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Effect of Different Doses of Mixed Fertilizer on Some Biogeochemical Cycling Bacterial Population in Carp Culture Pond

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Abstract

The effect of different doses of mixed fertilizer on the response of some biogeochemical cycling bacteria were examined using seven different doses (i.e, 105, 211, 422, 844, 1689, 3378 and 6757 g/tank/week) of mixed fertilizers with a fixed C : N : P ratio of 88.6 : 7.5 : 1. Advanced fry of *Catla catla* (1.2±0.04 g), *Labeo bata* (0.99±0.05 g), and *Cyprinus carpio* (1.3±0.06 g) were introduced at the rate of 16 fish/tank and reared for 120 days. Water samples were collected and monitored for bacterial population (HB, CDB, DNB and PSB), primary productivity, plankton, water quality parameters, and total phosphate in the sediment at ten days intervals. Number of bacteria (HB 15–270 x 10^3/ml, CDB 15–237 x 10^2/ml, DNB 5–221 x 10^2/ml and PSB 3–159 x 10^1/ml) increased directly with the increase in the fertilizer doses and showed maximum rate of bacterial growth at the fertilizer doses 48,000 kg/ha/yr. Three levels of responses to fertilizer doses - poor bacterial density and rate of fertilizer mineralization efficiency (D_{12} to D_{48}), moderate bacterial density and highest rate of fertilizer mineralization efficiency (>D_{48} to D_{192}) and highest bacterial density but slow rate of fertilizer mineralization efficiency (>D_{192} to D_{768}) were observed. The fish growth tended to rise with the increase in fertilizer dose. Maximum fish growth was at D_{96} (96,000 kg/ha/year) and decline thereafter. Though the bacterial load increased with fertilizer doses, the primary productivity of phytoplankton and fish growth did not increased when fertilizer doses increased. Therefore, it can be concluded that fertilizer dose 48,000 kg/ha/yr is required to manipulate the optimum bacterial population and productivity of the aquaculture pond and application of excessive doses of fertilizer increases production cost and causes environmental pollution.

Key words: mixed fertilizer dose, bacterial growth rate, fertilizer mineralization efficiency, fish growth.

Introduction

It is widely proposed the biological productivity in aquaculture ponds is limited by nutrients. Pond fertilization through organic and inorganic sources has become a management protocol in aquaculture. It compensates for the specific nutrient deficiency and augments of biological productivity mediated through autotrophic and heterotrophic pathways (Schroeder, 1974; Moav *et al.,* 1977; Debeljak *et al.,* 1990; Jhingran, 1995; Das and Jana, 1996). In aquatic system organic manures contribute a great amount of combustible matter, which is oxidised and produce carbon dioxide that helps in the algal photosynthesis and also helps in the reduction of dissolved atmospheric nitrogen to ammonia. The decomposition of organic fertilizer is carried out by bacteria, fungi, actinomycetes (Persson *et al*., 1980; Gaur *et al*., 1995; Boyd, 1995). As a result, the released essential nutrients help to sustain the biological productivity of the system.

The phosphorus flow is mainly related to phytoplankton activity in the water column and decomposition on the pond bottom, while nitrogen flow is mainly linked to heterotrophic (decomposition and nitrification) and autotrophic (photosynthesis) processes (Milstein *et al*., 2003). The biogeochemical cycling of bacteria therefore play a key role in the nutrient dynamics of fish ponds, where the biogeochemical microbial activities are regulated by various environmental factors in fish culture ponds managed under different stocking combinations (Jana and Patel, 1984; 1985; 1990; Jana and Roy, 1985a; 1985b; 1986; Jana and De, 1990; 1993).

