3 BIOMASS PRODUCTION: ROLE OF N-P CONCENTRATION AND RATIOS

3.1 INTRODUCTION

Production of algal biomass in a waterbody depends primarily on the concentration and ratio of the essential nutrients in the system (Ryding & Rast 1989). According to Borchart (1996) growth of an algal species can be limited by only one nutrient at a time. Algae may respond to nutrient limitation with sharp transitions between limitations of one nutrient or another. The transition occurs while the nutrients are at their ratio within the cell perfectly matching the growth requirements of the alga (Droop 1974, Borchart 1996). Whether the growth-limiting nutrient is N or P can be gauged roughly from the ambient and cellular N: P ratios (Borchart 1996). The optimal ratio of N: P varies among species, the typical atomic ratios being 16:1 (Redfield ratio) found in phytoplankton biomass (Redfield 1958, Redfield et al. 1963) and this corresponds to a mass ratio of 7.2 N: 1 P. In natural phytoplankton N: P critical supply ratios (atomic ratio) varies roughly from 7:1 to 45:1 i.e. 4.4:1 to 19.4:1 mass ratio (Suttle & Harrison 1988). Macroalgae tend to be enriched in N, with a median ratio of 30:1 (Atkinson & Smith 1983, Duarte 1992). Smith (1983) reported that lakes having epilimnetic TN: TP ratios >29 will typically exhibit low proportions of blue-green algae. However, they are generally found in lakes having N: P supply-ratios less than 10:1 (Flett et al. 1980). Low ratios of N: P (usually <10:1) may indicate N-limitation, whereas higher values (>20-30:1) may indicate P-limitation (Rhee 1978, Lapointe 1986, Fujita et al. 1989, Vymazal 1995, Borchart 1996); biomass having TN: TP ratios beyond the range of 5-10 are normally considered as limited of one of the nutrients.

While studying the interaction of N-P ratio with phytoplankton, there is high emphasis on the change in community composition (Costa 2003, Camara et al. 2009, McCarthy et al. 2009). Other studies also emphasized the growth limitation of phytoplankton (Chapelle et al. 2010, Varkitzi et al. 2010) or luxury uptake of certain nutrient (Portielje & Lijklema 1994, Littler et al. 2010) without much gain in biomass production. The other aspect with economic interest is the production of high biomass by the best combination of ratio and concentration of N & P. This aspect has been taken into consideration in this study. Thus, the study aims at finding strategy of nitrogen and

phosphorus addition in different ratio-concentration combinations for getting a good yield of phytoplankton biomass.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Set up

Stock algal solution was prepared the same way as described in Chapter 2. The same three ratios R4, R8 and R16 were taken for this study. Four concentrations, 100, 250, 500 and 1000 µg/l of P (as KH_2PO_4) and proportionately N (as KNO_3) were used to maintain these ratios (Table 3.1). The treatments have been denoted by 'R' prefixed with P concentration and suffixed with N: P ratio value. Sampling was done on every third day for 21 days. Analysis of nitrate, orthophosphate, chlorophyll content, algal community composition and growth was done as described in Chapter 2.

Table 3.1. Experimental set up for studying effect of different N-P ratio and concentration combinations on biomass multiplication of phytoplankton (R = N: P ratio)

	Nutrient addition on 0^{th} day	
Treatment	P (µg/l)	N (µg/l)
C	-	-
100R4	100	400
100R8	100	800
100R16	100	1600
250R4	250	1000
250R8	250	2000
250R16	250	4000
500R4	500	2000
500R8	500	4000
500R16	500	8000
1000R4	1000	4000
1000R8	1000	8000
1000R16	1000	16000

3.3 RESULTS

3.3.1 Growth

The phytoplankton density (measured as OD678) in the water samples of various treatments against days of incubation is presented in Figure 3.1. OD678 for the treatments were higher than that seen in control system. For any single ratio (say, R4, R8 or R16) the phytoplankton density had increased with increasing concentration of phosphorus. At lower concentrations of P (100 and 250 µg/l) with increasing ratio of the nutrients the phytoplankton density increased. At 500 µg/l P, 500R8 had higher values than 500R4. For 500R16 although initially the values were lower than 500R4 and 500R8 it also resulted in higher values towards the end of experiment. At 1000 µg/l P, the phytoplankton density was very close for all ratios throughout the incubation period.

