



J. Algal Biomass Utiln. 2009, **1** (1): 41 – 60

STUDIES ON KINETICS OF PHOSPHATE UPTAKE BY BLUE-GREEN ALGAE

Murali, R, Subramanian, V. V , ¹Sumathi, P and Sivasubramanian, V

Vivekananda Institute of Algal Technology (VIAT), RKM Vivekananda College,

Chennai-600 004, India.

¹Department of Biochemistry, SRM Arts and Science College, Kattankulathur – 603 203, India

E-mail: vsivasubramanian@gmail.com; vavesu@gmail.com

Abstract

Cylindrospermum sp collected from Chennai and *Nostoc* sp collected from *Thandurai*, near Chennai, the two fresh water filamentous blue-green algae, were made unialgal and used for the present study. Investigations on the growth, kinetics of PO₄ uptake, effect of PO₄ on growth, uptake of PO₄, and photosynthesis were carried out. Uptake of PO₄ followed Michaelis-Menton Kinetics in both the algae. *Cylindrospermum* sp showed a biphasic kinetics by changing its uptake kinetics in higher PO₄ concentration and it is well suited as a phycoremediation organism for PO₄ removal from wastewaters and effluents. *Nostoc* sp showed only a simple kinetics and very low K_s (high affinity) for PO₄ and it is well suited for nutrient poor waters. PO₄ uptake was very low in dark. Higher uptake rates were observed in lower light intensities (0.05 k lux) in both the blue-green algae. Na⁺ seems to enhance growth of blue-green algae along with PO₄. Na⁺ is also required for the uptake of PO₄. In the absence of Na⁺, PO₄ uptake was very low in blue-green algae studied

INTRODUCTION

The phytoplankton cells can survive and grow at concentrations below the detectable

limit of phosphorus (about 0.03 µg at /L). This observation has stimulated studies relating the rate of supply of phosphorus

under natural conditions to its rate of utilization. There are several sources of phosphorus available to the phytoplankton other than inorganic orthophosphate. Phosphorus is found in natural waters also in the form of organic and inorganic polyphosphates of low molecular weight and bound to colloidal particles in suspension and in sediments (*Strick et al.*, 1972). Another possible major source of phosphorus is excretion by zooplankton. *Pomeroy et al.*, (1963) calculated the demand for phosphate from the rate of photosynthesis in several regions and compared it to the phosphate available from zooplankton.

The total phosphorus for a body of water is usually reasonably constant value fixed by geochemical factors (*Chiou et al.*, 1974). If "cultural eutrophication" is occurring, then sewage, industrial waste; or runoff from agricultural lands usually is associated with an increase in the total standing crop of algae. Papers by *Schindler* and colleagues (1972) show conclusively that in a group of small lakes of various types phosphorus was the nutrient which could often be called the "limiting nutrient" for the growth of phytoplankton and thus for eutrophication.

There is no evidence that algae can assimilate any form of phosphorus other than orthophosphate. Algal cells have phosphatases in or near the outer membrane which allow them hydrolyse organic phosphates.

"Luxury consumption" of phosphate by algae results in the storage of polyphosphate granules in the cell. These may contain sufficient PO_4 for many cell divisions and help to carry phytoplankton through short periods of phosphorus depletion. Phosphate, in contrast to nitrate, is readily adsorbed to soil particle and thus not move easily with ground water.

Phosphorus is not needed for growth in large quantities like Carbon, Oxygen, hydrogen and nitrogen, but it is one of the most common limiting elements on land and in fresh water. Devices for overcoming phosphorus deficits have been evolved by algae. These are (i) luxury consumption (2) an ability to use phosphate at low level (low K_s) and (3) alkaline phosphatase production.

In oligotrophic lakes the growth of cyanobacteria and higher algae is usually determined by the quantity of inflowing phosphate (*Schindler*, 1977). When this nutrient is limiting, phosphate uptake by the

algae is accompanied by a decrease of the external phosphate concentration to nanomolar levels (Rigler, 1968 ; Pettersson, 1979).

Under these conditions transport of phosphate into the cell has to proceed against a considerable concentrating gradient of several orders of magnitude (nanomolar concentration outside the cell compared to approximately 100 μM in the cytoplasm (Wagner and Falkner, 1992). This is only possible if transport is coupled to an energy source. Investigation by *Falkner et al.*, (1980) on the energy dependent accumulation of orthophosphate by the bluegreen alga *Anacystis nidulans* have established.