Balanced bacterial growth requires substrates with carbon, nitrogen and phosphorus in an atomic ratio of 106 : 12 : 1 (Goldman *et al*., 1987), although bacteria have some capacity to tolerate these requirements (Tezuka, 1990). According to Ghosh and Chattopadhyay (2005), the optimum C : N and N : P ratio were 28-29 : 1 and 3-7 : 1 for maximum abundance of mineralizing bacterial population, respectively. The role of C : N : P ratio as well as N/P ratio of ambient water in regulating the food chain through selective pressure on the certain organisms has been emphasized (Jana *et al*., 2001). Direct dose dependent gradual rise N/P ratio of ambient waters of all the treatments despite application of different doses of input fertilizer with constant N/P ratio implied that nitrogen tended to rise gradually with increase in absolute phosphate fertilizer dose indicates that phosphorus is more favourable over nitrogen and can be used at a much faster rate compared to nitrogen (Jana *et al*., 2001). While examining the influence of different C : N : P (12 : 2 : 1 to 151 : 12 : 1) applied at the rate of 0.043 g/l/week on growth of certain biogeochemical cycling bacterial populations and nutrient status of the system, it was concluded that mixed fertilizer (C : N : P ratio of 88.6 : 7.5 : 1) comprising cattle manure (95%), poultry droppings (2.5%), urea (2%) and single superphosphate (0.5%) applied at the rate of 23,000 kg/ha/year was the suitable cost effective fertilization option for aquaculture practices under tropical climate (Jana *et al.*, 2001). Therefore, it appears that not only C/N ratio, but N/P ratio and dose of fertilizer are quite important in the microbial decomposition and mineralization of organic matter.

The manipulation of microbial population in aquaculture systems through the application of mixed fertilizer of appropriate dose is an important subject for the development of intensive fish culture. The purpose of the present study was to examine the effect of different doses of mixed fertilizer on the bacterial population in the fish farming tank using a fixed stocking density.

Materials and Methods

Twenty four outdoor cement tanks (4500 l; 3 x 1.5 x 1 m) were arranged in a completely randomized design, provided with dry soil on the bottom and filled with ground water (pH 7.2-7.4 and temperature 28.5-30°C) a week prior to experiment. Poultry droppings (PD), cattle manure (CM), single super phosphate (SSP) and urea (U) were procured and mixed in different proportions to formulate a mixed fertilizer of organic and inorganic ingredients maintaining the fixed CNP ratio of 88.6 : 7.5 : 1, which was found to be optimum for pond fertilization (Jana *et al.*, 2001). The fertilizer dose applied in the tanks varied in a two fold manner starting from 105 - 6757 g/tank/week (equivalent to 12,000–768,000 kg/ha/year) (herein called treatments D_{12}-D_{768}) during the period of experimentation. The total C, N and P of mixed fertilizer supplied to each tank per week under designated fertilizer dose D_{12} were 18.408 g, 1.566 g and 0.208 g, respectively. Likewise, the amount of C, N and P supplied in the remaining fertilizer doses (D_{24}, D_{48}, D_{96}, D_{192}, D_{384} and D_{768}) increased in a two fold manner. A set without fertilizer treatment was used as control. Each treatment had three replications.

Fry of catla (*Catla catla,* 1.2±0.04 g), Bata (*Labeo bata,* 0.99±0.05 g), and common carp (*Cyprinus carpio,* 1.3±0.06 g) were procured from a local fish farm, acclimated for a week and introduced at the rate of 16 fish (catla-4 : bata-6 : common carp-6) /tank or equivalent to 35,500 fish/ha and reared for 4 months. The selected stocking density was in accordance with the results obtained earlier (Bhakta, 2003) with the stocking density ranging from 17,777 to 142,222 fish/ha the results indicated the stocking density of 35,500 fish/ha is optimum. No supplementary feed was provided during the culture period.

Water and surface sediment samples for microbial examination were collected aseptically at a fixed hour of the day (09.00 h) at ten days intervals during the study period (June, 2001 - September, 2001). The pooled samples collected from randomly selected five sites in each tank and used as inoculum in a petri plate. Aliquots of ten fold dilution 10^{-1} to 10^{-3} for water and 10^{-2} to 10^{-4} for sediment samples were made in sterile distilled water. The suspension of surface sediment (3 cm) was prepared by mixing 1 g of wet sediment in 99 ml sterile distilled water. Conventional spread plate technique under aerobic conditions was used to enumerate viable counts of aerobic heterotrophic bacteria (HB), cellulose decomposing bacteria (CDB), denitrifying bacteria (DNB) and phosphate solubilizing bacteria (PSB), following the methods described by Rodina (1972) and Austin (1990) at an incubation temperature of 35°C for three days. Each dilution of the sample was plated in triplicate and arithmetical mean of the three counts was used in the present study.

