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Inhibition of bacterial perchlorate reduction by zero-valent iron

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Abstract

Perchlorate was reduced by a mixed bacterial culture over a pH range of 7.0–8.9. Similar rates of perchlorate reduction were observed between pH 7.0 and 8.5, whereas significantly slower reduction occurred at pH 8.9. Addition of iron metal, Fe(0), to the mixed bacterial culture resulted in slower rates of perchlorate reduction. Negligible perchlorate reduction was observed under abiotic conditions with Fe(0) alone in a reduced anaerobic medium. The inhibition of perchlorate reduction observed in the presence of Fe(0) is in contrast to previous studies that have shown faster rates of contaminant reduction when bacteria and Fe(0) were combined compared to bacteria alone. The addition of Fe(0) resulted in a rise in pH, as well as precipitation of Fe minerals that appeared to encapsulate the bacterial cells. In experiments where pH was kept constant, the addition of Fe(0) still resulted in slower rates of perchlorate reduction suggesting that encapsulation of bacteria by Fe precipitates contributed to the inhibition of the bacterial activity independent of the effect of pH on bacteria. These results provide the first evidence linking accumulation of iron precipitates at the cell surface to inhibition of environmental contaminant degradation. Fe(0) was not a suitable amendment to stimulate perchlorate-degrading bacteria and the bacterial inhibition caused by precipitation of reduced Fe species may be important in other combined anaerobic bacterial–Fe(0) systems. Furthermore, the inhibition of bacterial activity by iron precipitation may have significant implications for the design of *in situ* bioremediation technologies for treatment of perchlorate plumes.

Abbreviations: SEM – scanning electron microscope; XRD – X-ray diffraction; LEC – lactate enrichment culture; eeq – electron equivalent

Introduction

Over the past decade, perchlorate has been increasingly recognized as a potential hazardous subsurface contaminant. Advances in analytical chemistry have provided the ability to measure perchlorate in the $\mu g\ l^{-1}$ range, which has led to detection of perchlorate in drinking water supplies previously assumed to be uncontaminated (Urbansky 1998). In January 2002, the EPA recommended implementation of a maximum oral reference dose of perchlorate that would set a drinking water standard of approximately 1 $\mu g\ l^{-1}$ (EPA 2002). In addition to ecotoxicological investigation, parallel research continues to identify means by which perchlorate might be removed from soils and natural waters.

Perchlorate is a highly oxidized molecule and reduction to chloride is thermodynamically favorable (Coates et al. 1999; Herman & Frankenberger 1998; Sawyer et al. 2003; vanGinkel et al. 1995). This suggests that treatment scenarios

developed to promote reduction of highly oxidized pollutants such as chlorinated aliphatics or nitro-heterocyclic compounds may be useful for degrading perchlorate. Zero-valent iron (Fe(0)) and anaerobic bacteria have been shown to act separately and in concert to achieve many anaerobic transformations. In these combined systems, bacteria utilize hydrogen and possibly ferrous iron produced from corrosion of Fe(0) as electron donors (Daniels et al. 1987; Rajagopal & Legall 1989). Research conducted over the last decade has demonstrated that removal of redox sensitive pollutants can be enhanced by combining Fe(0) and bacteria (Gregory et al. 2000; Novak et al. 1998; Till et al. 1998; Weathers et al. 1997). For example, Weathers et al. (1997) showed that removal of chloroform was faster and more complete by combining Fe(0) and anaerobic bacteria than it was with Fe(0) alone or bacteria alone.

In addition to providing a source of electrons for bacteria, Fe(0) reacts directly with a variety of compounds. Fe(0) has already been demonstrated to abiotically reduce oxidized organic pollutants such as carbon tetrachloride or 1,1,1-trichloroethane and inorganics such as nitrate or hexavalent chromium (Alowitz & Scherer 2002; Gillham & Ohannesin 1994; Matheson & Tratnyek 1994). Abiotic reduction of perchlorate by Fe(0) has also recently been reported. Gurol and Kim (2000) reported that partial conversion of 0.001 mM perchlorate was achieved using 100 g l^{-1} Fe(0) (SA = 0.74 m^2 g^{-1}) with and without ultraviolet light, but not at lower concentrations of Fe(0). In addition, a recent study reported between 4% and 66% reduction of perchlorate by high concentrations (500–1250 g l^{-1}) of Fisher, Connelly, and Peerless Fe(0) (Moore et al. 2003). As such, the high Fe(0) concentrations required to reduce low concentrations of perchlorate suggest that abiotic treatment may not be an effective means of perchlorate reduction.

