

# Nitrate removal with low N<sub>2</sub>O emission by application of sulfur denitrification in actual agricultural field

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**Abstract** Sulfur denitrification was applied to the agricultural field and the characteristics of the treatment were evaluated from the viewpoints of nitrate removal efficiency and nitrous oxide (N<sub>2</sub>O) emission. Two actual sites where sulfur denitrification was performed were surveyed. One is a valley bottom field, where groundwater contaminated with nitrate is coming up as spring water. The nitrate concentration in influent was about 45 mgN/L. The other was wastewater from a plastic greenhouse. The nitrate concentration in inflow water was about 200 mgN/L. Nitrate was almost removed by the containers packed with sulfur (S<sup>0</sup>)-CaCO<sub>3</sub> blocks in both sites. Increase of sulfate indicated that nitrate was removed by sulfur denitrification. This was also estimated stoichiometrically from the relationships between the removed nitrate and produced sulfate. The N<sub>2</sub>O was supersaturated in water at most sampling points and the highest concentration of dissolved N<sub>2</sub>O reached 900 µgN/L in Saitama in March. It seemed that insufficient nitrate removal caused accumulation of intermediates during denitrification, such as nitrite and N<sub>2</sub>O, in this month. However, the emission ratio of N<sub>2</sub>O to the removed nitrate during these processes was kept low, ranging from 0.01 to 0.19%, at both two sites throughout all surveys.

**Keywords** Agriculture; autotrophic denitrification; groundwater; nitrous oxide

## Introduction

Contamination of groundwater with nitrate is a serious problem, particularly in agricultural areas where nitrogen loading is generally high. The drinking water criterion for nitrate content is set at 50 mg/L (= 11.3 mgN/L) by the World Health Organization (WHO) due to the adverse effects on human health, such as methaemoglobinemia and stomach cancer, triggered by nitrite produced from nitrate. However, we can easily find many aquifers contaminated with nitrate far exceeding the WHO standard all over the world (Agrawal *et al.*, 1999; Singh *et al.*, 1995; Zhang *et al.*, 1996).

The high concentration of nitrate is a serious problem in itself; moreover, it has an added feature. The discharged nitrate is reduced to dinitrogen gas by microorganisms under anoxic conditions, termed denitrification, and nitrous oxide (N<sub>2</sub>O) is produced during the process as an intermediate. N<sub>2</sub>O has 280 times greater global warming potential (GWP) than that of CO<sub>2</sub> (IPCC, 1996). Many researchers have shown that soil-water systems, such as a marsh, wetland, and paddy field, are significant N<sub>2</sub>O sources (Minami and Fukushi, 1984; Buresh and Austin, 1988; Reddy, 1989; Paludan and Mathiensen, 1996). This gas emission from the natural environment due to denitrification could be enhanced by nitrate discharge (Kawashima *et al.*, 1996). Actually, our previous work showed the high fluxes of N<sub>2</sub>O gas to the atmosphere from an agricultural soil-water system consisting of paddy fields and a small river receiving groundwater contaminated with large amounts of nitrate (Hasegawa *et al.*, 2000).

Many treatment methods for nitrate have been developed to date, such as biological denitrification, ion exchange (Hradil *et al.*, 1997; Lin and Wu, 1997), electro dialysis (Hell *et al.*, 1998; Kesore *et al.*, 1997), and catalytic reduction (Reddy and Lin, 2000). Among these processes, biological nitrate removal by heterotrophic denitrification has