Water temperature, pH and Dissolved oxygen was recorded on the spot with the help of specific probe of Multiline System (F/SET-3, Best-Nr. 400327, WTW Wissenschaftlich-technische Werkstatten 82362 Weilheim, Germany). Rest of the water and sediment quality parameters were examined following the standard methods of APHA (1995) and Jackson (1967). Primary productivity was determined using the light and dark bottle method described by Vollenweider (1974). Fertilizer Mineralization Efficiency (FME) for orthophosphate (OP) over control was calculated using the formula as follows:

$$\mathrm{FME}(\%) = [(P_T - P_C) / P_C] \times 100$$

Where,

P_T = Mean concentration of orthophosphate in water of the treatments

P_C = Mean concentration of orthophosphate in water of the control

Fish growth and their survival were recorded at the time of harvest after 4 months of culture.

All results obtained from the tanks were statistically interpreted. A one way ANOVA (Gomez and Gomez, 1984) was used to compare the treatment means. Before analysis, the assumptions of normal distributions and homogeneity of the variance were checked using Kolmogrov-Smirnov and Cochran's tests, respectively. If the main effect was found to be significant, the ANOVA was followed by a LSD (least significance difference) test. All statistical tests were performed at 5% probability level using statistical package EASE and M-STAT.

Results

Bacterial Population

In water : There was a dose-dependent response of different groups of bacteria to fertilizer application in water. The overall counts of HB (15-270 x 10^3/ml), CDB (15 – 237 x 10^2/ml), DNB (5-221 x 10^2/ml) and PSB (3 – 159 x 10^1/ml) tended to rise with the increase in fertilizer dose and showed the order of variations as follows: $D_{768} > D_{384} > D_{192} > D_{96} > D_{48} > D_{24} > D_{12}$ (ANOVA, F = 565.75, $P < 0.05$). The rate of increase in HB (90-557%), CDB (116-560%), DNB (153-993%) and PSB (62-358%) varied markedly in different doses of fertilizer (Figure 1). The density of various group of bacterial population showed a gradual rising trend with time and with different doses of fertilizer.

In sediment : The fertilizer dose responses were similar to those in water although the counts of HB (32-249 x 10^4/ml), CDB (31-236 x 10^3/ml), DNB (26-186 x 10^4/ml) and PSB (13 – 162 x 10^2/ml) remained higher in sediment than water. The rate of increase in populations occurring in all fertilizer doses were 50 – 242%, 16-225%, 73-325% and 68-338% in HB, CDB, DNB and PSB, respectively (Figure 1). Unlike the temporal change observed in water, there was cumulative rise in the bacterial population of sediment in response to input fertilizer.

Water and sediment quality

Water quality varied highly in different doses of fertilize as well as over time. Water temperature and pH ranged from 28.5-30°C and 7.5 to 8.22 in different treatments, respectively (Table 1). The concentration of dissolved oxygen in surface water varied between 2.8 and 18.8 mg/l and gradually increased till fertilizer dose D_{192} and decreased with further increase in fertilizer dose (D_{384} and D_{768}). Temporal changes showed an increasing trend. The chemical oxygen demand of water exhibit a rising trend with direct functions of the fertilizer dose and time ranging from 14.5 - 561 mg/l (Table 1).

Fertilizer dose differences were well marked (ANOVA, F=123.48, $P < 0.05$) for various nutrient parameters of water and sediment. The concentrations of ammonium-N (0.055 - 2.258 mg/l), nitrite-N (0.029 - 0.477 mg/l), nitrate-N (0.02 - 0.364 mg/l), orthophosphate (0.055 - 0.992 mg/l) and organic carbon of water (0.97 and 11.6%) registered a gradual rising trend with application of increasing doses of fertilizers (Table 1). All the nutrient parameters of water (Organic-C, ammonium-N, nitrite-N, nitrate-N, orthophosphate and soluble reactive phosphate) increased over the time.

The total phosphate content of surface sediment also exhibited a dose dependent function ranging from 0.115 to 2.77 mg/l among all the fertilizer doses employed (Table 1). Likewise, total phosphate of sediment revealed a gradual increasing trend with time.

Fertilizer mineralization efficiency (FME)

The values of FME for orthophosphate tended to increase as the fertilizer doses increased. The values ranged from 24 to 989 % (Figure 2).