Bacteria that respire perchlorate as an electron acceptor can be very efficient at perchlorate reduction and there is the potential for these bacteria to utilize hydrogen and ferrous iron as electron donors. Reports in the literature indicate that several perchlorate-degrading bacteria such as *Wolinella succinogenes*, *Dechloromonas* sp. HZ, and *Dechloromonas* sp. JDS5 can degrade perchlorate in the presence of hydrogen (Shrout 2002; Wallace et al. 1996; Zhang et al. 2002). Similarly, *Dechloromonas agitata* CKB can reduce perchlorate using ferrous iron as an electron donor (Bruce et al. 1999). Since perchlorate is a thermodynamically favorable electron acceptor and since hydrogen and ferrous iron can be effective electron donors for perchlorate reduction, combining Fe(0) and anaerobic bacteria may be an effective method for reducing perchlorate to chloride. As such, a bioaugmented permeable reactive barrier containing Fe(0) may be an effective way of intercepting and treating perchlorate-contaminated groundwaters *in situ*.

The original goal of this work was to evaluate the potential for Fe(0) to serve as a source of electron donor for bacterial perchlorate reduction and to enhance degradation by combining complementary biotic and abiotic reactions. Preliminary experiments, however, showed that perchlorate reduction by an anaerobic bacterial culture appeared to be inhibited by the presence of Fe(0) (Shrout & Parkin 2000). The objective of the work described herein was to determine if the presence of Fe(0) inhibited perchlorate degradation and, if so, the reason for this inhibition. We hypothesized that inhibition of bacterial perchlorate reduction by Fe(0) might be due to: (1) elevated pH values, and/or (2) accumulation of iron species at the surface of the bacterial cell. These hypotheses were investigated by measuring the rate and extent of perchlorate reduction by two anaerobic bacterial enrichment cultures in the absence and presence of Fe(0) and ferrous iron and characterizing bacterial and iron microcosms with SEM, Mössbauer spectroscopy, and XRD.

Materials and methods

Enrichment cultures

Two mixed bacterial cultures consisting of 'readily obtainable' anaerobic bacteria were used for this research. One culture was previously acclimated to perchlorate; this acclimated LEC received 0.25 mM perchlorate every 2 days. (This 0.25 mM perchlorate concentration represents a demand of 8.3% of the electrons supplied by 2 mM lactate.) The unacclimated LEC received no perchlorate prior to the batch experiments described herein. Both cultures were developed from anaerobic digester sludge from the Iowa City, Iowa, North

Treatment Plant. These lactate enrichment cultures (LECs) were maintained as draw-and-fill stock reactors operated on a 40-day hydraulic retention time (HRT) during which they received a reduced mineral nutrient medium and 2 mM lactate as an electron donor and carbon source every 2 days. The pH of these cultures was maintained near neutral using bicarbonate as described below. Both cultures produced appreciable quantities of methane and were generally anaerobic as evidenced by the visual observation of black ferrous sulfide precipitates. All cultures were operated under pseudo-steady state conditions for more than 2 years prior to conducting the microcosm experiments described below. The pseudo-steady state characteristics were defined as consistent utilization of all perchlorate and lactate added with relatively constant measured biomass concentrations.

Growth medium

The reduced, chloride-free, anaerobic medium used for the LECs contained NH_4HCO_3 (15.2 mM), $KHCO_3$ (5.40 mM), $Na_2S \cdot 9H_2O$ (1.40 mM), $FeO_4P \cdot 4H_2O$ (0.20 mM), $MgHPO_4 \cdot 3H_2O$ (3.30 mM), $CaCO_3$ (0.17 mM), $(NH_4)_2HPO_4$ (1.20 mM), $CoBr_2$ (0.011 mM), KI (0.016 mM), $(NaPO_3)_6$ (0.017 mM), $MnBr_2$ (0.002 mM), NH_4VO_3 (0.004 mM), $ZnBr_2$ (0.002 mM), $NaMoO_4 \cdot 2H_20$ (0.002 mM), H_3BO_3 (0.008 mM), $NiBr_2$ (0.002 mM), and cysteine (0.083 mM).

