238.07 µgml^{-1}) in 0N and minimumby *Anabaena* sp. BTA964 (25.95 µgml^{-1}) in 2N.Among non-heterocystous forms (*Phormidium* spp. BTA52, BTA75 and BTA1048), maximumammonium was excreted by *Phormidium* sp. BTA52 (134.09 µgml^{-1}) in 1N and minimum was excreted by *Phormidium* sp. BTA1048 (55.91 µgml^{-1}) in 0N concentrations of nitrate.

Regarding effect of phosphate onextracellular ammonium excretion, it was observed that the quantity was maximum in 1N followed by 2N, 0.5N and 0N concentrations of phosphate (Fig. 2a-2j). *Nostocmuscorum* BTA950 excrete maximum(233.79 µgml^{-1}) in 1N whereas minimum was observed in 0Nby *Anabaena* sp. BTA964 (11.09 µgml^{-1}).

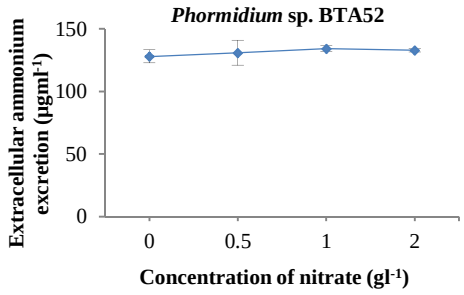
A primary effect of phosphate deficiency is inhibition of uptake and utilization of phosphate leading to depletion of nucleotide phosphate bonds. Cyanobacterial cells store polyphosphate reserves at high levels during exponential growth, mediated by polyphosphate synthetase, which requires energy [10]. These polyphosphate bodies were degraded during P-deficiency.During differentiation of a vegetative cell into a heterocyst, major structural and biochemical changes occur that affect nitrogen fixation. Under high concentrations, 2N and absence of phosphate, the inability to produce more amount ofammonia might be due to the absence of strategies to enhance P acquisition (uptake by the expression of highly efficient transporters) and conserve use i.e. mobilization of P by modified C and N metabolism.

With cyanobacteria, nitrate is taken up by a common high affinity transport system involving the NrtABCD permease (an ABC-type transporter) and to a lesser extent enters the cells by diffusion [11, 12,13].Once inside the cell, nitrate is reduced to nitrite by nitrate reductase, and nitrite is further reduced to ammonium by nitrite reductase [14].

Growth of many species of algae is limited by the availability of nitrogen and/or phosphate [15, 16,17]. Nutrient-enhanced (mixed N and P) cyanobacterial growth was also observed by [18]. Nitrate uptake can be influenced by availability of phosphate ion (PO_4^{3-}), which has an important role in cellular energetics as part of ATP and which influences the activity of many enzymes required for cell metabolism, including the nitrate reduction process [19].

It has been known that nitrogen-deprived cells generally show higher inorganic nitrogen uptake rates than nitrogen-sufficient cells in the short term [20,21]. However, after an initial nitrate uptake in nitrogen-deprived cells, these cells entered a lag phase in which no nitrate uptake occurred. Furthermore, the length of the lag phase increased with the time that cells were deprived of nitrate. In contrast, nitrogen-sufficient cells continued to take up nitrate.In addition, nitrogen deprivation may cause cell autolysis [22] and excretion of secondary metabolites [23, 24]. The conditions for the most efficient longterm nitrate uptake clearly include a rapidly growing, healthy, phosphate-sufficient cyanobacterial strain.

Our results demonstrate the potential of cyanobacteria to efficiently utilize and survive under different concentrations of nitrate and phosphate and subsequently ability to excrete extracellular ammonium into the surrounding medium. Moreover, potentially valuable biomass was produced as a by-product.Data generated during present investigations could be useful in understanding of a commercial or biotechnological potential of these strains. The results clearly prove that the extracellular ammonium content were specific characteristics of every individual cyanobacterial strain which is very dependent on growing conditions. The medium composition influences the normal growth of cyanobacteria and normal development of physiological processes.



