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Nutrient Removal, Growth Response and Lipid Enrichment by a Phytoplankton Community

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Abstract

The study aims at the utilitarian approach of managing nutrient and biomass. Removal of phosphorus (P) was faster as compared to that of nitrogen (N). Up to 90% of P-removal was achieved within 1.5 days at high phytoplankton density. Highest rate of P-removal was 72.4 µg/l/hr while that of N removal was 609.8 µg/l/hr. The trends in removal log phases of nutrients and growth log phase of phytoplankton indicates that N is essential for cellular multiplication and P-removal was associated with some additional process other than uptake by phytoplanktons. The exposure to different N: P ratio and the nutritional history of phytoplanktons influence their nutrient removal strategy. Lower N: P ratio increases cellular lipid content in the phytoplanktons. Cumulative addition of even lower amount of nutrients can produce higher biomass as compared to one time addition of higher amount of nutrients.

Keywords- Nitrate, Phosphate, Algae, Lipid

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Introduction

The process of nutrient removal from the aquatic medium is affected by physico-chemical as well as biological factors. Physical factors influencing uptake rates are pH, quality and amount of available light, the temperature (Straskraba, 1980) and water movement. Chemical factors include concentration of the nutrient in the medium, and nature of the ionic form of the element (Tilman et al., 1982; Vymazal, 1995). Similarly, the attributes of the aquatic plants like the type of tissue, the size and age of the plant, its past nutritional history, and interplant variability (Vymazal, 1995) are the important biological factors.

These factors are equally applicable for algal growth and nutrient uptake. Biotic interactions, more specifically, have the major role to play. The first biotic factor which significantly influences algal growth is the initial population density of the algae. The higher the algal density, the better the algal growth and nutrient removal efficiency. Higher algal density, however, leads to accumulation of auto-inhibitors, self-shading and a reduction in photosynthetic efficiency (Darley, 1982). The nutritional history of phytoplanktonic algae greatly affects the rate of nutrient removal from an aquatic medium (Lobban and Harrison, 1994).

Out of the various nutrients critical to algal growths, important ones usually include phosphorus and nitrogen (Bowie et al., 1985). These nutrients exist in aquatic systems in different forms such as dissolved inorganic, dissolved organic, particulate organic, particulate inorganic and biotic forms. Out of these only dissolved forms are directly available for algal growth. The major dissolved forms of nitrogen are ammonia, nitrate and nitrite, and that of phosphorus is orthophosphate. In natural phytoplankton, the critical supply ratios of nitrogen to phosphorus (N: P) varies roughly

from 7:1 to 45:1 atomic ratio i.e. 4.4:1 to 19.4:1 mass ratio (Suttle and Harrison, 1988). The optimal ratio of N: P varies among species. The typical atomic ratio of 16:1 (Redfield ratio) is found in phytoplankton (Redfield, 1958). Generally the mass ratio of 7.2 N: 1 P is used as optimal ratio, but not strictly. Macro-algae tend to be more enriched in N, with a median N: P ratio of 30:1 (Atkinson and Smith, 1983). Low ratios of N: P (usually <10:1) may indicate N-limitation, whereas higher values (>20-30:1) may indicate P limitation (Rhee, 1978; Vymazal, 1995). 5-10 TN: TP ratio is generally used (where TN means total nitrogen and TP means total phosphorus.)

Besides other factors, the nutrient supplies greatly affect the biochemical composition of algae as well as their growth rates. With an annual mean of ca. 17% (Rai et al., 1997), algae can increase lipid synthesis up to 30-40% of total production when grown in severely nutrient limited medium. The lipid content in naturally occurring autotrophic alga varies, depending upon species, nutrition, season and stage of algae (Becker, 1994; Kilham et al., 1997). This lipid content further can be increased artificially (Hu and Gao, 2006; Xu et al., 2006).

Nutrient removal by algae provides an ample opportunity to utilize this process for the efficient treatment of waste water. Moreover, algal biomass if harvested can be used for the purpose of food and bio-fuel production. With these two utilitarian considerations, the present study aims at understanding the complex dynamics of nutrient removal from water. The study precisely aims at: (i) investigating the strategy of phosphorus (P) and nitrogen (N) removal from aquatic medium by a natural community of phytoplankton, (ii) tracing the growth pattern and change in lipid content of phytoplankton in response to addition of

P and N in different ratios, and (iii) examining the probable effect of one-time addition and two-time addition of nutrient on production of phytoplankton.

Material and Methods

Algal inoculum preparation and nutrient addition

Oligotrophic water was collected from different water bodies of Delhi and was mixed in a tank to get a diverse population of phytoplanktons. After allowing 15 days for acclimatization, the algal community was collected on a GF/F filter paper and washed in distilled water to prepare a working stock of 4.3 x 10^6 cells/lt. The initial community composition of phytoplankton was noted using high power compound microscope. 50ml of the stock water was taken in nine conical flasks of 3lt capacity each. 500 µg/l P (as KH_2PO_4) and proportionate amount of NO_3-N (as KNO_3) were added to the flasks so as to prepare three ratios of N: P viz. 4:1, 8:1 and 16:1 in triplicates. The three ratios have been noted in the text ahead as R4, R8 and R16 respectively. Micronutrients were also added in accordance with the algal media as noted in APHA, 1998. On 19th day, again 250 µg/l P and proportionate amount of NO_3-N was added to the culture. Another set of experiment was run paralally maintaining the three N: P ratios at 1000 µg/l of P.

To see the effect of N: P ratio on cellular lipid content, tap water was kept in 45lt buckets in open field protected from rain and other disturbances (material and biological). After keeping it for 3 days for de-chlorination, 1lt of stock algal inoculum was added to each bucket. In a similar pattern as mentioned in previous section, nutrients (N and P) were added to these buckets to get treatment ratios as R4, R8 and R16. All the sets were maintained in triplicates. The phytoplanktons were allowed to grow for 15 days and then

harvested. The buckets were stirred manually once everyday.

