Application of Autotrophic Denitrification to Nitrate-Contaminated Industrial Wastewater

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An autotrophic denitrification system was applied to the treatment of industrial wastewater with very low C/N ratio. The experiments were conducted by both batch suspension and continuous column runs. The denitrification behavior in suspension runs obeyed the first-order reaction with the rate constants k_1 and k_2 of 0.014 and 0.004 m^3/g-SS•d, respectively. In the continuous runs, a column was packed with a mixture of granular sulfur and limestone. The microbes mostly composed of *Thiobacillus denitrificans* attached on the surface of sulfur particles like biofilm with an approximate thickness of 100 ìm. The denitrification rate in the packed column was expressed by a half-order equation with the rate constant of 0.172 g-N$^{1/2}$•m$^{1/2}$/(kg-S•d). Lower denitrification extent of industrial wastewater compared with synthetic wastewater was concluded to be caused by the lack of alkalinity.

Key Words: Autotrophic denitrification, Sulfur, Limestone, Packed column, *Thiobacillus denitrificans*

INTRODUCTION

The increasing of nitrate pollution in rivers and closed basins is partly caused by industrial activities. In many developing countries, problems in technologies and cost required for wastewater treatment bring about an illegal discharge of nitrate from plants, occasionally at a higher level than that regulated by the government. To suppress such a problem, development of simple and economical wastewater treatment technologies is required. Nitrate removal (hereafter *denitrification*) from industrial wastewater containing sufficient organic compounds is effectively performed by heterotrophic bacteria. However, for example, nitric acid-rinsing process wastewater, nitrate-contaminated groundwater, and nitrified landfill leachate have low organic carbon/nitrogen ratio,

and chemicals such as methanol are added to wastewater to promote the denitrification process. The cost of such chemicals lowers the efficiency of the heterotrophic denitrification method. In such a case, simple and economical denitrification with autotrophic bacteria is suitable. *Thiobacillus denitrificans* is a typical autotrophic bacteria which can reduce nitrate by employing various sulfur compounds as an electron donor without adding any organic compounds. Elemental sulfur is reported as the most economical electron donor because of its low price (Driscoll and Bisogni, 1978).

Autotrophic denitrification using elemental sulfur in a packed column has been applied for the treatment of nitrate-contaminated groundwater (Schippers et al., 1987), and of nitrified landfill leachate (Hano et al., 1996; Koenig and Liu, 2001; Koenig and Liu, 1996).

However, very few studies have reported on the denitrification of industrial wastewater. In this study, we investigated the feasibility of autotrophic denitrification system for nitrate-contaminated industrial wastewater. The basic kinetics of the denitrification reaction was evaluated both in batch suspension and continuous packed-column runs. Furthermore, the possibility of the present system in treating industrial wastewater was discussed.

MATERIALS AND METHODS

Acclimation of microbes

To obtain the sludge which can perform autotrophic denitrification, activated sludge taken from the municipal wastewater treatment plant was acclimatized by feeding synthetic wastewater under anaerobic condition for a long time. The composition of the synthetic wastewater for acclimation is shown in Table 1. Sodium thiosulfate was used as an electron donor and pH during the acclimation was maintained neutral by adding phosphate buffer.

Table 1. Composition of Synthetic Wastewater for Acclimation

Components	Concentration [g/m^3]
Nitrogen source	
KNO$_3$	144
Nutrient mineral	
NH$_4$Cl	148
MgSO$_4$.7H$_2$O	122
FeSO$_4$.7H$_2$O	10
NaHCO$_3$	1,000
Buffer component	
KH$_2$PO$_4$	1,500
Na$_2$HPO$_4$.12H$_2$O	3,500

Industrial wastewater characteristic

The industrial wastewater for treatment study supplied by a steel company was taken from an effluent of nitric-acid rinsing process after passing the coagulation and sedimentation treatment. The

composition of the industrial wastewater is described in Table 2. Trace amount of heavy metals such as nickel and chrome are also contained. In order to reduce inhibition effect during denitrification experiments, industrial wastewater was diluted ten-fold by tap water. In the experiments to elucidate the kinetics of denitrification, phosphate buffer and inorganic salts used for acclimation were added to the industrial wastewater.

