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Nitrate removal by *Thiobacillus denitrificans* immobilized on poly(vinyl alcohol) carriers

Zhenya Zhang[a], Zhongfang Lei[b,*], Xiaoyan He[a], Zhiyin Zhang[b], Yingnan Yang[a], Norio Sugiura[a]

[a] *Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba 305-8572, Japan*
[b] *Department of Environmental Science and Engineering, Fudan University, 220 Handan Road, Shanghai 200433, China*

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ABSTRACT

Nitrate contamination is becoming a widespread environmental problem, and autotrophic denitrification with *Thiobacillus denitrificans* is a promising process considering efficiency, cost and maintenance. The denitrification efficiencies of *T. denitrificans* were compared in batch reactors between free cells and cells immobilized on polyvinyl alcohol (PVA) carriers made with thrice freezing/thawing and boric acid methods. The results indicated that the free cell reactor of *T. denitrificans* added with 10% (v/v) of PVA carrier made by thrice freezing/thawing (PVA–TFT) exhibited faster in $S_2O_3^{2-}$–S consumption, SO_4^{2-} generation, and NO_3^-–N denitrification, with corresponding values being 165 mg ($S_2O_3^{2-}$–S)/L d, 491 mg (SO_4^{2-})/L d, and 44 mg (NO_3^-–N)/L d, which were increased by 50%, 61%, and 57% respectively compared to the control reactor with only free cells. Inhibition of denitrification by accumulated SO_4^{2-} in PVA–TFT reactor appeared at the concentration of approximately 6000 mg (SO_4^{2-})/L, and 75% of NO_3^-–N removal efficiency was achieved after 12 d operation under the condition of initial 700 mg/L NO_3^-–N concentration.

1. Introduction

Polyvinyl alcohol (PVA), a cheap and synthetic polymer, has been widely used now for cell and enzyme immobilizations mainly because of its high durability, high chemical stability and non-toxicity to microorganisms or enzymes. Among the reported PVA immobilization methods, PVA–boric acid and freezing/thawing methods are frequently used.

Although two main problems, e.g., agglomeration of PVA gel beads and toxicity of saturated boric acid, cannot be absolutely resolved, PVA–boric acid method is still adopted for immobilizations of activated sludge [1–6], some functional microorganisms [5,7–11], and enzymes [12,13]. To overcome these problems, several modifications have been found effectively to enhance the properties of formed PVA beads, such as adding alginate [3,10,11] or powdered activated carbon [5], being treated with sodium sulfate [13] or by phosphoylation [2–4], or using glutaraldehyde to reduce the hydration [9], etc. Meanwhile, PVA, a kind of hot water soluble polymer, can be transformed to a stable, macroporous hydrogel under low temperature, which forms the basis of freezing/thawing method. Because of no requirement of toxic boric acid and increased mechanical strength of formed beads, PVA–freezing/thawing method has been successfully used in cell immobilizations [14–19].

It has been noted that PVA beads made by these two methods exhibited porous structures from surface to interior [9,15,17,20], which is beneficial for adsorption of microorganisms and protection against detrimental conditions when used as carriers. However, little information can be found in literature about the application of porous PVA carrier directly in water or wastewater treatment.

On the other hand, nitrate contamination, originated from agricultural runoff, landfill leachate, leaking septic tanks, etc., is becoming a widespread environmental problem. And biological denitrification processes, including autotrophic denitrification and heterotrophic denitrification, have been commonly used for wastewater treatment. Compared with heterotrophic denitrification, autotrophic denitrification is attracting increasing interest in recent years due to the following three major advantages: (i) no residual organic problems because of the utilization of inorganic substances as electron donors; (ii) low operation and maintenance costs due to no external organic carbon needed; and (iii) lower cell yield, which minimizes sludge handling or lessens the risk of biological regrowth in distribution systems and disinfection by-product formation [21–27].

Among the denitrifying microorganisms, only a few species of autotrophic bacteria can reduce nitrate to nitrogen gas while oxidizing elemental sulfur or reduced sulfur compounds (S^{2-}, $S_2O_3^{2-}$, SO_3^{2-}) to sulfate, and *T. denitrificans* is the most frequently used.

