

# Use of autotrophic sulfur-oxidizers to remove nitrate from bank filtrate in a permeable reactive barrier system

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**“Capsule”:** *An in situ biological reactive barrier system treating nitrate-contaminated bank filtrate is evaluated.*

## Abstract

This study was conducted to evaluate the potential applicability of an in situ biological reactive barrier system to treat nitrate-contaminated bank filtrate. The reactive barrier consisted of sulfur granules as an electron donor and autotrophic sulfur-oxidizing bacteria as a biological component. Limestone was also used to provide alkalinity. The results showed that the autotrophic sulfur oxidizers were successfully colonized on the surfaces of the sulfur particles and removed nitrate from synthetic bank filtrate. The sulfur-oxidizing activity continuously increased with time and then was maintained or slightly decreased after five days of column operation. Maximum nitrate removal efficiency and sulfur oxidation rate were observed at near neutral pH. Over 90% of the initial nitrate dissolved in synthetic bank filtrate was removed in all columns tested with some nitrite accumulation. However, nitrite accumulation was observed mainly during the initial operation period, and the concentration markedly diminished with time. The nitrite concentration in effluent was less than 2 mg-N/l after 12 days of column operation. When influent nitrate concentrations were 30, 40, and 60 mg-N/l and sulfur content in column was 75%, half-order autotrophic denitrification reaction rate constants were  $31.73 \times 10^{-3}$ ,  $33.3 \times 10^{-3}$ , and  $36.4 \times 10^{-3} \text{ mg}^{1/2}/\text{l}^{1/2}/\text{min}$ , respectively. Our data on the nitrate distribution profile along the column suggest that an appropriate wall thickness of a reactive barrier for autotrophic denitrification may be 30 cm when influent nitrate concentration is less than 60 mg-N/l.

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**Keywords:** Bank filtration; Autotrophic denitrification; Nitrate; Permeable reactive barrier; Sulfur-oxidizing bacteria

## 1. Introduction

Nitrate, which causes methemoglobinemia in infants and poses other health-related problems (Bouchard et al., 1992), is highly mobile in soil and diffuses easily into the subsurface environment. Conventional nitrate treatment technologies including ion exchange, reverse osmosis, electrodialysis, and distillation are mechanically complex, require periodical maintenance, and generally cost-prohibitive. The use of heterotrophic denitrification can be an alternative as a biological

treatment. However, addition of organic substrates is often inevitable since denitrification requires an ample amount of organic matter and organic matter concentration in the subsurface water is usually very low. It requires additional treatment cost and, more importantly, may result in secondary pollution of a water system. In order to circumvent such problems, an autotrophic denitrification system using sulfur-oxidizing bacteria has been considered. Elemental sulfur is non-toxic, water-insoluble, and stable under ambient environmental conditions. The autotrophic bacteria oxidize reduced other sulfur compounds (i.e.,  $\text{S}^{2-}$ ,  $\text{S}_2\text{O}_3^{2-}$ ,  $\text{SO}_3^{2-}$ ) as well as elemental sulfur to sulfate while reducing nitrate to nitrogen gas.

Considerable research has been conducted on sulfur-based autotrophic denitrification (Gayle et al., 1989) including (1) the treatment of nitrate-contaminated

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groundwater and surface water (Flere and Zhang, 1999; Schippers et al., 1987; van der Hoek et al., 1992; Zhang and Lampe, 1999), (2) nitrate treatment in wastewater and landfill leachate (Koenig and Liu, 1996, 2001a), (3) the kinetic study (Batchelor and Lawrence, 1978a,b; Koenig and Liu, 2001b; Justin and Kelly, 1978a,b), and (4) the effects of environmental conditions (i.e., aerobic or anaerobic) on sulfur/limestone autotrophic denitrification performance (Zhang and Lampe, 1999). The autotrophic denitrification system with sulfur/limestone and *Thiobacillus* sp. has been successfully applied in polluted groundwater (Kruithof et al., 1988; Gayle et al., 1989; Hiscock et al., 1991), wastewater effluent from a septic tank (Sikora and Keeney, 1976), and landfill leachate (Koenig and Liu, 1996).

Permeable reactive barrier (PRB) is an emerging technology for groundwater remediation, which has advantages over conventional remediation means such as pump-and-treat system. The PRB system is placed in the path of a migrating plume of contaminated groundwater, and reactive materials within the barrier are selected to induce biological or geochemical reactions resulting in the removal or treatment of groundwater contaminants. Over the past decade, a variety of PRB systems has been developed to treat acid mine drainage and inorganic/organic pollutants such as metals, nutrients, and chlorinated aliphatic hydrocarbons in groundwater (Blowes et al., 2000).

In Korea, bank filtrate has become increasingly contaminated with nitrate originating from fertilizers

and stockbreeding facilities; thus in many cases, the quality of bank filtrate can not meet the Korean drinking water standard of 10 mg-N/l. The objective of this study was to evaluate the potential applicability of an in situ biological reactive barrier system consisting of sulfur/limestone and autotrophic denitrifiers to treat nitrate in bank filtrate. As a part of the study, we performed bench scale tests (1) to observe the feasibility of autotrophic denitrification using elemental sulfur, (2) to determine the autotrophic nitrate removal efficiency at various nitrate concentrations, and (3) to understand the spatial distribution of ions such as nitrate, nitrite, and sulfate throughout the reaction column.