Microcosm experiments

Batch microcosms were started from culture harvested from the LECs to investigate the potential for Fe(0) to serve as an electron donor for perchlorate reduction and the effect of iron corrosion on perchlorate reduction. Volumes of 50 ml LEC were transferred from a holding reservoir to 60-ml serum bottles in an anaerobic chamber with a $N_2/CO_2/H_2$ headspace (94/4/2, %volume). The average VSS (as described in Analyses) of acclimated-LEC and unacclimated-LEC were 210 and 280 mg l^{-1}, respectively. Iron filings (Fisher Scientific; surface area = 3.82 m^2 g^{-1}) or ferrous (Fe(II)) iron (Sigma Chemical; $FeCl_2 \cdot 4H_2O$) were added directly by mass to some microcosms while preparing serum bottles in the anaerobic chamber. A concentration of 20 g l^{-1} Fe(0) was estimated to be a potentially effective electron donor for these LECs. Lactate (as lactic acid) was also added to some microcosms (2 mM) from an aqueous concentrated stock solution. All bottles were sealed with butyl rubber stoppers and sparged with N_2 and/or CO_2 gas, depending upon desired pH to maintain anaerobic conditions at or above 1 atmosphere pressure to prevent the influx of external gases. The pH of some microcosms was controlled by lowering pH with the addition of 100% CO_2 gas or raising pH with a potassium hydroxide solution (2 M). The purpose was to poise pH at a non-circumneutral pH to investigate the effect of pH on perchlorate reduction. Some microcosms containing Fe(0) required addition of CO_2 gas one to three times daily to maintain the desired pH over the duration of the experiment. Abiotic controls consisted of 20 g l^{-1} Fe(0) incubated without LEC in reduced anaerobic growth medium. All bottles were incubated at 20 °C in the dark on a shaker table at 160 rpm. All conditions were performed in triplicate.

Iron species characterization

The iron precipitates formed in the perchlorate-degrading microcosms were characterized by Mössbauer spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM). Samples were collected after 120 h from acclimated-LEC microcosms started with 1 mM perchlorate and 20 g l^{-1} Fe(0), and with 1 mM perchlorate and 2 mM lactate. Additional samples were obtained from microcosms of acclimated-LEC with 1 mM perchlorate, 2 mM lactate and 100 mM added Fe(II) after 24 h incubation. For microcosms where Fe(0) was added, prior to freeze-drying, the cell suspension was decanted from serum bottles utilizing gravity settling to minimize collection of higher-density Fe(0). Approximately 25 ml of cell suspension from each reactor was freeze-dried under anoxic conditions, collected as dried flakes, and stored under N_2 headspace for sample preparation. Freeze dried samples analyzed with Mössbauer spectroscopy were placed in a hermitically sealed sample holder under an inert atmosphere (anaerobic chamber of N_2/H_2 (95/5, v/v)). Mössbauer spectra were collected at room temperature with a 50 mCi ^{57}Co source mounted on a transducer in constant

velocity mode. Calibration of the spectra was performed against an α-Fe foil also collected at room temperature. Spectra were fit with Voight based lines using the Recoil (Ottawa, Canada) software program (Rancourt & Ping 1991). Freeze-dried samples for XRD analysis were sieved to 325 mesh and admixed with a water/glycerol (1:1) solution to prevent oxidation during analysis. Scans were completed between 5° and 80° with a step of 0.02° every 2 s. SEM samples were prepared from the freeze-dried powders under an inert atmosphere. A portion of each freeze-dried sample was mounted on an aluminum stub using double-sided carbon tape. The only exposure of the samples to atmospheric conditions occurred while transferring the prepared stubs to the sputter coater and during the transfer from the sputter coater to the sample chamber of the SEM. Samples were sputter coated with gold in an argon vacuum headspace. Samples were viewed up to 25,000 times magnification using a Hitachi (San Jose, CA) S-4000 scanning electron microscope.

An estimation of expected soluble and precipitate Fe(II) species was performed using Environmental Research Software (Hallowell, ME) MINEQL 4.0 assuming a closed atmosphere and pH = 7.0.

Analyses

Analysis for perchlorate, chlorate, chlorite, and chloride was performed using a Dionex (Sunnyvale, CA) DX-500 ion chromatograph equipped with a Dionex ASRS suppressor operating in external water mode with a regenerant of 10 mM sulfuric acid. Separation was achieved with a Dionex AS11 or AS16 column maintained at 40 °C, by an eluent of sodium hydroxide flowing at 1 ml min^{-1} controlled by a Dionex GP50 gradient pump. A Dionex CD20 conductivity detector performed detection, and peak areas were integrated by Dionex PeakNet software. Injection volumes depended upon the initial concentration of perchlorate. The detection limit for perchlorate was approximately 0.0001 mM. The detection limits for chlorate and chlorite were approximately 0.01 mM.