* Corresponding author. Tel.: +86 21 65642018; fax: +86 21 65642018.
E-mail address: zflei@fudan.edu.cn (Z. Lei).

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Up to now, many researchers have paid more attention to the efficiencies and influence factors of autotrophic denitrification processes by *T. denitrificans* (enriched sludge or pure culture) with reduced sulfur compounds as electron donors in the treatment of nitrate-contaminated drinking water, groundwater or wastewaters [23–35]. Few of them demonstrated the efficiency of immobilized *T. denitrificans*, which might enhance the denitrification efficiency and protect the bacteria against detrimental environments.

The objective of this study was to investigate the denitrification efficiencies of *T. denitrificans* immobilized on PVA carriers formed by PVA–boric acid method and PVA–freezing/thawing method under the condition of thiosulfate used as reduced sulfur compound. And whether the immobilization processes could enhance the reactor performance or not compared with free cells was also discussed.

2. Materials and methods

2.1. Microorganisms and culture medium

T. denitrificans 3870 used in this study was obtained from the Institute of Physical and Chemical Research, Japan (RIKEN), which was separated from residual sludge of a domestic wastewater treatment plant.

The basal mineral medium utilized in this study contained (per liter): 1.8 g KH_2PO_4, 1.2 g Na_2HPO_4, 0.1 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4{\cdot}7H_2O$, 30 mg $FeCl_3{\cdot}6H_2O$, 5.0 g KNO_3, 0.5 g $NaHCO_3$, and 100 ml of 10% (10 g/100 ml) $Na_2S_2O_3{\cdot}5H_2O$, which was suggested by RIKEN. Before use, the basal mineral medium (except $Na_2S_2O_3$ solution) was autoclaved at 125 °C for 15 min and the $Na_2S_2O_3$ solution was sterilized by filtration.

The culture medium was composed of 90% (v/v) of basal mineral medium and 10% (v/v) of trace element solution. The latter solution contained (mg/L): EDTA (500.0), $CaCl_2$ (55.4), $CuSO_4{\cdot}5H_2O$ (15.7), $CoCl_2{\cdot}6H_2O$ (16.1), $MnCl_2{\cdot}4H_2O$ (50.6), $ZnSO_4{\cdot}7H_2O$ (220.0), $(NH_4)_6Mo_7O_{24}{\cdot}H_2O$ (11.0), and $FeSO_4{\cdot}7H_2O$ (49.9) [36].

All the chemicals used in the experiments were of chemical grade.

2.2. Experiment setup and efficiency assessment

All batch experiments were started at initial culture pH 7.0 (adjusted with 0.1 M NaOH) and conducted on a shaking table at 30 ± 2 °C in duplicate in 500 ml reactors sealed with butyl rubber stoppers. The working volume was 440 ml, composed of 400 ml of culture medium and 40 ml of the 4th-day *T. denitrificans* enrichment with the same culture medium. Before operation, the reactors were flushed with nitrogen gas for 3 min to exclude oxygen, and then all the outlets of the reactors were immersed in water.

The initial cell densities, indicated by optical density at 650 nm, were identical for all the reactors of the following experiments before startup (OD_{650} = 0.197). And the initial $S_2O_3^{2-}$–S and NO_3^-–N concentrations for the reactors were approximately 3850 mg ($S_2O_3^{2-}$–S)/L and 700 mg (NO_3^-–N)/L, respectively.

There are four kinds of reactors in this study. One is the control, with only free cells and no carrier added in the culture medium, the other three were labeled as PVA–TFT, PVA–BA1, and PVA–BA2 for the reactors with carriers added and prepared with the methods of PVA–thrice freezing/thawing, PVA–boric acid with water-washing, and PVA–boric acid without water-washing, respectively. And the volume ratios of added carrier to total volume (carrier fill) were approximately 10% for the three cell-immobilized reactors.