## 2. Materials and methods

### 2.1. Cultivation of microorganisms

*Thiobacillus denitrificans* was reported to exist as a facultative bacterium in a large variety of environments (Batchelor and Lawrence, 1978a,b). Sulfur-oxidizing bacterial consortium containing *Thiobacillus denitrificans* was kindly provided by Inha University (Inchon, Korea) for this study. The consortium was isolated from a tidal flat near Inchon, Korea. The culture was enriched in a liquid medium containing 2 g  $\text{KNO}_3$ , 5 g  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ , 2 g  $\text{K}_2\text{HPO}_4$ , 1 g  $\text{NaHCO}_3$ , 0.5 g  $\text{NH}_4\text{Cl}$ , 0.5 g  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , and 0.01 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ /l sterile distilled water at 30 °C (Koenig and Liu, 1996).

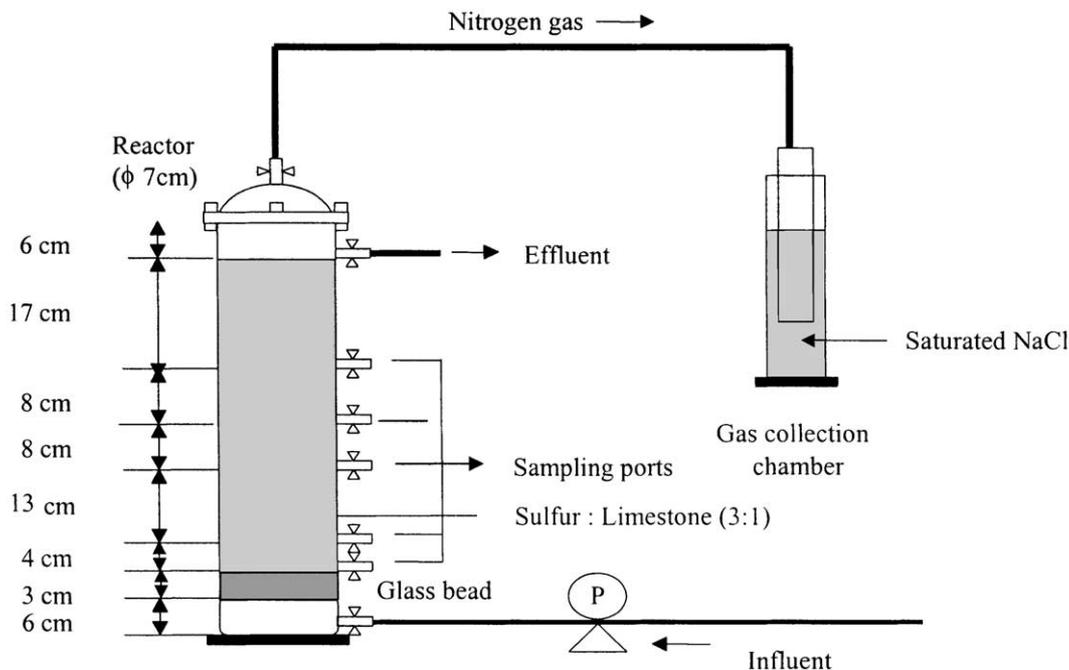


Fig. 1. A schematic of the column reactor used.

## 2.2. Experimental setup

The column reactors for autotrophic denitrification were made of Pyrex<sup>®</sup> with an inside diameter of 70 mm and a height of 700 mm. They were packed with elemental sulfur granules with 2-mm diameter and limestone with 2–5 mm-diameter at the volume ratio of 3:1 (Flere and Zhang, 1999). The granular elemental sulfur was provided from a local manufacturer (Miwon Commercial Co., Korea). Since autotrophic denitrification consumes alkalinity limestone was added to maintain the pH of the system at near neutral range. The enriched consortium prepared as described above was introduced into the packed bed reactors and recycled for three days for the microorganisms to attach on the sulfur particles. Colonization of the bacterial cells on the surfaces of sulfur particles was observed by scanning electronic microscopy (Ko et al., 1999).

Synthetic bank filtrate used for this study was a solution containing 0.217 g KNO<sub>3</sub> (30 mg-N/l), 0.05 g NH<sub>4</sub>Cl, and 0.06 g KH<sub>2</sub>PO<sub>4</sub>/l sterile distilled water. Limestone was the sole alkalinity source in the column. A column reactor was fed continuously in upflow mode with a seepage velocity of 1 m/day (flow rate: 1.0 ml/min) using a peristaltic pump to represent the flow of bank filtrate. All experiments were conducted at 20 °C. After the system approached at steady state, synthetic bank filtrate was artificially contaminated with nitrate at a level of 40 or 60 mg-N/l and introduced into column reactors from the bottom. A schematic diagram of the continuous flow packed-bed reactor is shown in Fig. 1.

