

LONG-TERM STABILITY OF METALS IMMOBILIZED BY MICROBIAL REDUCTION

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ABSTRACT

Recent research has demonstrated the ability of some types of anaerobic organisms to reduce a variety of metal and metalloid contaminants, resulting in subsequent removal of the contaminants from solution. This paper describes an investigation to determine the stability of these immobilized phases with respect to the RCRA toxicity characteristics leaching procedure (TCLP), and to consider their long-term stability under conditions that might be found in an aquifer system. The metals investigated were arsenic (As), chromium (Cr), and selenium (Se). They were immobilized in columns containing silica sand by a sulfate-reducing bacteria (*Desulfovibrio desulfuricans*) and by an iron-reducing bacteria (*Shewanella putrefaciens*). The sand containing the biomass and immobilized metals was subjected to leaching via the TCLP test, which involves aggressive mixing, and to leaching from undisturbed columns with simulated groundwater. The results of the TCLP leach procedure showed that soil containing these metals, which have been immobilized in place, probably will not constitute a hazardous waste; however, the concentrations in the leachate was close to RCRA standards. Leaching of the metals with aerated, simulated groundwater from undisturbed columns released some metals over a period of three weeks; however, most of the metals that were precipitated from solution remained on the sand media.

Key words: biological metal reduction, arsenic, selenium, chromium

INTRODUCTION

The presence of metals, metalloids, and inorganic radionuclides in soil and groundwater at contaminated sites presents special challenges to environmental managers because options for remediation are very limited. For example, a review of groundwater cleanup alternatives published by the National Research Council presented 13 case studies, yet none of them dealt with inorganic pollutants (NRC, 1994). Because these contaminants are not degradable, remediation alternatives are primarily limited to excavation of contaminated soil and pump-and-treat processes for contaminated groundwater.

Inorganic pollutants, which are present as anions in soil and groundwater systems, are a particular problem as most soil minerals have negative surface charge near neutral pH (Langmuir, 1997). Therefore, electrostatic

repulsion will limit sorption processes. This in turn results in high mobility for anionic contaminants, which exacerbates the problems associated with their presence in both saturated and unsaturated subsurface environments. Contaminants, which may be present as anionic species in soil and groundwater, include arsenic (As), chromium (Cr), molybdenum (Mo), selenium (Se), technetium (Tc), vanadium (V), and uranium (U). A common characteristic of each of these constituents is that they form stable anionic species under oxidizing conditions, but will form insoluble precipitates under reducing conditions. Table 1 provides a summary of the stable species expected under oxidizing and reducing conditions for these elements.

Reference to Table 1 reveals that while these elements are likely to be present as soluble anionic species under oxidizing condi-

tions, they each form insoluble precipitates under reducing conditions. Accordingly, much of the work on biological remediation of inorganic contaminants has focused on immobilization of metals through microbially mediated reduction. In the past two decades there has been a large number of studies which have demonstrated microbial reduction of As (Ahmann et al., 1994; Macy et al., 1996), Cr

(Fude et al., 1994; Shen and Wang, 1994; Lovley and Phillips, 1994), Mo (Tucker et al., 1997), Se (Oremland et al., 1989; Tomei et al., 1992; and Tomei et al., 1995), and U (Lovley et al., 1991; Lovley and Phillips, 1992a, 1992b). Thorough reviews of the literature on microbial reduction of metals have been published by Lovley, (1993a, 1993b). One potential application of this phenomenon would

Table 1. Representative species of common elements which form anions under oxidizing conditions (adapted from Brookins, 1988).

