Microbial reduction of perchlorate with zero-valent iron

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\begin{abstract}
Microbial reduction of perchlorate in the presence of zero-valent iron was examined in both batch and column reactors to assess the potential of iron as the electron donor for biological perchlorate reduction process. Iron-supported mixed cultures completely removed 65 mg/L of perchlorate in batch reactors in 8 days. The removal rate was similar to that observed with hydrogen gas (5%) and acetate (173 mg/L) as electron donors. Repeated spiking of perchlorate to batch reactors containing iron granules and microorganisms showed that complete perchlorate reduction by the iron-supported culture was sustained over a long period. Complete removal of perchlorate by iron-supported anaerobic culture was also achieved in a bench-scale iron column with a hydraulic residence time of 2 days. This study demonstrated the potential applicability of zero-valent iron as a source of electrons for biological perchlorate reduction. Use of zero-valent iron may eliminate the need to continually supply electron donors such as organic substrates or explosive hydrogen gas. In addition, iron is inexpensive, safe to handle, and does not leave organic residuals in the treated water.
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1. Introduction

Perchlorate is a major groundwater contaminant that is derived mostly from anthropogenic activities. It has been released into the environment primarily through the use of ammonium perchlorate as a propellant in missiles, rockets, and explosives, as a pyrotechnic in fireworks, in magnesium batteries, paint, and enamel production, and as an automobile air bag inflator (Espenson, 2000; Logan, 2001; Moore et al., 2003; Motzer, 2001; Urbansky, 1998; Xu et al., 2003). US EPA has identified perchlorate users and manufacturers in 44 states and reported that at least 15 million people in the United States may be affected by the perchlorate contamination in their drinking water supplies (US EPA, 1999, 2002).

Based on human health concerns, the Office of Environmental Health Hazard Assessment (OEHHA) in California tentatively set a public health goal (PHG) of 6 μg/L for perchlorate in 2004 (Council on Water Quality, 2004; OEHHA, 2004). Recently, the EPA revised the perchlorate reference dose to 0.0007 mg/kg/day in accordance with a finding of a National Academy of Science’s report (2005). The new reference dose corresponds to a drinking water equivalent level of 24.5 ppb (US EPA, 2005).

Perchlorate salts are not only chemically stable in natural water, but also extremely soluble and mobile. For example, sodium perchlorate and ammonium perchlorate have solubilities of 2010 and 220 g/L, respectively (Motzer, 2001). Consequently, only a limited number of technologies are capable of removing perchlorate from water: ion exchange, biological treatment, membrane filtration, and electro-dialysis (Council on Water Quality, 2004). Anion exchange is effective but has high operation and maintenance costs. Perchlorate removal by nano-filtration (NF) and reverse-osmosis (RO) was investigated by the Metropolitan Water District of Southern California, but these membrane processes appear to be more expensive than ion exchange (US EPA, 1999).
In contrast, microbial reduction of perchlorate has been recognized as a promising treatment technology in recent years (Motzer, 2001; Soltis, 1998; Xu et al., 2003). One central requirement of biological perchlorate removal is a supply of electron donors for perchlorate reducing bacteria (PRB). Coates et al. (1999) showed that perchlorate-reducing bacteria are ubiquitous and that they are capable of utilizing a variety of organic substrates as electron donors to reduce perchlorate. Several studies have demonstrated perchlorate reduction via addition of hydrogen or organic substrates such as acetate and ethanol (Condit et al., 2005; Greene and Pitre, 2000; Hatzinger et al., 2005). Of the frequently used organic electron donors, acetate has been shown to be effective for PRB (Brown et al., 2003; Kim and Logan, 2001; Min et al., 2004; Wu et al., 2001). A pathway has been proposed for microbial perchlorate reduction with acetate as the electron donor (Logan, 2001; Rikken et al., 1996). In this pathway, perchlorate is sequentially reduced via chlorate and chlorite to chloride. Although acetate has been shown to support perchlorate reduction in fixed bed treatment systems (Brown et al., 2003; Kim and Logan, 2001; Min et al., 2004), it is still questionable whether the use of organic substrates for groundwater and drinking water treatment is acceptable and economically feasible (Logan, 2001).