Plankton population and primary productivity

The temporal variability in the counts of plankton (2 - 26 x 10^4/l) was in parallel with that of gross primary productivity of phytoplankton (22 - 402.87 mg C/m^3/h). Both the parameters showed maximum and minimum values when the applied dose of fertilizer was D_{192} and D_{12}, respectively (Table 2). There was gradual temporal rise in the values of both plankton and primary productivity in the low doses (D_{12} – D_{192}), but a declining trend in the higher doses (D_{384} – D_{768}).

Fish growth, survival and yield

The average weight of fish ranged from 10 to 45

Table 1. Mean values (± S.E.) of water and sediment quality in different doses of mixed fertilizers employed. Same script among treatments (rows) revealed lack of significant difference

Parameters	Fertilizer Doses							
	Control	D_{12}	D_{24}	D_{48}	D_{96}	D_{192}	D_{384}	D_{768}
Temperature (°C)	29.5±1.5	29.8±1.5	29.7±0.4	30 ± 0.3	28.9 ± 0.2	29.5± 0.3	29.5± 0.3	29 ± 0.6
pH	7.79±0.002	7.8±0.003	7.84±0.001	7.73±0.005	7.71±0.003	7.75±0.001	7.68±0.004	7.59±0.002
DO (mg/l)	$6.1^{G}\pm0.45$	$7.6^{F}\pm14$	$9.02^{E}\pm 6$	$9.7^{D}\pm2.2$	$12.15^{B}\pm2.4$	$18.8^{A}\pm4$	$11.1^{C}\pm1.52$	$9.6^{DE}\pm3$
COD (mg/l)	$24.47^{H}\pm 5.5$	$44.15^{G}\pm12$	$53.56^{F}\pm 5$	$90.71^{E}\pm5$	$176.28^{D}\pm9$	$270^{C}\pm12$	$376^{B}\pm17$	$504.3^{A}\pm23$
NH_4-N (mg/l)	$0.094^{H}\pm0.005$	$0.162^{G}\pm0.01$	$0.266^{F}\pm0.05$	$0.451^{E}\pm0.03$	$0.566^{D}\pm0.04$	$0.874^{C}\pm0.08$	$1.385^{B}\pm0.06$	$1.804^{A}\pm0.04$
NO_2-N (mg/l)	$0.05^{H}\pm0.002$	$0.089^{G}\pm0.03$	$0.133^{F}\pm0.04$	$0.213^{E}\pm008$	$0.241^{D}\pm0.028$	$0.281^{C}\pm0.01$	$0.340^{B}\pm0.05$	$0.395^{A}\pm0.04$
NO_3-N (mg/l)	$0.046^{H}\pm0.001$	$0.063^{G}\pm0.05$	$0.102^{F}\pm0.01$	$0.158^{E}\pm0.06$	$0.169^{D}\pm0.025$	$0.180^{C}\pm0.04$	$0.234^{B}\pm0.04$	$0.266^{A}\pm0.046$
PO_4-P (mg/l)	$0.077^{D}\pm0.012$	$0.096^{D}\pm0.01$	$0.141^{D}\pm0.04$	$0.182^{CD}\pm0.02$	$0.328^{C}\pm0.022$	$0.623^{B}\pm0.07$	$0.752^{AB}\pm0.1$	$0.839^{A}\pm0.09$
Organic C (%)	$1.67^{G}\pm0.15$	$3.13^{F}\pm0.23$	$4.47^{E}\pm0.5$	$5.72^{D}\pm0.25$	$6.015^{D}\pm0.35$	$6.75^{C}\pm0.6$	$8.54^{B}\pm1$	$10.28^{A}\pm1.5$
Total-P content of sediment (mg/l)	$0.183^{F}\pm 0.002$	$0.287^{F}\pm0.02$	$0.414^{EF}\pm0.024$	$0.57^{DF}\pm0.07$	$0.768^{D}\pm 0.07$	$1.064^{C}\pm0.05$	$1.423^{B}\pm0.1$	$2.066^{A}\pm0.15$
Fertilizer mineralization efficiency (FME) (%)	-	$24^{G}\pm 11$	$83^{F}\pm8.5$	$136^{E}\pm5$	$325^{D}\pm3.5$	$709^{C}\pm1.2$	$876^{B}\pm1.1$	$989^{A}\pm0.54$

