Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © PHYCOSPECTRUM
ISSN: 2229 – 6905

Nutrients Kinetics and their effects on Bio-fertilizer *Nostoc spongiaeforme*

Bhagya Lakshmi Jyothi,K[1*] and TRK Reddy[2]

[1]*Department of Biotechnology, Dr. Lankapalli Bullayya College, Visakhapatnam-530 013, Andhra Pradesh, India*
[2]*Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India*
**Corresponding author, kbljyothi@gmail.com*

ABSTRACT

The percentage of nitrogen in Cyanobacteria would be 8-10% of the total dry weight and nitrogen becomes an important factor in controlling the luxuriant growth of blue-green algae. The growth of photosynthetic diazotrophs (blue-green algae) greatly depends on the environmental conditions such as physical and nutritional factors in flooded rice-fields and in other habitats. A study was conducted to investigate the effect of nitrogen on growth, nitrogen uptake, rate of uptake of nitrogen and changes in Km values in the culture of cyanobacterium *Nostoc spongiaeformae* collected from rice- field soils of Andhra Pradesh, India. An experiment was designed to find out the effect of different combined nitrogen sources(KNO_3, $NaNO_2$ and NH_4Cl) on the growth of algae when exposed to different concentrations (0.1,1.0,1.5and 2.0 mg/ml) and incubated for 72 hours. Based on the results potassium nitrate (KNO_3) and sodium nitrite ($NaNO_2$) were favorable for the growth of Cyanobacteria even at high concentrations whereas ammonium chloride (NH4Cl) has been proved to be toxic for growth at higher concentrations.

Key words: Biofertilizers; *Synechococcus aeruginosus*; *Nostoc spongiaeformae*; Nitrogen nutrients; Growth and Kinetics

INTRODUCTION

Nitrogen has been well documented and stated as an important nutrient for the growth and metabolism of plants and microorganisms and it has been recognized that high quantities of nitrogenous sources are being required for the growth of improved crops especially rice in India. Farmers have been employing the chemical nitrogen fertilizer $\left(NO_3^-, NO_2^-, NH_4^+\right)$ in the rice-fields which undergo denitrification and are degraded with nitrate reductase and nitrite reductase to NH_3 which on further transamination transform into amino acids (Fogg et al., 1973; Anand, 1990). Besides these inorganic chemical nitrogen sources, organic nitrogen compounds which are residues of plants and animals consisting of various complex organic nitrogenous compounds are required for better rice crop yield. These nitrogenous compounds are further denatured and degraded by microorganisms into nitrogenous salts, which are finally absorbed by the soil and enrich the soil.

The predominantly growing blue-green algae in tropical rice-fields are attributed with the role of augmenting the nitrogen status of the rice-field soil by nitrogen fixation in the presence of nitrogenase enzyme (Stewart, 1980). Besides fixing elemental nitrogen, the blue-green algae are also able to assimilate the extraneous or combined nitrogen supplied as ammonia, nitrate, nitrite and urea which could be incorporated in the synthesis of pigments and also aminoacids (Fogg and Wolfe, 1954; Neilson and Larsson, 1980). Different blue-green algae exhibited different levels of efficacy of absorption and assimilation at different concentrations (high or low) of nitrogenous compounds. Lower concentrations of inorganic nitrogen substances **/** fertilizers (lower than 50 kg**/**ha) would always promote the growth of blue-green algae while higher dose (100 kg**/**ha) would prove as toxic level and inhibited the growth (Goyal, 1985; Singh and Dash, 1994) in rice field soil.

