Effects of C/N, C/S and S/N ratios on TOC and nitrogen removal in the sulfate reduction-sulfur denitrification process

Ryoko YAMAMOTO-IKEMOTO and Tomoaki KOMORI

Department of Civil Engineering, Kanazawa University,
2-40-20 Kodatsuno, Kanazawa City, 920-8667, Japan

ABSTRACT

Effects of C/N, C/S and S/N ratios on TOC and denitrification removal were examined in the sulfate reduction-sulfur denitrification process using the two upflow biological filter reactors. High TOC and nitrogen removal ratio was obtained at C/N=2.2 and S/N=0.7 through the reactors. In the first reactor, sulfate reduction occurred predominantly. SRB mainly produced acetate at C/S=3.1. However, acetate was decomposed in the first reactor at C/S=1.6. In the second reactor, sulfur denitrification and heterotrophic denitrification occurred simultaneously. Sulfur denitrification occurred in the lower part of the reactor in S/N=0.7, and all sulfide produced in the first reactor was re-oxidized. On the other hand, sulfur denitrification also occurred in the upper part of the reactor in S/N=1.3, and 35 mg/gSS of sulfur granules were accumulated in the biofilm. The sulfur granules played an important role on denitrification. The activity of SDNB in the second reactor was about 3-4 times lower than the activity of DNB. When the S/N ratio increased, the activity of SDNB increased and those of DNB decreased. The number of SDNB was greater than those of DNB. The activity of SRB in the first reactor increased, when S/N ratio increased. SRB also grew in the anoxic second reactor, and the activity of SRB in the second reactor was relatively high. The sulfur cycle consist of sulfate reduction and sulfur denitrification in the second reactor might be established in the reactor.
KEYWORDS: Sulfur denitrification; sulfate reduction; nutrient removal, denitrification, anaerobic process.

INTRODUCTION

Nitrogen removal in the wastewater treatment process is very important to control of eutrophication in lakes and reservoirs. In the biological denitrification process, organic substances are sometimes insufficient for heterotrophic denitrification. Sulfur denitrification bacteria (SDNB), which utilize reduced sulfur for energy source, are also useful for nitrogen removal. However, since sulfide concentration in wastewater is low, the addition of reduced sulfur such as thiosulfate is need (Matsui and Yamamoto, 1986, Furumai et al., 1996, Koenig and Liu, 1996, Yamamoto-Ikemoto *et al*, 2000). On the other hand, sulfate reducing bacteria (SRB) grow in wastewater and activated sludge, and the sufficient concentrations of sulfate are contained in wastewater (Yamamoto *et al*, 1995, 1996). It is known that SRB can utilize many kinds of organic matters. When SRB reduce sulfate to sulfide, SDNB can utilize produced sulfide. Sulfate reduction-sulfur denitrification process is a new nitrogen and carbon removal process by using the sulfur cycle (Yamamoto-Ikemoto *et al*., 1998). The concepts of the process are shown in Fig. 1. The process consist of three phase, i.e.. sulfate reduction, denitrification and nitrification. In this process, since organic substances were converted to low molecular organic acids by SRB, wasted sludge decreased and TOC removal ratio increased. In this study, effects of denitrification and TOC removal on C/N and S/N ratio were examined in the sulfate reduction-sulfur denitrification process.

MATERIALS AND METHOD

Figure 1 shows the experimental set up. The process consisted of two upflow biological filter reactors packed with PP media. Returned sludge of a municipal plant was inoculated into the reactors. Synthetic wastewater consisted of acetate and peptone shown in Table 1 was fed to the bottom of the first reactor. When most of sulfate was reduced to sulfide in the first reactor, effluent of the first reactor was fed to the second reactor inoculated with activated sludge. Sodium nitrate solution was also added to the second reactor at the same flow rate of influent, assuming recycling water from the nitrification reactor followed by the second (denitrification) reactor. HRT of the first and second reactors were 1.5 and 3 hrs, respectively. Another experimental conditions are also described in Table 1.