Sampling strategy

Removal or uptake of nitrogen and phosphorus was measured every hour for first 12 hours of incubation. Afterwards measurements were taken at 3 hours interval till fifth day evening. Sixth day onwards measurements were taken once everyday till 19th day. After nutrient addition at noon on 19th day, measurement for nutrient removal was taken twice at an interval of 2 hours followed by once every day on 20th day and 21st day and the final measurement was taken on 33rd day. Growth measurements were made in the same pattern except that after 21st day the sampling was done once everyday till the 33rd day. At each sampling, 10ml sample was taken away from the medium for each parameter to be studied. After taking measurements the 10ml sample meant for growth measurement was poured back to the system. Chlorophyll measurements were taken once on 19th day (before nutrient addition) and once on 33rd day. Since the experimental waters were homogenous mixtures of nutrients and algal cells, it can be assumed that the loss of water (nutrient and algal cell) due to sampling will not affect the quality of water remaining in the experimental systems.

Growth measurement and calculations

Growth 'Gth' has been measured indirectly in terms of optical density (OD) of the water sample at 678nm (OD678) using a UV-VIS spectrophotometer. Nutrient availability has been denoted as 'Avl' and expressed as µg/l. Nutrient (N or P) removal rate has been denoted as 'Rrt' and expressed as µg/l/hr or µg/l/day. The removal of nutrients from water has been expressed as %CR, %R and %RA.

Percentage cumulative removal,

$$\%CRs = \frac{C_0 - C_S}{C_0} \times 100 \text{; s=1, 2, 3, …n}$$

Where, C_0 is the initial concentration of nutrients (N or P) and C_s is nutrient concentrations at sampling day 's'.

Percent removal, $\%Rs = \frac{C_{(S-1)} - Cs}{C_0} \times 100$; s=1, 2, 3 …n

Where, C_0 is the initial concentration of nutrients (N or P), C_s is concentration at sampling day 's' and C_{s-1} is that of the value previous to C_s.

Percent removal of the available concentration, $\%RA = \frac{C_{(S-1)} - Cs}{C_{(S-1)}} \times 100$

Where, C_s is concentration at sampling day 's' and C_{s-1} is that of the value previous to C_s.

Avl, Gth, Grt, Rrt, %CR, %R and %RA have been suffixed with the three ratios R4, R8 and R16 to express association of the parameters with the ratio e.g. Rrt R4 means rate of removal at the ratio of R4.

Nutrients and Chlorophyll measurement

Nitrate in water sample was measured spectrophotometrically (λ = 410nm) by Phenol Disulfunic Acid (PDA) method. Inorganic phosphorus was also measured spectrophotometrically (λ = 690nm) in presence of Stannous chloride and acidified Ammonium molybdate. Chlorophyll was measured spectrophotometrically using 90% acetone (APHA 1998). Specifications of chlorophyll measurement were as: GF/F (0.45μm) filter paper; centrifugation at 5000rpm for 20 minutes; extraction in 90% acetone for 24 hours at 4°C in dark; λ = 630, 647, 664 and 750nm.

Algal Community Composition

50ml of the water preserved with Lugol's solution was centrifuged in a 50 ml centrifuge tube at 2000 rpm (using R8C laboratory centrifuge of Remi equipments). Majority of the supernatant water was taken out using a dropper to ensure minimum disturbance to the pellet so that the resulting mixture in the tube delivers a suitable density (neither low nor very high density)

for counting. The concentration factor (initial volume/ resulting volume) was noted down. The algal community composition (Sinha and Naik, 1997; Prescott, 1951) in the solution was studied under a binocular compound microscope on a haemocytometer slide using 10x eye piece and 40x objective. Classes of algae with relatively low frequency of occurrence have been ignored deliberately. Phytoplankton community composition of the stock has been noted in table-1.

Table 1. Community composition of phytoplanktons in stock inoculum

	Class	Genera			
1	Chl.	Ankistrodesmus	15	Cyn.	Aphanothece
2	Chl.	Chlamydomonas	16	Cyn.	Chrococcus
3	Chl.	Chlorella	18	Cyn.	Merismopedia
4	Chl.	Chlorellidiopsis	19	Cyn.	Synechococcus
5	Chl.	Cosmarium	20	Cyn.	Synechocystis
6	Chl.	Crucigenia	21	Blc.	Amphora
7	Chl.	Cylindrocystis	22	Blc.	Cymbella
8	Chl.	Kirchneriella	23	Blc.	Fragilaria
9	Chl.	Oocystis	24	Blc.	Gomphonema
10	Chl.	Peridinium	25	Blc.	Navicula
11	Chl.	Staurastrum	26	Blc.	Nitzschia
12	Chl.	Scenedesmus	27	Eug.	Euglena
13	Chl.	Selenastrum	28	Eug.	Lagerheimia
14	Chl.	Tetraedron	29	Eug.	Phacus

Total number of individuals per liter of sample has been calculated as

Total no. of individuals/lt of sample =

$$\frac{Total\ individuals\ of\ all\ classes\ \ per\ slide}{Concentrationfactor} \times 10^7$$

Algae harvesting (with *Moringa oleifera* coagulant)

From dried samples of de-oiled *Moringa oleifera* (MO) seed powder, 10% (w/v) solution was prepared using distilled water. It was stirred for 30 minute and filtered through Whatman GF/F filter paper (0.45 μ) to prepare the crude extract (1 ml ≈ 100 mg of dry seed powder). 45ml of the extract was added to all the buckets, followed by rapid mixing (250 rpm) for 20seconds, slow mixing (40 rpm) for 2 minutes (Ghebremichael, 2004) and a settling period of 1 hour (Muyibi and Evison, 1995). Later on the supernatant was decanted carefully (avoiding any biomass loss) and the bottom material was filtered. Filtrate along with the filter paper was dried in hot-air oven at 70°C till constant weight. Ash was assessed in the harvested biomass following incineration in a muffle furnace at 525°C for 6 hours (APHA, 1998) for calculation of the ash free biomass.

