FINAL REPORT
U.S. Department of Energy

REDUCTION AND IMMOBILIZATION OF RADIONUCLIDES AND TOXIC METAL IONS USING COMBINED ZERO VALENT IRON AND ANAEROBIC BACTERIA

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Project Number: 55071
Grant Number: DE-FG07-96ER62318
Grant Project Officers: Dr. Jay Grimes
Project Duration: 9/96 – 5/01
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3. **Executive Summary**

The use of zero valent iron, permeable reactive barriers (PRBs) for groundwater remediation continues to increase. An exciting variation of this technology involves introducing anaerobic bacteria into these barriers so that both biological and abiotic pollutant removal processes are functional. This work evaluated the hypothesis that a system combining a mixed culture of sulfate reducing bacteria (SRB) with zero valent iron would have a greater Cr(VI) removal efficiency and a greater total Cr(VI) removal capacity than a zero valent iron system without the microorganisms. Hence, the overall goal of this research was to compare the performance of these types of systems with regard to their Cr(VI) removal efficiency and total Cr(VI) removal capacity. Both batch and continuous flow reactor systems were evaluated.

In batch studies using Cr(VI)-unacclimated and Cr(VI)-acclimated cultures, and an initial concentration of 2.0 mM for both cultures, Cr(VI) was removed most rapidly in the treatments in the following order: Fe(0)+SRB+Lactate > Fe(0)+SRB > Fe(0) > SRB+Lactate.

Two continuous-flow column studies were conducted, a short-term study for a period of 58 days and a long-term study for a period of 360 days. In each study, columns were packed with 5 g of steel wool and selected columns were seeded with a SRB stock culture. The columns (2.25 cm dia. x 9.2 cm long) were fed via a syringe pump at a flow rate of 10 mL/d. The results clearly showed that seeding the columns with the SRB culture greatly improved their Cr(VI) removal performance.
In the 58-day study, the influent Cr(VI) concentration ranged from 380 μM to 19.2 mM. The ratio of the overall performance of the column pairs, based on total mass of Cr(VI) removed during the study, was: Fe(0)+SRB compared to Fe(0) (143%); Fe(0)+SRB+Lactate vs. Fe(0) (178%); and Fe(0)+SRB+Lactate vs. Fe(0)+SRB (123%).

In the 360-day study, the influent Cr(VI) concentration ranged from 190 μM to 3.08 mM. The performance of the columns in terms of the ratio of the total mass of Cr(VI) removed by each was as follows: Fe(0)+SRB vs. Fe(0) (207%); Fe(0)+SRB+Lactate vs. Fe(0) (575%); Fe(0)+SRB+Lactate vs. Fe(0)+SRB (278%).
4. Research Objectives

Because of poor waste management practices in industries that utilize chromium, chromium discharge into the environment has often occurred. Chromium contaminated soils and waters have been reported in several studies. Large groundwater plumes contaminated with toxic metal ions such as chromium exist at several Department of Energy (DOE) facilities. The presence of the chromium in ground water represents a serious health threat due to its known toxicity and mutagenicity (Ajmal et al., 1984).

Chromium exists primarily in two oxidation states in natural waters and soil: hexavalent chromium [Cr(VI)] and trivalent chromium [Cr(III)]. Markedly different chemical behavior, bioavailability and toxicity characterize these species. Chromium speciation and distribution in aqueous systems is strongly dependent on pE and pH (Calder, 1988; Bartlett and James, 1988). Cr(VI), which predominates under oxidizing (high pE) conditions, is typically present as an anion, either chromate (\(\text{CrO}_4^{2-}\)) at pH > 6.5 or bichromate (\(\text{HCrO}_4^-\)) at pH < 6.5. Cr(VI) is toxic and mutagenic to organisms, and is very soluble over a wide pH range in natural waters. There is evidence that all forms of Cr(VI), both water-soluble and water-insoluble compounds, are respiratory carcinogens in humans (Nieboer and Shaw, 1988; Yassi and Nieboer, 1988). In ground water, Cr(VI) movement has been found to be unretarded or only slightly retarded by adsorption to aquifer material. Adsorption of Cr(VI) occurs at low pH (Calder, 1988; Kent et al., 1995). Therefore the presence of hexavalent chromium in the environment, especially in groundwater, represents a serious health threat to the humans.
In contrast, Cr(III), which predominates under more reducing (low pE) conditions, is less soluble, and precipitates as oxides and hydroxides at pH values > 5. Cr(III) is also less toxic than Cr(VI). The predominant species of Cr(III) in the pH range 6.5 - 10.5 is Cr(OH)₃ (Rai et al., 1987). Cr(III) has strong tendency to adsorb to surfaces (Sass and Rai, 1987). The present limit for total dissolved Cr in drinking water is 1 μM (0.05 mg/L), requiring the treatment of some industrial waste solutions before discharge to the environment (U.S. EPA, 1976).

Increasing concern of the impact of chromium on the environment and on human health requires improved techniques for the treatment of Cr(VI)-contaminated soil and water. Techniques for the treatment of Cr(VI)-contaminated waters involve reduction of Cr(VI) to Cr(III), followed by adjustment of the solution pH to near-neutral conditions to precipitate Cr(III) ions. Benefits of this process are twofold: the toxicity of chromium is reduced, and the transport of this metal in ground water is lessened. Reduction of Cr(VI) to Cr(III) followed by Cr(III) precipitation is an efficient technique for the treatment of Cr(VI) contaminated waters and soils.

The transformation of chromium in groundwater depends on the redox state and pH of the water. It has been found that Cr(VI) reduction can provide an effective means for the remediation of Cr(VI)-contaminated waters and soils (Kent et al., 1994; Davis and Olson, 1995; James, 1996; Palmer and Puls, 1994). Recent studies have shown that Cr(VI) can be reduced to Cr(III) by reductants such as zero valent iron (Fe(0))(Blowes et al., 1997; Cantrell et al. 1995; Powell, et al., 1995; Gould, 1982); Fe(II) (Pettine et
Iron containing minerals such as magnetite (Fe$_2$O$_4$), pyrite (FeS$_2$) (Palmer and Puls, 1994; Blowes et al., 1997) and hematite (Fe$_2$O$_3$) (Eary and Rai, 1989), hydrogen sulfide (Pettine et al., 1994); organic reductants such as $\alpha$-hydroxyl carboxylic acids (Deng and Stone, 1996); organic matter (Jardine et al., 1999); humic and fulvic acids (Wittbrodt and Palmer, 1995, 1996), or by microorganisms (Fude et al., 1994; Sulzbacher et al., 1997; Wang and Shen, 1997; Wang and Shen, 1995; Lovley, 1993). Reduction of Cr (VI) to Cr(III) by sulfate-reducing bacteria (SRB) proceeds in two ways: indirect microbial reduction, by stimulating SRB to produce hydrogen sulfide which subsequently serves as the reductant (Smillie et al., 1981; Saleh et al., 1989); and direct, enzyme-mediated reduction (Lovley and Phillips, 1994).