Comparing the various treatments listed above, the curves of 100R16 and 250R4 were almost overlapping. The curve of 250R16 traveled above the 500R4 curve till 15th day. On 18th day, the curve crossed the 500R4 curve, afterwards falling below. The 15th, 18th and 21st day phytoplankton densities values of 1000R4 were less than that of the 500R8.

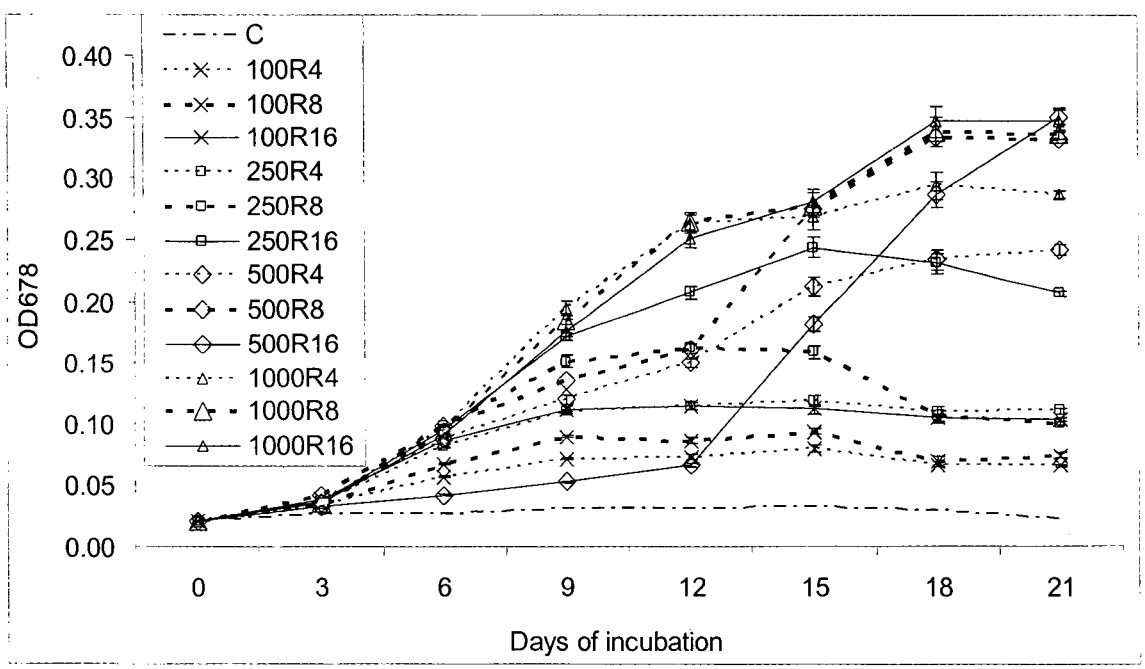

Figure 3.1. Growth of phytoplankton at different concentrations and ratios of P and N

3.3.1.1 Rates of growth (OD678/day)

In almost all treatments except 500R8 and 500R16, the highest growth rates (OD678/day) had occurred on 9th day of incubation (Figure 3.2). Control system had the lowest rates. The highest growth rate were in the decreasing order of 1000R4, 1000R8, 1000R16, 250R16, 250R8, 500R16, 500R8, 500R4, 250R4, 100R16, 100R8, 100R4 and control. This order indicates that at P concentrations of 100, 250 and 500 µg/l the maximum growth rate increased with increasing ratios for each individual concentration but reversed at 1000µg/l. Growth rates of 100P treatment were the lowest and 1000P were highest. Growth rates of 500P were sometimes lower and sometimes higher than the growth rates of 250P treatments.

