

Combined Microbial-Fe(0) Treatment System to Remove Nitrate from Contaminated Groundwater

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Abstract: Experiments were conducted to delineate the applicability and limitations of biologically active Fe(0) barriers to remove nitrate under various geochemical and hydraulic conditions. Microcosm studies showed that, while no Fe(0) treatment was needed to remove nitrate from a high-carbon soil, adding Fe(0) to a low-carbon soil supplemented the electron donor pool and enhanced nitrate removal. Montmorillonite, an acidic aluminosilicate mineral, enhanced Fe(0) corrosion and nitrate removal (from about 1 to 3 mg/L NO₃⁻-N per day), and reduced the transient accumulation of nitrite. Combining autotrophic denitrifiers (e.g., *Paracoccus denitrificans*) with Fe(0) significantly reduced the amount of nitrite eluted from aquifer columns, from up to 7 to less than 1 mg/L NO₂⁻-N. Bacteria were observed to preferentially colonize the Fe(0) surface, which produces cathodic H₂ when corroded by water. The preferential colonization of Fe(0) suggests that hydrogenotrophic consortia are likely to develop around Fe(0) walls to exploit cathodic depolarization as a metabolic niche.

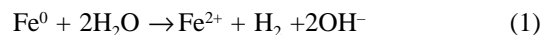
Introduction

Modern agricultural practices and increased use of fertilizers have resulted in increased amounts of nitrate entering groundwater systems (Nolan et al., 1997). Nitrate in drinking water supplies is of concern because of health risks such as methemoglobinemia. To prevent this, a maximum contaminant level (MCL) for drinking water of 10 mg/L NO₃⁻-N was set in the United States.

Although numerous physical-chemical processes have been proposed for the removal of nitrate from water, many of these are marginally cost-effective and/or have detrimental side-effects on water quality (Kapoor and Viraraghavan, 1997). The use of autotrophic, H₂-utilizing denitrifiers is an attractive technique, due primarily to the cleanliness of the process. Because carbon dioxide is the carbon source for au-

totrophic growth, there is no need to remove residual organic substrates. Hydrogen gas is innocuous and is only slightly soluble in water (0.78 mg/L at 1 atm, 25°C). Autotrophic growth also leads to lower cell yield and less biomass production. Nevertheless, the relatively high cost and low solubility of hydrogen represents a limitation for delivering this energy source to autotrophic denitrifying bacteria.

Research with hydrogenotrophic, anaerobic bacteria suggests a method by which iron corrosion could be exploited to overcome limitations associated with hydrogen delivery in autotrophic denitrifying systems. Daniels et al. (1987) demonstrated that when zero-valent iron (Fe(0)) is submerged in anoxic water, hydrogen gas is produced via cathodic depolarization:



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The use of this cathodic hydrogen as an energy source to support bacterial growth has been demonstrated for several types of anaerobic pure cultures, including autotrophic denitrifiers (Till et al., 1998). In such cases, nitrate is eventually reduced via nitrite to N_2 gas. Abiotic reduction of nitrate by Fe(0) has also been studied, and proceeds also via nitrite through a different pathway that leads to the less desirable end product, NH_4^+ (Huang et al., 1998; Till et al., 1998).

This work investigated the applicability and limitations of combining hydrogenotrophic denitrifiers with Fe(0) to remove nitrate under various geochemical and hydraulic conditions. The following variables were considered: (1) the organic carbon content of the aquifer material, which represents a potential electron donor pool to sustain denitrification; (2) the presence of aluminosilicate minerals, which may buffer the Fe(0)-corrosion-induced increase in pH that inhibits microorganisms; and (3) the limits of hydraulic loadings that can be handled by a biologically active Fe(0) barrier to intercept and treat nitrate plumes.

Materials and Methods

Aquifer microcosms were prepared in duplicate using 250-mL serum bottles capped with Mininert⁺ valves. All microcosms were amended with 90 g of either top garden soil (2.5% organic C) or sand (0.1% organic C) plus 100 mL of bicarbonate-buffered mineral medium containing inorganic nutrients and 30 mg/L NO_3^- -N. The medium recipe is described elsewhere (Till et al., 1998). Some microcosms were amended with montmorillonite clay. These contained 80 g of low-carbon soil and 10 g of montmorillonite (Fisher Scientific). Biological and abiotic transformations were studied separately and interactively by including or excluding Fe(0) (10 g Fisher Fe-filings, 0.1410 m^2/g) and denitrifying hydrogenotrophs (10 mL of *Paracoccus denitrificans*, ATTC# 17741, $OD_{600} = 0.006$). Abiotic removal was assessed per NH_4^+ accumulation (Till et al., 1998), and biological denitrification was quantified per N_2O accumulation in acetylene-blocked microcosms (Yoshinari and Knowles, 1976).