Oil Extraction from Algae

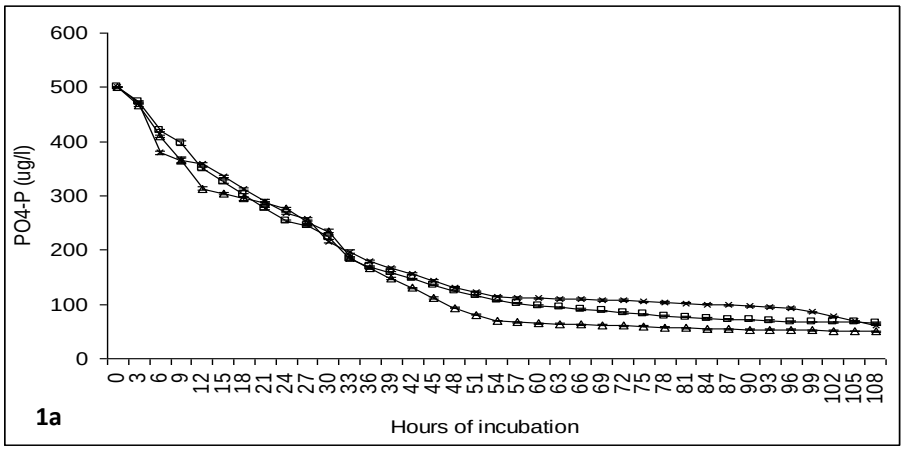
Weighed amount of dry algal biomass (actual biomass after subtracting the ash fraction) of each treatment were extracted separately in soxhlet apparatus (AOAC, method) using petroleum ether (boiling point 60-80°C). The extract was concentrated in rotavapour; the residual oil was cooled and weighed. Percent oil content was calculated as:

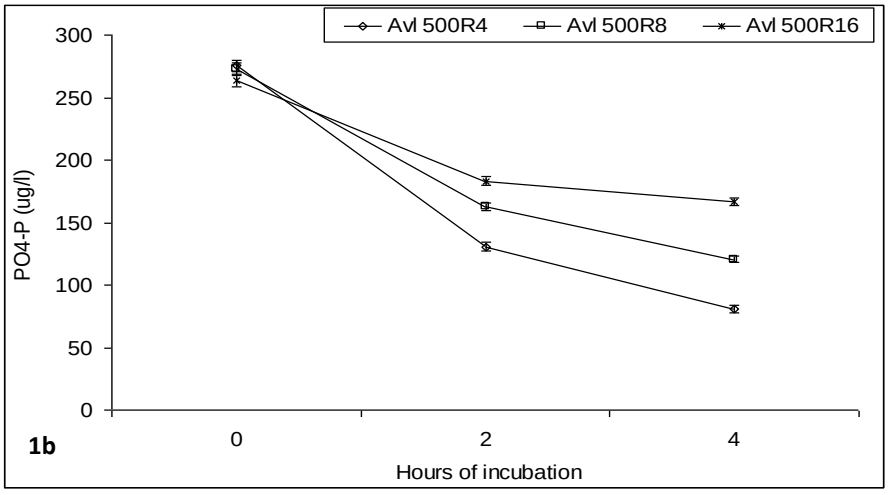
$$\%\ Oil\ content = \frac{Weight\ \ of\ \ oil\ (g)}{Weight\ \ of\ \ biomass\ (g)} \times 100$$

Results

Removal of phosphate by phytoplankton with time

Both hour-wise and day-wise analysis of PO_4-P (µg/l) status (figure-1) in water samples of experimental systems have shown that with passing time the values have decreased and approached to zero but never touched zero. The minimum values of PO_4-P (µg/l) were observed on 19th day and on 33rd day. In figure-1a, it is visible that the log-phase of P-removal has occurred within the first 48 hours (2 days) of incubation and stationary phase after 60 hours. The starting concentration of 500µg/l of PO_4-P reached to 400µg/l within 5 hours (R16), 300µg/l within 12 hours (R8) and below 90µg/l (apprx.) within 48 hours (R8) of incubation (figure-1a). In general, decrease in PO_4-P at night was lower than that of during day time.