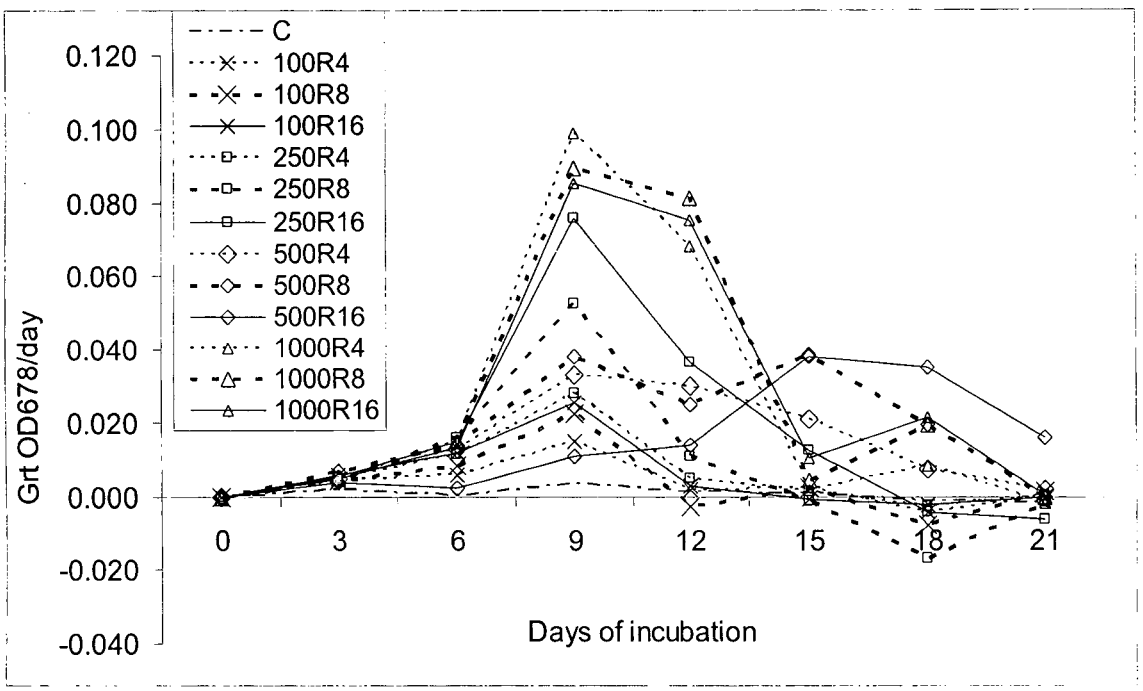

Figure 3.2. Growth rates of phytoplankton at different N-P concentrations and ratios

3.3.1.2 Growth maximum values

Growth maximum values (OD678 max.) of the treatments (Figure 3.3) showed that all treatments had higher values than the control set. For each concentration the values increased with increasing ratio. Between ratio-concentration combinations, the maximum OD for 100R16 was close to that of 250R4; 250R16 had slightly higher value than 500R4. Similarly, the value for 500R16 was higher than all three ratios of 100 µg/l P.

Growth maximum study for the four concentrations against the three ratios (Figure 3.4) showed that 100P, 250P and 500P had distinctly separate values from each other being higher in the order of concentration. For 1000P, value at R4 was distinctly higher than other treatments whereas very close to 500P at R8 and R16 (higher at R8 and lower at R16).

As observed from figure 3.5, maximum growth at R4 was distinctly separate from other treatments and the values were higher for higher concentrations. At R8 the growth maximum values were in the order of 1000P≥ 500P> 250P> 100P whereas at R16 they were in the order of 250P> 500P≥ 1000P> 100P. The ANOVA test (α-0.05) for OD678 of water samples of various treatments (Table 3.2) showed significant difference (p = 0.001; LSD = 0.863).

