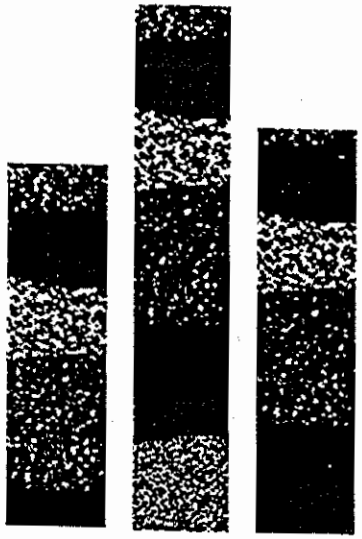
Bioremediation of Metals and Inorganic Compounds



Editors

Andrea Leeson
and Bruce C. Alleman
Battelle

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CHROMIUM ELIMINATION WITH MICROBIALLY REDUCED IRON – REDOX-REACTIVE BIOBARRIERS

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Frank Caccavo Jr. (University of New Hampshire, Durham, New Hampshire)

ABSTRACT: The research presented herein addresses the need to explore strategies for the cost-effective development of permeable reactive barriers in the deep subsurface. The metabolic capabilities of Dissimilatory Metal-Reducing Bacteria (DMRB) appear to have the potential to create zones of reduced indigenous metals (e.g. ferrous iron) in the path of a groundwater contaminant plume forming redox-reactive barriers. Microbially reduced iron can reductively dechlorinate organic pollutants, reduce nitroaromatics, and/or precipitate heavy metals and radionuclides from contaminated waters. Starvation of bacteria has shown to enhance bacterial transport and distribution through porous media, thus providing a possible means for the delivery of bacteria into the subsurface. To demonstrate the potential of starved bacteria for *in situ* metal-reduction, starved bacteria were resuscitated with surface associated iron minerals in batch and column experiments. In both batch and column studies, the microbially produced surface-associated Fe(II) reduced Cr(VI), a common groundwater contaminant, to Cr(III). Cr(III) precipitated on the existing surfaces, forming stable end products and thereby eliminating the chromium compounds from the water phase.

INTRODUCTION

The heavy metal contamination of groundwater can occur together with chlorinated solvents since metal plating facilities often use chlorinated solvents as degreasing agents before the plating process. Also, some U.S. Department of Energy sites are contaminated with complex mixtures of heavy metals, radionuclides, and chlorinated solvents. The remediation of these sites presents a challenge due to the mix of contaminants.

Zero-valent iron based redox-reactive subsurface barriers have been successfully employed for the elimination of heavy metals and chlorinated solvents from groundwaters (Cantrell et al., 1995; Fortner, 1995). However, the costs of establishing such a zero-valent iron based redox-reactive subsurface barrier by excavation and placement of zero-valent iron filings into the aquifer can be prohibitively high depending on the necessary depth of the barrier (Scott et al., 1998; Fortner, 1995).

Ferrous iron (Fe(II)), produced from indigenous ferric iron (Fe(III)), has shown to also mediate the reactions described above leading to the dechlorination of chlorinated solvents, and the precipitation of heavy metals and radionuclides from contaminated groundwater (Scott et al., 1998; Buerge and Hug, 1997; Gorby et al., 1994).

Recently, a technology involving the injection of a chemical reductant (dithionite) to reduce indigenous ferric iron producing redox-reactive ferrous iron was successfully demonstrated (Scott et al., 1998). The use of chemical reductants however, could become very expensive depending on the subsurface conditions. If other reducible compounds are present besides ferric iron (e.g. humics), the amount of chemical required to reduce the indigenous ferric iron could increase significantly without contributing to the desired contaminant reduction.

Therefore, we are currently evaluating the feasibility of using dissimilatory metal-reducing bacteria (DMRB) to selectively reduce indigenous metals, such as ferric iron. DMRB have been shown to facilitate degradation of chlorinated organics and the precipitation of heavy metals (Caccavo et al., 1996; Caccavo and Olson, 1995; Gorby et al., 1994). DMRB can directly reduce these contaminants enzymatically or alternatively DMRB can enzymatically generate ferrous iron from naturally occurring ferric iron minerals. The ferrous iron can in turn chemically dechlorinate chlorinated organics, and reductively immobilize metals and radionuclides (e.g. Cr^{6+}, U^{6+}).