been investigated as well and applied in actual wastewater treatment plants. However, external organic matter is needed when applying this process in the natural environment where the level of organic matter is usually low. Instead of the heterotrophic process, sulfur denitrification has attracted increasing interest for nitrate removal. This process is based on autotrophic denitrification by sulfur-oxidizing bacteria (Baalsrud and Baalsrud, 1954; Kuenen, 1989; Kuenen *et al.*, 1992; Lee and Sublette, 1990; Robertson and Kuenen, 1992), and therefore is applicable in areas where organic materials are limited. During this process, nitrate is reduced as an electron acceptor, whereas reduced sulfur is oxidized to sulfate as an electron donor. Practical use of sulfur denitrification in several off-site plants for nitrate removal, such as a sulfur-limestone bed reactor, has been tried (Hashimoto *et al.*, 1987; Xiushan *et al.*, 1993; Furumai *et al.*, 1996; Koenig and Liu, 1997; Wang, 1998). On the other hand, there were few reports about on-site application of sulfur denitrification (Zhang and Flere, 1998). Moreover,  $N_2O$  emission during sulfur denitrification was not investigated well (Koenig and Liu, 1997).

We have already demonstrated the efficiency of sulfur addition into soil for nitrate removal through batch and column experiments. Sufficient removal of nitrate using a column packed with a mixture of soil and sulfur powder indicated the applicability of sulfur addition to an actual soil environment.  $N_2O$  emission was also measured and it was indicated that the emission of  $N_2O$  during sulfur denitrification was kept lower compared to that from the process using organic matter as an electron donor (Hasegawa *et al.*, 2000, 2001).

In order to understand applicability of sulfur denitrification process, surveys were conducted at two actual sites where sulfur denitrification was performed. The seasonal changes were investigated and the efficiency of the system was evaluated from the viewpoints of nitrate removal efficiency and  $N_2O$  emission.

## Materials and methods

### Field survey

Two actual fields where sulfur denitrification was performed for nitrate removal were surveyed. One is a valley-bottom field (Ibaraki, Japan), where groundwater contaminated with nitrate appears as spring water. Two 40 L containers packed with sulfur ( $S^0$ )- $CaCO_3$  blocks (SC11, Nittitsu, Japan) were connected in series and the spring water was led into the containers at about 200 mL/min. The air influx to the containers was prevented by using a lid. Surveys were conducted in June, October and December of 2001.

The other site was used for treatment of wastewater discharged from a plastic greenhouse, inside of which nitrate was used as fertilizer for growing roses (Saitama, Japan). The wastewater was led into a 12 m-long channel (width: 33.5 cm, height: 25 cm) at 150–350 mL/min. Most (0–10 m) of the channel was packed with sulfur- $CaCO_3$  blocks (SC11, Nittitsu, Japan) and the remaining part (10–12 m) was packed with zeolite. Lids full of pores were used so as not to cut air influx. Surveys were conducted in November and December of 2001, and in February and March of 2002.

The shapes of the sulfur- $CaCO_3$  blocks were not uniform, but the diameters of the blocks used at the two sites were similar, about 1 cm. During sulfur denitrification, pH decreases. Therefore,  $CaCO_3$  was mixed in a sulfur- $CaCO_3$  block. The weight ratio of elemental sulfur to  $CaCO_3$  was 1:1.2 in each block.

### $N_2O$ measurement

$N_2O$  gas flux from the water surface was measured using the closed chamber method (Minami and Fukushi, 1984; Ambus *et al.*, 1993). The chamber used in Ibaraki was cuboid (40.3 L, 0.32 m  $\times$  0.42 m  $\times$  H0.30 m) made of polyethylene. The chamber was placed on the

container and a 1 L sample was taken from the gas phase into a sampling bag (Tedlerbag, GL Science, Japan) by an air pump at 15 minute intervals. In Saitama, an acrylic cuboid chamber (31.5 L, 0.42 m × 0.25 m × H0.30 m) was used in the same way.