Dry cell mass was determined by measuring the volatile suspended solids (VSS) by standard wet chemistry techniques (APHA et al. 1989). Aliquots of a known volume of cell suspension, ranging from 25 to 100 ml, were removed from growth vessels, and liquid was removed from these samples by vacuum filtering through a 1.2 μm pore glass filter that was then dried at 103 °C.

Samples of acclimated-LEC were harvested by centrifugation, resuspended in distilled water, and stained to verify the presence of living cells using a Molecular Probes (Eugene, OR) LIVE/DEAD BacLight Bacterial Viability Kit. Nucleic acids in live cells stained with SYT09 dye were green in color and nucleic acids from dead cells stained with propidium iodide were red in color. Prepared stains were viewed using a confocal microscope. Stains of *Staphlococcus aureus* were used as a positive control.

Solution pH was measured using Merck ColorPHast (Darmstadt, Germany) pH strips. Surface area of Fe(0) was determined by BET analysis performed under anoxic conditions. Soluble Fe(II) was measured spectrophotometrically using the 1,10-Phenanthroline method (APHA et al. 1989). The detection limit for Fe(II) was approximately 5 μM.

Results and discussion

After an initial lag period of about 100 h, an unacclimated lactate-enriched culture (LEC) removed 0.05 mM of perchlorate (Figure 1). Complete removal of all perchlorate was observed

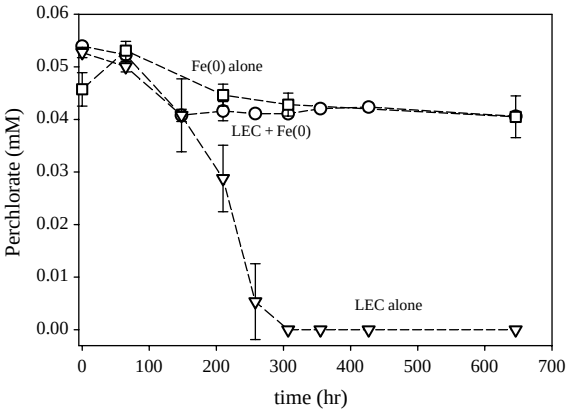

Figure 1. Effect of Fe(0) on the reduction of perchlorate by an unacclimated lactate enrichment culture (LEC). This unacclimated LEC was not previously acclimated to perchlorate. Experimental conditions: 20 g L^{-1} Fe(0) in reduced medium, 20 gL^{-1} Fe(0) with LEC, and LEC alone (unfed−resting cells). Error bars represent ± one standard deviation for three replicate reactors.

by 300 h. Previous studies of perchlorate reduction by pure-culture bacteria have shown chloride as the only end-product (Coates et al. 1999; Rikken et al. 1996). Due to the complex medium and low concentration of perchlorate used, production of chloride could not be confirmed in this experiment, but in experiments using a similar LEC fed higher concentrations of perchlorate, recovery of chloride was near 100% (Shrout 2002).

Abiotic experiments with Fe(0) alone (containing the same reduced growth media used for LEC) resulted in negligible perchlorate reduction. The negligible reduction of perchlorate by Fe(0) indicated that perchlorate removal during the biotic experiments was due to bacterial reduction alone. Despite the favorable thermodynamics of perchlorate reduction by Fe(0) (Equation (1)),

$$4Fe^0 + ClO_4^- + 8H^+ = 4Fe^{2+} + Cl^- + 4H_2O;\ \Delta G^{\circ\prime} = -133.495\ \text{kJ eeq}^{-1} \quad (1)$$

the lack of perchlorate reduction observed in the presence of 20 g l^{-1} Fe(0) is consistent with previous studies that observed little or no perchlorate reduction with Fe(0) (Gurol & Kim 2000; Moore et al. 2003). Other reports for abiotic-Fe(0) reactivity showed that 10 g l^{-1} Fe(0) (100 mesh, 0.74 m^2 g^{-1}) was unable to reduce perchlorate and a concentration of 1250 g l^{-1} Fe(0) (40 mesh, 2.50 m^2 g^{-1}) was required to reduce 66% of 0.1 mM perchlorate to chloride (over a 2 week time period) (Gurol & Kim 2000; Moore et al. 2003).