According to *Brownell* (1979) when Na^+ is present, Cyanobacteria have higher nutrient uptake efficiencies and lower loss rates of fixed organic matter. *Seale et al.*, (1987) evaluated the effects of NaCl and phosphorus enrichments on natural phytoplankton assemblages. Sodium chloride from anthropogenic sources, such as road run off and industrial wastes, may alter fresh water phytoplankton communities. Increased salinity should favour growth of cyanobacteria because these are the only phototrophic plankton

taxa requiring Na^+ for growth (Allen and Arnon, 1955; Bostwick *et al.*, 1968). Phosphate uptake by cyanobacteria was enhanced most by Na^+ additions in the laboratory when $\text{PO}_4\text{-P}$ levels exceeded 50 $\mu\text{g/L}$ (Mohleji and Verhoff, 1980).

In the present investigation two fresh water blue-green algae, *Cylindrospermum sp* and *Nostoc sp* were selected for studying the kinetics of PO_4 uptake. The effect of Na^+ on the growth, uptake of phosphate and photosynthesis has also been investigated.

MATERIALS AND METHODS

Blue-green algae, culture medium and growth measurement

The blue-green algae, *Nostoc sp.* isolated from Chitheri Lake (Thandurai, Chennai.) and *Cylindrospermum sp.* isolated from R.K.M. Vivekananda College Campus (Mylapore, Chennai) were made unialgal and grown in Allen and Arnon medium (1955) without nitrate. Cultures were grown at 28 ± 1 °C in a growth chamber provided with continuous illumination by white fluorescent lamps (Philips, 40W). Growth was measured by fresh weight determination after centrifugation. Division rates were calculated using the fresh weight.

Pigment Analysis

i. Extraction and Estimation of chlorophyll a.

Algal culture was centrifuged at 8000 rpm for 5 minutes and pellet obtained was re-suspended in cold 90% acetone and left in the dark, inside the refrigerator for 10-12 hours. This was again centrifuged at 8000 rpm for 5 min and the clear supernatant was used for estimation of chlorophyll a. Optical density measurements were made using Spectronic 20. Chlorophyll a was estimated from extinction co-efficients given by Jeffrey *and* Humphrey, (1975).

ii. Extraction and estimation of phycobilin pigments

The pellet after acetone extraction was again resuspended in phosphate buffer (pH 7.5) and phycobilins were extracted by repeated freezing and thawing. After complete extraction, the clear supernatant was obtained by centrifugation and was subjected to spectrophotometric analysis of phycobilin pigments by employing the formula of Bennett and Bogorad,

Measurement of O₂ uptake and evolution

Photosynthesis measurements were made using PE 135 DO analyser, (Elico). Illumination was provided by a Philips 60W bulb. Light intensity was measured using a LUTRON LX-101 digital Luxmeter. Respiratory O₂ uptake measurement was taken after covering the reaction vessel with a black cloth. Photosynthesis rate were expressed as $\mu\text{g O}_2$ evolved/ $\mu\text{g chl a/h}$.

Phosphate deficient cultures and uptake measurement (Murphy and Riley, 1962)

The culture was grown in Allen Arnon medium with 10 $\mu\text{M PO}_4$ for a week. After the phosphate in the medium was completely depleted the culture was centrifuged and the pellet was resuspended in PO_4 free medium and incubated under light for 30 min to ensure complete depletion of PO_4 and used for uptake studies. Aliquots enriched with PO_4 were incubated for 30 min at room temperature under 500 lux light intensity with occasional shaking and at the end of incubation period, the aliquots were centrifuged. Cell-free supernatants were analyzed for PO_4 and uptake was calculated as the difference between the concentration added and that remaining at the end of experiment.

RESULTS

Growth and pigment composition of *Cylindrospermum* sp and *Nostoc* sp in different PO₄ concentrations.

