Nitrate and Nitrite Reduction by Fe$^0$: Influence of Mass Transport, Temperature, and Denitrifying Microbes

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ABSTRACT

To evaluate how mass transport, temperature, and denitrifying micro-organisms affect the relative rates of nitrate and nitrite reduction by iron metal (Fe$^0$), nitrate and nitrite reduction rates were measured over a range of mixing rates and temperatures. The effect of mixing rate was studied at a polished Fe$^0$ rotating disk electrode (RDE) in an electrochemical cell, and the effect of temperature was studied in batch reactors with granular Fe$^0$ in the absence and presence of Paracoccus denitrificans. Electrode rotation rate had little influence on the cathodic current measured in the presence of nitrate, whereas higher rotation rates resulted in significant increases in current in the presence of nitrite. The heterogeneous reaction rate coefficient ($k_{rxn}$) for nitrite reduction at the Fe$^0$ RDE is several orders of magnitude faster than the surface-area normalized rate coefficient ($k_{SA}$) for nitrite reduction by granular Fe$^0$. Activation energies for nitrate and nitrite reduction by granular Fe$^0$ were similar (21.7 $\pm$ 9.3 kJ mol$^{-1}$ for nitrate and 23.8 $\pm$ 1.8 kJ mol$^{-1}$ for nitrite). Addition of P. denitrificans to reactors containing Fe$^0$ resulted in faster nitrate removal compared to treatments with Fe$^0$ alone at all temperatures tested (5 to 50°C). Nitrite removal rates measured in both batch reactors and the electrochemical cell were typically 1.5 to 15 times faster than those measured for nitrite, depending on the electrode rotation rate, pH, temperature, and presence of microbes. Results from a simple first-order kinetic model based on sequential reduction of nitrate $\rightarrow$ nitrite $\rightarrow$ ammonium suggest that differences in the relative rates of nitrate and nitrite reduction may explain why nitrite appears as an intermediate product from nitrate reduction in some studies and not in others.

Key words: iron metal; zero-valent iron; nutrients; rotating disk electrode; reactive barrier; denitrifiers

INTRODUCTION

In a recent survey of 1,255 drinking-water wells and 242 public supply wells, nitrate was the compound most frequently detected at concentrations exceeding a regulatory standard [such as U.S. EPA’s maximum concentration level (MCL)] or a health advisory (Squillace et al., 2002). Of the 1,497 wells tested (for volatile organic compounds, pesticides, and nitrate), water in 9.4% of the wells exceeded the U.S. EPA’s nitrate MCL of 10

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mg/L as nitrogen (0.71 mM). The high nitrate concentrations are due to the rising use of nitrogenous fertilizers, changes in land-use patterns from pasture to arable, and increased recycling of domestic wastewater in low-land rivers (Kapoor and Viraraghavan, 1997; Nolan and Stoner, 2000). Aquifers exposed to high nitrogen loading and aquifers comprised of well-drained surficial soils over unconsolidated sand and gravels have been shown to be more likely to have nitrate contamination (Nolan et al., 2002).

Numerous physical-chemical and biological processes have been developed for the removal of nitrate from water. Ion exchange, reverse osmosis and biological denitrification are the three most common nitrate removal techniques (Kapoor and Viraraghavan, 1997). A promising alternative treatment technology for nitrate removal is reduction by iron metal (Fe$^{0}$) in permeable reactive barriers (PRBs). Fe$^{0}$ PRBs have been more commonly used for chlorinated solvents and heavy metals (Powell et al., 1998; Scherer et al., 2000; U.S. Environmental Protection Agency, 2002), but the common occurrence of nitrate as a cocontaminant has led to numerous laboratory studies of nitrate reduction by Fe$^{0}$ (Siantar et al., 1996; Cheng et al., 1997; Rahman et al., 1997; C.P. Huang et al., 1998; Till et al., 1998; Zawaideh and Zhang, 1998; Devlin et al., 2000; Kielemoes et al., 2000; Schlicker et al., 2000; Alowitz and Scherer, 2002; X.H. Huang and Zhang, 2002; Miehr et al., 2003; Westerhoff and James, 2003). Nitrate reduction has also been reported in a field-scale Fe$^{0}$ PRB designed to treat trichloroethene (TCE) (Yabusaki et al., 2001). In batch and column studies, however, the presence of nitrate has been shown to inhibit rates of TCE reduction (Farrell et al., 2000; Schlicker et al., 2000), 1,2-dibromo-3-chloropropane reduction (Siantar et al., 1996), and arsenic and arsenite removal (Su and Puls, 2001). Inhibition in the presence of nitrate has been attributed to competitive effects, as well as increased buildup of corrosion products.