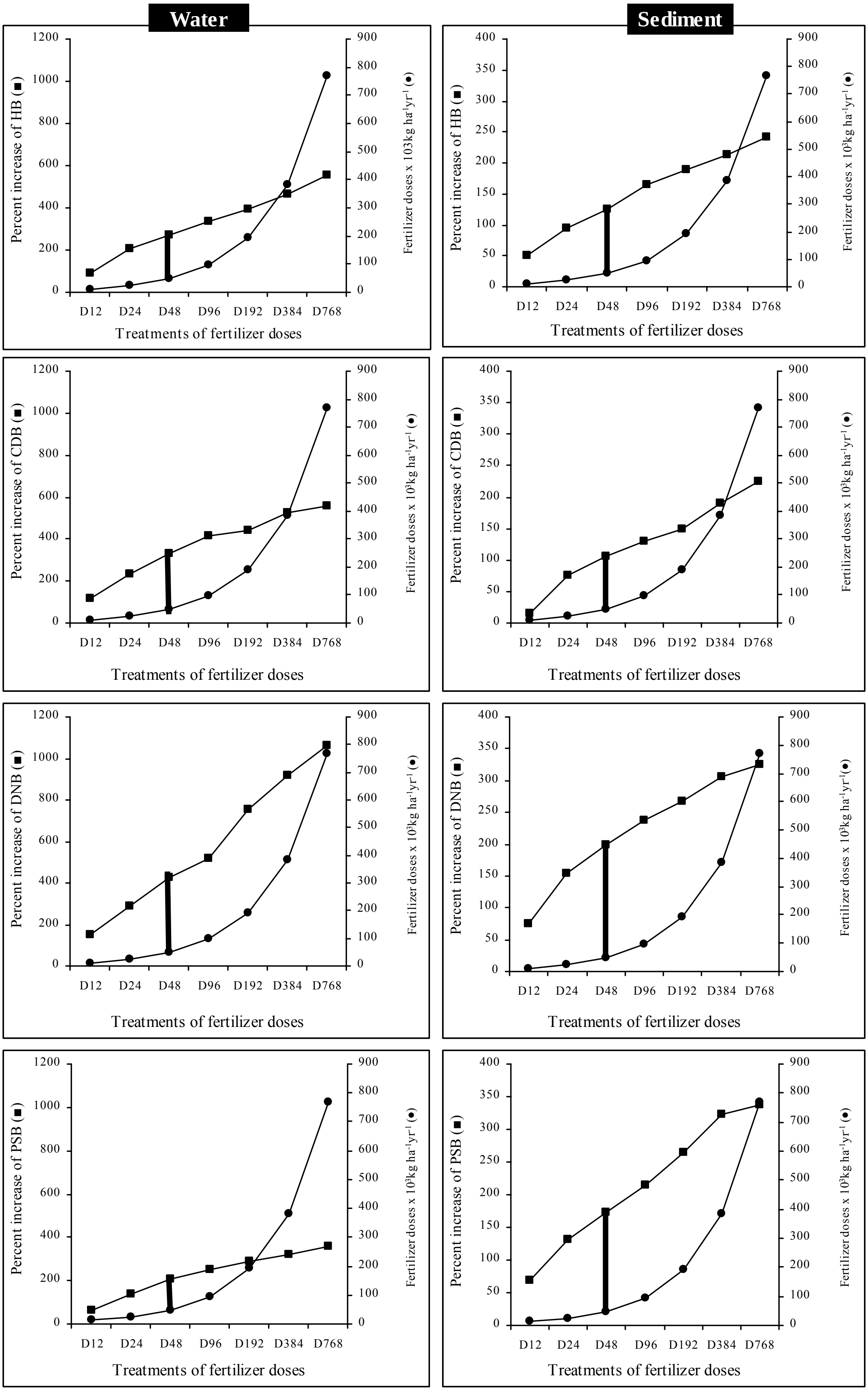


Figure 1. Percent increase of biogeochemical cycling bacterial (HB, PSB, CDB and DNB) population with increasing fertilizer doses in water and sediment. Shaded part depicts the maximum percent increase of biogeochemical cycling bacterial population.