Metal	Oxidation State	Important Soluble Species	Representative Insoluble Forms
As	-II		As ₂ S _{3(s)} , AsS _(s)
	0		FeAsS _(s) , As _(s)
	III	H ₃ AsO ₃ , H ₂ AsO ₃ ⁻ , HAsO ₃ ²⁻ , AsO ₃ ³⁻	As ₂ O _{3(s)}
	V	H ₃ AsO ₄ , H ₂ AsO ₄ ⁻ , HAsO ₄ ²⁻ , AsO ₄ ³⁻	
Cr	III		Cr ₂ O _{3(s)}
	VI	H ₂ CrO ₄ , HCrO ₄ ⁻ , CrO ₄ ²⁻	
Mo	IV		MoS _{2(s)} , MoO _{2(s)}
	VI	H ₂ MoO ₄ , HMoO ₄ ⁻ , MoO ₄ ²⁻	MoO _{3(s)}
Se	-II	H ₂ Se, HSe ⁻ , Se ²⁻	
	0		Se _(s)
	IV	H ₂ SeO ₃ , HSeO ₃ ⁻ , SeO ₃ ²⁻	SeO _{2(s)}
	VI	H ₂ SeO ₄ , HSeO ₄ ⁻ , SeO ₄ ²⁻	
Tc	IV		TcO ₂ , 2H ₂ O _(s)
	VI	TcO ₄ ⁼	
U	IV		UO _{2(s)} , USiO _{4(s)}
	VI	UO ₂ ²⁺ , UO ₂ (CO ₃) ⁰ , UO ₂ (CO ₃) ₂ ²⁻ , UO ₂ (CO ₃) ₃ ⁴⁻	
V	II		VO _(s) , V ₂ O _{4(s)}
	V	VO ₂ ⁺ , HVO ₃ , VO ₃ ⁻ , H ₃ VO ₄ , H ₂ VO ₄ ⁻ , HVO ₄ ²⁻ , VO ₄ ³⁻	V ₂ O _{5(s)}

involve the use of a permeable reactive barrier in which reduction and precipitation of the contaminants occur in a reducing zone down gradient of the contaminant plume, created by stimulating growth of anaerobic bacteria (Thomson et al., 1991).

One of the most significant shortcomings of permeable barrier technology for inorganic pollutants is that potential users of this technology are unable to answer the question, "What is the ultimate fate of the contaminants that are immobilized in the barrier?" To date there has been almost no consideration given to the long-term stability (i.e., time scale of decades or centuries) of these immobilized contaminants. Previous work has focused on achieving immobilization of the contaminants, with the implicit assumption that water quality standards would be met as a result of the low solubility of the reduced phases. Potential re-mobilization of the contaminants through complexation or oxidation has not been evaluated through use of either extended period experimental testing or through a comprehensive theoretical evaluation which considers all potential release mechanisms.

The objective of this research project was to evaluate the long-term stability of metal contaminants immobilized by microbial reduction. The contaminants considered were As, Cr, and Se, as these are representative of pollutants found at many industrial and mining sites as well as at U.S. Department of Energy installations. This paper describes the results of a laboratory investigation in which these metals were immobilized on an inert substrate (sand) by a sulfate-reducing bacteria and a metal-

reducing bacteria. The stability of the immobilized contaminants was then investigated through a series of leaching tests.

METHODS AND MATERIALS

The research was conducted in two phases. The first phase consisted of microbial reduction in column studies where the objective was to generate quantities of immobilized contaminants through microbial reduction. The second phase of the research used these solids in a series of leaching experiments to evaluate their stability.

Experimental System

Microbial reduction was conducted in plastic columns filled with acid-washed silica sand. The coarse sand (10 - 20 mesh size) was washed with 10% HNO₃, then rinsed with deionized water. Two different types of columns were used. Large columns (10 cm diameter by 1.0 m length) constructed of clear PVC were used to generate kg quantities of sand with immobilized metals on it. They were operated for approximately six months, after which the contents, consisting of sand, microbial culture, and associated precipitates were removed for leach testing. A second series of columns was also run which consisted of a number of small polycarbonate columns (1 cm diameter by 10 cm length) also filled with sand. Anaerobic microbial cultures were grown in these columns and contaminants allowed to precipitate on the sand. They were then subjected to leaching tests without removing the solid media to determine stability of the solids in the absence of physical disturbance.