The four reactors were compared and assessed with SO_4^{2-} generation rate, NO_3^-–N denitrification rate, and $S_2O_3^{2-}$–S consumption rate, respectively. These values at *j*th d were calculated as follows:

$$SO_4^{2-}\ \text{generation rate (mg/L d)} = \frac{[M]_j - [M]_i}{j - i} \quad (1)$$

$$S_2O_3^{2-}\ -\ \text{S consumption rate or } NO_3^-\text{–N denitrification rate (mg/L d)} = \frac{[M]_i - [M]_j}{j - i} \quad (2)$$

where [*M*] (mg/L) is the concentration of SO_4^{2-}, $S_2O_3^{2-}$–S or NO_3^-–N in the reactor, and *i* (d) or *j* (d) is the operation time ($j > i$, $i \geq 0$). In addition, $j = i + 2$ (d) ($i = 0, 2, 4, \ldots$) for the calculations of daily values, and $j = 12$ or 18 (d) and $i = 0$ (d) for average values in this study.

Besides, NO_3^-–N removal efficiency, calculated as Eq. (3), was also used.

$$NO_3^-\text{–N removal efficiency (\%)} = \frac{[M]_0 - [M]_t}{[M]_0} \times 100 \quad (3)$$

where $[M]_0$ and $[M]_t$ are the initial and terminal NO_3^-–N concentrations in the reactor, respectively.

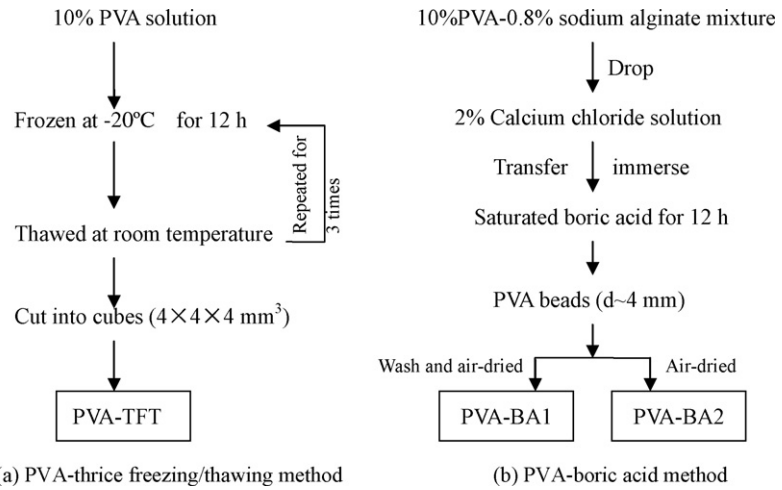

Fig. 1. Flowchart of carrier preparations with PVA–thrice freezing/thawing (PVA–TFT) (a) and PVA–boric acid (PVA–BA (b) methods.

2.3. Preparation of PVA carriers

The PVA carriers were prepared following the procedures depicted in Fig. 1. PVA, with average polymerization degree of 2000, and sodium alginate were provided by Wako Pure Chemical Inc. Ltd., Japan.

The 10% (m/v) PVA solution was obtained by adding 10 g PVA to 100 ml of distilled water, which was heated to 60 °C to dissolve PVA.

In PVA–thrice freezing/thawing (PVA–TFT) method, the 10% PVA solution was first frozen at −20 °C for 12 h and later thawed at room temperature. After three cycles of freezing/thawing process, the resulting solidified PVA was cut into 4 mm × 4 mm × 4 mm cubes.

In PVA–boric acid (PVA–BA) method, the 10% PVA solution was kept at 4 °C for 24 h before 0.8% (m/v) sodium alginate being added. The mixture was extruded as drops into a 2% (m/v) calcium chloride solution, and later transferred into saturated boric acid and immersed for about 12 h to complete solidification. All these operations were carried out at room temperature under gently stirring, and the resulting beads were approximately 4 mm in diameter. As for PVA–BA1, the beads were completely washed with distilled water to remove any excess boric acid after solidification and added into reactor after air-dried; and for PVA–BA2, the beads were air-dried and added into reactor without water washing after solidification in boric acid.

