MEASUREMENT OF DENITRIFICATION ACTIVITY OF THE BOTTOM SEDIMENT AT NOGAWA RIVER
(Natural purification of an urban river ecosystem)

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ABSTRACT

Nitrogen removal by denitrification in sediment and artificial substrate was measured at Nogawa River by continuous method, based on acetylene inhibition technique, during one year from December 1987 to November 1988. The quality of artificial substrate is an important factor for denitrification rate in a river bed sample. The denitrification rate per unit area in the river bed composed of concrete (13.8gN m^{-2} $year^{-1}$) was higher than that in the gravel and sand (7.4gN m^{-2} $year^{-1}$) river bed. The proportion of denitrificated nitrogen to the input nitrogen in the river was calculated. The average value for the concrete river bed was 0.3%, and 7.4% in the river bed of gravel and sand. From these results, if all area of Nogawa River was changed to a concrete bed, the proportion of denitrificatied nitrogen to input nitrogen is estimeted to be 1.7%, and about 3.1% in the case of a gravel and sand. Denitrification activity in the river bed of a small urban river was found to be dependent on the type of river bed.

Key words: denitrification, acetylene inhibition, natural purification, attached microbial community, Nogawa River.

INTRODUCTION

Denitrification process is important for natural purification in an aquatic environment, because it releases nitrogen compounds as vapor molecules such as N_2, N_2O to the atmosphere. Recently, it was found that the denitrification process occurs inside supended particles in the aerobic river environment or in the anaeroic microlayer in the river bed sediment (6). Recently, river beds of many urban river have been changed to concrete for the purpose of preventing flooding. Nogawa river is a typical urban river for the study of the denitrification process because a part of the river bed is composed of concrete. The rate of purification activity is expected to be high in the Nogawa River as the nitrate concentration is high and there is domestic sewerage inflow in the upper reaches of the river. In this study, the denitrification activity was measured at Nogawa River, in order to estimate the role of the denitrification process for natural purification and the effect of river beds on the denitrification rate were discussed.

MATERIALS AND METHODS

River water sampling were carried out from September 1987 to November 1988 at three sites (N-2, N-2A, N-3) in Nogawa River, at Koganei City, Tokyo (Fig. 1). River bed was made by concrete, water flow was high at

N-2, N-2A was loacated between N-2 and N-3, and river bed was similar to that at N-2.

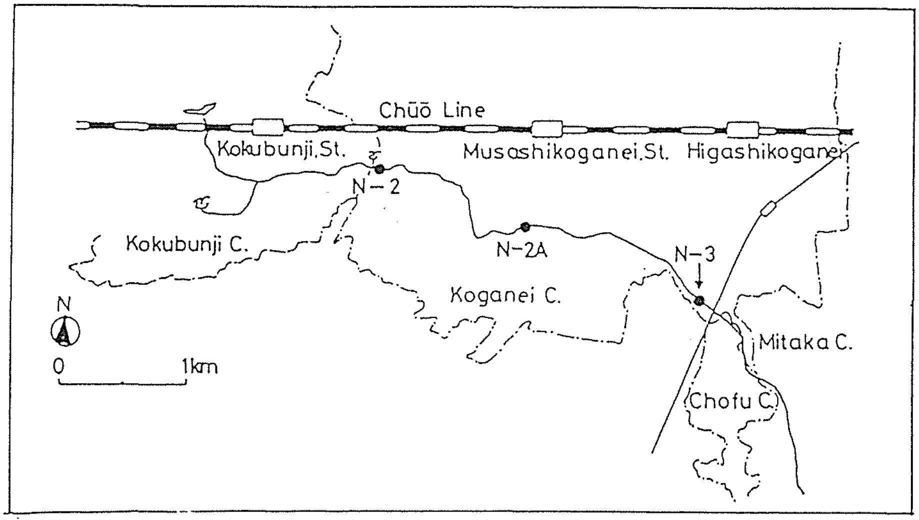

Fig. 1 Sampling site at Nogawa River

Water analysis.
The river water was collected at the surface layer. The pH value and the dissolved oxygen concentration (DO) were measured by a glass electrode and Winkler's method. The concentrations of suspended solid (SS) and chlorophyll a (Chl. a) were determined with a residue on Whatman GF/C filter after filtration. The organic carbon concentration was analyzed by the method of Menzel and Vaccaro (5). Total nitrogen concentration was determined by the method of Solorzano and Sharp (11). Ammonium, nitrite and nitrate nitrogens were measured with filtrate after the method of Solorzano(10), Bendschneider and Robinson (2), and Wood et al(13), respectively.