Table 2. Composition of Nitric Acid-Rinsing Process Wastewater

Components	Concentration [g/m^3]
NO$_3$-N	430
NO$_2$-N	0.98
SO$_4$-S	14.5
PO$_4$-P	0.95
F	890
COD	2.5
Al	0.055
Fe	0.015
Cr	0.043
Cu	0.015
Zn	0.027
Ni	3.96
pH	6.9

Batch denitrification in suspension runs

The batch denitrification runs were performed in a 300 mL Erlenmeyer flask by mixing 100 mL of industrial wastewater and 100 mL of acclimatized denitrification sludge. Sodium thiosulfate was used as an electron donor. To keep the anaerobic condition, a flask was equipped with rubber lid after purging with nitrogen gas and shaken at 30°C during reaction. After sampling nitrogen gas was purged into a flask for 3 minutes to provide anaerobic condition.

Continuous operation of packed column

A column made of acrylic pipe, 42 mm in diameter and 620 mm in height, was filled with a mixture of granular sulfur and limestone (Fig. 1). Limestone was used to suppress the decrease of

alkalinity caused by proton released during autotrophic denitrification. The volume ratio of sulfur to limestone was set at 1:1. Both sulfur and limestone particles ranged in size from 2.8 to 4 mm. The weight of sulfur packed in the column was 423 g. Six sampling ports were installed on the wall of the column with 80 mm distance interval. After the denitrification activity of acclimatized sludge became appreciable, the sludge was loaded in and recycled through the column to settle microbes on the granules. At the beginning of the continuous column operation, synthetic wastewater was fed into the column at low flow rate to avoid the washout of microbes from the column. After the growth of biofilm

appeared and denitrification activity became steady, the feed was changed to industrial wastewater. Samples were taken from the inlet and outlet as well as from the sampling ports on the column wall. The temperature was maintained at 30°C in all runs.

ANALYSES

Nitrate, nitrite, and sulfate were analyzed by using an ion chromatography model Dionex AS4A-SC, equipped with CD and UV-detector. The alkalinity of the wastewater was determined by titration using 0.01 M H_2SO_4. Methyl red-bromcresol green solution was used as indicator.
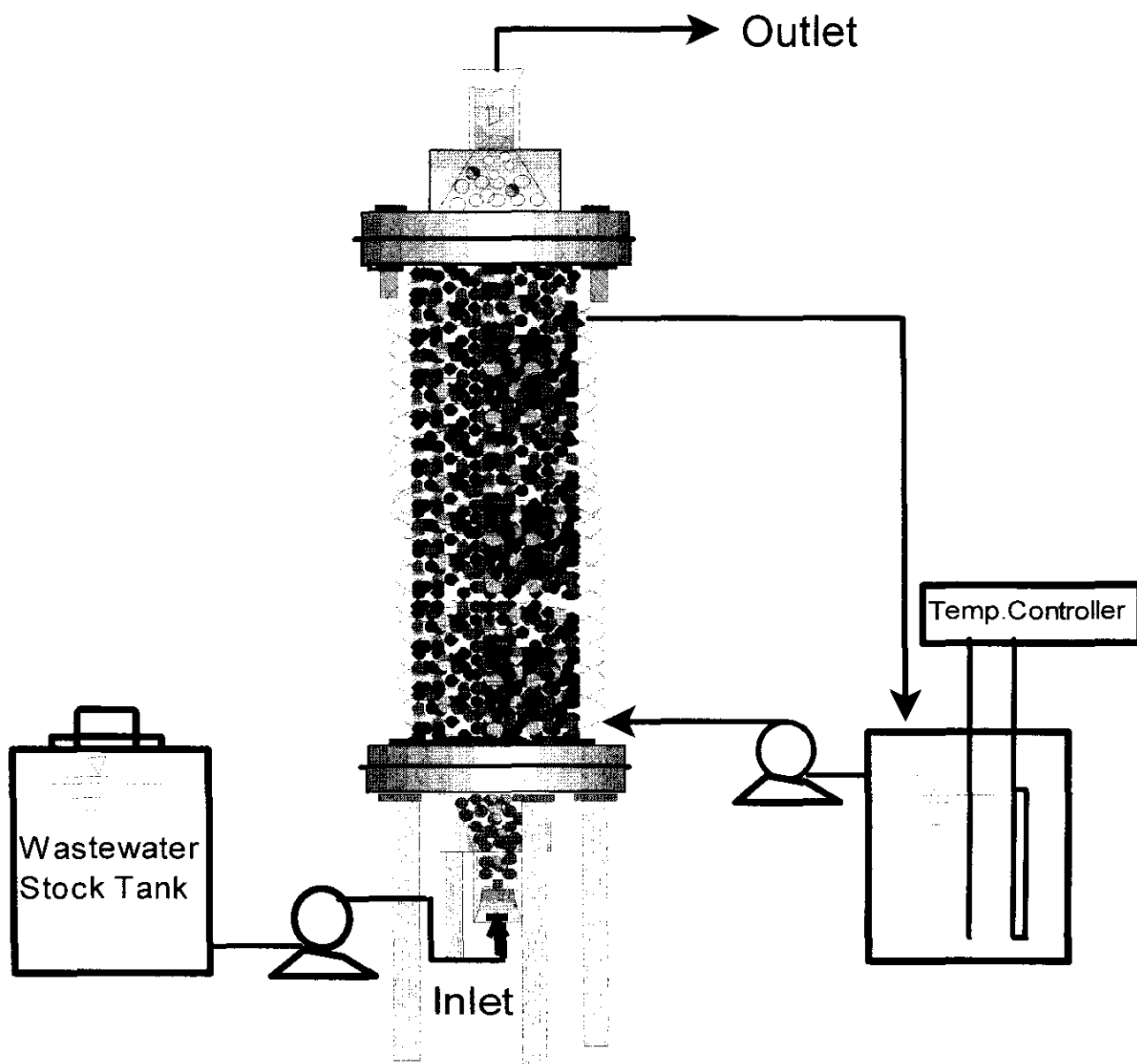