Influent wastewater, effluent of the first rector, middle part of the second reactor and effluent of the second reactor were collected. Effluent of the sampling tap at the lower part of the second rector was also collected. The concentrations of nitrate, sulfate, sulfide and organic components in the filtered samples were measured. In the final of each Run, the batch experiments in the BOD bottles were carried out in the several conditions shown in Table 2. The activity of SRB was measured by the anaerobic batch experiment, using artificial wastewater as the substrate (S-1). To estimate the metabolism of SRB, sodium molybdate which was inhibiter of SRB, was added to the substrate (S-2). The activities of heterotrophic denitrification bacteria (DNB) and SDNB were measured by the anoxic batch experiments using acetate (S-3) and sulfide (S-5) and thiosulfate (S-6), respectively. In Run 3, S-4, in which no electron donor was added, was also used. The numbers of SRB, DNB and SDNB were measured by the MPN method.

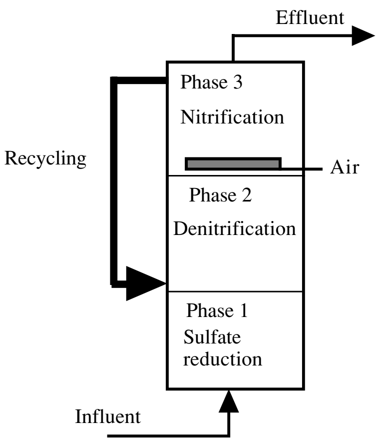


Fig. 1 Concept of sulfate reduction-sulfur denitrification process.

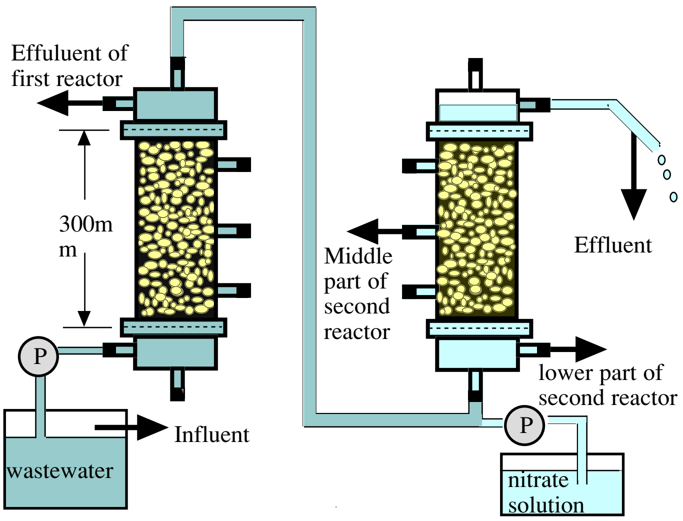


Fig. 2 Experimental set up and sampling points.

Table 1 Composition of artificial wastewater

Run	Run 1	Run 2	Run 3
Period	0-136	137-179	180-214
CH_3COOK	100	100	100
Polypeptone	200	200	200
Yeast Extract	20	20	20
$NaHCO_3$	71	71	71
KCl	174	174	174
$MgSO_4 \cdot 7H_20$	157	157	314
$CaCl_2$	51	51	51
KH_2PO_4	91	91	91
$MgCl_2$	61	61	61
$NaNO_3$*	110	220	220
C/N	4.4	2.2	2.2
C/S	3.1	3.1	1.7
S/N	1.4	0.7	1.3

*added in the bottom of the second rector.

