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ORIGINAL ARTICLE

Bioremediation of Farm Ponds for Improving Water Quality and Fish Productivity in Bastar Plateau

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ABSTRACT
This study was conducted with two ponds, one was treated with Nitrosomonas and Nitrobacter nitrifying bacterial inoculants and another pond was kept as control. Dynamics of physico-chemical parameters of water, nitrifying bacterial loads and fish yields were examined. Bioremediation of fish pond with nitrifying bacterial inoculants decreased the concentrations of ammonium nitrogen, nitrite nitrogen and phosphates and increased the dissolved oxygen, nitrate nitrogen and nitrifying bacterial loads as compared to control. Nitrifying bacterial loads were higher throughout the culture period in treated pond than control. Performance of fish was recorded higher in bio-remediated pond (29.22 t/ha) than in control pond (25.41t/ha). Nitrifying bacterial inoculants are found useful for the better water quality and higher fish production in ponds.
Keywords: Bioremediation, *nitrifying bacteria*, fish pond, pond water quality.

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INTRODUCTION

Chhattisgarh is blessed with water resources in the form of reservoirs (83,873 ha), ponds (70, 893 ha) and rivers (3,573 km). There are 2 to 3 multi-purpose ponds in every village totaling to 53,965 in Chhattisgarh. Average density of pond per village is estimated to be 2.2 numbers [15]. Chhattisgarh is among top six states in the production of fish in the country. About 2 lakh 84 thousand metric ton fish was produced in the year 2013-14 in various irrigation waterways and fish tanks. The main species of fish production are *Rohu, Katla and Mrigal* in the state [3]. Poor water quality is one of the important constraints to aquaculture system and thereby affects fish productivity and socio-economic status of farmers.

Fish live, breed and grow in water. They are wholly dependent on water where they live. For fish, water quality is therefore the most important factor affecting their health and performance. In aquaculture ponds, the quality of water during the culture period is deteriorate mainly due to the accumulation of metabolic wastes, decomposition of unutilized feed and decay of biotic materials. Beneficial bacteria directly uptake or decompose the organic matter or toxic material in the water, thus improving the water quality. The use of beneficial microbes which control pathogens through a variety of mechanisms is viewed as an alternative to antibiotics and become a major field in the development of aquaculture. In recent years, there is a great interest in the use of beneficial bacteria in aquaculture to enhance mineralization of organic matter to improve water quality, inhibit pathogens and promote the growth of farmed fish [17, 13, 14, 8, 2, 4, 10, 16, 6, 9]. Looking to the beneficial effects of bioremediation of fish ponds with some beneficial bacteria, the present study was undertaken to study the effect of microbial cultures on water quality and fish yield in ponds of Bastar region of Chhattisgarh and compared the results with untreated pond.

MATERIAL AND METHODS

The experiment was conducted with two farm ponds at Tahkapal village; block Tokapal in Bastar district of Chhattisgarh state in India. The farm ponds were stocked with white carp, *mrigal* (*Cirrhinus mrigala*) and Indian major carp, catla (*Catla catla)* and rohu (*Labeo rohita*). The fish were fed with rice bran, groundnut oil cake and FYM at the rate of 2% body weight of fish per day. Farm yard manure, poultry manure, compost and inorganic fertilizer, superphosphate was applied, prior and after stocking of fish, in both the ponds. One pond was treated with nitrifying bacterial cultures keeping another pond as control. The study was carried out for a culture period from July, 2014 to June, 2015. Physico-chemical parameters of water, nitrifying bacterial loads, and fish yield were recorded. Two nitrifying bacterial cultures *Nitrosomonas* species @ 2 kg ha^{-1} and *Nitrobacter* species @1 kg ha^{-1} respectively were used in treated pond.

Water samples were collected in morning hours at monthly intervals in well cleaned, dried and sterile bottles for analysis of physico-chemical parameters and beneficial bacterial loads of water by following the methods suggested in Golterman and Clymo [7], Wetzel and Likens [18] and APHA [1]. The isolation and enumeration of beneficial bacterial species in the ponds was carried out. The observations were made in triplicates.