The DMRB metabolism could be used to generate a redox-reactive biobarrier *in situ*. Such a barrier could be generated by stimulating indigenous DMRB populations or inoculating contaminated environments with pre-adapted or genetically engineered DMRB. The economic feasibility of bioaugmenting large volumes of soil *in situ* depends on the ability to transport DMRB over large distances in aquifers. Starvation has been shown to significantly enhance bacterial transport and distribution of bacteria through porous media (Sharp et al. 1999) and holds promise for a field scale application.

The objective of this work was to demonstrate that starved DMRB can be resuscitated and grown on surface associated iron and that the microbially produced Fe(II) provides sufficient reducing power for the reduction of Cr(VI) to Cr(III). The distribution of Cr-species within the experimental systems was also determined.

MATERIAL AND METHODS

Preparation of Starved Cells. Cells of *Shewanella alga* BrY were aerobically grown to the late exponential or early stationary phase. The cells were harvested by centrifugation, washed 3 times, and finally re-suspended in a phosphate buffered saline solution (PBS). The cells were starved by aseptically stirring on a magnetic stir plate at room temperature.

Fe-Coating of Quartz Sand. Quartz sand (40 mesh) was washed with 1 N HNO_3 and rinsed with deionized water. To 100 g of washed sand, 200 mL of a solution of amorphous Fe(III)-hydroxide was added. The amorphous iron solution was freshly prepared by dissolving $FeCl_3$ in deionized water (34.5 g/L) and slowly adjusting to pH 7 using NaOH. The Fe(III)-hydroxide-sand mixture was vigorously shaken and allowed to settle for several hours. The overlying water was then decanted and replaced with a 0.1 mM NaCl-solution and allowed to settle again overnight. The next morning, the Fe(III)-hydroxide-NaCl solution was discarded completely and replaced with freshly made Fe(III)-hydroxide solution. This process was repeated four times before the coated sand was allowed to air dry. The dried sand was sieved to break up large aggregates and remove fine material.

Batch Experiments. Vials containing 9 mL of anaerobic minimal medium (Caccavo et al., 1996) and 1 g of Fe(III)-coated sand were prepared. All anaerobic media and a lactate stock solution were boiled and cooled down under an oxygen free N_2 (80 vol %)-CO_2 (20 vol %) atmosphere, sealed gas tight and sterilized by autoclaving. Starved bacteria were injected into each vial (except for the bacteria free controls). The initial cell concentration was approximately 5×10^6 colony-forming units per mL (CFU/mL). All additions were made maintaining anoxic conditions using sterile syringes and needles; the vials were incubated statically in the dark. After a sufficient amount of Fe(II) had been produced, 0.1 mL of a K_2CrO_4 solution was injected into the vials to give a final concentration of approximately 5 mg/L as Cr(VI). Cr(VI) concentrations in the aqueous phase were monitored over time. K_2CrO_4 was also injected into autoclaved vials and control vials. At the end of the experiments, the vials were opened and analyzed for Cr(VI) and total chromium in the aqueous phase and on the sand.

Column Studies. Column studies utilizing PTFE-stainless steel assemblies were used to investigate the capability of starved BrY cells to reduce surface associated Fe(III) in a more field realistic setting. The columns were filled with approximately 7 g of Fe-coated sand and covered with aluminum foil to prevent photo-oxidation. Anaerobic minimal medium supplemented with 1 mM lactate was pumped through the columns once a day, ensuring the exchange of approximately 2 pore volumes in each column. Initially each column was inoculated with approximately 5×10^7 CFU of starved BrY cells. After Fe(II) had been produced in the columns, a K_2CrO_4 solution was continuously injected into the columns, and influent and effluent concentrations of Cr(VI) were monitored. At the end of the experiment, the columns were destructively sampled and the sand was analyzed for Cr(VI) and total Cr.

Analytical Techniques. The concentration of Fe(II) and total Fe were determined spectrophotometrically using ferrozine (Lovley and Phillips, 1987). The assay was slightly modified and utilized 2.5 N HCl or 0.25 NH_2OH-HCl in 2.25 N HCl for the extraction.

Cr(VI) and total chromium were determined using methods modified from Greenberg et al. (1992) (Cr(VI) aqueous, and total Cr in aqueous samples and from sand), and Vitale et al. (1994) (Cr(VI) from sand). Sym-diphenylcarbazide was utilized for the spectrophotometrical determination of chromium at 540 nm.

