Physico-Chemical Factors and Bacteria in Fish Ponds

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Abstract

Analyses of pond water and mud samples show that nitrifying bacteria (including ammonifying bacteria, nitrite bacteria, nitrobacteria and denitrifying bacteria) are in general closely correlated with various physico-chemical factors; ammonifying bacteria are mainly correlated with dissolved oxygen; denitrifying bacteria are inversely correlated with phosphorus; nitrite bacteria are closely correlated with nitrites, nitrobacteria are inversely correlated with ammoniac nitrogen. The nitrifying bacteria are more closely correlated with heterotrophic bacteria. Nitrobacteria are inversely correlated with anaerobic heterotrophic bacteria. The correlation is quite weak between all the nitrite bacteria which indicates that the nitrite bacteria have a controlling and regulating function in water quality and there is no interdependence as each plays a role of its own. The paper also discusses how the superficial soil (pond mud down to 3.5 cm deep) and different layers of the mud affect the biomass of bacteria. The study shows that the top superficial layer (down to 1.5 cm deep) is the major area for decomposing and converting organic matter.

Introduction

The nitrogen cycling process in a pond is mainly carried out by ammonifying bacteria, nitrobacteria, nitrifying bacteria and denitrifying bacteria (generally called nitrite bacteria). These nitrifying bacteria play a vital role in water quality control. Water quality is an important indicator in appraising the eutrophic situation, primary productivity and fish yield potential. The relationship between bacteria and the water environment has received the attention of researchers, but the studies undertaken have been mostly limited to the relationship between the aerobic heterotrophic bacteria or total bacteria and the water environment (Guo et al. 1988; Fang et al. 1989; Zhang et al. 1989; Liu et al. 1992(a)). There is not much information on the relationship between anaerobic bacteria and major physico-chemical factors, particularly the nitrifying bacteria affecting water quality.

Studies were undertaken to assess the relationship between bacteria and nitrogen in fishponds. The results of these studies are presented in this paper. These studies also analyzed the topsoil of the pond mud (Fang et al. 1993). Other studies have been made to find out the activation of the pond mud. The results showed that the superficial pond mud down to 1.5 cm deep is where most of the decomposition and conversion of nitrogenous organic matter takes place. Use of modern microbiology to increase the activation of pond mud and improve water quality is a new approach to reducing outbreaks of diseases and sustaining the development of aquaculture.

Materials and Methods

The study was undertaken in an intensive polyculture pond of 0.48 ha with a water depth of 2.5 m, situated in No. 1 Fishery Team, Helei Village in Wuxi, China. The pond was stocked with black carp (*Mylopharyngodon piceus*), grass carp (*Ctenopharyngodon idella*), Wuchang fish (*Megalobrama amblycephala*), common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*) and crucian carp (*Carassius carassius*). They were fed from March to October with grass (rye grass, land grass and cabbage), shellfish (snails) and commercial feeds (wheat, rice dregs, bean cakes and pellet concentrates).

Soil and water sampling was undertaken between April and October. The samples were collected at three spots in the pond and mixed together. The mud was collected in cylindrical glass bottles (5 cm in diameter) at two levels: 0-1.5 cm depth (layer 1) and 1.5-3.5 cm depth (layer 2). Samples of 50 g each collected from the three sites were mixed and diluted in 450 ml of germ-free distilled water for analysis.

Table 1. Bacteria biomass in the first layer of pond mud.

