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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.

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* 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:

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

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

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.

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

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

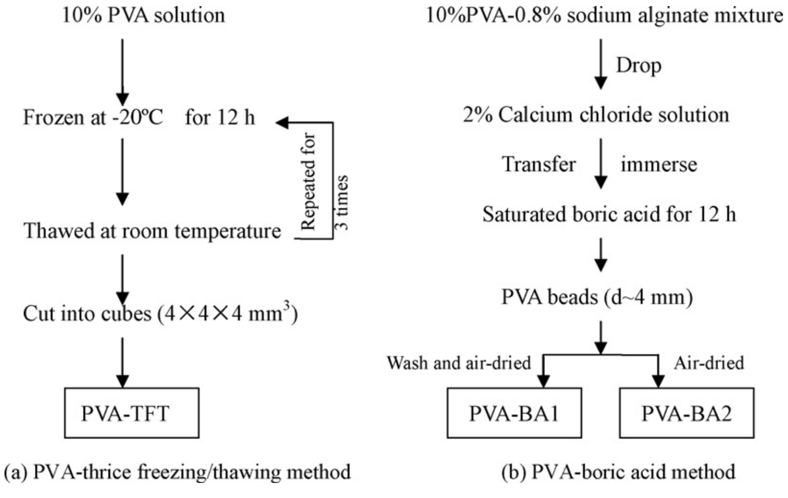

Fig. 1. Flowchart of carrier preparations with PVA–thrice freezing/thawing (PVA–TFT) (a) and PVA–boric acid (PVA–BA (b) methods.