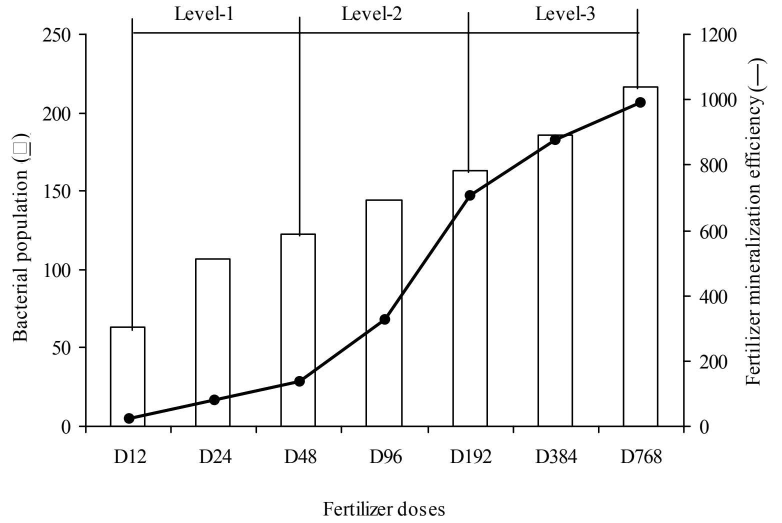


Figure 2. Relationship of heterotrophic bacteria (HB) and fertilizer mineralization efficiency (FME) in different doses of fertilizers employed.

Table 2. Plankton population, primary productivity and Growth criteria of carps in different doses of mixed fertilizers employed. Same script among treatments (rows) revealed lack of significant difference

Parameters	Fertilizer Doses							
	Control	D_{12}	D_{24}	D_{48}	D_{96}	D_{192}	D_{384}	D_{768}
Plankton population (x 10^4/l)	2.5	5	8	14	17.5	21	13	10
Primary productivity (mg C/m^3/h)	29.5	49.1	68.6	88	141	327.4	116	68.3
Final weight of fish (g)	6.8-10.25	9.5-14.25	14-24	20.3-32	24.5-45	16-21.6	0	0
Daily growth rate (g/d)	0.046-0.085	0.079-0.118	0.12-0.21	0.197-0.36	0.196-0.316	0.126-0.172	0	0
Survival (%)	100	100	100	100	100	100	0	0
Fish yield (g/tank)	$124.5^F\pm7.5$	$186^E\pm11$	$264^D\pm8.2$	$369^B\pm9$	$465^A\pm13$	$290^C\pm19$	0	0

g in catla, 6.6 to 28.66 g in bata and 6.7 to 24.53 g in common carp (Table 2). Daily growth rate of carps was maximum (0.197 - 0.36 g/d) in D_{48} and minimum (0.079 to 0.118 g/d) in D_{12}. Survival of fish varied markedly and ranged from 0 to 100% in different treatments. There was no fish mortality when the fertilizer dose remained between D_{12} and D_{192}, but cent percent mortality was encountered beyond that level (Table 2).

The fish yield tended to rise as a direct function of the fertilizer dose till the dose of D_{96} (465 g/tank), further rise in fertilizer dose (D_{192} - D_{768}) resulted in as much as 37% decline in fish yield which was up to 100% in the treatments D_{384} and D_{768} (Table 2).

Discussion

Results obtained from the study clearly revealed that different groups of bacterial population increased with fertilizer doses (HB 50 – 557%, CDB 16 – 560%, DNB 73 – 993% and PSB 62 – 358%). The relationship between the rate of increase of bacterial population and fertilizer dose implies that the biogeochemical cycling bacterial populations were more responsive to application of quantitatively different doses of fertilizer (Figure 1). Growth rate of biogeochemical cycling bacterial populations was higher in lower doses of fertilizer than the higher doses. In response to increasing fertilizer doses, the favourable and maximum growth rate of HB, CDB, DNB and PSB population was found in the treatment received with fertilizer dose 48,000 kg/ha/yr (Figure 1), furthermore excessive application of fertilizer doses (D_{96} - D_{768}) was not microbiologically more effective. Goldman *et al.* (1987) suggested that bacterial growth efficiency decreases with increasing C : N and C : P ratio in the substrate. From this study, it was evident that the different biogeochemical cycling bacteria contribute differently in the functional response of treatments with various fertilizer doses (Figure 3). Of the four bacterial population the maximum contribution (water 62 – 216 x 10^3/ml and sediment 86 – 195 x 10^4/ml) was found in HB followed by CDB (water 5.4 – 17.5 x 10^3/ml and sediment 6.2 – 17.1 x 10^4/ml), DNB (water 3.6 – 16.4 x 10^3/ml and sediment 5.6 – 13.6 x 10^4/ml) and PSB (water 0.39 – 1.1 x 10^3/ml and sediment 0.43 – 1.14 x 10^4/ml) in all doses of fertilizer. From this result it is obvious that the HB poses a major role to decompose the organic matter.

