THE POTENTIAL FOR IN SITU PERCHLORATE DEGRADATION

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ABSTRACT: Perchlorate-respiring microorganisms (PRMs) occur naturally in soil, although their concentrations are site specific and depend on a history of perchlorate contamination. The persistence of perchlorate in groundwater aquifers results primarily from a combination of aerobic conditions and a lack of a sufficient quantity of electron donor for microbiological perchlorate removal. PRMs can grow using acetate and lactate as a carbon source. A polylactate compound specially formulated for the slow release of lactic acid upon hydrolysis (HRC™) has been used in past studies to aid in the in situ removal of chlorinated aliphatic compounds. It is shown here that HRC™ can also be used for perchlorate degradation. Lactate is used directly as a carbon and energy source by PRMs and does not need to be converted to hydrogen. The growth rates of bacterial isolates in laboratory experiments demonstrated complete removal in as little as 1.5 days.

INTRODUCTION

Perchlorate is highly soluble in water and poorly sorbs to mineral surfaces, but perchlorate contamination persists in many groundwater soils and aquifers. Perchlorate can be used as an electron acceptor by some bacteria. Under controlled conditions in the laboratory, we have previously shown that perchlorate can be microbiologically degraded under conditions analogous to flow in an aquifer. Using sand columns bioaugmented with acclimated perchlorate-degrading cultures, perchlorate was reduced to non-detectable levels (<4 ppb) through addition of excess acetate and trace nutrients at flow velocities typical of those in ground water (Logan and Kim 1998). The specific biochemical pathways necessary for perchlorate reduction are not well known, but there is good evidence that the pathway proceeds via \( \text{ClO}_4^- \rightarrow \text{ClO}_3^- \rightarrow \text{ClO}_2^- \rightarrow \text{Cl}^- + \text{O}_2 \). In this last step, a chlorite dismutase produces oxygen. This is an interesting development for perchlorate degradation versus the degradation of other chemicals since oxygen is generated in the process of perchlorate degradation. Oxygen is a preferred electron acceptor and under fully aerobic conditions perchlorate is not degraded. However, dissolved oxygen does not accumulate in solution and therefore its production during perchlorate degradation does not inhibit the overall reaction (Logan 1998).

In situ microbial reduction is a feasible approach to perchlorate degradation, but there are several factors that may be limiting intrinsic perchlorate removal rates that include: insufficient biomass concentrations or presence of perchlorate reducing microorganisms (PRMs); insufficient oxidizable substrate; and inhibition by high dissolved oxygen concentrations. To determine factors that
can limit intrinsic rates, and to develop information for initiating in-situ perchlorate degradation, we have been conducting different laboratory batch and column experiments using pure and mixed perchlorate-reducing cultures.

PRM abundances are highly variable in soil samples, but it appears that PRMs are widely distributed in nature (Coates et al. 1999, Logan et al. 1999, Wu 2000). At sites we examined, soils contained $10^7$ to $5 \times 10^9$ cells/g-soil based on acridine orange counts (Wu 2000). Soil testing at several sites not previously known to be contaminated with perchlorate indicated that there were few soil bacteria intrinsically capable of perchlorate degradation (<25 PRMs per $10^9$ soil bacteria). However, at a site known to have perchlorate contamination, Wu (2000) found 0.9 to 1.7 PRM per gram of soil. This suggests that abundances of PRMs are highly site specific. Accumulation times for perchlorate reduction in these soil samples typically took approximately a month. Therefore, depending on the site or perchlorate levels, and the need for immediate perchlorate degradation, in situ bioaugmentation could be helpful in obtaining more rapid bioremediation at a specific site.

The addition of a source of oxidizable substrate is required for perchlorate degradation for two reasons. First, the aquifer must be anoxic because oxygen inhibits bacterial respiration of perchlorate. Second, a substrate must be supplied for bacterial growth. In this study, we examined the use of HRC™, a polylactate compound specially formulated for the slow release of lactic acid upon hydrolysis. This material was originally developed to stimulate hydrogen production by in situ microorganisms via fermentation of the lactate (Koenigsberg and Norris, 1999). However, here we tested the ability of HRC™ to directly support bacterial reduction of perchlorate.

MATERIALS AND METHODS

Batch experiments were conducted using two perchlorate-degrading isolates (KJ and PDX), and a mixed culture obtained from a perchlorate-degrading reactor. The perchlorate reducing bacterium KJ was originally isolated from an acetate-fed column packed with sand and inoculated with a wastewater consortium of bacteria acclimated for perchlorate degradation. Isolate PDX was obtained by standard enrichment techniques with lactate and perchlorate using a sample from a wastewater treatment plant (Mulvaney 1999). The mixed culture was taken from the effluent of a packed bed reactor degrading perchlorate supported by acetate. The reactor was originally inoculated with KJ but it was not maintained as a pure culture during the month long experiment when samples were taken for the experiments reported here.

Batch experiments were conducted by inoculating sterilized media containing HRC™ or lactate, and perchlorate. Media was autoclaved and degassed in an anaerobic chamber to remove oxygen. HRC™ was scraped onto a small piece of aluminum foil and added directly into the sample. Lactate was added as a concentrated solution. Cell growth was measured by monitoring solution optical density at an absorbance of 600 nm (A600). The medium consisted the following 165 mg/L NaClO₄ (unless indicated otherwise) and the following: 1.55 g/L K₂HPO₄; 0.85 g/L NaH₂PO₄; 0.5 g/L NH₄H₂PO₄; and a

![FIGURE 1. Mixed culture degradation of perchlorate in the presence of excess HRC™. Perchlorate was not detectable after 38 hours.](image)

trace mineral solution consisted of 50 mg/l MgSO₄•7H₂O, 3 mg/l EDTA, 2 mg/l ZnSO₄•7H₂O, 1 mg/l CaCl₂•2H₂O, 4 mg/l FeSO₄•7H₂O, 0.4 mg/l Na₂MoO₄•2H₂O, 0.2 mg/l CuSO₄•5H₂O, 0.4 mg/l CoCl₂•6H₂O, 1 mg/l MnCl₂•2H₂O, 0.1 mg/l NiCl₂•6H₂O, 0.1 mg/l NaSeO₃, and 0.6 mg/l H₂BO₃.