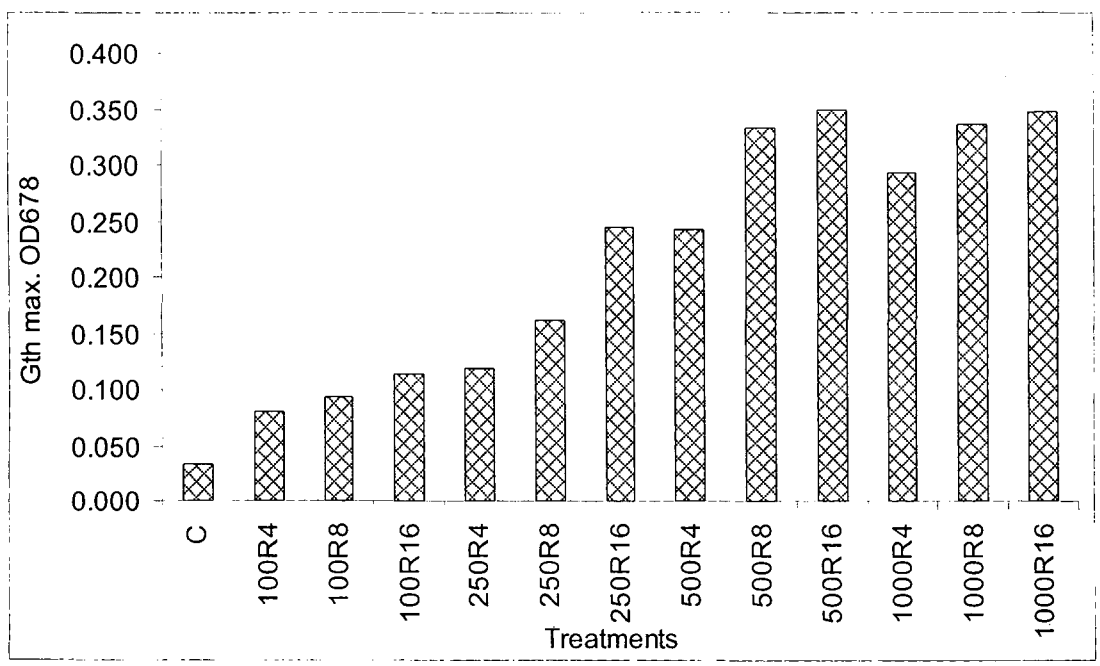

Figure 3.3. Maximum growth (OD678) achieved by phytoplankton subjected to different nutrient concentration-ratio treatments

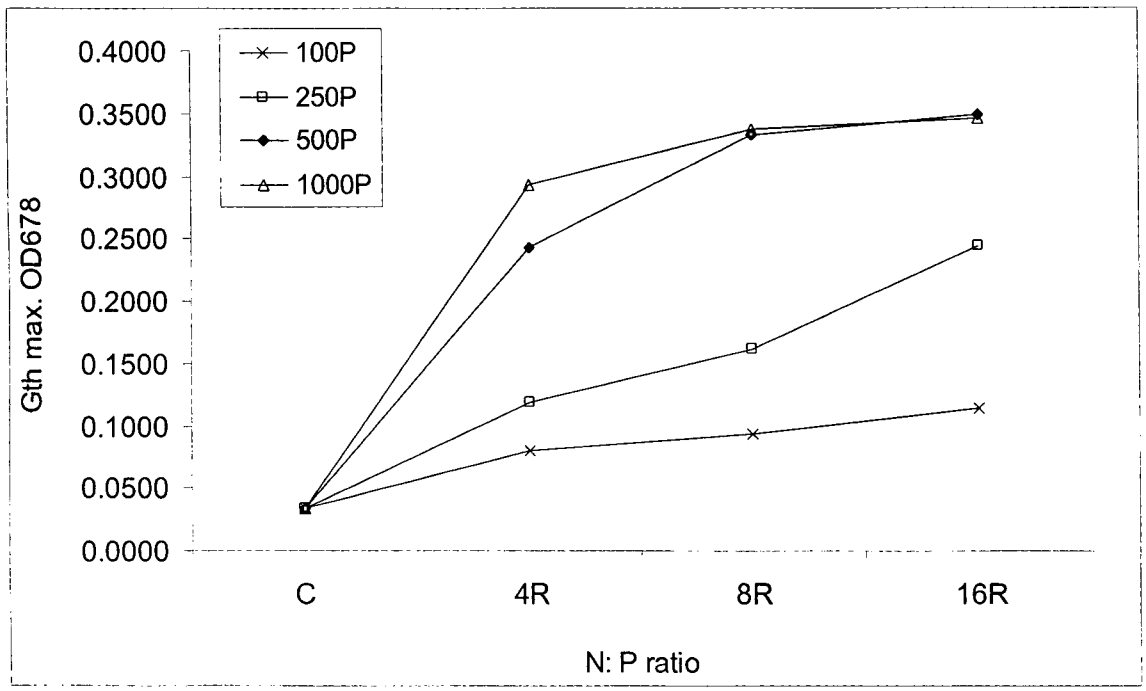

Figure 3.4. Phytoplankton growth at different P concentration with varying N-P ratios