Blue-green algae showed wide variation in their preference for the available nitrogen source in the ambient media. Nitrate nitrogen was evidenced as the most suitable nitrogen source for the growth of *Nostoc muscorum* (Kratz and Myers, 1955), *Westiellopsis prolifia* and *Hapalosiphon welvitchin* (Kaushik, 1986) in basal media. Ammonia has been found to be very good source of nitrogen in strains of *Anabaena* sp. (Bottomley et al, 1979), *Anabaena flos-aquae* (Rhee and Lederman, 1983) and *Aphanizomenon flos-aquae* (Ward and Wetzel, 1980). The maximum nitrite and. nitrate concentration for growth of *Anabaena doliolum* was 0.02 M and further increase of nitrite and nitrate showed less growth beyond 0.02 M, however the concentrationsof ammonium- nitrate above 0.4 M were toxic (Singh and Srivastava, 1968), may cause cell lysis in *Phormidium persicinum* (Pinter and Provasoli, 1958) and also in *Calothrix scopulorum* (Stewart, 1964).

The above literature on nitrogen sources had been mainly confined to the effect of different combined nitrogen sources on the growth of algae on supplying with various combined nitrogen sources. In the present study the effect of various nitrogen sources i.e. sodium nitrite ($NaNO_2$), potassium nitrate (KNO_3) and ammonium chloride (NH_4Cl) on the growth in terms of chlorophyll-a and protein contents after 72 hours of inoculation of *Nostoc spongiaeforme*, besides the kinetics and Km values (Michaelis constant) of nitrogen sources recorded.

MATERIALS AND METHODS

The filamentous, heterocystous and nitrogen fixing *Nostoc spongiaeforme* are very commonly found in rice fields were isolated from the local rice-fields of Andhra Pradesh, India. Isolation and purification were performed by the usual protocols of dilution and plating. Axenic and experimental algal cultures of *Nostoc spongiaeforme* were maintained and grown in Chu. No. 10 medium as modified by Gerloff et al., (1960). The pH was adjusted to 8.5 and stocks and experimental cultures were maintained in culture chambers at 28±2^{0}C and illuminated with fluorescent light emitting 1600 lux and shaken twice a day. Short-term experiments (72 hours incubation period) were conducted to study the effect of various types of nitrogen sources $\left(NO_3^-, NO_2^-, NH_4^+\right)$ on growth and cellular metabolites and uptake and their kinetics were studied in this algae. The estimation method of nitrate $\left(NO_3^-\right)$ (Brucine sulphanilic method mentioned by Brown et al., 1974), nitrite $\left(NO_2^-,\right)$ (Sulphanilamide and N-(1- naphthyl)- ehtylenediamine - dihydrochloride method, APHA, 1980) and ammonia $\left(NH_4^+\right)$ (Nessler's reagent method, APHA, 1980) were employed . For chlorophyll- a estimation, 10 ml algal culture were centrifuged at 5000g for 5 min and O.D. measured to calculate chlorophyll as per the formulae of MacLachlan and Zalik (1963). Proteins were determined by the method of Lowry et al., (1951).

Different concentrations (0.1, 1.0, 1.5 & 2.0 mg per ml) of nitrogen sources i.e. sodium nitrite ($NaNO_2$), potassium nitrate (KNO_3) and ammonium chloride (NH_4Cl) were supplied in growth medium. pH of the growth media and control cultures(BM-NO3) were adjusted to 8.5 with 0.1 N NaOH and 0.1 N HCl, were sterilized and inoculated with equal number of nitrogen starved small vegetative filaments of 2-4 cells (125 x 10^4 per ml which was equivalent to 100 mg fresh weight) of *Nostoc spongiaeforme* grown in NO_3 free basal medium. (For obtaining starved inoculums, the growing cultures were transferred to the required nutrient deficient media and were allowed to grow for one week in that medium. The starvation was observed by the reduction of pigment in the algal cultures). After 72 hours of inoculation of algae, growth was measured in terms of chlorophyll-a and protein. The uptake of nitrogenous substances was measured as the difference between nitrogen content before and after inoculation with algal cells (72 hours).

RESULTS:

In order to examine the effect of the nitrogen sources of potassium nitrate (KNO_3), sodium nitrite ($NaN0_2$) and ammonium chloride (NH_4Cl) on growth and their kinetics in *Nostoc spongiaeforme* , short-term experiments were conducted where the results were recorded after 72 hours after inoculation of algae.