Most laboratory studies report ammonium (NH$_4^+$) as the major product from nitrate reduction (Siantar et al., 1996; Cheng et al., 1997; Rahman et al., 1997; C.P. Huang et al., 1998; Till et al., 1998; Westerhoff and James, 2003; Zawaideh et al., 1998; Devlin et al., 2000; Kielemoes et al., 2000; Schlicker et al., 2000; Alowitz and Scherer, 2002; Huang and Zhang, 2002). In large-scale columns packed with Fe$^{0}$, ammonium accounted for most of the influent nitrate at early times; however, at later times, less than 25% of the nitrate could be accounted for by ammonium (Westerhoff and James, 2003). Potential explanations for the poor mass balance at later times included sorption of ammonium or nitrate onto oxides (formed from Fe$^{0}$ corrosion) or loss of ammonium as ammonia gas (due to the high pH in the columns). Adsorption of nitrate on oxide coated Fe$^{0}$ has also been inferred based on faster nitrate removal rates observed for oxide coated Fe$^{0}$ compared to relatively clean Fe$^{0}$ (Miehr et al., 2003) and site saturation effects observed in the presence ferrous iron and nitrate (Huang and Zhang, 2002).

In a few studies, however, nitrite has been observed as an intermediate product from nitrate reduction (Siantar et al., 1996; Rahman et al., 1997; Su and Puls, 2001). The appearance of nitrite as an intermediate product is significant because of the concerns about health effects associated with nitrite (Fan and Steinberg, 1996). Ammonium has also been reported as an end product from nitrite reduction (Rhaman et al., 1997; Kielemoes et al., 2000; Alowitz and Scherer, 2002), and interestingly, Hu and co-workers recently reported N$_2$ gas as a primary endproduct from abiotic nitrite reduction by Fe$^{0}$ (Hu et al., 2001). The formation of nitrite as an intermediate product from nitrate reduction means that the accumulation of nitrite may be controlled by the relative kinetics of nitrate and nitrite reduction.

For example, solution pH has been shown to affect the kinetics of both nitrate and nitrite reduction by Fe$^{0}$. A pH values relevant to natural systems and Fe$^{0}$ PRBs (i.e., pH 6.5 to 9.0), slower rates of nitrate and nitrite reduction have generally been observed at higher pH values (Hu et al., 2001; Alowitz and Scherer, 2002; Miehr et al., 2003; Westerhoff, 2003). Of the limited data available with which to compare nitrite and nitrate reduction rates, it appears that nitrite is reduced faster than nitrate at pH values less than 8.0 (Rahman et al., 1997; Alowitz and Scherer, 2002).

Above a pH of 8.0, similar rates of nitrate and nitrite reduction are observed, suggesting that differences in pH values may explain why nitrite appears as an intermediate in some studies and not in others. Other factors that may control the relative rates of nitrate and nitrite reduction include mass transport and the presence of denitrifying bacteria. Indeed, the addition of autotrophic (hydrogenotrophic) denitrifiers was found to increase nitrate removal rates in Fe$^{0}$ PRBs and offer an alternative pathway that improves the end product distribution, favoring N$_2$ over abiotically produced NH$_4^+$ (Till et al., 1998; Dejournett and Alvarez, 2000). The recent observation that indigenous microorganisms can colonize Fe$^{0}$ PRBs (Alvarez et al., 1999; Phillips et al., 2000), presumably to feed on water-derived H$_2$ that is produced during Fe$^{0}$ corrosion:

$$\text{Fe}^{0} + 2\text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + 2\text{OH}^- + \text{H}_2 \ \ \ \ (1)$$

suggests denitrifying bacteria may influence the relative rates of nitrate and nitrite reduction.

To evaluate how mass transport, temperature, pH, and denitrifying micro-organisms affect the relative rates of
nitrates and nitrites by Fe0, we measured nitrates and nitrites over a range of electrode rotation rates, solution pH values, and temperatures. The effect of rotation rate was studied at a polished Fe0 rotating disk electrode (RDE) in an electrochemical cell, and the effect of temperature and solution pH was studied in batch reactors with granular Fe0 in the absence and presence of Paracoccus denitrificans.