RESULTS AND DISCUSSION

Microbial Fe(III)-reduction. The resuscitation of starved *S. alga* BrY cells with artificially Fe(III)-coated sand is shown in Figure 1. After an initial lag phase, the

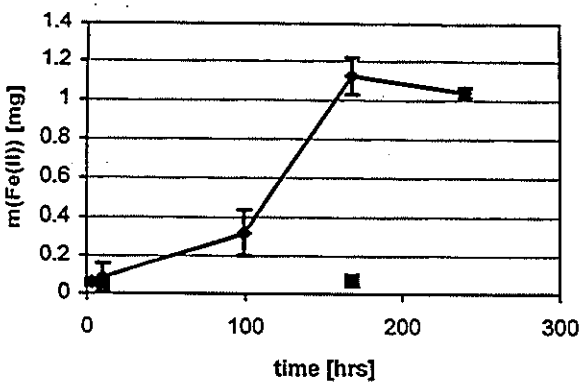


FIGURE 1. Resuscitation of starved *Shewanella alga* BrY cells on amorphous Fe(III)-oxyhydroxide coated sand. ◆ setups containing starved bacteria, ■ bacteria-free controls. The error bars represent the standard error of the mean.

injected cells of *S. alga* BrY reduced the surface associated Fe(III)-minerals, resulting in increasing Fe(II)-concentrations over time. The final mass of Fe(II) produced in batch - experiments (~1.1 mg Fe^{2+}) is equivalent to more than 80% of the total amount of Fe(III) added (~1.3 mg). Most of the Fe(II) produced remained associated with the sand surface as observed by Gorby et al. (1994). No increase in Fe(II)-concentrations was observed in bacteria-free controls or controls containing bacteria but no lactate, suggesting that the Fe(III)-reduction was due to microbial activity.

In columns inoculated with starved BrY cells and operated in semi-batch mode up to 80 % of the surface associated Fe(III) was reduced to Fe(II) after approximately 8 weeks and remained associated with the sand surface.

Cr(VI)-reduction by microbially reduced Fe(II). Batch vials containing microbially produced Fe(II), regardless of whether autoclaved (data not shown) or not, reduced the injected Cr(VI) rapidly while controls containing no or little Fe(II) (< 0.1 mg) did not show any detectable Cr(VI)-reduction (Figure 2). Even at the first sampling event, 30 seconds after injection of the K_2CrO_4-solution, the Cr(VI) concentrations in vials containing microbially reduced Fe(II) had decreased rapidly and continued to decrease over the duration of the experiment. The controls, containing no or little Fe(II), showed relatively constant Cr(VI)-levels throughout the experiment (up to 2 days). This indicates that the reduction of Cr(VI) is due to the presence of microbially reduced Fe(II).

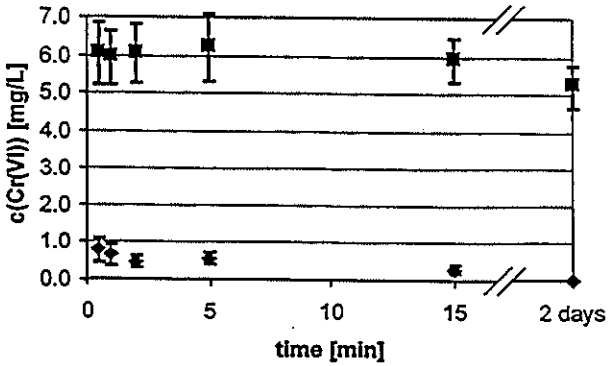


FIGURE 2. Aqueous phase Cr(VI) concentrations over time in vials containing microbially reduced Fe(II) (◆, n=6) and controls with no or little microbially reduced Fe(II) (< 0.1 mg) (■, n=2). The error bars represent the standard error of the mean.

The columns containing microbially reduced Fe(II) showed a noticeable delay in the breakthrough of injected Cr(VI) as compared to a non-retarded tracer (Figure 3). The amount of Cr(VI) reduced was calculated as the difference between the area of the breakthrough curve for Cr(VI) and the integral over the Cr(VI) injection-profile, divided by the flow rate. The amount of Cr(VI) reduced in the column was calculated to be approximately 35 % of the total capacity for Cr(VI)-reduction (assuming a 3:1 ratio for Fe(II):Cr(VI), Blowes et al., 1997). This difference may be due to blocking of potential reaction sites by Fe-Cr-precipitates or the reaction of reduced Fe(II) with other constituents of the injection fluid (e.g. residual oxygen). More than 95 % of the chromium recovered was recovered as Cr(III) from the column sand (data not shown).