Denitrification assay.
The denitrification activity was measured by acetylene inhibition technique (1, 14). Figure 2 shows an experimental device for measuring of denitrification rate. A concrete plate and a plastic box containg sediment, which were left in the river bed for several weeks at N-2 and N-3, were used to determine in situ denitrification. The samples were incubated in a plastic container (13 litter) with the river water, which was sataurated at in situ temperature with acetylene, for several hours. Water container was always mixed by a recirculating pump during the incubation period. The N_2O production was calculated from the volume of the water and the difference in the concentration between influent and effluent. The denitrification rate was represented by aerial basis. The concentration of N_2O was analyzed by a gas chromatograph (Shimadzu GC-7A) equipped with ^{63}Ni electron capture detector after purge-trap method. The fluctuation of incubation temperature was less than ±1.0°C.

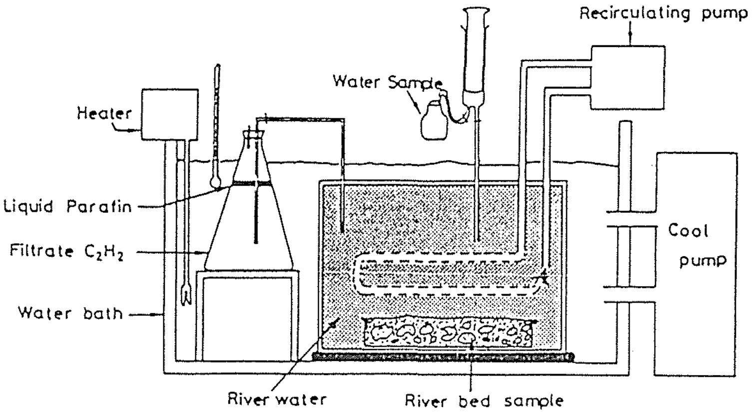

Fig. 2 Experimental chamber used for measurement of denitrification rate of river bed sample under in situ condition

RESULTS AND DISCUSSION

Water quality.

Table 1 shows the chemical components in river water at each station during measurement period. The concentration of nitrate, which is an electron acceptor for denitrifying becteria, slightly decreased in concentration as moving of water from N-2 to N-3. The trend of increase in concntration of dissolved oxygen and chlorophyll a, were observed. this is because of the exfoliation of peiphyton and big aquatic type plants which had developed near N-2A and N-3.

TABLE 1 Chemical composition of river water at Nogawa

		N-2	N-2A	N-3
pH		7.2	7.4	8.1
DO	$(mg \cdot l^{-1})$	6.7	8.1	11.3
SS	$(mg \cdot l^{-1})$	12	10	65
Chl.a	$(\mu g \cdot l^{-1})$	5.8	7.5	9.7
TOC	$(mg \cdot l^{-1})$	6.9	5.2	6.3
POC	$(mg \cdot l^{-1})$	2.3	2.2	3.4
DOC	$(mg \cdot l^{-1})$	4.6	3.0	2.9
TIN	$(\mu gat \cdot l^{-1})$	577	540	490
NH_4-N	$(\mu gat \cdot l^{-1})$	88	74	45
NO_2-N	$(\mu gat \cdot l^{-1})$	17	17	13
NO_3-N	$(\mu gat \cdot l^{-1})$	472	449	432

Denitrification rate at Nogawa River.

The result of measurement of the denitrification rate at each site is shown in Figure 3. The denitrification rate in N-2 varied from 4.3 $mgN\ m^{-2}.\ h^{-1}$ to 0.04 $mgN\ m^{-2}.\ h^{-1}$, and the mean value was calculated to be 37.8 $mgN\ m^{-2}.\ day^{-1}$. This was a little higher than that reported for an attached bacterial community of 8-16 $mgN\ m^{-2}.\ day^{-1}$ by Nakajima(6). The extremely high rate during January to March 1988, which are similar to the value reported by Ogawa and Ogura(7), can be explained by the existence of a large epilithic community in the river. The low activities in October and November might be due to the significent decrease in standing crop of the attached microbial community, corresponding to the abundant rainfall during the period. Conversely a good relationship between denitrification rate and water temperature at N-3 site was observed. At this site the denitrification rate varied from 2.0 $mgN\ m^{-2}.\ h^{-1}$, to 0.2 $mgN\ m^{-2}.\ h^{-1}$, and the