The results recorded in Table 2 evidenced the effect of different concentrations (0.1, 1.0, 1.5 and 2.0 mg per ml) of nitrogen sources i.e. potassium nitrate (KNO_3), sodium nitrite ($NaNO_2$) and ammonium chloride (NH_4Cl) on the growth in terms of chlorophyll-a and proteins, nitrogen uptake and Km values of nitrogen sources in *Nostoc spongiaeforme*. As shown in the Table 1, the total contents of chlorophyll-a and proteins were conspicuously reduced in the nitrogen depleted medium (BM-NO_3) as compared to nitrogen supplemented cultures i.e. potassium nitrate (KNO_3), sodium nitrite ($NaNO_2$) and ammonium chloride (NH_4Cl) and indicated the role of nitrogen in controlling the growth (chlorophyll-a and proteins). Among the three nitrogen sources, potassium nitrate (KNO_3) showed growth promotory effect when compared to sodium nitrite ($NaNO_2$) and ammonium chloride (NH_4Cl).

Graded concentrations of the three nitrogen sources exert quantitative effects on growth in terms of chlorophyll-a and proteins in *Nostoc spongiaeforme*. The levels of chlorophyll-a and proteins increased with the gradual increase in concentration of potassium nitrate (KNO_3) up to 2.0 mg per ml concentration. Except 0.1 mg per ml potassium nitrate (KNO_3) cultures, high quantities of chlorophyll-a and proteins were evidenced in all the other concentrations (1.0, 1.5 and 2.0 mg per ml) as compared to control (BM-NO_3). The cultures grown with graded concentrations of sodium nitrite ($NaNO_2$) also showed the similar pattern like that of potassium nitrate (KNO_3) by showing the increased levels of these cellular components (chlorophyll-a and protein) up to a concentration of 2.0 mg per ml but the amounts of these cellular metabolites were less than those found in the cultures grown with potassium nitrate (KNO_3) at the corresponding concentrations. Increasing concentrations of ammonium chloride (NH_4Cl) also enhanced the growth (chlorophyll-a and proteins) up to 1.5 mg per ml, thereafter it fell with 2.0 mg per ml concentration. However, the quantities of chlorophyll-a and proteins were nearly the same as in 1.5 mg per ml concentration of ammonium chloride (NH_4Cl) as well as in the basal medium.

Based on these results, potassium nitrate (KNO_3) and sodium nitrite ($NaNO_2$) were favourable for the growth of *Nostoc spongiaeforme* even at high concentrations whereas ammonium chloride (NH_4Cl) has been proved to be toxic for growth at higher concentrations. Among the three nitrogen sources, potassium nitrate (KNO_3) was found to influence the algal growth than sodium nitrite ($NaNO_2$) and ammonium chloride (NH_4Cl) as it significantly enhanced the growth of *Nostoc spongiaeforme*. Similar observations were reported on the requirement of nitrogen sources for the growth of blue-green algae both in laboratory and field conditions (Goyal and Marwaha, 1985; Adhikary and Mishra, 1999).

The uptake of nitrogen capacity of *Nostoc spongiaeforme* was greater in ammonium chloride (NH_4Cl) among the employed nitrogen sources. The uptake of nitrogen from different nitrogen supplemented [i.e. potassium nitrate (KNO_3), sodium nitrite ($NaNO_2$) and ammonium chloride (NH_4Cl)] cultures was gradually increased with increasing concentrations upto 2.0 mg per ml.