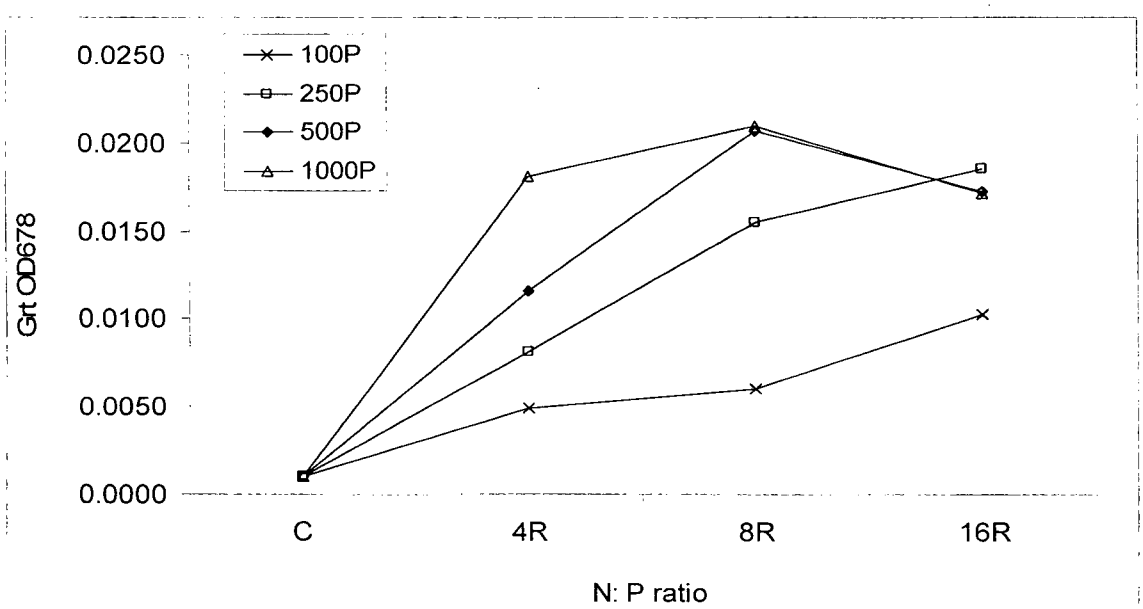

Figure 3.5. Phytoplankton growth rates at different P conc. with varying N-P ratios

3.3.2 Chlorophyll content in waters:

Chlorophyll-a (chl-a) and total chlorophyll (chl-t) for all treatments were higher than control (Figure 3.6). For each concentration, the chl-a and chl-t values increased with increasing ratio. At different concentration for the same ratio, the chlorophyll values increased with increasing concentration. 100R8 had values close to 250R4 and 250R8

and were less than that of 100R16. According to chl-a values, the C, and 100R4 were mesotrophic in nature; 100R8, 100R16, 250R4, 250R8 and 500R4 were eutrophic in nature whereas other treatments were hypertrophic in nature. The ANOVA test (Table 3.3; α-0.05) showed significant difference between various treatments (p = 0.048; LSD = 126.354).

Table 3.2. Single factor ANOVA test (α-0.05) for OD678 of waters of various treatments.

Treatment	Average	Class
C	0.028	A
100R4	0.059	AB
100R8	0.067	ABC
100R16	0.087	ABC
250R4	0.088	ABCD
250R8	0.104	ABCDE
500R16	0.129	BCDEF
500R4	0.138	BCDEF
250R16	0.152	CDEF
500R8	0.175	DEF
1000R4	0.183	EF
1000R16	0.194	F
1000R8	0.194	F
P-value	0.001	
LSD	0.863	

Treatments belonging to one group do not significant differ from one another.