2.4. Analytical methods

All the following indices were analyzed in duplicate, and the data present were the average values of duplicate experiments.

Biomass was indicated by optical density at the wavelength of 650 nm (OD_{650}). OD_{650} (DR4000U spectrophotometer, Hach Co.) and pH (Twin B-212, Horiba) were measured directly with sampled cultures.

For the determinations of nitrate nitrogen (NO_3^-–N), sulfate (SO_4^{2-}), and thiosulfate sulfur ($S_2O_3^{2-}$–S), the culture was sampled once every other day and centrifuged at 5000 rpm for 15 min, and the supernatant was used for the corresponding measurements.

The concentration of NO_3^-–N was obtained with UV spectrophotometry (DR4000U, Hach Co.) by measuring the absorbance of supernatant at 220 nm and 275 nm, respectively [37]. SO_4^{2-} was determined with Sulfaver 4 turbidimetric kit following the procedures developed by Hach Company [38]. In addition, a classical iodimetry [39] was used to quantify the amount of $S_2O_3^{2-}$–S remained in the culture.

2.5. Statistical analysis

One-way ANOVA and *F*-test were used for statistical analysis, and $P < 0.1$, $P < 0.05$, $P < 0.01$, and $P < 0.001$ denoted significance levels of differences between cell-immobilized and control reactors.

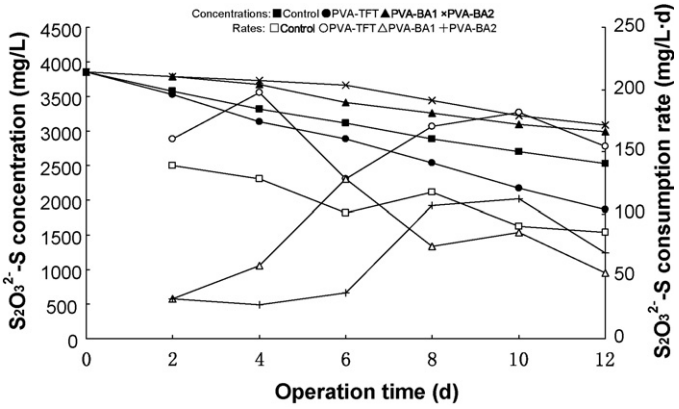

Fig. 2. Variations of $S_2O_3^{2-}$–S concentrations and consumption rates in the four reactors.

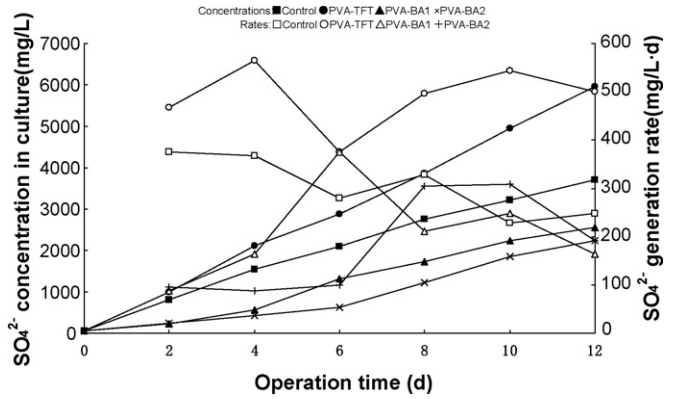

Fig. 3. Variations of SO_4^{2-} concentrations and generation rates in the four reactors. The SO_4^{2-} concentration introduced by the addition of basal mineral medium and trace element solution has been excluded from the results.

3. Results and discussion

3.1. $S_2O_3^{2-}$ consumption and SO_4^{2-} generation

The variations of $S_2O_3^{2-}$–S and SO_4^{2-} concentrations with the corresponding consumption or generation rates are illustrated in Figs. 2 and 3, respectively.