## 2.3. Analytical methods

At intervals, the samples were collected from various points of the column through the sampling ports and filtered through a 0.45- $\mu$ m filter membrane for anion analysis. A Dionex 500 Ion Chromatography equipped with an AS14A anion column and a CD20 conductivity detector was used to analyze nitrite (NO<sub>2</sub><sup>-</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), and sulfate (SO<sub>4</sub><sup>2-</sup>) concentrations. Alkalinity and pH of the system were also monitored because the amount of sulfate produced by denitrification activity may exceed the regulation level of the ion and its accumulation may inhibit denitrifying activity of the consortium.

## 3. Results and discussion

### 3.1. Observation of microorganisms

A bacterial consortium containing *Thiobacillus denitrificans* is likely to be found on the surfaces of sulfur particles; hence, the surfaces were observed by

scanning electronic microscopy. As shown in Fig. 2, microorganisms were successfully colonized on the surfaces of the sulfur granules. A lot of microorganisms were found around the pores of sulfur particles. *Thiobacillus denitrificans* formed white colonies on a selective agar medium containing thiosulfate when grown for seven days at 30 °C. The chemical composition of the medium was the same as described in Section 2.1.

### 3.2. Effect of initial pH

The efficiency of denitrification is very sensitive to pH and an optimum pH of most denitrifying bacteria is known to be around 7 and 8 (Oh et al., 1999). Koenig and Liu (2001a) also reported that the highest autotrophic denitrification efficiency was observed at pH 7.0–8.0. Pure strains of *Thiobacillus denitrificans* showed an optimum growth at pH 7.5–8.0 when thiosulfate was used as a sole energy source (Claus and Kutzner, 1985). Since denitrification is a hydrogen ion (H<sup>+</sup>)-producing reaction, alkalinity should be provided to maintain the pH of the system and keep removing nitrate. Most effective and commonly used alkalinity source is sodium bicarbonate (NaHCO<sub>3</sub>). However, NaHCO<sub>3</sub> seems to be an unsuitable constituent for a reactive barrier because it is not a cost-effective and moreover, is provided as powder, which is not appropriate for a barrier-packing material. Therefore, limestone has been widely used as an alternative alkalinity (Kruithof et al., 1988; van der Heek et al., 1992; Wang, 1998; Flere and Zhang, 1999; Zhang and Lampe, 1999; Zhang and Shan, 1999).

Batch tests were conducted to investigate the effect of the initial pH on an autotrophic denitrification reaction. The initial pH values of systems were controlled by HCl and NaOH, and limestone was used as an alkalinity source. Fig. 3 showed nitrate removal profiles when initial pH varied from pH 5 to 9 at an initial nitrate

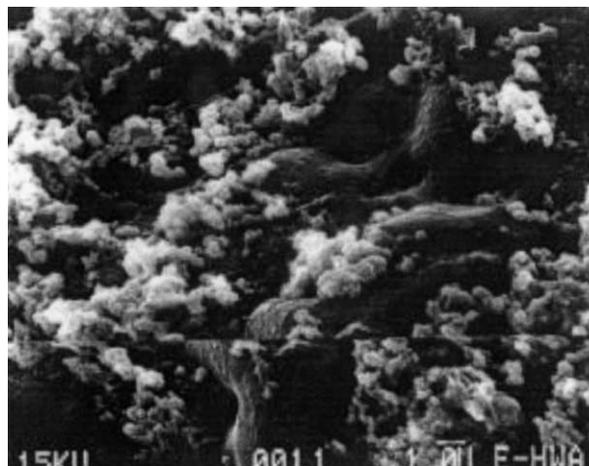


Fig. 2. Scanning electron microscopic image of a consortium containing *T. denitrificans* on sulfur granules ( $\times 5400$ ).

concentration of 40 mg-N/l. Significant decreases were observed at the whole pH range tested. The lowest nitrate concentration was observed at pH 7 and 8. Oh et al. (1999) reported that denitrification reaction was completely stopped at pH 6 and 9. Liu and Koenig (2002) also observed that nitrate removal was severely

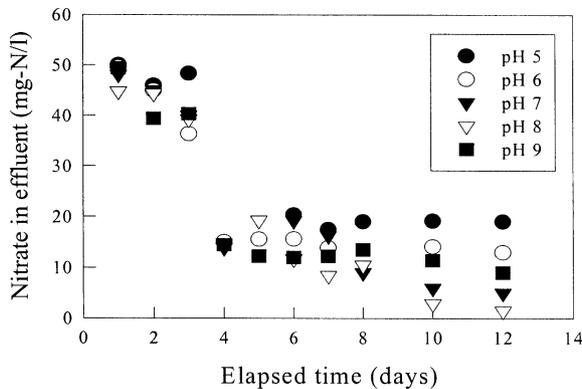


Fig. 3. Nitrate removal profiles with time at different initial pH values (initial nitrate concentration; 40 mg-N/l).