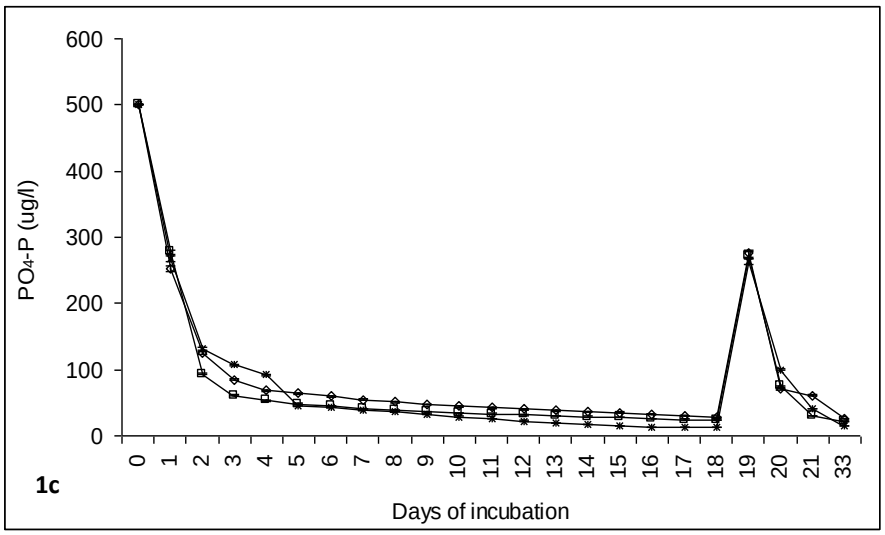


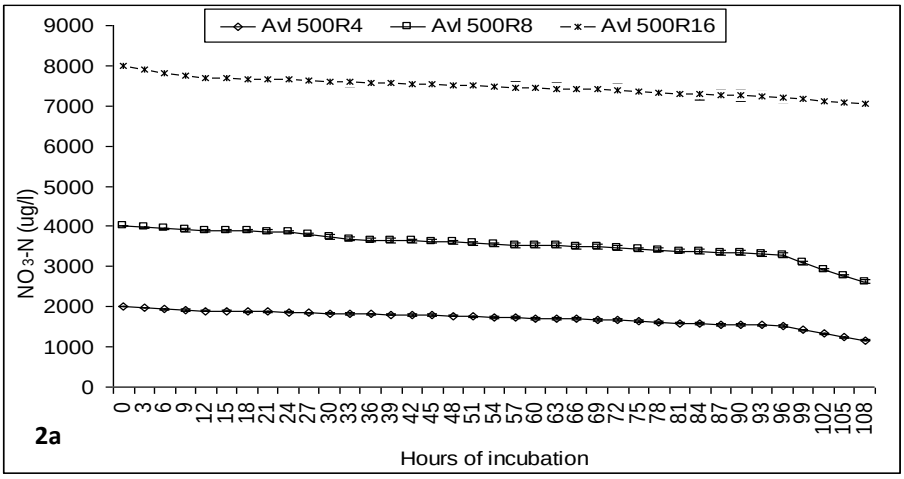
Figure 1. Changes in amount of PO_4-P (µg/l) in water sample with respect to time: (1a) hours of incubation at low initial phytoplankton density (1st event of nutrient addition); (1b) hours of incubation at high phytoplankton density (2nd event of nutrient addition, 19th day); (1c) days of incubation

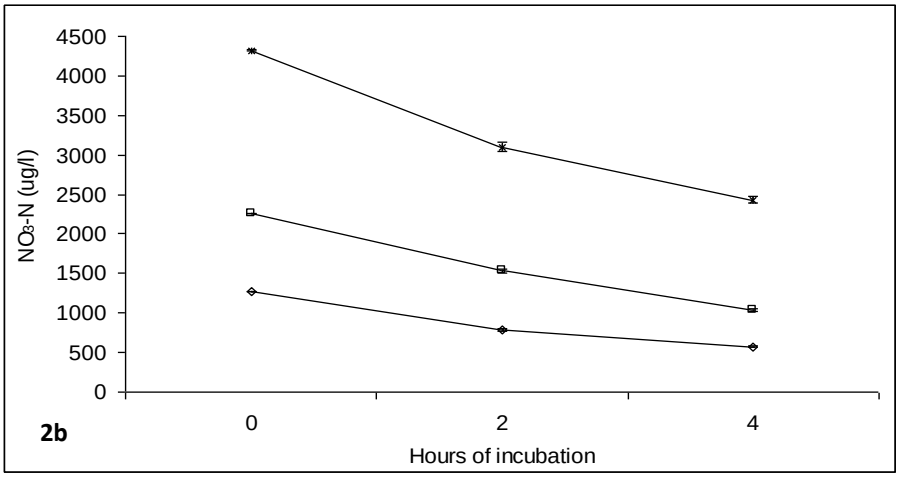
Figure-1b shows that after the 2nd day (log phase) the decrease in PO_4-P (µg/l) concentration was gradual. The minimum level of available P (PO_4-P µg/l) was 10 µg/l (apprx.) by 19th day (in R16). The peak at 19th day noon corresponds to addition of PO_4-P (µg/l) into the systems. This peak again declined afterwards. An observable difference in the first and the second event of nutrient addition was that after the second event of nutrient addition the PO_4-P (µg/l) values have declined below 80 µg/l from a starting value of 275 µg/l within only 4 hours (figure-1c).

Removal of Nitrate by phytoplankton with time

The pattern of NO_3-N (µg/l) availability in the systems was different from that of phosphorus (figure-2). Within the first 4 days the removal of N was very low in all cases as evident from figure-1. After fourth day, the values of nitrogen removal have, however, increased in case of

R4 and R8; it was still low for R16. The lag phase, log phase and stationary phase of decrease in NO_3-N availability in the three curves corresponding to the three ratio treatments were distinct from each other. In case of the R4 and R8, the log phase was visible on the fourth day while in case of R16 it was observed on the eighth day (figure-2a). The steepest point on R16 curve was observed on the twelfth day and the log phase ended on the fifteenth day.





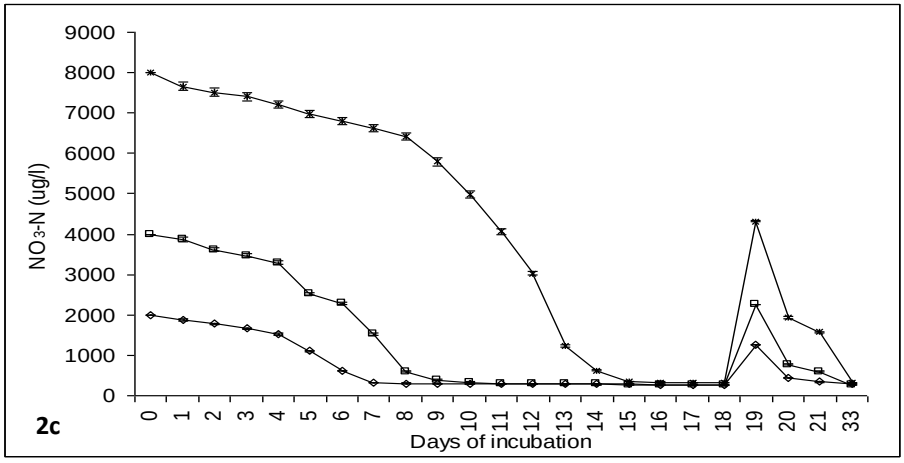


Figure 2. Changes in amount of NO3-N (µg/l) in water sample with respect to time: (2a) hours of incubation at low initial phytoplankton density (1st event of nutrient addition); (2b) hours of incubation at high phytoplankton density (2nd event of nutrient addition, 19th day); (2c) days of incubation

By the second event (nineteenth day) of nitrogen addition, concentration of the residual NO_3-N was found between 260-330 µg/l (from a starting concentration range of 2000-8000 µg/l). The peak at 19th day was due to fresh addition of NO_3-N. Similar to phosphorus removal curve, the nitrogen removal curve has shown steep decline at the 2nd event of nitrogen addition (figure-2b and 2c). The residual values of nitrate for the three treatments in both the first and the second event of nutrient additions were very similar (around 200-300 µg/l) and the variations were negligible as compared to the initial inputs.