It can be seen that the $S_2O_3^{2-}$–S concentrations showed similar decreased trends in the four reactors, and the largest decreases were observed in the PVA–TFT reactor from the 4th day on (Fig. 2). The total decreases of $S_2O_3^{2-}$–S concentrations after 12 d operation were 1323, 1984, 864, and 768 mg ($S_2O_3^{2-}$–S)/L in the control, PVA–TFT, PVA–BA1, and PVA–BA2 reactors, respectively.

The variations of the $S_2O_3^{2-}$–S consumption rates seemed to be more complicated than $S_2O_3^{2-}$–S concentrations (Fig. 2). The *T. denitrificans* immobilized on PVA–BA carriers exhibited much lower activities, indicated by $S_2O_3^{2-}$–S consumption rate, than free cells and those immobilized on PVA–TFT carrier before the 6th day,

Table 1
Results of $S_2O_3^{2-}$–S consumption, SO_4^{2-} generation, and NO_3^-–N denitrification rates obtained in the four reactors

Rates	Control	PVA–TFT	PVA–BA1	PVA–BA2
$S_2O_3^{2-}$–S consumption rate (mg ($S_2O_3^{2-}$-S)/L d)	110.25 ± 21.37	165.33 ± 23.93^{c}	72.00 ± 33.03 ^{b}	64.00 ± 38.21 $^{b, 0.71}$
SO_4^{2-} generation rate (mg (SO_4^{2-})/L d)	304.67 ± 62.20	491.33 ± 66.33 ^{d}	208.67 ± 98.09 ^{a}	181.33 ± 103.74 $^{b, 0.65}$
NO_3^-–N denitrification rate (mg (NO_3^-–N)/L d)	28.00 ± 6.20	44.00 ± 4.38 ^{d}	19.33 ± 9.61 ^{a}	15.33 ± 8.16 $^{b, 0.46}$

Rate data expressed as mean ± S.D. Superscripts a, b, c, and d denote significance of differences between the cell-immobilized reactor and the control reactor at $P < 0.1$, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. And the numerical superscripts indicate the approximate significance levels of differences between PVA–BA1 and PVA–BA2 reactors obtained by ANOVA analysis.

although complete washing in the making process of PVA beads might have some beneficial effect on eliminating the toxicity of boric acid (Fig. 2; $P < 0.05$ for both PVA–BA reactors compared to the control, Table 1). In this study, the average $S_2O_3^{2-}$–S consumption rates were approximately 110, 165, 72, and 64 mg ($S_2O_3^{2-}$–S)/L d for the control, PVA–TFT, PVA–BA1, and PVA–BA2 reactors, respectively (Table 1). And significant increase was observed for $S_2O_3^{2-}$–S consumption rate in the PVA–TFT reactor compared to the control ($P < 0.01$), while no significant difference was found for $S_2O_3^{2-}$–S consumption between the two PVA–BA reactors ($P = 0.71$) (Table 1).

The results of SO_4^{2-} concentrations and generation rates in the reactors were in accordance with those of $S_2O_3^{2-}$–S (Figs. 2 and 3). The total increases of SO_4^{2-} concentration were 3656, 5896, 2504, and 2176 mg/L, with average SO_4^{2-} generation rates approximately 305, 491, 209, and 181 mg/L d for the control, PVA–TFT, PVA–BA1, and PVA–BA2 reactors, respectively (Table 1). Still, significant increase was observed for SO_4^{2-} generation rate in the PVA–TFT reactor compared to the control ($P < 0.001$), and no significant difference was obtained between the two PVA–BA reactors ($P = 0.65$) (Table 1). In addition, the SO_4^{2-} generation rate seemed to vary in the same way as $S_2O_3^{2-}$–S consumption rate in each reactor, resulting from the sulfur balance of the denitrification process.