The media fed to the columns was a simulated groundwater at pH 7.5 and is listed in Table 2. Contaminants were added to the solution at 5 mg/L as As(V) ($\text{Na}_2\text{AsO}_4 \cdot 7\text{H}_2\text{O}$), Cr(VI) (KCrO_4), and Se(VI) (Na_2SeO_4). All media were prepared using reagent-grade salts.

Total metal concentrations were measured by graphite furnace atomic absorption (GFAA) spectroscopy. Anions were measured by ion chromatography. Sulfide was determined colorimetrically by the methylene blue method.

Pairs of both the large- and small-diameter columns were inoculated with two different types of anaerobic bacteria. One set of columns was inoculated with a sulfate-reducing bacteria (SRB), *Desulfovibrio desulfuricans* (strain Dd 642), while the other was inoculated with an iron-reducing bacteria (IRB), *Shewenella putrefaciens* (strain CN32). Ten mM of lactate was added to the simulated groundwater to serve as the electron donor for microbial metabolism. Ten mM of Fe(III) was added to the feed of *S. putrefaciens* columns to serve as an electron acceptor.

Approximately 100 pore volumes of feed solution were passed through the large-diameter

SRB columns, and approximately 60 pore volumes were passed through the IRB columns. At that point, feeding of the columns was discontinued, and leaching studies were conducted on the solid media. The small-diameter columns were operated as shown in Table 3.

Leaching Studies

Several different leaching procedures were used to measure the stability of contaminants immobilized by microbial reduction. These tests include 1) determination of total metals on sand media by acid leach, 2) deionized water (DI water) leach from disturbed samples, 3) toxicity characteristics leaching procedure (TCLP) leach test from disturbed samples, and 4) oxidized groundwater leaching from undisturbed samples. The distinction between disturbed and undisturbed samples refers to their origin; loose samples of media from the large columns were collected and leached in vials with varying degrees of mixing, whereas the sand media in the small columns containing the biomass and immobilized metals were leached in place with no physical disturbance whatsoever.

Total metals were determined by leaching samples with concentrated 10% HNO_3 . The

Table 2. Composition of simulated groundwater fed to columns.

Constituent	Conc. (mg/L)	Conc. (meq/L)	Constituent	Conc. (mg/L)	Conc. (meq/L)
Na	566.	24.6	Cl	60.	1.7
K	78.	2.0	SO_4	1000.	20.8
Ca	20.	1.0	NO_3	30.	.5
Mg	2.5	.2	HCO_3	300.	4.9

acid was then analyzed by GFAA spectroscopy. The DI water leach involved placing 5.3 g of wet sand containing the biomass and immobilized metals in a sealed centrifuge tube. Thirty mL of DI water was added to the tubes and gently mixed by hand for 30 seconds every four hours for the first 12 hours, then mixed again at 24 hours. Eight mL samples were collected periodically during the leach test, passed through a 0.45 μm filter, and preserved with 1% HNO_3 .

The TCLP test procedure (Method 1311, Appendix II, 40CFR Part 261) involved use of an acidic leach solution prepared by adding 5.7 mL of glacial acetic acid and 64.3 mL of 1N NaOH to 1 L of DI water. This solution has a pH of 4.9. A slurry was prepared consisting of 10g of sand media and 200 g of leach solution. This slurry was then mixed for 18 ± 2 hours at 30 rpm. The leachate was filtered and analyzed for contaminants by GFAA spectroscopy.

The undisturbed sand media in the small columns was simply leached by passing simulated groundwater without the metals through the columns and measuring the contaminant concentrations in the leachate. This was intended to simulate passage of uncontaminated groundwater through the contaminants immobilized in place in a subsurface environment. The leachate was kept in tightly capped collapsible containers and therefore was not oxygenated.