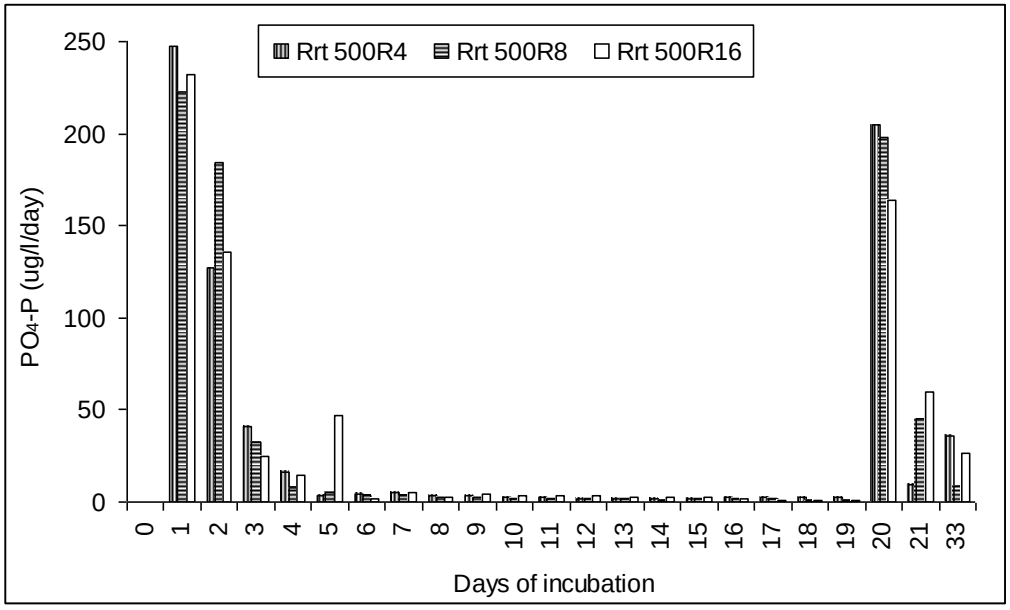
Nitrate and phosphate removal rates

PO_4-P removal rates (figure-3a) were highest on first day (247.3µg/l/day; 49.5%) followed by second (36.8%), third (8.2%) and fourth (3.3%) day, beyond which the rates were very low (around 1%). This decreased rate corresponds to low availability of P in the systems by then. Among the different ratios, the highest rate

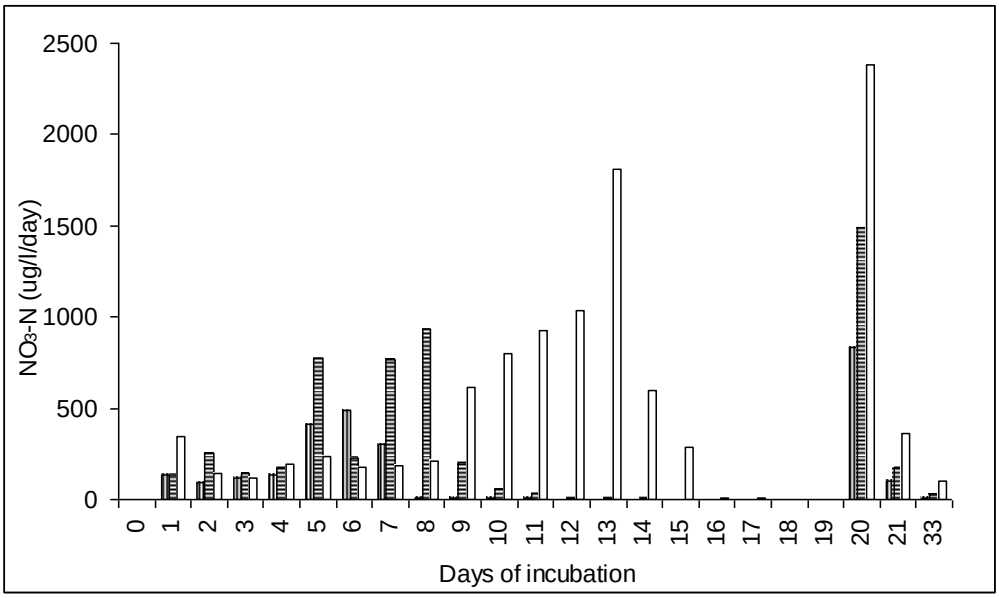
on the first day was observed for R4 while on the second day it was observed for R8. On fifth day, nutrient removal rate was the highest for R16 (9.3%). In case of nitrogen removal (figure-3b), in spite of higher availability of the nutrient the rates were low in all cases for the first four days. For R4, nitrogen uptake increased till 6th day (24.5%) and decreased gradually and almost finished by 8th day. For R8, the uptake increased till 8th day (23.3%) and decreased suddenly to reach almost negligible by 12th day. In case of R16, the uptake rates were, however, constantly low for the first 7 days, after which it increased gradually till morning of the 12th day and reached to 1807 µg/l/day at afternoon of the 12th day(35.5%). After that it decreased and reached to negligible amounts by the 19th day. In lag phase of removal the values ranged between 4.6-6.9% for R4, 3.3-6.4% for R8 and 1.4-4.3% for R16. In log phase the values varied between 15.1-24.5%, 19.1-23.3%, 7.7-35.5% for R4, R8 and R16 respectively. In the stationary phase values were mostly below 0.5%.