The selection of Fe(0) and SRB for the reduction of Cr(VI) in this research was based on previous research indicating that both Fe(0) and SRB can be used to reduce Cr(VI) independently. Fe(0) has been reported to reduce chlorinated solvents (Charlet et al., 1998; Johnson et al., 1996; Matheson and Tratnyek, 1994) and uranium (Charlet et al., 1998; Cantrell et al., 1995). Fe(0) has been intensively used as a reactive medium in subsurface permeable reactive barriers (PRBs) to remediate groundwater plumes contaminated with reducible pollutants such as chlorinated solvents (Tratnyek et al., 1997) and Cr(VI) (Puls et al., 1996). In addition, it is known that SRB are able to grow on hydrogen, which is a byproduct of iron corrosion under anaerobic conditions (Rajagopal and Legall, 1989). Removal of hydrogen by SRB not only can increase the rate of
corrosion but also can enhance the reactivity of Fe(0), therefore increasing the abiotic Cr(VI) reduction by Fe(0). Since Cr(VI) is known to be toxic to microorganisms at certain levels, bacterial viability can be further enhanced by abiotic reduction of Cr(VI) by Fe(0). Using a combined Fe(0)-SRB system may synergistically combine abiotic and biological processes to maximize Cr(VI) reduction.

To date, no study has been conducted to evaluate chromium reduction by a combined abiotic/microbial (Fe(0)/SRB) system. Similar research by Weathers et al. (1997), however, established the beneficial effects of combining anaerobic, methanogenic microbial communities and Fe(0) for the degradation of chlorinated organic compounds. The hypothesis of this work was that a system combining a mixed culture of sulfate reducing bacteria with zero valent iron would have a greater Cr(VI) removal efficiency and a greater total Cr(VI) removal capacity than a zero valent iron system without the microorganisms. Hence, the overall goal of this research was to compare the performance of these types of systems with regard to their Cr(VI) removal efficiency and total Cr(VI) removal capacity.

The specific objectives of this research were to:

- Determine the rate of hydrogen production for various types of metallic iron, using acid-washed and unwashed iron.
- Examine the impact of Fe(0) on sulfate reduction and Cr(VI) reduction by SRB.
• Compare Cr(VI) reduction in batch systems containing Fe(0) only, SRB only, and Fe(0) combined with SRB.

• Examine the impact of solids (aquifer materials) on Cr(VI) reduction in batch systems containing Fe(0).

• Conduct long term column studies to compare the reduction of Cr(VI) in flow through systems containing Fe(0) only, or Fe(0) and SRB, amongst others.

• Following the long-term column studies, to gain insight into the behavior of the columns by

  (1) investigating the surface of the Fe(0) from the columns using electron microscopy and molecular microscopy techniques,

  (2) investigating the microbial population that colonized the columns using electron microscopy and most probable number analysis, and

  (3) comparing the ability of a Cr(VI)-unacclimated and Cr(VI)-acclimated culture (developed from a seed culture taken from a column containing microorganisms) to reduce Cr(VI).
5. Methods and Results

A. Methods

Hydrogen and methane in headspace were measured using a Gow-MAC Series 600 gas chromatograph (GC) with N₂ as the carrier gas at a flow rate of 30 ml/min. The column was operated at 65°C, the injection temperature was set at 250°C and the thermal conductivity detector (TCD) was set at 250°C. The standard curve was established by measuring a range of concentrations using methane and H₂ standards (Scott Specialty Gases). The sample injection volume was 100 μl. The minimum detection limit (MDL) for hydrogen was 0.93 μmole/bottle and the MDL for methane was 2.79 μmole/bottle.

Sulfate was measured using a DIONEX Model DX 500 ion chromatography (IC) equipped with a Dionex AS4A column and a AG4A guard column. The flow rate was 2 ml/min. This instrument used a regenerant solution of 0.018mM Na₂CO₃ and 0.017 mM NaHCO₃. The calibration range was routinely from 5 to 50 mg/L. All samples were pre-filtered using 0.2 μm-pore size syringe-tip filters. Since sulfate concentrations typically ranged from 0 to 1000 mg/L, most of samples above the calibration range were diluted to appropriate levels. The MDL for sulfate was 0.31 mg/L. Total dissolved sulfide (TDS) was measured on filtered samples using an iodometric method, Standard Method 4500-S²⁻ (APHA, 1992). Samples were filtered through 0.45μm-pore size syringe tip filters. The MDL for sulfide was 6.4 mg/L.

Lactate and acetate were measured with a Hewlett Packard Series 1050 high performance liquid chromatograph (HPLC) using a PRP X300 column (Hamilton) with a
flow rate of 5 ml/min. The injection volume was 100 µL. The mobile phase used for detection of organic acid was 0.04 N H₂SO₄. All samples were filtered using 0.2 µm syringe tip filter prior to measurement. The standard curve was established by measuring a range of known concentrations. The MDLs for lactate and acetate were 2.05 mg/L and 4.47 mg/L respectively.

Cr(VI) was measured using the colorimetric diphenylcarbazide method (Franson, 1992). Samples were filtered using 0.2 µm-pore size, PTFE, syringe tip filters. Standard curves were established by measuring a range of known Cr(VI) concentrations. The MDL was 1.35 mg/L.