Addition of 20 g l^{-1} Fe(0) to unacclimated-LEC halted perchlorate reduction after about 20% of initial perchlorate had been reduced (at about 200 h) (Figure 1). A substantial increase in pH was observed when Fe(0) was added to unacclimated-LEC (from 7.1 to 8.6 in one bottle and from 7.1 to 9.2 in a second bottle) relative to unacclimated-LEC alone and Fe(0) alone which stayed near neutral (pH 7.1 and 7.4 for two bottles). The pH of the Fe(0) alone treatments remained near neutral over the observed period due to sufficient buffering (with CO_2/HCO_3^-). The rise in pH observed with Fe(0) and unacclimated-LEC was most likely due to corrosion of Fe(0) (driven by hydrogen utilization by unacclimated-LEC as compared to abiotic conditions) and release of hydroxide ions and ferrous iron (Fe(II)) (Equation (2)).

$$Fe^0 + 2H_2O = Fe^{2+} + 2OH^- + H_{2(g)} \quad (2)$$

Effect of pH

To explore whether elevated pH was responsible for the apparent Fe(0) inhibition of bacterial perchlorate reduction, the pH of acclimated-LEC microcosms with and without Fe(0) were controlled by manual addition of carbon dioxide and potassium hydroxide. Acclimated-LEC was used to remove the lag in perchlorate reduction (e.g., Figure 1) and to allow us to use higher concentrations of perchlorate without potential toxicity to bacteria. All treatments received lactate (2 mM) as an exogenous electron donor to avoid potential electron donor limitations and to minimize the effect of electron donor preferences (i.e., a preference for lactate over H_2 produced from Fe(0) corrosion). Microcosms with and without Fe(0) were maintained at four different pH values. Perchlorate reduction was slower when acclimated-LEC was incubated with Fe(0) at each of the four pH values tested than when Fe(0) was absent (Figure 2). In general, higher pH resulted in slower perchlorate removal rates in all microcosms containing Fe(0). Fe(0) treatments were affected by each pH increase, whereas lactate-only fed treatments were unaffected until the pH was raised to 8.9. The effect of pH on perchlorate-reducing bacteria was consistent with reports in the literature where elevated pH slowed or stopped perchlorate reduction. For example, Attaway & Smith (1993) reported growth of a mixed

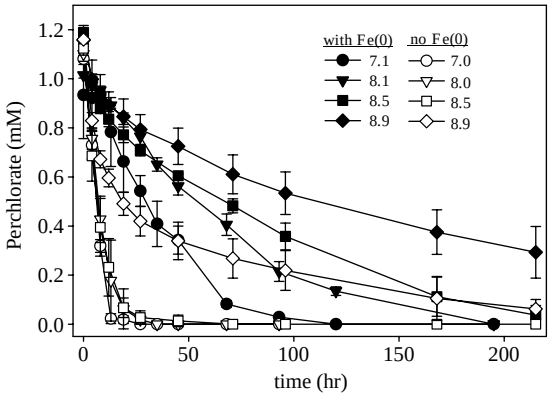

Figure 2. Effect of pH and Fe(0) addition on perchlorate reduction by acclimated-LEC fed 2 mM lactate. Error bars represent ± one standard deviation for three replicate reactors.

perchlorate-degrading culture from pH 6.0 to above 8.5 and perchlorate reduction from pH 6.6 to 7.5 and *Dechloromonas agitata* CKB, a well-characterized perchlorate-degrading bacteria, has been shown to grow between pH 6.5 and 8.5 (Bruce et al. 1999). Thus, the elevated pH created from Fe(0) corrosion (Equation (2)) may be a problem for perchlorate-degrading bacteria. However, in these experiments, treatments with Fe(0) performed poorly compared to lactate-only treatments held to the same pH suggesting that the inhibition observed in the presence of Fe(0) is not due solely to inhibition of bacterial activity by increased pH.