Table 2 Substrates using the batch experiments

Substrate	S-1	S-2	S-3	S-4	S-5	S-5
CH_3COOK	300	300	300	0	0	0
Polypeptone	200	300	300	0		
Yeast Extract	40	40	40	0	0	0
$MgSO_4 \cdot 7H_20$	256	256	0	0	0	0
$Na_2S.9H_2O$	0	0	0	0	748	0
$Na_2S_2O_3.5H_2O$	0	0	0	0	0	750
$NaNoO_4$	0	48	48	48	48	48
$NaNO_3$	0	0	411	411	411	411
$NaHCO_3$	71	71	71	71	71	71
$CaCl_2$	51	51	51	51	51	51
KH_2PO_4	91	91	91	91	91	91
$MgCI_2$	61	61	61	61	61	60
condition	anaerobic		anoxic			

RESULTS AND DISCUSSIONS

Effect of C/N and S/N ratios on the reactor performance

Figure 3 shows the courses of the sulfate, nitrate and TOC concentrations. After 30 day's cultivation, sulfate in the effluent of the first reactor decreased and those of second reactor increased. These results indicated that sulfate was reduced in the first reactor and re-oxidized in the second reactor. Nitrate added to the bottom of the second reactor disappeared in the reactor, indicated that sulfur denitrification occurred in the second reactor in Run 1 (C/N=4.4, S/N=1.4). Just after increasing of nitrate concentration in Run 2 (C/N=2.2, S/N=0.7), sulfate increased over theoretical value, which was a half of influent concentrations, and nitrate disappeared in the reactor. It was considered that during Run 1, sulfur granules were accumulated in the biofilm, and the accumulated sulfur was oxidized in initial of Run 2, in which nitrogen concentration increased. Accumulated sulfur played an important role on nitrogen removal. However, when accumulated sulfur decreased, nitrate was remained in the effluent. When the sulfate in the wastewater increased in Run 3 (C/N=2.2, S/N=1.3), nitrate was removed in the reactor 2. TOC in the effluent of the first reactor decreased gradually. However most of sulfate was reduced in the reactor. TOC was also decreased in the second reactor by heterotrophic denitrification. Figure 3 shows the change of the organic compositions thorough the reactors. In Runs 1 and 2, sulfate was reduced and protein was decomposed to acetate in the first reactor. However in Run 3, acetate was decomposed in the first reactor. In the second reactor, heterotrophic denitrification and sulfur denitrification occurred simultaneously and most of nitrate was removed in Run 1. When the nitrate concentration increased in Run 2, mainly sulfur denitrification occurred in the lower part of the reactor, and heterotrophic denitrification occurred