Alternatively, hydrogen gas has been used to support autotrophic perchlorate reduction (Giblin et al., 2000; Logan and LaPoint, 2002; Miller and Logan, 2000; Nerenberg et al., 2002; Nerenberg and Rittmann, 2002; Zhang et al., 2002). Hydrogen gas (5%), along with carbon dioxide, was provided to achieve 30–39% perchlorate reduction in an autotrophic bioreactor (Logan and LaPoint, 2002; Miller and Logan, 2000). However, the use of hydrogen as an electron donor has a number of drawbacks. Hydrogen is relatively expensive and dangerous to handle because of its explosive nature.

Perchlorate-reducing bacteria are ubiquitous and diverse (Coates et al., 1999; Waller et al., 2004; Zhang et al., 2002). The majority of PRB was identified by Achenbach et al. (2001) as β-subclass of proteobacteria. Several bacteria that are capable of reducing perchlorate with hydrogen as the sole electron source have been isolated. Miller and Logan (2000) isolated Dechloromonas sp. JM from hydrogen-utilizing autotrophic consortium and report that the isolate was capable of reducing perchlorate with hydrogen, but unable to use CO2 as the carbon source. Dechloromonas sp. HZ, on the other hand, can reduce perchlorate with hydrogen as the electron donor while requiring CO2 as the carbon source (Yu et al., 2006; Zhang et al., 2002). Recently two autotrophic perchlorate degrading bacteria, Dechloromonas sp. JDSS and Dechloromonas sp. JDS6, were isolated from a perchlorate-contaminated site (Shrout et al., 2005a).

Fe(0) is a strong reducing agent (E° = −0.44 V), and has been used in recent years to treat oxidized pollutants such as nitroaromatics, nitramines, and azo dyes through reductive transformation (Agrawal and Tratnyek, 1996; Singh et al., 1998; Oh et al., 2001; Perey et al., 2002). Thermodynamically, perchlorate is readily reducible by Fe(0). However, studies have shown that this reaction is very slow under ambient conditions, suggesting that the energy barrier to the reaction is large (Espenson, 2000; Moore et al., 2003). Under anaerobic conditions, iron corrosion produces hydrogen gas through the reduction of protons. In the presence of hydrogenotrophic microorganisms, the cathodic hydrogen may be utilized to degrade perchlorate, as has been shown previously for nitrate, chlorinated solvents, and sulfate (Daniels et al., 1987; DiStefano et al., 1992; DeWeerd et al., 1991; Lampron et al., 2001; Smith et al., 1994; Weathers et al., 1997).

Recently, Shrout et al. (2005a) studied microbial perchlorate reduction in the presence of zero-valent iron using a mixed culture obtained from an anaerobic digester. However, they reported that the addition of Fe(0) to the anaerobic culture resulted in slower rate of perchlorate reduction. The inhibitory effect of zero-valent iron on perchlorate was attributed to the increase in pH and encapsulation of bacteria by iron precipitates (Shrout et al., 2005a). High concentrations of bicarbonate (1260 mg/L) and phosphate (430 mg/L) in the culture media resulted in the formation of vivianite, Fe3(PO4)2, and siderite, FeCO3. In contrast, Yu et al. (2006) showed that zero-valent iron was capable of serving as electron donor for perchlorate reduction by providing hydrogen to a hydrogen-utilizing autotroph (Dechloromonas sp. HZ). Initially, the activity of Dechloromonas sp. HZ pure culture was strongly dependent on solution pH; however, once perchlorate reduction was established, microbial reduction process was sustained even at pH 9 (Yu et al., 2006). While Yu et al. successfully demonstrated the feasibility of microbial perchlorate reduction supported by zero-valent iron, their experimental conditions were not typical of environment commonly found in natural and engineered systems. As the logical extension to the proof-of-concept studies employing batch pure cultures studies, flow-through column studies with environmentally relevant mixed cultures are needed to further promote and enhance the potential of the integrated Fe(0)-cell system for perchlorate remediation.

The objectives of this study are to investigate the feasibility of integrated iron-biological perchlorate reduction through both batch and column reactor experiments. We hypothesize that, in an integrated iron–biological system, iron could serve as a precursor of hydrogen gas, which could be used as electron donor for mixed cultures to reduce perchlorate. The sustainability of the iron–biological system was evaluated with a flow-through column seeded with mixed cultures obtained from a municipal wastewater treatment plant.