B. Results

Two continuous-flow column studies were conducted, a short-term study for a period of 58 days and a long-term study for a period of 360 days. Tables 3.1 and 3.2 show the results from the short-term study. Of note, by the end of the study period on day 58, the instantaneous % removal had decreased to 20% in the Fe(0) only column, to 25% in the Fe(0)+SRB column, and to 90% in the Fe(0)+SRB+Lactate column. The cumulative % removal values at the end of the study were 57% for the Fe(0) only column, 80% for the Fe(0)+SRB column, and 97% for the Fe(0)+SRB+Lactate column. The ratio of the overall performance of the column pairs, based on total mass of Cr(VI) removed, was: Fe(0)+SRB compared to the Fe(0) column (143%); Fe(0)+SRB+Lactate compared to Fe(0) (178%); and Fe(0)+SRB+Lactate compared to Fe(0)+SRB (122%). The decreased performance of the Fe(0)+SRB+Lactate column coincided with an apparent decrease in
sulfate reduction activity and lactate oxidation activity; sulfate rose to greater than 6 mM and lactate to an average of about 2.5 mM by the end of the study. The effluent pH of all experimental columns was higher than the control pH. The pH increased to about 10 for the Fe(0), Fe(0)+SRB and Fe(0)+SRB+Lactate columns.
Table 3.1: Steady-state influent and effluent Cr(VI) concentrations in the short-term column study.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Days</th>
<th>Target Influent (mM)</th>
<th>Column</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control (mM)</td>
</tr>
<tr>
<td>I</td>
<td>0 - 14</td>
<td>0.36</td>
<td>0.35 ± 0.00</td>
</tr>
<tr>
<td>II</td>
<td>14 - 30</td>
<td>0.96</td>
<td>0.84 ± 0.04</td>
</tr>
<tr>
<td>III</td>
<td>30 - 46</td>
<td>9.6</td>
<td>8.93 ± 0.70</td>
</tr>
<tr>
<td>IV</td>
<td>46 - 58</td>
<td>19.2</td>
<td>18.80 ± 0.47</td>
</tr>
</tbody>
</table>

(-) Steady state was not reached.

Table 3.2: Average influent and effluent Cr(VI) concentrations in the short-term column study.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Days</th>
<th>Target Influent (mM)</th>
<th>Column</th>
</tr>
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<td>IV</td>
<td>46 - 58</td>
<td>19.2</td>
<td>18.80 ± 0.47</td>
</tr>
</tbody>
</table>

Control: mineral medium; Fe(0): steel wool and mineral medium; Fe(0)+SRB: steel wool seeded with SRB cell suspension; Fe(0)+SRB+Lactate: steel wool seeded with SRB cell suspension and fed with lactate.
The lactate concentration in the effluent from all steel wool columns and the glass bead column for the first 100 days of the long-term study was almost always nondetectable (not shown). Also, the acetate concentration in the effluent from all columns was mostly nondetectable after the first 40 days (not shown). Sulfate concentrations from the Fe(0)+SRB+Lactate column were lowest of all the columns, averaging 0.6 mM (Fig. 3.1). The concentration of sulfate in the effluent from the Fe(0)+SRB column was similar to that from the Fe(0)+SRB+Lactate column for the first 40 days, while the columns were operated identically. After this time, the sulfate concentration rose to an average value of 3.4 mM for the Fe(0)+SRB column. In the absence of lactate as an electron donor added in the influent, cathodic hydrogen most likely served as the electron donor to support the reduction of sulfate.

The effluent concentration of Cr(VI) from all treatment columns was nondetectable during Phases II, III and IV (day 100 to 186) of the long-term study, corresponding to influent concentrations of 0.19, 0.38 and 0.77 mM Cr(VI), respectively (Fig. 3.2). During Phase V, from day 186 until 216, the target influent Cr(VI) concentration was 1.54 mM. During this time, for all columns with steel wool, only the effluent concentration of Cr(VI) from columns Fe(0)+SRB and Fe(0)+SRB+Lactate were on all occasions nondetectable; the average Cr(VI) effluent concentration for column Fe(0) was 0.49 mM, and for column Fe(0)+Soil was 0.07 mM. The high Cr(VI) influent concentration impacted sulfate reduction and lactate oxidation in the biological columns: the average effluent sulfate concentration rose to an average concentration of 4.64 mM for column Fe(0)+SRB, and 3.17 mM for column Fe(0)+SRB+Lactate. The average effluent lactate concentration increased to 0.99 mM for column Fe(0)+SRB+Lactate. As for the glass bead column (SRB+Lactate), during this time the average Cr(VI) concentration was 0.20 mM, and the average effluent sulfate and lactate concentration rose to an average concentration of 4.86 mM and 0.55 mM respectively.
Figure 3.1: Sulfate breakthrough curves in the long-term column study. Phases represent influent Cr(VI) concentrations: 0 μM (I); 192 μM (II); 385 μM (III); 769 μM (IV); 1.54 mM (V); 3.08 mM (VI) Columns contained the following: Control: mineral medium; Fe(0): steel wool and mineral medium; Fe(0)+Soil: steel wool and Oak Ridge Soil; Fe(0)+SRB: steel wool seeded with SRB cell suspension; Fe(0)+SRB+Lactate: steel wool seeded with SRB cell suspension and fed with lactate.
Figure 3.2: Cr(VI) breakthrough curves in the long-term column study. Phases represent influent Cr(VI) concentrations: 192 μM (II); 385 μM (III); 769 μM (IV); 1.53 mM (V); 3.08 mM (VI). Columns contained the following: Control: mineral medium; Fe(0): steel wool and mineral medium; Fe(0)+Soil: steel wool and Oak Ridge Soil; Fe(0)+SRB: steel wool seeded with SRB cell suspension; Fe(0)+SRB+Lactate: steel wool seeded with SRB cell suspension and fed with lactate.
During Phase VI, from day 216 until 360, the target influent Cr(VI) concentration was 3.08 mM. During this time, for the steel wool columns, only for column Fe(0)+SRB+Lactate was the Cr(VI) concentration in the effluent always nondetectable. The average Cr(VI) concentration in the effluent was 2.98 mM from column Fe(0), 2.85 from column Fe(0)+Soil, and 2.39 from column Fe(0)+SRB. The instantaneous Cr(VI) removal efficiency for all columns other than the Fe(0)+SRB+Lactate column decreased to less than 10%. The cumulative Cr(VI) removal efficiencies for the columns at the end of the study were 17% for column Fe(0), 23% for Fe(0)+Soil, 36% for Fe(0)+SRB, 100% for Fe(0)+SRB+Lactate (Fig. 3.3). The performance of these columns in terms of the ratio of the total mass of Cr(VI) removed by each was as follows: Fe(0)+SRB vs. Fe(0) (207%); Fe(0)+SRB+Lactate vs. Fe(0) (575%); Fe(0)+SRB+Lactate vs. Fe(0)+SRB (278%) (Fig. 4.35). The high Cr(VI) influent concentration during Phase VI impacted sulfate reduction and lactate oxidation in the biological columns: the average effluent sulfate concentration rose to an average concentration of 5.79 mM for column Fe(0)+SRB, 4.19 mM and for column Fe(0)+SRB+Lactate. The average effluent lactate concentration increased to 2.99 mM for column Fe(0)+SRB+Lactate. Although the Cr(VI) removal efficiency was 100% for column Fe(0)+SRB+Lactate, the biological processes in this column appeared to be impacted by the high Cr(VI) concentration. In the glass bead column (SRB+Lactate), the average Cr(VI) concentration during this time was 2.88 mM. The instantaneous Cr(VI) removal efficiency in this column also decreased to less than 10 % and the cumulative Cr(VI) removal efficiency at the end of the study was 22%. The average effluent sulfate and lactate concentration rose to 5.95 mM and 6.17 mM respectively in this column.
Figure 3.3: Cumulative percent Cr(VI) removal versus mass Cr(VI) loaded in the long-term column study. Phases represent influent Cr(VI) concentrations: 192 μM (II); 385 μM (III); 769 μM (IV); 1.53 mM (V); 3.08 mM (VI).