Figure 1. *Experimental apparatus – Packed Column Operation*

RESULTS AND DISCUSSION
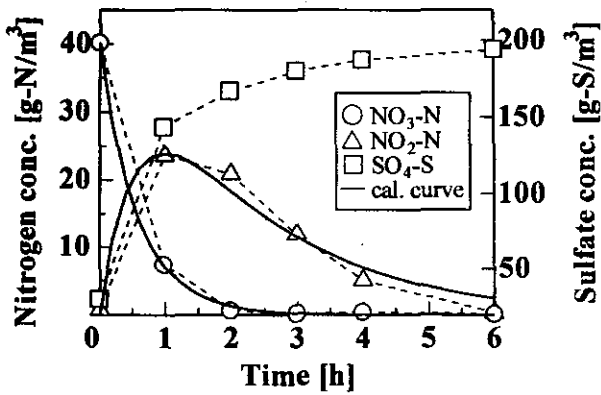
Kinetics of denitrification

Figure 2. Denitrification of Industrial Wastewater in Batch Suspension Run pH 7, MLSS 3,050 g/m³

The denitrification kinetics of industrial wastewater was evaluated both in batch suspension and continuous packed column runs. Fig. 2 shows the basic behavior of denitrification in batch suspension runs. Nitrate decreased rapidly from 40 to 8 g-N/m³ after 1 hour, while nitrite as an intermediate was produced and accumulated at a maximum concentration of about 20 g-N/m³. After 2 hour, nitrate concentration reached almost zero and nitrite gradually decreased to almost zero after 6 hour. Sulfate was produced in accordance with the nitrogen removal. Matsui and Yamamoto (1986) reported that both nitrate and nitrite reduction in autotrophic denitrification using thiosulfate as an electron donor follow the first-order reaction when synthetic wastewater was employed. Supposing the same kinetics for industrial wastewater; that is, nitrate is first reduced to nitrite by the rate constant k_1 and nitrite to nitrogen gas by the rate constant k_2 as shown in Eq. (1), the concentration of both components can be expressed by Eqs. (2) and (3).