MATERIALS AND METHODS

Electrochemical experiments

Experiments were carried out in a custom three-electrode glass cell, which has been described previously (Scherer et al., 1997, 2001). The cell consisted of an Fe0 RDE working electrode (geometric surface area = 0.071 cm²), a double-junction saturated calomel reference electrode, a Pt mesh counter electrode, and a pH 8.4 borate buffer electrolyte. Pretreatment of the electrode has been described previously (Scherer et al., 1997, 2001). Following pretreatment, nitrate or nitrite was introduced into the cell by injection of a known volume of deaerated aqueous stock solution through a septum. Linear sweep voltammograms (LSVs) were obtained with and without nitrate or nitrite with a potentiostat (EG&G Model 283A). All LSVs were obtained by scanning anodically at a scan rate of 0.2 mV s⁻¹. Initial experiments at slower scan rates verified that 0.2 mV s⁻¹ provided sufficient time for steady-state conditions to develop. Potentials are reported relative to the standard hydrogen electrode (SHE), and currents are reported in accord with IUPAC convention (anodic current is positive and cathodic current is negative).

Batch experiments with Fe0 fillings

Batch reactors were used to investigate the effect of temperature on the kinetics of nitrate and nitrite reduction by Fe0 in the presence and absence of autotrophic denitrifying bacteria. Reactors were 120-mL bottles capped with an aluminum crimp top and butyl rubber septum. Abiotic reactors were filled with 100 mL of buffered mineral medium with 10 mg/L as N nitrate or nitrite and 7 g untreated, and sieved (between 0.5 and 1 mm) Master Builders (Cleveland, OH) iron fillings. The BET specific surface area of the sieved iron fillings was 1.06 m² g⁻¹. Biotic reactors were filled with 80 mL of buffered mineral medium and 20 mL of a stock culture of P. denitrificans (ATCC 17741, optical density at 600 mm = 1.25). Reactors were assembled in an anaerobic chamber before being brought outside. Reactors were mixed by rotating end over end. All reactor headspace was purged with a H2/CO2 gas mixture (80:20 v/v) following inoculation to ensure anaerobic condition and to provide a carbon source for the microbes used in the biotic systems. Abiotic reactors were run in duplicate and biotic reactors were run in triplicate.

Chemical and microbial analysis

Nitrate and nitrite were analyzed using a Dionex (Sunnyvale, CA) BioLC ion chromatograph. Separation was achieved by ion exchange (Dionex AS4A column with 2.2 mM carbonate and 0.74 mM bicarbonate eluant) with chemical suppression (25 mM H2SO4) and conductivity detection. The method detection limit was approximately 0.5 mg/L for nitrate and nitrite as N. Ammonium was measured using a Dionex DX-100 ion chromatograph, fitted with a Dionex Ionpac CS12A column and a Dionex CSRS-Ultra 4 mm suppressor. The eluant used was a 22 mM H2SO4 solution. The eluant also contained 1 mM oxalic acid to prevent the adsorption of ferrous iron to the column. Eluant was pumped through the system at 1 mL min⁻¹. Optical density was used to determine the biomass concentration in stock cultures of P. denitrificans. The absorbance was measured of a sample in a 1-mL plastic cuvette at a wavelength of 600 nm using a Spectronic Genesys 5 Spectrophotometer (Rochester, NY). Solution pH was measured in the electrochemical cell with an Orion pH electrode, whereas pH measurements for the batch reactors were made with colorpHast pH strips with a detection range of pH 6.5 to 10 ± 0.03.

RESULTS AND DISCUSSION

Nitrite and nitrate reduction at an Fe0 RDE

The cathodic current measured at an Fe0 RDE (jₜₖₑₐₜ, A cm⁻²) was larger in the presence of nitrate and nitrite compared to borate buffer alone (Fig. 1). Larger cathodic currents in the presence of nitrate and nitrite are consistent with our expectation that these compounds are acting as oxidants. Nitrite increases the cathodic current about 10 times more than the addition of an equivalent amount of nitrate (about 46 mM), suggesting that the rate of nitrite reduction at the Fe0 RDE is faster than the rate of nitrate reduction (even assuming likely differences in the number of electrons transferred).