Date	*Aerobic bacteria x10^5 (ind/ml)	*Anaerobic bacteria x10^5* (ind/ml)	Aerobic bacteria x10^5 (ind/ml)	Anaerobic bacteria x10^5 (ind/ml)	Ammonifying bacteria x10^5 (ind/ml)	Nitrobacteria x10 (ind/ml)	Nitrifying bacteria x10 (ind/ml)	Denitrifying bacteria x10^5 (ind/ml)
4/9			2.4		2.5			0.25
4/23	0.12		1.9	20.00	2.5			0.95
5/7	0.75	0.78	53	110.00	1.5			4.50
5/21	8.70	8.40	140	5700.00	1.5			4.50
6/4	0.19	0.29	60	110.00	25.0	2	2.5	25.00
6/18	0.16	0.24	16	15.00	11.0	25	0	2.50
7/30	0.90	0.50	9.6	0.36	2.5	250	40	0.15
8/13	0.19	0.10	28	28.00	11.0	1.5	12	2.50
8/27	0.08	0.17	26	19.00	4.5	25	2.5	2.50
9/10	0.07	0.15	9.5	7.90	25.0	2.5	2.5	1.50
9/24	0.01	0.03	13	10.00	4.5	25	45	2.50
10/8	0.015	68.00	78	65.00	25.0	25	2.5	11.00
10/23	0.19	0.17	24	24.00	110.0	25	0	0.95

*indicates the bacteria in the water body, the rest from the first layer of the mud.

The plate method was used for the determination of aerobic and anaerobic heterotrophic bacteria and MPN (Most Probable Numbers) method was used for ammonifying bacteria, nitrifying bacteria, nitrobacteria and denitrifying bacteria. A CO_2 incubator was used for the culture of anaerobic and denitrifying bacteria and a common incubator for ammonifying bacteria.

The total nitrogen and ammoniac nitrogen were determined by colorimetric analysis; the nitrate nitrogen was determined by the phenoldi-acid method; nitro-nitrogen by the a-Naphthylamine-p-Aminobenzene sulfonic acid anhydrous colorimetric method; total phosphorus and dissolved phosphates were determined by the molybdenum blue colorimetric method and the dissolved oxygen content was determined by a YS 157 oxygen meter.

Results

Aerobic heterotrophic bacteria in the pond water fluctuated between 0.01-8.7 x 10^5 ind/ml, the anaerobic heterotrophic bacteria fluctuated between 0.03-68 x 10^5 ind/ml, which is slightly higher than the former (mean value is about 2 times greater) (Table 1). The result seems to indicate that even if the dissolved oxygen is sufficient in the water, apart from the aerobic bacteria, there might be also anaerobic heterotrophic bacteria. In the reduced oxygen condition, the anaerobic bacteria maintain their existence and balance through denitrifying bacteria and denitrification (Fang et al. 1993; Molongoski and Clug 1980).

Bacteria Biomass in Pond Mud

The aerobic heterotrophic bacteria in the first layer of pond soil fluctuated between 1.9-140 x 10^5 ind/ml and the anaerobic heterotrophic bacteria between 0.4-5 700 x 10^5 ind/ml. Ammonifying bacteria fluctuated between 1.5-110 x10^5 ind/ml, denitrifying bacteria between 0.15-25 x 10^5 ind/ml; the nitrifying bacteria between 0-450 ind/ml; and the nitrobacteria between 15 -2500 ind/ml (Table 1).

Table 2 shows that the aerobic heterotrophic bacteria in the second layer of pond soil fluctuated between 0.6-390 x 10^5 ind/ml, anaerobic heterotrophic bacteria 0.7-14 x 10^5 ind/ml. Ammonifying bacteria fluctuated between 0.025-4.5 x 10^5 ind/ml, denitrifying bacteria between 0.25-7.0 x 10^4 ind/ml; nitrobacteria between 0-250 ind/ml, and nitrifying bacteria between 0-45 ind/ml.