RESULTS AND DISCUSSION

In aquaculture ponds, the quality of water during the culture period is deteriorate mainly due to the accumulation of metabolic wastes, decomposition of unutilized feed and decay of biotic materials. Nitrifying bacteria directly uptake or decompose the organic matter or toxic material in the water, thus improving the water quality. In the present study, the effect of bioremediation of farm pond with *Nitrosomonas* and *Nitrobactor* bacterial inoculants were tested in terms of changes in physico-chemical properties of pond water and nitrifying bacterial loads during different months, their correlations and fish yield.

The levels of dissolved oxygen (Table 1) were ranged between 4.2-7.7 ppm with a mean value of 5.3 ppm in treated pond whereas in case of control the range of dissolved oxygen was 3.3-6.1 ppm with a mean value of 3.3 ppm. The levels of dissolved oxygen (Figure 1) increased from the start of rainy season to the month of December- January in both the ponds. However, the levels were higher in treated pond throughout the culture period as compared to control. The dissolved oxygen (Table 2 and 3)was significantly and negatively correlated with phosphate concentration at 0.05 α level and positively correlated with Nitrite-N at 0.01 α level in both treated and control pond.

The pH (Table 1) of pond water treated with nitrifying bacteria was found in the range of 7.30-7.92 with a mean of 7.61 whereas it was ranged between 7.34-7.96 with a mean value of 7.62 in control pond. The pH varied among the months during the year (Figure 2). No significant relationship (Table 2) was observed with pH and other physicochemical properties of water analyzed in case of treated pond however, in case of control pond, pH was significantly and positively correlated with nitrite-N (Table 3). The rainfall brings down the pH of water resulting in slow mineralisation [11]. The largest fish crops are usually produced in water, which are just on the alkaline side of neutrality between pH 7-8 [12]. The limit above or below pH 4.8 and 10.8 are harmful for fish.

The levels of ammonium-N (Table 1) were ranged between 0.19-0.48 ppm with a mean value of 0.31 ppm in treated pond whereas in case of control the range of ammonium-N was 0.29-0.62 ppm with a mean value of 0.43 ppm. The levels of ammonium-N (Figure 6) increased from the start of rainy season to the month of April-May in both the ponds. However the levels were lower in treated pond throughout the culture period as compared to control. The ammonium-N (Table 2 and 3) was significantly and positively correlated with nitrobactor load in treated pond whereas no such effect was seen in case of control pond.

The levels of nitrite-N (Table 1) were ranged between 0.01-0.05 ppm with a mean value of 0.03 ppm in treated pond whereas in case of control the range of nitrite-N was 0.03-0.08 ppm with a mean value of 0.05 ppm. The levels of nitrite-N (Figure 4) increased from the start of rainy season to the month of January in both the ponds. However the levels were lower in treated pond throughout the culture period as compared to control. The nitrite-N level (Table 2 and 3) was significantly and positively correlated with nitrosomonas load in treated pond whereas no significant correlation was found in control pond.

The levels of nitrate -N (Table 1) were ranged between 0.32-0.63 ppm with a mean value of 0.47 ppm in treated pond whereas in case of control the range of nitrate -N was 0.24-0.51 ppm with a mean value of 0.37 ppm. The levels of nitrate -N (Figure 5) decreased from the start of rainy season to the month of April in both the ponds. However the levels were higher in treated pond throughout the culture period as compared to control. The nitrate-N (Table 2 and 3) concentration was significantly and negatively correlated with ammonium-N and nitrobactor load in both the ponds.