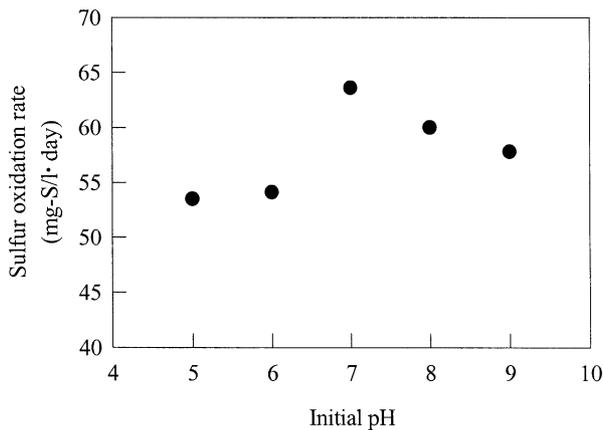


Fig. 4. Sulfur oxidation rates at various initial pH values (initial nitrate concentration; 40 mg-N/l).

inhibited at pH lower than 5.5 due to the shortage of limestone and the normalized specific denitrification rate was highest in the neutral pH range above 7.0. However, in this study, a remarkable decrease in nitrate concentration at those pH values was observed since we provided enough amount of limestone for adequate buffering capacity.

The sulfur oxidation rates were determined by conducting linear regression analyses on the at least three points from sulfate production profiles with time in each batch test. Fig. 4 showed that sulfur oxidation rate, an indirect indication of denitrification efficiency, was also the highest at pH 7 (64 mg-S/l-day). Our data suggest that maintaining pH at near neutral is one of the key aspects to obtain optimal nitrate removal efficiency in a biological PRB system using an autotrophic denitrification reaction.

### 3.3. Effect of initial nitrate concentration

In order to determine the effect of nitrate concentration on its removal efficiency, influent nitrate concentrations varying from 30 to 60 mg-N/l were tested with a hydraulic retention time (HRT) of 12 h. HRT is an important factor affecting denitrification efficiency because adequate contact time is needed among nitrate, sulfur-oxidizing bacteria, and sulfur particles. Claus and Kutzner (1985) reported a minimum HRT of 1.7 h for a sulfur/limestone autotrophic denitrification system was taken to achieve 80% of denitrification. Fig. 5 shows overall profiles of nitrate, nitrite, and sulfate concentrations in effluent at different influent nitrate concentrations. At a level of 30 mg-N/l, the nitrate was almost completely transformed to nitrite during the first four days of column operation, and nitrite accumulation was observed. After two days of the accumulation, however, the nitrite concentration slowly decreased, and the compound was finally detected less than 0.5 mg-N/l in 14 days. Transient nitrite accumulation was also

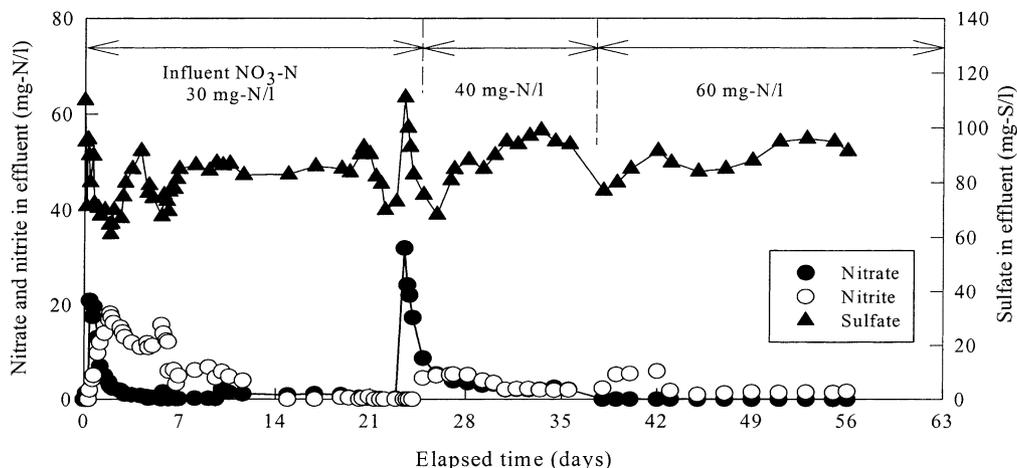


Fig. 5. Profiles of nitrate, nitrite, and sulfate concentrations with time at different initial nitrate concentrations.

reported previously (Furumai et al., 1996). Bisogni and Discoll (1977) and Matsui and Yamamoto (1986) reported that nitrate conversion to nitrogen gas was limited and the accumulation of nitrite was observed when the ratio of thiosulfate to nitrate was low. Oh et al. (1999) also reported that nitrate was incompletely denitrified due to a shortage of thiosulfate as an electron donor when the ratio of  $S_2O_3^{2-}/NO_3-N$  was less than 6.51.

After 23 days of column operation, the influent nitrate concentration was augmented to 40 mg-N/l while maintaining the other experimental conditions identical. As shown in Fig. 5, nitrate concentration rapidly decreased and detected less than 5 mg-N/l after initial two days of column operation. As before, effluent nitrite concentration increased and then decreased afterwards. Compared to the case of influent nitrate concentration of 30 mg-N/l, the rate of nitrate reduction increased. It is probably because the metabolic activity of the surface-attached consortium was more stabilized

or enhanced during the column operation. The pattern of nitrate removal was also very similar when 60 mg-N/l of nitrate were inflow. The effluent nitrate concentration was less than 3 mg-N/l after three days and further decreased. However, nitrite was not detected after one day of operation. Although influent nitrate concentration was increased from 30 to 60 mg-N/l, nitrate removal efficiency did not decrease and was maintained above 90% (Fig. 6). This indicates that the consortium was successfully adapted and its nitrate transformation activity was not significantly affected by an increase in nitrate concentration.

