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Fe(0)-Supported Autotrophic Denitrification

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Proof of concept was obtained that Fe(0) can stoichiometrically reduce nitrate to ammonium and that cathodic hydrogen [produced during anaerobic Fe(0) corrosion by water] can sustain microbial denitrification to reduce nitrate to more innocuous products (i.e., N_2O and N_2). Autotrophic, denitrifying growth on Fe(0) was proven through the use of a dual-flask apparatus. Cathodic H_2 from a flask containing Fe(0) was allowed to diffuse to another (anoxic) flask containing a pure culture of *Paracoccus denitrificans,* where denitrification and microbial growth were observed. Nitrate reduction and end product distribution were studied in batch reactors amended with either steel wool or Fe(0) powder. Steel wool, with a smaller specific surface area, was less reactive, and its corrosion did not significantly increase the pH of the solution. This allowed for a greater participation of denitrifiers in the nitrate removal process, which increased nitrate removal rates and transformed a greater portion of the added nitrate to innocuous gases rather than to ammonium. Combining denitrifiers with the more reactive Fe(0) powder did not increase removal rates or decrease the proportion of nitrate reduced to ammonium. This was attributed to a corrosion-induced increase in pH above the tolerance range of the bacteria (pH > 10). Nitrate removal was sustained over 4 months in flow-through columns packed with steel wool and seeded with autotrophic denitrifiers. Increasing the hydraulic retention time from 0.67 to 2.33 days increased the nitrate removal efficiency and decreased the fraction of nitrate reduced to ammonium. The finding that Fe(0) can sustain autotrophic denitrification may have practical applications to treat nitrate-contaminated waters in ex-situ or in-situ reactive filters.

Introduction

Nitrate is a priority pollutant due to its potential to cause methemoglobinemia. There is also circumstantial evidence linking ingestion of nitrate to gastric cancer and birth defects (*1*). Nitrate contamination is a major water quality problem in agricultural regions (*2*). In Iowa alone, 1 million ton of nitrogen is applied each year, and 18% of the private wells contain nitrate above the drinking water standard of 10 mg/L NO_3^--N. Another 37% of the wells have levels greater than 3 mg/L NO_3^--N, typically considered indicative of anthropogenic pollution (*3*). 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 (*4*).

Nitrate-contaminated waters are commonly treated by ion exchange or by reverse osmosis. These traditional treatment processes, however, are relatively expensive to operate and are limited by the production of nitrate-concentrated waste streams that may pose a disposal problem (*4*). Thus, there is considerable interest in developing alternative treatment approaches. One novel chemical process that is currently undergoing evaluation is abiotic nitrate reduction by zero-valent metals (*5*, *6*). This method holds great potential for use in in-situ reactive barriers of the type currently intercepting and treating groundwater plumes containing Cr(VI) (*7*) or chlorinated solvents (*8*). Such semipermeable reactive walls are particularly attractive 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. Nevertheless, reductive treatment of nitrate with zero-valent metals is constrained by the production of ammonium as the primary end product (*5*). Ammonium has an adverse aesthetic impact on drinking water and may interfere with subsequent disinfection processes (*9*).

Biological denitrification is another alternative to remove nitrate from drinking water sources. Denitrification reduces nitrate to innocuous nitrogen gas rather than to ammonium and generally results in lower operating costs as compared to ion exchange and reverse osmosis (*4*). Denitrification, however, can produce excessive biomass and soluble microbial products that require subsequent treatment, especially when heterotrophic bacteria are used. This has awakened considerable interest in the use of autotrophic, hydrogenotrophic denitrifiers, which can remove nitrate more cleanly with production of less residual organics.

Autotrophic denitrification using hydrogen as an electron donor has been studied by numerous researchers (*10*−*14*) and proceeds by the following reaction (*10*):

$$2NO_3^- + 5H_2 \rightarrow N_2 + 4H_2O + 2OH^- \qquad (1)$$

Hydrogen is one of the most thermodynamically favorable electron donors for nitrate-based respiration, and its high diffusivity through biofilms is conducive to enhanced nitrate removal. Nevertheless, the use of hydrogen in engineered denitrification systems is limited by its relatively high cost, low solubility, and hazardous (explosive) properties during handling and storage.

