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Effects of bacteria on nitrogen and phosphorus release from river sediment

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Abstract

To better understand the mechanisms of eutrophication, we addressed the microbial processes that influence many key aspects of water-sediment systems. In this study, a large column experiment was conducted for 30 d. Along the column, solution samples were collected at different locations at different time. The samples were analyzed for physical, chemical, and biological properties of the sediment and overlying water. The results showed that the amount of nitrogen transforming bacteria was higher than that of phosphorous bacteria. The amount of nitrogen transforming bacteria was in the order: ammonifier > denitrifying bacteria > nitrobacteria and nitrosomonas. Principal component analysis indicated that the three main factors accounted for more than 90% overall contributions for bacterium growth, which represented nutrition, organics and oxygen, and pH and redox potential (Eh) of the environment. Corresponding to the bacteria, the concentrations of nitrogen in the system was in the order: ammonia (NH_4^+-N) > nitrate (NO_3^--N) > nitrite (NO_2^--N). The fluxes of N and P clearly showed a temporal release and adsorption processes in the water-sediment system. The large magnitude of N fluxes suggested that N might act as an important contamination source for the water quality. However, P exchange between the sediment and overlying water was less intensive during the experiment.

Key words: eutrophication; sediment; P and N transforming bacteria

Introduction

Pollution of phosphorus (P) and nitrogen (N) is well known to contribute to eutrophication problems of the surface water, such as rivers and lakes. Human activities have long-term influence on the global pollution (Peierls *et al*., 1991; Fisher and Reddy, 2001). For example, losses of P and N through surface run-off and drainage from agricultural land can impair river water quality and may pose a potential health hazard (Withers and Lord, 2002). Exterior pollutants adsorbed onto the sediment still can result in water eutrophication. Therefore, the polluted sediment may serve as an interior contaminated source (Morin and Morse, 1999; Hu *et al*., 2001; Petticrew *et al*., 2001; Kim *et al*., 2004).

Research on the interior contaminated source of sediments has received increased attention to address the eutrophication problem of surface water. Rydin (2000) performed a P release experiment by maintaining steep diffusion gradients in flow-through chambers under oxic and anoxic conditions. He pointed out that the release of P from shallow sediments depended not only on the concentration of mobile P, but also on transport processes and primarily diffusion. Phosphate adsorption and release from sediments were related to pH, oxic and anoxic conditions, and the redox potential (Gomez *et al*., 1999). Gardolinski *et al*. (2004) investigated the release of organic and inorganic phosphorus from a riverine sediment subjected to salinity conditions typical of estuarine mixing. Usui *et al*. (2001) studied the mechanisms regulating N_2O production in an estuarine sediment by comparing the change in N_2O production with those in nitrification and denitrification activities. Using formulated and natural sediments, Verrhiest *et al*. (2002) highlighted chemical and bacterial changes of the sediments under a laboratory condition. Using water and sediment from a basin, Fabiano *et al*. (2003) carried out a laboratory experiment to study the main ecological mechanisms involved in bioremediation. Their results suggested that the increase of organic matter and benthic bacterial density in the overlying water indicated a strong association between the sediments and water column processes. Through a study of depth distributions of active bacteria and bacterial activity in lake sediment, Haglund *et al*. (2003) concluded that bacterial abundance and total activity decreased with increasing sediment depth, but the viable fraction of the total bacteria was similar throughout the sediment profile. Turly and Dixon (2002) explored the relationships between nitrogen transforming bacterial numbers vs. DNA synthesis (a measure of bacterial growth), the concentration of bulk particulate organic carbon, and total nitrogen in deep-sea surficial sediments. They found statistically significant positive exponential relationships between nitrogen transforming bacterial numbers vs. 3H-thymidine incorporation

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rates (DNA synthesis), phytodetritus particulate organic carbon (%), and total nitrogen (%) from samples collected in the deep NE Atlantic.

The effects of eutrophication are well known, including algal blooms, fish kills and bad tasting water; however, the mechanisms governing the effects are poorly understood. In particular, effects of microbial processes are key to many aspects of the functioning of the water-sediment system, and commonly are inadequately addressed (Meyer-reil and Koster, 2000). The increase of concentrations of P and N in the environment and its effects on the surface water do not follow a linear or direct relationship (Pagliosa *et al*., 2005). We need to explore the complex relationship by studying the interactions of water and sediments that adsorb and release the pollutants, and chemical as well as bacterial processes in the system. In the mechanism studies of water eutrophication, limited experiments have been designed to characterize the factors that affect the exchange processes of P and N among the sediment, pore water, and the overlying water, and to quantify temporal and vertical distributions of N and P, microbial biomass, rates of nitrification and denitrification, and chemical fluxes between the sediment and overlying water.

The objectives of this research were to: (1) characterize temporal and vertical distributions of nitrogen transforming bacteria and phosphorus bacteria in a water-sediment system; (2) identify the most important factors that affect bacterium growth in the system; (3) quantify temporal and vertical distributions of N and P in the system, as well as temporal fluxes of N and P exchange between the sediment and overlying water. The study was conducted under laboratory conditions with a large water-sediment column, which permitted good control of the environmental parameters.

1 Materials and methods

Sediment samples were collected from a tide affected section of the Pearl River near the Foshan City, Guangdong Province, China. This river section was narrow and seriously contaminated by municipal and industrial wastewaters. Polluted mainly by organic contaminants, the river sediment was oily, dark, and with irritant odor. During the ebb tide period, the river sediment near banks was exposed to the air, where sediment was sampled to a depth of 20 cm using a shovel. After air-dried, the sediment was ground and sieved with a 2-mm sieve. A total of 127 kg of sediment was prepared for the experiment.