To estimate the number of electrons transferred and confirm that the increased cathodic currents observed in Fig. 1 are due to the reduction of nitrite and nitrate by the Fe0 RDE, the concentrations of reaction products in the cell solution were compared to those expected from
the total cathodic charge passed \( (q, C) \). From Faraday’s law, the amount of product \( (N, \text{mol}) \) is related to the amount of charge \( (q, C) \) by \( N = q/nF \) (Bard and Faulkner, 2001). For nitrate reduction, the rate of reaction was too slow to accumulate enough products to detect over a 48-h time period. For nitrite reduction, the only product observed was ammonium. The amount of ammonium measured in the cell (0.121 mmol) accounted for 107% of the total cathodic charge \( (q_{\text{tot}} = 65.6 \, C) \) in a long-term electrolysis experiment (24 h with \( E_{\text{appl}} = -0.64 \, V, n = 6, \) and initial \([\text{NO}_2^-] = 100 \, \text{mM}\) ). The reasonable agreement between cathodic charge and ammonium measured in the cell solution suggests that ammonium is the primary product of nitrite electrolysis at the \( \text{Fe}^0 \) electrode.

To evaluate the influence of mass transport on the kinetics of nitrate and nitrite reduction at an \( \text{Fe}^0 \) RDE, we measured currents at different electrode rotation rates. An applied potential of \( -0.64 \, V \) was selected to minimize the formation of an oxide layer on the \( \text{Fe}^0 \) electrode (Scherer et al., 1997). Electrode rotation rate had little effect on the current measured in the presence of 135 mM nitrate (Fig. 2). The negligible change in current observed as the rotation rate increased from 400 to 8100 rpm indicates that the rate of nitrate reduction was limited by reaction at the \( \text{Fe}^0 \) electrode surface, rather than mass transport to the surface. Compared to our previous work with carbon tetrachloride (CCl\(_4\)) (Scherer et al., 1997) and nitrobenzene (ArNO\(_2\)) (Scherer et al., 2001), significantly higher concentrations of nitrate were required to achieve a current that could be reproducibly distinguished from the background current (due to \( \text{Fe}^0 \) oxidation and water reduction). The large concentration required to observe a current due to nitrate reduction suggests that the kinetics of nitrate reduction at the \( \text{Fe}^0 \) RDE are slower than CCl\(_4\) and ArNO\(_2\) reduction even considering differences in number of electrons transferred.

Currents measured in the presence of nitrite were significantly larger than those measured in the presence of nitrate despite the larger concentration of nitrate (Fig. 2). As expected, higher nitrite concentration results in increased currents. Unlike nitrate, however, electrode rotation rate strongly influenced the current measured in the presence of nitrite. Changes in current were observed even at the highest rotation rates (6,400 and 8,100 rpm), indicating that nitrite is reduced at the electrode surface faster than it is transported to the surface (Levich, 1962). The reproducibility of currents as the rotation rate is stepped from 400 to 8,100 rpm and then back down again is another indication that the reduction of nitrite at the \( \text{Fe}^0 \) electrode is limited by a physical diffusion step rather than a chemical reaction step.

For a first-order process, currents measured at the RDE are proportional to the first-order rate coefficient (i.e., \( j = nFkC \)) and can be approximated by the Koutecky Levich equation (Bard and Faulkner, 1980):

\[
\frac{1}{j} = \frac{1}{j_{rxn}} + \frac{1}{0.62 \, nFD^{2/3} \, \omega^{1/2} \, \nu^{-1/6} \, C}
\]  

(2)

where \( j \) is the current at the electrode, \( j_{rxn} \) is the current due to chemical reaction at the electrode, \( D \) is the mo-
molecular diffusion coefficient, \( \nu \) is the kinematic viscosity of the electrolyte solution, \( \omega \) is the angular velocity of the rotating disk (\( \omega = 2\pi f \), where \( f = \text{rpm}/60 \), rotation rate in \( \text{s}^{-1} \)), and \( C \) is the aqueous bulk concentration. For a reaction that is mass transport limited, a plot of \( 1/j \) vs. \( 1/\text{rpm}^{1/2} \) (known as a Koutecky Levich plot) should be linear [Equation (2)] with the slope proportional to \( D^{2/3} \) (Bard and Faulkner, 2001). For reduction of nitrite at the \( \text{Fe}_0 \) RDE, linear relationships between \( 1/j \) and \( 1/\text{rpm}^{1/2} \) are observed for nitrite concentrations ranging from 30 to 100 mM (Fig. 3). Regression of the linear regions results in slopes within about 20% for the three higher concentrations \((4.2 \pm 0.7 \text{ rpm}^{1/2} \text{ mA}^{-1}) \) and within about 40% for all four concentrations \((5.0 \pm 1.8 \text{ rpm}^{1/2} \text{ mA}^{-1}) \). The linearity of the plot and reasonable agreement among the slopes confirms that diffusion to the electrode surface limits the amount of current measured from nitrite reduction at the \( \text{Fe}_0 \) RDE. Consistent Koutecký-Levich slopes also provide additional reassurance that the migrational component of the current is negligible. Extrapolation of the linear regions to high rotation rates (i.e., \( \text{rpm} \to 0 \)) yields an estimate of the current in the absence of mass transfer effects. For nitrite reduction, extrapolating to \( \text{rpm}^{-1/2} = 0 \) results in a positive \( y \)-intercepts for each of the four concentrations studied. The inverse of the \( y \)-intercept represents the current that would flow if mass transfer were sufficiently fast to keep the concentration of nitrite at the electrode surface equal to the bulk nitrite concentration (i.e., \( j_{\text{rxn}} \), the reaction limited current). The reaction limited currents for nitrite reduction are proportional to the nitrite concentration and indicates that nitrite reduction at the \( \text{Fe}_0 \) RDE is a first-order process (Fig. 3 inset).