### 3.4. Characteristics of effluent

Changes in pH, sulfate concentration, and alkalinity in effluent were monitored with time when influent nitrate concentration was 30 mg-N/l and hydraulic retention time was 12 h (Fig. 7). Sulfate concentration

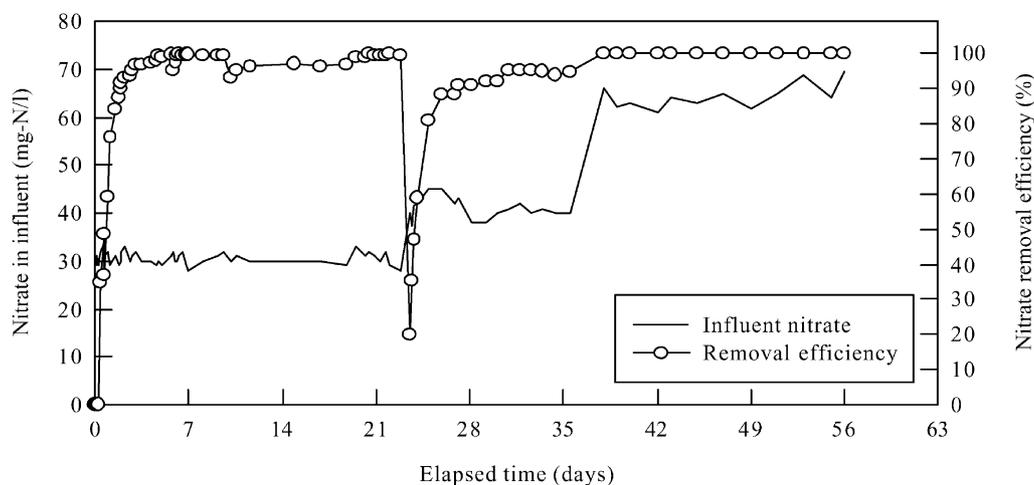


Fig. 6. Profile of nitrate removal efficiency with time at different nitrate concentrations.

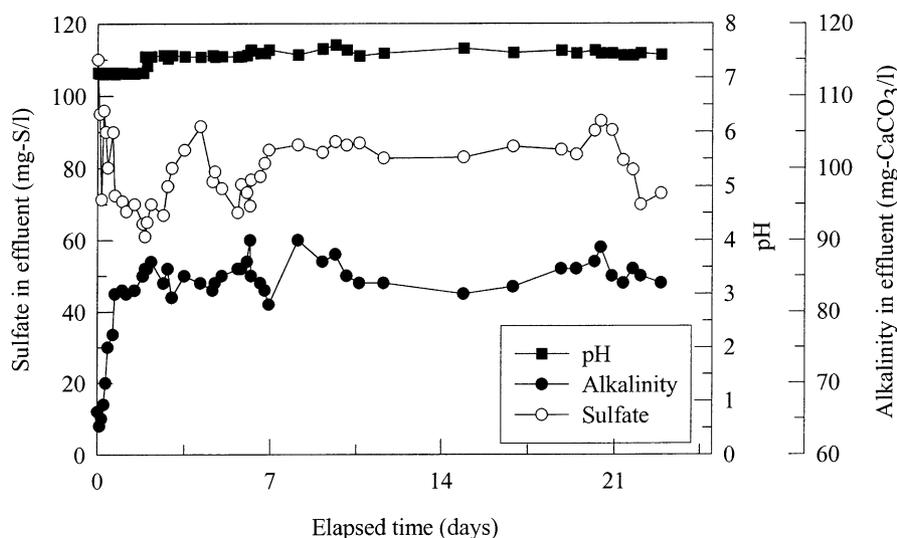
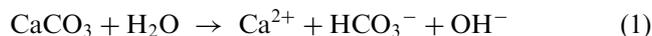


Fig. 7. Changes in pH, sulfate concentration, and alkalinity in effluent with time (initial nitrate concentration; 30 mg-N/l).

in effluent was between 70–90 mg-S/l. In Korea, the standard level of sulfate concentration for drinking water is 67 mg-S/l (i.e., 200 mg-SO<sub>4</sub><sup>2-</sup>/l), which is a little lower than the sulfate level obtained from this study.

Alkalinity and pH did not significantly change with time; moreover, their changes were positively related in response to the increase of nitrate concentration. Alkalinity and pH were maintained almost constantly after four days of operation; pH was stable at about 7.5 and alkalinity was 85–90 mg-CaCO<sub>3</sub>/l. Other researchers also observed a stable pH range (pH 6.9–7.5) in an autotrophic denitrification system (van der Hoek et al., 1992). The reason can be attributed to the use of limestone. Limestone was used both to neutralize the hydrogen ions produced during the autotrophic denitrification reaction and to provide an inorganic carbon source necessary to autotrophs. Since the pH range in autotrophic denitrification system falls between 7 and 8, dominant mechanism of limestone dissolution can be expressed as follows (Zhang and Shan, 1999).