$$NO_3^- \xrightarrow{k_1} NO_2^- \xrightarrow{k_2} N_2 \qquad (1)$$
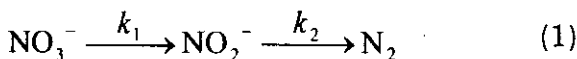
$$\frac{1}{X}\left(\frac{dC_{NO_3}}{dt}\right) = -k_1 C_{NO_3} \qquad (2)$$

$$\frac{1}{X}\left(\frac{dC_{NO_2}}{dt}\right) = k_1 C_{NO_3} - k_2 C_{NO_2} \qquad (3)$$

where X is mixed liquor suspended solid (MLSS) of denitrifying sludge. By integration of Eqs. (2) and (3), the concentration of nitrate and nitrite are derived as follows:

$$C_{NO_3} = C_{NO_{3_0}} e^{-k_1 Xt} \qquad (4)$$

$$C_{NO_2} = C_{NO_{3_0}} \frac{k_1}{k_2 - k_1}(e^{-k_1 Xt} - e^{-k_2 Xt}) \qquad (5)$$

where $C_{NO_{3_0}}$ is nitrate concentration at 0 h. By curve fitting procedure with Eqs. (4) and (5), the rate constants k_1 and k_2 were determined as 0.014 and 0.004 m³/(g-SS•d), respectively. Consequently, it is clear that the accumulation of nitrite is caused by the high reduction rate of nitrate compared with that of nitrite to nitrogen gas.
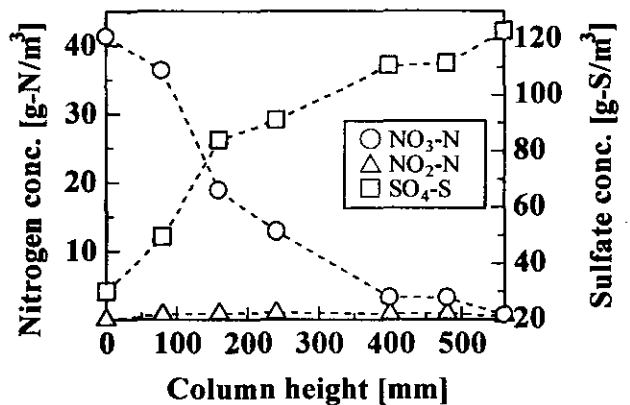

Figure 3. Denitrification of Industrial Wastewater in Packed Column Run HRT 3.5 h
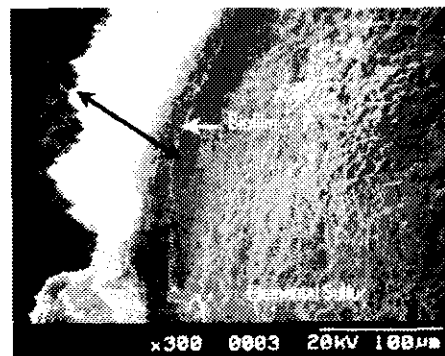

Figure 4. Biofilm Attached on the Surface of Elemental Sulfur

Figure 3 shows the nitrate, nitrite, and sulfate distribution in the column under empty-bed hydraulic retention time of 3.5 h. Nitrate concentration decreased along the axial distance of the packed column and reached almost zero at the exit. Sulfate gradually increased as the denitrification proceeded. Fig. 4 shows the scanning electron micrograph (SEM) of used granular sulfur sampled from the column. The biofilm that clearly formed on the surface of sulfur particles had a significant thickness of approximately 100 ìm even in dry state. Therefore, we assumed that the diffusion of nitrate through biofilm controlled the denitrification process. In this case, the apparent reaction order is $(n + 1)/2$, where n is the apparent reaction order in suspension runs. The denitrification reaction in the suspension runs discussed above was first-order. However, it was obtained by using thiosulfate as electron donor. To determine the reaction order of denitrification when sulfur particles were used in place of thiosulfate, separate batch runs were conducted by suspending sulfur particles in various initial nitrate concentrations. As a result, the denitrification rate was almost constant independent of the initial nitrate concentration, which led to the zero order kinetics (data not shown). Therefore, experimental results of packed-column were examined with an equation derived from the mass balance and apparent half-order rate equations. Reactor residence time is expressed as W_s / v_o, with the equation as follows:

$$\sqrt{C_{NO_3}} = \sqrt{C_{NO_{3_0}}} - \frac{1}{2}k\frac{W_s}{v_0} \qquad (6)$$