On the 2nd event of nutrient addition (19th day), the removal rates for phosphorus and nitrogen were very high on the same day. Just after nutrient addition, the removal rate of P for R4 reached 204.5µg/l/day (70.6%) followed by R8 and R16 with values 198.2 (55.8%) and 163.6µg/l/day (36.7%) respectively. Whereas, the P-removal on the 1st day was the resultant of 24 hours of removal, the values at 19th day were that of only 20 hours, given that the amount of the nutrients added at 2nd event was half that of at 1st event. As evident from figure-3a, on the 2nd event, a major part of P has been removed within the first 4 hours of incubation, although the available nutrient at 19th day was much lower than that of on the 1st day of incubation. Afterwards the values decreased, with R16 having highest value (47.9%) on the 21st day among the three

(R8=36.7%; R4=7.3%). In figure-3a, the values on the 33rd day need not be confused for single day P-removal rather it is a sum of 12 days P-removal (thus, the actual per day removal is very low). Contrary to P-removal, the highest value for N-uptake is shown by R16, (figure-3b) reaching up to 2384 µg/l/day (43.7%) just after nutrient addition. R8 and R4 ratio treatments have uptake values as 1490 (51.8%) and 832µg/l/day (54.7%) respectively. On the 2nd day %R values were almost equal (range: 18.7-19.9%) for all treatments. The trend remained the same on the 21st day also, but with much lower uptake values.



(3a)



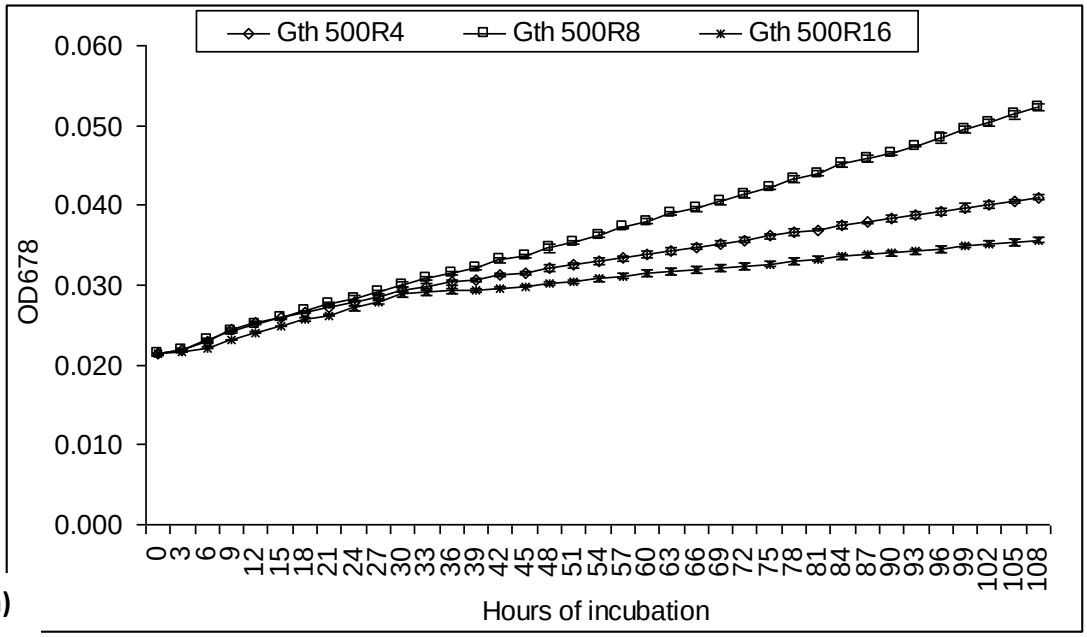
(3b)

Figure 3. Rate of removal (Rrt; µg/l/day) of nutrients by phytoplanktons from water column with respect to days of incubation: (3a) PO_4-P; (3b) NO_3-N

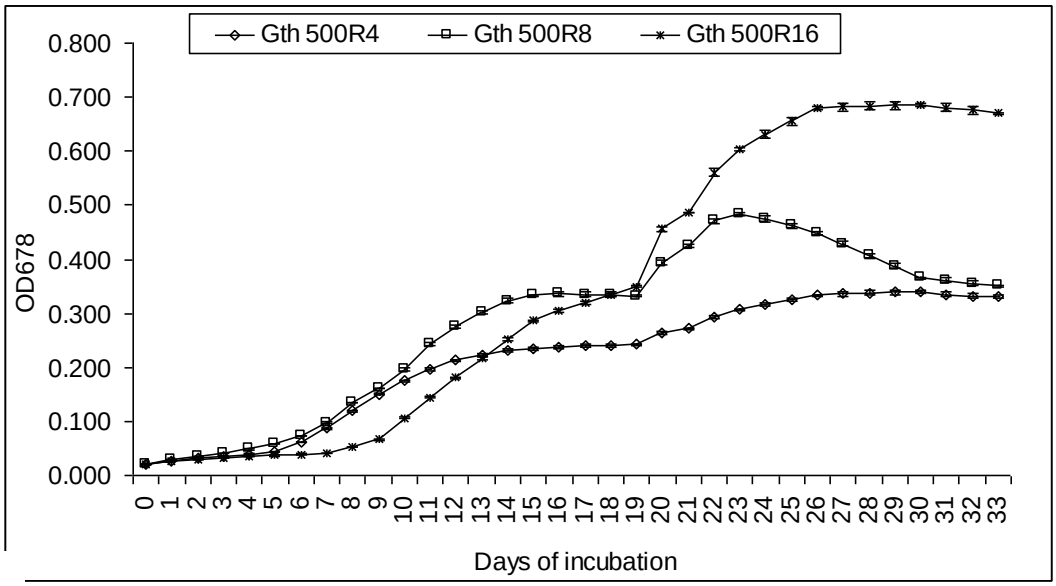
Study of cumulative percentage of removal (%CR) shows that up to 81% removal was achieved (R8) by the 2nd day, whereas it took another 3 days to reach 90% removal. The maximum of phosphorus removal was 98% (R16) and it took 19 days to occur. In case of the 2nd event of nutrient addition, up to 70.6% (R4) removal was observed within first 4 hours and around 90% removal was recorded by the 2nd day. In general, when the phosphorus availability was high the removal rate was also very high. The removal of phosphorus was still higher when the initial phytoplankton density was high. Percent cumulative removal (%CR) values, by the 19th day were 87, 94 and 96 for R4, R8 and R16 respectively. Similarly, at the 2nd event of nutrient addition, the highest % CR values (by 14th day after the 2nd addition) were 78, 89 and 93 respectively.