Actively growing PO₄ depleted cultures of *Cylindrospermum* sp and *Nostoc* sp were inoculated into conical flasks containing media amended with different concentration of PO₄ (5, 20 and 40 μM PO₄ (supplied as K₂HPO₄)). Fresh weight determinations were made on the 7th day. The results are given in Table 1 and Fig.1. a,b. *Cylindrospermum* sp did not show any major increase in growth when the PO₄ concentration was increased. Whereas *Nostoc* sp showed a maximum growth only with lowest concentration supplied (5 μM PO₄). At 20 and 40 μM PO₄ levels the growth of *Nostoc* sp was retarded by 75% and 80% respectively.

Table 2 and Figs.2 a,b and 3 a,b show the results of pigment analysis of cultures grown in different concentrations of PO₄. In *Cylindrospermum* sp there was a slight increase in chl a levels at higher PO₄ levels. Whereas the phycobilins, especially, phycocyanin, showed a significant increase at higher PO₄ levels. In *Nostoc* sp there was a significant increase in chl a at higher

Phosphate uptake by blue-green algae

PO₄ levels. Whereas the phycobilins did not increase except for phycoerythrin which showed a marginal increase at higher PO₄ levels.

Effect of PO₄ and NO₃ on the heterocyst frequency

Actively growing cultures of *Cylindrospermum* sp and *Nostoc* sp were inoculated into the media amended with different concentrations of PO₄ and NO₃ separately and incubated for 7 days. On the 7th day the heterocyst frequency was determined and the results are given in Fig.4 a,b and 5. Increasing the PO₄ level increased the heterocyst frequency whereas NO₃ showed the opposite effect. The results of *Cylindrospermum* sp were also showed the same trend and so the data are not given here.

Uptake of PO₄ by PO₄- depleted and PO₄- treated cultures of blue-green algae

Actively growing cultures (PO₄- depleted) of *Cylindrospermum* sp and *Nostoc* sp were centrifuged and pellet was resuspended in 200 ml of PO₄- free Allen Arnon medium. To 100 ml of the culture 20 μM PO₄ was added and incubated for 1 hr under light. This was taken as

PO₄ treated culture and the remaining 100 ml of sample was treated as PO₄ depleted culture. Uptake experiment was started by adding 6 μM PO₄ as the initial concentration and incubated under light. PO₄ analysis was done for every 5 minutes for 30 minutes. The results are shown in Table 5 and Fig.5, a,b. In *Cylindrospermum sp* PO₄ treated culture showed a faster rate of uptake than the PO₄ depleted. The saturation was reached within 15 min. Whereas in *Nostoc sp* both PO₄ depleted and treated cultures showed some rate of uptake.

Light and dark uptake of PO₄

Actively growing PO₄ depleted cultures were used for present study. 2μM PO₄ was added as initial concentration and samples were incubated for 30 min under dark and under different light intensities (0.5, 1.0, and 1.5 k lux) PO₄ uptake rates were calculated and the results are given in Table 6 and Fig. 6 a and b. *Cylindrospermum sp* showed a lower dark uptake (31% of light uptake) and under light (0.5 k lux) showed a maximum uptake and a slight decrease of uptake was noticed under higher light intensities (1.0 & 1.5 K lux). In *Nostoc sp* dark uptake was very low (only 5.3% of light uptake). Uptake was maximum in lower light intensities (0.5 K lux).

KINETICS OF PO₄ UPTAKE

Determination of V max and Ks

PO₄ –depleted cultures of *Cylindrospermum sp* and *Nostoc sp* were used for short- term (<30 min) uptake studies. Methods for preparing PO₄ depleted culture for uptake measurements are already given in Materials and Methods. The uptake experiments were carried out with different concentrations of PO₄. Velocity of PO₄ uptake (V) was plotted against substrate concentration (S) and from the hyperbola V max has obtained. The results are given in Figs 7 a,b and 8 a, b and Table 7. *Cylindrospermum sp* showed a biphasic kinetics. Whereas *Nostoc sp* showed only one V max (71.0). The Ks value of *Nostoc sp* was very low (2.0).