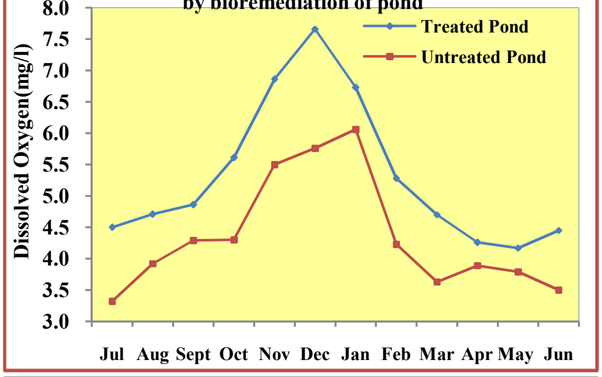

Fig. 1. Dynamics of dissolved oxygen as influenced by bioremediation of pond

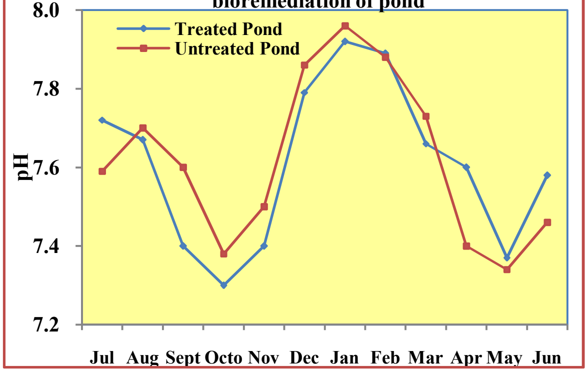

Fig. 2. Dynamics of pH as influenced by bioremediation of pond

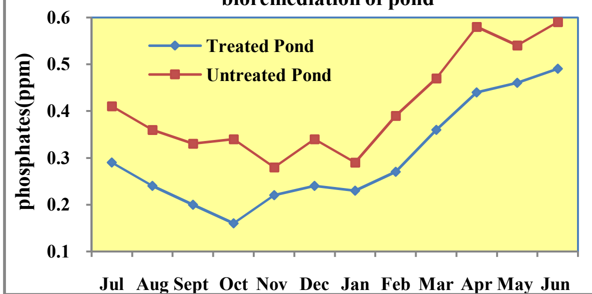

Fig. 3. Dynamics of Phosphate as influenced by bioremediation of pond

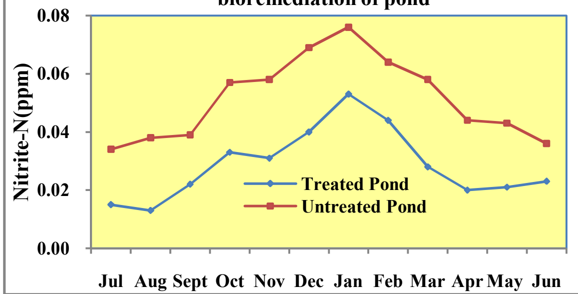

Fig. 4. Dynamics of Nitrite-N as influenced by bioremediation of pond

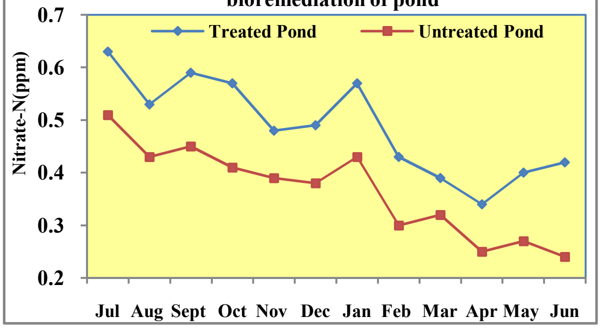

Fig. 5. Dynamics of Nitrate-N as influenced by bioremediation of pond

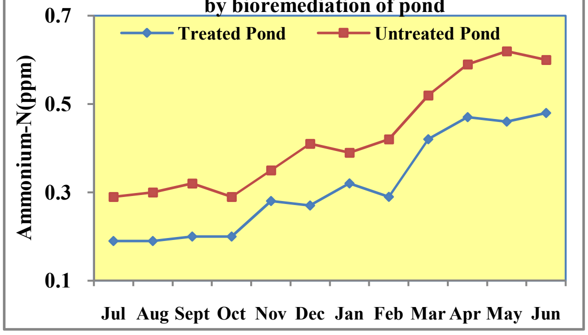

Fig. 6. Dynamics of Ammonium-N as influenced by bioremediation of pond

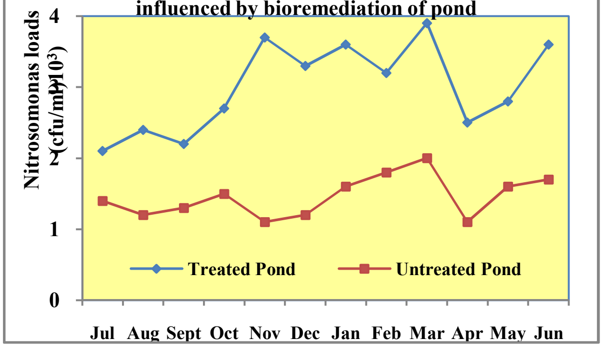

Fig. 7. Dynamics of Nitrosomonas load as influenced by bioremediation of pond

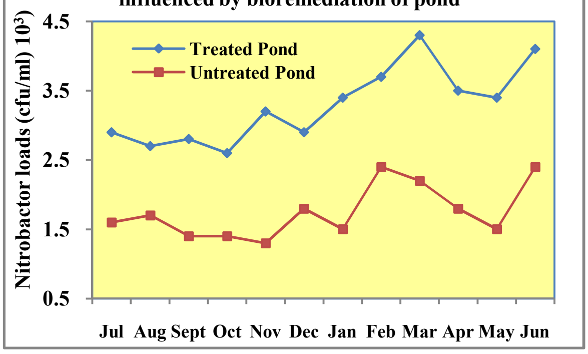

Fig. 8. Dynamics of Nitrobactor load as influenced by bioremediation of pond

The nutrients, nitrate-N, nitrite-N, and ammonia-N in the pond water showed varied distribution which might be due to biological or chemical reactions or combination of these two. In fish ponds, mineralization of fertilizers, feed wastes and excreta often increases the ammonia concentration, which is harmful to fish above 0.1 mg/l. Hence it is a critical water quality parameter to be maintained at optimal level in fish ponds. The nitrogen cycle involves the oxidation of ammonia to nitrite by bacteria of the genus *Nitrosomonas* and the subsequent oxidation of the nitrite to nitrate by *Nitrobacter*. Inputs of ammonia cannot be eliminated from the water body. However, it can be converted to non-toxic nitrate by nitrifying bacteria which can be accomplished by means of microbial treatment. The levels of ammonia and nitrites were relatively low in treated than in