Columns contained the following: Fe(0): steel wool and mineral medium; Fe(0)+Soil: steel wool and Oak Ridge Soil; Fe(0)+SRB: steel wool seeded with SRB cell suspension; Fe(0)+SRB+Lactate: steel wool seeded with SRB cell suspension and fed with lactate.
6. Relevance, Impact, and Technology Transfer

A. The scientific discoveries made in this research focus on the problem of management of Cr(VI). The results of this research significantly improved the understanding of the reduction and immobilization of Cr(VI) in environments containing Fe(0) and sulfate reducing bacteria.

B. The principal benefit to DOE of the new information generated is that the efficiency and longevity of zero valent iron permeable reactive barriers used to reduce and immobilize Cr(VI) can be significantly extended in the presence of sulfate reducing bacteria.

C. The information is immediately useable in the field.

D. This research shows that when zero valent iron reactive permeable barriers are emplaced in subsurface environments containing sulfate, the introduction of sulfate reducing bacteria improves the efficiency and extends the life of the barrier. The results can be used immediately.

E. Large scale trials would be the next reasonable step with this information.

F. The graduate students working on this project received training in analytical methods, sampling methods and data analysis.

G. This research has advanced our understanding of the impact of sulfate reducing bacteria in environments containing Cr(VI) and elemental iron. It has advanced our knowledge of benefits to be gained by SRB in these environments for the purpose of Cr(VI) reduction and immobilization.
H. Additional scientific hurdles include (1) the development of appropriate kinetic equations describing the reduction and immobilization of Cr(VI) in environments containing SRB and metallic iron, and (2) incorporating these kinetic equations into groundwater transport models.

I. No other government agencies or private enterprises have expressed interest in the project.

7. Project Productivity

Significant progress was made in determining the impact of SRB on Cr(VI) reduction and immobilization in environments containing iron metal. Similar work with radionuclides was not conducted because of time and personnel constraints, which was partly due to the lack of suitable additional research assistants for the project. Funding that was provided for the purchase of equipment required to conduct radionuclide research was returned to DOE.

8. Personnel Supported

The following professional personnel were supported by the research effort: Dr. Lenly J. Weathers (PI), Dr. Lynn E. Katz (co-PI), and Dr. Cynthia Henny (Ph.D. student). The following M.S. students provided assistance to Dr. Henny: John Merrifield, Anthony Naples, Andrew Reid, Charlene Graham and Rebecca Pollis. Ms. Therese Anderson
(Laboratory Manager) and Ms. Mary Burton (Administrative Assistant) were also compensated for the time spent assisting with the project.

9. Publications

- Henny C., L.J. Weathers, L.E. Katz, and J.D. MacRae. Cr(VI) reduction and immobilization under sulfate reducing conditions in the presence of iron metal. To be submitted. Included in Appendix A.


10. Interactions

- Henny C., L.J. Weathers, L.E. Katz, and J.D. MacRae. Abiotic and biotic Cr(VI) reduction in a laboratory-scale permeable reactive barrier. Presented at The Sixth International In Situ and On-Site Bioremediation Symposium, San Diego, CA, June 4-7, 2001.

Anaerobic Bacteria. Lenly J. Weathers, Lynn E. Katz, Cynthia Henny, Anthony Naples, Andrew Reid, and Rebecca Pollis.


11. Transitions

None known.

12. Patents

This work partly supported this patent application:

Application Serial No.: 60/044,810; 09/446,581

Title: Fe(0)-Based Bioremediation of Aquifers Contaminated with Mixed Wastes

13. Future Work

Future work will focus on methods to increase the longevity of reactive PRBs.

14. Literature Cited


APHA. (1992), Standard Methods for the Examination of Water and Wastewater. 18th ed.


15. Appendices

A. Henny C., L.J. Weathers, L.E. Katz, and J.D. MacRae. Abiotic and biotic Cr(VI)
reduction in a laboratory-scale permeable reactive barrier. In: A. L. Leeson, B.
Alleman, P. Alvarez, and V. Magar (eds.), Bioaugmentation, Biobarriers and
Biogeochemistry, Battelle Press, 6(8):139.

B. Henny C., L.J. Weathers, L.E. Katz, and J.D. MacRae. Cr(VI) reduction and
immobilization under sulfate reducing conditions in the presence of iron metal. To
be submitted.
ABSTRACT: The removal of Cr(VI) from the following column reactors was compared: (1) a column containing 5 g steel wool only (column Fe(0)), (2) a column containing 5 g steel wool which was seeded with a mixed culture of sulfate-reducing bacteria (SRB) and which was continuously supplied with 20 mM lactate (column Fe(0)+SRB+Lactate), (3) a column containing 5 g steel wool which was seeded with SRB but not supplied with lactate (column Fe(0)+SRB), and (4) a sterile control column packed with 5 mm-diameter glass beads. The influent sulfate concentration to all columns was 12.4 mM. Influent Cr(VI) concentrations of 380 and 960 μM, and 9.6 and 19.2 mM were investigated. By the end of the study on day 58, the instantaneous Cr(VI) removal efficiency had decreased to 20% in the Fe(0) only column, to 25% in the Fe(0)+SRB column, and to 90% in the Fe(0)+SRB+Lactate column. Based on the total mass of Cr(VI) removed during the study, the ratio of the overall performance of the column pairs was: Fe(0)+SRB compared to Fe(0): 143%; and Fe(0)+SRB+Lactate compared to Fe(0): 178%. Thus, seeding the columns with the SRB culture greatly improved their Cr(VI) removal performance.