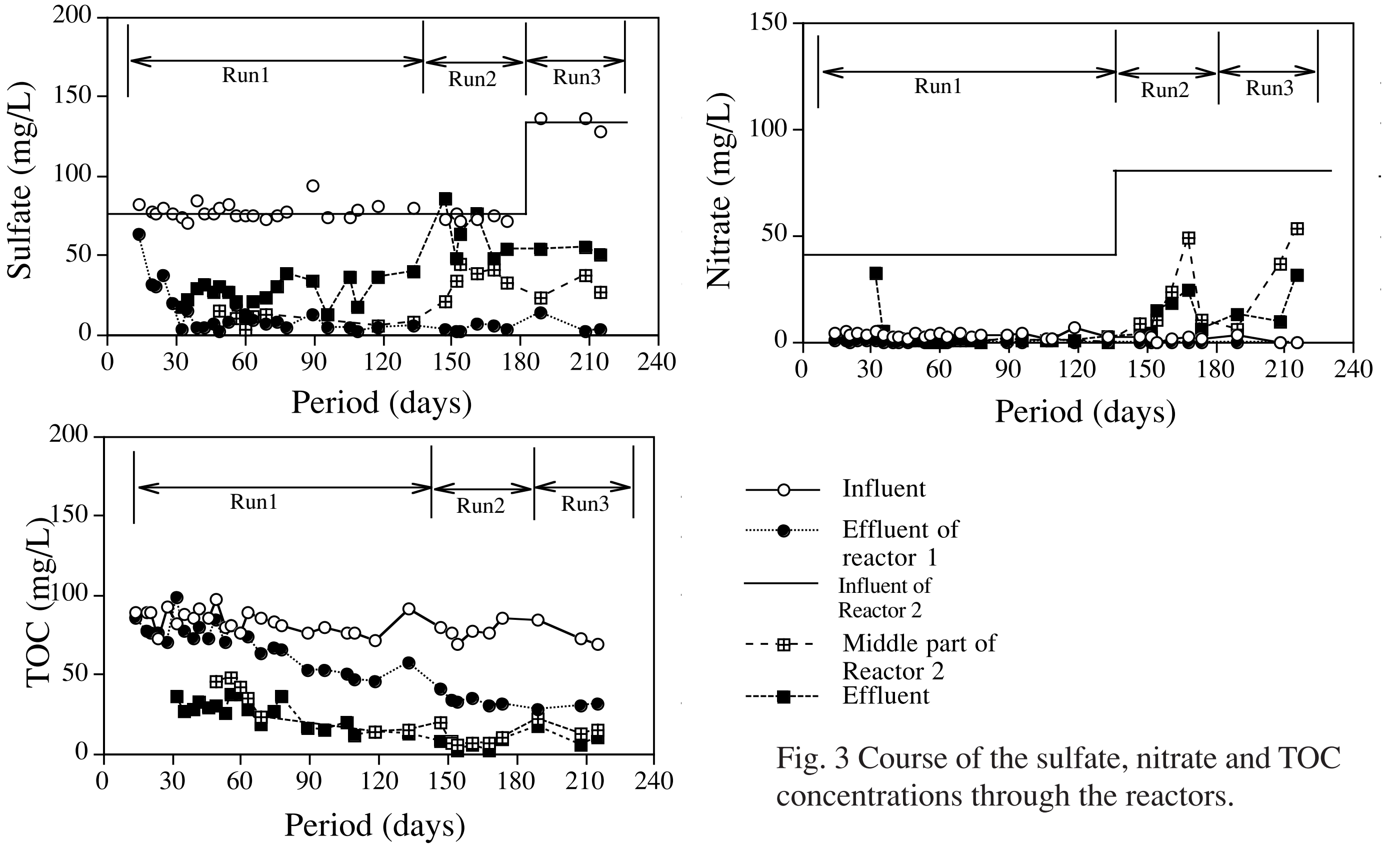
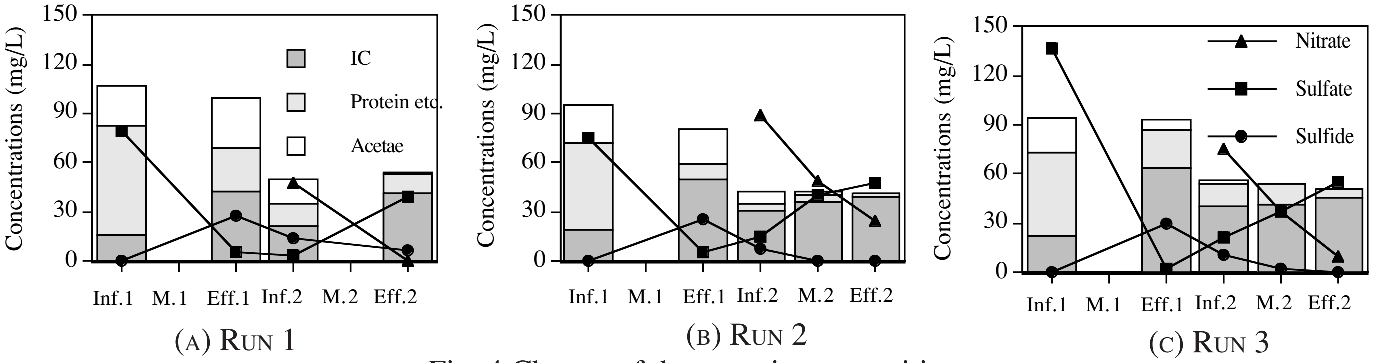


Fig. 3 Course of the sulfate, nitrate and TOC concentrations through the reactors.