The feasibility of bioaugmenting Fe(0) in sandy soil was also investigated in continuous-flow columns. Glass columns (26.5 cm long, 2.5 cm diameter) were packed with a mixture of sand and Fe(0) filings (15% by weight). One column was autoclaved to discourage growth of indigenous microbes, while the other was seeded with *P. denitrificans*. Mineral medium containing 33 mg/L NO_3^- -N was pumped through the columns in an upflow mode using a Masterflex[®] (Barrington, IL) 7523-30 peristaltic pump with a 519-

15 pump head. The flowrate was varied from 0.02 to 0.16 mL/min (i.e., hydraulic retention time [HRT] = 0.25 to 2 d). The influent reservoirs were 5000-mL Pyrex jars topped with Teflon[®]-lined delivery caps. These reservoirs were continuously purged with an N_2/CO_2 (80:20 v/v) gas mixture using tubing with a stone diffuser. At the end of the 152-d experiment, samples were taken from the soil/Fe(0) matrix and examined under a scanning electron microscope.

Nitrate and nitrite were analyzed using a Dionex BioLC ion chromatograph, and ammonium was measured with a Dionex DX-100 ion chromatograph as described elsewhere (Till et al., 1998).

Results and Discussion

Semi permeable reactive Fe(0) barriers are particularly attractive for groundwater remediation in that they conserve energy and water, and through long-term low operating and maintenance costs have the potential to be considerably less costly than conventional clean up methods. Early studies of Fe(0) barriers found little microbial contribution to contaminant degradation, and research has focused primarily on abiotic processes. Nevertheless, the potential for microorganisms to enhance reductive treatment with Fe(0) has been demonstrated in recent laboratory experiments (Till et al., 1998; Weathers et al., 1997). This work corroborates that microbial utilization of cathodic hydrogen is a critical link between biogeochemical interactions and enhanced contaminant removal.

Batch Aquifer Microcosms

Microcosms prepared with high-carbon (2.5%) top soil removed nitrate relatively fast (approximately 30 mg/L as N within 4 d), regardless of whether they were amended with Fe(0), *P. denitrificans*, both, or none. Apparently, indigenous microorganisms utilized naturally occurring organic matter as electron donors to denitrify. Thus, neither Fe(0) nor bacterial amendment enhanced NO_3^- removal in high-carbon topsoil. In contrast, adding Fe(0) to low-carbon (0.1%) sandy soil had a beneficial effect compared with control microcosms without Fe(0). These controls exhibited no nitrate removal, possibly due to a low concentration of suitable electron donors in the sandy soil. Adding Fe(0) supplemented the electron donor pool and enhanced nitrate removal below the MCL (10 mg/L as N) within 20 d (Figure 1).

Adding *P. denitrificans* to Fe(0)-amended, low-carbon microcosms did not enhance nitrate removal. These microcosms experienced a large increase in pH

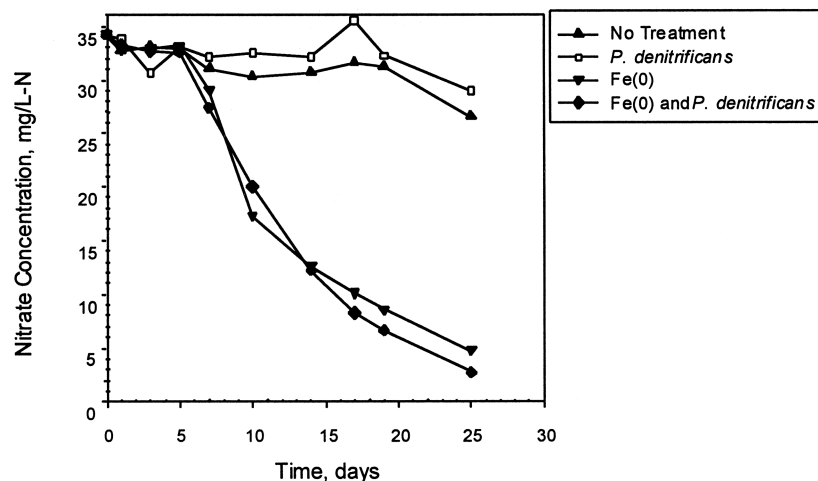


Figure 1. Nitrate removal in low-carbon (0.1%) aquifer microcosms.