Research with hydrogenotrophic, anaerobic bacteria suggests a method by which iron corrosion could be exploited to overcome limitations associated with hydrogen delivery in denitrifying systems. When iron metal is immersed in water, its oxidation is coupled with the reduction of water-derived protons to form cathodic hydrogen (*15*):

$$Fe(0) + 2H_2O \rightarrow H_2 + Fe^{2+} + 2OH^- \qquad (2)$$

Cathodic hydrogen has been used as an energy source for the autotrophic growth of pure cultures of methanogenic, homoaceotogenic, and sulfate-reducing bacteria (*15*, *16*) and as an electron donor for reductive dechlorination by a mixed

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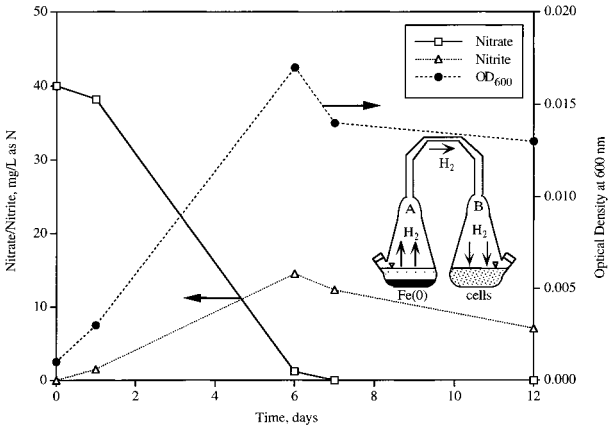

FIGURE 1. Autotrophic growth of *Paracoccus denitrificans* in a dual-flask apparatus. Growth was coupled to Fe(0) corrosion and nitrate reduction. Flask A contained metallic iron and water, and flask B contained nitrate-amended, carbonate-buffered medium inoculated with bacteria.

methanogenic culture (*17*). This suggests that cathodic hydrogen could also be used to sustain autotrophic denitrification.

This study addresses the feasibility of supporting autotrophic denitrification using Fe(0) (via cathodic hydrogen) as the sole energy source. Emphasis was placed on investigating the effect of pH, Fe(0) surface area concentration, and hydraulic retention time on nitrate removal efficiency and end product distribution. In doing so, information was obtained to provide a basis for screening process applicability and limitations.

Materials and Methods

Experimental Design. *Batch Reactors.* A dual-flask apparatus was constructed to demonstrate that Fe(0) (via cathodic hydrogen) can serve as the original electron donor and energy source for biological denitrification. Two 250-mL Pyrex Erlenmeyer flasks (Fisher Scientific) were fused at the top with glass tubing as described by Belay and Daniels (*18*). An opening was made on the side of each flask for sample addition or removal. The opening was extended by fusing a short section of glass tubing onto it and projecting it as a side arm and was sealed with Teflon-lined septa and aluminum crimps. One of the flasks (A) contained 10 g of acid-washed Fe(0) powder (2.02 m^2/g, Aldrich Chemical Co.) and 100 mL of deionized water (Figure 1 inset). The other flask (B) contained 100 mL of mineral medium with 40 mg/L NO_3^--N and was seeded with 5 mL of *Paracoccus denitrificans* cell suspension from a stock culture reactor. In addition to nitrate, the mineral medium contained the following (in mg/L): $NaHCO_3$ (250), KH_2PO_4 (50), and a trace "metals" solution comprised of $CuCl_2 \cdot 2H_2O$ (0.0392), $ZnCl_2$ (0.1363), $NiCl_2$ (0.013), $FeCl_2 \cdot 4H_2O$ (0.7016), $AlCl_3$ (0.1106), $MnCl_2 \cdot 4H_2O$ (0.2807), $CoCl_2 \cdot 6H_2O$ (0.0382), $Na_2MoO_4 \cdot 2H_2O$ (0.0254), H_3BO_4 (0.0382), and Na_2SO_4 (0.1420). The flasks were purged with N_2/CO_2 (80:20, v/v) following inoculation to remove dissolved oxygen and to provide an inorganic carbon source for autotrophic growth. Controls lacking either Fe(0), nitrate, or inocculum were similarly prepared. The flasks were incubated for 12 days at 21 °C under quiescent conditions.