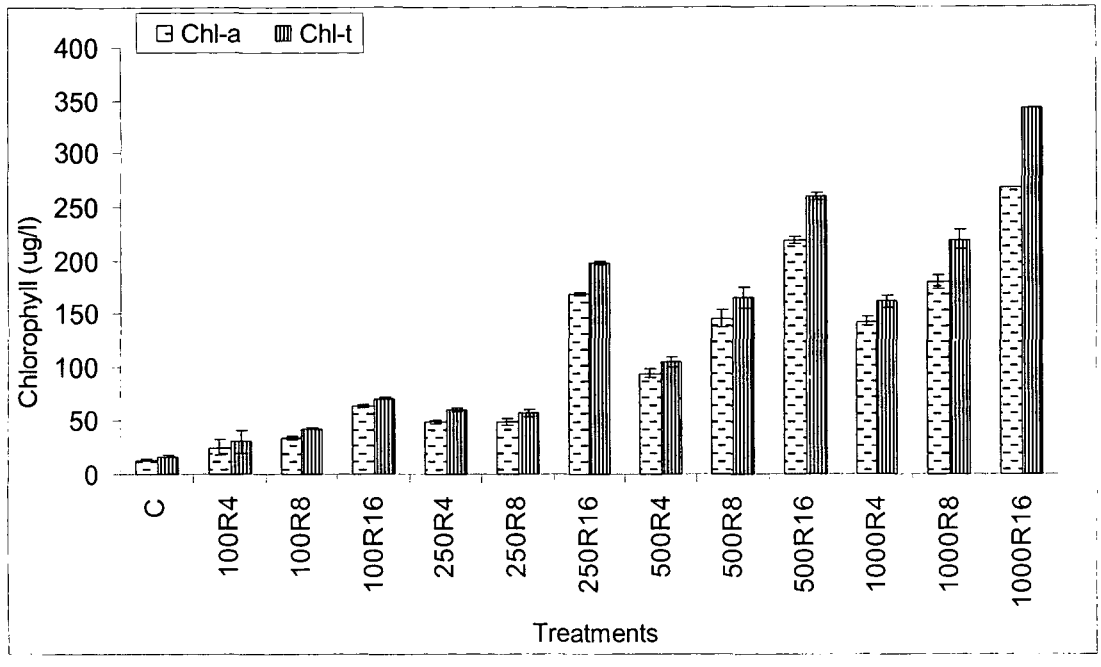

Figure 3.6. Chlorophyll content (chl-a and chl-t) in water samples of different treatments

Table 3.3. Single factor ANOVA test (α-0.05) for total chlorophyll (chl-t) in water column of various treatments

Treatment	Average	Class
100P	50.091	A
250P	105.151	AB
500P	176.262	AB
1000P	212.521	C
P-value	0.048	
LSD	126.354	

Treatments belonging to one group do not have any significant difference.

3.3.3 Residual nutrients

Residual nutrient analysis (Figure 3.7) in the systems showed that the nutrients in treatment sets were higher than that in the control experiment. Residual P in treatment systems varied only between 10-34 µg/l. Values beyond 20µg/l were seen in 100R16, 250R8, 500R4 and 500R8. Comparing the residual P levels with that of the experiment discussed in chapter-2 (time period of incubation was 33days), it seems that given a higher period of incubation time these values would have declined still further. Except

for 1000R16, where the nitrate value was 994 µg/l, the values had varied between 223 and 321 µg/l in other treatments.

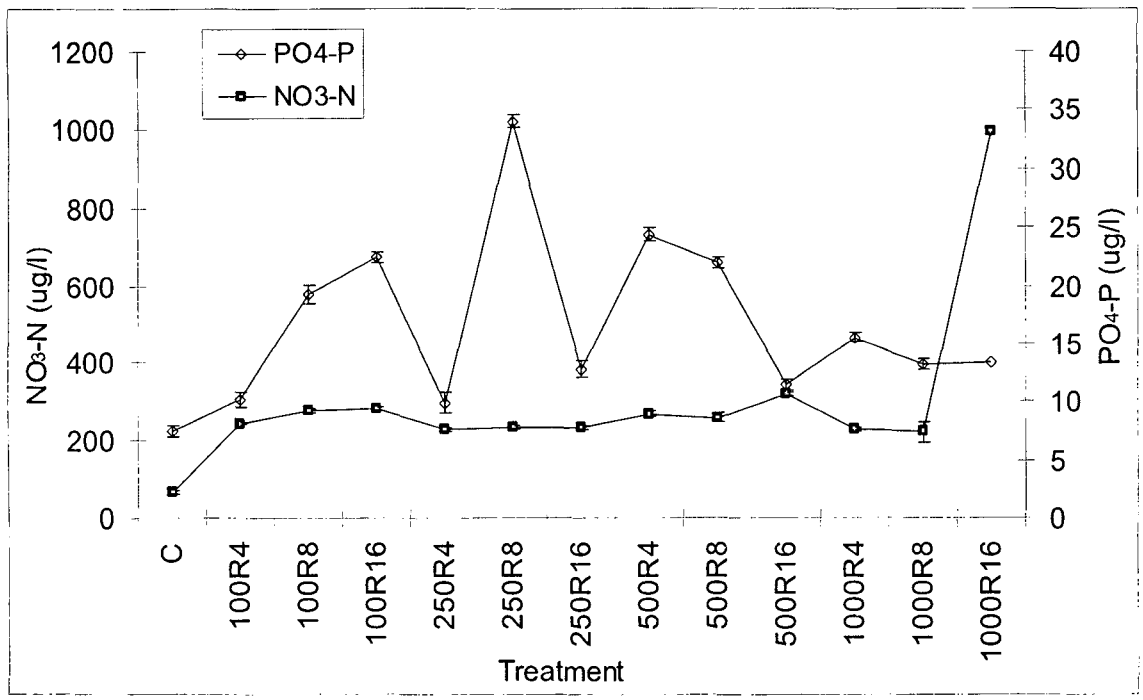

Figure 3.7. Residual nitrate and phosphate (µg/l) in water samples from different treatments