(A) Run 1 (B) Run 2 (C) Run 3

Fig. 4 Change of the organic composition.

in upper part of the reactor. The sulfate concentrations in effluent were recovered to the theoretical value. On the other hand, in Run 3, sulfur denitrification occurred in the all part of the reactor. The sulfate concentration did not recovered, indicating that the sulfur granules were accumulated in the reactor. These results suggested that S/N ratio influenced on the competition of DNB and SDNB, and also sulfur accumulation.

Results of the batch experiments

Figure 5 shows the typical results of the batch experiments in the anaerobic conditions using the biofilm grown in the first reactor. Sulfate decreased and sulfide increased. The sulfate reducing rate was calculated from the decrease of sulfate. Figure 6 shows the typical results of the batch experiments of the second reactor. Although the reactor was operated in the anoxic conditions, sulfate reduction occurred in the anaerobic batch experiment using the substrate S-1. In the anaerobic batch experiments, sodium molybdate which was a toxicant of SRB, was also introduced in the substrate. When sulfate reduction was suppressed by sodium molybdate, the acetate concentration decreased and the propionate concentration increased. These results suggested that SRB play an important role in decomposition of propionate to acetate. It was reported (Yamamoto-Ikemoto *et al.* 1998) that in the sulfate reducing reactor of C/S=3.1, sulfate reducing bacteria produced acetate according to following equation,

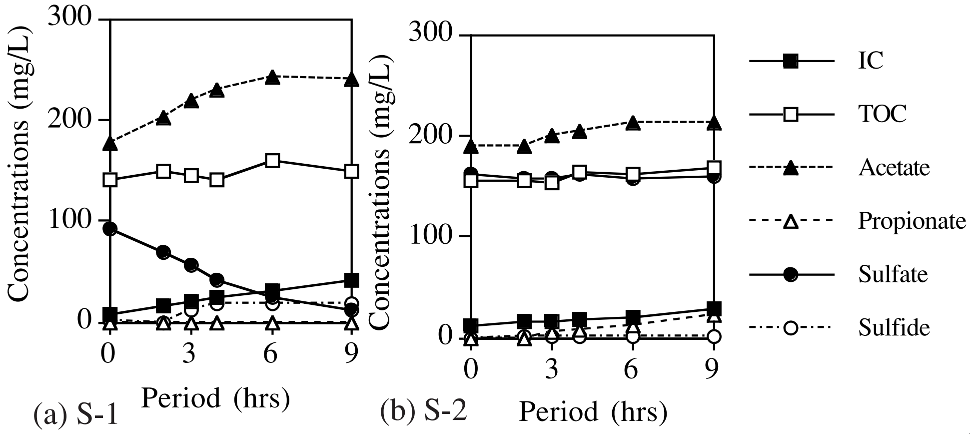


Fig. 5 The results of the batch experiment in the anaerobic conditions using the biomass in the first reactor of Run 2.

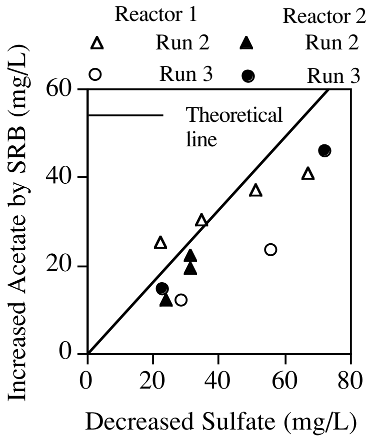


Fig. 7 Relationship between reduced sulfate and produced acetate by sulfate reduction.