The effect of pH on the denitrification activity of *P. denitrificans* was studied in 250-mL serum bottles fed hydrogen gas as the electron donor. Duplicate reactors containing 100 mL of mineral medium, 25 mg/L NO_3^--N, 20 mL of H_2/CO_2 (80/20, v:v) gas mix, and 5 mL of *P. denitrificans* cell suspension were set up at pH 5, 6, 7, 9, 10, and 11. The initial pH was adjusted with HCl and NaOH, and pH values increased less than 0.2 unit during this experiment.

Batch experiments were also conducted to compare the fate of nitrate in abiotic versus biological treatment systems. Duplicate treatments were prepared inside an anaerobic chamber with 250-mL serum bottles containing 150 mL of deoxygenated (N_2/CO_2 purged) mineral medium plus nitrate (50 mg/L as N). Abiotic reactors were amended with 10 g of either steel wool [medium 1, 0.0075 m^2/g, Rhodes American] [0.4 m^2/L Fe(0) surface area concentration] or acid-washed Fe(0) powder [135 m^2/L Fe(0) surface area concentration]. Biological reactors were seeded with 5 mL of *P. denitrificans* from a stock culture and were fed 40 mL of H_2 gas (1 atm) as electron donor. Combined treatment reactors were also prepared with iron plus bacteria using the same quantities described above. Sterile controls contained 200 mg/L of the biocide $HgCl_2$.

An acetylene block technique was performed on seeded reactors to quantify biological denitrification. Acetylene blocks nitrous oxide reductase and prevents nitrate reduction beyond N_2O. This enables the quantification of denitrification per N_2O accumulation (*19*). Seeded reactors were injected with 10 mL of acetylene gas. All reactors were quiescently incubated at 21 °C and were periodically sampled until all of the nitrate and nitrite were removed from the seeded reactors.

Samples were taken with 5-mL disposable syringes and filtered through 0.2-μm nylon Acrodisc filters (Gelman Sciences) prior to storage and analysis. All glassware, lids, and media were autoclaved at 240 °C for 15 min prior to use.

Column Reactors. Continuous-flow columns were used to investigate the ability of hydrogenotrophic denitrifiers to sustain nitrate removal in the presence of Fe(0) in a flow-through system. Two glass columns (2.5 cm diameter × 26.5 cm long) were packed with 8 g of steel wool. One column was seeded with a mixed culture of hydrogenotrophic denitrifiers and was maintained under no-flow conditions for 7 days to permit the bacteria to colonize the steel wool. The other column was kept sterile to control for the effect of the added bacteria. This was accomplished by adding 200 mg/L $HgCl_2$ to the influent medium. Mineral medium containing 50 mg/L NO_3^--N was continuously purged with N_2/CO_2 (80:20, v/v) gas in 25-L polyethylene carboys and fed to the columns in an up-flow mode using a Masterflex 7523-30 peristaltic pump. The columns had a porosity of 0.9 and an advection-dominated hydraulic regime (Peclet number = 396), based on a bromide tracer study conducted as described by Chen et al. (*20*). Both columns were operated at two different flow rates (10 and 3 mL/h) corresponding to hydraulic retention times (HRT) of 0.67 and 2.33 days, respectively. These retention times were chosen to obtain Darcy velocities representative of in-situ conditions ($5.7 \times 10{-}4$ and $1.7 \times 10{-}4$ cm/s) (*21*).

Stock Culture Reactors. The source of organisms for all experiments were two 250-mL glass reactors containing 100 mL of cell suspension and 150 mL of H_2/CO_2 gas (80:20, v/v). An axenic culture of *Paracoccus denitrificans* (American Type Culture Collection 17741) was used for batch experiments, while a mixed culture of hydrogen-oxidizing, denitrifying (HOD) aquifer microorganisms (*13*) was used for column experiments. The reactors were incubated at 21 °C on an orbital shaker table rotating at 100 rpm and maintained using a semi-batch feed and waste mode with a 20-day mean cell residence time. The optical density of both suspensions was 0.03 at 600 nm (i.e., about 10 mg/L as total suspended solids).