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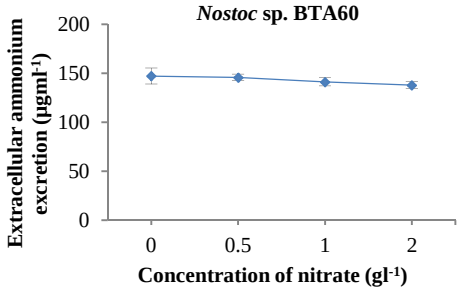

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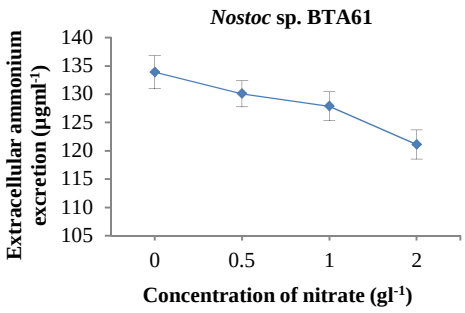

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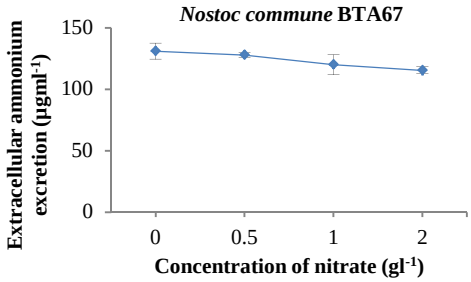

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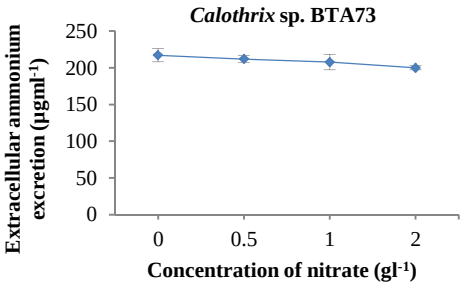

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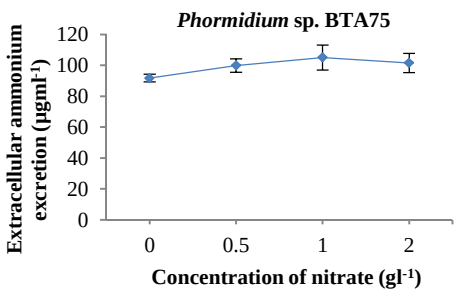

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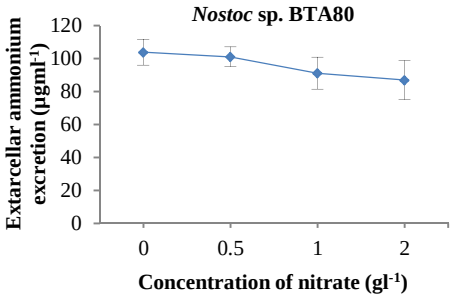

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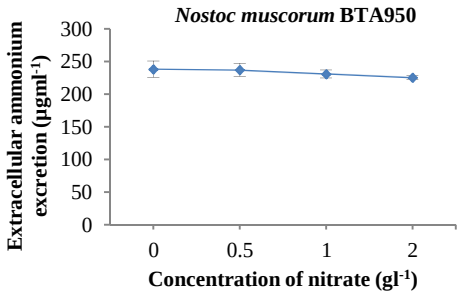

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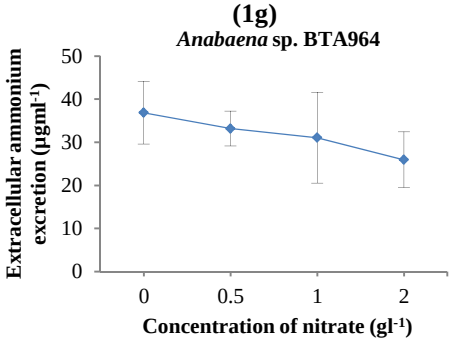

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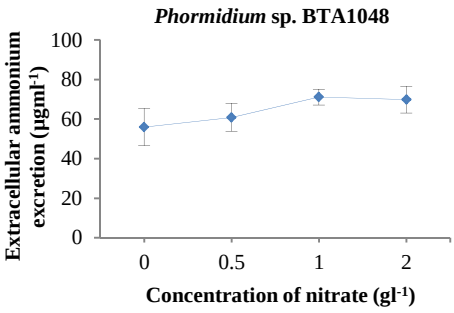
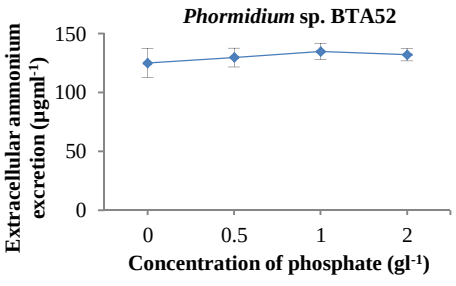


(1j)

Fig. 1a-1j: Production of extracellular ammonium excretion in different concentrations of nitrate