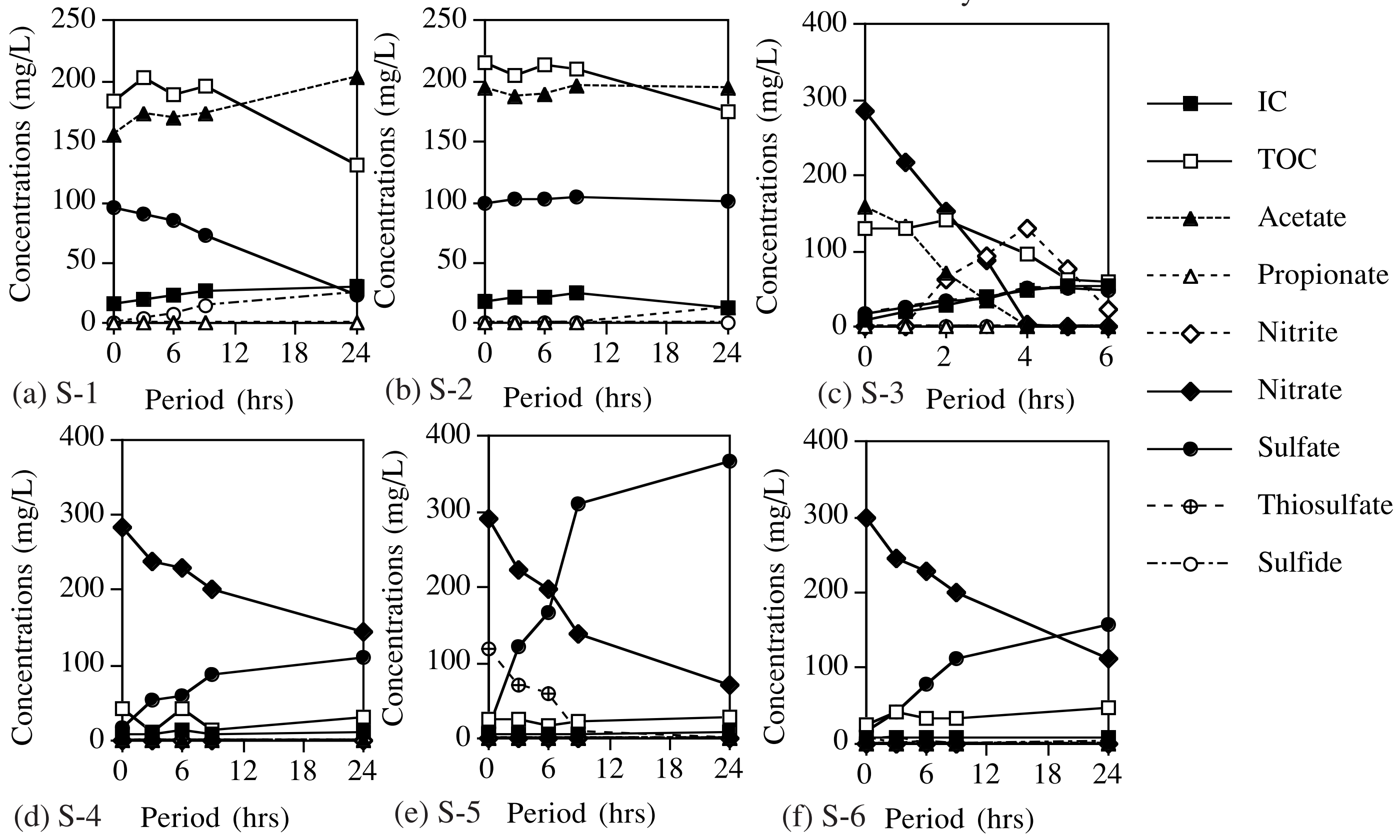


Fig. 6 The results of the batch experiment in the anoxic conditions using the biomass of the second reactor of Run 3.

$$4CH_3CH_2COO^- + 3SO_4^{2-} \rightarrow 4CH_3COO^- + 4HCO_3^- + H^+ + 3HS^- \quad (1)$$

From the deference of the increased acetate without molybdate and those with molybdate, acetate produced by SRB in the anaerobic batch experiment could be obtained. Figure 6 shows the relationship between reduced sulfate and produced acetate by SRB in the batch experiments. The line represent the theoretical line, at which SRB produced acetate by equation (1). The experimental results were agreed to the theoretical line, except of microorganisms in the reactor 2 in Run 3. These results suggested that propionate and/or hydrogen utilized SRB was predominant in the first reactor of Run 1 and 2 (C/S=3.1), and the second reactor. However in Run 3 of low C/S ratio (C/S=1.6), the acetate utilizing SRB increased in the reactor.