Analytical Methods. Nitrate and nitrite were analyzed with a DioneX BioLC ion chromatograph. Ammonium was analyzed on an Alltech cation system with an ERIS 1000 autosupressor and Hewlett-Packard 3396 series integrator. Total Kjeldahl nitrogen (TKN) was analyzed by the University of Iowa Hygienic Laboratory using Standard Method 4500-NB. Nitrous oxide was measured with a Hewlett-Packard

TABLE 1. Fate of Nitrate in Batch Reactors, as a Percentage of the Added Nitrate[a]

treatment	unreacted (NO_3^--N), %	denitrified biologically (N_2O-N), %	reduced abiotically by Fe(0) (NH_4^+-N), %	assimilated by bacteria (organic N), %	mass balance closure (total N), %
Fe(0) powder	0	0	98 ± 0.6	0	98 ± 0.6
Fe(0) powder and bacteria	0	2 ± 0.8	94 ± 3.0	2 ± 0.2	98 ± 1.8
steel wool	68 ± 3.2	0	30 ± 2.6	0	98 ± 0.6
steel wool and bacteria	0	64 ± 10.8	28 ± 8.0	2 ± 2.0	94 ± 1.2
H_2 gas and bacteria	0	93 ± 2.4	0	1 ± 0.4	94 ± 0.8

[a] The initial concentration was 50 mg/L NO_3^--N. Incubation times were 8 days for reactors prepared with Fe(0) powder and 12 days for reactors prepared with steel wool or with H_2 gas. Seeded reactors were acetylene blocked and had about 1 mg/L of organic N associated with *P. denitrificans*. Values represent the average of duplicate treatments ± 1 SD. No nitrite was detected in any of the reactors (LOD < 0.5 mg/L NO_2^--N).

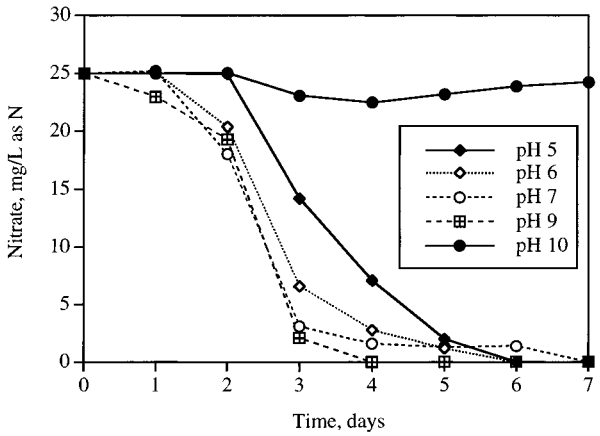

FIGURE 2. Effect of pH on nitrate removal by *Paracoccus denitrificans*. Reactors were fed H_2 and incubated at 21 °C on a rotary shaker table at 100 rpm. Data represent the average of duplicate treatments.

5890 Series II gas chromatograph equipped with a thermal conductivity detector and an Alltech packed Haysep Q molecular sieve column. Detection limits were 0.5 mg/L as N for nitrate, nitrite, and ammonium and 0.1 mg/L as N for nitrous oxide.

Dissolved oxygen (DO) was measured with a MI-730 membrane oxygen electrode and 02 ADPT adapter (Microelectrodes, Inc.). The response of the electrode was measured using a Fluke 77 Series II Multimeter. The DO detection limit was approximately 0.1 mg/L. Biomass concentrations were measured by optical density at 600 nm using a Milton Roy Spectronics 601A spectrophotometer. A Beckman Model F 71 pH meter was used for pH measurement.

Total organic carbon was measured with a Shimadzu TOC 5000 system. The detection limit was approximately 0.3 mg/L of carbon.

Results

Batch Reactors. A dual-flask apparatus seeded with *P. denitrificans* was used to demonstrate that Fe(0) (via cathodic hydrogen) could sustain autotrophic denitrification. Following hydrogen generation in flask A (Figure 1, inset), nitrate was removed below the detection limit within 7 days with a transient accumulation of nitrite and a concomitant increase in microbial concentration in flask B (Figure 1). No nitrate removal or microbial growth occurred in control runs lacking Fe(0), nitrate, or inoculum (data not shown).

The effect of pH on the denitrification activity of *P. denitrificans* was studied without Fe(0) using batch reactors fed H_2 gas. Experiments conducted at different (stable) pH values showed that pH did not have a statistically distinguishable effect ($p > 0.05$) on nitrate removal in the range of pH 6−9 (Figure 2). Denitrification occurred at pH 5, although at a slower rate. No nitrate was removed within two weeks at pH 10 or greater.