The sediment was used to conduct particle size analysis (Gee and Or, 2002) and measure pH value, cation exchange capacity (CEC) (Rhoades, 1982), organic matter (Org) (Schnitzer, 1982), total nitrogen (TN) (Bremner and Mulvaney, 1982), and total phosphorus (TP) (Olsen and Sommers, 1982).

A column experiment was set up to simulate the chemical and biological processes in the sediment. The column was composed of PVC pipes with the outer diameter of 22 cm, the inner diameter of 20 cm, and the height of 150 cm (Fig.1). At each end of the top and bottom, a ring flange

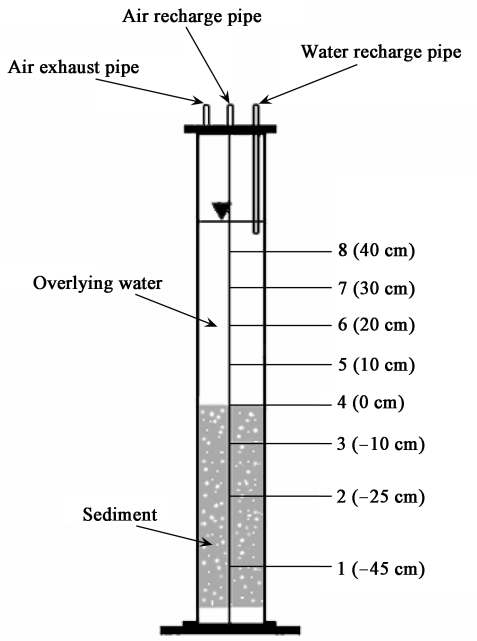

Fig. 1 Set up of column experiment. The numbers in the figure indicate the locations of suction cups.

with an air-tight washer was fixed with 8 bolts. On the top, three glass pipes (the inner diameter of 10 mm) were installed, serving as the water recharge pipe, air recharge pipe, and air exhaust pipe, respectively. Above the bottom of 10 cm, there was a plate with many tiny holes for water to go through. A valve was installed between the bottom and plate.

After filling 55 cm of sediment above the plate in the column, distilled water was recharged from the bottom valve to saturate the sediment gradually until the water depth above the water-sediment interface reached 65 cm. Suction cups were installed at locations of –45, –25, –10, 0 (at the water-sediment interface), 10, 20, 30, and 40 cm (Fig.1) to collect solution samples from the sediment and overlying water during the experiments. To improve the representativeness and quality of the mixed sediments, we waited for 7 d as a conditioning period before starting to take measurements (Suedel *et al*., 1994). Similar to Verrhiest *et al*. (2002), the monitoring of physico-chemical and biological parameters of sediment and water column was carried out over a 30-d incubation period in the experiment. The duration of the sediment monitoring was chosen according to current durations of sediment toxicity tests (SETAC, 1993). During the experiment, distilled water was added through the recharge pipe to keep a constant water level. Air of an oxygen-nitrogen mixture (with 4% of oxygen) was continuously input through the air recharge pipe at a rate of 0.5 L/min, which was conducive to minimize the disturbance of sediment. Samples of solution were collected at the locations with the suction cups daily. The amount of each sampled solution was 100 ml. The solution samples were used to analyze contents of bacteria, N, P, pH, redox potential (Eh), chemical oxygen demand (COD), and dissolved oxygen (DO) at the different locations and time. Because of the limited amount of solution sample, only PO_4^{3-}-P was analyzed using the

standard method (Olsen and Sommers, 1982), while pH, Eh, DO, NH_4^+-N, NO_3^--N, and NO_2^--N using the probe methods (Thermo Orion, USA). The methods to measure bacteria were described as follows.

Bacteria measurement included ammonifier, nitrobacteria, nitrosomonas, denitrifying bacteria, inorganic phosphobacteria, organic phosphobacteria, and heterotrophic bacteria. The MPN (most probable number) method (Woomer, 1994) was used to measure ammonifier, nitrobacteria, nitrosomonas, denitrifying bacteria. The plate count method (Zuberer, 1994) was used to measure inorganic phosphobacteria, organic phosphobacteria, and heterotrophic bacteria. The bacterial community was characterized by determination of the bacterial density in total bacteria number or colony forming units (CFU).