INTRODUCTION

Chromium exists primarily in two oxidation states in natural waters and soil: hexavalent chromium [Cr(VI)] and trivalent chromium [Cr(III)]. Cr(VI), which predominates under oxidizing conditions, is typically present as an anion, either chromate ($CrO_4^{2-}$) at pH > 6.5 or bichromate ($HCrO_4^-$) at pH < 6.5. Cr(VI) is toxic and mutagenic to organisms, and is very soluble over a wide pH range in natural waters. In ground water, Cr(VI) movement has been found to be only slightly retarded by adsorption to aquifer material. Therefore the presence of hexavalent chromium in the environment, especially in groundwater, represents a serious health threat to humans. Cr(III), in contrast, predominates under more reducing conditions, is less soluble, and precipitates as oxides and hydroxides at pH values > 5. Cr(III) is also less toxic than Cr(VI). The predominant species of Cr(III) in the pH range 6.5 - 10.5 is Cr(OH)$_3$ (Rai et al., 1987). Cr(III) has a strong tendency to adsorb to surfaces (Sass and Rai, 1987).

Techniques for the treatment of Cr(VI)-contaminated waters involve reduction of Cr(VI) to Cr (III), followed by adjustment of the solution pH to near-neutral conditions to precipitate Cr(III) ions. Benefits of this process are twofold: the toxicity of chromium is reduced and the transport of this metal in ground water is lessened.
To date, no study has been conducted to evaluate chromium reduction by a system combining zero valent iron and sulfate reducing bacteria (SRB) although previous research has indicated that Cr(VI) is reduced by Fe(0) (Blowes et al., 1997; Gould, 1982; Powell et al., 1995) and SRB (Lovley and Phillips, 1994). Similar research by Weathers et al. (1997) has established the beneficial effects of combining anaerobic, methanogenic microbial communities and Fe(0) for the degradation of chlorinated organic compounds. The hypothesis of this work was that a system combining a mixed culture of sulfate reducing bacteria with zero valent iron would have a greater Cr(VI) removal efficiency and a greater total Cr(VI) removal capacity than a zero valent iron system without the microorganisms. Hence, the overall goal of this research was to compare the performance of these types of systems with regard to their Cr(VI) removal efficiency and total Cr(VI) removal capacity.

**MATERIALS AND METHODS**

A 58-day column experiment was conducted which compared the ability of the following column reactors to remove Cr(VI): (1) a column containing steel wool (SW) only, (2) a column containing SW which was inoculated with a lactate-enriched SRB stock culture and continuously supplied with lactate, (3) a column containing SW which was inoculated with the SRB stock culture but which was not supplied with lactate, and (4) a column packed with autoclaved, 5 mm-diameter glass beads which served as a control for the loss of Cr(VI) by sorption. The operation of the two columns containing SW and SRB promoted different modes of growth: the lactate-fed column promoted the growth of heterotrophic, autotrophic and, possibly, mixotrophic SRB. In contrast, the organic carbon-free environment of the lactate-starved column required the SRB to immediately switch to an autotrophic mode of growth in order to remain viable.

Experiments were conducted at 30°C using adjustable glass columns with an inside diameter of 2.25 cm and an adjusted length of 9.2 cm (ACE Glass). Three of the columns were packed with 5 g of superfine steel wool (Rhodes, Chicago, IL). Two of these columns were then seeded with sulfate reducing bacteria by injecting 40 mL of stock reactor cell suspension (200 mg/L volatile suspended solids) into each. The columns were maintained in a no-flow mode for 7 days to permit the bacteria to colonize the steel wool. Following this, influent was pumped into the columns through Teflon tubing from 50-mL glass, gas-tight syringes (Hamilton) with a syringe pump (Harvard Apparatus). The columns were fed in an upflow mode at a volumetric flow rate of 10 mL/d. The porosity of the steel-wool-packed columns was measured to be 0.90, resulting in a hydraulic residence time of 4 days. After six days, Cr(VI) was added to the feed solution at a concentration of 380 μM. The Cr(VI) concentration was increased to 960 μM after 14 days total, to 9.6 mM after 28 days total, and to 19.2 mM after 46 days total. Table 1 summarizes the Cr(VI) influent concentrations to the columns. Effluent samples were collected periodically for 58 days and analyzed for Cr(VI), sulfate and lactate. Cr(VI) was measured using the colorimetric diphenylcarbazide method (APHA, 1992). Sulfate was measured using an ion chromatograph. Lactate was measured by high performance liquid chromatography.
TABLE 1. Cr(VI) influent concentrations to the columns.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Time period (days)</th>
<th>Cr(VI) influent conc. (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0-6</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>6-14</td>
<td>0.38</td>
</tr>
<tr>
<td>II</td>
<td>14-28</td>
<td>0.96</td>
</tr>
<tr>
<td>III</td>
<td>28-46</td>
<td>9.6</td>
</tr>
<tr>
<td>IV</td>
<td>46-58</td>
<td>19.2</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

During phases I and II of the study, corresponding to influent Cr(VI) concentrations of 380 and 960 μM, respectively, Cr(VI) was not detected in the effluent from any of the treatment columns (Figure 1). Thus the instantaneous Cr(VI) removal efficiency was 100% for all columns.

During Phase I and II, the effluent sulfate concentration from columns Fe(0) and Fe(0)+SRB was about equal to that of the control, indicating the lack of sulfate reduction in these columns (Figure 2). In contrast, the sulfate concentration from the lactate-fed column during Phase II averaged about 38% of the control value, indicating that sulfate reduction was occurring in this column. Also, the lactate concentration in the effluent from this column was nondetectable on all but one sampling event during Phase II, even though the influent lactate concentration was about 20 mM (data not shown). Taken together, this data
indicated that SRB were active in the lactate-fed column, but not the Fe(0)+SRB column, during Phase II.

During Phase III, corresponding to an influent Cr(VI) concentration of 9.6 mM, Cr(VI) levels in the effluent from the Fe(0) column increased starting on the sixth day at this influent concentration (Figure 3). As a result, the instantaneous Cr(VI) removal efficiency fell to about 45% and the cumulative Cr(VI) removal efficiency fell to 80% for the Fe(0) column during this period. In contrast, Cr(VI) was not detected in the effluent from the other treatment columns (Figure 3). Figure 4 indicates that columns Fe(O)+SRB and Fe(O)+SRB+Lactate performed 125% better than the Fe(0) column by the end of Phase III.

Sulfate levels decreased in the Fe(O)+SRB+Lactate column, reaching non-detectable levels on day 42 of the study (Figure 2), while the lactate concentration in the effluent from this column remained less than 1 mM throughout this period and was non-detect during half of this time (data not shown). Hence, as in Phase II, the data indicated that the SRB were biologically active in the Fe(O)+SRB+Lactate column, but not in the Fe(O)+SRB column.