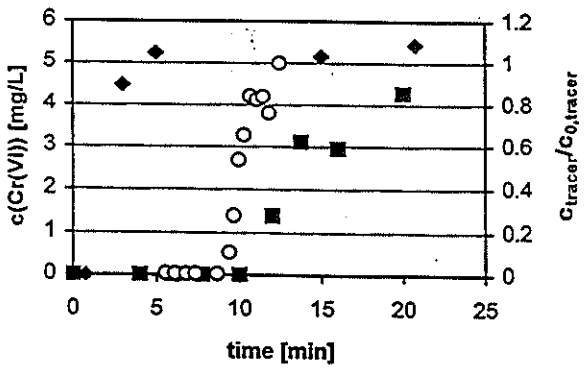


FIGURE 3. Cr(VI) concentrations in influent (◆) and effluent (■) of column containing microbially reduced Fe(II). Tracer breakthrough data (○) correspond to right y-axis.

CONCLUSIONS

Starved cells of *Shewanella alga* BrY can be resuscitated with artificially Fe(III)-coated sand as the electron acceptor, resulting in the production of surface associated Fe(II). The microbially produced Fe(II) almost instantaneously reduces the common groundwater contaminant Cr(VI) to Cr(III), which precipitates on the sand. The structure and stability of the precipitated chromium has been assessed by many authors (Blowes et al., 1997; Peterson et al., 1997). The precipitates have been described as $Cr(III)_xFe(III)_{1-x}$-(oxy)hydroxides. The Cr(III) ions appear to be stable under most conditions encountered in natural groundwater systems. These results suggest that metal-reducing bacteria can be used to reduce indigenous metals in or downstream of a contaminated aquifer to create a zone of redox-reactive Fe(II), which can in turn chemically reduce common groundwater contaminants such as heavy metals, radionuclides and chlorinated aliphatics. To further develop this technology, studies will be conducted that address the re-activation of depleted reactive iron zones. Column studies will be utilized to investigate whether this is possible and how long it will take to re-activate such zones by stimulating microbial Fe(III)-reduction.

The current results suggest that a permeable redox-reactive biobarrier technology based on the bioaugmentation with DMRB is possible.

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REMEDIATION OF A HEAVY METAL PLUME USING A REACTIVE WALL

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ABSTRACT: Historical storage of sulfide mineral ore concentrate at an industrial site in Western Canada has resulted in widespread contamination of underlying soil and groundwater. The oxidation of sulfide minerals has released significant quantities of heavy metals including Cd, Cu, Ni, Pb, and Zn into the groundwater. The impacted groundwater currently threatens a nearby marine ecosystem. In March 1997, a pilot-scale compost-based sulfate-reducing porous reactive wall was installed within a dissolved heavy metal plume at the site. The porous reactive wall uses sulfate-reducing bacteria to convert dissolved sulfate in the heavy metal plume to sulfide. As a consequence, heavy metals are precipitated out as insoluble metal sulfides. During the trenching phase of construction, biodegradable (guar gum) slurry was used to stabilize trench walls to allow for effective emplacement of the reactive media. The guar gum was observed to disappear from within the wall over a 3-4 week period following installation. Monitoring of the wall to date has shown significant decreases in heavy metal concentrations within and down-gradient of the wall suggesting that sulfate reduction and metal sulfide precipitation are effectively occurring. Dissolved copper concentrations have been observed to decrease from approximately 2200 µg/L to less than 5 µg/L following treatment within the wall. Cd, Ni, Pb, and Zn concentrations have shown similar decreases with treatment concentrations generally being observed below instrument detection limits.

INTRODUCTION

Soils and groundwater at an industrial site in Greater Vancouver, Canada are extensively contaminated with heavy metals including cadmium, copper, nickel, lead and zinc. The heavy metal contamination has resulted from the oxidation of sulfide mineral ore concentrate historically stored on site. Heavy metals and acidity in the groundwater currently pose a potential threat to a nearby marine ecosystem located immediately down-gradient of the site.

A permeable reactive pilot test wall was installed within the groundwater plume in late March 1997 to evaluate the potential application of reactive wall technology at the site. Permeable reactive treatment wall technology is an emerging technology which is being increasingly employed for treatment of contaminated groundwater (Blowes and Ptacek, 1992; Gillham and O'Hannesin, 1992; Robertson and Cherry, 1995; Powell et al., 1995; Baker et al., 1996; USEPA, 1998). The technology involves the placement of a permeable wall