



Short communication

Controlling denitrification accompanied with nitrite accumulation at the sediment-water interface



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ABSTRACT

Nitrate removal efficiencies of ecological treatment systems could be significantly improved by carbon addition, however, occasionally leading to the accumulation of nitrite. Simulation batch experiments were conducted to investigate effects of ethanol, as a carbon source, and temperature on denitrification at the sediment-water interface. At a C/N below 1.57, our results show nitrate removal rates were reduced as the nitrite accumulation reached a level of 7.8 mgL⁻¹. Nitrate was removed with nitrite accumulations less than 20% at C/Ns of 1.57 and above at 20, 25 and 35 °C. According to the two-step zero-order kinetic model, both nitrate and nitrite reduction rates increased with C/Ns and temperature, but the former was greater than the latter throughout the experiment. The difference between two reduction rates led to nitrite accumulations, possibly due to electron competition and reductase activity. The feasibility of using a carbon source and increasing temperature was further confirmed in a mesocosm experiment, with a nitrate removal efficiency of 73.7% and a nitrite accumulation ratio of 0.8%. Our study suggests the addition of an external carbon source combined with a longer hydraulic retention time (HRT) would be an effective approach to achieve an ideal nitrate removal efficiency with low nitrite accumulation for an ecological treatment system.

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1. Introduction

The effluents from the wastewater treatment plant (WWTP) are usually high in nitrate and likely to have detrimental impacts on the quality of the water supply and the health of the aquatic ecosystems (Liu et al., 2013; Zhang et al., 2015). In many countries, wetlands, ponds, and riparian buffer strips have been artificially restored or constructed to remove nitrate from effluents (Ingersoll and Baker, 1998; Zhao et al., 2013). However, traditional denitrification in these ecological treatment systems is limited by carbon deficiency (Wen et al., 2010; Yang et al., 2012), as bioavailable organics in wastewater are depleted after anoxic/aerobic biological treatment.

The sediment-water interfaces within ecological treatment systems are considered essential to eliminate nitrate from WWTP effluents. It is a shallow, oxic-anoxic layer separating the sediment from overlying water and providing an anoxic environment and

organic matter for denitrification. The sediment-water interface is also an immense substrate for denitrifying bacteria to attach on. Recent studies illustrated that taking advantages of denitrification at the sediment-water interface could artificially strengthen nitrate removal and shorten HRTs by wetlands or ponds (Jensen et al., 1993; Richardson et al., 2004; Chen et al., 2015).

The denitrification pathway by heterotrophic bacteria can be expressed as a stepwise process: NO₃⁻-N → NO₂⁻-N → NO → N₂O → N₂, in which nitrates were electron acceptors and carbon sources were electron donors (Vidal-Gavilan et al., 2014). As an intermediate, nitrite accumulation occurs frequently and is troublesome because of toxic effects on nitrate removal and human health (Ge et al., 2012; Wang et al., 2014). So far, most studies have investigated nitrite accumulation with a pure denitrifying culture or a full-scale activated sludge bioreactor (Van Rijn et al., 1996; Carrera et al., 2003; Pan et al., 2013). Nitrite accumulation was determined quite relevant with carbon sources and temperature variation. Unlike wastewater, the WWTP effluents or agricultural non-point pollutions are characteristic with low C/Ns and large quantities (Compton et al., 2011; Xu et al., 2016). The

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occurrence and fate of nitrite in ecological treatment systems is exerting a tremendous fascination.

In this study, we investigate enhanced denitrification at the sediment-water interface with the following objectives: to figure out whether nitrite accumulation occurs at the sediment-water interface; to determine the functions of carbon sources and temperature on denitrification; to reveal the mechanism of nitrite accumulation; to illustrate feasible control strategies for engineering applications.

2. Materials and methods

2.1. Sediment source and synthetic wastewater

The sediments were obtained from a river located at Shanghai Jiao Tong University. After removing residues of fauna and flora, they were transferred to reactors uniformly. The synthetic wastewater was prepared by dissolving NaNO_3 into tap water without NaNO_2 . The buffer solution with KH_2PO_4 (1.18 gL^{-1}) and Na_2HPO_4 (7.29 gL^{-1}) was utilized to keep pH in range of 7.5 ± 0.1 (Cao et al., 2013).

2.2. Denitrification simulation experiments

2.2.1. Batch experiments

The reactor was an open cylinder with a height of 25 cm and a diameter of 16 cm, maintaining a sediment layer of 3 cm and overlying water of 15 cm (Fig. S1(a)). The sediments were acclimated for about 20 d until nitrate removal rates in each reactor tend to be uniform and constant. The supernatant was waived after each cycle and the synthetic wastewater was supplemented again with the initial C/N of 2.09.