In the anoxic condition, denitrification occurred in the all batch experiments. Using the substrate S-4, in which no electron donor contained, sulfate increased by denitrification. It was understood that accumulated sulfur granules in the biofilm was utilized as an electron donor by SDNB. Accumulated sulfur was estimated as 35 mg/gSS. When acetate and peptone were added to the substrate (S-3), acetate and nitrate decreased immediately. Sulfate also increased, indicating that heterotrophic denitrification and sulfur denitrification occurred simultaneously. Therefore, the heterotrophic denitrification rate was calculated considering the utilized nitrate by SDNB form the amount of increased sulfate, according to the following equation.

$$5S^0 + 6NO_3^- + 2H_2O \rightarrow 5SO_4^{2-} + 3N_2 + 4H^+ \quad (2)$$

Figure 8 shows the relationship between utilized nitrate by DNB and decreased acetate in both batch experiments and reactor experiments. The experimental values well agreed with the theoretical line of following equation,

$$5CH_3COO^- + 8NO_3^- + 13H^+ \rightarrow 10CO_2 + 4N_2 + 14H_2O \quad (3)$$

These results suggested that DNB grown in the second reactor mainly utilized acetate for energy source. In this process, sulfate reduction, sulfur denitrification and heterotrophic denitrification were occurred stoichiometry. It might be important in the first reactor to promote acetate production by SRB.

When sulfide was added to the substrate (S-5), nitrate decreased and sulfate increased. Sulfur denitrification occurred theoretically in the following equation,

$$5HS^- + 8NO_3^- + 3H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O \quad (4)$$

Figure 9 shows the produced sulfate and decreased nitrate in the batch experiments using substrate S-5. Sulfur denitrification occurred according to Eq. (2). The denitrification rate coefficients were obtained from the nitrate decreasing rate form the decreasing of nitrate. When thiosulfate was added (S-6), sulfur denitrification also occurred stoichiometry according to following equation until thiosulfate was removed, as shown in Fig. 10.

$$5S_2O_3^{2-} + 8NO_3^- + H_2O \rightarrow 10SO_4^{2-} + 4N_2 + 3H^+ \quad (5)$$

After disappearing thiosulfate, sulfur granule also oxidized. Sulfur denitrification rate using thiosulfate was almost the same as those using sulfide.

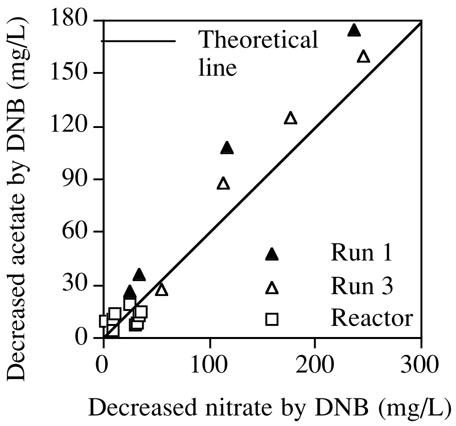


Fig. 8 Relationship between utilized nitrate by heterotrophic denitrification bacteria (DNB) and decreased acetate.