During the limestone dissolution [Eq. (1)], one mole of CaCO<sub>3</sub> produces one mole of bicarbonate (HCO<sub>3</sub><sup>-</sup>) alkalinity and the hydroxyl ions (OH<sup>-</sup>) neutralize the hydrogen ions generated from an autotrophic denitrification reaction. Based on the stoichiometric equation of autotrophic denitrification, alkalinity of 4.57 mg-CaCO<sub>3</sub> is to be consumed for the removal of 1 mg NO<sub>3</sub><sup>-</sup>-N.

### 3.5. Spatial distribution of nitrate, nitrite, and sulfate

The spatial distributions of nitrate, nitrite, and sulfate during the denitrification process throughout the column were shown in Fig. 8. We determined the average ion concentrations after the reaction had approached to steady state at different initial nitrate concentrations. Almost all nitrate was removed in the area within 25 cm from the bottom of the column. In case of 30 mg-N/l

influent nitrate, a sharp accumulation of nitrite occurred up to the 17 cm of the column, and the nitrite level rapidly decreased to less than 2 mg-N/l. At 60 mg-N/l of initial nitrate, the highest nitrite concentration was observed at around 0 cm from the bottom. This shows that the introduced nitrate was immediately transformed to nitrite, but the nitrite concentration rapidly decreased and reached near zero at 33 cm of the column. The data suggest that nitrite reduction mainly occurred between 17 and 33 cm region of the column.

From the results of nitrate distribution at different column heights, we predicted autotrophic denitrification reaction rates in the test column at different initial nitrate levels using a mathematical model. The predominant phenomena associated with the autotrophic denitrification in sulfur-packed bed biofilm reactors are: (1) sulfur dissolution into the biofilm clinging to the surface of sulfur particles and diffusion through the biofilm; (2) nitrate diffusion from bulk liquid into the biofilm; (3) occurrence of autotrophic denitrification inside the biofilm; and (4) diffusion of reaction products (i.e., SO<sub>4</sub><sup>2-</sup>, N<sub>2</sub>, etc.) from the biofilm to bulk liquid (Koenig and Liu, 2001b).

Since the Monod saturation constant K<sub>s</sub> for autotrophic denitrification is very low [e.g., 0.2 mg/l as NO<sub>3</sub><sup>-</sup> (Claus and Kutzner, 1985); 0.03 mg/l as NO<sub>3</sub><sup>-</sup>-N (Batchelor and Lawrence, 1978a)], the intrinsic reaction inside the biofilm can be taken as zero-order reaction (Koenig and Liu, 2001b).

Based on the assumptions that the system is at steady state and substrate transport into biofilm follows Fick's diffusion law, the following equations for a substrate removal can be obtained using a simplified pore diffusion model (Harremoes, 1976; Jasen and Harremoes, 1985):

Zero-order bulk reaction:

$$R_a = k_{0a} \text{ valid for } \beta = \sqrt{\frac{2DC}{k_{0a}\delta}} \geq 1 \quad (2)$$

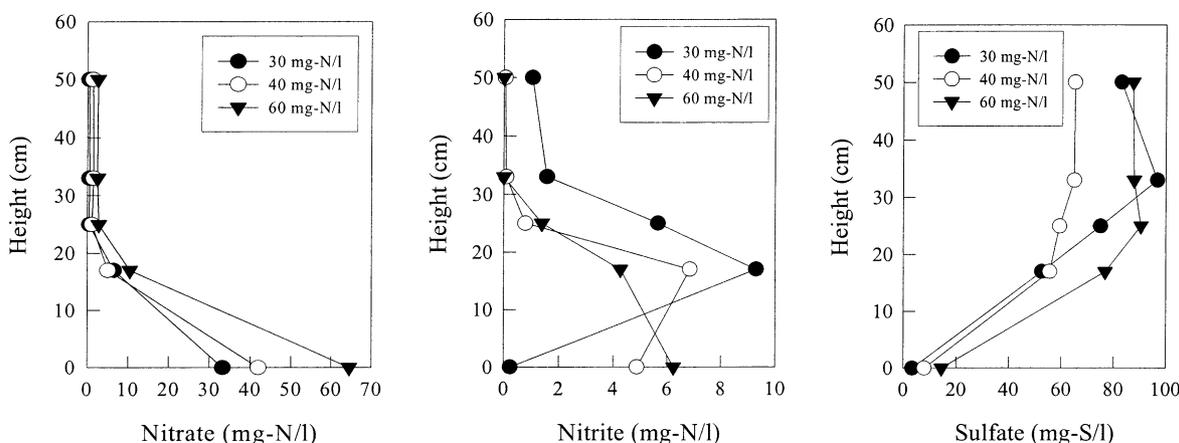


Fig. 8. Spatial distribution of nitrate, nitrite, and sulfate at different column heights.