The medium for ammonifier was prepared by dissolving 5 g albumen gel, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.5 g K_2HPO_4 in 1000 ml deionized water at pH 7.0. After incubation at 25–30°C for 7 d, Nessler reagent was added into the medium for detection of ammonifier. The medium for nitrobacteria consisted of (in 1000 ml deionized water): 1.0 g $NaNO_3$, 0.03 g $MgSO_4{\cdot}7H_2O$, 0.75 g K_2HPO_4, 1.0 g Na_2CO_3, 0.01 g $MnSO_4{\cdot}4H_2O$, and 1.0 g $NaHPO_4$ at pH 7.0. After incubation at 25–28°C for 14 d, Griess reagent was added into the medium for measurement of nitrobacteria. The medium for nitrosomonas contained (in 1000 ml deionized water): 2.0 g $(NH_4)_2SO_4$, 0.03 g $MgSO_4{\cdot}7H_2O$, 0.75 g K_2HPO_4, 1.0 g Na_2CO_3, 0.03 g $MnSO_4{\cdot}4H_2O$, 5.0 g $CaCO_3$, and 0.25 g NaH_2PO_4 at pH 7.0. After incubation at 25–28°C for 10–14 d, Griess reagent was added into the medium to measure nitrosomonas. The medium for denitrifying bacteria was prepared by dissolving 20 g albumen gel, 1.0 g glucose, 0.75 g K_2HPO_4, 1.0 g agar, 2.0 g NaH_2PO_4, and 1.0 g KNO_3 in 1000 ml deionized water with pH 7.0. After incubation at 25–28°C for 10–14 d, Nessler reagent was added into the medium for detection of denitrifying bacteria. The medium for organic phosphobacteria consisted of (in 1000 ml deionized water): 0.5 g $(NH_4)_2SO_4$, 0.3 g $MgSO_4{\cdot}7H_2O$, 10.0 g glucose, 0.3 g NaCl, 0.03 g $MnSO_4{\cdot}4H_2O$, 5.0 g $CaCO_3$, 0.03 g $FeSO_4{\cdot}7H_2O$, 0.2 g lecithin, 16 g agar, and 0.3 g KCl with pH 7.0–7.5. For each of three different diluted solutions, after incubation at 25–30°C for 1–2 d, the plate count method was used to measure organic phosphobacteria. The medium for inorganic phosphobacteria contained (in 1000 ml deionized water): 0.5 g $(NH_4)_2SO_4$, 0.3 g $MgSO_4{\cdot}7H_2O$, 10.0 g glucose, 0.3 g NaCl, 0.03 g $MnSO_4{\cdot}4H_2O$, 10.0 g $Ca_3(PO_4)_2$, 0.03 g $FeSO_4{\cdot}7H_2O$, 16 g agar, and 0.3 g KCl with pH 7.0–7.5. For each of three different diluted solutions, after incubation at 25–30°C for 1–2 d, the plate count method was used to measure inorganic phosphobacteria. The medium for heterotrophic bacteria was prepared by dissolving 10.0 g albumen gel, 3.0 g beef extract, 5.0 g NaCl, 1.0 g Na_2CO_3, and 5–18 g agar in 1000 ml deionized water with pH 7.0–7.2. For each of 3 different diluted solutions, after incubation at 25–30°C for 1–2 d, the plate count method was used to measure heterotrophic bacteria.

The nutrients (N and P) in the sediment can be released to pore water and move into the water column through dispersion. The temporal nutrient flux between the sediment and water is calculated by (Fisher and Reddy, 2001):

$$J_{\mathrm{D}} = \frac{V(C_i - C_{i-1})}{A(t_i - t_{i-1})} \tag{1}$$

where, J_{D} is the temporal nutrient flux (mg/(m^2·d)), C_{i-1} and C_i are nutrient concentrations (mg/L) in the water column at time t_{i-1} and t_i (d), respectively, V is the water column volume (L), and A is the interface area (m^2). The flux was calculated based on concentrations at different layers in the water column. Since the fluxes came from the sediment through dispersion upward and the concentrations along the water column were different, we divided the water column into different layers. Then fluxes were calculated for the layers and the sum of the fluxes represented the flux of water column. Specifically, the water column was divided into 5 layers based on sample locations 8, 7, 6, 5, and 4 (at the interface). For example, at time t_i, using C_{i-1} and C_i measured at location 8 and Eq.(1), we calculated J_{D1} (the first layer from the water surface to the depth of 20 cm. Similarly, using concentrations measured at locations 7, 6, 5, 4 at t_{i-1} and t_i and Eq.(1), we calculated J_{D2}, J_{D3}, J_{D4}, and J_{D5}. The sum of J_{D2}, J_{D3}, J_{D4}, and J_{D5} was the flux of water column at time t_i.

The principle component analysis of bacterium growth was conducted using the SAS/STAT package (SAS Institute, 1990).

2 Results

2.1 Solution chemistry and solid analysis

Measured particle sizes of the sediment were 54% of sand, 24% of silt, and 22% of clay. The measured chemical properties included pH 4.74, CEC 31.8×10^{-4} mg/g soil, Org 6.20%, TN 0.46%, and TP 0.27%.

Based on the monitoring results, variations of pH, Eh, and temperature were not significant. During the experiment, pH in the water was 6.4–6.5 and pH in sediment was around 7.0. Eh was also in the intermediate state. At the beginning of experiment, Eh in the sediment was 14–20 mV. After 7 d, Eh decreased to be 10 mV. The temperature reached the peak (30–31.5°C) on day 7, then decreased, and kept at about 29°C for the rest of time.

At the beginning of experiment, the concentration of DO was relatively high. With the experiment going on, DO in the system was consumed quickly. On day 8, DO in the water was 0.91 mg/L and DO in the sediment was only 0.47 mg/L at on day 15.

2.2 Nitrogen transforming bacteria and nitrogen characteristics

During the 30 d experiment, nitrogen transforming bacteria, including ammonifier, nitrobacteria, nitrosomonas, and denitrifying bacteria, changed with time and depth. In general, the amount of nitrobacteria, nitrosomonas was much smaller than that of ammonifier and denitrifying

bacteria. Except at the deeper sediment with short time periods, the amount of nitrosomonas was negligible (Table 1).