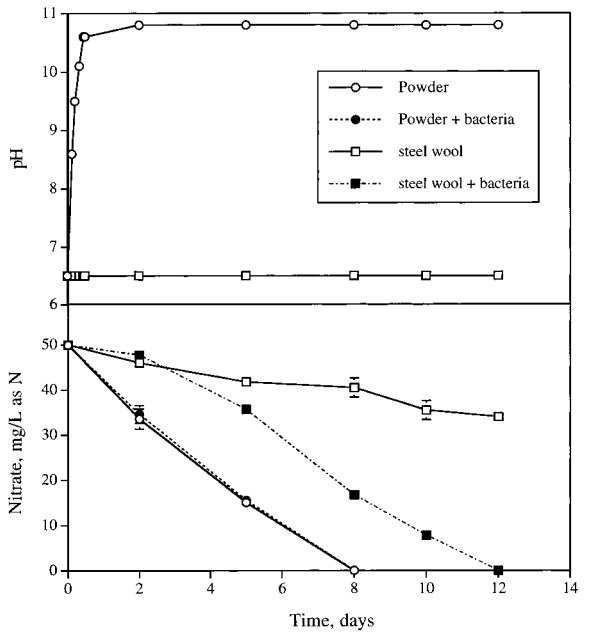

FIGURE 3. Nitrate removal and pH change in batch reactors amended with steel wool or Fe(0) powder. *P. denitrificans* enhanced nitrate removal in reactors containing steel wool but not iron powder. Corrosion of the more reactive iron powder increased the pH above the tolerance range of *P. denitrificans*.

Nitrate was removed in abiotic reactors amended with either Fe(0) powder or steel wool, and (within experimental error) all of the nitrate removed was reduced to ammonium (Table 1). Reactors with Fe(0) powder removed all of the nitrate within 8 days, while abiotic reactors amended with steel wool removed only 20% of the added nitrate within this time (Figure 3). Reactors containing Fe(0) powder experienced a rapid increase in pH, from 6.5 to 10.6 units within 12 h, and the pH stabilized at 10.8 within 2 days (Figure 3). In contrast, the pH remained constant at 6.5 in the reactors prepared with the less reactive steel wool or with H_2 gas.

Adding bacteria did not significantly affect the rate of nitrate removal (Figure 3) nor the fate of nitrate (Table 1) in reactors prepared with Fe(0) powder. Similar to the abiotic reactors, seeded reactors with Fe(0) powder reduced nearly all of the added nitrate to ammonium and only 2% was biologically denitrified to N_2O (Table 1). In contrast, bacteria had a significant effect on the fate of nitrate in reactors prepared with steel wool. While 68% of the added nitrate remained after 12 days in abiotic reactors prepared with steel wool alone, all of the nitrate was removed within this time in reactors with steel wool plus bacteria (Figure 3). Furthermore, while the abiotic reactors converted nitrate to ammonium as the end product, the seeded (acetylene-blocked) reactors denitrified most of the added nitrate (64%) to N_2O and reduced only 28% of it to ammonium (Table 1). No ammonium was detected in seeded reactors prepared