2. Materials and methods

2.1. Microorganisms

A perchlorate reducing seed culture was prepared by mixing biomass samples from an anaerobic digester and activated sludge in the Wilmington wastewater treatment plant (Wilmington, DE, USA). The biomass concentration of the seed culture was adjusted to approximately 400 mg/L based on the total solids contents of anaerobic digester sludge and activated sludge. Both batch and column experiments were inoculated with mixed cultures without acclimation or enrichment.

The culture medium contained 100 mg/L NH4H2PO4, 2 mg/L MgSO4·7H2O, 400 mg/L NaHCO3, 40 μg/L NiCl2·6H2O and 40 μg/L Na2SeO3·5H2O. HEPES buffer in both acid (C6H12N4O7S)
2.2. Batch Degradation Experiments

Batch experiments were conducted using 250-mL amber bottles at room temperature under anoxic and light-excluded conditions. Liquid volume in each reactor was 125 mL. Table 1 summarized different experimental conditions examined in triplicate batch experiments. Each iron-amended bottle contained 2 g of iron fillings. Zero-valent iron used in this study was cast iron filings obtained from Master Builders, Inc. (Aurora, OH) and was used as received. In addition to batch reduction experiments with cast iron, we conducted a batch experiment with high-purity iron granules (Alfa Aesar, Ward Hill, MA) to determine the product of perchlorate reduction to verify that the reduction of perchlorate to chloride is the dominant mechanism responsible for the removal of perchlorate. Pure iron granules were used for this study because of the presence of chloride impurities in cast iron granules.

To compare perchlorate reduction kinetics in the presence of different electron donors, acetate and hydrogen were introduced to separate batch reactors. The headspace of H2-fed bioreactor was filled with 95% N2 and 5% H2 gas in a glove box (Coy, Glass lake, MI). The acetate-fed bioreactors contained 173 mg/L of sodium acetate as the sole electron donor. Bottles were sealed with screw-top Minitert™ caps (Alltech, Deerfield, IL) and low-permeability vinyl tape to maintain the solution pH at 7.

2.3. Column study

A laboratory-scale column bioreactor was established with iron and seed culture to demonstrate the feasibility of continuous and long-term perchlorate removal by the iron-mediated mixed culture. A cylindrical glass column (2.5 cm ID × 30 cm/L, Ace Glass, Vineland, NJ) with Teflon® end-caps, was packed with a mixture of iron fillings and glass beads (3 mm ID, Fisher Scientific, Pittsburgh, PA) and seeded with a mixture of activated sludge and anaerobic digester cultures obtained from Wilmington wastewater treatment plant. Zero-valent iron used in the column was cast iron filings obtained from Master Builders, Inc. (Aurora, OH) and was used as received. Glass beads and iron granules were homogeneously mixed in at 19:1 ratio (vol:vol) to avoid pore-clogging due to interparticle bridging of iron granules. The porosity of packed column was 0.26 and pore volume was 38 mL. The influent containing perchlorate and pH-buffered nutrient media was continuously pumped to the bottom of the column at a flow rate of 1 mL/h (column residence time = 2 days) using a Masterflex peristaltic pump (Cole-Palmer, Vernon Hills, IL). The flow rate was kept constant during experiments for 1 month. The influent vessel was continuously purged with nitrogen gas to strip any residual dissolved oxygen in the influent solution.

The iron–biocolumn was inoculated with the mixed culture during the packing of column in an anaerobic glove box. An abiotic control column was also packed in the same manner except for the inoculation with microbial culture. After inoculation with the culture, both biocolumn and abiotic columns were purged with 99.99% nitrogen gas to eliminate oxygen in the column pores. The abiotic iron column was operated in parallel with the biotic column to quantify possible physico-chemical reduction of perchlorate by iron granules.