Effect of aqueous Fe(II) additions

Both the solubility and speciation of Fe(II) are strongly influenced by pH and the known production of Fe(II) from Fe(0) corrosion (Equation (2)) led to the exploration of whether Fe(II) was responsible for the inhibition of perchlorate reduction observed in the presence of Fe(0). Direct addition of Fe(II) as $FeCl_2$ resulted in decreased perchlorate reduction in treatments of acclimated-LEC fed 2 mM lactate (Figure 3), suggesting that Fe(II) might be responsible for Fe(0) inhibition. The pH of all treatments was near 7.0 and required

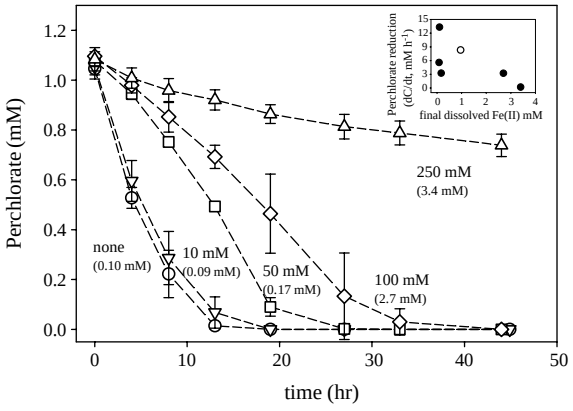

Figure 3. Effect of Fe(II) on perchlorate reduction by acclimated-LEC fed 2 mM lactate. Fe(II) was added as $FeCl_2 \cdot 4H_2O$. Final dissolved Fe(II) concentrations are reported in parentheses. Error bars represent ± one standard deviation for three replicate reactors. Inset: Plot of perchlorate reduction rate (estimated by the initial decrease in perchlorate per hour) versus final concentration of dissolved Fe(II). Open symbol represents a treatment with 20 g L^{-1} Fe(0) + 2 mM lactate (i.e., no Fe(II) was added).

no adjustment for the duration of the experiment. Although soluble Fe(II) has been shown to inhibit anaerobic biological activity (Andrews & Novak 2001; Ram et al. 2000), final dissolved Fe(II) concentrations did not correlate well with perchlorate reduction rate in treatments with acclimated-LEC fed lactate with or without Fe(0) or Fe(II) (Figure 3 – inset). The lack of correlation with dissolved Fe(II) indicated that solid Fe(II) species might be responsible. Interactions of solid iron species with bacteria have been described by others. Both Fe(II) and Fe(III) solids have been shown to precipitate on bacterial cell surfaces and even be incorporated into cell walls (Glasauer et al. 2001; Warren & Ferris 1998), and sorption of Fe(II) to cell surfaces has been shown to inhibit dissimilatory iron reduction by *Shewanella putrefaciens* and *Geobacter metallireducens* (Liu et al. 2001; Roden & Urrutia 2002). While inhibition observed in these experiments could also be due to a nutrient limitation caused by precipitation of required minerals (e.g., phosphate) with Fe(II), this is unlikely. The ability of acclimated-LEC microcosms to ultimately degrade three 1 mM perchlorate doses when incubated with up to 100 mM Fe(II) implies nutrients were not limited (data not shown). Most Fe(II) produced during Fe(0) corrosion will precipitate. The predominant anions available in the nutrient medium used in these experiments (carbonate, phosphate, and sulfide) are theoretically capable of precipitating approximately 30 mM Fe(II) (as $FeCO_3$, $Fe_3(PO_4)_2$, and FeS).

Characterization of Fe precipitates

To evaluate whether Fe(II) species accumulated at cell surfaces, freeze-dried culture samples exposed to 1 mM perchlorate in the absence and presence of Fe(0) (pH = 7.0) were characterized. Recall that in the absence of Fe(0), perchlorate reduction occurred, whereas in the presence of Fe(0), significant inhibition was observed (Figure 2). Mössbauer spectra of a sample of acclimated-LEC alone (no Fe(0) added) showed some accumulation of ferrihydrite, an Fe(III) precipitate, in the culture sample after 120 h (Figure 4a). In the presence of Fe(0), however, significantly more accumulation of reduced Fe precipitates was observed at 120 h (as evidenced by the almost 10-fold increase in % absorption in Figure 4b). The Mössbauer

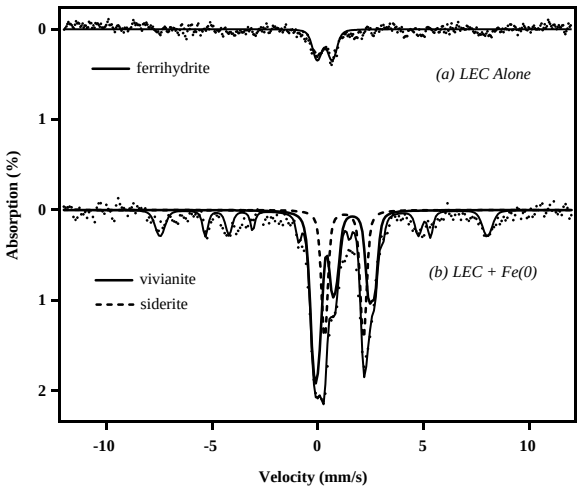

Figure 4. Mössbauer spectra of solids collected from acclimated-LEC incubated with 1 mM perchlorate and 2 mM lactate for 120 hours in the (a) the absence and (b) presence of 20 g L-1 Fe(0). Subspectra for only the Fe(II) precipitated species, vivianite and siderite, are shown. Trace Fe(0) and a mixture of magnetite/maghemite were also observed as magnetic sextets.