The better performance of the Fe(O)+SRB column compared to the Fe(O) only column might have been due to the added sulfide in this column. The column was seeded with cell suspension from the stock reactor that contained sulfide and FeS. The lack of measurable sulfate reduction in the Fe(O)+SRB column appeared to discount the involvement of SRB in the reduction of Cr(VI) in this column. Because lactate was not provided to column Fe(O)+SRB, the microorganisms
apparently did not have enough time to switch from heterotrophic to autotrophic growth using H₂ as electron donor and HCO₃⁻ as carbon source.

![Graph showing effluent Cr(VI) concentrations during Phases III and IV.]

**FIGURE 3. Effluent Cr(VI) concentrations during Phases III and IV.**

During Phase IV, corresponding to an influent Cr(VI) concentration of 19.2 mM, Cr(VI) levels in the effluent from the Fe(0)+SRB column increased starting on the second day at this influent concentration and on the eighth day in the effluent from the Fe(0)+SRB+Lactate column (Figure 3). Also, the Cr(VI) level in the Fe(0) only column reached a steady-state value of 14 mM during this time. As a result, by the end of the study period on day 58, the instantaneous Cr(VI) removal efficiency had decreased to 20% in the Fe(0) only column, to 25% in the Fe(0)+SRB column, and to 90% in the Fe(0)+SRB+Lactate column. The cumulative Cr(VI) removal efficiency values at the end of the study were 57% for the Fe(0) only column, 80% for the Fe(0)+SRB column, and 97% for the Fe(0)+SRB+Lactate column. The ratio of the overall performance of the column pairs, based on total mass of Cr(VI) removed, was: Fe(0)+SRB compared to the Fe(0) column: 143%; Fe(0)+SRB+Lactate compared to Fe(0): 178%; and Fe(0)+SRB+Lactate compared to Fe(0)+SRB: 122% (Figure 4), which indicated that seeding the columns with the SRB culture greatly improved their Cr(VI) removal performance.
In Situ and On-Site Bioremediation

FIGURE 4. Performance ratio comparison for different column pairs, based on the total mass of Cr(VI) removed by each column.

ACKNOWLEDGEMENTS
This project has been funded by the United States Department of Energy (DOE) as part of the Environmental Management Science Program. The contents do not necessarily reflect the views and policies of the USDOE.

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In Situ and On-Site Bioremediation


Cr(VI) Reduction and Immobilization under Sulfate Reducing Conditions in the Presence of Iron Metal

A Research Paper Submitted to

Water Research

by

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ABSTRACT

Cr(VI) can be reduced to Cr(III) by zero-valent iron and by sulfate reducing bacteria (SRB). In addition, select SRB are able to grow on hydrogen, a byproduct of iron corrosion in water. The hypothesis of this project was that a laboratory-scale, zero-valent iron, permeable reactive barrier seeded with a mixed culture of hydrogenotrophic SRB would have a greater total Cr(VI) removal capacity than a similar, unseeded system. This hypothesis was evaluated in a continuous-flow column study for 360 days using steel wool-packed columns. One column was seeded with a lactate-enriched SRB stock culture that demonstrated hydrogenotrophic ability. The influent Cr(VI) concentration ranged from 190 μM to 3.08 mM. At the end of the study, the chromium removed in the seeded column was 207% of that removed by the unseeded column. These findings could be significant for zero-valent iron PRBs designed to treat Cr(VI)-contaminated groundwater.

KEY WORDS: chromium, iron, sulfate reducing bacteria

SHORT TITLE: Cr(VI) Reduction by Fe(0) and SRB
INTRODUCTION

Chromium exists primarily in two oxidation states in natural waters and soil: hexavalent chromium [Cr(VI)] and trivalent chromium [Cr(III)]. Cr(VI) is toxic and mutagenic to organisms and is very soluble over a wide pH range in natural waters. In contrast, Cr(III) is less toxic than Cr(VI) and precipitates as oxides and hydroxides at pH values > 5. Large groundwater plumes contaminated with chromium exist at several U.S. Department of Energy (DOE) facilities. Cr(VI) reduction can provide an effective means for the remediation of Cr(VI)-contaminated water. Cr(VI) can be reduced to Cr(III) by chemical reductants such as iron metal (Blowes et al., 1997; Cantrell et al. 1995; Powell, et al., 1995; Gould, 1982); Fe(II) (Pettine et al.,1998; Buerge and Hug, 1997; Eary and Rai, 1988, 1989); iron containing minerals such as magnetite (Fe₂O₄), pyrite (FeS₂) (Palmer and Puls, 1994; Blowes et al., 1997) and hematite (Fe₂O₃) (Eary and Rai, 1989); hydrogen sulfide (Pettine et al., 1994); or by anaerobic bacteria (Schmieman et al., 2000; Fude et al., 1994; Sulzbacher et al., 1997; Wang and Shen, 1997; Wang and Shen, 1995; Lovley, 1993; Yamamoto et al., 1993). Research with sulfate reducing bacteria (SRB) has revealed that SRB reduce Cr(VI) by two mechanisms: directly, via enzyme-mediated reduction (Lovley and Phillips, 1994), and indirectly, by the production of hydrogen sulfide which subsequently serves as the reductant (Smillie et al., 1981; Saleh et al., 1989). Research has also shown that SRB are able to grow on hydrogen, which is a byproduct of iron corrosion under anaerobic conditions (Gu et al., 1999; Shokes and Moller, 1999; Rajagopal and Legall, 1989).

Groundwater remediation using Fe(0)-based permeable reactive barriers (PRBs) has recently become more common. Solid iron particles and steel wool have both been used as a PRB iron source. A promising modification of this technology involves seeding PRBs with anaerobic
bacteria so that pollutants are removed by abiotic processes as well as biological processes mediated by autotrophic, hydrogenotrophic anaerobes. Research investigating chromium reduction by combined Fe(0)/microbial systems is limited, although the beneficial effects of combining microbial communities and Fe(0) for the degradation of other pollutants has been demonstrated previously (Weathers et al., 1997; Till et al., 1998; Gregory et al., 2000; Wildman and Alvarez, 2001). The hypothesis of this work was that a system combining a mixed culture of SRB with Fe(0) would have a greater total Cr(VI) removal capacity than a purely abiotic Fe(0) system. Hence, the overall goal of this research was to compare the performance of these two systems with regard to their total Cr(VI) removal capacity.

MATERIALS AND METHODS

Experimental Design

The response of the following column reactors was compared: (1) a column containing steel wool only [designated column Fe(0)], (2) a column containing steel wool that was inoculated with an SRB stock culture and supplied with lactate for 44 days before it was discontinued [designated column Fe(0)+SRB], and (3) a column which served as a control for the loss of Cr(VI) by sorption.

