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Elucidation of the nitrogen-transformation mechanism for nitrite removal using a microbial-mediated iron redox cycling system

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ABSTRACT

Given the abundance of iron on the Earth, nitrogen-contaminated water is a key region for the biogeochemical cycling of N and Fe under anaerobic conditions. However, the biogeochemical processes between N and Fe remain uncertain, as do the understanding of nitrogen-transformation pathways. In this study, the nitrogen balance for the nitrite removal process in the system containing *Shewanella oneidensis* MR-1 and the iron oxide of either ferrihydrite or magnetite was elucidated by microbially mediated biotic and abiotic reactions with the participation of Fe. Gas chromatography results showed that during the subsequent operation phase with the re-addition of nitrite, nitrite reduction by biogenic Fe(II) was the sole process to produce gaseous nitrogen (N_2O), and yields of which were achieved to 65.56 % and 23.13 % with the ferrihydrite and magnetite systems, respectively. Meanwhile, both ferrihydrite and magnetite remained relatively stable with a small amount of phosphosiderite. Accompanied with biogenic Fe(II) formation in the start-up phase, reduction of nitrite to ammonia coupled with lactate oxidation could be driven by *Shewanella oneidensis* MR-1. Thus, in order to remediate nitrogen pollution in aquatic environment, given the intimate association between the microbial-mediated Fe mineralization process and the biogeochemical cycling of N, it is essential to truly understand the nitrogen-transformation mechanism under the coexistence of both processes.

1. Introduction

Inorganic compounds of nitrogen are widespread water contaminants [1]. Particularly, nitrite can accumulate through denitrification under anaerobic environment, causing a public health problem [2,3]. Moreover, the abundance of iron oxide in aquatic environment makes biogeochemical cycling of N associated with dissimilatory iron reduction possible [4–7]. Yet the actual biogeochemical redox process under such conditions remains uncertain [8]. The inextricable linkages between N and Fe biogeochemical processes have promising potential for nitrite/nitrate removal in aquatic environment. Currently, chemical denitrification, due to its high reactivity and low cost as compared to biological and physicochemical treatments, has been widely applied; however, the presence of ammonium as the reduction end-product of nitrite makes nitrogen removal incomplete in chemical denitrification [9]. Among gaseous nitrogen products (N_g) from denitrification, such as nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2), N_2O is more readily produced than N_2 in nitrite denitrification in Fe(II)-containing systems [10,11]. In general, the composition of the gaseous nitrogen emission in denitrification can be calculated according to corresponding chemical balance equations; however, it would be more informative to elucidate the N_g formation pathways through analytical detection.

Microbial-mediated reducing of nitrite and Fe (III) have drawn considerable attention over the past several years [12–14]. The reactions could be expressed in Eqs. (1)–(6) below, which can generally be happened in anoxic environment at 22–37 °C over a pH range of 6–8.5 [10,11,15–17]. Accompanied by the biogenic Fe (II) released from iron mineral systems with iron-reducing microbes, the fate of nitrite can be influenced greatly due to either the accumulation of dissolved Fe (II) or the formation of secondary mineral phase, the new iron mineral generated under the bioreduction process [18–20]. Moreover, the differences in bioavailability of iron mineral may be largely responsible for the discrepancy of biogenic Fe(II) generation [21], about which few studies have been reported. Simultaneously, although it has been recognized that both abiotic and biotic pathways for nitrite-reduction are involved in biogeochemical N cycle [22], questions still remain on 1) the relative contribution of chemical and microbial reactions and 2) the

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interaction of these two reactions [23].

$$4Fe(OH)_3 + CH_3CHOHCOO^- + 7H^+ = 4Fe^{2+} + CH_3COO^- + HCO_3^- + 10H_2O \quad (1)$$

$$2Fe_3O_4 + CH_3CHOHCOO^- + 11H^+ = 6Fe^{2+} + CH_3COO\text{-} + HCO_3^- + 6H_2O \quad (2)$$

$$6Fe^{2+} + NO_2^- + 16H_2O = 6Fe(OH)_3 + NH_4^+ + 10H^+ \quad (3)$$

$$4Fe^{2+} + 2NO_2^- + 9H_2O = 4Fe(OH)_3 + N_2O + 6H^+ \quad (4)$$

$$9Fe^{2+} + NO_2^- + 10H_2O = 3Fe_3O_4 + NH_4^+ + 16H^+ \quad (5)$$

$$6Fe^{2+} + 2NO_2^- + 5H_2O = 2Fe_3O_4 + N_2O + 10H^+ \quad (6)$$

Multiple nitrite removals accompanied by Fe redox cycles have been achieved in the ferrihydrite- and magnetite-associated *Shewanella oneidensis* MR-1 systems [24]. The objectives of this research were to detail the nitrogen-transformation pathways in these iron redox cycling systems and illustrate the influence of biogenic Fe(II) on the end-products in those systems.

2. Material and methods

2.1. Iron (hydr)oxides preparation and incubation of Shewanella oneidensis MR-1

Ferrihydrite and magnetite were synthesized according to the previously published method [24]. *Shewanella oneidensis* MR-1, a high efficiency type strain, was provided by the Marine Culture Collection of China (MCCC). Cells were grown aerobically in Luria-Bertani medium at 30 °C for 20 h to reach the mid-log phase, after the cells were collected by centrifugation at 10,000 rpm for 10 min and then washed three times with sterilized deionized water. Cells were re-suspended in the sterile and anaerobic (99.99 % He) mineral medium to harvest a cell suspension with an optical density (λ = 600 nm) of approximately 1.0 for the following experiments.