EFFECT OF Na⁺ ON GROWTH AND PO₄ UPTAKE BY BLUE-GREEN ALGAE

Effect of Na⁺ on growth

Actively growing PO₄ depleted cultures of *Cylindrospermum sp* and *Nostoc sp* were inoculated into Allen Arnon medium amended with different concentration of Na⁺ (0.01, 0.05 and 0.01 mM). The cultures were incubated for 7 days and fresh weight

determination were done on 7th day. The results are shown in Table 8 and Fig 9 a,b. Increasing Na^+ increased growth in both the algae. Saturation of growth was reached at 0.1 mM Na^+ in *Cylindrospermum sp.*, where as growth showed saturation at 0.05 mM Na^+ in *Nostoc sp.*

Effect of Na^+ and PO_4 combinations on growth and pigment composition

Actively grown PO_4 depleted cultures of *Cylindrospermum sp* and *Nostoc sp* were inoculated in Allen Arnon medium amended with different combination of Na^+ and PO_4 and incubated for 7 days. On the 7th day fresh weight determination was done. Division rates (divisions/day) were calculated and the results are shown in Table 9 and Fig 10 a,b. Chla and phycobilins were analyzed. The results are shown in Table 10 and 11 and Figs 11 a,b and 12 a,b. Chl a levels were higher in high PO_4 grown cultures of *Cylindrospermum sp* and *Nostoc sp*. Phycobilins were higher in higher PO_4 and high Na^+ combinations in both the cultures. Of the three phycobilins analysed, phycoerythrin was produced in higher concentrations in *Nostoc sp* where as *Cylindrospermum sp* produced higher amount of phycocyanin and lesser amounts

of phycoerythrin. Na^+ alone did not enhance the pigments.

Effect of Na^+ on the uptake of PO_4 by blue-green algae

PO_4 uptake (short term) experiments were conducted using PO_4 and Na^+ depleted cultures of *Cylindrospermum sp* and *Nostoc sp* amended with different Na^+ concentrations (0.01, 0.05 and 0.1mM) with 5 μM PO_4 as the initial concentration. Cultures without Na^+ served as control. The results are given in Table 12 and Fig 13 a,b. The uptake of PO_4 was very low when Na^+ was not included in both the organisms (3.6 & 18%). Even with a little Na^+ (0.01 mM) the uptake reached the maximum.

DISCUSSION

Effect of PO_4 on growth and pigment composition of blue-green algae

Attempts to model algal succession under nutrient limitation are often based on the assumption that the uptake of the limiting nutrient is in equilibrium with the use of the nutrient for cellular growth and development (Tilman, 1977, 1981., Tilman *et al.*, 1982; Cembella *et al.* , 1984 a). The growth rate of the algae may then be described by the Monod model or the Droop

model as a function of the ambient or the cellular concentration of the limiting nutrient, respectively (Cembella *et al.*, 1984 b). The two models are both applicable for steady state situations (Tilman *and* Kilham, 1976, Goldman, 1977). Other workers have decoupled the processes of uptake and growth to study the effect of transients in the supply of the limiting nutrient and its consequences for growth (Grenney *et al.*, 1973 ; Lehman, *et al.*, 1975 ; Nyholm 1976 & 1978).

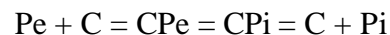
Of the two blue-green algae tested in the present study *Nostoc* sp., behaved like a low nutrient species. The growth was maximum under very low PO₄ levels. Whereas *Cylindrospermum* sp. showed similar growth rates in all the concentrations tested (Table 1 and Fig.1 a,b). This was also reflected in the levels of pigments in both the blue-green algae.

Olsen *et al.*, (1989) showed that the ability of the species to compete for PO₄ depended on the Pi uptake characteristics and their capability to retain the accumulated Pi. High affinity (low K_s) in uptake at low Pi concentrations contributed considerably to the growth efficiency. Kuenzler and Ketchum (1962) showed that algal growth rates plotted

against PO₄ concentrations from batch culture show a linear relationship at very low concentrations and virtual independence at concentrations above 10 µg P/L.

Kinetics of PO₄ uptake by blue-green algae

Falkner *et al.*, (1995) working with the blue-green alga *Anacystis nidulans* observed that the PO₄ uptake did not obey Michaelis - Menton equation due to existence of a threshold value. (Olson, 1989). The simplest explanation for the existence of a threshold concentration is that transport of phosphate is catalyzed by a carrier until the external PO₄ concentration has attained an equilibrium value (P_e)_A, the threshold value. In such a case, the uptake process can be depicted by the following simplified model:



Which is composed of reversible reaction steps that allow for some kind of product control (Rhee 1973). Accordingly, the external PO₄, (P_e), reacts with a carrier C to give a phosphocarrier complex that releases PO₄ at the inner side of the membrane (Droop, 1974).