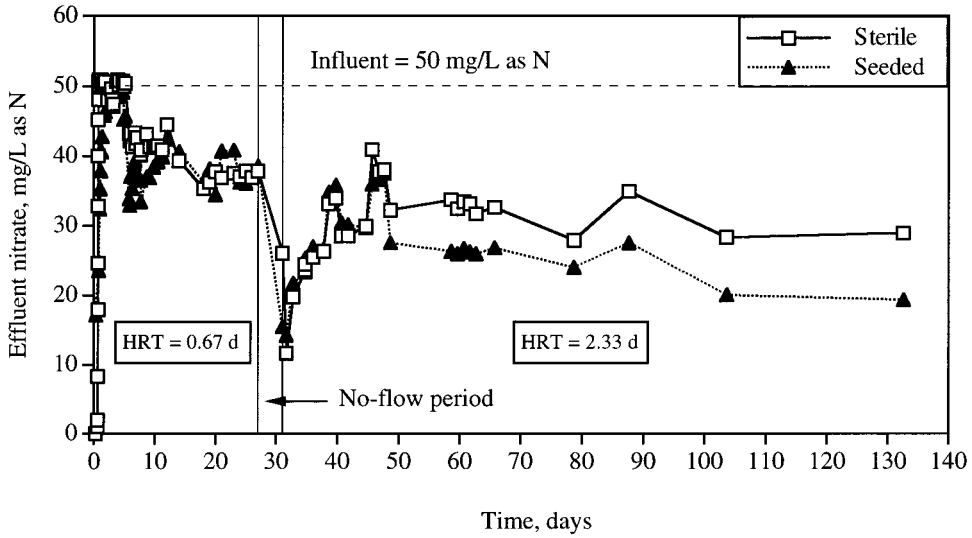

FIGURE 4. Effluent nitrate concentration from steel wool columns.

TABLE 2. Chemical Characteristics of Effluent from Seeded versus Control Column[a]

		effluent	
parameter	influent	seeded	control
nitrate, mg/L as N	50	19.6 ± 0.5	28.6 ± 0.4
nitrite, mg/L as N	<0.5	<0.5	<0.5
ammounium, mg/L as N	<0.1	15.5 ± 0.5	19.5 ± 0.2
pH	6.7	9.1	9.0
dissolved oxygen, mg/L	3.0	<0.1	<0.1
total organic carbon, mg/L	<0.3	2.2	2.3

[a]Samples were taken on day 132, while operated at 3 mL/h (HRT = 2.33 d), after effluent nitrate concentrations reached a steady level.

without Fe(0) and fed H_2 gas. These reactors biologically denitrified 93% of the initial nitrate to N_2O. All seeded reactors assimilated 1−2% of the added nitrate into organic nitrogen associated with microbial biomass, as measured by TKN. In all cases, 94−98% of the added nitrate was accounted for at the end of the experiment.

Continuous-Flow Column Studies. Columns packed with steel wool were used to investigate the ability of hydrogenotrophic denitrifiers to enhance nitrate removal in a flow-through system. The columns were fed 50 mg/L NO_3^--N at an initial flow rate of 10 mL/h (HRT = 0.67 d). The effluent nitrate concentration from both seeded and sterile (control) columns leveled off at approximately 37 mg/L as N after 24 days (Figure 4), representing a nitrate removal efficiency of 26%. Flow was stopped after 27 days, and following a 4-day no-flow reseeding period, flow was resumed at 3 mL/h (HRT = 2.33 d). Under these conditions, the seeded column exhibited a higher nitrate removal efficiency, which stabilized at 61% after 100 days of operation (Figure 4). The control column steadily removed 43% of the influent nitrate at this time. No nitrite was ever detected in the effluent of either column.

The steady-state effluent quality was higher for the seeded column (Table 2). Not only was the nitrate concentration lower (19.6 ± 0.5 versus 28.6 ± 0.4 mg/L) but also a smaller fraction of the nitrate removed was converted to ammonium (50% versus 91%). Nitrogenous gases such as N_2, the end product of biological denitrification, were not analyzed in this experiment. Consequently, mass balance closures for nitrogen were looser for the seeded column (about 30% of the influent nitrate unaccounted) than for the control column (4% unaccounted). No significant differences were found between the two columns in pH (which increased from an influent pH 6.7 to an effluent pH 9), in dissolved oxygen (which decreased from an influent of 3 mg/L to less than 0.1 mg/L), or in effluent total organic carbon (which was about 2 mg/L for both columns). The source of organic carbon in the unseeded (control) column is unclear and was not investigated in this study. Other researchers, however, have proposed that hydrocarbons in Fe(0)−water systems can be formed by the reduction of aqueous carbon dioxide by Fe(0) (*22*) or by the release of carbide carbon impurities from Fe(0) (*23*).