The concentration of dissolved N<sub>2</sub>O in water was analyzed by the head-space method. A water sample (30 mL) was collected from each sampling point gently into a 50 mL glass vial without release of N<sub>2</sub>O from the water phase. Fixed quantity (1 mL) of Hibitane (5 w/v% gluconate solution, Sumitomo Pharmacy, Japan) was added for inactivating bacteria. Then the vial was sealed with a butyl rubber septum and an aluminum seal and shaken. After letting it stand for 1 hour in 20°C, it was used for analysis. When N<sub>2</sub>O was analyzed, 0.5 mL of the sample was taken from the sampling bag or gas phase of the vial and injected into GC-ECD (8AIE, Shimadzu). The analytical condition of the GC was as follows. A glass column (I.D. 2.6 mm, 3.0 m) packed with Polapak Q (80/100 mesh) was used. The temperature of the detector and the column was 340°C and 70°C, respectively. Nitrogen gas was supplied as a carrier gas at 30 mL/min.

### Other analysis

Water samples were filtrated using a cellulose-acetate filter (pore size: 0.45 µm, Advantec, Japan) in the field. Nitrate (NO<sub>3</sub><sup>-</sup>), nitrite (NO<sub>2</sub><sup>-</sup>), and sulfate (SO<sub>4</sub><sup>2-</sup>) were determined using a capillary ion analyzer (CIA, Q-4000, Waters, USA). Total organic carbon (TOC) in the filtrate was analyzed using TOC-500 (Shimadzu, Japan). Dissolved oxygen (DO) and pH were measured using electrodes (TOA, Japan) in the field.

### Results and discussion

Table 1 shows the results of field surveys in Ibaraki. The concentration of nitrate in the inflow water was kept at around 45 mgN/L. The nitrate concentration decreased with an increase in sulfate concentration along the water flow. This indicated that nitrate was removed by the process of sulfur denitrification. Inflow nitrate was almost removed through this process. However, removal efficiency of nitrate seemed to be influenced by water temperature. When the water temperature was reduced in December, 38% of inflow nitrate remained in outflow water, whereas 89.2% of inflow nitrate was removed in June. It was indicated from pH values that CaCO<sub>3</sub> worked well as buffer and prevented pH decline due to sulfur denitrification.

The concentration of dissolved N<sub>2</sub>O in water in equilibrium with ambient air is approximately 0.3 µgN/L. However, it is often observed that a much higher concentration of N<sub>2</sub>O exists in water, because N<sub>2</sub>O easily dissolves into water. As shown in the samples in December, N<sub>2</sub>O had already existed in the inflow water and decreased along the water flow. However, even in the outflow water, it was supersaturated. N<sub>2</sub>O gas flux was kept at around

**Table 1** Results of field surveys in Ibaraki

		June 25, 2001				October 16, 2001				December 3, 2001			
		in	1st	2nd	out	in	1st	2nd	out	in	1st	2nd	out
NO <sub>3</sub> <sup>-</sup>	(mgN/L)	44.4	29.6	12.8	4.8	46.4	33.9	16.3	10.9	44.1	40.0	22.3	17.2
NO <sub>2</sub> <sup>-</sup>	(mgN/L)	0.0	0.0	0.0	0.0	0.0	0.0	13.2	0.0	0.0	0.0	0.0	0.0
SO <sub>4</sub> <sup>2-</sup>	(mgS/L)	0.0	32.5	68.3	80.6	0.0	23.6	62.6	76.7	0.0	11.6	48.8	62.8
Dissolved N <sub>2</sub> O	(µgN/L)	-	-	-	-	-	24.3	-	-	14.0	12.2	6.7	6.3
N <sub>2</sub> O flux	(µgN/m <sup>2</sup> /min)	-	-	-	-	-	3.8	1.4	-	-	2.6	2.7	-
TOC	(mgC/L)	-	-	-	-	-	-	-	-	3.8	4.2	5.4	6.0
DO	(mg/L)	-	1.9	1.3	-	-	1.1	2.3	-	10.2	4.6	1.7	3.8
pH	(-)	-	6.0	-	-	-	6.0	-	-	6.0	6.5	6.0	6.8
Water temp.	(°C)	-	17.6	18.5	-	-	16.1	16.1	-	11.7	12.5	12.1	11.4

-: not measured

2.6  $\mu\text{gN}/\text{m}^2/\text{min}$ . In our previous work, it was shown that the  $\text{N}_2\text{O}$  flux from the paddy field receiving high concentration of nitrate (21.3  $\text{mgN}/\text{L}$ –24.5  $\text{mgN}/\text{L}$ ) ranged from 0.0 to 2.6  $\mu\text{gN}/\text{m}^2/\text{min}$ . The fluxes detected in Ibaraki coincided with these values (Hasegawa *et al.*, 2000).