2.2. Experimental setup

The final concentration of approximate 5×10^7 cells/mL of *S. oneidensis* MR-1 were incubated with He, 2500 mg/L Fe (total) (Fe(II) and Fe(III)) of ferrihydrite ($Fe(OH)_3$) or magnetite (Fe_3O_4) and 30 mg/L NO_2^--N. Nitrite, as an intermediate product in the nitrogen cycle, usually can accumulate through denitrification under anaerobic environment. Furthermore, it attracted the less attention even though the more toxic for microbe than nitrate. In anaerobic experiments, lactate ($C_3H_5O_3Na$, 50 mM) and sodium anthraquinone-2, 6-disulfonate (AQDS, 100 μM) were added as the electron donor and electron shuttle, respectively. The pH, measured after all components had been mixed, was 6.8. Serum bottle reactors were incubated in thermostatic shaker (100 rpm) maintained at 30 ± 0.5 °C in the dark. The batch tests for nitrogen transformation and mineral conversion analysis were conducted within 75 h and 180 h, respectively. And the second addition of nitrite was at 30 h. All materials were autoclaved at 121 °C for 30 min before use.

2.3. Chemical analysis

Sampling was conducted with sterile syringes and needles, and then filtered through 0.22 μm PTFE filters. Dissolved NO_2^--N, NO_3^--N and NH_4^+-N were measured by spectrophotometry [25–27]. The concentrations of Fe(II), containing adsorbed and dissolved Fe(II), were analyzed using the ferrozine method [28].

Gaseous nitrogen was detected by GC (GC 2010 plus, Shimadzu, Japan). The standard curve was drawn by an external standard method, which was prepared with a gas standard including 400 ppm of NO_2, 1000 ppm of N_2 and 600 ppm N_2O formulated with He. The gas stream

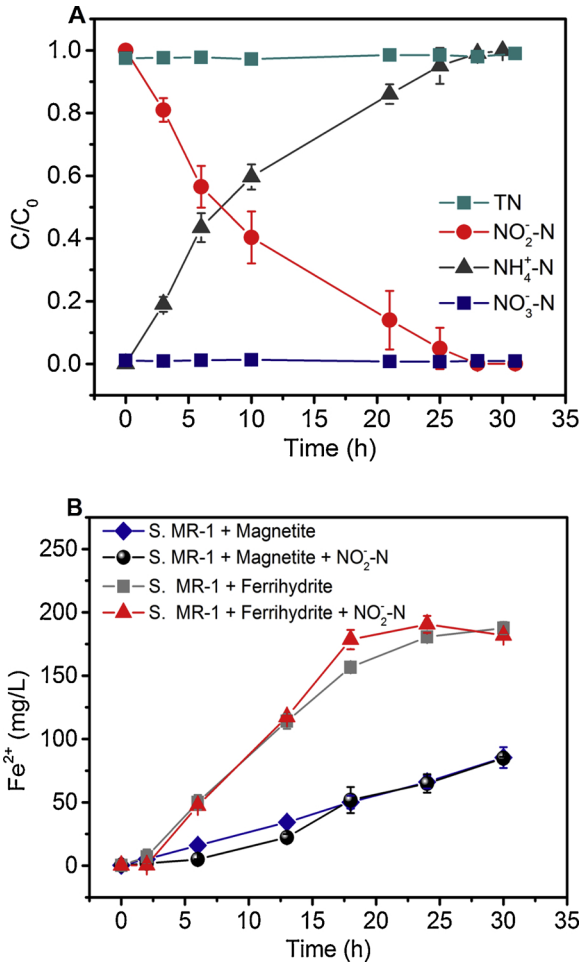

Fig. 1. Products of nitrite reduction by *S. oneidensis* MR-1 (A) and the accumulation of Fe^{2+} in various systems with incubation time (B). Values are averages of triplicate samples. Error bars represent the standard deviation of the mean (n = 3).

was manually sampled for the analysis of GC. According to the standard curve, the concentrations of NO_2, N_2 and N_2O can be calculated. The XRD patterns of magnetite, ferrihydrite and the solid-phase products were recorded on a Rigaku D/max RBX X-ray diffractometer with Cu-Kα radiation (λ = 0.154 nm). The scanning rate was 8°/min in the 2θ range of 5-80°.

Each treatment in the experiments was replicated three times. The data were statistically analysed by Origin (version 9.0, Massachusetts, OriginLab, USA), which were expressed as the mean ± standard deviation (SD).

3. Results and discussion

3.1. Nitrogen-transformation profiles for nitrite removal by S. oneidensis MR-1

Although nitrite was reported to be toxicity to microorganisms, previous experiment found *S. oneidensis* MR-1 could be survived at the concentration of 30 mg/L [24]. Results showed that during the process of nitrite reduction by *S. oneidensis* MR-1 (Fig.1A), NO_2^--N was completely removed within 30 h of the reaction, whereas NH_4^+-N gradually formed as the final product. Nitrite respiration involves either of denitrification and nitrate ammonification pathways [29]. Respiratory denitrification is catalyzed by two distinct enzymes, the NirK and NirS enzymes, which reduce nitrite to nitric oxide; the NrfA enzyme reduces nitrite to ammonium in respiratory nitrate ammonification [30,31].