Discussion

Decomposition of Nitrogenous Organic Matter

Pond soil is an important component in pond ecology and can absorb nitrogen and phosphorus and some undecomposed organic matter that has settled on the bottom. The nutritional elements from the soil are further released into the water body. Sun Yao et al. (1997) reported that the contact layer between pond mud surface and water is the major source of nutrition. The organic nitrogen is decomposed to NH_4^+-N which adheres to the surface of the mud before being released in the water where it continuously rises to the surface of the water and escapes to the air (Mei et al. 1995; Blackburn and Henriksen. 1985). Several scholars have agreed that the nutrients NH_4^+-N and PO_4^{3-}-P are released only from mud 0-2 cm thick (Mei et al. 1995; Blackburn and Henriksen 1985; Maeda et al. 1987). Mei et al. (1995) reported that the decomposition depth of organic carbon reaches 4 cm, the organic nitrogen can reach 4-6 cm, but major organic content is within 0-2 cm. Carignan and Flett (1981) reported that sulphuret within only 2 cm can be oxygenation-reduced before settling down. It is considered that nitrogen is first converted to ammonia-nitrogen by the ammonifying bacteria during the decomposition of nitrogenous organic matters. Then, ammonia-nitrogen is converted to nitrite by nitrobacteria. Then, nitrite is converted to nitrate by nitrifying bacteria. Finally, nitrate is converted to nitrite, N_2 and N_2O by denitrifying bacteria. There is a widespread distribution of ammonifying bacteria and denitrifying bacteria, but it is concentrated in the pond mud. The nitrifying bacteria and nitrobacteria are only available on the superficial mud. The present studies also determined the quantity of the bacteria. t-tests show that nitrite bacteria is different in biomass with the variation of the pond mud stratification, the contact between water and mud has a higher quantity of bacteria compared to the upper water levels and deeper mud. In general, the decomposition of the nitrogenous organic matters is mainly at the water-mud surface, which is also a key area of energy flow.

Nitrifying Bacteria and Physico-chemical Factors

There are many studies on the relationship between aerobic heterotrophic bacteria and water quality (Guo et al. 1988; Fang et al. 1989; Liu et al. 1992(a)**;** Liu et al. 1992(b)). However, information on the relationship between nitrite bacteria and major physico-chemical factors is limited.

Table 3 indicates that ammonifying bacteria have high correlation with dissolved oxygen content ($r=0.693$, $p<0.05$). The major role of ammonifying bacteria is to break down nitrogenous organic matter to ammoniac nitrogen. Increase in dissolved oxygen content accelerates the decomposition process of organic matters. Denitrifying bacteria have an inverse correlation with phosphorus ($r=-0.648$, $p<0.05$). Denitrifying bacteria convert the nitrate into nitrite, N_2 and N_2O. The increase of phosphorus content inhibits the activity of denitrifying bacteria and increases the nitrite in the pond. Nitrobacteria have inverse correlation with ammoniac nitrogen ($r=-0.694$, $p<0.05$). The main role of nitrobacteria is to break down ammoniac nitrogen into nitrite. Frequent feeding, aeration and fish excreta increase the level of ammoniac nitrogen. Thus, the enhancement of ammoniac nitrogen content inhibits the activity of nitrobacteria. Nitrifying bacteria have direct correlation with nitrite ($r=0.638$, $p<0.05$). The nitrifying bacteria convert the nitrite to nitrate. In general, the nitrite content is the lowest among the content of ammonia nitrogen, nitrite and nitrate in pond. Thus, the amount of nitrifying bacteria is the lowest among the ammonifying bacteria, nitrobacteria, nitrifying bacteria and denitrifying bacteria.

Theoretically speaking, the aerobic bacteria should have a direct correlation with dissolved oxygen content and an inverse correlation

Table 2. Bacteria biomass in the second layer of pond mud.

Date	Aerobic bacteria $x10^5$ (ind/ml)	Anaerobic bacteria $x10^5$ (ind/ml)	Ammonifying bacteria $x10^5$ (ind/ml)	Nitrobacteria $x10^5$ (ind/ml)	Nitrifying bacteria $x10^5$ (ind/ml)	Denitrifying bacteria x10 (ind/ml)
4/9	1.2	0.8	0.25			0.095
4/23	0.6	1.1	0.025			0.025
5/7	9.2	1.4	0.45			0.03
5/21	390		0.07			0.095
6/4	15	0.9	0.45	0.9	1.4	0.45
6/18	1.1	3.1	0.75	4.5	0	0.0095
7/30	3.5	5.1	0.45	25	4.5	0.11
8/13	4.2	3.6	2.5	4.5	0.6	0.095
8/27	13	14	1.1	0	0.4	0.45
9/10	1	0.7	0.04	2.5	2.5	0.025
9/24	7.3	6.6	1.1	25	0.4	0.15
10/8	2.5	1.4	0.45	2.5	0.4	0.025
10/23	11	8.3	8.3	3	0.4	0.7