Half-order bulk reaction:

$$R_a = k_{(1,2)a} C^{1/2} \text{ valid for } \beta < 1 \quad (3)$$

where,  $C$ : the bulk concentration of substrate at the surface of the biofilm (mg/l),  $D$ : the diffusion coefficient of substrate ( $\text{dm}^2/\text{h}$ ),  $k_{0a}$ : the zero-order reaction rate constant per unit biofilm area ( $\text{mg}/\text{dm}^2\text{h}$ ),  $k_{(1/2)a}$ : the half-order reaction rate constant per unit biofilm area, ( $\text{mg}^{1/2}/\text{dm}^{1/2}\text{h}$ ),  $R_a$ : the removal rate per unit biofilm area ( $\text{mg}/\text{dm}^2\text{h}$ ),  $\beta$ : the penetration ratio, and  $\delta$ : the thickness of the biofilm (dm).

Since the penetration efficiency of substrate into the biofilm pores is likely to be less than 100%, i.e.  $\beta$  is less than 1 [Eq. (3)], a zero-order reaction inside the biofilm translates into a half-order reaction at the exposed surface of the biofilm (Koenig and Liu, 2001b). Therefore, a half-order reaction was applied to predict the autotrophic denitrification in our system. This is also true for heterotrophic denitrification (Harremoos, 1976). Wang (1998) reported that when a mean nitrate bulk concentration was less than 43 mg/l, the data regression curve was parabolic indicating half-order reaction kinetics and when over than 43 mg/l, the curve was a straight line indicating zero-order kinetics.

Therefore, we assumed that a denitrification rate in the test column followed a half-order reaction and developed a mathematical mass transport model consisting of advection, dispersion, and biological reaction terms [Eq. (4)].

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} - r \quad (4)$$

where,  $v$ : average seepage velocity [ $\text{LT}^{-1}$ ],  $D$ : dispersion coefficient [ $\text{L}^2\text{T}^{-1}$ ],  $r$ : biological reaction rate [ $\text{ML}^{-3}\text{T}^{-1}$ ].

Before the column experiment was conducted, we determined the dispersion coefficient in the absence of bacterial activity. By fitting the points, we obtained the hydrodynamic dispersion coefficient of  $2.38 \times 10^{-4} \text{cm}^2/\text{sec}$  and the seepage velocity of  $1.05 \times 10^{-3} \text{cm}/\text{sec}$ , and the effective porosity of 0.38 (Fig. 9). Column Peclet number, which was determined from the seepage velocity, column length, and hydrodynamic dispersion coefficient of the column reactor, was 212, meaning that advection dominated the hydrodynamic of the column reactor (Shackelford, 1994). Therefore, dispersion seemed not to be a significant factor at the applied upflow velocity.

Using the least square method, we determined the half-order autotrophic denitrification reaction rate constants at different initial nitrate concentrations. As shown in Fig. 10, predicted and observed nitrate concentration profiles matched very well at different column heights and at various initial nitrate concentrations. When influent nitrate concentrations were 30, 40, and 60 mg-N/l and sulfur content in

column was 75%, half-order autotrophic denitrification reaction rate constants were  $31.7 \times 10^{-3}$ ,  $33.3 \times 10^{-3}$ , and  $36.4 \times 10^{-3} \text{mg}^{1/2}/1^{1/2} \text{min}$ , respectively. Koenig and Liu (1997, 2001b) obtained half-order autotrophic denitrification reaction rate constants of  $39.3 \times 10^{-3}$ – $60 \times 10^{-3} \text{mg}^{1/2}/1^{1/2}\text{min}$  in a packed column reactor filled with 100% sulfur with 2.8–5.6 mm of particle diameter.

### 3.6. Relationship between removed nitrate and produced sulfate

In this study, we observed that an increase of influent nitrate concentration did not always result in an increase of sulfate production. This is not consistent with the stoichiometric relationship describing a sulfur-based denitrification reaction (Batchelor and Lawrence, 1978a).

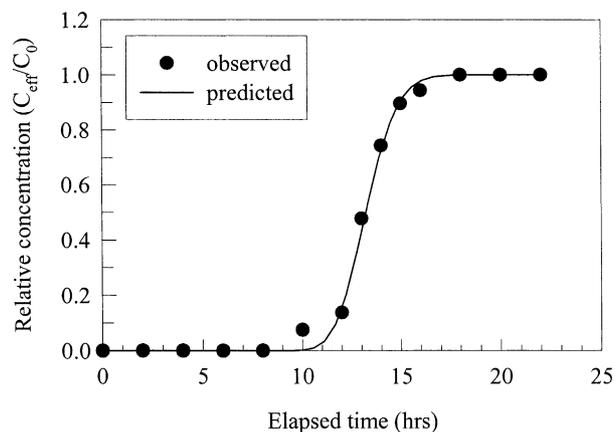


Fig. 9. Breakthrough curve of  $\text{NO}_3^-$  in column reactor.