Experiments were conducted at 30°C using adjustable glass columns with an inside diameter of 2.25 cm and an adjusted length of 9.2 cm (ACE Glass). Two of the columns were packed with 5 g of fine steel wool (Rhodes, Chicago, IL). The chemical composition of the steel wool, as reported by the manufacturer, was (in %): Fe (52), Si (30), C (16), Mn (1.25), P (0.7), and S (0.05). One steel wool column was then seeded with SRB by injecting 40 mL of stock
reactor cell suspension (185 mg VSS/L) into it. The column was maintained in a no-flow mode for 7 days to permit the bacteria to colonize the steel wool. Following this, influent was pumped into the columns through Teflon tubing from 50-mL glass, gas-tight syringes (Hamilton) with a syringe pump (Harvard Apparatus). The column was fed in an upflow mode at a volumetric flow rate of 10 mL/d. The porosity of the steel-wool-packed columns was measured to be 0.9, resulting in a hydraulic residence time of 3.3 days. The hydraulic residence time of the empty control column was 3.7 days.

The concentration of various constituents (in mg/L) of the medium provided to each column was as follows: MgSO₄ • 7H₂O (1502), NH₄Cl (400), MgCl₂ (400), KCl (400), CaCl₂ • 2H₂O (25), (NH₄)₂HPO₄ (80), (NaPO₃)₆ (10), CoCl₂ • 4H₂O (2.5), KI (2.5), MnSO₄ (2.5), NH₄VO₃ (0.5), ZnCl₂ (0.5), Na₂MoO₄ • 4H₂O (0.5), H₃BO₃ (0.5), NiCl₂ • 6H₂O (0.5), cysteine (10), NaHCO₃ (4200). After 100 days, Cr(VI) was added to the feed solution at a concentration of about 190 μM (9.9 mg/L). In general, a given influent Cr(VI) concentration was maintained until the effluent Cr(VI) concentration in all columns stabilized within 20%. The Cr(VI) influent concentration was then increased. The study was thus broken up into six phases, which were defined on the basis of the influent Cr(VI) concentration (Table 1). Effluent samples were collected throughout the study and analyzed for Cr(VI), sulfate and pH.

Stock Culture Reactor

A lactate-enriched, mixed culture was developed under sulfate-reducing conditions in a completely-mixed, flow (CMF) reactor as the primary inoculum source. The stock reactor was a magnetically-stirred, 20-L glass vessel containing 15 L of cell suspension. The culture was seeded using sludge from the anaerobic digester at the Ellsworth, Maine wastewater treatment plant. The reactor was maintained at 30°C with a 15-d hydraulic retention time by continuously pumping
Cr(VI)-free medium into the reactor at a flow rate of 1 L/d. The medium recipe was the same as that provided to the columns, except that it contained the following (in mg/L): FeCl₂•4H₂O (40), MgSO₄•7H₂O (6207), NaCH₃CHOHCOO (2206). Medium was autoclaved and purged of oxygen by bubbling argon gas through it prior to use.

**Analytical Methods**

Cr(VI) was measured using the colorimetric diphenylcarbazide method (APHA, 1987). The method detection limit (MDL) was 1.35 mg/L (26.0 μM). Sulfate was measured with a DIONEX Model DX 500 ion chromatograph (IC) equipped with a Dionex AS4A column and an AG4A guard column. The MDL for sulfate was 0.31 mg/L. Lactate and acetate were measured with a Hewlett Packard Series 1050 high performance liquid chromatograph (HPLC) using a PRP X300 column (Hamilton). The MDLs for lactate and acetate were 2.05 mg/L and 4.47 mg/L, respectively. Biomass was measured as volatile suspended solids (VSS) (APHA, 1987). The pH was measured using a Ross combination microelectrode (Orion Instruments) and pH meter.

**RESULTS AND DISCUSSION**

For the first 40 days after column Fe(0)+SRB was seeded, the feed to this column was prepared by adding pure lactate to the influent medium, a procedure which produced an influent lactate concentration that varied greatly from the target value of 8.0 mM (Fig. 1). After 40 days, lactate was no longer added to this column. The lactate concentration in the effluent from column Fe(0)+SRB for the first 50 days of the study was almost always nondetectable, indicating 100% removal of the added lactate (Fig. 1). Acetate, a lactate fermentation byproduct, was monitored for the first 50 days of the study. Acetate was generally nondetectable after 15 days (Fig. 1).
The influent target sulfate concentration for all columns was 6 mM. During the first 40 days, the sulfate concentration in column Fe(0)+SRB effluent was erratic, possibly due to the variability in the influent lactate concentration (Fig. 2). After lactate was no longer added to column Fe(0)+SRB, the effluent sulfate concentration rose to an average value of 3.4 mM. In the absence of lactate, cathodic hydrogen most likely served as the electron donor to support sulfate reduction, based on batch experiments with Fe(0) (data not shown; Henny, 2000). The concentration of sulfate from column Fe(0) was essentially equal to that of the control column (Fig. 2), indicating that sulfate was only reduced by biological processes.

The pH in the effluent from the columns containing Fe(0) was elevated compared to the control (Fig. 3), averaging about 8.6. An elevated pH in solutions containing Fe(0) results from the reduction of water-derived protons to molecular hydrogen:

\[ Fe(s) + 2H_2O \rightarrow Fe^{2+} + 2OH^- + H_2(g) \]  

During Phase II, the target influent concentration of Cr(VI) to all columns was 0.19 mM. During this time, the effluent concentration of Cr(VI) from both treatment columns was nondetectable (Fig. 4). Concurrently, the concentration of Cr(VI) in the effluent from the control column was 0.18 mM, indicating that the removal of Cr(VI) in the active columns was not due to sorption of Cr(VI) onto the columns. Fig. 5 presents the immediate removal efficiency for each column versus the total mass loaded where the total mass loaded is defined as

\[ m_{\text{loaded}} = \sum Q \times C_{\text{in}} \]  

where \( Q \) is the influent flowrate and \( C_{\text{in}} \) is the influent Cr(VI) concentration, and where the immediate removal efficiency is defined as

\[ \text{Immediate removal efficiency} = \left( \frac{C_{\text{in}} - C_{\text{out}}}{C_{\text{in}}} \right) \times 100\% \]
where $C_{\text{out}}$ is the effluent Cr(VI) concentration. The immediate Cr(VI) removal efficiency for this period was 100% for both columns. Fig. 5 also presents the total removal efficiency for each column versus the mass loaded. The total removal efficiency is defined as:

$$\text{Total removal efficiency} = \left( \frac{m_{\text{loaded}} - m_{\text{out}}}{m_{\text{loaded}}} \right) \times 100\%$$  \hspace{1cm} (4)

where $m_{\text{out}}$ was defined as the total mass of Cr(VI) leaving a column:

$$m_{\text{out}} = \sum Q \times C_{\text{out}}$$  \hspace{1cm} (5)

The total Cr(VI) removal efficiency was 100% for both active columns at the end of this phase.