**Comparison of \( \text{Fe}_0 \) RDE and batch reactors containing granular \( \text{Fe}_0 \)**

The overall rate of reduction by \( \text{Fe}_0 \) can be represented as a series of resistances due to mass transport and reaction (Scherer et al., 1997, 2001; Arnold et al., 1999; Nam and Tratnyek, 2000):

\[
\frac{1}{k_{\text{SA}}} = \frac{1}{k_{\text{rxn}}} + \frac{1}{k_{\text{mt}}} \tag{3}
\]

where \( k_{\text{SA}} \) is the overall surface area-normalized rate coefficient \((\text{m s}^{-1})\), \( k_{\text{mt}} \) is the mass transport coefficient \((\text{m s}^{-1})\), and \( k_{\text{rxn}} \) is the first-order heterogeneous reaction rate coefficient \((\text{m s}^{-1})\). Since rates of reduction by \( \text{Fe}_0 \) vary considerably over the range of treatable contaminants, it is possible that there is a continuum of kinetic regimes from purely reaction controlled (i.e., \( k_{\text{rxn}} < k_{\text{mt}} \) and thus \( k_{\text{SA}} \approx k_{\text{rxn}} \)), to intermediate (i.e., \( k_{\text{rxn}} \approx k_{\text{mt}} \)), to purely mass transport controlled (i.e., \( k_{\text{mt}} < k_{\text{rxn}} \) and thus
The larger values of $k_{ran}$ measured for nitrite reduction at an Fe$^0$ RDE compared to $k_{SA}$ observed in batch reactors with granular Fe$^0$ is consistent with our previous work with CCl$_4$ and ArNO$_2$ (Scherer et al., 1997, 2001). The magnitude of the increase, however, is significantly greater for nitrite reduction. One explanation may be that it is more appropriate to compare $k_{mt}$ estimates with geometric surface area normalized rate coefficients, rather than BET surface area normalized rate coefficients (Arnold et al., 1999). The larger surface areas expected from BET measurements compared to geometric estimates (particularly for Fe$^0$ particles that contain a layer of oxide corrosion products) would underestimate $k_{SA}$. Indeed, a 25-fold increase in a geometric $k_{SA}$ relative to a BET $k_{SA}$ for reduction of chlorinated methanes by granular zinc metal was reported by Arnold et al. (1999). It seems unlikely that surface area estimates alone could account for the large discrepancy observed between nitrite reduction rates in batch reactors and at the RDE. Although it seems plausible that the 10- and 50-fold differences observed for CCl$_4$ and ArNO$_2$ can be attributed to differences in mass transport in batch experiments and/or the absence of an oxide layer that is typically present on granular Fe$^0$, the larger discrepancy observed for nitrite suggests that there is a fundamental difference in the reaction occurring on the Fe$^0$ RDE compared to on an oxide-coated granular Fe$^0$. For example, it was recently suggested that both indirect reduction by H$_2$ (formed from corrosion of Fe$^0$), as well as direct reduction by Fe$^0$ were important in nitrite reduction by Fe$^0$ in the presence of oxygen (Hu et al., 2001). Alternatively, the difference may be due to the ionic nature of nitrite, which will significantly alter the interaction with both oxide surfaces and electrodes.

