Nitrate reduction in the interactive reaction system of L17 and soil minerals

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Abstract

In this study, the potential for microbially catalyzed NO\textsubscript{3}\textsuperscript{-} reduction with iron oxide was examined using \textit{Klebsiella pneumoniae} strain L17 and four types of iron oxides under anaerobic conditions. The results showed that L17 had the capacity of nitrate reduction, and iron oxides can accelerate the reduction rate significantly. The biogenic Fe(II) contributed to the acceleration slightly, which was not enough to reduce so much nitrogen. To investigate the role of iron oxide for the nitrate reduction, a series of minerals but iron oxides with L17 were combined for nitrate reduction, and the results showed all the oxides can accelerate the reduction rate, indicating that the electron might transfer to nitrate through the oxides but not experiencing iron oxide’s reduction. Hence, besides the well-known mechanism: direct microbial reduction and reduction by the biogenic Fe(II), a new mechanism was proposed whereby soil minerals can mediate electron transfer to accelerate the microbial nitrate reduction. This study could be helpful in understanding the relationship between the redox cycles of Fe and N in subsurface sedimentary environments.

Key Words

Nitrogen cycle, iron cycle, electron transfer, denitrification, dissimilatory iron reduction.

Introduction

Nitrogen is an essential element for living organisms, and the availability of a suitable nitrogen source often limits primary productivity in both natural environments and agriculture (Cabello \textit{et al.} 2004). It is well known that the natural nitrate reduction is mainly attributed to the biotic process of direct enzyme catalysis by nitrate reduction bacteria (Gonzalez \textit{et al.} 2006), while various abiotic and biotic-abiotic combined processes have also been reported to be responsible for the natural reduction of nitrate in anoxic environment (Jørgensen \textit{et al.} 2009). Firstly, the biotic processes involve (i) the anaerobic reduction of NO\textsubscript{3} to N\textsubscript{2}O and N\textsubscript{2} (denitrification), (ii) the conversion of NO\textsubscript{3} into ammonia (dissimilatory ammonification), and (iii) the conversion of nitrate to ammonia, which is used by the cell to incorporate nitrogen into biomolecules (assimilation) (Gonzalez \textit{et al.} 2006). Secondly, it is suggested that ferrous iron as electron donors is capable of reducing nitrate in anaerobic, sedimentary environments (Jørgensen \textit{et al.} 2009). Reduction of nitrate to ammonia can proceed at appreciable rates in abiotic systems in the presence of green rust compounds at circumstance pH (Ottery \textit{et al.} 1997). The presence of crystalline iron oxide (lepidocrocite and goethite) surfaces accelerates low-temperature reduction of NO\textsubscript{3} coupled to Fe(II) oxidation at pH values greater than 8.0 (Hansen \textit{et al.} 2009). Thirdly, microbially catalyzed nitrate reduction coupled to Fe(II) oxidation under anaerobic environment has also been reported (Straub \textit{et al.} 1996; Weber \textit{et al.} 2001), and the role of biogenic Fe(II) was taken into consideration for nitrogen cycling.

The occurrence of biological Fe(II)-dependent nitrate reduction in a variety of natural systems suggests that this reaction may play a significant role in coupling the redox cycles of Fe and N in sedimentary environments. However, it is also observed that the molar ratios of NO\textsubscript{3} consumed to Fe(II) oxidized exceeded the theoretical stoichiometry (Straub \textit{et al.} 1996; Weber \textit{et al.} 2001; Nielsen \textit{et al.} 1998). The reason for this disagreement is unclear in these previous studies. Hence, the study was aim at explaining the reason of above disagreement in the simulated system of bacteria/soil mineral/nitrate, and the mechanism will be further discussed.

Methods

Materials

NaN\textsubscript{O\textsubscript{3}} (>99.0\%) were purchased from Sigma-Aldrich without further purification. Other chemicals being of analytical grade were purchased from Guangzhou Reagent Factory, China. \textit{Klebsiella pneumoniae} strain L17 was a dissimilatory iron reducing bacterium (DIRB), isolated subterranean forest sediment in Zhaoqing, China (Li \textit{et al.} 2009). Goethite (α-FeOOH), Lepidocrocite (γ-FeOOH), hematite (α-Fe\textsubscript{2}O\textsubscript{3}), and Maghemite (γ-Fe\textsubscript{2}O\textsubscript{3}) were synthesized according to procedures as previously described (Li \textit{et al.} 2009),

