Iowa State Water Resources Research Institute

Annual Technical Report

FY 1998

Introduction

Research Program

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Treatment of nitrate-contaminated groundwater using zero-valent iron and autotrophic denitrification
Project Number	R-0007
Start Date	09/01/1998
End Date	08/30/2000
Research Category	Biological Sciences
Focus Category #1	Groundwater
Focus Category #2	Nitrate Contamination
Focus Category #3	Treatment
Lead Institution	Iowa State University

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Pedro J. Alvarez	Unknown	University of Iowa	01

Problem and Research Objectives

Nitrate is a priority pollutant due to its potential to cause methemoglobinemia. There is also circumstantial evidence linking nitrate ingestion to gastric cancer and birth defects (Mirvish, 1985).

Nitrate contamination is a major water quality problem in the United States, especially in the North Central Region (Nolan et al., 1997). In Iowa alone, 1 million tons of nitrogen are applied each year, and 18% of the private wells contain nitrate above the drinking water standard of 10 mg/l as N. Another 37% of the wells have levels greater than 3 mg/l as N, typically considered indicative of anthropogenic pollution (Kross et al., 1993). The ubiquity of the nitrate contamination problem is reflected in a 1985 AWWA survey, which found that 23% of all primary drinking water standard violations in the United States were due to high nitrate concentrations (Kapoor and Viraraghavan, 1997). Without appropriate cleanup measures, nitrate can persist in subsoils and endanger groundwater resources and public health. Therefore, there is considerable public interest and regulatory pressure to clean up nitrate-contaminated aquifers. Several physical-chemical and biological processes have been proposed for this purpose (e.g., reverse osmosis and ion exchange). However, traditional treatment processes are relatively expensive to operate and are limited by the production of nitrate-concentrated waste streams that may pose a disposal problem. This provides a strong motivation to explore novel nitrate-removal alternatives that addresses both physical-chemical and microbiological advantages and constraints.

The general goal of this project is to exploit favorable biogeochemical interactions in Fe(0) barriers to enhance the remediation of aquifers contaminated with nitrate and nitrite. Laboratory experiments that address critical knowledge gaps will be performed during two years. Specific objectives include:

1) To delineate the applicability and limitations of biologically-active semipermeable Fe(0) barriers to intercept and remove nitrate and/or nitrite from groundwater plumes, and to obtain basic criteria for the design and operation of biologically active Fe(0) barriers.

2) To determine the effect that Fe(0) has on the activity of denitrifying bacteria, and to evaluate the effect that the bacteria have on the abiotic reduction of nitrate by Fe(0).

3) To determine how environmental factors and substrate interactions affect the efficiency of bioaugmented Fe(0) barriers to attenuate nitrate migration under various hydraulic regimes, and to evaluate the effect of microbial growth on the permeability of the barrier.

Methodology

Aquifer microcosms will be used to evaluate feasibility of Fe(0)-based denitrification, to obtain basic criteria on its applicability and limitations, to assess how different contaminant mixtures, temperature, and redox conditions affect removal kinetics and end product distribution, and to gain a better understanding of the interactions between Fe(0) and microorganisms. Flow-through aquifer columns will be used to evaluate how environmental factors and hydraulics affect nitrate removal efficiency and product distribution, and how microbial growth affects the permeability of the barrier. Please refer to the original proposal, and to the following section, for details on experimental and analytical methods.

Principal Findings and Significance

Summary: 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 high-carbon soils, adding Fe(0) to low-carbon soil can supplement the electron donor pool and enhance nitrate removal. Montmorillonite, an acidic aluminosilicate mineral, enhanced Fe(0) corrosion and nitrate removal, 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. Bacteria were observed to preferentially colonize the Fe(0) surface, which produces cathodic H_2 when corroded by water. The preferential colonization of Fe(0) suggest that hydrogenotrophic consortia are likely to develop around Fe(0) walls to exploit cathodic depolarization as a metabolic niche.

Background: Although numerous physical-chemical processes have been proposed for removal of nitrate from water, many of these have proven marginally cost-effective and/or have detrimental side-effects on water quality. The use of autotrophic, hydrogen-utilizing denitrifiers is an attractive technique, due primarily to the cleanliness of the process. Since CO_2 is the carbon source for autotrophic growth, there is no need to remove residual organic substrates. H_2 gas is inherently clean and only slightly soluble in water (0.78 mg/L at 1 atm, 25^{o}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. 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 (Huang *et al.*, 1998; Till *et al.*, 1998), and proceeds through a different pathway leading to the less desirable end product, NH_4^+.

This past year, we investigated the applicability and limitations of combining hydrogenotrophic denitrifiers with Fe(0) to remove nitrate under various geochemical and hydraulic conditions. Variables considered included (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.

Methods: Aquifer microcosms were prepared in duplicate using 250 mL serum bottles capped with Mininert valves. All microcosms were amended with 90 grams of either 2.5% org-C soil or 0.1% org-C sand plus 100 mL of mineral medium containing 30 mg/L NO_3-N. Some microcosms were amended with montmorillonite clay. These contained 80 grams of low-carbon soil and 10 grams of montmorillonite. Biological and abiotic transformations were studied separately and interactively by including or excluding Fe (0) (10g Fisher Fe-filings) and denitrifying hydrogenotrophs (10 mL of *Paracoccus denitrificans*, ATTC# 17741, OD_{600}=0.006). Abiotic removal was assessed per NH_4^+ accumulation, 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 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 a liquid culture of *P. denitrificans* (OD_{600}=10). 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 mL/min to 0.16 mL/min (i.e., HRT = 2 to 0.25 days). 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 day study, samples were taken from the soil/Fe(0) matrix and examined under a scanning electron microscope. All anions were measured using a DioneX BioLC ion chromatograph. Ammonium was measured using a Dionex DX-100 ion chromatograph.

