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Bioinhibitory effect of hydrogenotrophic bacteria on nitrate reduction by nanoscale zero-valent iron

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HIGHLIGHTS

- The presence of HTB can limit the occurrence of nitrate removal by nZVI.
- The pathway of bioinhibition from HTB is preventing chemical reduction happening.
- FeOOH was the product of iron corrosion in the presence of HTB.

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ABSTRACT

Hydrogenotrophic bacteria (HTB) were introduced into a nitrate removal system, which used nanoscale zero-valent iron (nZVI) as reductant, to investigate its bioinhibitory effect. Based on the results, it was noted that addition of HTB culture (10–50 mL) led to 58.9–91.4% decrease in the first observed rate constant (k_{obs1}), which represented the nitrate removal rate by nZVI, and a reduction in the generated poisonous by-products from 94.9% to 38.5%. In other words, HTB had a significant inhibitory effect on nitrate reduction by nZVI. However, the pathway of this bioinhibition only prevented the occurrence of chemical reduction, but not competition for nitrate. Furthermore, FeOOH coating was observed on the surface of nZVI, instead of Fe_3O_4 or Fe_2O_3, which could prevent electron transmission from nZVI to nitrate. Considering that FeOOH was the product of iron corrosion, the result indicated that HTB could inhibit chemical reduction by enhancing the reaction between nZVI and water.

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1. Introduction

Nitrate pollution in subsurface water has become a serious environmental problem in many parts of the world (Chen et al., 2005; Yang et al., 2006). During the last decades, a number of studies have paid attention to nitrate removal by nanoscale zero-valent iron (nZVI) due to its high surface reactivity (Choe et al., 2000; Liou et al., 2006; Sohn et al., 2006). Many studies have shown that ammonium accounted for more than 90% of the reaction products under the condition of no pH control (Choe et al., 2000).

With regard to the reaction between nZVI and nitrate, numerous researchers have focused on optimization of the final products (Liou et al., 2005; Lee et al., 2007) and predominant influence of environmental conditions such as pH (Chen et al., 2004), dissolved oxygen (Zhang, 2003), and other ions in groundwater (Su and Puls, 2004). Recently, many reports (Till et al., 1998; Kielemoes et al., 2000; Biswas and Bose, 2005) have suggested that certain kinds of hydrogenotrophic bacteria (HTB) can lower the poisonousness of final products formed from nitrate reduction by Fe^0, and the competition of nitrate has been considered to be the main reason for this phenomenon, as described in Eqs. (1) and (2).

$$4Fe^0 + NO_3^- + 7H_2O \rightarrow 4Fe^{2+} + NH_4^+ + 10OH^- \quad (1)$$

$$0.33NO_3^- + H_2 + 0.08CO_2 + 0.34H^+ \rightarrow 0.015C_5H_7O_2N + 0.16N_2 + 1.11H_2O \quad (2)$$

Although this kinetic competition between chemical and biological nitrate removal has been verified in our previous work (An et al., 2010), some unreasonable phenomena have been noted: (i) Nitrate removal using HTB should be strongly restricted by nZVI due to its high poisonousness; however, there was no difference in the magnitude of effect between nZVI and HTB on nitrate removal rate (Nel et al., 2006; Pan et al., 2007); (ii) The small amount of ionic nitrogen loss in the first few days indicated that biological denitrification could hardly create sufficient competitive pressure

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on chemical reduction by nZVI (Till et al., 1998; An et al., 2009). Thus, there must be some other mechanism for the bioinhibitory effect of HTB on chemical reduction of nitrate in this integrated system. The aim of the present study was to investigate the bioinhibitory mechanism of HTB on nitrate removal by nZVI, focusing on the changes in the nitrate removal rate and its reductive product.

2. Material and methods

2.1. Cultivation of microorganisms

Alcaligenes eutrophus was purchased from China Center of Industrial Culture Collection (Beijing, China). The seed culture employed in our previous work (An et al., 2010) was used in the present study, and was incubated as described previously. The nitrate concentration and biomass growth of this seed culture are presented in Fig. SI 1.

2.2. Experiment design

The integrated nZVI-bacteria denitrification system was prepared in 175-mL serum bottles. Initially, 10 mL of the medium, containing 50 mg L^{-1} of nitrate and other nutrients (An et al., 2010), and a certain amount of the *A. eutrophus* cell suspension (10, 25, or 50 mL, the optical density at 422 nm was about 0.007) were added to each bottle and then diluted to 100 mL with deionized water. Subsequently, this culture was purged with Ar gas for 20 min to remove residual oxygen, and then transferred into another deoxidized serum bottle containing 0.056 g of nZVI particles (80 nm, 54.25 m^2 g^{-1}), synthesized as described by Wang et al. (2006) (Fig. SI 2) using the standard Schlenk and vacuum line techniques. After adjusting the initial pH to 7.0 with HCl, the bottles were sealed with Teflon septa and aluminum crimps, followed by mixing at 150 rpm using a rotary shaker at 30 °C. Parallel experiments were also performed without HTB or nZVI particles.