spectrum confirms the presence of Fe(0) and indicates a complex mixture of precipitates, including almost half as vivianite, $Fe_3(PO_4)_2$, about a third as siderite, $FeCO_3$, and trace amounts of a magnetic sextet (most likely magnetite, Fe_3O_4, or maghemite, γ-Fe_2O_3). XRD patterns corroborate the presence of vivianite and siderite in the sample (data not shown). A sample taken at $t = 0$ from the same culture (immediately after the addition of Fe(0)) showed only trace amounts of Fe(0), ferrihydrite, and the magnetic sextet, confirming that the Fe precipitates formed during the 120 h of the experiment (data not shown). The clear presence of precipitates in the Fe(0) treatments is consistent with our hypothesis that inhibition of perchlorate-reducing activity in the presence of Fe(0) may be due to encapsulation of bacteria by reduced Fe precipitates.

SEM images collected from the same samples provides additional visual evidence that the bacteria exposed to Fe(0) became encapsulated with some type of precipitate (Figure 5b). In acclimated-LEC samples not treated with Fe(0), structures that look like bacterial flocs containing two morphologies (small cocci shapes and long filamentous rod shapes) are observed after 120 h (Figure 5a). Shapes observed in acclimated-LEC were less than 0.5 μm in size, and while bacteria as small as 0.1 μm in size have been identified by

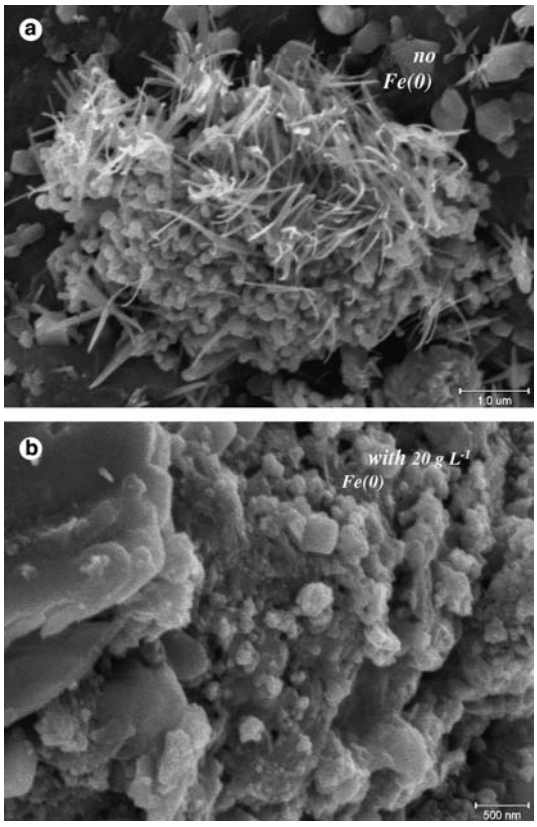

Figure 5. SEM images of samples collected from an acclimated LEC incubated with 1 mM perchlorate and 2 mM lactate in the (a) absence and (b) presence of 20 g L^{-1} Fe(0).

others (Madigan et al. 1996), we also conducted nucleic acid staining of acclimated-LEC separately to provide additional evidence that similar shaped and sized components from acclimated-LEC observed in SEM photographs were bacteria (data not shown). Similar bacterial flocs could not be identified in acclimated-LEC samples treated with Fe(0) despite a thorough investigation of the sample.