3.3.4 Increments in biomass:

The increment in biomass (Table 3.4) was highest (17.2 times on 21st day) in the case of the treatment 500R16 and lowest (1.6 times) in the case of control. Increment in biomass of different treatments in decreasing order was as follows: 500R16 (17.2 times on 21st day), 1000R16 (17.0 times on 18th day), 1000R8 (16.5 times on 18th day), 500R8 (16.3 times on 18th day) 1000R4 (14.4 times on 18th day), 250R16 (12.0 times on 15th day), 500R4 (11.9 times on 21st day), 250R8 (7.9 times on 12th day), 250R4 (5.8 times on 15th day), 100R16 (5.6 times on 12th day), 100R8 (4.6 times on 15th day), 100R4 (3.9 times on 15th day) and C(1.6 times on 15th day. In the treatment 500R8, biomass doubled by 3rd day of incubation. By 6th day the biomass increased 4.0 to 4.8 times in all treatments except 100R4, 100R8 and 500R16. Similarly, by 9th day 1000R4 had 9.5 times of biomass increment and in general there was 1.5 to 2 times increase in biomass within a day. But, with depletion of available nutrients the biomass increment decreased progressively with time. By 12th day the highest increment of 13.0 times was commenced in 1000R8. By 18th day the value went up to 17.0 times in 1000R16 and the

highest value of increment (17.2) occurred in 500R16 by 21st day. The mean doubling time was lowest (2.1 day) in case of 1000R16 and highest (7.6 days) in case of 100R4.

Table 3.4. Biomass increments of phytoplankton in response to different treatments

	Number of folds the initial biomass increased along the incubation period								Mean doubling time (days)
Treatments	0th	3rd	6th	9th	12th	15th	18th	21st	
C	1.0	1.3	1.3	1.5	1.6	1.6	1.4	1.1	-
100R4	1.0	1.6	2.8	3.5	3.6	3.9	3.3	3.2	7.6
100R8	1.0	1.7	3.3	4.4	4.3	4.6	3.5	3.7	6.5
100R16	1.0	1.9	4.2	5.5	5.6	5.5	5.2	5.1	4.3
250R4	1.0	1.8	4.0	5.4	5.6	5.8	5.4	5.4	5.1
250R8	1.0	1.7	4.8	7.4	7.9	7.8	5.3	4.9	3.0
250R16	1.0	1.8	4.7	8.4	10.2	12.0	11.4	10.1	2.5
500R4	1.0	1.7	4.3	5.9	7.3	10.4	11.5	11.9	3.5
500R8	1.0	2.0	4.8	6.6	7.9	13.5	16.3	16.3	2.2
500R16	1.0	1.6	2.1	2.6	3.3	8.9	14.0	17.2	2.4
1000R4	1.0	1.8	4.7	9.5	12.9	13.2	14.4	14.1	2.5
1000R8	1.0	1.7	4.6	9.0	13.0	13.6	16.5	16.5	2.2
1000R16	1.0	1.8	4.4	8.6	12.3	13.8	17.0	17.0	2.1

3.4 DISCUSSION

Nitrogen and phosphorus play a major role in primary production of biomass. Limitation of one nutrient or the other affects the biomass production of phytoplankton (Turner et al. 1990, Panigrahi et al. 2009). Significant relationship of biomass production in response to nitrate addition was observed in Danish coastal waters (Carstensen & Henriksen 2009). Xiao-long et al. (2007) observed that total phosphorus (TP) and water temperature plays a governing role in phytoplankton dynamics in most seasons in their study and also found a complex relation between phytoplankton biomass and nitrogen concentration.

For any single ratio (say, R4, R8 or R16) the phytoplankton density increased with increasing concentration of phosphorus. At a single concentration of P (e.g. 100 µg/l, or

500 µg/l) if the ratios were changed (nitrogen concentration were varied), the change in phytoplankton density showed no discernable trend. At different concentration of P every individual ratio of N: P had different effects on phytoplankton growth. It had also been found that a system with higher N: P ratio and relatively lower concentration of phosphorus can result in similar growth values in contrast to another system with lower N: P ratio and higher concentration phosphorus. For instance, the curves of 100R16 and 250R4 were almost overlapping; the 15th, 18th and 21st day phytoplankton densities values of 1000R4 were less than that of the 500R8. The lower growth values of 1000R4 and 100R16 would have happened due to the developing secondary limitation of N and P, respectively.