Figure 1 shows the spatial changes of  $\text{N}_2\text{O}$  gas flux to the atmosphere, dissolved  $\text{N}_2\text{O}$ , and nitrate concentration in Saitama. As shown in Figure 1(c) and Table 2, nitrate was reduced along the water flow. Increase of sulfate indicated the occurrence of sulfur denitrification. The concentration of nitrate in inlet water was much higher than that of Ibaraki. More than 95% of inflow nitrate was removed in the 0–8 m part of the channel in November, December, and February. In March, the removal efficiency was dropped and 86.2% of inflow nitrate was removed in the 0–10 m part.

The temperature of the inlet water in Saitama was kept at around 18°C, because the wastewater was warmed inside the plastic greenhouse and discharged. However, it declined to about 10–12°C along the water flow. As shown in Table 2, pH values were kept at around 6.0 throughout the channel. The concentration of TOC in inlet water was around 9  $\text{mgC}/\text{L}$ . It increased at the fore part of the channel in November, whereas it was kept at around 7  $\text{mgC}/\text{L}$  in December and February. Although the air influx to the channel was not shut out, DO dropped below 1.0  $\text{mg}/\text{L}$  at the fore part of the channel.

Spatial changes of  $\text{N}_2\text{O}$  gas flux (a), dissolved  $\text{N}_2\text{O}$  (b), and  $\text{NO}_3^-$  (c) in Saitama are shown.  $\text{N}_2\text{O}$  gas flux was not measured in November of 2001.

The concentration of dissolved  $\text{N}_2\text{O}$  in the inlet water ranged from 8.8 to 251.0  $\mu\text{gN}/\text{L}$ . The concentration increased and reached a maximum value at around 4 m in the channel and decreased along the water flow.  $\text{N}_2\text{O}$  flux to the atmosphere ranged from 0.3 to 32.7  $\mu\text{gN}/\text{m}^2/\text{min}$  and showed the highest value at 4 m. Among four surveys, the highest and the lowest concentrations of dissolved  $\text{N}_2\text{O}$  were observed in March and February, respectively. The highest value of nitrite, 8.6  $\text{mgN}/\text{L}$ , was detected at the 4 m point in March. Therefore, it seemed that the insufficient nitrate removal caused the accumulation of intermediates, such as nitrite and  $\text{N}_2\text{O}$ , in March. The positive correlation between nitrite accumulation and  $\text{N}_2\text{O}$  production was particularly shown in wastewater treatment processes (Itokawa *et al.*, 2001; Schulthess and Gujer, 1996). The other indexes of water quality, such as water temperature and DO, were no different between March and the other months. Therefore, it was not clear what caused the decline of nitrate removal efficiency in March.

At both sites, the decrease of nitrate with increase of sulfate indicated that nitrate was removed by sulfur denitrification. The correlation between nitrate and sulfate is shown in