where W_s is weight of sulfur particles and v_o is influent flow rate. Figure 5 shows the correlation between square root of nitrate concentration in the column and W_s/v_o. Experimental results clearly satisfied Eq. (6). The half-order reaction rate constant k was calculated from the slope of linear correlation in Fig. 4 as 0.172 g-N$^{1/2} \cdot$m$^{1/2}$/(kg-S$\cdot$d). Such half-order denitrification behavior in a packed column was also reported by Harremous (1976) using heterotrophic bacteria, and by Koenig and Liu (2001) for the denitrification of landfill leachate in a sulfur-packed column.
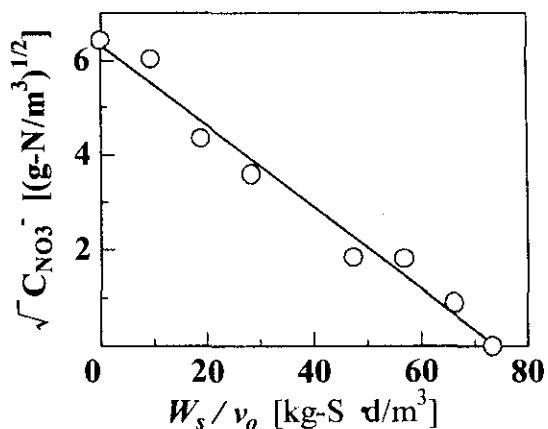

Figure 5. Correlation Between Square Root of Nitrate and Weight of Sulfur Particles in Packed Column
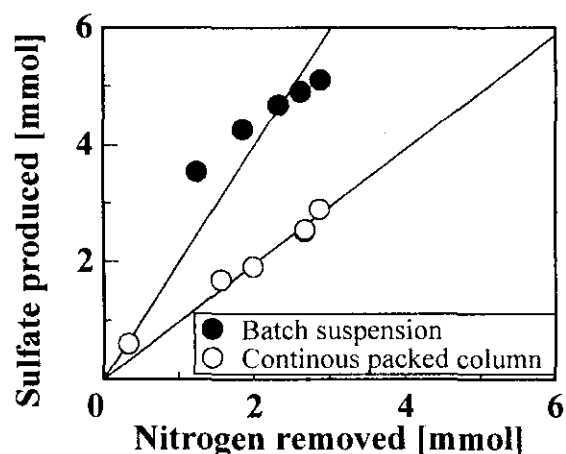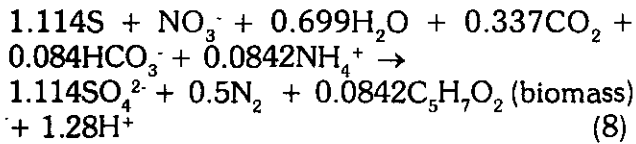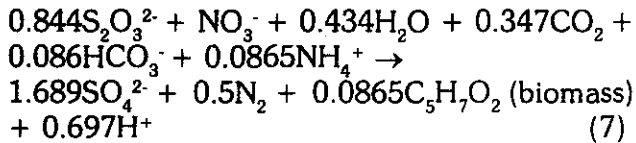
Stoichiometry of autotrophic denitrification

Figure 6. Correlation Between Nitrogen Removed and Sulfate Produced

In the autotrophic denitrification using *Thiobacillus denitrificans*, sulfate is produced with the progress of nitrogen removal. Fig. 6 shows the relation between sulfate produced and nitrogen removed in both batch suspension and continuous packed column runs. The slopes of the correlating line were about 1.9 and 0.98, respectively. In the denitrification reaction using *Thiobacillus denitrificans*, it is reported that the mole ratio of sulfate produced to nitrogen removed was 1.69 when thiosulfate was used as an electron donor, as predicted by Eq. (7) (Bisogni and Driscoll, 1977). However, when the electron donor was elemental sulfur, the ratio was 1.114, as by Eq (8) (Driscoll and Bisogni, 1978).

$$0.844S_2O_3^{2-} + NO_3^- + 0.434H_2O + 0.347CO_2 +$$
$$0.086HCO_3^- + 0.0865NH_4^+ \rightarrow$$
$$1.689SO_4^{2-} + 0.5N_2 + 0.0865C_5H_7O_2 \text{ (biomass)}$$
$$+ 0.697H^+ \tag{7}$$

$$1.114S + NO_3^- + 0.699H_2O + 0.337CO_2 +$$
$$0.084HCO_3^- + 0.0842NH_4^+ \rightarrow$$
$$1.114SO_4^{2-} + 0.5N_2 + 0.0842C_5H_7O_2 \text{ (biomass)}$$
$$+ 1.28H^+ \tag{8}$$

The results of experiments are close to the values reported in the literatures. This finding suggests that the dominant microbe existing in our sludge is *Thiobacillus denitrificans*. This microbes which known to have high resistance against pollutants such as heavy metals (Claus and Kutzner, 1985), is suitable for the treatment of industrial wastewater involving many kinds of pollutants.