control pond. This might be because of the use of nitrifying bacteria in the treated pond. As these bacteria are known to convert ammonia to nitrite and then to nitrate, low levels of ammonia and nitrite observed in treated pond as compared to control can be supported. The oxidation of various forms of inorganic nitrogen in the well oxygenated surface water might have resulted in the increased concentration of nitrates.

The levels of phosphate (Table 1) were ranged between 0.16-0.49 ppm with a mean value of 0.30 ppm in treated pond whereas in case of control the range of phosphate was 0.28-0.59 ppm with a mean value of 0.41 ppm. The levels of phosphate (Figure 3) decreased during rainy period thereafter increased in both the ponds. However the levels were lower in treated pond throughout the culture period as compared to control. Phosphate concentration (Table 2 and 3) was significantly and negatively correlated with nitrate-N at 0.01 α level and positively correlated with ammonium-N at 0.01 α level in both the ponds and positively correlated with nitrobactor load at 0.05 α level in treated pond.

Table 1: Effect of bioremediation of pond on physico-chemical properties and nitrifying bacterial loads of water.

Physicochemical properties	Treated Pond		Untreated pond	
	Mean ± SD	Range	Mean ± SD	Range
Dissolved oxygen (ppm)	5.32±1.16	4.17-7.66	4.35±0.92	3.32-6.06
pH	7.61±0.21	7.30-7.92	7.62±0.21	7.34-7.96
Ammonia-N (ppm)	0.31±0.12	0.19-0.48	0.43±0.13	0.29-0.62
Nitrite-N (ppm)	0.03±0.01	0.01-0.05	0.05±0.01	0.03-0.08
Nitrate-N (ppm)	0.47±0.11	0.32-0.63	0.37±0.09	0.24-0.51
Phosphate (ppm)	0.30±0.11	0.16-0.49	0.41±0.11	0.28-0.59
Nitrobactor loads (cfu/ml) 10^3)	3.29±0.55	2.60-4.30	1.75±0.39	1.30-2.40
Nitrosomonas loads (cfu/ml) 10^3)	3.00±0.63	2.10-3.90	1.46±0.29	1.10-2.00