The addition of Cr(VI) to the influent appeared to impact sulfate reduction in column Fe(0)+SRB, as the average effluent sulfate concentration increased 21% to 3.51 mM (Table 1).

During Phase III, the target influent Cr(VI) concentration was 0.38 mM. During this time, the effluent concentration of Cr(VI) from both treatment columns was nondetectable while the average concentration of Cr(VI) in the effluent from the control column was 0.36 mM (Fig. 4). The immediate Cr(VI) removal efficiency during this period was 100% for both columns, as was the total Cr(VI) removal efficiency at the end of this phase (Fig. 5). Cr(VI) at this influent concentration had minimal impact on sulfate reduction in column Fe(0)+SRB (Fig. 2, Table 1).

During Phase IV, the target influent Cr(VI) concentration was 0.77 mM. During this time, the effluent concentration of Cr(VI) from all treatment columns was nondetectable (Fig. 4), such that the Cr(VI) removal efficiencies continued to be 100% for both columns (Fig. 5). Cr(VI) at this influent concentration had minimal impact on sulfate reduction in column Fe(0)+SRB (Fig. 2, Table 1).

During Phase V, the target influent Cr(VI) concentration was 1.54 mM. During this time, the effluent concentrations of Cr(VI) from column Fe(0)+SRB were on all occasions
nondetectable while the average Cr(VI) effluent concentration for column Fe(0) was 0.49 mM (Fig. 4). The failure of column Fe(0) caused the immediate Cr(VI) removal efficiency for this column to decrease to less than 20% and the total Cr(VI) removal efficiency to fall to 89% (Fig. 5). The pH in column Fe(0) effluent also began to drop at this time, signaling a loss of reactivity of the iron (Fig. 3). The high Cr(VI) influent concentration also impacted sulfate reduction in the biological column, as the average effluent sulfate concentration rose 21% to an average value of 4.64 mM for column Fe(0)+SRB (Fig. 2, Table 1). Inhibition of sulfate reduction by high levels of Cr(VI) have been reported previously (Chirwa and Wang, 1997; Mazierski, 1994).

During Phase VI, from day 216 until 360, the average influent Cr(VI) concentration was 3.08 mM and the average concentration of Cr(VI) in the effluent from the control column was 3.03 mM. During this time, the average Cr(VI) concentration in the effluent from column Fe(0) was 2.98 mM, and 2.39 mM from column Fe(0)+SRB (Fig. 4). The immediate Cr(VI) removal efficiencies of columns Fe(0) and Fe(0)+SRB decreased to less than 10% (Fig. 5). The decrease in the reactivity of the columns is also apparent in the drop in the pH in the effluent from these columns (Fig. 3). The total Cr(VI) removal efficiencies for the columns at the end of the study were 17% for column Fe(0) and 36% for Fe(0)+SRB (Fig. 5). The high Cr(VI) influent concentration impacted sulfate reduction in the biological column, as the average effluent sulfate concentration rose to an average concentration of 5.79 mM for column Fe(0)+SRB, such that only 5% of the sulfate was reduced. The ratio of the total Cr(VI) mass removed by column Fe(0)+SRB to that removed by column Fe(0) as a function of the mass loaded is presented in Fig. 5. At the end of the study, column Fe(0)+SRB had removed over twice as much chromium (207%) as column Fe(0).
CONCLUSION

This study clearly illustrates the beneficial impact of seeding an iron metal, permeable reactive barrier with anaerobic microorganisms for the treatment of Cr(VI)-contaminated water. At the end of the study, the steel wool-packed column that was seeded with a mixed culture of sulfate reducing bacteria had removed over twice the chromium as the unseeded column. As a result, we have added to the growing body of research demonstrating the positive effects of seeding zero-valent iron PRBs with anaerobic bacteria for the removal of pollutants, including nitrate (Till et al., 1998), chlorinated organics (Weathers et al., 1997; Gregory et al., 2000) the nitramine explosive RDX (Wildman and Alvarez, 2001) and, with this study, hexavalent chromium.
ACKNOWLEDGMENTS

This work was funded by the U.S. Department of Energy through the Environmental Management Science Program (EMSP Project No. 55071).
FIGURE CAPTIONS

Fig. 1. Lactate and acetate in the influent and effluent of column Fe(0)+SRB.

Fig. 2. Sulfate in the effluent from the various columns. Phases represent influent Cr(VI)
concentrations: 0 mM (I); 0.19 mM (II); 0.38 mM (III); 0.77 mM (IV); 1.54 mM (V);
3.08 mM (VI). Columns contained the following: Control: empty; Fe(0): steel wool;
Fe(0)+SRB: steel wool seeded with lactate-enriched, SRB culture.

Fig. 3. pH of the effluent from the various columns. Phases represent influent Cr(VI)
concentrations: 0 mM (I); 0.19 mM (II); 0.38 mM (III); 0.77 mM (IV); 1.54 mM (V);
3.08 mM (VI). Columns contained the following: Control: empty; Fe(0): steel wool;
Fe(0)+SRB: steel wool seeded with lactate-enriched, SRB culture.

Fig. 4. Cr(VI) in the effluent from the various columns. Phases represent influent Cr(VI)
concentrations: 0 mM (I); 0.19 mM (II); 0.38 mM (III); 0.77 mM (IV); 1.54 mM (V);
3.08 mM (VI). Columns contained the following: Control: empty; Fe(0): steel wool;
Fe(0)+SRB: steel wool seeded with lactate-enriched, SRB culture.

Fig. 5. Immediate and total Cr(VI) removal efficiencies in the various columns; and the
performance ratio for the mass of Cr(VI) removed by column Fe(0)+SRB to that
removed by column Fe(0). Phases represent influent Cr(VI) concentrations: 0 mM (I);
0.19 mM (II); 0.38 mM (III); 0.77 mM (IV); 1.54 mM (V); 3.08 mM (VI). Columns
contained the following: Control: empty; Fe(0): steel wool; Fe(0)+SRB: steel wool
seeded with lactate-enriched, SRB culture.
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