Table 1: Kinetics and effect of nitrogen sources on growth of *Nostoc spongiaeforme*

Concen-tration (mg/ml)	Chlorophyll-a (mg/g)	Protein (µg/ 100mg fw)	**Nitrogen uptake rate/ hr (mM x10^{-5})**	**S / V (mM)**	Km (mM)
BM – NO_3^- Control	0.5182	35.00	0	0	
NH_4Cl					
0.1	0.0147	31.66	52.20	0.035	
1.0	0.0313	37.50	520.62	0.035	0.03
1.5	0.0523	33.33	771.48	0.036	
2.0	0.0172	15.00	1010.05	0.037	
$NaNO_2$					
0.1	0.0273	18.33	40.42	0.035	
1.0	0.0609	31.66	402.23	0.036	0.06
1.5	0.0687	36.66	585.02	0.037	
2.0	0.0754	38.33	804.42	0.036	
KNO_3					
0.1	0.0272	5.83	27.50	0.035	
1.0	0.0782	36.66	280.52	0.035	0.07
1.5	0.0825	40.00	403.27	0.036	
2.0	0.0873	46.66	534.12	0.037	

KNO_3 = Potassium nitrate; $NaNO_2$ = Sodium nitrite; NH_4Cl = Ammonium chloride; mM = Milli Molar, f.w. = fresh weight; S / V = Rate of uptake of nitrogen, Km = Michaelis constant.

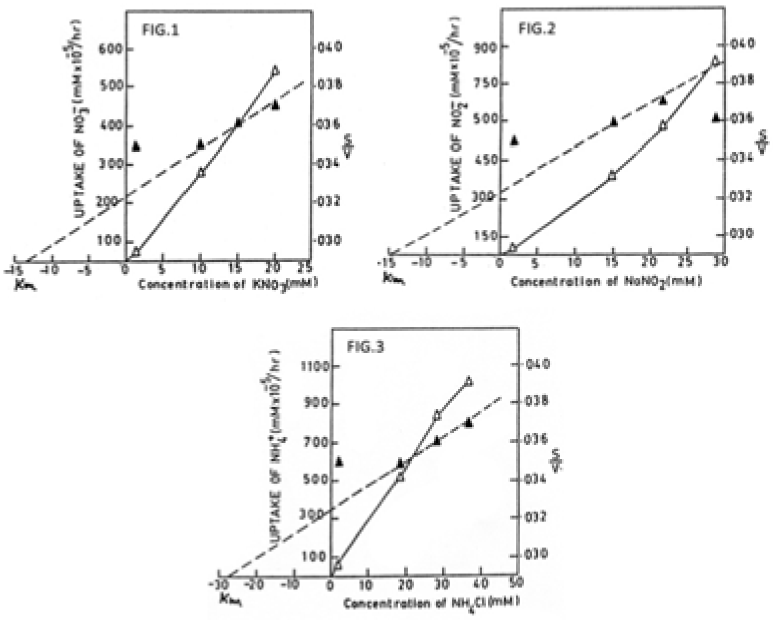

Fig 1 to 3. The Uptake of NO^-_3 NO_2^- and NH_4^+ (mM x 10^{-5} / hr.) by ***Nostoc spongiaeforme*** in different concentrations (0.1, 1.0, 1.5 and 2.0 mg per ml) of KNO_3, $NaNO_2$ and NH_4Cl cultures after 72 hours incubation
Δ – Δ : Uptake of NO^-_3 NO_2^- and NH_4^+ (mM x 10^{-5} / hr.) NO^-_3 NO_2^- and NH_4^+ (mM x 10^{-5} / hr.) in KNO_3, $NaNO_2$ and NH_4Cl cultures.
▲- ▲ :S/V (rate of uptake) of NO^-_3 NO_2^- and NH_4^+ in KNO_3, $NaNO_2$ and NH_4Cl cultures.

The S/V values indicating the rate of uptake of nitrogen in *Nostoc spongiaeforme* at 0.1, 1.0, 1.5 and 2.0 mg per ml concentrations of potassium nitrate (KNO_3), sodium nitrite ($NaNO_2$) and ammonium chloride (NH_4Cl) were 0.035, 0.035, 0.036, 0.037 mM; 0.035, 0.036, 0.037, 0.036 mM; 0.035, 0.035, 0.036, 0.037 mM respectively after 72 hours.