In almost all treatments except 500R8 and 500R16, the highest growth rates (OD678/day) had occurred on 9th day of incubation. Control system had the lowest rates. The growth rate increased with increasing ratios for each individual concentration of P except when it was 1000µg/l with which a reverse trend was seen. 500 µg/l had overlapping values with 250 µg/l and sometimes 250 µg/l treatment had value higher than that of 500 µg/l. Similarly, the maximum phytoplankton density for 100R16 was close to 250R4; 250R16 had slightly higher value than 500R4; similarly the density of phytoplankton in 500R16 was higher than with all other ratios of 1000 µg/l P. These observations of growth maximum showed that even at lower inputs of P high biomass yield can be achieved by appropriately manipulating N input. A moderate P concentration (250-500 µg/l) and moderate N: P ratio (R8) can produce optimal biomass economically. As evident from the Analysis of variance test, the pairs 100R8-100R16, 500R16-500R4, 1000R16-1000R8 treatments did not significant differ in between.

Chlorophyll-a (chl-a) and total chlorophyll (chl-t) at each concentration of P increased with increasing ratio whereas at each ratio, increased with increasing concentration. With reference to open trophic state boundaries (OECD, 1982) the control, and 100R4 are mesotrophic in nature; 100R8, 100R16, 250R4, 250R8 and 500R4 are eutrophic in nature whereas other treatments are hypertrophic in nature. From economic point of view the eutrophic systems and hypertrophic systems are of interest.

The residual N and P in the treatment systems showed that these treatments (in terms of ratio and concentration) do not have significant effect on residual nutrients except for

1000R16. The higher residual nitrate in 1000R16 is due to higher initial nutrient load. The presence of negligible amount of residual nutrients in the treatment systems shows them to be nutrient efficient.

The role of phosphorus along with grazing pressure and irradiance in regulating algal biomass has been studied by Kjeldson (1996). Growth limitation of phytoplankton biomass by phosphorus has been reported by Lee et al. (2010). The changes in phytoplankton standing crop have been attributed to nutrient concentration in Suwannee River and plum region of USA by Bledsoe & Phlips (2000).

The biomass increment shows that although in the lag phase of growth the doubling time was around 3-4 days while it was only 1-2 days during the log phase of growth. The highest number of increments of 17.2 times in 500R16 which is higher than 17.0 in 1000R16 shows that it is not necessary to have a higher concentration of P to have a better biomass yield. Normally, with higher input of P and higher N: P ratio the increment in biomass went up. In addition, the highest increase in biomass occurred invariably during log phase of growth i.e. with a higher population density of algae the time duration of biomass increment decreased. However, the rate of increment decreased later on as nutrients depleted due to removal. These two results indicates that if a continuous supply of nutrients is provided, a much faster biomass doubling rate can be achieved (provided that space does not become limiting i.e. if continuous harvesting is ensured). Olive and Higgins (1981) noted a 0.6 time doublings a day of *Selenastrum capricornutum* (Printz.) in bioassay of Portage Lake water, Ohio. Nutrient enrichments with PO_4-P and NO_3-N indicated that P was usually the major limiting nutrient. Under unbalanced N: P ratio, Vartikzi et al. (2010) noted a lower growth rate (0.11-0.22 divisions per day) for *Prorocentrum lima.*

The initial increase in optical density and decline at the end of the incubation period indicate that for a continuous high biomass production in an aquatic system continuous supply of nutrient is needed. This view is supported by the increase in optical density of experimental waters with response to sequential addition (Chapter 2) of nitrogen and phosphorus. Moreover, at the end of incubation period either or both of the available N and P were very low to support any further growth in the medium. This is analogous to

natural aquatic systems where a high phytoplankton biomass prevails when either there is a continuous nutrient supply from external source or there is internal loading.

3.5 CONCLUSIONS

- For all ratios of nutrient, experimented in the present study, phytoplankton density increases with increasing concentration of phosphorus.
- With all concentration of P (or N), the individual ratio of N: P had different effects on phytoplankton growth.
- A treatment having higher N: P ratio and low P concentration can have growth similar to another treatment with smaller N: P ratio and high P concentration.
- Normally, with higher input of P and higher N: P ratio the biomass yield increases.
- Notable increase in biomass occurs invariably during log phase of growth. At a higher population density of algae, the time required for biomass multiplication decreases (doubling time: around 1-2 day).