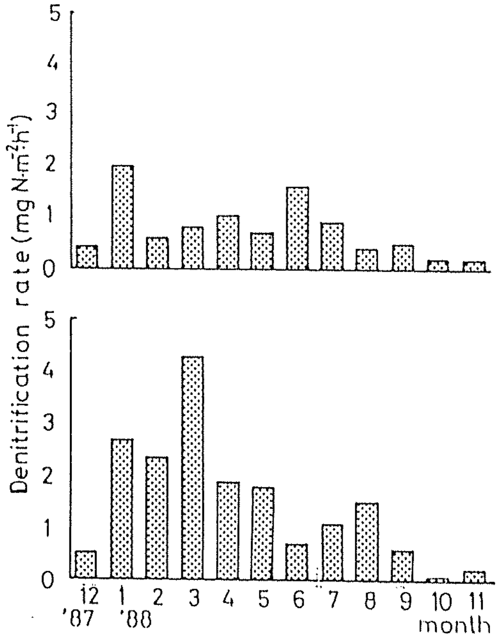

Fig. 3 Seasonal change in denitrification rate measured with river bed sample from Nogawa

TABLE 2 Numbers of denitrifying and total bacteria in the attached subsance (N-2) and the sediment (N-3)

		N - 2		N - 3	
		cells.dryg^{-1}	cells.mgC^{-1}	cells.dryg^{-1}	cells.mgC^{-1}
Denitrifying bacteria	Apr.	3.3 x 10^7	0.2 x 10^6	2.1 x 10^5	0.8 x 10^3
	Jun.	1.3 x 10^7	0.9 x 10^4	1.2 x 10^6	0.8 x 10^3
	Nov.	2.4 x 10^6	2.2 x 10^4	1.1 x 10^4	2.4 x 10^3
Total bacteria	Apr.	5.0 x 10^7	1.9 x 10^7	2.0 x 10^6	3.4 x 10^7
	Jun.	4.0 x 10^7	2.7 x 10^6	1.0 x 10^7	8.2 x 10^7
	Nov.	1.1 x 10^9	1.0 x 10^7	4.9 x 10^7	1.1 x 10^7

mean value was 18.6 mgN m^{-2}. day^{-1}. The denitrification rate has no relation with the carbon and nitrogen contents in the sediment of the river bed. Table 2 shows the number of the denitrifying bacteria and total bacteria at N-2 and N-3 sites. The number of denitrifying bacteria at N-2 site was one to three orders of magnitude larger than that at N-3.

Environmental factor

It is well known that the denitrification rate depends upon temperature and the availabilities of electron donor and acceptor (4). A good relationship between the incubation temperature and the denitrification rate was also observed in this study (Fig. 4). However, the poor correlation coefficients between the measured temperature of river water and *in situ* denitrification rate of r=0.48 at N-2 site and r=0.3 at N-3 site, indicate that there might be more important environmental factors which affects the denitrification other than water temperature in Nogawa River.

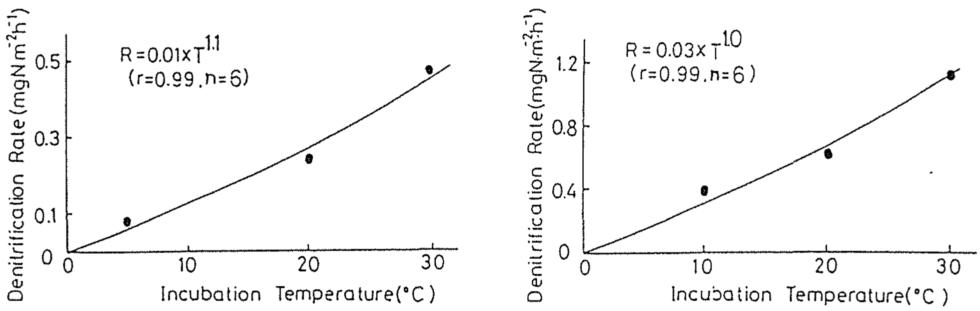

Fig. 4 Relationship between denitrification rate and incubation temperature

It was observed that denitrification is related to the change in dissolved oxygen concentration (0-16 mg. l^{-1}). An increase of denitrification rate was observed to correspond with the increase of glucose concentration up to 100 mg C. l^{-1}. However, DOC concentration in Nogawa River was 0.8 to 10 mg C. l^{-1}. This suggested that organic matter concentration was not a major controlling factor which affecting the denitrification rate. Furthermore, it is suggested that denitrification rate dose not depend on the organic matter concentration in attached microbial community on the river bed because the organic matter is sufficient in the river bed.