Growth of phytoplanktons

Growth of phytoplankton (Figure-4a) in the three systems in response to nutrient addition has shown that an observable increase in phytoplankton density has taken place after about 60 hours (2.5 days). Log phase of growth in case of R4 and R8 treatments started after 5th day of incubation whereas in case of R16 it started after the 8th day (figure-4b). In response to nutrient addition, there was an increase in the algal density (OD) in the order of 16, 23 and 32 folds of the initial value respectively, for the corresponding ratios R4, R8 and R16. The growth curves when compared with nutrient removal curves indicate a luxury uptake of phosphorus without showing any substantial increase in the phytoplankton density. This shows that most of the available phosphorus (PO_4-P) has been taken up from the system within the lag phase of growth and the nitrogen uptake has been speeded up only after initiation of log phase of growth. The second log phase of growth has followed the second event of nutrient addition.



(4a)



(4b)

Figure 4. Changes in growth (Gth) of phytoplankton measured in terms of optical density at 678nm (OD678) with respect to incubation period: (a) hours; (b) days

Comparison between cumulative addition and onetime addition of nutrients

As depicted in the table-2, the experimental systems with nutrient concentrations 500P and 1000P status have been achieved by one time addition of required P and N whereas that of 750P has been achieved by cumulative addition of P as 500 µg/l (for 19 days) followed by 250 µg/l (for 14 days) and proportionate amount of nitrate. Total time of incubation in case of 500P and 1000P are 19 days and that of 750 is 33 days. The total chlorophyll (chl-t) analysis for the three ratios against 'one-time addition' and 'cumulative addition' of nutrients is showing that 750P has higher values than 500P and 1000P for R4 and R16. For R8, however, 750P has higher values than 500P but lower value (not significantly) than 1000P. Between the one-time addition systems, 500P has lower values of chl-t than 1000P for each individual ratio. Similar trends were observed for growth-maximum (Gth. Max.)

values as well as for chl-t values, except that 1000P has higher value than 500P at R8 and lower value than the same at R16.

Table. 2. Comparision between 'cumulative addition' and 'one time addition' of nutrient for algal chlorophyll and growth maximum

	Total Chlorophyll (µg/l)			Growth maximum (OD678)		
	500P 19d	1000P 19d	750P 33d	500P 19d	1000P 19d	750P 33d
R4	104	161	210	0.2423	0.2937	0.3392
R8	165	219	206	0.3328	0.3371	0.4833
R16	260	343	462	0.3500	0.3472	0.6844

Measurement has been taken at highest growth value

Luxury/limitation of nitrogen and algal lipid content

Similar to growth response, the cellular lipid content (% w/w) of phytoplankton is affected by luxury/limitation of nitrogen. Algae grown at 500R8 have a cellular lipid content of 6.47±0.09%. At 500R16 the lipid content decreased slightly (6.32±0.13%) but with decreasing nitrogen input the lipid content has increased (500R = 7.72±0.11%; 500R1= 8.27±0.16). The cellular lipid content of algae was in the order of 500R1>500R4>500R8>500R16.

Discussion

Removal of nutrients by phytoplanktons

At low population density of phytoplankton, phosphate-removal log phase started as soon as there was input of nutrients into the experimental systems, whereas nitrate-removal log phase started at

a later stage corresponding to increasing phytoplankton density. On the other hand at the 11-16 times higher phytoplankton density level (on 2nd event of nutrient addition), both phosphate and nitrate removal had log phase as soon as there was nutrient addition. At low phytoplankton density, phosphate took 2 days to attain 80% and 5 days to attain 90% removal; at the 11-16 times higher density level of phytoplankton, however, it took only 2 days to attain 90% removal. This shows that phosphate-removal was affected by some other factor besides phytoplankton density i.e., luxury uptake *(need to clarify more)*. In contrast to above, nitrate-uptake was very much density dependent as nitrate-uptake increased with increasing phytoplankton density till depletion of nutrient, on both the events of nutrient addition.

Sicko-Goad and Jensen (1976) found that algal cultures that had been deprived of adequate P exhibited a 26-fold increase in cellular P contents (primarily as polyphosphates) within 1 h of exposure to phosphate. Gonzalez et al. (2008) found an 80% phosphate removal by a consortium of algae-bacteria from swine manure within 3 weeks of inoculation. The density driven nutrient uptake has been reported by Lau et al. (1995), they observed that an initial algal density of 1 X 10^7 ml^{-1} performed 80% P and 90% ammonia removal in 7 days, while the same degree of removal was achieved in 10 day with an algal density of 1 X 10^6 ml^{-1}. Lafarga-De la Cruz et al. (2006) got up to 99% P removal and 92% N removal from the culture media, similar to the present study. Sorokin and Dallocchio (2008) reported in the lagoon of Vincia that the DIP uptake rate depends upon bloom density with a range of 20 and 73 nM/min.

While comparing surface washed phytoplankton with non-washed phytoplankton, Sanudo-Willhelmy et al. (2004) found that surface adsorption is a

major process in nutrient removal from water column by phytoplankton (Trichodesmium). Their finding supports the trends of P-removal of the present study. This adsorption to the surface of phytoplankton rather than biological uptake is the cause of deviations from the Redfield ratio. This additional non-biological process of nutrient removal (scavenging) could affect the interpretation of marine nutrient inventories and ecosystem models. Fu et al. (2005) examined how surface bound P pools affected Redfield stoicheometry and P uptake kinetics in cultures and natural blooms of different algae. They found that amount of surface adsorbed P on exponential growth phase cultures was 14% of the total cellular P.