From Figs. 2 and 3, *T. denitrificans* immobilized on PVA–TFT carrier seemed to use thiosulfate more efficiently than the others, including free cells. It was reported that PVA beads made by freezing/thawing with or without cell addition possessed distinctive heterogenous macropores [15,17,20,40]. The average inner dimensions of the macropores obtained by Szczesna-Antczak and Galas [17] and Hatakeyema et al. [40] were approximately 2–10 μm, which is close to the dimension of microorganisms and beneficial for cell adsorption and immobilization. It was reported that microorganisms immobilized on porous carriers sintered with coal fly-ash could tolerate 15% of salinity and exhibited 99% of phenol removal in 16 h [41], suggesting that porous structure might provide protection for microorganisms from adverse conditions, e.g., high concentration of SO_4^{2-} in this study. Its detailed protection mechanism needs further investigations. Compared with the control reactor, the average $S_2O_3^{2-}$–S consumption and SO_4^{2-} generation rates in the PVA–TFT reactor were increased by 50% and 61%, respectively. Although the total SO_4^{2-} concentration increased to 5896 mg/L in the PVA–TFT reactor after operation for 12 d, no apparent inhibition was observed. The obtained SO_4^{2-} concentration, higher than the initial inhibition concentration (5000 mg/L) observed by Claus and Kutzner [22] for a pure culture of *T. denitrificans*, might be resulted from the protection effect of porous PVA structures. In addition, this SO_4^{2-} concentration is much higher than 1500 mg/L (500 mg SO_4^{2-}–S/L) [42] and 2000 mg/L [43] obtained in enriched mixed cultures of autotrophic organisms, indicating that *T. denitrificans* could tolerate higher sulfate concentration conditions when immobilized on porous materials.

Unlike in cell-immobilized reactors, the SO_4^{2-} generation rates in the control seemed to decrease with the increase of SO_4^{2-} concentration. However, that the inhibition caused by SO_4^{2-} accumulation started at which SO_4^{2-} level could not be obtained according to our experiments because of the complexity of denitrification process and multi-factors involved.

3.2. Denitrification efficiency

Seen from Fig. 4, the NO_3^-–N concentrations in the reactors decreased with time going on. The PVA–TFT reactor with *T. denitrificans* immobilized exhibited the highest NO_3^-–N removal among the four reactors, about 75% of NO_3^-–N being removed in all after 12 d operation under the same initial NO_3^-–N concentration (~700 mg (NO_3^-–N)/L). Compared with the control reactor

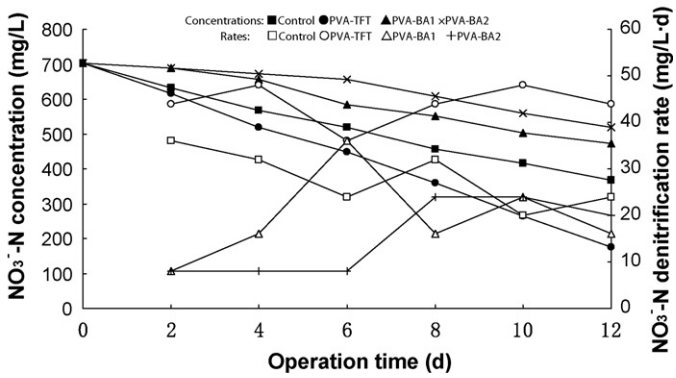

Fig. 4. Variations of NO_3^-–N concentrations and denitrification rates in the four reactors.

(free cells), in which 52% of NO_3^-–N removed after 12 d operation, the total NO_3^-–N removal efficiency of the PVA–TFT reactor was increased by 27%.

The total decreases of NO_3^-–N concentration were 336, 528, 232, and 184 mg/L, and the average NO_3^-–N denitrification rates were 28, 44, 19, and 15 mg (NO_3^-–N)/L d, respectively, for the control, PVA–TFT, PVA–BA1, and PVA–BA2 reactors (Table 1). Compared with free cells, *T. denitrificans* exhibited higher denitrification activity on PVA–TFT carrier, with the average NO_3^-–N denitrification rate increased by 57%. A significant increase was also observed for NO_3^-–N denitrification rate in the PVA–TFT reactor in comparison with the control ($P < 0.001$), and no significant difference was observed between the two PVA–BA reactors ($P = 0.46$) (Table 1). The average denitrification activities for those cells immobilized on PVA–BA carriers decreased ($P < 0.1$ for PVA–BA1 and $P < 0.05$ for PVA–BA2, respectively) (Table 1), although some recovery from the toxicity of boric acid was observed after operation for 6–8 d (Fig. 4). The variations of NO_3^-–N in the four reactors were in agreement with the results of $S_2O_3^{2-}$–S and SO_4^{2-} obtained in this study.