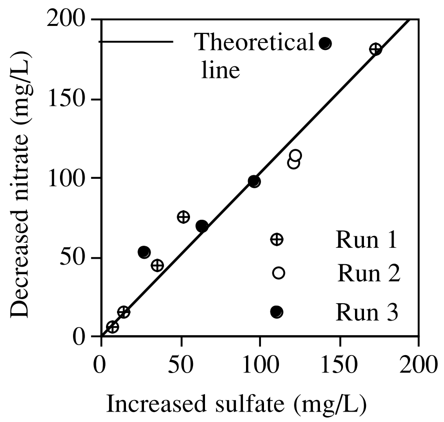


Fig. 9 Relationship between increased sulfate and decreased nitrate in the batch experiments using S-5 (Sulfide).

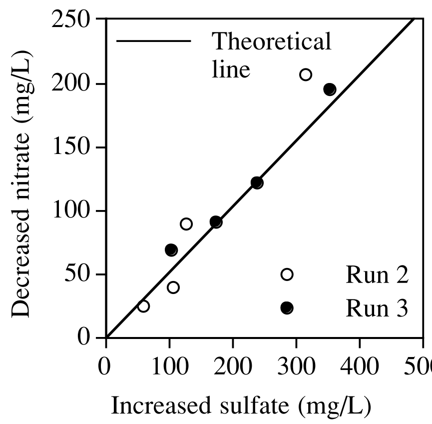


Fig. 10 Relationship between increased sulfate and decreased nitrate in the batch experiments using S-6 (thiosulfate).

Population change in the reactors

Table 3 shows the summery of the presence and activity of SRB, DNB and SDNB. The activity of SRB in the first reactor increased followed by decrease in C/S. In the second reactor, although the heterotrophic denitrification activity was four times as high as the sulfur denitrification rate, SDNB could coexist with DNB. The number of SDNB were greater than those of DNB. When C/N ratio decreased in Run 2, heterotrophic denitrification activity increased. When S/N ratio increased, sulfate reducing activity increased and heterotroph denitrification activity somehow decreased. The activity of SRB in the second reactor was almost the same as those in the first reactor. In the second reactor, sulfur cycle was established, and sulfur denitrification bacteria played an important role on nitrogen removal.

Table 3 Summery of the presence and activity of SRB, DNB and SDNB

Run No.	C/N	C/S	S/N	Presence ($x10^6$ MPN/gSS)				Activity ($mgSO_4$/gSS.hr, $mgNO_3$/gSS.hr)			
				Column 1	Column 2			Column 1	Column 2		
				SRB	DNB	SDNB	SRB	SRB	DNB	SDNB	SRB
Run 1	4.4	3.1	1.4	-	0.7	-	13.0	-	28.6	7.4	4.2
Run 2	2.2	3.1	0.7	-	-	13.0	84.0	9.1	41.6	6.5	3.5
Run 3	2.2	1.7	1.3	46.0	8.3	44.0	25.0	12.6	32.9	16.1	3.1

CONCLUSIONS

The results are summarized as follows:

1) High TOC and nitrogen removal ratios were obtained at C/N=2.2 and S/N=0.7 in the sulfate reduction-sulfur oxidation process

2) Sulfate reduction occurred predominantly in the first reactor. SRB mainly produced acetate at C/S=3.1. However, acetate decomposed in the first reactor at C/N=1.7.

3) Sulfur denitrification and heterotrophic denitrification occurred simultaneously in the second reactor. Sulfur denitrification occurred in the lower part of the reactor in S/N=0.7, and all sulfide produced in the first reactor was re-oxidized. On the other hand, sulfur denitrification also occurred in the upper part of the reactor in S/N=1.3, and 35 mg/gSS of sulfur granules were accumulated in the biofilm.

4) The activity of SDNB in the second reactor was about 3-4 times lower than the activity of DNB. When the S/N ratio increased, the activity of SDNB increased and those of DNB decreased. The number of SDNB was greater than those of DNB.

5) The activity of SRB in the first reactor increased, when S/N ratio increased. SRB also grew in the anoxic second reactor, and the activity of SRB in the second reactor was relatively high.

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