In the water column (e.g., at 20 cm above the interface), the general patterns changing with time were similar for ammonifier, nitrobacteria, and denitrifying bacteria. The bacteria increased with time first, reached the maximum values of similar magnitude on about day 15, and then decreased. However, the temporal variation of nitrobacteria was larger than that of ammonifier and denitrifying bacteria.

As shown in Table 1, at the interface, ammonifier changed relatively fast with time, reaching the maximum of 4.0×10^6 CFU/ml on about day 8 and then decreased. After 22 d, the ammonifier increased again to the end of the experiment. Denitrifying bacteria gradually increased, reached the maximum 4.5×10^5 CFU/ml on about day 15, then decreased. The temporal change of nitrobacteria was similar to that of denitrifying bacteria, but reaching the maximum 2.0×10^5 CFU/ml on day 22.

At 10 cm below the interface (–10 cm), ammonifiers and denitrifying bacteria in the sediment reached the maximum values (9.0×10^6 and 11.0×10^6 CFU/ml, respectively) on day 8 and day 22, respectively, then decreased. Nitrobacteria reached the maximum (4.5×10^5 CFU/ml) on about day 22. At –25 cm in the sediment, the amount of ammonifier was higher than that of denitrifying bacteria. With a small variation at the beginning, the ammonifier increased rapidly after 14 d and on day 29 reached the largest value of 11.0×10^7 CFU/ml in the experiment. The change of denitrifying bacteria was relatively smooth, reached the maximum about on day 15, then decreased. At –45 cm, the amount of ammonifier reached the maximum on day 8, then decreased, and increased again after 22 d. The change of denitrifying bacteria was relatively large with two peaks on day 15 and 29 (Table 1).

Table 1 Temporal and vertical distributions of nitrogen transforming bacteria in the water-sediment system (unit: $\times10^4$ CFU/ml)

Depth (cm)	Nitro-bacteria	Nitro-somonas	Denitrifying bacteria	Ammo-nifier	Total
			1 d		
20	0.1	-	4.0	20.0	24.1
0	0.1	-	0.2	110.0	110.3
–10	0.2	-	0.5	20.0	20.7
–25	0.4	-	4.5	140.0	144.9
–45	0.7	11.5	15.0	15.0	42.2
			8 d		
20	0.2	-	1.0	10.0	11.2
0	0.5	-	25.0	400.0	425.5
–10	0.2	-	25.0	900.0	925.2
–25	0.2	-	95.0	100.0	195.2
–45	0.4	-	25.0	3,000.0	3,025.4
			15 d		
20	95.0	-	75.0	95.0	265.0
0	6.5	-	45.0	25.0	76.5
–10	3.0	-	150.0	400.0	553.0
–25	3.5	-	950.0	95.0	1,048.5
–45	140.0	-	450.0	45.0	635.0
			22 d		
20	20.0	-	25.0	25.0	70.0
0	20.0	-	25.0	25.0	70.0
–10	45.0	4.5	1,100.0	45.0	1,194.5
–25	-	6.5	250.0	2,500.0	2,756.5
–45	-	9.5	45.0	95.0	149.5
			29 d		
20	-	-	15.0	75.0	90.0
0	0.1	-	25.0	200.0	225.1
–10	0.3	-	250.0	250.0	500.3
–25	-	-	160.0	11,000.0	11,160.0
-45	-	-	450.0	2,000.0	2,450.0

Table 1 also clearly shows the vertical distributions of nitrogen transforming bacteria. On day 1 (from the first sampling) nitrobacteria in the system increased with the depth (10^3–10^4 CFU/ml). Except at –45 cm, the amount of nitrosomonas was negligible. Denitrifying bacteria were minimal at the interface. Ammonifier at the interface and –25 cm was higher than at other depths. On day 8, the distribution of nitrobacteria along the column was relatively uniform. The amount of nitrosomonas was negligible. Denitrifying bacteria and ammonifier in the water were much fewer than in the sediment. Except at –25 cm, the distribution of denitrifying bacteria in the sediment was quite uniform. Ammonifier repeated the increase-decrease patterns in the sediment. On day 15, the amount of nitrobacteria at 20 and –45 cm was larger than other depths. The amount of nitrosomonas was negligible. Denitrifying bacteria increased with depth and decreased at –45 cm. The amount of ammonifier was minimal at the interface, maximal at –10 cm, and then decreased. On day 22, the amount of bacteria in the water was the same as that at the interface. The distribution of nitrobacteria reached the maximum at –10 cm and then became negligible in the deeper sediment. The amount of nitrosomonas increased with depth in the sediment. Denitrifying bacteria reached the maximum at –25 cm and then decreased. Ammonifier increased below the interface, reached the maximum at –25 cm, and then decreased. On day 29, the amount of nitrobacteria in the system became very small. Denitrifying bacteria increased with the depth gradually. Ammonifier at the most active stage also increased with the depth, reached the maximum at –25 cm, and then decreased.