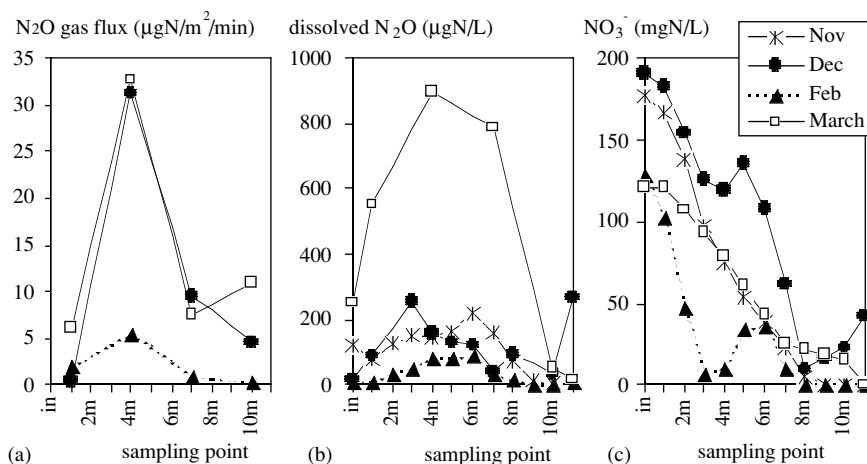


Figure 1 Results of field surveys in Saitama

**Table 2** Results of field survey in Saitama in February

		February 19, 2002											
		in	1 m	2 m	3 m	4 m	5 m	6 m	7 m	8 m	9 m	10 m	out
NO <sub>3</sub> <sup>-</sup>	(mgN/L)	129.5	102.5	48.1	7.0	10.6	34.5	36.8	10.4	0.0	0.0	0.0	0.0
NO <sub>2</sub> <sup>-</sup>	(mgN/L)	0.0	0.0	3.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SO <sub>4</sub> <sup>2-</sup>	(mgS/L)	33.1	39.1	75.8	149.6	193.5	213.5	213.3	284.4	307.5	314.8	304.2	312.6
Dissolved N <sub>2</sub> O	(μgN/L)	8.8	14.2	36.4	53.0	87.1	84.6	91.4	36.0	15.3	3.0	2.5	10.9
N <sub>2</sub> O flux	(μgN/m <sup>2</sup> /min)	-	2.0	-	-	5.6	-	-	1.0	-	-	0.3	-
TOC	(mgC/L)	-	-	2.2	4.6	4.2	4.4	3.2	6.9	7.7	7.5	7.8	7.9
DO	(mg/L)	7.0	5.2	2.4	0.8	2.6	1.5	1.9	0.3	0.2	-	-	-
pH	(-)	6.3	6.5	6.0	5.8	5.9	5.9	5.9	5.9	6.2	-	-	6.3
Water temp.	(°C)	19.0	12.4	12.0	13.0	14.2	12.4	13.6	12.8	13.4	-	-	12.0
NH <sub>4</sub> <sup>+</sup>	(mgN/L)	-	-	-	2.1	-	-	-	3.4	-	-	4.1	-

-: not measured

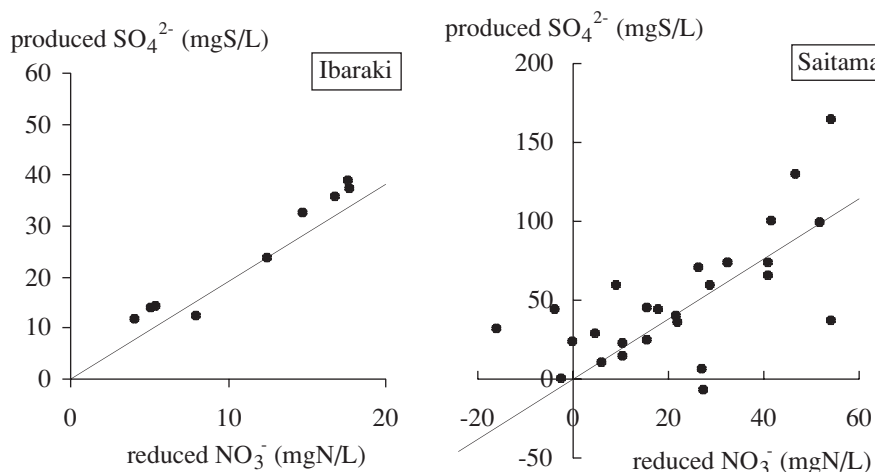
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Figure 2. Removed nitrate and produced sulfate were estimated for each sampling point in both sites. Theoretically, for reducing 6 mol NO<sub>3</sub><sup>-</sup> to N<sub>2</sub>, conversion of 5 mol S<sup>0</sup> to SO<sub>4</sub><sup>2-</sup> is needed. The inserted line in Figure 2 shows this theoretical ratio, 1.9. Actual data taken from two sites showed a similar trend to the theoretical ratio, whereas some data were placed on the upper part of the theoretical lines. This indicated that some amount of sulfur was oxidized by oxygen instead of nitrate by sulfur oxidizing bacteria.