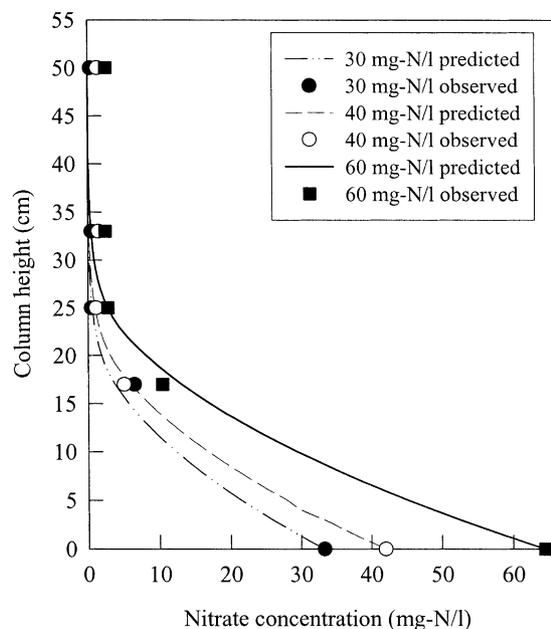


Fig. 10. Predicted and observed nitrate concentrations at different column heights.

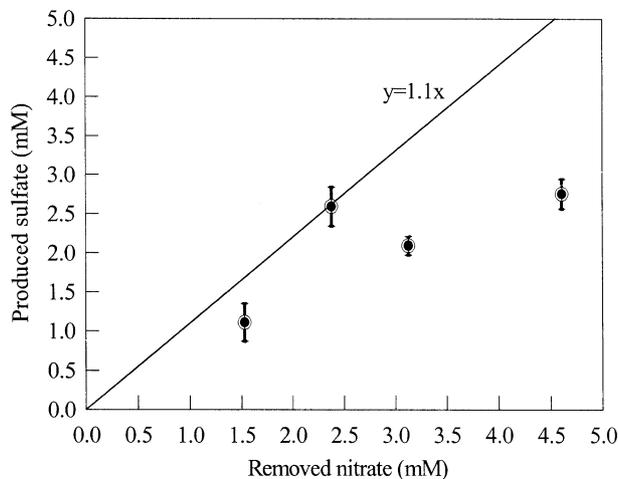
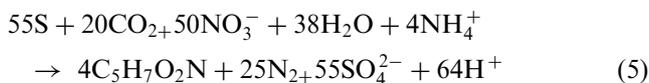


Fig. 11. Relationship in concentration between removed nitrate and produced sulfate (1.5, 2.5, 3.0, and 4.5 mM are equivalent to 20, 30, 40, and 60 mg-N/l, respectively).



At this moment, we cannot explain such discrepancy. However, one plausible reason is that some of the produced sulfate might have been converted to hydrogen sulfide after nitrate in influent had been exhausted. This view is in part supported by Fig. 8 showing the spatial distribution patterns of nitrate and sulfate.

In autotrophic denitrification, *Thiobacillus denitrificans* oxidizes elemental sulfur to sulfate while reducing nitrate to nitrogen gas under anoxic conditions. The stoichiometric equation [Eq. (5)] shows that 7.5 mg  $\text{SO}_4^{2-}$  is produced for every 1 mg  $\text{NO}_3^-$ -N reduced (Batchelor and Lawrence, 1978a,b). Other researchers presented similar ratios of  $\text{SO}_4^{2-}$  production to nitrogen removal such as 6.39 (Sikora and Keeney, 1976), 7.75 (Hashimoto et al., 1987), and 7.89 (Koenig and Liu, 1996). In this study, the ratio of produced sulfate to removed nitrogen ranged from 4.32 to 8.32 with an average of 5.74. Fig. 11 shows the relationship in concentration between removed nitrate and produced sulfate expressed in millimolar concentrations. The stoichiometric molar ratio of sulfate to nitrate based on Eq. (5) is 1.1, shown as the straight line in Fig. 11. Obtained experimental values were close to the theoretical values when the nitrate concentrations were 1.5 (i.e., 20 mg-N/l) and 2.5 mM (i.e., 30 mg-N/l). However, as nitrate concentration increased to 3 (i.e., 40 mg-N/l) and 4.5 mM (i.e., 60 mg-N/l), the amounts of produced sulfate significantly decreased, and they were plotted far below the straight line. As already discussed, the difference can be explained by the production of hydrogen sulfide, which is consistent with the detection of fouling odor through some sampling ports located at the end part of the column. The conversion of sulfate to sulfide

by sulfate-reducing bacteria requires organic matter; for instance, 1.33 mol of organic carbon are needed for the reduction of one mole of sulfate when methanol is used as a substrate (Maier, 2000). In our system, however, organic matter was not provided externally since the main reaction (i.e., denitrification coupled with sulfur oxidation) was performed autotrophically. Therefore, the carbon sources used for the sulfide production may have been supplied from organic acids produced by the sulfur-oxidizing bacteria present in the column (Zhang and Shan, 1999) and the organic debris originating from natural bacterial lyses.

#### 4. Conclusions

This laboratory study shows a biological permeable reactive barrier using the sulfur-based autotrophic denitrification process can successfully remove nitrate from synthetic bank filtrate. In addition, data suggest that barrier thickness of about 30 cm is appropriate when nitrate concentration in the influent is less than 60 mg-N/l at 1 m/day of a seepage velocity. However, it is worthwhile noting that the appropriate barrier thickness may vary depending on environmental factors, nitrate concentration, and bacterial activity so that it should be carefully determined on a site-by-site basis before field application. We believe that this is a cost-effective and viable technology to treat nitrate in bank filtrate and groundwater, and a year-round feasibility study is underway to draw kinetic parameters for a successful field application.

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