Figure 2 shows temporal and vertical distributions of N (NH_4^+-N, NO_3^--N, and NO_2^--N) in the water-sediment system. In the water column, the concentration of NH_4^+-N increased with time. At the interface, the concentration of NH_4^+-N initially increased to the peak on day 8, then decreased. After 15 d, the concentration increased again close to the peak value and kept the level to the end of the experiment. In the sediment, the concentration increased with time. Along the column, NH_4^+-N concentration in the water was much lower than those at the interface and in the sediment. The concentration of NO_3^--N in the water was low, changing between 0.09–1.27 mg/L. The concentration at the interface was higher than in the water but lower than in the sediment, reached the maximal within 21 d, and then decreased quickly. The changes of NO_3^--N at –10 and –45 cm in the sediment were similar to that at the interface. The concentrations of NO_3^--N at –25 cm increased with time continuously. The concentration of NO_2^--N in the water changed between 0.03–0.05 mg/L. The distributions at the interface and –10 cm were similar, which reached the peak on day 15 and then decreased. The distributions at –25 and

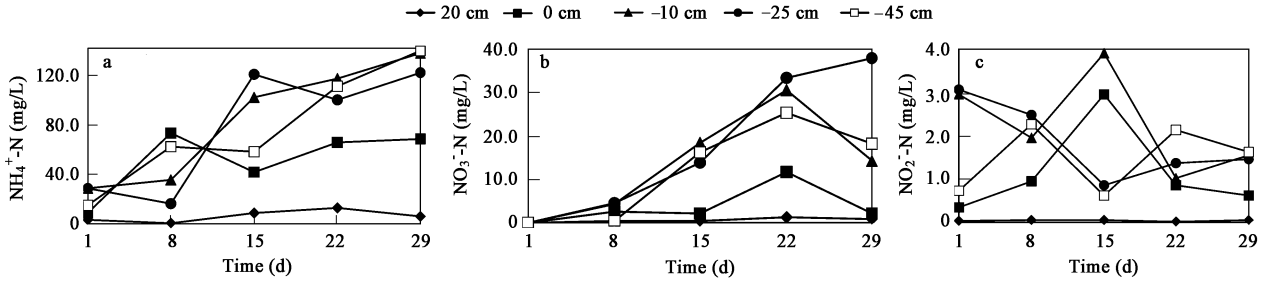

Fig. 2 Temporal and vertical distributions of (a) NH_4^+-N, (b) NO_3^--N, and (c) NO_2^--N in the water-sediment system.

–45 cm were similar with the minimum values at 15 d.

2.3 Phosphorous bacteria and phosphorous distributions

As shown in Fig.3a, inorganic phosphobacteria in the water (e.g. at 20 cm) varied slowly. At beginning, the number of colony forming units (CFU) of the inorganic phosphobacteria was 630 CFU/ml, increased to 2900 CFU /ml on day 15, and decreased to 1800 CFU/ml at the end of experiment. On the contrary, organic phosphobacteria decreased from 1.7×10^5 CFU/ml at the beginning to 1.2×10^5 CFU/ml on day 15 and 5.2×10^3 CFU/ml at the end of experiment (Fig.3b). At the interface, inorganic phosphobacteria decreased from 2.6×10^4 CFU/ml at the beginning to 770 CFU/ml on day 29. Organic phosphobacteria increased from 3.9×10^3 CFU/ml at the beginning to 3.6×10^6 CFU/ml on day 15, and then decreased to 1.5×10^4 CFU/ml at the end of experiment.

At –10 cm in the sediment, inorganic phosphobacteria increased from 200 CFU/ml to 8.3×10^3 CFU/ml at 15 d and kept the level for the rest of time (Fig.3a). Organic phosphobacteria also reached the maximum at 15 d and slightly decreased after that, ranging between 2.0×10^3–2.4×10^5 CFU/ml (Fig.3b). At –25 cm, inorganic phosphobacteria were 4.0×10^2 CFU/ml on day 1, reached the maximum of 1.4×10^4 CFU/ml on day 15, and then gradually decreased to 7.8×10^2 CFU/ml on day 22. Similarly organic phosphobacteria reached the maximal at 15 d and then decreased, ranging from 1.6×10^4 to 4.3×10^5 CFU/ml. At –45 cm, inorganic phosphobacteria decreased from 6.3×10^3 to 1.3×10^3 CFU/ml within the first 8 d, increased to 7.6×10^4 CFU/ml between 8–15 d, and then decreased to 1.0×10^4 CFU/ml. Organic phosphobacteria deceased from 1.6×10^5 CFU/ml to 6.0×10^3 CFU/ml, then increased to 3.3×10^5 CFU/ml on day 15, and kept the level after 22 d. After 15 d, the phosphorus bacteria seemed to approach the equilibrium.

Figure 4 shows the temporal and vertical distributions of PO_4^{3-}-P in the water-sediment system. The time average values of P concentration were 0.271, 2.004, 1.202, 0.965, 0.978 mg/L at 20, 0, –10, –25, –45 cm, respectively. With the maximum value of 0.612 mg/L on day 15, P concentration in the water was smaller than in the sediment. The larger concentrations along the depths were at the interface and –10 cm. The highest concentrations appeared at the interface (5.16 mg/L) and –10 cm (3.77 mg/L) on day 8 and day 22, respectively. At other depths in the sediment, the initial concentration was about 0.2 mg/L, the highest concentration was 2.0 mg/L on day 15, and the final concentration was about 1.0 mg/L.