**Effect of temperature on rates of NO$_3^-$ and NO$_2^-$ reduction by granular Fe$^0$**

Given the potential difference in reaction mechanism for nitrite reduction at a bare Fe$^0$ electrode and oxide-coated granular Fe$^0$, we conducted additional experiments to evaluate mass transfer limitations by measuring the effect of temperature on both nitrate and nitrite removal in batch reactors with granular Fe$^0$. Nitrite was rapidly removed in the presence of 7 g L$^{-1}$ of Fe$^0$ over a temperature range of 5 to 50°C (Fig. 4). Nitrite removal was more rapid at higher temperatures, and appeared first order at early times. Most often, ammonium has been reported as the major product from nitrite reduction by Fe$^0$ (Rahman et al., 1997; Kielemoes et al., 2000), but recently nitrogen gas has also been observed in significant quantities (Hu et al., 2001). In our electrochemical experiments, we observed ammonium as the major end product, but in the batch reactors, the high ferrous iron...
concentration made it difficult to accurately quantify ammonium concentrations, and we did not analyze for N₂(g). At the higher temperatures, the solution pH increased above 8.5 at later times and inhibited the rate of nitrite removal. The significantly slower rates observed at high pH values are consistent with previous work evaluating the effect of pH on nitrite reduction by granular Fe⁰ (Alowitz and Scherer, 2002). Nitrate followed a similar trend with faster removal rates generally observed at higher temperatures (Table 1). Nitrite was not observed as an intermediate from nitrate reduction.

Rates of contaminant reduction by Fe⁰ measured in batch experiments have been shown to exhibit a temperature dependency consistent with the Arrhenius equation (Sivavec and Horney, 1995; Scherer et al., 1997; Deng et al., 1999):

\[ k = A \exp \left( -\frac{E_A}{RT} \right) \]  

(4)

where \( E_A \) is the activation energy (kJ mol⁻¹), \( A \) is the preexponential factor (same units as \( k \)), and \( R \) is the gas constant (8.314 J K⁻¹ mol⁻¹). A linearized form of the Arrhenius equation can be obtained by taking the natural logarithm of both sides of Equation (4):

\[ \ln k = \ln A - \frac{E_A}{RT} \]  

(5)

The linear relationships observed for plots of \( \ln k \) vs 1/T indicates that the temperature dependency of nitrate and nitrite reduction by Fe⁰ metal is consistent with the Arrhenius equation (Fig. 5). The slope and intercept of the linear regression lines in Fig. 5 can be used to estimate both an \( E_A \) and \( A \) for nitrate and nitrite reduction (slope = \(-E_A/R \) and y-intercept = \( \ln A \)). Similar activation energies are computed for nitrate (\( E_A = 21.7 \pm 9.4 \) kJ mol⁻¹) and nitrite (\( E_A = 23.9 \pm 1.8 \) kJ mol⁻¹) suggesting a similar temperature dependency for the two compounds. The nitrate value of 21.7 kJ mol⁻¹ is remarkably similar to the value of 22 kJ mol⁻¹ (based on measurements at 8 and 28°C) previously reported for nitrate reduction by Master Builders’ Fe⁰ (Westerhoff, 2003). The values in

Table 1. Effect of temperature and microbes on \( k_{obs} \) (h⁻¹) for NO₃⁻ and NO₂⁻ reduction by granular Fe⁰a.

<table>
<thead>
<tr>
<th></th>
<th>5°C</th>
<th>25°C</th>
<th>35°C</th>
<th>50°C</th>
<th>( E_A ) (kJ mol⁻¹)</th>
<th>( \ln A )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe⁰ + NO₂⁻</td>
<td>0.081 ± 0.007</td>
<td>0.140 ± 0.010</td>
<td>0.250 ± 0.012</td>
<td>0.334 ± 0.051</td>
<td>23.9 ± 1.8</td>
<td>7.77 ± 0.73</td>
</tr>
<tr>
<td>Fe⁰ + NO₃⁻</td>
<td>0.011 ± 0.002</td>
<td>0.041 ± 0.001</td>
<td>0.020 ± 0.001</td>
<td>0.045 ± 0.002</td>
<td>21.7 ± 9.4</td>
<td>4.64 ± 3.76</td>
</tr>
<tr>
<td>Fe⁰ + NO₃⁻ + P. denitrif.</td>
<td>0.025 ± 0.002</td>
<td>0.101 ± 0.007</td>
<td>0.146 ± 0.003</td>
<td>0.192 ± 0.018</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>( k_{NO3}/k_{NO3} ) + P. denitrif.</td>
<td>2.3</td>
<td>9.2</td>
<td>7.3</td>
<td>4.3</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

aBatch reactors contained 7 g Master Builder iron filings (not acid washed) in buffered mineral medium containing either 0.7 mM NO₃⁻ or NO₂⁻. All batch reactors were incubated in the dark while continuously rotated. Standard deviations based on triplicate reactors. NA = not applicable. Initial pH of the reactor solutions was near neutral.
resulted in higher NO$_3^-$ compared to treatments with Fe$_0$ alone suggesting NO$_2^-$ accumulation in this experiment, which precludes corroborating the potential for bacteria to reduce the accumulation of this undesirable intermediate of nitrate reduction, as reported for iron column experiments (Dejournett and Alvarez, 2000). No nitrite accumulation was observed in this experiment, which precludes corroborating the potential for bacteria to reduce the accumulation of this undesirable intermediate of nitrate reduction, as reported for iron column experiments (Dejournett and Alvarez, 2000).