Studies by Falkner *et al.*, (1980) in *Anacystis nidulans* has shown that the rate limiting step for the incorporation of phosphate is the transport through the membrane, which appears to be strictly regulated by bivalent cations. They have proposed that the activation of the transport by illumination may be mediated by changes in the cytoplasmic levels of Mg^{2+} via an activation of the carrier from the inner side of the membrane.

In the present investigation when the PO_4 depleted cultures of blue-green algae were given a short period of PO_4 treatment for 20 min. *Cylindrospermum* sp. could change its uptake capability and show an enhanced PO_4 rates when compared to control. Whereas *Nostoc* sp. could not change its uptake mechanism and both the PO_4 -depleted and PO_4 - treated cultures showed the same trend.

In *Cylindrospermum* sp. and *Nostoc* sp. PO_4 uptake was almost 80 to 90% light dependent and *Nostoc* sp. showed a very low dark uptake. PO_4 dependent velocity of PO_4 uptake by these two blue-green algae followed the Michaelis - Menton Kinetics on *Nostoc* sp. showed (low K_s) high affinity for PO_4 than *Cylindrospermum* sp. *Cylindrospermum* sp. showed a

biphasic kinetics (Fig.7 a,b and 8 a,b). This is considered to be an efficient method of adaption to higher ambient PO_4 levels and will help the organism to withstand eutrophication. This also makes *Cylindrospermum* sp as a suitable organism for removal of nutrients from wastewaters and effluents.

Effect of Na^+ on growth and PO_4 uptake

Seale *et al.*, (1987) have studied the effect of Na^+ and phosphate on growth of cyanobacteria. They have observed that as single factor NaCl enrichments had no significant effect on growth rates of cyanobacteria. But Na^+ in combination with high PO_4 could enhance the growth of cyanobacteria. *Anabaena* growth rates (Allen and Arnon, 1955 ; Ward and Wetzel, 1975) and biomass yields (Brownell and Nicholas, 1967) were maximized at 5 - 40 ppm NaCl. Phosphate uptake by cyanobacteria was enhanced most by Na^+ additions when PO_4 - P levels exceeded 50 $\mu g/L$ (Mohleji and Verhoff, 1980). These laboratory studies imply that cyanobacteria may be stimulated when Na^+ concentration exceed 5 $\mu g/L$, particularly if phosphate is also increased. Increased salinity appears to be correlated with filamentous cyanobacteria

(Stoermer and Ladewski, 1976 ; Baybutt and Makarewicz, 1981).

In both the organisms tested, in the present investigation, Na⁺ alone could not enhance the growth and pigment composition. Enhanced growth rates and higher pigment compositions were observed in PO₄ and Na⁺ combinations.

In *Cylindrospermum sp* and *Nostoc sp* the PO₄ uptake was completely reduced in the absence of Na⁺. This shows that the uptake and transport systems for PO₄ is Na⁺ dependent. Even with a very low Na⁺ (0.01 mM) the uptake reached the maximum.

To sum up, of the two organisms tested *Nostoc sp* is well suited to grow in low phosphate environments and it cannot adapt to higher levels of phosphate due to

eutrophication. Whereas *Cylindrospermum sp* could adapt to increasing PO₄ levels by changing its uptake kinetics. Na⁺ ions are necessary for the uptake of PO₄ in these organisms. The PO₄ uptake mechanism seems to be Na⁺ dependent. Further studies by employing metabolic inhibitors, and other divalent cations like Mg₂₊ and alkaline phosphatase will throw more light on the kinetics of PO₄ uptake and mechanism involved in transport of PO₄ across the membrane and also the actual role played by Na⁺ in enhancing uptake of PO₄ by these blue-green algae.

Acknowledgements

The authors would like to thank The Secretary, R K M Vivekananda College, Chennai, India, for facilities.