2.4 Factors for bacterium growth and fluxes of N and P

To identify the most important factors that affect bacterium growth, principal component analyses were carried out in three representative conditions: in the water column (20 cm), at the interface (0 cm), and in the sediment (−25 cm). Nine factors were used in the analyses, including water temperature, DO, COD, PO_4^{3-}-P, NH_4^+-N, NO_3^--N, NO_2^--N, Eh, and pH. In the water column, three main components accounted for 91.4% of the overall factor contributions to bacterium growth. The three components made almost equal contributions. The first component (DO, COD, and NO_2^--N) represented the condition of oxygen and organic matter for bacterium growth. The

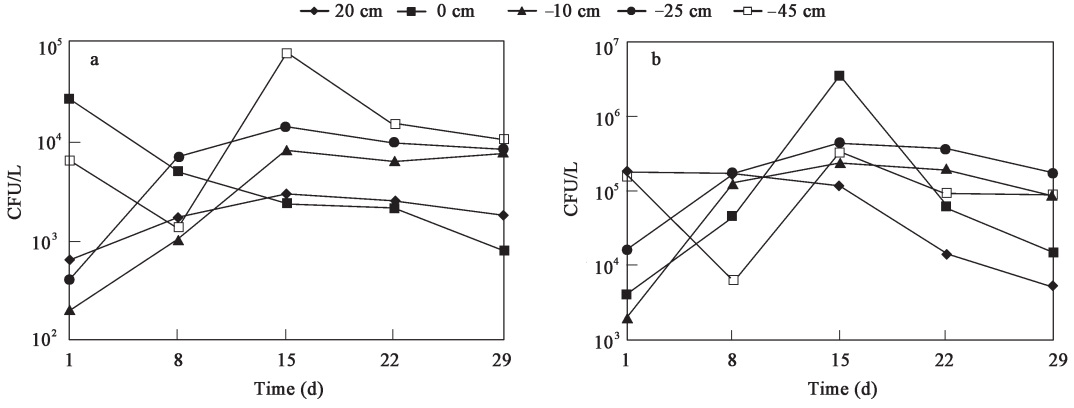

Fig. 3 Temporal and vertical distributions of (a) inorganic phosphobacteria and (b) organic phosphobacteria.

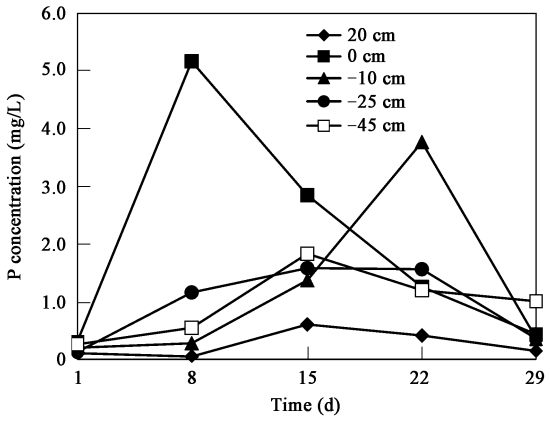

Fig. 4 Temporal and vertical distributions of PO_4^{3-}-P in the water-sediment system.

second component (water temperature, PO_4^{3-}-P, NH_4^+-N, NO_3^--N) represented the nutrition for bacterium growth. The third component (Eh and pH) represented the condition of living environment. At the interface, three main components accounted for more than 90% of the overall factor contributions. The first component (DO, COD, NO_2^--N, and NH_4^+-N) represented the condition of oxygen and nutrition for bacterium growth. The second component (water temperature and PO_4^{3-}-P) represented the need of water temperature and P nutrition. The third component (NO_3^--N, Eh, and pH) represented the condition of bacterium living environment. The first component made the largest contribution of 36.3%. In the sediment, three main components accounted for 100% of the overall factor contributions. The first component (NO_3^--N, NH_4^+-N, Eh, and pH) represented the need for N nutrition and the living environment. The second component (DO, COD, and NO_2^--N) represented the requirement of oxygen and organic matter. The third component (water temperature and PO_4^{3-}-P) represented the condition of water temperature and P nutrition for bacterium growth. The first component made the largest contribution up to 55%.

Using the method above (Eq.(1)), we calculated temporal fluxes of NH_4^+-N, NO_3^--N, NO_2^--N, and PO_4^{3-}-P as shown in Fig.5. The amount of positive fluxes of NH_4^+-N was much larger than that of negative fluxes and the total flux was 5528.30 mg/(m^2·d), indicating that the release process was dominated. The fluxes varied between −2500 and 4400 mg/(m^2·d) with the largest variation between 8 and 11 d. The fluxes of NO_3^--N were between −600 and 600 mg/(m^2·d). The positive and negative values indicated that the release process and adsorption process occurred alternatively. The total flux was 338.63 mg/(m^2·d) with the maximum value on day 8–10 and the minimum (the most negative) value on day 14. The fluxes of NO_2^--N were between –81 and 127 mg/(m^2·d) with a relatively large variation within the first on day 20. The total flux of NO_2^--N was 116.03 mg/(m^2·d). The positive and negative values of PO_4^{3-}-P fluxes changed alternatively with the largest variation between 3 and 6 d. After 13 d, the amount and variation of P fluxes became small. The total flux was 39.69 mg/(m^2·d).