Based on a combination of the spectroscopic and visual evidence, it appears that perchlorate inhibition in the presence of Fe(0) is at least partially due to accumulation of reduced Fe species at cell surfaces that appeared to encapsulate bacterial cells. This inhibition may have not been observable in other experiments that combined Fe(0) and bacteria (e.g., Gregory et al. 2000; Novak et al. 1998; Till et al. 1998; Weathers et al. 1997) because most of the contaminants investigated are susceptible to abiotic reduction by reduced Fe species (e.g., Kim & Picardal 1999; Klausen et al. 1995; McCormick et al. 2002; Weber et al. 2001). Therefore, the inhibition of bacteria may be masked by reaction of the contaminant with the reduced Fe species. Since perchlorate is not reduced by Fe(0) under the conditions of our experiments, the inhibition caused by Fe species interaction with bacteria could be observed. Although the SEM and Mössbauer data add to the compelling evidence that Fe precipitates inhibited bacterial activity by physically coating the cell surfaces, an alternative explanation may be related to the enzymatic physiology of these organisms. For example, Kersters & Verstraete (1996) assessed the effect of high levels of Fe(II) on *Aeromonas hydrophila* and attributed the observed toxicity to over-production of free radical species. The production of free radicals by Fe(II) acting upon oxygen produced from chlorite dismutation by perchlorate-reducing bacteria could lead to bacterial inactivation.

Fe(0) as an electron source

For acclimated-LEC that required no lag period prior to perchlorate reduction, Fe(0) could serve as a source of electrons with sufficient control. Under controlled-pH conditions, acclimated-LEC reduced perchlorate faster when incubated with Fe(0) compared to acclimated-LEC given no exogenous source of electrons (Figure 6). The pH

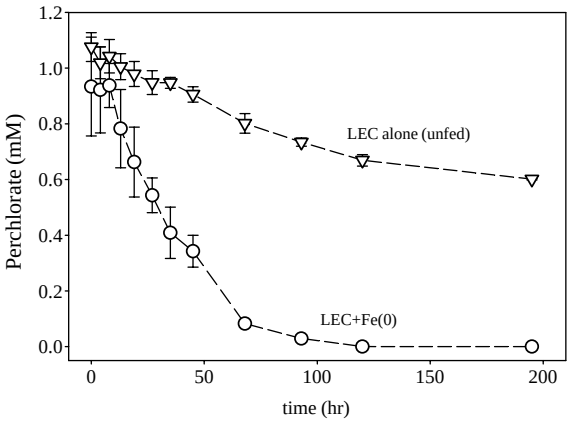

Figure 6. Effect of Fe(0) on the reduction of perchlorate by acclimated-LEC. The pH of the reactors was held constant at a pH of 7.1 ± 0.2, the initial concentration of perchlorate was 1 mM, and the concentration of Fe(0) was 20 g L^{-1}. Lactate was not added to either reactor. Error bars represent ± one standard deviation for three replicate reactors.

of all treatments was held to 7.1 ± 0.2. The faster rates of perchlorate reduction in the presence of Fe(0) (with pH control and no other source of electrons) were most likely due to hydrogen gas and ferrous iron produced from Fe(0) corrosion (Equation (2)) that have been previously shown to serve as electron donors for perchlorate-reducing bacteria (Daniels et al. 1987; Rajagopal & Legall 1989; Weathers et al. 1997). This emphasizes the need for an electron donor to drive bacterial perchlorate reduction. When concentrations of perchlorate are low, these electrons have the potential to be produced endogenously or from residual organics (e.g., unacclimated-LEC, Figure 1); however when perchlorate concentrations are elevated, an exogenous source of electrons is required. This result suggests perchlorate reduction could be temporarily stimulated by addition of Fe(0) with adequate pH control. Nonetheless, these benefits will most likely be temporary due to production of hydroxide and reduced Fe precipitates that can inhibit bacterial cells.

Conclusions

The ability of LEC to reduce perchlorate was unexpectedly inhibited by the addition of Fe(0) in batch microcosm experiments. We expected abiotic and biotic reactions to act synergistically as

has been previously reported with other highly oxidized contaminants (Gregory et al. 2000; Novak et al. 1998; Till et al. 1998; Weathers et al. 1997). Additionally, Fe(II) sorbed on iron oxides or bacteria can greatly enhance the reduction of highly oxidized pollutants (Klausen et al. 1995; McCormick et al. 2002). We found that abiotic reduction of perchlorate is negligible (unless the iron to perchlorate ratio is very large). Our results indicated that Fe(0) is not a good choice for use in perchlorate treatment as Fe(0) caused inhibition of bacterial reduction. Fe(0) corrosion resulted in production of insoluble, reduced, Fe species that appeared to inhibit perchlorate reduction by encapsulating the bacteria. This is the first evidence linking accumulation of solid iron species at the cell surface to inhibition of environmental contaminant degradation by bacteria. These results suggest the potential for inhibition of bacterial activity with the addition of Fe(0).

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