Table: 2. Correlation coefficient between physico-chemical parameters and beneficial microbial loads of treated pond water during different months

-	DO	pH	Phosphate	Nitrite	Nitrate	NH_4-N	Nitrosomonas
pH	0.261	1					
Phosphate	-0.601*	-0.028	1				
Nitrite-N	0.725**	0.474	-0.370	1			
Nitrate-N	0.348	0.079	-0.849**	0.111	1		
Ammonium-N	-0.337	-0.014	0.896**	-0.025	-0.914**	1	
Nitrosomonas	0.460	0.249	0.146	0.599*	-0.418	0.455	1
Nitrobactor	-0.271	0.269	0.688*	0.154	-0.745**	0.807**	0.672*

** Significant at 0.01 α level, *Significant at 0.05 α level

Table: 3. Correlation coefficient between physico-chemical parameters of control pond water during different months

-	DO	pH	Phosphate	Nitrite	Nitrate	NH_4-N	Nitrosomonas
pH	0.507	1					
Phosphate	-0.694*	-0.509	1				
Nitrite	0.797**	0.632*	-0.514	1			
Nitrate	0.260	0.303	-0.771**	0.021	1		
Ammonium	-0.285	-0.301	0.853**	-0.104	-0.912**	1	
Nitrosomonas	-0.309	0.227	0.277	0.197	-0.321	0.341	1
Nitrobactor	-0.383	0.275	0.529	0.005	-0.600*	0.493	0.602*

** Significant at 0.01 α level, *Significant at 0.05 α level

Though phosphorus is considered as the most important critical factor in the maintenance of pond fertility (Boyd, 1982), high levels lead to eutrophication and water deterioration. It was observed that orthophosphate concentrations were maintained at relatively low levels in nitrifying bacteria treated ponds than in control ponds. Rao [13] reported that the beneficial bacteria utilize phosphate for their body metabolic activities and thus diminish this nutrient in pond waters.

Nitrosomonas loads (Table 1) ranged from 2.10 $\times10^3$ to 3.90 $\times10^3$ with a mean of 3.00×10^3 cfu/ml in treated pond, and 1.10×10^3 to 2.00×10^3 with a mean of 1.46×10^3 cfu/ml in control pond, whereas, *Nitrobacter* loads in treated and untreated pond ranged from 2.60×10^3 to 4.30×10^3 and 1.30×10^3 to 2.40×10^3 with mean values of 3.29×10^3 and 1.75×10^3 cfu/ml. The dynamics of relative loads of *Nitrosomonas* and *Nitrobacter* in two ponds are shown in Figure 7 & 8. *Nitrosomonas* and *Nitrobacter* loads are relatively higher in treated pond than in control pond. The abundance of nitrifying bacteria in treated pond is seems the manifestation of treatment. Nitrifying bacterial loads were also observed to be

gradually increasing by the end of the culture period. As these bacteria are known to convert ammonia to nitrite and then to nitrate, low levels of ammonia and nitrite observed (Table 2) in treated pond as compared to control. The use of nitrifying bacterial inoculants resulted in maintaining pond water quality in terms of dissolved oxygen, ammonia, nitrite, nitrate and phosphate concentrations. The nitrosomonas load (Table 2 and 3) was significantly and positively correlated with nitrobactor load. The nitrobactor load was significantly and positively correlated with phosphate and ammonium-N concentration in treated pond only and negatively correlated with nitrate-N concentration in both the ponds.
The number of fish stocked and harvested with their biomass and gross and net yields are shown in Table 4. The gross yields obtained in treated and control pond is 31.00 and 27.20 t/ha. However, net yields in the ponds are 29.22 and 25.41 t/ha, respectively. The fish yield indicates that the fish grow well in nitrifying bacteria treated pond than in control pond might be due to improved water quality in the treated pond (Table 1).

Table 4: Effect of bioremediation of ponds on performance of fish

Parameters	Treated Pond	Control Pond
Fish stocked (no./ha)	18,800	18,800
Total Biomass of fish Stocked (kg)	1786	1786
Fish harvested (no./ha)	18110	17990
Gross Yield (t/ha)	31.00	27.20
Net Yield (t/ha)	29.22	25.41

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