Performance of packed column in the treatment of industrial wastewater
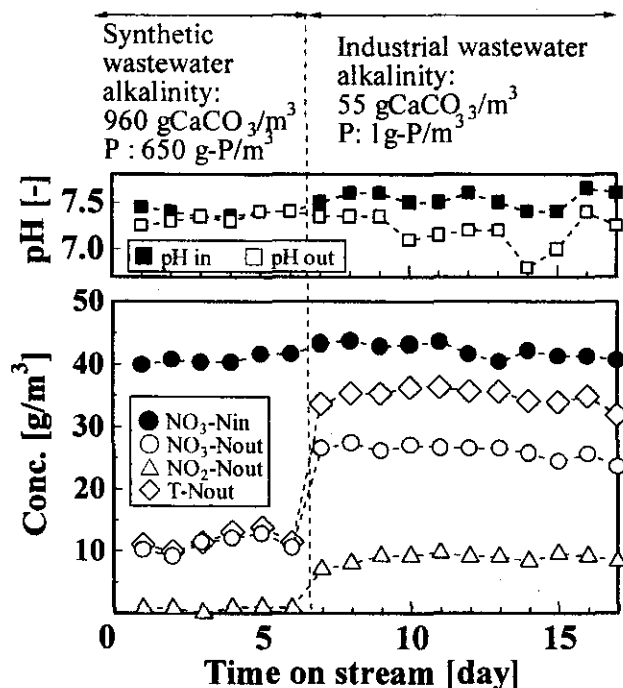

Figure 7. *Continuous Denitrification of Industrial Wastewater Under Low Initial Alkalinity HRT 2.3 h*

The performance of the packed column in treating industrial wastewater was evaluated by comparing with the treatment performance of synthetic wastewater which involved nitrate and

some minerals with phosphate buffer. The industrial wastewater for the present study was prepared by adding only KH_2PO_4 (1 g-P/m^3) to the original wastewater. The wastewater was fed into the packed column at a flow rate of 6 cm^3/min after finishing the runs with synthetic wastewater. Fig. 7 shows the transient behavior during feed change. After switching to the industrial wastewater, the total concentration of nitrate and nitrite nitrogen (hereafter referred to as *T-N*) in the effluent drastically increased, indicating a significant decrease of denitrification activity. Nitrite, which was not produced in treating the synthetic wastewater, was observed at constant level till the end of experiment. The differences of treatment conditions between synthetic and industrial wastewater are pH, phosphate concentration, and alkalinity. In the separate experiments, the effect of pH and phosphate concentration on denitrification were investigated. The optimum pH range observed was between 7 and 7.8. Under the constant pH, phosphate concentration, which ranged from 1 to 650 g-P/m^3, did not affect the denitrification activity. Therefore, both pH and phosphate concentration might not be the cause of the different denitrification behaviors in Fig. 7. The alkalinity of industrial wastewater was as low as 55 g-CaCO$_3$/m^3 under the condition of 40 g-N/m^3 nitrate. It is known that autotrophic denitrification consumes the alkalinity. According to Eq. (8), the consumption of alkalinity is calculated as 4.38 g alkalinity (as CaCO$_3$) per g-N of nitrate reduced. Therefore, it is difficult to provide high denitrification extent without supplying enough alkalinity. The limestone packed in the column was expected to supply the alkalinity consumption; however, findings revealed that denitrification rate of industrial wastewater was very low. Thus, the supplying of alkalinity by limestone was insufficient. To confirm this assumption, the substances deposited on the surface of limestone before and after use in the packed column were analyzed by energy dispersive X-ray (EDX). Table 3 shows that the fraction of calcium on the surface of limestone decreased drastically after runs due to the formation of phosphorus, sulfur, and ferrous deposits. Consequently, we considered that the formation of such deposits on the surface of limestone inhibited the supply of alkalinity.