Table 3. Correlation between (i) different bacteria; and (ii) bacteria and major physio-chemical factors in the first layer of pond soil.

Variables	Aerobic bacteria		Anaerobic bacteria		Ammonifying bacteria		Denitrifying bacteria		Nitrobacteria		Nitrifying bacteria	
	r	p	r	p	r	p	r	p	r	p	r	p
Anaerobic bacteria	0.929	0.000	-	-	-	-	-	-	-	-	-	-
Ammonifying bacteria	-0.382	0.276	-0.247	0.491	-	-	-	-	-	-	-	-
Denitrifying bacteria	0.832	0.003	0.944	0.000	-0.283	0.428	-	-	-	-	-	-
Nitrobacteria	-0.678	0.031	-0.76	0.009	0.458	0.183	-0.598	0.068	-	-	-	-
Nitrifying bacteria	-0.545	0.103	-0.596	0.069	-0.023	0.949	-0.413	0.235	0.586	0.075	-	-
Dissolved oxygen	-0.665	0.036	-0.469	0.172	0.693	0.026	-0.440	0.203	-0.369	0.294	0.146	0.688
Chlorophyll-a	0.404	0.247	0.412	0.237	-0.000	1.000	0.258	0.472	-0.421	0.226	-0.189	0.602
Available phosphorus	-0.371	0.291	-0.610	0.061	-0.249	0.488	-0.648	0.044	0.439	0.205	0.347	0.327
Ammoniac nitrogen	0.382	0.276	0.266	0.458	-0.557	0.095	0.149	0.682	-0.694	0.026	-0.448	0.194
Nitrates	-0.356	0.313	-0.184	0.612	0.238	0.508	-0.059	0.871	0.172	0.634	-.210	0.561
Nitrites	-0.452	0.189	-0.560	0.093	-0.309	0.385	-0.491	0.15	0.377	0.283	0.638	0.048

r = Correlation p = Probability-value

Table 4. t-test on bacteria from the two layers of soil.

Name	Soil layer	Times of determination	Standard deviation	Degree of freedom	P value
Ammonifying bacteria	1	13	1.328		
	2	13	1.526	24	0.001
Denitritying bacteria	1	13	1.621		
	2	13	1.144	24	0.001
Nitrobacteria	1	9	1.648		
	2	8	1.154	15	0.139
Nitrifying bacteria	1	7	1.356		
	2	7	0.997	12	0.008
Aerobic bacteria	1	13	1.063		
	2	13	1.69	24	0.009
Anaerobic bacteria	1	13	2.181		
	2	12	1.015	23	0.002

All bacteria, except nitrobacteria, in the first layer of pond soil have a significant difference ($p<0.01$) from those in the second layer of the soil.

with anaerobic bacteria. In fact aerobic bacteria are directly proportional to anaerobic bacteria ($r=0.929$, $p=0.000$) and in inverse proportion to dissolved oxygen content ($r=-0.665$, $p=0.036$). A likely explanation for this is that both aerobic and anaerobic bacteria have a double function in relation to changes in dissolved oxygen content. Because of the low oxygen content in the contact surface between the water and the soil, the heterotrophic bacteria are more suitable for anaerobic conditions. The nitrifying bacteria have weak correlation between themselves ($r<0.60$, $p>0.05$).

The contact surface between the water and the soil is dominated by the heterotrophic bacteria. The nitrifying bacteria are strongly correlated with physio-chemical factors, indicating that they have an important role in the management of water quality.

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