In addition, the terminal pH values after 12 d operation were 6.53, 6.31, 6.66, and 6.78 in the control, PVA–TFT, PVA–BA1, and PVA–BA2 reactors, respectively, under the same initial pH of 7.0, which lay between 6 and 9, the optimum pH range for *T. denitrificans* [44].

3.3. Stoichiometric analysis

Eq. (4) can be used to represent the stoichiometric reaction when thiosulfate being used as reduced sulfur compound in denitrification process [42,45]:

$$0.844S_2O_3^{2-} + NO_3^- + 0.347CO_2 + 0.086HCO_3^- + 0.086NH_4^+ + 0.434H_2O \rightarrow 1.689SO_4^{2-} + 0.500N_2 + 0.086C_5H_7O_2N + 0.697H^+ \quad (4)$$

According to the above reaction, the theoretical stoichiometric values of $\Delta S_2O_3^{2-}$–S/ΔNO_3^-–N, $\Delta SO_4^{2-}/\Delta NO_3^-$–N and $\Delta SO_4^{2-}/\Delta S_2O_3^{2-}$–S ratios were calculated as 3.86 g/g, 11.58 g/g, and 3.00 g/g, respectively, which were quite similar to the results obtained in this study (Table 2).

It can be seen that all the relative errors were less than ±10%. The relative errors for SO_4^{2-} quantifications with turbidimetric method and NO_3^-–N determinations with UV spectrophotometry were pointed out to be 0.2–7.0% and 2.6–5.1%, respectively [37]. Therefore, taking the errors possibly brought by sampling and the analytical methods into consideration, the obtained results suggested that the denitrification processes completed by

Table 2
Average ratios for $\Delta S_2O_3^{2-}$–S/ΔNO_3^-–N, ΔSO_4^{2-}/ΔNO_3^-–N, and ΔSO_4^{2-}/$\Delta S_2O_3^{2-}$–S obtained in the four reactors

Ratios	Values (g/g)				Relative errors (%)[a]			
	Control	PVA–TFT	PVA–BA1	PVA–BA2	Control	PVA–TFT	PVA–BA1	PVA–BA2
$\Delta S_2O_3^{2-}$–S/ΔNO_3^-–N	3.94	3.75	3.72	4.17	2.07	−2.85	−3.63	8.03
ΔSO_4^{2-}/ΔNO_3^-–N	10.88	11.17	10.79	11.83	−6.04	−3.54	−6.82	2.11
ΔSO_4^{2-}/$\Delta S_2O_3^{2-}$–S	2.76	2.97	2.90	2.83	−8.00	−1.00	−3.33	−5.67

[a] The relative errors (RE) were calculated according to the following equation: $RE(\%) = (V_d - V_t)/V_t \times 100$, in which V_d and V_t were the determined and theoretical values, respectively.

T. denitrificans were progressing thoroughly and other nitrogenous by-products might not generated in the reactors.

3.4. Efficiency of the PVA–TFT reactor after prolonged-time operation

The above results showed the PVA–TFT reactor performed more efficiently than others in the denitrification process and 75% of 700 mg/L NO_3^-–N could be removed after 12 d operation. To assess the denitrification potential of this kind of immobilized *T. denitrificans* and the effect of generated SO_4^{2-} concentration on denitrification, a prolonged-time operation was carried out in another PVA–TFT reactor and all the related indices were analyzed and determined as above (Fig. 5).