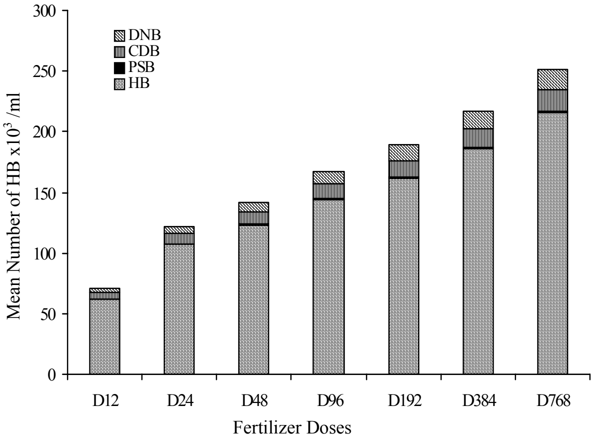
Critical appraisal of data of bacterial density and fertilizer mineralization efficiency clearly revealed three levels of responses to fertilizer doses applied (Figure 2). Bacterial density and rate of fertilizer mineralization efficiency was poor under level 1 (D_{12} to D_{48}), moderate bacterial density and highest rate of fertilizer mineralization efficiency under level 2 (>D_{48} to D_{192}) and highest bacterial density but slow rate of fertilizer mineralization efficiency under level 3 (>D_{192} to D_{768}).

The HB population showed a strong correlation with ammonium-N, orthophosphate and organic carbon in all doses of fertilizer conducted in tanks. It is apparent that HB growth induction by these nutrients is definitive. In fish pond, organic carbon, inorganic nitrogen, water temperature and phosphorus are the important attributes causing seasonal changes of DNB population (Jana and Patel, 1985).

Wolny (1967) observed that the N/P ratio favouring high productions in fish ponds varied between 4 and 8. Since there was gradual rise of N/P ratio from 2.46 to 4.52 till the fertilizer dose D_{48} (48,000 kg/ha/yr) then decreased, it is suggested that availability of phosphate was high at the fertilizer doses 48,000 kg/ha/yr due to PSB population. According to Jana *et al.* (2001), N/P ratios between 2 and 6.7 were favourable for highest density of PSB population. Ghosh and Chattopadhyay (2005) proposed that the population of denitrifying and phosphate solubilizing bacteria decreased with increasing C/N and N/P ratio.

Maximal productivity and greater abundance of plankton were found in the fertilizer dose D_{192} when HB population was 163 x 10^3/ml. The relationship between bacterial population, primary productivity and fish growth clearly indicated that though the

(a) Water



(b) Sediment

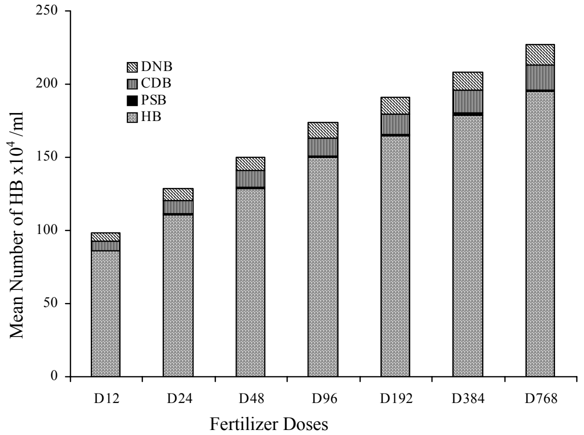


Figure 3. Contribution by four biogeochemical cycling bacteria (HB, PSB, CDB and DNB) in total growth of bacterial population occurred in (a) water and (b) sediment in different doses of fertilizers employed.

bacterial load increased with fertilizer doses, the productivity and fish growth were not increased with the fertilizer doses. Therefore, it can be concluded that a fertilizer dose 48,000 kg/ha/yr is required to manipulate the optimum bacterial population and productivity of the aquaculture pond and application of excessive doses of fertilizer causes production cost and environmental pollution.

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