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Experimental procedure

To avoid the interference of other inorganic anions in detection, the anaerobic NaHCO₃-buffered (30 mM, pH 6.8, N₂:CO₂ (80:20) atmosphere) medium only contained 7.5 mM NaN₃ or NaNO₂ as electron accepter and 5 mM glucose as electron donor, and harvested cells of K. pneumoniae L17 were added with final concentration of ca. 10⁷ cells/ml. Cells were grown in nutrient broth under aerobic conditions on a rotary shaker at 180 rev/min at 30°C, and harvested by centrifugation at 6,900 × g for 10 min at 4°C when it approached the exponential phase. The pellets were washed three times and resuspended in sterile fresh basal medium to an optical density of 0.7 to 1.1 (λ = 600 nm). The density of 1.1 corresponded to approximately 1.4 × 10⁸ cells/ml, based on preliminary experiments that correlated culture optical density with viable cell counts determined by serial dilution and plating. Several batch experiments for NO₃⁻/NO₂⁻ reduction including controls were conducted in this study: (1) Fe²⁺ (0.3 mM); (2) α-FeOOH (25 mM); (3) α-FeOOH (25 mM) + Fe²⁺ (0.3 mM); (4) L17; (5) L17 + α-FeOOH, γ-FeOOH, α-Fe₂O₃, γ-Fe₂O₃, Bi₂O₃, Al₂O₃, Nd₂O₃, ZrO₂, or TiO₂ (4.5 g/L). Standard anaerobic techniques were used throughout all experiments as previously described (Li et al. 2009). Inoculation and sampling were conducted by using sterile syringes and needles. All vials were conducted in duplicate and incubated in a BACTRON Anaerobic/Environmental Chamber II (SHELLAB, Sheldon Manufacturing Inc.) at 30°C in dark.

Analytical methods.

To remove the cells and oxide, samples for determination of NO₃⁻/NO₂⁻ must be filtrated using a 0.22-µm syringe filter after centrifugation at 8,500 × g for 20 min. The concentration of NO₃⁻/NO₂⁻ was determined by ion chromatography (Dionex ICS-90) with an ion column (IonPac AS14A 4 × 250 mm). A mobile phase consisting of Na₂CO₃ (8.0 mM) and NaHCO₃ (1.0 mM) solutions was operated at a flow rate of 1.0 mL/min. The total concentration of Fe(II), including dissolved and sorbed Fe(II), was determined by extracting Fe(II) from the samples using 0.5 mol/L HCl for 1.5 h and assaying the extract using 1,10-phenanthroline colorimetric assay. Dissolved Fe(II) was determined by removing the mineral and adsorbed Fe(II) from the aqueous phase using a 0.22-µm syringe filter and then assaying the filtrate by 1,10-phenanthroline. Adsorbed Fe(II) was calculated as the difference between the total and dissolved Fe(II).

Results

NO₃⁻ reduction

Figure 1a showed that L17 can reduce nitrate efficiently from 7.5 mM to 0 mM in 4 days, while the NO₃⁻ reduction rate was obviously accelerated by the addition of iron oxides, and the total NO₃⁻ (7.5 mM) disappeared completely just in 2 days. The first-order-rate constants (k) in Figure 1c suggested that the k values of L17/α-FeOOH, L17/γ-FeOOH, L17/α-Fe₂O₃, and L17/γ-Fe₂O₃ were 2.1732/d, 2.3132/d, 2.6806/d, and 2.8828/d, much higher than that of L17 alone (0.6503/d). To illustrate the role of biogenic Fe(II) from L17, to confirm this hypothesis, a series of minerals but not iron oxides (non-IOs) were used in this reaction system, such as Bi₂O₃, Al₂O₃, Nd₂O₃, ZrO₂, and TiO₂. As shown in Figure 1b, in comparison with L17 alone, the NO₃⁻ reduction rate was accelerated by the addition of non-IOs, and The first-order-rate constants (k) in Figure 1c suggested that the k values of L17/Bi₂O₃, L17/Al₂O₃, L17/Nd₂O₃, L17/ZrO₂, and L17/TiO₂ were 0.766/d, 1.1243/d, 1.2695/d, 1.4583/d and 1.6038/d, higher than that of L17 alone (0.6503/d). The above important finding suggested that the acceleration of nitrate reduction might be attributed to the possible mechanism that the electron from L17 can be transferred through the semiconductor, which can lead an increase of electron transfer.

Proposed mechanisms and reduction pathways

Based on the above discussion, the electron transfer from cell to nitrate/nitrite may have three ways (Figure 2), (i) the direct reduction via the metabolism of the bacterium, (ii) the reduction by biogenic adsorbed Fe(II) of dissimilatory iron reduction, besides these two ways, a new way was proposed as (iii) the semiconductor-
mediated electron transfer process. It must be clear that the electron transfer for the reduction of nitrate and Fe(III) oxide is originally driven by the microbe L17. And the biogenic adsorbed Fe(II) can also contribute to the denitriﬁcation slightly, while a large fraction of electron from L17 was directly transferred to nitrogen through the iron oxide, which lead a signiﬁcant enhancement of nitrate/nitrite reduction. Regarding to semiconductor-mediated electron transfer process, an important question was raised how the electron from the L17 transfer through the semiconductor. Herein we proposed another hypothesis, which was described as: the electron from cells can be injected to the conduction band of semiconductors, and then transferred to the surface, ﬁnally, it can be accepted by the surface reducible species, including nitrate, Fe(III) and so on. Next work will be focused on proving this hypothesis.

**Conclusion**

The study showed that L17 had the capacity of nitrate reduction, and iron oxides can accelerate the reduction rate signiﬁcantly. But the biogenic Fe(II) contributed to the acceleration slightly, which was not enough to reduce so much nitrogen. A series of non iron oxides with L17 were combined for nitrate reduction, and the results showed all the oxides can accelerate the reduction rate, indicating that the electron might transfer to nitrate through the oxides but not experiencing iron oxide’s reduction. Hence, besides the well-known mechanism: direct microbial reduction and reduction by the biogenic Fe(II), a new mechanism was conﬁrmed as soil mineral can mediate electron transfer to accelerate the microbial nitrate reduction.

**References**