(i.e., $\text{pH} > 10$ after 25 d) due to Fe(0) corrosion (Equation 1). Such high pH levels are known to inhibit microbial denitrification (Till et al., 1998). Therefore, abiotic nitrate reduction by Fe(0) outcompeted the biological denitrification pathway. This notion is supported by the stoichiometric reduction of nitrate to ammonium, which is the end product of the abiotic reaction, and by the absence of (microbially produced) N_2O in acetylene-blocked microcosms (data not shown). This indicates that pH control may be important to sustain Fe(0)-based denitrification.

Montmorillonite clay has been reported to buffer against the corrosion-induced pH increase in Fe(0) systems. This is accomplished through a series of dissolution and proton-generating reactions at the Fe(0) surface (Powell and Puls, 1997). Thus, the presence of such acidic aluminosilicate minerals may improve the reactivity of Fe(0) barriers. This is illustrated in Figure 2, which depicts the ability of montmorillonite to enhance nitrate removal in Fe(0)-amended microcosms. These microcosms were sterilized to isolate the effect of montmorillonite on iron reactivity. Interestingly, adding montmorillonite (11% by weight) also prevented the accumulation of the more toxic intermediate, nitrite. This was attributed to enhanced Fe(0) reactivity due to a lower pH at the Fe(0) surface. Enhanced Fe(0) reactivity in montmorillonite-amended microcosms was also indicated by higher H_2 evolution (data not shown).

A 5-day lag period was observed in Fe(0)-amended microcosms (Figures 1 and 2). During this period, little nitrate removal was observed. The reason for this lag period is unknown, although the fact that it also occurred in sterile (poisoned) microcosms suggests that it was related to iron chemistry rather

than to microbial activity. Some researchers suggest that H_2 adsorbed to the Fe(0) surface, rather than the Fe(0) itself, may be responsible for contaminant reduction (Grittini et al., 1995; Schreier and Reinhard, 1995). According to this untested hypothesis, Fe(0) may act as a catalyst, activating H_2 in a manner similar to metals such as palladium. Perhaps the lag period reflects the time required to accumulate enough H_2 at the Fe(0) surface to facilitate the reaction. Alternatively, the Fe(0) used in this experiment was not acid washed, which raises the possibility that there was an oxide layer acting as a barrier to electron transfer (Scherer et al., 1998). Thus, the lag period could also reflect the time required to “activate” this layer by either reducing it with electrons from the inner core of the iron, or by building up aqueous concentrations of ferrous iron to form a reactive oxide. Interestingly, adding montmorillonite eliminated this lag period (Figure 2), which reiterates the potential for acidic minerals to enhance the reactivity of iron barriers.

Column Studies

The influent solution to both columns had a pH of about 7.0 units and a dissolved oxygen (DO) concentration of about 1 mg/L. The effluent pH was about 8.0 units for both columns, and no DO was detected in the effluents ($\text{DO} < 0.1$ mg/L). Figure 3 depicts effluent nitrate concentrations for different hydraulic retention times (HRT). Nitrate removal efficiencies were similar for bioaugmented and unamended columns. Decreasing the HRT had an adverse effect on nitrate removal efficiency. By day 123, at an HRT of 0.25 day, the nitrate concentra-