2.3. Characterization

The surface morphologies of nZVI were characterized by using a scanning electron microscope (SEM, Shimadzu ss-550) operating at an acceleration voltage of 15 kV. The crystal structures of the nanoparticles were examined with a Rigaku D/max-2500 X-ray diffractometer with Cu Kα radiation, voltage of 40 kV, and current of 100 mA (k = 0.1541 nm). The X-ray photoelectron spectroscopy (XPS) spectra of the nanoparticles were recorded by using a Kratos Axis Ultra DLD multi-technique XPS employing a monochromated Al Kα X-ray source (hv = 1486.6 eV), hybrid (magnetic/electrostatic) optics, and a multi-channel plate and delay line detector (DLD).

2.4. Analysis methods

Samples from batch experiments were taken every day with a 1-mL syringe and filtered through a 0.22-μm Millipore filter.

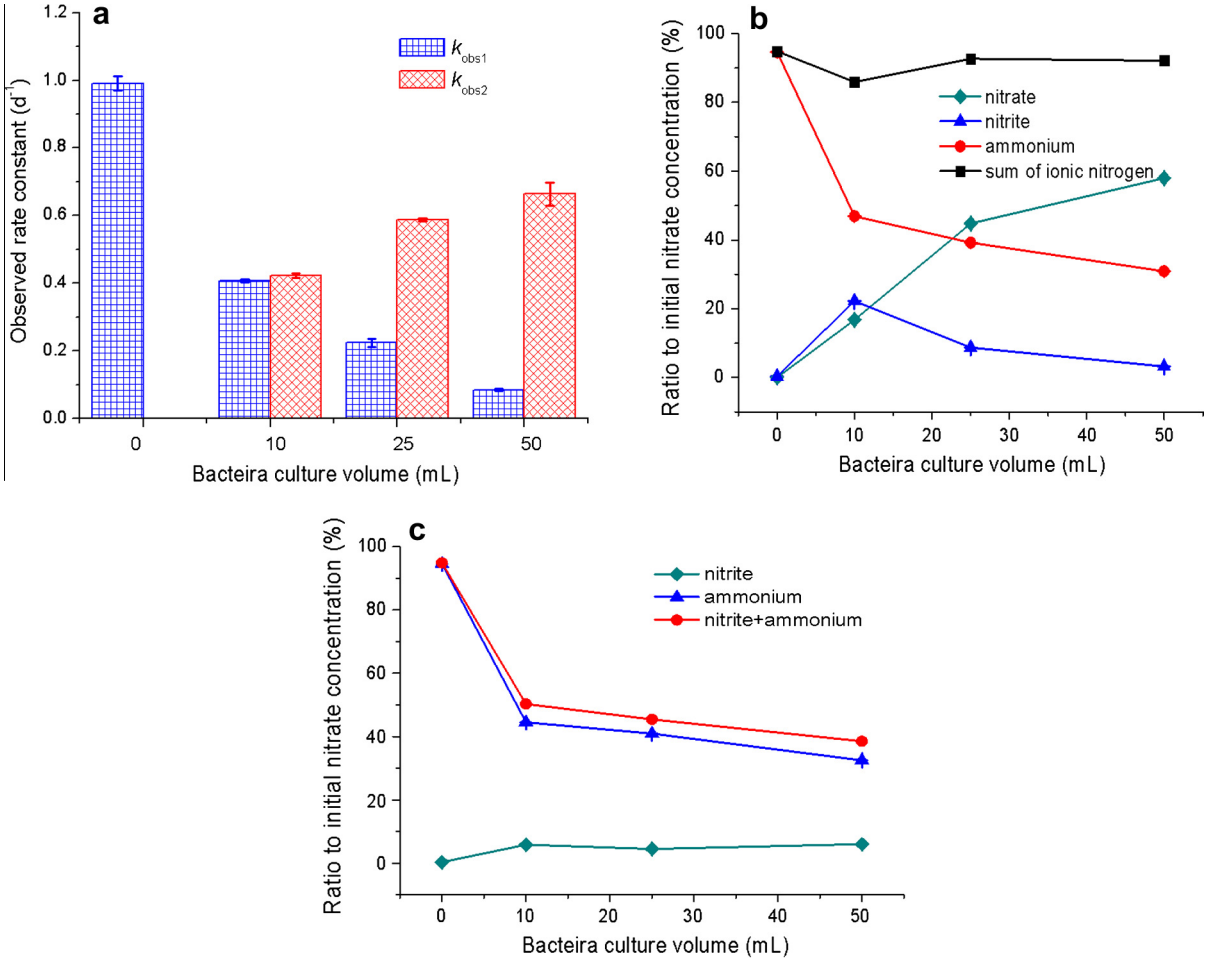

Fig. 1. The effect of HTB on (a) nitrate removal rate, (b) distribution of ionic nitrogen under the steady state (3-day reaction), and (c) poisonous by-products after completion of reaction.

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