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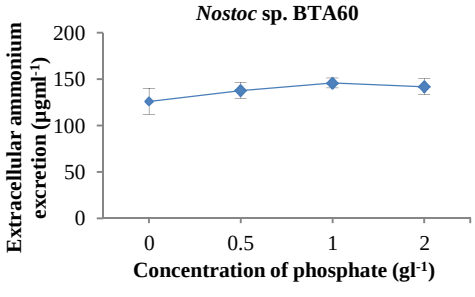

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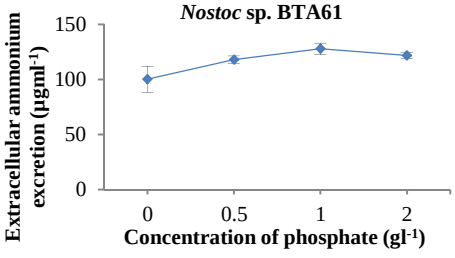

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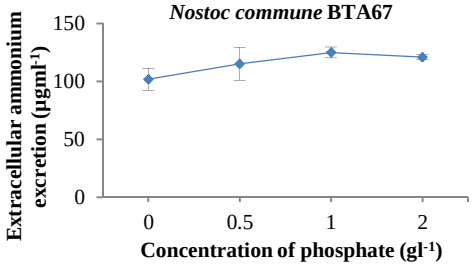

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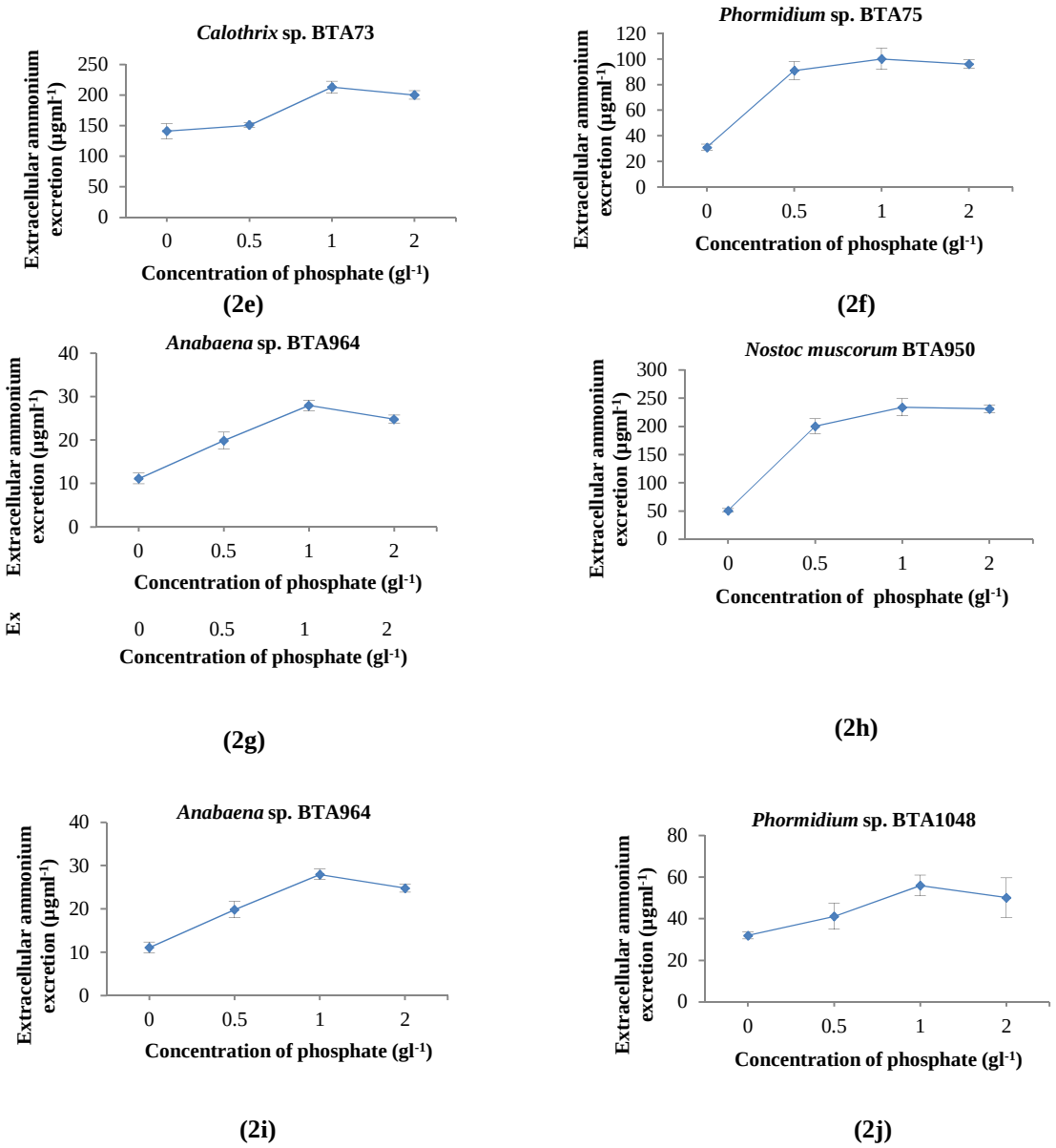