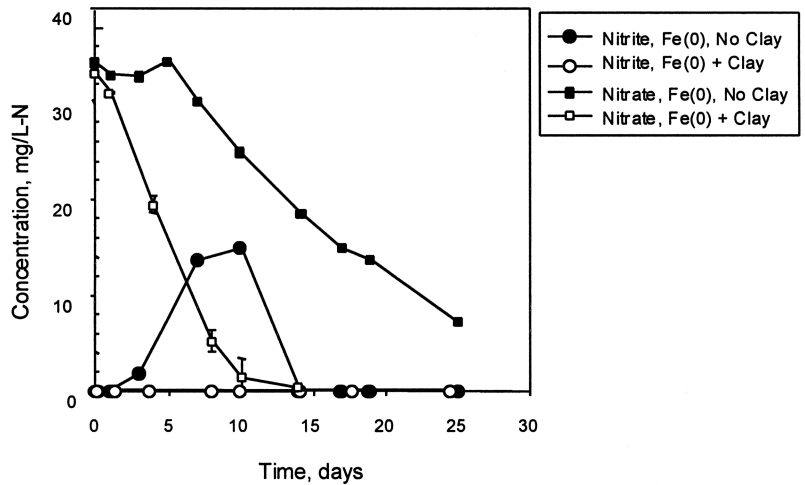


Figure 2. Effect of montmorillonite clay (11% by weight) on abiotic nitrate removal by Fe(0) and transient accumulation of nitrite. Data correspond to microcosms that were poisoned with 300 mg/L HgCl₂. Error bars depict one standard deviation from the mean of triplicate microcosms.

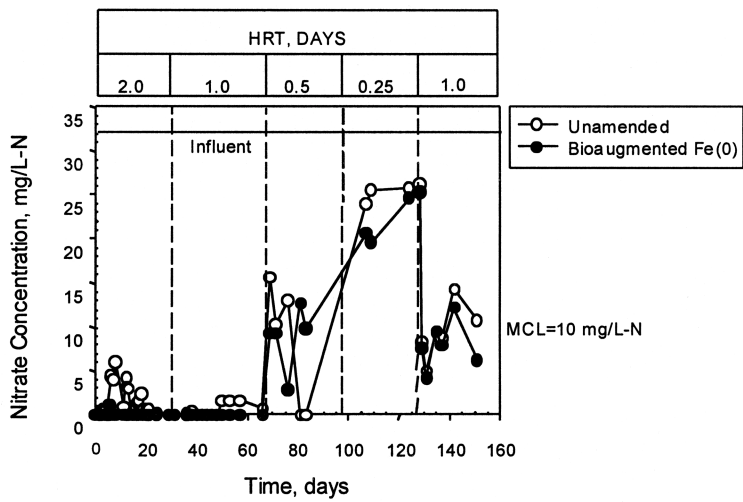


Figure 3. Effect of hydraulic retention time (HRT) on the effluent nitrate concentration from aquifer columns.

tion in both columns increased to 25 mg/L NO_3^- -N. This corresponds to relatively low (17%) removal efficiency. Yet, increasing the HRT back to 1.0 day decreased the effluent nitrate concentration below 10 mg/L (as N) for both columns (73% removal efficiency). This indicates that the loss of nitrate removal capacity was due to hydraulic overloading, which results in HRTs that are too short for efficient nitrate removal, rather than to exhaustion of Fe(0) reactivity over time. The typically slow flow velocity of groundwater would ensure long retention times conducive to high removal efficiencies.

Nitrite is a toxic intermediate of nitrate reduction by both the abiotic and biological pathways. Figure 4 depicts the effluent nitrite concentration in this same experiment. Effluent nitrite concentrations from the unamended column increased to as high as 7 mg/L NO_2^- -N early in the study, while nitrite concentrations in the effluent of the bioaugmented column generally remained below the MCL (1 mg/L NO_2^- -N). This was attributed to microbial denitrification of nitrite to N_2 . As the columns acclimated over time, nitrite was eliminated from the effluent of both columns. Acclimation of the autoclaved column was attributed to the fact that microbial activity was not completely eliminated, reflecting the difficulty to sterilize soil successfully and to keep such column systems sterile over extended periods of time.

The effluent ammonium concentrations for both columns followed similar trends, peaking at about 30 mg/L NH_3 -N after 7 weeks when the HRT was 1.0 day. Ammonium concentrations decreased subsequently in both columns below 10 mg/L NH_3 -N, suggesting an increase in nitrate removal by microbial denitrification.

The soil and Fe(0) filings from the columns were analyzed at the end of the experiment using scanning electron microscopy. Bacteria were found to selectively colonize the Fe(0) surfaces (over soil grains) in both columns. This suggests that indigenous denitrifiers may colonize Fe(0) barriers to exploit cathodic depolarization as a metabolic niche. The selective colonization of Fe(0) surfaces would facilitate microbial uptake of relatively insoluble cathodic H_2 .

In summary, biologically active Fe(0) barriers hold great promise for treating groundwater contaminated with nitrate, and should also be considered to treat other redox-sensitive priority pollutants. Bioaugmentation of the barrier matrix with autotrophic denitrifiers can shorten the acclimation period required for indigenous bacteria to colonize the Fe(0) surface. Alternatively, an indigenous hydrogenotrophic consortium is likely to eventually develop around an H_2 -producing Fe(0) barrier. Once microbial populations are established, the bacteria may enhance the treatment process by reducing the amount of nitrite accumulating as an intermediate of abiotic nitrate reduction and improve Fe(0) corrosion kinetics by removing the passivating H_2 layer from the Fe(0) surface. Thus, appropriate bacteria can enhance both the rate and extent of transformation and yield a more favorable end product distribution.