The Km values of potassium nitrate (KNO_3), sodium nitrite ($NaNO_2$) and ammonium chloride (NH_4Cl) were 0.07, 0.06 and 0.03 mM successively (Fig 1 to 3), which varied with the nitrogen substrate (S) and showed the induced enzymes activities and their affinities towards nitrogen substrate (S). Therefore ammonium chloride (NH_4Cl) exhibited lower Km values than potassium nitrate (KNO_3) and sodium nitrite ($NaNO_2$), indicating the enzymes involved in uptake of ammonium chloride (NH_4Cl) had higher affinity towards ammonium chloride (NH_4Cl). Among these three nitrogen sources studied in the present investigation, ammonium chloride (NH_4Cl) cultures showed lower Km values and high uptake of ammonium chloride (NH_4Cl) rate.

DISCUSSION

Although the biomass of blue-green algae increased without employment of chemical fertilizers, farmers of under developing countries in the Eastern Hemisphere such as India have been supplementing the rice-fields with inorganic fertilizers consisting nitrogen, phosphorus, ammonium sulphate and potassium to obtain high crop yield which enhance the growth of cyanobacteria (Dhaliwal et al., 1995), which possesses a characteristic selective action in utilizing the nitrogen fertilizers (Subramanian et al., 1965) for the growth and the absorbed nitrogen source could be reduced to ammonia before incorporation into organic metabolite such as glutamate (Thomas et al., 1977). Magee and Burris (1954) studied the nitrogen metabolism in diazotrophic cyanobacteria by incubating with $^{15}N_2$, $^{15}NH_4$, and $^{15}NO_3^-$, and concluded that the amino acid composition of the proteins were the same irrespective of the nitrogen source and incorporated into cellular proteins and cell wall material. Probably the nitrogen substances absorbed by active uptake through a carrier system as mentioned in Anacystis nidulans by Flores et al. (1983) and or simple diffusion process into cytoplasm where they preceded the nitrogen assimilation pathway, and incorporated into amino acids, proteins and cell materials and thereby increased the biomass and protein content of the algae as reported by Wohlhueter et al. (1973). Similarly, Sivasubramanian and Rao (1988) studied the kinetics of nitrogen uptake and assimilation involving the consumption of energy derived from photosynthesis and part of it is coming from respiration and also through passive diffusion in marine diatoms.

As evidenced by the above studies, currently it is of great interest to understand the relationship between kinetics of uptake of nutrients by cyanobacteria and nutrient levels.
The present experimental studies of short-term experiments on the effects of different concentrations of nitrogen sources were recorded on growth, nitrogen uptake, rate of uptake of nitrogen and changes in Km values in the cultures of algae *Nostoc spongiaeforme*. Normally, the growth and uptake of nitrogen sources by blue-green algae were dependent upon the type of nutrient source, physical factors, chemical interactions and physiological status of the algal cells. The recorded results on the effect of nitrogen sources suggest the augmentation of growth of algae in terms of chlorophyll-a and proteins of *Nostoc spongiaeforme* with increasing concentrations of nitrate and nitrite nitrogen (KNO_3 and $NaNO_2$) upto 2.0 mg per ml dose than in the nitrogen depleted basal medium (BM-NO_3). At lower concentrations of ammonia, the growth increased up to 1.0 mg per ml and beyond this concentration reduction in growth was noticed (Table1). Similarly, Singh and Srivastava (1968) reported that the augmentation of growth of *Anabaena doliolum* was recorded with increasing concentrations of potassium nitrate (KNO_3) and sodium nitrite ($NaNO_2$) while ammonium chloride (NH_4Cl) reduced the growth than the control. Ammonia may actually suppress overall growth when concentrations are sufficiently high (Glibert et al., 2016).