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 cleanup 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.

Microcosms prepared with high-carbon (2.5%) top soil removed nitrate relatively fast (ca. 30 mg/L as N within 4 days), 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 top soil. In contrast, the addition of Fe(0) to low-carbon (0.1%) sandy soil had a beneficial effect, resulting in the removal of nitrate below the MCL (10 mg/L as N) within 20 days (Figure 1). The no-treatment 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. Adding *P. denitrificans* to Fe(0)-amended microcosms did not enhance nitrate removal. Fe(0) corrosion increased the pH in these microcosms to a level that inhibited denitrifiers (pH after 25 days). This notion is supported by the absence of N_2O in acetylene-blocked replicates.

FIGURE 1: Nitrate removal in low-carbon aquifer microcosms. Fe(0) enhanced nitrate removal to a greater extent than bioaugmentation, possibly due to a corrosion-induced increase in pH that inhibited bacteria.

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). In order to provide a more hospitable environment for microbial growth, montmorillonite was used as a buffer against pH increase. Figure 2 depicts the ability of montmorillonite to enhance nitrate removal by Fe(0) in soil microcosms. Montmorillonite also prevented the accumulation of the more toxic intermediate, nitrite. These effects were attributed to enhanced Fe(0) corrosion at a lower pH. Enhanced Fe(0) reactivity in montmorillonite-amended microcosms was also indicated by higher H_2 evolution. Thus, the presence of naturally occurring aluminosilicate minerals may improve the performance of Fe(0) barriers.

FIGURE 2: Effect of montmorillonite clay (11% by weight) on abiotic nitrate removal by Fe(0) and transient accumulation of nitrite.

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 days, the nitrate concentration 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 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.

Figure 4 depicts the effluent nitrite concentration in this same experiment. Nitrite concentrations in the effluent of 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 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.

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

FIGURE 4. Effluent nitrite concentration from continuous flow columns.

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

In summary, biologically-active Fe(0) barriers hold great promise as a cost-effective alternative 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.

References Blowes D.W., Ptacek, C.J., Cherry, J.A., Gillham, R.W. & Robertson, W.D. (1995), Passive Remediation of Groundwater Using *IN Situ* Treatment Curtains. In: Yalcin B. A. and D. E. Daniel (eds.). Geoenvironment 2000: Characterization, Containment, Remediation, and Performance in Environmental Geotechniques, New York, N.Y.

Challis, B. (1973). "Rapid Nitrosation of Phenols and its Implications for Health Hazards from Dietary Nitrites." *Nature*. 244: 466.

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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." *Environmental Science and Technology*. 31: 2244-2251.

Till, B. A., L. J. Weathers, and P.J. Alvarez, "Fe(0)-Supported Autotrophic Denitrification." *Environ. Sci. Technol*. (1998), 32, 654-659.

Descriptors

Biological Treatment, Groundwater, Indigenous Microorganisms, In Situ Remediation, Nitrate, Nitrite, Reactive Barriers

Articles in Refereed Scientific Journals

Dejournett T. and P.J.J. Alvarez (1999) Combined Microbial-Fe(0) System to Treat Nitrate-Contaminated Groundwater. Bioremediation Journal (submitted).

Book Chapters

Dissertations

Dejournett, Todd. 1999. Combined Microbial-Fe(0) System to Treat Nitrate-Contaminated Groundwater. M.S. Thesis. Department of Civil and Environmental Engineering. University of Iowa.

Water Resources Research Institute Reports

Conference Proceedings

Other Publications

Information Transfer Program

Results of Dr. Alvarez' project were presented at an international conference, entitled In Situ and Onsite Bioremediation, The Fifth International Symposium sponsored by Battelle, San Diego, California, April 19-22, 1999.

The results of the Bear Creek research have been utilized in several educational workshops sponsored this year including; 1) four, one-day riparian management workshops for USDA-NRCS professionals, 2) a one day workshop for USEPA Region 6 Nonpoint Source Pollution Conference in Arkansas, and 3) a half-day workshop for the Sustainable Agriculture Research and Education Program, Facing a Watershed: Managing Profitable and Sustainable Landscapes in the 21st Century Conference. Research Team members have made over 60 presentations to public and private groups plus presentations at professional meetings during the past year. They also hosted more than 30 tours of the Bear Creek research and demonstration sites.

The results of the research project by Schultz et al. have been utilized in three educational workshops sponsored this year including; 1) a two day riparian management workshop for professionals in government and non-profit organizations, 2) a one day workshop for Missouri Agroforestry Conference, and 3) a half-day workshop at National Arbor Convention in Atlanta. Research Team members have made over 60 presentations to public and private groups plus presentations at professional meetings during the past year. They also hosted more than 30 tours of the Bear Creek research and demonstration sites. The Bear Creek project has been recognized by the U.S. Natural Resources Conservation Service as one of the finest riparian buffer systems in the nation, and has been selected as a National NRCS Training Site.

Another source of information is our webpage for the Institute and interdepartmental graduate major. We are currently in the process of updating this site.

As in the past, the Institute continues to offer complimentary copies of its publications to the public. The Institute continues to cooperate with the ISU Parks Library in establishing a collection of water resources literature and also maintains a small collection in the Agronomy Department Reading Room.

USGS Internship Program

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	0	5	0	0	5
Masters	0	5	0	0	5
Ph.D.	0	2	0	0	2
Post-Doc.	0	1	0	0	1
Total	0	13	0	0	13

Awards & Achievements

Publications from Prior Projects

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Other Publications