Acknowledgments

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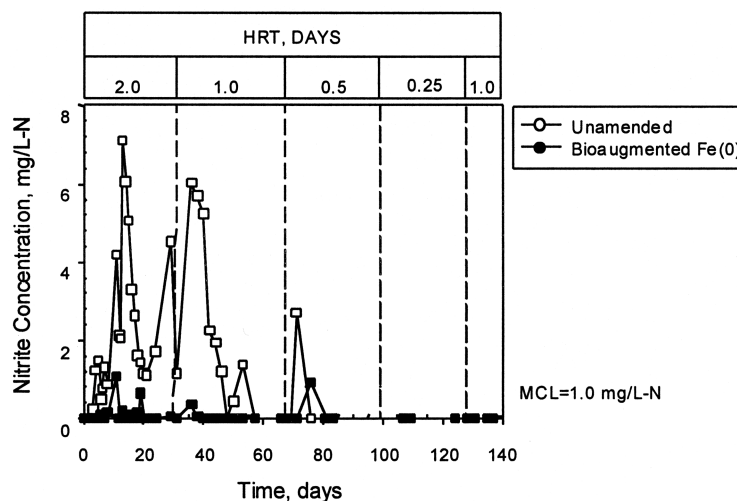


Figure 4. Effluent nitrite concentration from continuous flow columns.

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References

- Daniels, L., N. Belay, B. Rajagopal, and P. Weimer. 1987. Bacterial Methanogenesis and Growth from CO₂ with Elemental Iron as the Sole Source of Electrons. *Science* 23: 509-511.
- Grittini, C., M. Malcomson, Q. Fernando, and N. Korte. 1995. Rapid Dechlorination of Polychlorinated Biphenyls on the Surface of a Pd/Fe Bimetallic System. *Environ. Sci. Technol.* 29: 2898-2900.
- Huang, C.-P., H.-W. Wang, and P.-C. Chiu. 1998. Nitrate Reduction by Metallic Iron. *Wat. Res.* 32: 2257-2264.
- Kapoor, A. and T. Viraraghavan. 1997. Nitrate Removal from Drinking Water-Review. *J. Environ. Eng.* 123(4): 371-380.
- Nolan, B.T., B.C. Ruddy, K.J. Hitt, and D.R. Helsel. 1997. Risk of Nitrate in Groundwaters of the United States — A National Perspective. *Environ. Sci. Technol.* 31: 2229-2236.
- Powell, R.M. and R.W. Puls. 1997. Proton Dissolution of Intrinsic or Augmented Aluminosilicate Minerals for In Situ Contaminant Remediation by Zero-Valence-State Iron. *Environ. Sci. Technol.* 31: 2244-2251.
- Scherer, M.M., B.A. Balko, and P.G. Tratnyek. 1998. The Role of Oxides in Reduction Reactions at the Metal-Water Interface. In *Mineral-Water Interfacial Reactions: Kinetics and Mechanisms*. pp. 301-322. (D. Sparks and T. Grundl, Eds.). 1998 ACS Symposium Series No. 715, American Chemical Society: Washington, DC.
- Schreier, C.G. and M. Reinhard. 1995. Catalytic Hydrodehalogenation of Chlorinated Ethylenes Using Palladium and Hydrogen for the Treatment of Contaminated Water. *Chemosphere* 31(6): 3475-3487.
- Till, B.A., L.J. Weathers, and P.J. Alvarez. 1998. Fe(0)-Supported Autotrophic Denitrification. *Env. Sci. Technol.* 32: 654-659.
- Weathers, L.J., G.F. Parkin, and P.J.J. Alvarez. 1997. Utilization of Cathodic Hydrogen as Electron Donor for Chloroform Cometabolism by a Mixed Methanogenic Culture. *Environ. Sci. Technol.* 31(3): 880-885.
- Yoshinari, T. and R. Knowles. 1976. Acetylene Inhibition of Nitrous Oxide Reduction by Denitrifying Bacteria. *Biochem. Biophys. Res. Comm.* 69:7 05-710.