The C/N tests were conducted at 25°C with nitrate of 30 mgL^{-1} . C/Ns (0, 0.52, 1.04, 1.57, 2.61 and 4.17) were achieved by adding 0, 0.114, 0.228, 0.342, 0.570 and 0.913 mL ethanol, respectively. The temperature tests were conducted at 20, 25 and 35°C to represent different seasons with the optimal C/N. The heating rods and miniature submersible pumps were placed in water bath to control the temperature.

2.2.2. Electron competition experiments

Electron competition experiments were conducted in the same reactor as batch tests at 25°C , with nitrate added halfway of the nitrite reduction. The initial nitrite concentration was controlled at 20 mgL^{-1} with NaNO_2 . C/Ns of 1.04 and 4.71 were set and regulated by ethanol amounts. After 110 min, nitrate of 20 mgL^{-1} was added.

2.2.3. Mesocosm studies

The mesocosm system consisted of two main units. The first anaerobic unit with duckweed acted for denitrification. It was $45 \text{ cm} \times 60 \text{ cm} \times 70 \text{ cm}$ (inner width, length and height) as a scale-down system (Fig. S1(b)). Considering water and sediments in natural ponds respectively are 1–2 m and 20–30 cm depths, a sediment layer of 10 cm and a water depth of 60 cm were set. The second aerobic unit was filled with gravels (diameters of 20–30 mm) and perforated pipes for aeration to oxidize residual carbon.

The microbial acclimation started with a C/N of 1.57 and an HRT of 1 d. After 20 d, the nitrate removal efficiency reached a steady state. Three operation scenarios were applied including high C/N and low temperature mode (M1), high C/N and high temperature mode (M2), low C/N and high temperature mode (M3). The overlying water with nitrate of 30 mgL^{-1} was pumped into mesocosm.

2.3. Sample analysis

Test durations and sampling intervals depended on the concentration variations. Water samples in mesocosm were collected twice a day for about five times in each scenario. The mixed water samples collected from the upper, middle and lower layer of the overlying water were filtered through a membrane ($0.45 \mu\text{m}$ pore size). Nitrate, nitrite and ammonia nitrogen were analyzed with an auto discrete analyzer (Easychem 300, SYSTEA, Italy). TN and total organic carbon (TOC) were measured with a TN/TOC analyzer (Multi N/C 3100, Analyticjena, Germany). Temperature and pH were detected with a pH meter (pH 3310, WTW, Germany). Dissolved oxygen (DO) was determined with an oxygen meter (HQ30d, HACH, America). All chemical reagents were of analytical grade. All chemical analysis was conducted in duplicate.

2.4. Denitrification kinetics

The denitrification kinetic analysis adopted a simplified two-step model: $\text{NO}_3^- \text{-N} \rightarrow \text{NO}_2^- \text{-N} \rightarrow \text{N}_2$ (Ni and Yu, 2008; Boulétreau et al., 2012). This model only focused on nitrate and nitrite because the target points in this research were reductions of these two species and nitrite accumulation. The zero-order kinetics could be used for reduction when the carbon source is enough. The denitrification rates would not be nitrate-limited unless less than 0.3 mgL^{-1} (Glass and Silverstein, 1998). The two-step reactions considered nitrate and nitrite reduction rates were completely independent.

$$-\frac{dC_{\text{NO}_3}}{dt} = k_1 \quad (1)$$

$$-\frac{dC_{\text{NO}_2}}{dt} = k_1 - k_2 \quad (2)$$

where k_1 and k_2 are the zero-order reaction coefficient for nitrate and nitrite ($\text{mgL}^{-1} \text{ h}^{-1}$), respectively. The coefficients were determined by linear regression to the zero-order model during constant denitrification rates. The modeling results were graphed with data points averaging from all profiles.

3. Results

3.1. Batch experiments

Denitrification occurring at the sediment-water interface is highly significant for nitrogen removal. A preliminary experiment was conducted in a reactor with/without sediments to confirm the role of the sediment-water interface. It was observed that nitrate and TOC remained unchanged without sediments in the reactor, while with sediments, nitrate and TOC decreases apparently in 10 h (Fig. S2).

3.1.1. Effects of C/N on denitrification and nitrite accumulation

Denitrification rates increased with C/N only when the initial carbon source is limited (Fig. 1(a)). At the C/N of 0, nitrate decreased slowly as ethanol in microbial acclimation remained in sediment. In all treatments with carbon addition, nitrate was almost reduced and denitrification rate increased with C/N until reaching 1.57.

Fig. 1(b) describes nitrite accumulation at different C/Ns. Nitrite increased to 7.8 and 3.6 mgL^{-1} at C/Ns of 0.52 and 1.04, respectively, and could not be further decreased for carbon deficiency. Nitrite all reached its peak (below 2.6 mgL^{-1}) before 5 h and decreased to 0 after 20 h while C/Ns were 1.57 and above. The nitrite accumulation ratio was calculated by "nitrite generation/nitrate consumption $\times 100\%$ " (Wang et al., 2014). At a C/N below 1.57, the nitrite accumulation ratio exceeded 40%, while it was only about 20% when C/N was higher than 1.57.

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