Tables

Table 1 Growth of *Cylindrospermum sp* and *Nostoc sp* in different PO₄ concentrations

PO ₄ μM	Fresh weight on 7 th day (g/L)	
	<i>Cylindrospermum sp</i>	<i>Nostoc sp</i>
5	14	103
20	10	26
40	14	21

Table 2. Effect of PO₄ on chl a level in *Cylindrospermum sp* and *Nostoc sp*

PO ₄ μM	Pigments μg/g fresh wt	
	<i>Cylindrospermum sp</i> Chl a	<i>Nostoc sp</i> Chl a
5	43.0	25
20	60.0	107
40	62.5	213

Table 3. Effect of PO₄ on phycobilins in *Cylindrospermum sp* and *Nostoc sp*

PO ₄ μM	Pigments μg/g fresh weight					
	<i>Cylindrospermum sp</i>			<i>Nostoc sp</i>		
	PC	APC	PE	PC	APC	PE
5	0.150	0.06	0.09	0.009	0.0350	0.876
20	0.480	0.25	0.12	0.0374	1.354	3.518
40	0.530	0.27	0.15	0.469	0.740	4.76

Table 4(a) Effect of Phosphate on heterocyst frequency in *Nostoc sp*

PO ₄ μM	Heterocyst frequency %
12.5	9.87
25.0	18.18
50.0	21.80
100.0	27.40
200.0	152.27

Table 4(b) Effect of nitrate on heterocyst frequency in *Nostoc sp*

NO ₃ μM	Heterocyst frequency (%)
0	25.2
0.14	17.5
0.20	16.58
0.50	11.63
1.0	7.38
2	3.5

Table 5 Uptake of PO₄ by PO₄ - depleted and PO₄ - treated cultures of *Cylindrospermum sp* and *Nostoc sp*

S.No.	Time (min)	Concentrations of PO ₄ (µmole/L)			
		<i>Cylindrospermum sp</i>		<i>Nostoc sp</i>	
		PO ₄ -depleted	PO ₄ -treated	PO ₄ -depleted	PO ₄ -treated
1	0	6	6	6	6
2	5	4.8	3.25	4.50	4.25
3	10	3.6	1.5	3.25	3.00
4	15	3.0	0.7	2.0	1.60
5	20	2.4	0.5	1.10	0.75
6	25	1.5	0.35	0.95	0.5
7	30	1.0	0.4	1.0	0.5

Table 6 Effect of Light and dark uptake of PO₄ in *Cylindrospermum sp* and *Nostoc sp*

Light Intensity (K Lux)	Uptake rate (n moles PO ₄ /g fresh weight/min) (V)	
	<i>Cylindrospermum sp</i>	<i>Nostoc sp</i>
0 (Dark)	3.22 (31)	0.08 (5.3)
0.5	10.39	1.5
1.0	9.40	0.75
1.5	6.93	0.7

(Values in parenthesis denote % over light uptake)

Table 7 Vmax and Ks for PO⁴ uptake by *Cylindrospermum sp* and *Nostoc sp*

Blue-green algae	Vmax (n moles PO ⁴ /g fresh wt/m)	Ks μM
<i>Cylindrospermum sp</i>	I 27.0 II 76.0	I 3.5 II 5.5
<i>Nostoc sp</i>	71.0	2.0

Table 8 Effect of Na⁺ on growth of *Cylindrospermum sp* and *Nostoc sp*

Na ⁺ mM	Growth (Fresh weight g/L)	
	<i>Cylindrospermum sp</i>	<i>Nostoc sp</i>
0 (control)	25	45.0
0.01	23	50.0
0.05	31	52.5
0.1	47	52.5

Table 9 Growth of *Cylindrospermum sp* and *Nostoc sp* with Na⁺ and PO₄ combinations

Treatment	Growth rate (divisions/day)	
	<i>Cylindrospermum sp</i>	<i>Nostoc sp</i>
Control (NO PO ₄ , NO Na)	0.46	0.48
BM + 5 μM PO ₄ (Low)	0.76	0.52
BM + 0.01mM Na ⁺ (Low)	0.47	0.48

BM + 10 μ M PO ₄ (High)	0.73	0.54
BM + 0.1 mM Na ⁺ (High)	0.49	0.50
BM + 10 μ M PO ₄ + 0.1 mM Na ⁺	0.75	0.55

(BM = Basal medium)