3 Discussion

3.1 Nitrogen transforming bacteria and nitrogen transformation in the system

Concentration changes of NH_4^+-N, NO_3^--N, and NO_2^--N in the system were closely related to the concentration of dissolved oxygen (DO). At the beginning of experiment,

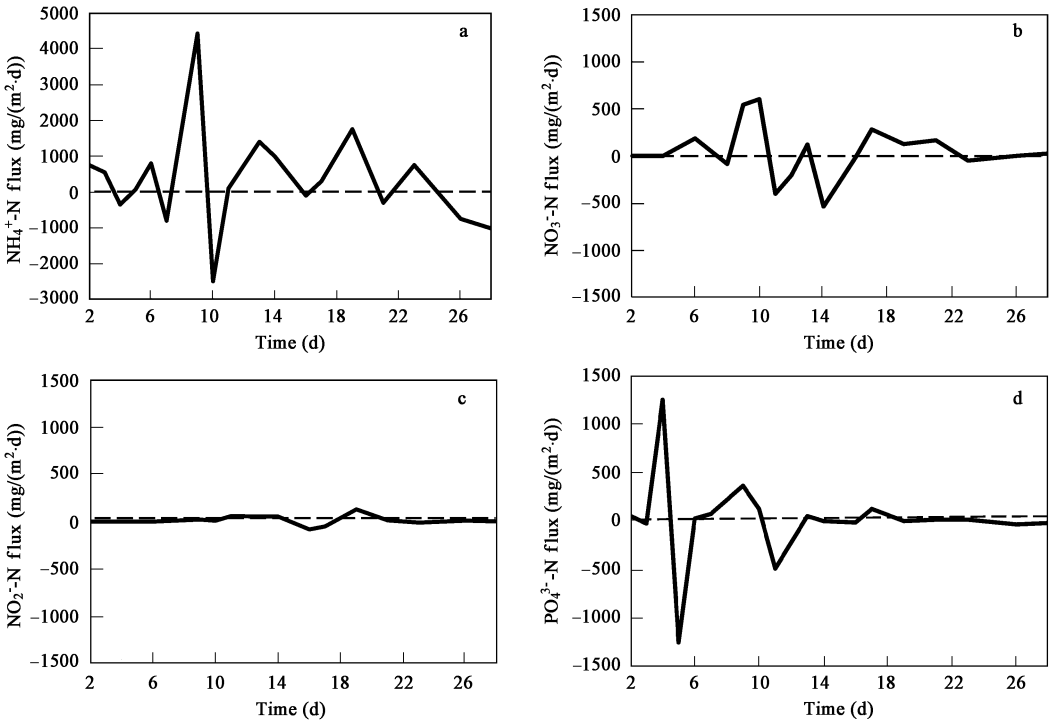

Fig. 5 Temporal chemical fluxes of NH_4^+-N (a), NO_3^--N (b), NO_2^--N (c), and PO_4^{3-}-P (d).

the concentration of DO was relatively high. Under the action of aerobic bacteria, organic matter in the sediment was decomposed to produce NH_4^+-N. Through the pore water, some NH_4^+-N moved into the water column to raise its NH_4^+-N concentration. Meanwhile, because of the action of nitro-bacteria, some NH_4^+-N was transformed to NO_3^--N and NO_2^--N.

As the experiment going on, DO in the system was consumed quickly. On day 8, DO in the water was 0.91 mg/L and DO in the sediment was only 0.47 mg/L on day 15. However, the input of a mixture of oxygen (4%) and nitrogen through the top of column may result in increase of concentrations of NH_4^+-N and NO_3^--N, and decrease of the concentration of NO_2^--N, which became negligible.

Available organic matter was consumed by increasing bacteria in the sediment. The bacteria began to transform the inorganic N materials and released NH_4^+-N. The anaerobic environment enhanced the denitrifying process and some NO_3^--N replaced oxygen as the electron accepter. Except in the water column, nitro-bacteria in other depths decreased quickly after 15 d, which resulted in large amount of NH_4^+-N in the pore water.

During the final period (22–29 d) of the experiment, the NH_4^+-N concentration kept increasing and NO_3^--N concentration decreased significantly except at −25 cm. NO_3^--N was transformed to NH_4^+-N through denitrification. However, NO_3^--N in pore water of sediment was limited to exchange and transform. As the result, after 15 d, NO_3^--N in the pore water did not reduce greatly. On the other hand, after the carbon source was consumed by the process of denitrifying, the left NO_3^--N was used by polyphosphate accumulation microorganisms (PAM) as electron acceptors for P adsorption. Nevertheless, the result of NO_3^--N concentration at −25 cm increasing with time needs for further study. Different from others (Verrhiest *et al*., 2002), the sediment used for the experiment was with sufficient carbon sources. Therefore, to the end of experiment, metabolism nutrients in the bacteria were released, which promoted increase of biomass. Microbial decomposition of organic N in the sediment produced more NH_4^+-N, which moved into the water and kept a high NH_4^+-N concentration. The result also suggested that it would be challenging to treat seriously contaminated water through the self-purification process.

The most active region of bacteria was from –10 to –25 cm in the sediment. Because of the rich organic matter in the sediment, bacteria in the sediment nearby the interface were also rich. The results were consistent with others (Haglund *et al*., 2003). In this region, significant microbial reaction made an anaerobic and reductive environment. Bacteria involved stronger ammonifying and denitrifying processes, which resulted in more NH_4^+-N in the pore water. On the other hand, NH_4^+-N in this region was easier to move into the water and air, which reduced NH_4^+-N concentration in the sediment. The less oxygen content in the deeper sediment was favorable to anaerobia activities as well as ammonifying and denitrifying processes. Because of the limitation for exchange between pore water and the overlying water, more NH_4^+-N was kept in the pore water. In this experiment, the concentrations of inorganic N in the pore water were much higher than that in the water column.