Relative rates of NO$_3^-$ and NO$_2^-$ reduction (i.e., $k_{NO_2^-}/k_{NO_3^-}$)

Nitrate removal rates measured in both batch reactors and the electrochemical cell ranged from 1.5 to 15 times faster than those measured for nitrate, depending on the electrode rotation rate, pH, temperature, and microbial activity (Figs. 2 and 6, and Table 1). The trend of faster nitrite reduction kinetics is consistent with previous reports of nitrite and nitrate reduction (Rahman et al., 1997; Alowitz and Scherer, 2002). As expected, based on the similar activation energies measured for nitrite and nitrate, temperature had only a modest effect on the relative rates of nitrite and nitrate reduction ($k_{NO_2^-}/k_{NO_3^-}$ ranged from about 8 to almost 14). In contrast, solution pH had a significant effect on $k_{NO_2^-}/k_{NO_3^-}$ with an almost sevenfold change observed over a pH range of 5.5 to 8.5. The highest $k_{NO_3^-}/k_{NO_2^-}$ ratio was observed at pH 6.5 to 7 with much smaller ratios observed at the higher pH values (pH > 7.5) typical of Fe reactive bar-

Effect of P. denitrificans on rates of NO$_3^-$ reduction by granular Fe$_0$

To assess the potential of autotrophic denitrifiers to enhance the performance of Fe$_0$ PRBs over a broad range of temperatures, nitrate removal was compared in batch reactors amended with Fe$_0$ alone or with P. denitrificans. Nitrate removal followed first-order kinetics and bioaugmentation of Fe$_0$ with P. denitrificans resulted in higher rate coefficients ($k_{obs}$) compared to treatments with Fe$_0$ alone at all temperatures tested (5 to 50°C) (Table 1). The pH also increased more rapidly with increasing temperature (data not shown). When the pH rose above 8.5, nitrate reduction by Fe$_0$ became slower and deviated from first-order kinetics, consistent with previous observations (Alowitz and Scherer, 2002). Therefore, data with pH values greater than 8.5 were excluded from the determination of first-order rate coefficients.

Whereas nitrate removal rates increased with temperature for both treatments (Table 1), the effect of bioaugmentation was most pronounced at 25°C (i.e., an almost 10-fold increase in nitrate reduction rate was observed), which is close to the reported optimum growth temperature of 26°C (American Type Culture Collection, 2003) for this strain (Table 1). Presumably, P. denitrificans participated in nitrate removal by using H$_2$ as electron to denitrify over a wide range of temperatures (up to 50°C); although experiments with P. denitrificans alone suggest that an adaptation period of a few days may be required for this strain to perform at 5°C (data not shown).

Overall, these results corroborate the notion that inoculating Fe$_0$ PRBs with autotrophic denitrifiers could enhance nitrate removal efficiency, although the establishment of a microbial population could require provision of sufficient buffering capacity to preclude an inhibitory corrosion-induced increase in pH [Equation (1)]. Sufficient buffering capacity may be achieved by incorporating solid buffers within the Fe$_0$ PRB, such as acidic aluminosilicate minerals (Dejournett and Alvarez, 2000). No nitrite accumulation was observed in this experiment, which precludes corroborating the potential for bacteria to reduce the accumulation of this undesirable intermediate of nitrate reduction, as reported for iron column experiments (Dejournett and Alvarez, 2000).
riers. Previous work showed that nitrate and nitrite have a similar sensitivity to pH with an almost 100-fold decrease in $k_{obs}$ over a pH range of 5.5 to 9.0 (Alowitz and Sherer, 2002). Biological denitrification of nitrate through nitrite to dinitrogen gas, on the other hand, appears to be differentially sensitive to pH. As pH increases beyond 8.5, nitrite reduction generally slows down to a greater extent than nitrate reduction, causing nitrite accumulation (Thomsen et al., 1994; Glass and Silverstein, 1998).