Concentrations of perchlorate and acetate were measured using either a Dionex 100 or 500 Ion Chromatograph using an AS-11 column. For perchlorate measurements, we followed procedures in Dionex Application Note 121. Cell dry weights were measured using 25 mm diameter filters (0.2 μm pore diameter; Osmonics Corp.) dried at 103°C for 2 h, and weighed on a Mettler Balance (UMT2) to within 0.1 μg.

RESULTS AND DISCUSSION

Mixed culture growth on HRC™. The polylactate compound (~0.34 g) was dissolved in media (500 mL) containing 165 mg/L of perchlorate in a 1 L flask. The solution was left overnight in an anaerobic chamber to allow the HRC™ to dissolve, resulting in an initial and stable lactate concentration of 404 mg/L. The mixed culture was originally grown on acetate but was acclimated to lactate by two serial transfers before inoculation. Upon inoculation with the mixed culture there was a negligible lag in growth (Figure 1) The cell growth was constant at a rate of 0.076 hr⁻¹ over the first 13 hours, resulting in a doubling time of 9.1 hr. Cell growth was complete between 21 and 33 hours. There was no change in perchlorate (162±3, n=11) or lactate (404±4, n=11) in the abiotic control.

Cell yield and Utilization ratios for HRC. Perchlorate was completely removed, while the final lactate concentration was 240±7 (based on the average of the last three measurements in Figure 1). This translates to an overall lactate utilization
rate of 2.42 mg-lactate/mg-perchlorate. The cell dry weight at the end of the experiment was 76.1±1.6 μg (n=3) for a 10 mL sample, resulting in a cell yield of 0.46±0.02 g-cell DW/g-lactate. and a ratio of 0.47±0.01 g-cell DW/g-perchlorate.

Growth of pure and mixed cultures on lactate. The growth rate of the mixed culture was compared to that of the perchlorate-reducing isolate KJ in separate batch experiments. Poly lactate compound (~0.42 g) was dissolved in 400 mL of media and distributed to six 28 mL anaerobic test tubes (Belco Glass Inc.). Samples were degassed as above and run in triplicate. Growth was measured by optical density using a spectrophotometer (Spec20, Milton Roy). The results shown in Figure 2 indicate that the mixed culture reached a higher optical density than that of the pure culture. The growth rate of KJ over the first 100 hr was 0.020 hr⁻¹, resulting in a doubling time of 34 hr. The mixed culture also achieved a higher perchlorate removal (280 mg/L) versus that of the pure culture (202 mg/L), suggesting a larger amount of growth.

Growth of PDX at high concentrations of lactate. Bacterial isolate PDX was originally isolated using a medium containing lactate as the sole carbon and energy source and perchlorate (Mulvaney 1999). Because the hydrolysis of the poly lactate compound can produce very high concentrations of lactate (grams/liter), we tested the ability of this isolate to grow at high concentrations of lactate. Chlorate (ClO₃⁻) was used as an electron acceptor in order to avoid any toxic effects that might be due to perchlorate. Chlorate removal was complete even in the presence of 100 to 4000 mg/L of lactate. Maximum doubling times of PDX range from 3.5 hours on perchlorate and acetate to 7 hours on lactate and chlorate (Zhang 2000).

Implications for In Situ Removal of Perchlorate using HRC™. These experiments demonstrate that pre-acclimated mixed and pure cultures can degrade perchlorate to non-detectable levels using lactate produced by the hydrolysis of HRC™. PRMs that can grow using lactate have been found to be relatively abundant in soils at sites known to be contaminated with perchlorate. Wu (2000) examined the abundance of PRMs in soils from a site in Texas that was contaminated with perchlorate, and pristine soils from the State College area. Results were normalized by the number of PRM per million bacteria. Using most-probable-number techniques and lactate as a growth substrate, Wu (2000) estimated that there were 0.2 PRM/10⁶ in the soil Assuming all cells were extracted from soil samples, this would translate to 0.9 PRM/g-soil at that site. The number of PRMs at that site is probably higher due to incomplete extraction of the cells from the soil samples. In contrast, soils from the State College area were below the detection limit (0.0025 PRM/10⁶) in a pristine soil sample. Re-analysis of the soil sample for a lower detection limit, and using acetate as a carbon source, it was determined that there were 7.7×10⁶ PRM/10⁶. Wu's studies therefore demonstrate that PRMs are present at perchlorate contaminated sites and that they can grow using lactate, but that at pristine sites these PRMs are far less abundant. It is therefore believed that HRC™ can be used for in situ remediation of contaminated soils without bioaugmentation, but that using pre-acclimated bacteria would help stimulate more rapid remediation at contaminated sites.

CONCLUSIONS
It has been demonstrated that perchlorate can be removed to below detectable concentrations using HRC™ and lactate as growth substrates. When acclimated to perchlorate, cell growth rates were rapid with doubling times of approximately 9 hours. Maximum growth rates of bacteria on lactate ranged from 3 to 35 hours depending on culture conditions (mixed versus pure cultures). These studies suggest that HRC™ can be an effective method for stimulating in situ reduction of perchlorate.

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