3.2 Phosphorous concentrations and bacteria distributions

The phosphorous balance between the water and the sediment is not a simple physical process, but closely related to the biological circulation of P. In the sediment from the severely contaminated river, there was a large amount of organic matter. In the column experiment, the most active biological activities occurred at the interface. Based on the concept that the P release is equal to the internal P loading, it was expected that at the beginning of the experiment, a large amount of P be released from the sediment to the water column, which would eliminate the P concentration difference between the sediment and the water column. However, the experiment results showed that the P concentration in the water was much lower than in the sediment. We explained the paradox as follows.

At the interface organic phosphobacteria increased at the fastest rate and reached the largest amount in the system during the experiment, while in the water column the amount of organic phosphobacteria was large at the beginning and then continuously decreased. The experiment results indicated that the small amount of organic P in the water from the sediment surface was quickly consumed by organic phosphobacteria, which resulted in decrease of organic phosphobacteria in the water. Below the sediment surface, the large amount of organic matter with P resulted in dramatic increase of organic phosphobacteria, which generated a large amount of inorganic P. Therefore, the P concentration at the interface was much higher than in the water column and the high concentration prevented upward P dispersion from the deeper sediment. As suggested by Morin and Morse (1999), sediments can buffer environmental nutrient concentrations in overlying waters.

The P concentration in the pore water increased first, then decreased corresponding to P release and adsorption, respectively. In the experimental system, although organic phosphobacteria produced a large amount of phosphate, inorganic phosphobacteria were limited. Although the sediment used for the experiment was with sufficient carbon sources, the organic matter in the water and on the top of sediment was limited. The lack of carbon source became a limited factor for the growth of inorganic phosphobacteria in the water and on the top of sediment. Lacking supply of organic carbon source was supported by the minimal number of heterotrophic bacteria in the water and on the top of sediment. For example, on day 15 the number of heterotrophic bacteria was only about 7.0×10^2 CFU/ml in the water and on the top of sediment. The number of heterotrophic bacteria increased with the depth and reached the maximum value of 4.3×10^6 CFU/ml at –45 cm.

In the system, the dominant bacteria were PAM, which played an important role in P transformation. Under the anaerobic sediment with sufficient carbon source, PAM can utilize the carbon source to compose polymers in the cells.

Meanwhile, PAM produced energy through hydrolysis of polyphosphates stored in the cells. The process of hydrolysis of polyphosphate released phosphate into the solution. Under anaerobic conditions with NO_3^--N, since the denitrifying rate was faster than the P release rate by PAM, denitrifying bacteria utilized the available substrates faster than PAM, which decreased the rate and amount of released P. After the carbon source was consumed by the process of denitrifying, the left NO_3^--N was used by PAM as electron acceptors for P adsorption. This explained why the concentrations of NO_3^--N and P decreased at the last period of the experiment.

3.3 Analyses of N and P release

Adsorption and release of N and P from sediments were influenced by various factors (Gomez *et al*., 1999). Except the factors discussed above, the changes of pH, Eh, and temperature in this experiment were small. The pH value in the water was between 6.4 and 6.5, while the pH value in the sediment was about 7.0. The Eh values varied between 14 and 20 mV for the first 7 d, then kept at 10 mV for the rest of time. The temperature varied between 30–31.5°C for the first 7 d, then kept at 28.5°C for the rest of time. These conditions were favorable to the N circulations and transformations, resulting in large fluxes of NH_4^+-N, NO_3^--N, NO_2^--N with the main form of NH_4^+-N (Figs.5a, b, c). However, the experimental conditions limited P release. At the beginning, sufficient DO increased the formation of iron and aluminum bound phosphorus, which affected the release of soluble phosphorus. The P fluxes were small and processes of release and adsorption occurred only at the beginning of the experiment (Fig.5d). Fluxes of N and P changed positively and negatively, suggesting obvious exchanging processes between the sediment and overlying water. However, the fluctuation of positive and negative fluxes decreased with time, indicating that the dynamic process at the interface changed gradually from an unstable state to a stable state, which was related to the status of bacteria in the system (Table 1 and Fig.3).

4 Conclusions

A large column experiment was conducted to study temporal and vertical distributions of N and P in a water-sediment system, which was related to the distributions of nitrogen transforming bacteria and phosphorus bacteria in the system. In the 30-d experiment, the amount of nitrogen transforming bacteria increased with time with dominant denitrifying bacteria and ammonifier. In the vertical direction, the amount of bacteria in the water and at the interface was lower than that in the sediment. Corresponding to the bacteria populations, the N distributions were in the order: NH_4^+-N > NO_3^--N > NO_2^--N.

Phosphorous release was closely related to the phosphorus bacteria. Compared with other depths, the larger amount of organic phosphobacteria at the interface resulted in a higher P concentration there. The experimental conditions and the relatively high P concentration at the interface limited upward P dispersion from the sediment and the P release process into water.

The processes of release and adsorption of NH_4^+-N, NO_3^--N, and NO_2^--N were active, whereas the processes of PO_4^{3-}-P occurred only at the beginning of the experiment. The fluxes with alternatively positively and negatively changes suggested significant release and adsorption processes in the water-sediment system. Relating to the bacterium equilibrium, the dynamic process at the interface varied gradually from an unstable state to a stable state.

With 9 factors (water temperature, DO, COD, PO_4^{3-}-P, NH_4^+-N, NO_3^--N, NO_2^--N, Eh, and pH), 3 principal components were shown dominantly to affect bacterium growth in the water column, at the interface, and in the sediment. The three main components accounted for more than 90% of the overall factor contributions to bacterium growth, among which the condition of nutrition was of the most importance.

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