Results from a simple first-order kinetic model based on sequential reduction of nitrate $\rightarrow$ nitrite $\rightarrow$ ammonium with no mass loss [Equation (6) through (9)] suggest that a similar explanation based on differences in the relative rates of nitrate and nitrite reduction may explain why nitrite appears as an intermediate product from nitrate reduction in some studies with granular Fe$^0$ (Siantar et al., 1996; Rahman et al., 1997) and not in others (Alowitz and Scherer, 2002; Westerhoff, 2003; Westerhoff and James, 2003).

$$\text{NO}_3^- \xrightarrow{k_{\text{NO}_3}} \text{NO}_2^- \xrightarrow{k_{\text{NO}_2}} \text{NH}_4^+$$ (6)

$$\frac{d[\text{NO}_3^-]}{dt} = -k_{\text{NO}_3}[\text{NO}_3^-]$$ (7)

$$\frac{d[\text{NO}_2^-]}{dt} = k_{\text{NO}_3}[\text{NO}_3^-] - k_{\text{NO}_2}[\text{NO}_2^-]$$ (8)

$$\frac{d[\text{NH}_4^+]}{dt} = k_{\text{NO}_2}[\text{NO}_2^-]$$ (9)

For ratios of $k_{\text{NO}_2}/k_{\text{NO}_3}$ approaching 1, such as those observed at high pH values, the sequential reduction model predicts nitrite will be observed as a significant intermediate product (Fig. 6). As the ratio of $k_{\text{NO}_2}/k_{\text{NO}_3}$ increases, less nitrite is predicted to accumulate.

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**Figure 6.** Effect of $k_{\text{NO}_2}/k_{\text{NO}_3}$ ratio on nitrite appearance (solid lines) predicted by a first-order kinetic model for sequential reduction of nitrate to nitrite to ammonium. $k_{\text{NO}_2}/k_{\text{NO}_3}$ ratios are given in labels on line. For reference, the disappearance of nitrate and appearance of nitrite and ammonium for $k_{\text{NO}_2}/k_{\text{NO}_3} = 1$ are shown as dashed lines.

**Figure 7.** Effect of pH and temperature on ratio of nitrite and nitrate first-order rate coefficients measured in batch reactors. Temperature data are given in Table 1 and pH data are taken from Alowitz and Scherer (Alowitz and Scherer, 2002). The temperature data was collected with pH values less than 8.5 and the pH data was collected at room temperature (about 22°C).
and by the time \( k_{\text{NO}_2^-}/k_{\text{NO}_3^-} \) reaches 10, negligible nitrite is predicted to appear. Given the wide range of sensitivity of \( k_{\text{NO}_2^-}/k_{\text{NO}_3^-} \) ratios observed within this study (Fig. 7), it seems reasonable that whether or not nitrite appears as an intermediate is simply due to the relative kinetics of nitrate and nitrite reduction.

**CONCLUSIONS**

A promising alternative treatment technology for nitrate removal is reduction by iron metal (Fe\(^0\)) in permeable reactive barriers (PRBs). The appearance of nitrite as an intermediate product from nitrate reduction, however, is of significant concern because of negative health effects associated with nitrite. Our results show that:

1. Based on a comparison of estimated mass transfer coefficients and reduction rates observed in batch reactors, it is unlikely that mass transfer will be important for nitrate and nitrite reduction in Fe\(^0\) PRBs.
2. Rates of nitrate and nitrite reduction by Fe\(^0\) have similar temperature dependencies.
3. Denitrifying bacteria enhance nitrate removal efficiency in the presence of Fe\(^0\) over a wide range of temperatures.
4. Under most conditions, the reduction of nitrite by Fe\(^0\) is faster than the reduction of nitrate. At high pH values, however, nitrite reduction is only slightly faster than nitrate reduction, and may result in nitrite appearing as an intermediate product from nitrate reduction.

**ACKNOWLEDGMENTS**

We would like to thank Johna Leddy (Chemistry Dept., University of Iowa) for her helpful comments on an early version of this manuscript. Support for this work was generously provided by the National Science Foundation under Grant BES-9983719 and the Iowa State Water Resources Research Institute (ISWRRI).

**REFERENCES**


GINNER

AU1
No Alvarez et al., 1999 in list.

AU2
No Phillips et al. 2000 in list.

AU3
Please update Miehr reference.

AU4
Verify spelling Rahman or Rhaman for references, also dates of these 2 references? Is this the same reference? Same page numbers? Please see that both are cited in text.