The relationship between the concentration of electron acceptor and the denitrification rate can be expressed by the Michaelis-Menten's equation when the concentration of electron donor does not contribute to denitrifiction rate (3). The half-saturated concentrations (Ks) were calculated to be 191 umol. l^{-1} at N-2 site and 111 umol. l^{-1} at N-3 site. The value obtained in this study was nearly equal to the result, 129 umol l^{-1}, obtained at the Nakanoumi by Seike et al (9). and was greater than that 25 umol l^{-1}, obersed at Teganuma by Ueda and Ogura (12). The average value of Ks at both sites obtained in this study was higher than the both these results. The high value observed at Nogawa River may be due to high nitrate concentration (Table 1). It was found that denitrification rate was affected by the water temperature, the availability electron acceptor and electron donor at Nogawa River. But the factor most affecting denitrification rate at Nogawa river was the quality of the artificial substrate which induces to anerobic particle formation in river bed.

<u>Estimation of nitrogen removal from river water by denitification at Nogawa</u>

Table 3 shows that a comparsion of the total load of inorganic nitrogen to the nitrogen removed by denitrification from water at Nogawa River. The annual average rate of denitrification of N-2 site (13.8 gN $m^{-2}.yr^{-1}$) was significantly larger than that of N-3 site (7.3 gN $m^{-2}.yr^{-1}$). On the other hand, the average proportion of the amount of denitrified nitrogen estimated from the rate, the area of river bed and residence time of the water, and input nitrogen was smaller at N-2 (0.3%) than at N-3 (7.4%). Accordingly, it may be expected that the concrete river bed area was higher in the rate of denitrification than the gravel and sand river bed area, and in case of the proportion of nitrogen removal opposite occured. This relationship was recoginized from the fact that the flow rate of the water was faster at the concrete river bed than that at the gravel and sand, resulting the difference in the residence time of the river water.

TABLE 3 Removal ratio of nitrogen by denitrification

DATE	CONCRETE (N-2~N-2A:1.8km, 2.7km²)				SAND & STONE (N-2A~N-3: 1.7km, 10.2km²)			
	(A) (kgN)	(B) (gN)	(B/A) (%)	VELOCITY ($m.s^{-1}$)	(A) (kgN)	(B) (gN)	(B/A) (%)	VELOCITY ($m.s^{-1}$)
2.DEC.87	3.4	2	0.1	0.28	4.6	66	2.1	0.03
20.JAN.88	3.1	18	0.6	0.20	2.9	323	11.0	0.03
22.FEB.88	3.2	13	0.3	0.24	2.2	213	9.9	0.01
11.MAR.88	2.0	24	1.2	0.24	1.5	65	4.3	0.06
23.APR.88	1.7	13	0.7	0.21	2.1	240	11.4	0.02
26.MAY.88	1.5	12	0.3	0.20	2.4	360	15.1	0.01
24.JUN.88	2.4	4.1	0.2	0.24	2.3	790	33.7	0.01
13.JUL.88	2.4	3.5	0.2	0.42	3.2	45	1.4	0.1
25.AUG.88	4.8	2.1	0.05	1.00	7.4	4	0.1	0.55
13.SEP.88	5.9	0.8	0.02	1.04	6.8	5	0.1	0.49
29.OCT.88	7.4	0.07	0.00	0.70	8.4	5	0.1	0.2
17.NOV.88	6.1	0.44	0.01	0.70	7.5	5	0.1	0.22
MAX.	7.4	24	1.2	1.04	8.4	790	33.7	0.55
MIN.	1.5	0.07	0.00	0.20	1.5	4	0.1	0.01
AVERAGE	3.7	7.8	0.3	0.46	4.3	177	7.4	0.14

* (A) : Amount of total inorganic nitrogen (NH_4-N+NO_2-N+NO_3-N)
(B) : Amount of denitrified nitrogen
(B/A) : Removal ratio of nitrogen

From the results. If all river beds of Nogawa River are converted to a concrete, the proportion of denitrified nitrogen to the total input nitrogen is 1.7%. If all river beds of Nogawa River are converted to a gravel and sand, the proportion is about 3.1%.

CONCLUSIONS

The denitrification activity as the natural purification process was investigated at two sites (concrete river bed, gravel and sand river bed) in Nogawa River, an urban small river. Denitrification rate in the river bed made of concrete was higher than that in the river bed composed of gravel and sand. However, the proportion of nitrogen removed by denitrification, which was calculated from the denitrification rate *in situ* and the amount of nitrogen in the river, was higher in river bed of gravel and sand than that in river bed of concrete. In this study, it was found that the denitrification in the river bed of urban small river depended on the type of river bed.

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