Comparing the uptake of nitrogen in short-term experiments, the uptake of ammonium nitrogen was greater from ammonium chloride (NH_4Cl) cultures in *Nostoc spongiaeforme* . Similar findings were observed by Hii et al (2011) in *Nannochloropsis* sp. The uptake of nitrogen from three nitrogen sources in *Nostoc spongiaeforme* was gradually increased with increasing concentrations. From these experiments, the results reflecting the relationship between the nitrogen substance as substrate (S) versus velocity of nitrogen uptake (V) and rate of uptake of nitrogen (S/V) were studied to observe the involvement of these parameters during the uptake of nitrogen sources by the algae (Table 1 & Fig 1, 2, 3). In these graphs, Michaelis constant (Km) was given as the negative S-intercept of the linear regression of (S/V) versus substrate (S) and comparing the recorded results during 72 hours period of life cycle suggested that the low Km values indicating the high affinity of the enzymes and higher Km values could also suggest the very low affinity of the enzyme involved in the metabolism of the nitrogen substrate and these changes revealed the modification in the rate of the uptake of nitrogen sources. Among these three nitrogen sources studied in the present investigation, *Nostoc spongiaeforme* showed lower Km values and high uptake of ammonium chloride (NH_4Cl) rate than potassium nitrate (KNO_3) and sodium nitrite ($NaNO_2$), indicating the enzymes involved in uptake of ammonium chloride (NH4Cl) had higher affinity towards ammonium chloride (NH_4Cl).

CONCLUSION:

Normally, Cyanobacteria prefers to utilize lower concentrations of nitrogenous fertilizers for optimum growth and nitrogen fixation (Anand, 1990).The present study suggests that the introduction of nitrogen-fixing Cyanobacteria in paddy-fields as biofertilizer in combination with lower concentrations of nitrogen fertilizer was found to increase the chlorophyll content and also grain yield of rice, and the soil fertility besides the liberation of photosynthetic O_2 into the standing waters during their active growth.

ACKNOWLEDGEMENT

The authors would like to thank to the Dept. of Botany, Andhra University, Visakhapatnam for providing necessary laboratory facilities.

References

Adhikary, S.P and Mishra, A. (1999). Interaction of Cyanobaceteria with fertilizers. In “cynobacetrial and algal metabolism and environmental biotechnology”.(Ed.) Tasmeem Fatme Narosa publication house, New Delhi. pp. 120-139.

Anand, N. (1990). Physiological responses of nitrogen fixing blue-green algae (cyanobacteria) to commercial fertilizers. In : Proc. Natl. Symp. Cyanobacterial nitrogen fixation. (Ed.) Kaushik, B.D. Associated Publishing Co., New Delhi. pp 83-90. 7.

Bottomley, P.J., Grillo, J.F. Van Baalen, C. and Tabita, F.R. (1979). Synthesis of nitrogenase and heterocysts by Anabaena sp. CA in the presence of high levels of ammonia. J. Bacteriol. **140**: 938-943.

Dhaliwal, M.K., Pandher, M.S., Gupta, R.P. Garcha, H.S. and Gagneja, M.R. (1995). Effect of chemical nitrogen on the growth and nitrogen fixation by blue-green algae in basmati rice. Ind. J. Ecology. **22**: 7-10.

Flores, E., Guerrero, M.G. and Losada, M. (1983). Photosynthetic nature of nitrate uptake and reduction in the cyanobacterium *Anacystis nidulans*. Biochim. Biophys. Acta. **722:** 408-416.

Fogg, G.E. Stewart, W.D.P., Fay, P. and Walsby, A.E. (1973). Nitrogen metabolism. In *"The blue-green algae."* Academic Press, London- New York. pp.180-213.

Fogg, G.E. and Wolfe, M. (1954). The nitrogen metabolism of the blue-green algae (Myxophyceae). In "Autotrophic Micro-organisms". (Ed.) Fry, B.A. and Peel, J.L. 4^{th} Symp. Soc. Gen. Mcirobiol. University Press, Cambridge **4**: 99-125.

Goyal, S.K. and Marwaha, T.S. (1985). Effect of ammonium nitrogen on growth absorption and fixation of nitrogen by Anabaena fertilissima. Phykos. **25** : 152-155.