Table 10 Effect of Na and PO₄ on chl a level in
Cylindrospermum sp and Nostoc sp

S.No.	Treatment	Chl a (μ /g fresh wt)	
		<i>Cylindrospermum</i> sp	<i>Nostoc</i> sp
1.	Control (NO PO ₄ , NO Na ⁺)	41.39	54.27
2.	BM + 5 μ M PO ₄ (Low)	47.5	65.82
3.	BM + 0.01mM Na ⁺ (Low)	40.4	57.82
4.	BM + 10 μ M PO ₄ (High)	77.7	87.06
5.	BM + 0.1 mM Na ⁺ (High)	41.2	51.02
6.	BM + 10 μ M PO ₄ + 0.1 mM Na ⁺	62.5	78.9

Table 11 Effect of PO₄ and Na⁺ on Phycobilins level in *Cylindrospermum* sp and *Nostoc* sp

S.No	Treatment	Phycobilins µg/g fresh weight					
		<i>Cylindrospermum</i> sp			<i>Nostoc</i> sp		
		PC	APC	PE	PC	APC	PE
1	Control (NO PO ₄ , NO Na ⁺)	0.123	0.026	0.066	0.05	0.10	0.9
2	BM + 5µM PO ₄ (Low)	0.390	0.061	0.093	0.06	0.20	1.10
3	BM + 0.01mM Na ⁺ (Low)	0.230	0.037	0.07	0.25	1.25	3.5
4	BM + 10 µM PO ₄ (High)	0.482	0.262	0.117	0.04	0.12	0.8
5	BM + 0.1mM Na ⁺ (High)	0.369	0.044	0.072	0.06	0.15	0.9
6	BM 10µM PO ₄ + 0.1mM Na ⁺	0.530	0.270	0.108	0.4	1.50	4.5

Table 12 Effect of Na⁺ on Uptake of PO₄ in *Cylindrospermum* sp and *Nostoc* sp

Conc. of Na ⁺ mM	Uptake rate n moles PO ₄ /g fresh weight/min (V)	
	<i>Cylindrospermum</i> sp	<i>Nostoc</i> sp
0 (control)	0.32 (3.6%)	1.02 (18%)
0.01	8.87	4.52
0.05	8.87	4.72
0.1	8.87	5.62

The values in parenthesis denote the % over maximum uptake

Table 13 Photosynthetic rate of *Cylindrospermum sp* and *Nostoc sp* under different light intensities

Light intensity K Lux	Rate of Photosynthesis (µg O ₂ evolved /µg chl a/h)	
	<i>Cylindrospermum sp</i>	<i>Nostoc sp</i>
0.25	0.25	9.23
0.5	0.373	8.72
1.0	2.61	7.75
1.5	1.49	6.44

References

ALLEN, M.B. & ARNON, D.I. 1955. Studies on nitrogen fixing bluegreen algae.

II. The sodium requirements of *Anabaena cylindrica* *Physiol.Pl.*8:653-660.

BAYBUTT, R.I & MAKAREWICZ, J.C. 1981. Multivariate analysis of Lake Michigan Phytoplankton Community at Chicago. *Bull.Torrey.Bot.Club* 108 : 255 : 267.

BENNETT, A. AND BOGORAD, L. 1973. Complimentary chromatic adaptations in filamentous bluegreen algae *J.Cell.Biol.* 58 : 419 - 435.

BOSTWICK, CD., BROWN, L.R. & TISCHER, R.G.1968 Some observations on the sodium and potassium interactions in the blue-green algae *Anabaena flos-aquae* *Pl.physiol.*21 : 466-469.

BROWNELL, P.F. 1979. Sodium as an essential micronutrient for plants and its

J. Algal Biomass Utiln. 2009, **1** (1): 41 – 60

possible role in metabolism. *Adv.Bot.Res.* 7:117-225.

CEMBELLA, A.D., ANTIA, N.J. & Harrison, P.J.1984 a. The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae a multidisciplinary perspective. Part 1. *CRC crit.Rev.Microbiol* 10 : 317-391.

CHIOU, CHARNG-JY1, BOYD, C.E. 1974. *Hydrobiologia* 45 : 345-55.

DROOP, M.R.1974. The nutrient status of algal cells in continuous culture. *J.Mar.BioLAsso.U.K.* 54 : 825-855.