Table 3. Composition of deposits on the surface of limestone before and after the use in packed column

Substances	Before use [%]	After use [%]
Ca	88.52	25.32
P	8.15	34.93
S	2.84	37.34
Fe	0.49	2.40

In order to confirm this idea, column runs were carried out by adjusting the alkalinity of the synthetic and industrial wastewater to the same strength. The composition of synthetic wastewater was the same with that used in Fig. 7, except with that with addition of phosphate buffer. Phosphorus concentration was set at 1 g-P/m^3. The nutrient minerals were added to industrial wastewater at the same concentration with those in the synthetic wastewater. Fig. 8 shows the comparison of the denitrification behavior between these two kinds of wastewater. The almost the same denitrification extent observed confirmed that insufficient alkalinity is the main reason for the drop of denitrification extent shown in Fig. 7.
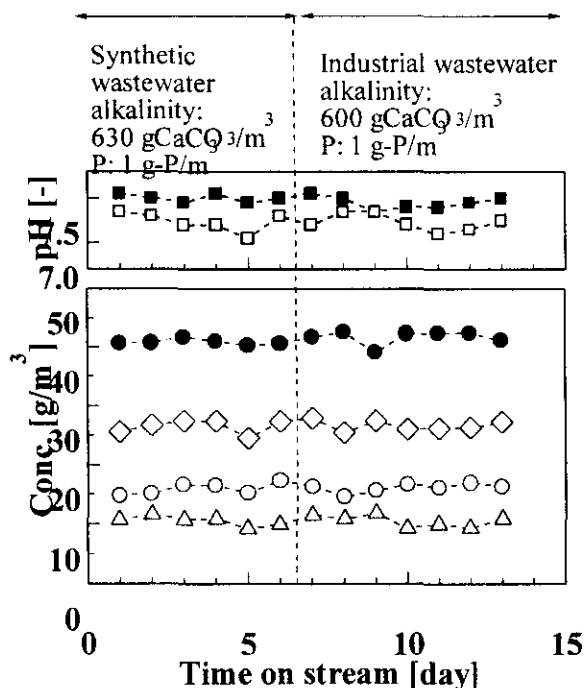

Figure 8. Continuous Denitrification of Industrial Wastewater Under High Initial Alkalinity HRT 2.3 h (Symbols are the same as those in Fig. 7)

Effect of coexisting components in industrial wastewater

As mentioned above, industrial wastewater from acid-rinsing process of steel company contains high concentration of nitrate, fluoride, and trace amount of heavy metals. To clarify the effect of such coexisting components, the batch experiments were conducted in suspension runs by mixing synthetic and industrial wastewater in various ratios. The industrial wastewater was used without dilution. The nitrate concentration in the synthetic wastewater was adjusted to the same level as that of the industrial wastewater. The initial alkalinity was set at 900 g-CaCO$_3$/m^3 in all runs. The fraction of industrial wastewater in mixed wastewater was changed from 0 to 100% by volume. The denitrification activity in the various mixing ratios is shown in Fig. 9. As clearly shown in this figure, T-N removal rate was almost the same for all mixing ratios, suggesting that any existing components in the industrial wastewater did not inhibit denitrification.

In separate batch experiments with synthetic wastewater, the effect of initial concentration nitrate, fluoride, and nickel were investigated. As the results showed, these components did not inhibit the denitrification at a level close to the actual concentration in the industrial wastewater (data not shown). It could be concluded that no inhibitors were present in wastewater from the acid-rinsing process of the steel company, and that autotrophic denitrification of industrial wastewater was possible even without dilution if the alkalinity is sufficient.

CONCLUSIONS

Denitrification system with autotrophic bacteria could satisfactorily remove nitrate from industrial wastewater. The kinetics of the denitrification reaction in suspension runs using sodium thiosulfate as an electron donor obeyed the first-order reaction. The stoichiometry of nitrogen removed and sulfate produced was close to that reported for *Thiobacillus denitrificans* in past studies. In the continuous runs using sulfur and limestone-packed column, the denitrification sludge formed a biofilm with a thickness of about 100 fÊm on the surface of granular sulfur. The decrease of nitrate along the axial distance of the

packed column obeyed the half-order kinetics with the slope proportional to the weight of the granular sulfur. The components in the present industrial wastewater did not inhibit the denitrification. Lower denitrification extent of industrial wastewater than that of synthetic wastewater, however, was due to lack of alkalinity. Finally, autotrophic denitrification of industrial wastewater was possible even without dilution, if the alkalinity was sufficient.

ACKNOWLEDGMENTS

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