The nitrogen to phosphorus ratio and nutritional history of the phytoplankton also influence nutrient uptakes. The trends of P and N removal hints that when the proportion of N become low (treatment R4), there will be a tendency of higher removal for both N and P by a natural community of phytoplanktons. But the maximum %CR values of P in these treatments were in the sequence of R4<R8<R16. Moreover, it is assumed that if the algae have a nutrition history of exposure to low N: P ratio, they may have a greater tendency of higher nutrient uptake. This was observed in the present study as by the 2nd event of nutrient addition the nutrient deprivation due to differential N: P ratio had prompted R4 to bear distinctly higher CR values. However, low N: P ratio had lower maximum values of percent uptake. The common happenings in both events of nutrient addition were the highest P-removal by R4 on the 1st day and the maximum P-removal by R16 treatment by end of the phase. In contrast to this, nitrogen uptake had shown that the 1st event of nutrient addition had encountered lower %CR values for R16 invariably up to 15 days, but like P-removal again R16 bore the

maximum %CR value. Here, luxury uptake can not be claimed as the N-removal is closely associated with phytoplankton density. In the initial days at both the events of nutrient addition, R4 had higher values of N-uptake (similar to the case of P-removal). The 2nd event of nutrient addition depicted similar trend as that of the 1st event. The residual N and P values were very near to each other for all the ratios.

Valenzuela-Espinoza et al. (1999) in a 7 day study of uptake by *Isochrysis* aff. *galbana* (Clone T-ISO) found similar trends of PO_4-P and NO_3-N removal from the medium. Maximum removal of phosphate happened within the initial 3 days after which nitrate removal was observed. The high rate of P removal as 204.5 µg/l/day and N removal as 2384 µg/l/day by the natural phytoplankton community of treatments R4 and R16 respectively is showing the potential of such cultures for scavenging nutrients from wastewaters.

Thus, phosphorus has been given preference in uptake over nitrogen irrespective of their supply ratio. This is clear from the fact that in case of P, maximum removal took place in the first 4 days while in case of nitrogen, higher uptakes started after 4th day. On the other hand, the occurrence of higher removal rates of nitrogen for the three ratios corresponding to growth log phase for the three respective ratios is suggesting that nitrogen is more essential for cellular multiplication as compared to phosphorus. The longer period of lag phase in case of R16 may have occurred due to acclimatization of phytoplankton community as per nutrient availability.

Phytoplankton growth in response to addition of N and P

Whereas, there was a time lag for visible growth response by the phytoplanktons in the 1st event of nutrient addition, it was not the same in case of 2nd

event. This indicates density dependency of biomass accumulation. Furthermore, the steeper growth curves of R16 and R8 after 2nd event of nutrient addition show that a continuous addition of nutrient may give a higher biomass accumulation. Remarkably, much before this point of time R4 had attained stationary phase in contrast to R8 and R16.

Ratio of N: P also affects the growth of the algae. Whereas log phase of growth in case of R4 and R8 started at 5th day, R16 responded at the 12 day. Similarly, the maximum OD678 value of water sample for the treatments was in the order of R16>R8>R4 which were 32, 23 and 16 folds of the initial value for these treatments respectively; that means an average value of 0.97, 0.70 and 0.48 doublings/day. Valenzuela-Espinoza et al. (1999) in a 7 day study got doubling rates of 0.65 and 0.67 day^{-1} respectively in f/2 media and an alternate media for *Isochrysis* aff. *galbana* (Clone T-ISO). Similarly, Lafarga-De la Cruz et al. (2006) achieved a growth rate of 0.68 day^{-1} at exponential stage of culture with *Rhodomonas* sp. in a seven day study.

On the basis of the observed pattern of nutrient removal and phytoplankton growth, it can be said that the status of available P in a natural water-body at a point of time may not be a true predictor of future growth of the algae. If there is a high standing crop of phytoplankton in an aquatic system, it means that there was a better nutritional history even if it may show lower amounts of available nutrients by then. On the other hand, if there is a high available P in the systems but high standing biomass then either P supply rate is higher than removal rate or there is deficiency of any other essential nutrient (macro or micro). High removal rate and high storage of P by the algae support the fact that total P in the standing/sedimented biomass is more important parameter as compared to others

for achieving success in nutrient interruption programmes meant for lake restoration. Reynolds (1992) also proposed similar views about nutrient interruption. It is also suggested that the autotrophs should either be removed or inactivated soon after sampling for getting the actual value of nutrients corresponding to the time of sampling. Moreover, total P and/or total N rather than available forms are better indicators of the eutrophication potential of a water-body.

Wang et al. (2008) in The Songhua Lake, Northeast China found that total phosphorus and total nitrogen were the main risk factors to have impact on the eutrophication, and that influence of the phosphorus on the lake eutrophication was larger than that of nitrogen.

Change in ratio of nitrogen to phosphorus and algal lipid content

Variation in ratio of nitrogen to phosphorus has affected the cellular lipid content (% w/w) of phytoplankton. While algae grown at 500R8 had a cellular lipid content of 6.47%, this value has increased when there was reduction in nitrogen input, but decreased when nitrogen input was increased. The cellular lipid content of algae was in the order of 500R4>500R8>500R16. Hu & Gao (2006) in *Nannochloropsis* sp. have noted changes in cellular lipid composition and content with changes in nitrogen and phosphate concentrations, salinity and temperature (in elevated CO_2 concentration). Shifrin and Chisholm (1981) reported that nitrogen (N) deprivation of 9 days could increase cellular lipid up to 72% in *Monallantus salina* (strain GSB Sticho).

Conclusion

1. Phytoplankton growth and nutrient uptake are affected by density of phytoplanktons. The removal is also affected by nutritional history of the

algae and amount and ratio of N and P in the aquatic medium.

2. The N: P ratio also affects cellular lipid content.
3. A higher biomass production can be achieved by continuous addition of nutrients and regular harvesting.
4. For avoiding erratic results, particularly in case of P measurement, algal biomass should be removed from the sample as soon as possible.
5. By controlled eutrophication, phytoplankton can be used for scavenging nutrient from eutrophified waters (wastewaters) and the resulting high biomass can be used resourcefully for production of various value added products like biofuels, biofertilizer, pharma-nutraceuticals etc.

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