FALKNER.G. HORNER, F., & SIMONIS, W. 1980. The regulation of the energy dependent phosphate uptake by the blue-green alga *Anacystis nidulans*. *Planta* 149 : 138-143.

FALKNER.G., WAGNER, F. SMALL, J.V and FALKNER, R.1995. Influence of fluctuating phosphate supply on the regulation of phosphate uptake by the blue-green alga *Anacystis nidulans* *J.phycol.* 31.745-753.

GOLDMAN, J.C. 1977 Steady-State growth of phytoplankton in continuous culture.

Phosphate uptake by blue-green algae

Comparison of internal and external nutrient equations. *J.phycol.*13:251-258.

GRENNEY, W.J., BELLA, D.A and CURL, Jr.H.C. 1973. A theoretical approach to interspecific competition in phytoplankton communities. *Am.Nat.*107 : 405-425.

JEFFREY S.W. and HUMPHREY G.F. 1975 Newspectrophotometric equation for determining chlorophylls a,b,c and C₂ in higher plants, algae and natural phytoplankton. *Biochem.physiol.Pflanz.*, 167 : 191 - 194.

KUENZLER, E.J and KETCHUM, B.H. 1962. Rate of phosphorus uptake by *Phaeodactylum tricornutum* *Biol.bull.* 123 : 34-35.

LEHMAN, J.T., BOTKIN, D.B and LIKENS, G.E. 1975. The assumptions and rationales of a computer model of phytoplankton population dynamics. *Limnol Oceanogr* 20 : 343-364.

MOHLEJI S.C. and VERHOFF, F.H. 1980 Sodium and potassium loss effects on phosphorus transport in algal cells. *J.Wat.Pollut. Control.Fed* 52 : 110-125.

J. Algal Biomass Utiln. 2009, **1** (1): 41 – 60

MURPHY and RILEY 1962 Determination of reactive phosphorus *Anal chim. Acta.* 27 : 31.

NYHOLM, N, 1976, A mathematical model of microbial growth under limitations by conservative substrates *Biotechnol. Bioeng.* 18: 1043 – 56.

OLSEN, Y., VADSTEIN, O., ANDERSON, T and JENSEN, A 1989, Competition between *Stauratrum luethemuellarii* (Chlorophyceae) and *microcystis aeruginosa* (Cyanophyceae) under varying modes of phosphate supply *J. Phycol* 25: 499 – 508.

POMEROY, L. R., H. M. MATHEWS, AND H. S. MIN. 1963. Excretion of phosphate and soluble organic phosphorus compounds by zooplank- ton. *Limnol. Oceanogr.* 8: 150-155.

PROVASOLI, L. 1958. Nutrition and ecology of RHEE.GY., 1973. A continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus sp.* *J.phycol.*9 : 495-506.

RIGLER, F.H. 1956. A tracer study of phosphorus cycle in lake water. *Ecology* 37 : 550 - 562.

Phosphate uptake by blue-green algae

SCHINDLER, D. W. 1977. Evolution of phosphorus limitation in lakes. *Science* 195: 260-262.

SEALE, D.B., BORAAS., M.E. and WARREN G.J. 1987. Effects of sodium and phosphate on growth of Cyanobacteria *Wat.Res.*21 625-631

STOERMER, E.F., AND LADEWSKI, T.B. 1936. Apparent optimal temperatures for the occurrence of some common phytoplankton species in Southern Lake Michigan. Great, Lakes. Research Div.Pub. No. 18, University of Michigan, Ann. Arbor.Mich.

STRICKLAND, J.D.H., PARSONS, T.R.1972. A practical Handbook of Sea-water Analysis *Bull. Fish.Res.Bd.Can* 167.311pp.

THOMAS, W.H. 1977. Effect of copper phytoplankton standing crop and productivity. Controlled ecosystem pollution experiment. *Bulletin of Marine Science*, 27 : 34-43.

TILMAN, D. 1987. Resource competition between planktonic algae, an experimental and theoretical approach *Ecology* 58 : 338-348.

WAGNER, F and FALKNER, G.1992. Concomitant changes in phosphate uptake photophosphorylation in the bluegreen alga *Anacystis nidulans* during adaptation to phosphate deficiency *J.Plantphysiol.* 140 : 163-167.