Discussion

Although it has long been known that Fe(0) could reduce nitrate during the corrosion cracking processes of steels in nitrate solutions, only recently have researchers attempted to exploit this chemical process to treat nitrate-contaminated water (*6*). In this work, both Fe(0) powder and steel wool effectively removed nitrate in the absence of bacteria. Fe(0) powder reduced nitrate in less time than steel wool (Figure 3). Because reductive treatment with Fe(0) is directly related to surface corrosion of the metal, the faster reaction with Fe(0) powder can be attributed to the higher surface area available for reaction. Yet, while reaction rates can be directly proportional to the Fe(0) surface area concentration (*24*), the faster rate with Fe(0) powder was not commensurate to its much higher Fe(0) surface area concentration (i.e., 135 versus 0.4 m^2/L for steel wool). This suggests that other processes besides chemical reaction at the Fe(0) surface were limiting in these quiescent reactors, such as mass transport to the surface, adsorption of reactants, and desorption and mass transport of products from the surface.

Both Fe(0) powder and steel wool stoichiometrically reduced nitrate to ammonium with no other nitrogenous products detected (Table 1). The overall reaction, which is thermodynamically favorable under standard conditions and pH 7, is proposed to proceed as follows:

$$4Fe(0) + NO_3^- + 7H_2O \rightarrow 4Fe^{2+} + NH_4^+ + 10OH^- \qquad \Delta G^{o\prime} = -620 \text{ kJ} \quad (3)$$

Stoichiometric nitrate reduction to ammonium has also been shown to occur using iron in green rust as the reductant (*25*). Nevertheless, other researchers have reported a significant yield of nitrite when treating nitrate-contaminated water with Al(0) powder (*5*) or with Fe(0) powder in the presence of 1,2-dibromo-3-dichloropropane (*6*). Nitrite, the

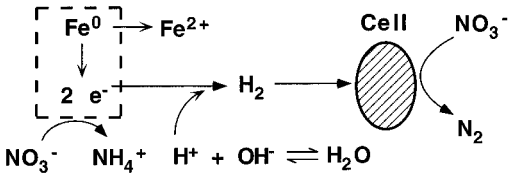

FIGURE 5. Concept of Fe(0)-supported denitrification.

first intermediate of nitrate reduction, accumulates when it is subsequently reduced at a slower rate than nitrate. This is undesirable because nitrite is more toxic than nitrate. While nitrite accumulation can sometimes be reduced by increasing the reaction time, further research would be required to determine why nitrite accumulates in some Fe(0) treatment systems but not in others.

To treat nitrate-contaminated waters, Fe(0) can be used not only as a direct reductant but also as a continuous source of cathodic hydrogen to sustain biological denitrification (Figure 5). This was demonstrated using a dual-flask apparatus that permitted the diffusion of cathodic hydrogen from one flask to the other while avoiding potential microbial inhibition by iron corrosion species (*18*). *P. denitrificans* simultaneously reduced nitrate and grew as Fe(0) corroded (Figure 1). Yet, no nitrate removal or microbial growth occurred in control runs lacking Fe(0), nitrate, or inoculum. Thus, cathodic hydrogen generation during Fe(0) corrosion was coupled to nitrate reduction by hydrogenotrophic denitrifiers. The overall reaction, which is given by combining eqs 1 and 2, is thermodynamically more feasible than abiotic nitrate reduction with Fe(0):

$$5Fe(0) + 2NO_3^- + 6H_2O \rightarrow 5Fe^{2+} + N_2 + 12OH^- \quad \Delta G^{\circ\prime} = -1147 \text{ kJ} \quad (4)$$

Interestingly, eq 4 also suggests that autotrophic denitrifiers, via cathodic depolarization, may contribute to the corrosion of iron and steel structures that are buried in nitrate-contaminated anaerobic soils or sediments.

Combined microbial-Fe(0) treatment systems offer significant potential advantages over approaches where either process is used alone, especially when nitrate is present with other reducible contaminants. Aerobic Fe(0) corrosion rapidly induces anoxic conditions favorable for denitrification and for other anaerobic processes (*26*). The production of cathodic hydrogen during Fe(0) corrosion increases the availability of an excellent electron donor for denitrification and for other reductive biotransformations. Fe(0) can also enhance microbial transformations by removing potentially co-occurring inhibitory pollutants such as Cr(VI) (*27*). In turn, bacteria can further degrade byproducts of abiotic transformations, such as dichloromethane which is a dead-end product from hydrogenolysis of carbon tetrachloride with Fe(0) (*17*, *28*). Furthermore, the removal of the passivating cathodic H_2 layer from the Fe(0) surface by bacteria enhances the corrosion of Fe(0) and, thus, the flow of electrons (i.e., cathodic depolarization) (*18*).