2.4. Analytical procedures

Perchlorate concentration was determined by a Dionex DX-500 ion chromatograph (Dionex, Sunnyvale, CA) equipped with an IonPAC AS 11 column and a guard column. The detection limit for perchlorate was 20 µg/L. Samples from batch experimental bottles were collected through the Minitert™ cap with 10-mL disposable syringe. Effluent samples from the column reactor were collected in vials daily. Both column and batch reactor samples were passed through a 0.22-µm syringe filter (Fisher Scientific, Pittsburgh, PA) for analysis. Since perchlorate analysis by IC required a strong base eluent (80 mM NaOH), samples containing soluble and colloidal Fe²⁺ and Fe³⁺ species may precipitate as hydroxides during IC analysis. To avoid contamination and damage to IC equipment, sodium hydroxide was added in excess to filtered samples and then a red precipitate formed over 1 h was removed by syringe filter before IC analysis. In addition, the concentration of chloride was analyzed using 10 mM NaOH eluent. Effluent pH was measured using a pH meter (Cole-Palmer, Vernon Hills, IL).

3. Results and discussion

3.1. Perchlorate reduction in batch reactors

Perchlorate concentration profiles in batch reactors under the different conditions are shown in Fig. 1. Complete disappearance of perchlorate was only observed in the presence of Fe(0), H2, or acetate. Perchlorate (65 mg/L) in the reactors containing both Fe(0) and cells was completely removed to
below the detection limit of 0.02 mg/L in 8 days. In contrast, only 15% of the initial perchlorate was removed in the Fe(0)-only reactors (no cells) over 10 days, indicating iron alone was not effective in removing perchlorate. Perchlorate reduction in the cell-only control, which contained the seed culture but no external electron source (i.e., no added iron, acetate, or H2), was slow but more marked than the abiotic control. The 25% reduction in perchlorate concentration over the first 4 days was most likely due to fermentable organic materials carried over from the seed culture. Perchlorate removal was not observed in the medium control containing only sterile medium under N2 gas.

A chloride balance performed in the batch experiments using high-purity iron (Alfa Aesar, Ward Hill, MA) indicated that perchlorate (ClO4-) was reductively transformed to chloride (Cl-) (Fig. 2). Perchlorate (0.55 mM) was completely recovered as chloride in 12 days. Repetitive spiking of the Fe(0)-cell reactors was performed to assess if perchlorate reduction can be sustained over time (Fig. 3). Perchlorate stock solution was spiked into the Fe(0)-cell reactors after the disappearance of the initial perchlorate. Fig. 3 illustrates that complete biodegradation of perchlorate was not only sustained throughout the experimental period but the rate of perchlorate reduction was enhanced, possibly due to the increase in PRB density.

3.2. Perchlorate reduction in iron column

Reduction of perchlorate by iron-mediated anaerobic culture was also examined in a bench-scale iron column with an influent solution containing 16 mg/L of perchlorate. Perchlorate concentrations in the column effluent were consistently below the detection limit (0.02 mg/L) over the 20-day experimental period (Fig. 4). Perchlorate concentration in the effluent of abiotic iron column (control), which was operated in parallel, was 15 ± 0.94 mg/L suggesting that (1) iron granules were not able to remove perchlorate without microorganisms and (2) there was no significant adsorption of perchlorate by iron granules and glass beads. Effluent pH ranged from 6.8 to 7.0 in both biotic and abiotic columns.

This study clearly demonstrated that cathodic H2 from anaerobic iron corrosion supported the growth of an anaerobic culture that was capable of completely reducing aqueous perchlorate. The integrated iron–microbial system was effective.
in removing perchlorate in both batch and column reactors. Integration of iron and microorganisms resulted in much more effective removal of perchlorate than that with iron or microorganism alone. The rate of perchlorate removal in the iron-fed system was comparable to that in acetate- and hydrogen-fed systems suggesting that iron can be an alternative source of electrons for biological perchlorate treatment processes. Use of zero-valent iron may eliminate the need to continually supply electron donors such as organic substrates and the explosive hydrogen gas. Therefore, the integrated iron–microbial process may be more economical and environmentally friendly. Iron granules are inexpensive, safe to handle, and does not generate hazardous by-products. In addition, in contrast to the acetate-fed system, the proposed iron system does not leave organic residuals in treated water.

4. Conclusions

Iron-supported mixed cultures completely removed 65 mg/L of perchlorate in batch reactors in 8 days. The removal rate was similar to that observed with hydrogen gas or acetate as an electron donor. Complete removal of perchlorate by iron-supported anaerobic culture was also achieved in a bench-scale iron column with a hydraulic residence time of 2 days. This study demonstrated the potential application of zero-valent iron as the source of electrons for microbial perchlorate reduction.

R E F E R E N C E