The results indicated that the persistent increase trend of SO_4^{2-} concentration slowed down and the SO_4^{2-} generation rate began to decrease largely from the 12th day on, which was in agreement with the decrease trends of NO_3^-–N concentration and NO_3^-–N denitrification rate (Fig. 5). The average daily SO_4^{2-} generated and NO_3^-–N removed were about 490 mg (SO_4^{2-})/L d and 40 mg (NO_3^-–N)/L d respectively before the 12th day, which were approximately 5.5 times of the corresponding daily values obtained during the period of the 12th to 18th days. Moreover, the terminal pH of the reactor after 18 d operation was 5.8, which lay out of 6–9, the optimum pH range for *T. denitrificans* [44]. Thus, we concluded that SO_4^{2-} started to inhibit the denitrification of *T. denitrificans* at approximately 6000 mg/L, which is much higher than the findings of Campos et al. (1500 mg (SO_4^{2-})/L or 500 mg (SO_4^{2-}–S)/L) [42] and Oh et al. (2000 mg (SO_4^{2-})/L) [43] in mixed cultures of enriched autotrophic organisms, and also higher than 5000 mg (SO_4^{2-})/L obtained by Claus and Kutzner [22] in a pure culture of *T. denitrificans*. The enhancement of sulfate tolerance could be attributed to the protection of porous PVA structures for immobilized *T. denitrificans*.

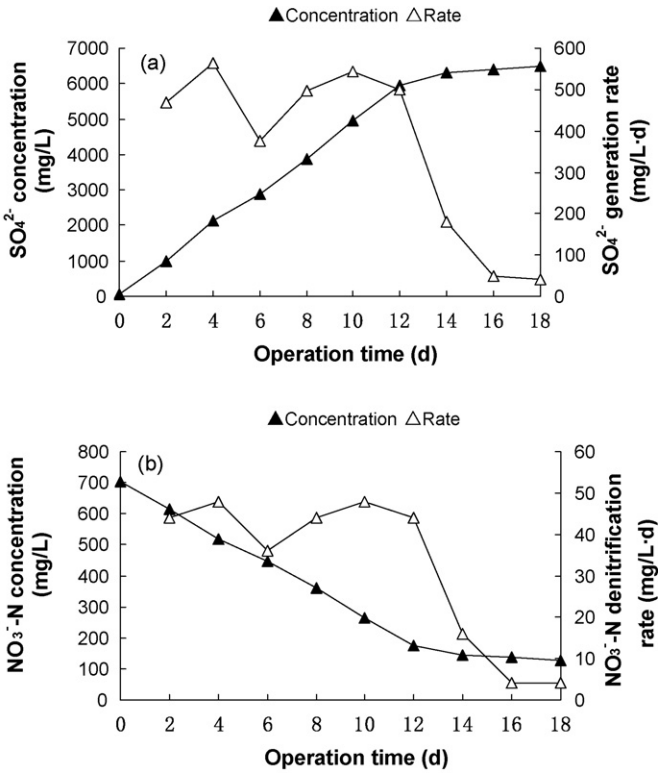

Fig. 5. SO_4^{2-} (a) and NO_3^-–N (b) profiles in the PVA–TFT reactor during a prolonged operation.

4. Conclusions

The present study showed that thrice freezing/thawing method could be used for the making of PVA carrier to immobilize *T. denitrificans* in the treatment of NO_3^-–N contained wastewaters. The *T. denitrificans* immobilized on PVA–TFT carrier could remove 75% of NO_3^-–N after 12 d operation when 700 mg/L of initial NO_3^-–N concentration being applied, and the average NO_3^-–N denitrification rate was 44 mg (NO_3^-–N)/L d. The results of prolonged operation of PVA–TFT reactor indicated that *T. denitrificans* exhibited excellent denitrification activities when generated SO_4^{2-} concentration less than 6000 mg/L, implying that PVA–TFT carriers might perform better in continuously operated reactors in flow-in and flow-out mode, provided that appropriate design (including wastewater characteristics and operation parameters) being guaranteed. To ensure this kind of PVA carrier being put into practice, detailed investigations, including the inner structures of the carrier, effect of porous structure on sulfate or nitrate diffusion, duration of the immobilized cells, and optimized design of the reactor, should be followed.

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