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Nitrate bioreduction in redox-variable low permeability sediments

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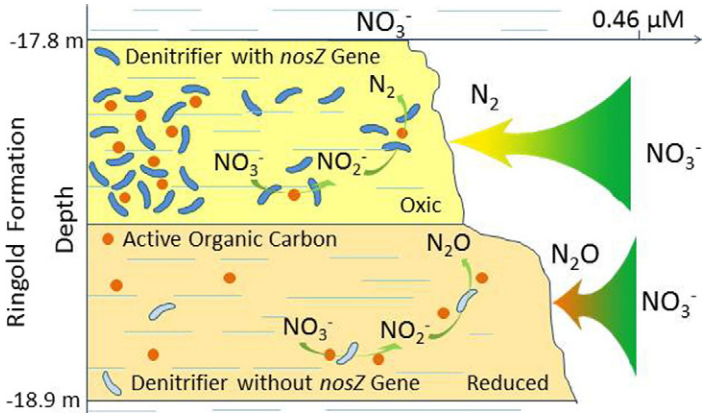
HIGHLIGHTS

- Low permeability zones (LPZ) can microbially remove nitrate in groundwater.
- The rate and end product of nitrate bioreduction vary within LPZ.
- Greenhouse gas N_2O can be the end product of nitrate bioreduction in LPZ.
- Organic carbon, denitrifier mass, and gene expression are the controlling factors.

GRAPHICAL ABSTRACT



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ABSTRACT

Low permeability zone (LPZ) can play an important role as a sink or secondary source in contaminant transport in groundwater system. This study investigated the rate and end product of nitrate bioreduction in LPZ sediments. The sediments were from the U.S. Department of Energy's Hanford Site, where nitrate is a groundwater contaminant as a by-product of radionuclide waste discharges. The LPZ at the Hanford site consists of two layers with an oxidized layer on top and reduced layer below. The oxidized layer is directly in contact with the overlying contaminated aquifer, while the reduced layer is in contact with an uncontaminated aquifer below. The experimental results showed that nitrate bioreduction rate and end-product differed significantly in the sediments. The bioreduction rate in the oxidized sediment was significantly faster than that in the reduced one. A significant amount of N_2O was accumulated in the reduced sediment; while in the oxidized sediment, N_2O was further reduced to N_2. RT-PCR analysis revealed that *nosZ*, the gene that codes for N_2O reductase, was below detection limit in the reduced sediment. Batch experiments and kinetic modeling were performed to provide insights into the role of organic carbon bioavailability, biomass growth, and competition between nitrate and its reducing products for electrons from electron donors. The results revealed that it is important to consider sediment redox conditions and functional genes in understanding and modeling nitrate bioreduction in subsurface sediments.

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The results also implied that LPZ sediments can be important sink of nitrate and a potential secondary source of N_2O as a nitrate bioreduction product in groundwater.

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1. Introduction

Nitrate is a major contaminant in groundwater worldwide (Rivett et al., 2008). Nitrate attenuation in groundwater is primarily controlled by biogeochemical processes that reduce nitrate (NO_3^-) to gaseous nitrous oxide (N_2O), dinitrogen (N_2), or ammonium (NH_4^+) (Zumft, 1997). In aquatic systems, nitrate bioreduction predominately occurs in sediments (Laverman et al., 2006), removing nitrate entering rivers (Laverman et al., 2010) and oceans (Ward, 2013). Extensive research has been performed to understand various processes and factors controlling the rate, extent, and intermediate and end products of nitrate bioreduction in environments (Rivett et al., 2008; Seitzinger et al., 2006). The rate and extent of nitrate bioreduction are affected by dissolved oxygen concentration (Rassamee et al., 2011), organic carbon speciation and contents (Babbin et al., 2014; Lu and Chandran, 2010), and denitrifier abundance and gene expression (Zhang et al., 2014). Other factors including nitrate concentration, nutrient availability, pH, and temperature can also affect the rate and intermediate products of nitrate bioreduction (Firestone et al., 1980; Pan et al., 2012; Rivett et al., 2008). In reduced sediments, the presence of sulfide and Fe(II) species may stimulate nitrate bioreduction via sulfide or Fe(II) oxidation (Vaclavkova et al., 2014; Zhang et al., 2009). A recent study found that the end product of nitrate bioreduction in oligotrophic lake sediments is largely determined by sediment redox conditions (Small et al., 2014). However, the mechanisms and process-level understanding on the effect of redox conditions on nitrate bioreduction rate in the subsurface and its relationship with microbial functional gene are not well-understood.