The nitrate removal rate and N<sub>2</sub>O emission ratio are summarized in Table 3. Hydrological retention time (HRT) was estimated from the division of water volume by flow rate without the volume of sulfur-CaCO<sub>3</sub> blocks in the channel. Total N<sub>2</sub>O emission from the channel was sum of the emission at each point estimated by multiplication of the flux and the area of water surface at the point. N<sub>2</sub>O emission ratio was derived from division of the total N<sub>2</sub>O emission by the total amount of removed nitrate in the channel.

As shown in Table 3, HRT in Saitama was almost 10 times larger than that in Ibaraki. This seemed to cause a higher removal rate of nitrate in Saitama compared to Ibaraki. Although the HRT values were similar in Ibaraki among three surveys, the nitrate removal rate in December was much lower compared to the values in the other two months. This was caused by the decline of the water temperature in December.

Two figures show the results in Ibaraki (left) and Saitama (right). Closed circles show calculated data from sampling points. Inserted line shows the theoretical ratio of produced sulfate (mgS) to reduced nitrate (mgN), 1.9.

**Figure 2** Relationships between produced sulfate and reduced nitrate

**Table 3** Nitrate removal efficiency and N<sub>2</sub>O emission ratio at two sites

		Ibaraki			Saitama			
		June	Oct.	Dec.	Nov.	Dec.	Feb.	March
Flow rate	(mL/min)	200	220	219	345	145	219	240
Real HRT	(h)	4.1	3.7	3.8	24.9	59.3	39.3	35.8
Removal rate of NO <sub>3</sub> <sup>-</sup>	(mgN/min)	7.9	7.8	5.9	61.3	26.2	28.4	29.0
NO <sub>3</sub> <sup>-</sup> removal rate/container volume	(mgN/h/L)	1.2	1.3	1.0	1.5	0.3	0.4	0.5
Total N <sub>2</sub> O emission	(μgN/min)	–	0.70	0.71	–	45.1	8.2	53.7
N <sub>2</sub> O emission ratio to removed NO <sub>3</sub> <sup>-</sup> (%)		–	0.01	0.01	–	0.17	0.03	0.19

–: not measured, real HRT: estimated without volume of sulfur-CaCO<sub>3</sub> blocks in the channel

The N<sub>2</sub>O emission ratio ranged from 0.01 to 0.19% in Ibaraki and Saitama. This value was much lower compared to the other reports about N<sub>2</sub>O emission ratio during heterotrophic denitrification in soil-water environment and wastewater treatment processes (Spector, 1998; Hasegawa *et al.*, 2000). Koenig and Liu (1997) also reported small N<sub>2</sub>O emission ratio (<0.8%) during sulfur denitrification process in a packed bed reactor system.

### Conclusions and perspectives

The concentration of nitrate in the influent was much higher than the WHO standard at both sites. This nitrate was sufficiently removed by the sulfur denitrification system, consisting of containers packed with sulfur-CaCO<sub>3</sub> blocks. Decrease in nitrate with increase in sulfate indicated that the inflow nitrate was removed by sulfur denitrification. N<sub>2</sub>O existed in the water in supersaturated condition at both sites. However, the flux to the atmosphere was kept low. The N<sub>2</sub>O emission ratio to the removed nitrate was extremely low, ranging from 0.01 to 0.19%. Therefore, for nitrate removal with low N<sub>2</sub>O emission, application of sulfur denitrification is effective.

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