Iron corrosion (eq 2), abiotic nitrate reduction (eq 3), and iron-supported denitrification (eq 4) could increase the pH beyond the tolerance range of common denitrifiers. To screen for such potential limitations when Fe(0) is combined with bacteria, we investigated the effect of pH on the (hydrogenotrophic) denitrification activity of *P. denitrificans* (Figure 2). While pH had no significant effect on nitrate removal between pH 6 and pH 9, *P. denitrificans* abruptly ceased to denitrify at pH 10 or higher. High pH values also hinder Fe(0) reactivity by inducing oxide deposition at the metal surface. This suggests a potential need to buffer the system against large pH increases, which could be accomplished by adding carbon dioxide (*4*) or by combining Fe(0) with aluminosilicate minerals that enhance proton generation at the Fe(0) surface and accelerate corrosion (*27*).

When considering a combined Fe(0)-microbial treatment system, the abiotic and biological processes represent competing pathways for reducing nitrate, with the biological pathway having more desirable end products. The surface area concentration of Fe(0) plays an important role on the ability of bacteria to enhance nitrate removal, especially in environments that are not highly buffered. A high surface area concentration may result in fast rates of abiotic nitrate reduction and produce high concentrations of hydrogen, the substrate for hydrogenotrophic denitrifiers. Nevertheless, such conditions may also increase the pH beyond the tolerance range of common denitrifiers (pH > 10), as seen when *P. denitrificans* was combined with Fe(0) powder (Figure 3). In a buffered system, it is also possible that a high surface area concentration of Fe(0) results in abiotic reduction kinetically outcompeting denitrification. Thus, while a high surface area concentration of Fe(0) may yield faster abiotic nitrate removal rates, it may hinder the ability of bacteria to improve the end product distribution. For example, adding bacteria to Fe(0) powder did not prevent the conversion of at least 94% of the added nitrate to ammonium (Table 1). On the other hand, reactors prepared with steel wool had a much lower Fe(0) surface area concentration and permitted a greater participation of bacteria in the treatment process. This increased the nitrate removal efficiency from 33% to 100% for the 12-day incubation period (Figure 3) and resulted in end products that more closely resembled the ideal H_2-amended denitrifying reactors (Table 1). This suggests that sustaining autotrophic denitrification with a lower surface area concentration of Fe(0) may represent a trade off between nitrate removal rates and more desirable end products.

Proof of concept was presented that Fe(0) and hydrogenotrophic denitrifiers can sustain nitrate removal in a flow-through system. The extent of both biotic and abiotic nitrate removal in flow-through columns was influenced by the hydraulic retention time. Increasing the hydraulic retention time from 0.67 to 2.33 days increased both the nitrate removal efficiency and the relative importance of biological denitrification (Figure 4), leading to a better end product distribution (Table 2). Longer retention times are a concern due to increased reactor volume requirements. However, Fe(0)-supported denitrification could also be implemented as a buried reactive barrier to treat contaminated groundwater. The typically slow flow velocity of groundwater (1 cm/d) (21) would ensure long retention times conducive to high removal efficiencies as contaminated groundwater flows through typical Fe(0) barriers (50−150 cm thick).

Hydrogenotrophic denitrifiers are ubiquitous (*29*), which suggests the potential for an indigenous denitrifying consortium to eventually develop around iron barriers to fill a metabolic niche associated with cathodic depolarization. Alternatively, bioaugmentation of Fe(0) barriers with hydrogenotrophic denitrifiers could shorten the adaptation period and enhance the short-term denitrification potential. Nevertheless, more research is needed to evaluate the effect of organic substrates on the ability of hydrogenotrophic denitrifiers to compete with other microorganisms.

In summary, the finding that Fe(0) can stoichiometrically reduce nitrate to ammonium and that cathodic hydrogen can support autotrophic denitrification may have practical applications to remove nitrate in ex-situ or in-situ reactive filters. Because of kinetic competition between the biological and the abiotic nitrate reduction pathways and because of the inhibition of autotrophic denitrifiers at high pH, sustaining autotrophic denitrification with a lower surface area concentration of Fe(0) might be more beneficial to enhance the end product distribution.

Acknowledgments

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