Fig. 2a-2j: Production of extracellular ammonium excretion in different concentrations of phosphate

CONCLUSION

In contrast with marine environment, freshwater sources have been less explored. Our studies will establish the rich cyanobacterial diversity of Loktak Lake and also help conserve and utilize them in bioindustry. Improvement in the ammonium excretion with changes of environmental factors (especially concentration of nutrients) could be a good basis for the exploitation of studied cyanobacterial strains as a source of valuable products. Since, these isolates were found to excrete more of extracellular ammonium, they are considered as potent candidates for alternative resources for biofertilizers applications.

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REFERENCES

[1]MA Vargas; H Rodriguez; J Moreno; H Olivares; JA Del Campo; J Rivas; MG Guerrero.*J.Phycol.*, **1998**, 34(5), 812-817.

[2] R Subhashini; K Kumar; S Kannaiyan. *Indian J.Microbiol*., **2003**, 43, 165-169.
[3] IP Maslova; EA Mouradyan; SS Lapina; GL Klyachko-Gurvich; DA Los.*J. Plant Physiol*.,**2004**, 51(3), 353-360.
[4] N Rosales; J Ortega; R Mora; E Morales. *Cienc.Mar*., **2005**, 31, 349-355.
[5] T Song;L Martensson;T Eriksson; W Zheng;U Rasmussen. *Microbiol.Ecol*., **2005**, 54, 131-140.
[6] GS Venkataraman.*Curr.Sci*., **1981**, 50, 253-256.
[7] CSReynolds. The ecology of freshwater phytoplankton-Cambridge studies in ecology, Cambridge University Press, Cambridge, **1984**, pp. 374.
[8] RY Stainer; RKunisawa;M Mandel; G Cohen-Bazire.*Bacteriol.Rev*., **1971**, 32,171-205.
[9] L Solorzano. *Limnol.Oceanogr*.,**1969**, 4, 799-801.
[10] G Falkner; R Falkner; D Graffius; P Strasser. *Arch. Hydrobiol*., **1984**, 101, 89-99.
[11] E Flores; A Herrero. In: The molecular biology of cyanobacteria. Kluwer, Dordrecht, The Netherlands, **1994**, pp. 487-517.
[12] M Lomas; PM Glibert; GM Berg. *J. Phycol*., **1996**, 32, 907-916.
[13] I Luque; E Flores; A Herrero. *Biochim.Biophys.Acta*.,**1994**, 1184, 296-298.
[14] A Herrero; E Flores. In: Cyanobacterial nitrogen metabolism and environmental biotechnology. Narosa Publishing House, New Delhi, India, **1997**, pp. 1-33.
[15] BE Lapointe.*Bull. Mar. Sci*.,**1989**, 44, 312-323.
[16] ST Larned.*Mar.Biol*., **1998**, 132, 409-421.
[17] GR Russ, LJ McCook. *J. Exp. Mar. Biol. Ecol*.,**1999**, 235, 237-254.
[18] MW Miller;ME Hay;SL Miller;D Malone;EE Sotka;AM Szmant. *Limnol.Oceanogr*.,**1999**, 44, 1847-1861.
[19] G Ahlgren.*Hydrobiologia*,**1988**, 170, 191-210.
[20] C Garbis; DO Hall; J Serra. *J. Appl. Phycol*., **1992**, 4, 139-148.
[21] A Lavoie; J de la Noue.*Water Res*.,**1985**, 19, 1437-1442.
[22] RJ Daley; SR Brown.*J. Phycol*.,**1973**, 9, 395-401
[23] S Boussiba. In: Cyanobacterial nitrogen metabolism and environmental biotechnology. Narosa Publishing House, New Delhi, India, **1997**, pp. 35-72.
[24] CPD Brussaard; AAM Noordeloos; R Riegman.*J. Phycol*., **1997**, 33, 980-987.