Nitrate contaminant in the subsurface can be attenuated via denitrification (Rivett et al., 2008), which refers to an assemblage of nitrate, nitrite, NO, and N_2O respiration in the absence of oxygen (Zumft, 1997). N_2O can be the end product of incomplete denitrification (Zumft, 1997). Various factors have been identified that lead to N_2O emission as the end product of nitrate bioreduction. The presence of trace oxygen, minor free nitrous acid species, low pH, high initial nitrate, and nitrite concentrations can individually or collectively inhibit N_2O reduction to N_2 (Firestone et al., 1980; Kampschreur et al., 2009; Laverman et al., 2010; Pan et al., 2012; Rassamee et al., 2011). On the other hand, high organic carbon concentration, low nitrate and nitrite concentration, and long duration of anaerobiosis favor N_2 as the end product (Butterbach-Bahl et al., 2013; Kampschreur et al., 2009; Weymann et al., 2010). These findings were primarily derived from studies in wastewater treatment systems, agricultural soils, streams and rivers. Limited studies indicated that groundwater systems are also important sources or sinks of N_2O (Ronen et al., 1988; Weymann et al., 2010). The relative importance of N_2O production in groundwater to land/surface N_2O flux, the mechanisms leading to N_2O accumulation in groundwater, and reactive transport of N_2O in sediment-groundwater system have not been well studied.

Low permeability zones (LPZs) are important subsurface units that can retard and attenuate contaminant migration in groundwater (Arighi et al., 2005; Robertson et al., 1996; Liu and Ball, 2002). Nitrate bioreduction in LPZ sediments is, however, an under-studied area (Rivett et al., 2008). Limited studies indicate that LPZ sediments may strongly affect the fate and transformation of nitrate in groundwater (Lin et al., 2012a; Lee et al., 2012). These studies revealed that nitrate bioreduction primarily resulted from heterotrophic activities, and the effect of lithoautotrophic Fe(II)- and sulfide-oxidizing microorganisms (such as *E. shelobolina*) was negligible (Percak-Dennett and Roden, 2014). A diffusion-based reactive transport model had been established by incorporating nitrate bioreduction kinetics that provided important insights into nitrate migration and attenuation in LPZ (Percak-Dennett and Roden, 2014). These previous studies in the LPZ sediments, however, focused on the loss of nitrate without considering the reduction kinetics of nitrite and N_2O. Field monitoring at the U.S. Department of Energy's Hanford site, however, found the accumulation of N_2O in groundwater in LPZ and nearby aquifer zones, indicating that it is important to consider the bioreduction of nitrate and its reduction products. In addition, the redox potential of LPZ sediments often varies as a function of distance from nearby aquifer. Consequently, the objective of this study is to investigate the bioreduction kinetics of nitrate and its reduction products in LPZ sediments with different redox potentials. Batch experiments using both the oxidized and reduced sediments were performed to characterize the bioreduction rates of nitrate and its reduction products, and to determine the end product of nitrate bioreduction (e.g., N_2O, N_2, or NH_4^+). Real-time polymerase chain reaction (RT-PCR) analysis was performed to investigate the mechanisms controlling N_2O or N_2 as the end product of nitrate bioreduction. Controlled experiments and kinetic modeling were performed to provide insights into the effect of organic carbon content and speciation, microbial growth, and various model parameters on nitrate bioreduction. The results provided important insights into the effect of sediment redox state, indigenous organic carbon, and long-term adaption of microbial community on the rate and end product of nitrate bioreduction in LPZ sediments as subsurface units.

2. Materials and methods

2.1. Sediments

The sediments used in this study were obtained from a LPZ at the U.S. Hanford 300 Area Integrated Field Research Challenge (IFRC) site (http://ifchanford.pnl.gov), where nitrate is a contaminant in the overlying aquifer (Bjornstad et al., 2009). The LPZ consists of an upper layer of yellow-brown Miocene–Pliocene-aged lacustrine fine-grained oxidized sediment (OS) and a lower layer of dark gray to black Miocene–Pliocene-aged lacustrine fine-grained reduced sediment (RS). The LPZ separates the overlying aquifer formation from the underlying Ringold formation (Lin et al., 2012b). The OS and RS were collected aseptically from ca. 18.0–18.3 m depth and ca. 18.6–18.9 m depth of core C-6209 recovered during drilling. The sediment core was stored in a −80 °C freezer and thawed at 4 °C before use for the experiments. The geochemical and microbial community properties in the sediments have been characterized previously as described elsewhere (Lee et al., 2012; Lin et al., 2012a, 2012b; Percak-Dennett and Roden, 2014; Peretyazhko et al., 2012). Important properties from the previous characterization and the present study are summarized in Table 1. Briefly, the RS contains over 9 times of organic carbon and HCl-extractable Fe(II) than the OS. The biomass and microbial diversity in the RS are much lower than those in the OS. The biomass numbers determined by most probable number culture method (Lin et al., 2012a) show that denitrifiers, aerobes, and fermenters reside in both OS and RS with higher abundance in the OS. Sulfate reducer population is similar in the OS and RS.

2.2. Nitrate reduction experiments

2.2.1. Batch experiments

Batch experiments were performed in duplicates to determine nitrate bioreduction rate and to identify factors controlling the rate and end product of nitrate bioreduction in the sediments. Sediment suspensions were prepared in 160 mL glass bottles, by mixing a