Goyal, S.K. (1985). Effect of different sources of combined nitrogen on algalization. Phykos. **24**: 149-151.

Hii, Y.S., Soo, C.L., Chuah, T.S., Mohd-Azmi and Abol-Munafi, A.B. (2011). Journal of Sustainability Science and Management. **6** : 66-68.

Kaushik, B.D. (1986). Response of cyanobacterial nitrogen fixation to physiological stress. In "Current status of Biological nitrogen fixation research". Nat. Symp. Haryana Agricult. Hissa, Haryana.

Kratz, W.A. and Myers, J. (1955). Nutrition and growth of several blue-green algae. Am. J. Bot. **42:** 282-287.

Magee, W.E. and Burris, R.H. (1954). Fixation of N_2 and utilization of combined nitrogen by *Nostoc muscorum. Am. J Bot*. **11**: 777-782.

Neilson, A.H. and Larsson, T. (1980). The utilization of organic nitrogen for growth of algae: physiological aspects. Physiol. Plant. **48** : 542-553.

Patricia M. Glibert, Frances P. Wilkerson, Richard C. Dugdale, John A. Raven, Christopher L. Dupont, Peter R. Leavitt, Alexander E. Parker, JoAnn M. Burkholder, Todd M. Kana. (2016). Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. Limnol. Oceanogr. **61:** 165-197.

Pinter, I.J. and Provasoli, L. (1958). Artificial cultivation of a red-pigmented marine bluge-green alga, *Phormidium persicinum*. J. Gen. Microbiol. **18**: 190-197.

Rhee, G.Y. and Lederman, T.C. (1983). Effect of nitrogen sources on phosphorus limited growth on Anabaena flosaquae. J. Phycol. **19**: 179-185.

Singh, P.K. and Dash, H.P. (1994). Blue-green algal growth and N_2-fixation at nitrogen and phosphorus fertilization in rice-field. In "Recent Advances in phycology" (Ed.) Kashyap, A.K. and Kumar, H.D. Rastogi and Company, Meerut. pp: 203-201.

Sivasubramaniyan, V. and Rao, V.N.R. (1988). Uptake and assimilation of nitrogen by marine diatoms-11. Kinetics of nitrogen assimilation. Plant Science. **2:** 89**-**98.

Srivastava, B.S. and Singh, H.N. (1968). Studies on morphogenesis in a blue-green alga. 1. Effect of inorganic nitrogen sources on developmental morphology of Anabaena doliolum. Can. J. Microbiol. **14:** 1341-1346.

Stewart, W.D.P. (1964). The effect of available nitrate and ammonium nitrogen on the growth of two nitrogen fixing blue-green algae. J. Exp. Bot. **15**: 138-145.

Stewart, W.D.P. (1980). Some aspects of structure and function in N_2 fixing cyanobacteria. Ann. Rev. Microbiol. **34**: 497-536.

Subramanyan, R., Relwani, L.L and Mann, G.B. (1965). Fertility build-up of rice-field soils by blue-green algae. Proc. Ind. Acad. Sci. **6B**: 252-277.

Thomas, J., Meeks, J.C., Wolk, C.P. Shaffer, P.W., Austin, S.M. and Chien, W.S. (1977). Formation of glutamine from [^{13}N] dinitrogen and [^{14}C] glutamate by heterocysts isolated from *Anabaena cylindrica*. J. Bacteriol. **129**: 1545-1555.

Ward, A.K. and Wetzel, R.G. (1980). Interaction of light and nitrogen source among planktonic blue-green algae. Arch. Hydrobiol. **90**: 1-125.

Wohlhueter, R.M., Schutt, H. and Holzer, H. (1973). Regulation of glutamine synthetase in vivo in E. Coli. In “The Enzymes of Glutamine Metabolism”. (Ed.) Prusiner, S. and Stadiman, E.R. Academic Press, New York pp. 45-64.