RESULTS

Immobilization by Microbial Reduction

Figures 1 and 2 plot the effluent concentrations from the large-diameter columns and summarize the removal of the contaminants by *D. desulfuricans* and *S. putrefaciens*, respectively. Recall that the influent concentrations of each metal (As, Cr, and Se) were 5 mg/L and that the columns were operated at a flow rate of approximately 1 pore volume/day. In the *D. desulfuricans* column, consistent removal was achieved for As and Cr, with erratic removal of Se. The *S. putrefaciens* column demonstrated excellent removal of Cr up through 30-pore volumes of feed solution, when its performance suddenly decreased. This is believed to be due to stress on the culture which resulted from a leak that allowed most of the water to drain from the column one weekend. Since the focus of this project was on the stability of the immobilized metals, the causes of reduced performance were not thoroughly investigated. The total mass of metals immobilized on the sand was 42.7 mg/kg, 6.4 mg/kg, and 4.1 mg/kg for Cr, As, and Se, respectively. The total concentration of metals immobilized on the sand containing the *S. putrefaciens* culture was 34.4 mg/kg for Cr and 4.3 mg/kg for Se.

Table 3. Summary of small-column immobilization procedures.

Column ID	Organism	Contaminants in Feed	No. of Pore Vols.of Feed
A1, A2, A3	<i>D. desulfuricans</i>	As	68
C2, C3	<i>D. desulfuricans</i>	As, Cr, Se	56

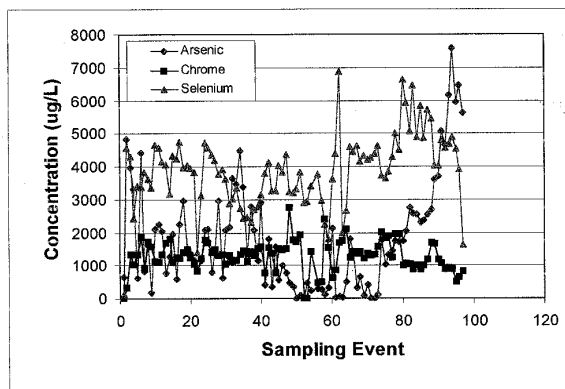


Figure 1. Effluent concentrations from 10-m diameter column inoculated with *Desulfovibrio desulfuricans*.

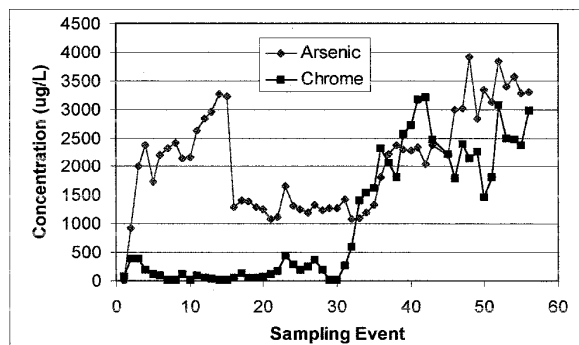


Figure 2. Effluent concentrations from 10-m diameter column inoculated with *Shewanella putrefaciens*.

Leach Tests

The TCLP was developed to define the toxic characteristics of a waste under RCRA. Samples of the sand media containing the microbial culture and the precipitated pollutants were taken from the large-diameter columns and subjected to leaching by a weak acetic acid solution (Method 1311, Appendix II, 40CFR Part 261). In order to pass the TCLP test, the leachate from the extraction must contain less than 5.0 mg/L for As and Cr, and 1.0 mg/L for Se. The TCLP results are summarized in Table 4. These results show that the immobilized contaminants will probably pass the TCLP criteria and therefore do not possess hazardous waste characteristics as defined by RCRA.

However, the values reported in Table 4 do not give much of a margin of safety as they are all close to the maximum concentrations established by RCRA regulations.

Kinetic leaching rates were measured using DI water and the TCLP acetic acid solution. Samples of the sand media containing the microbial culture and the immobilized pollutants were taken from the large-diameter columns and subjected to sequential leaching with samples being collected periodically throughout a 24-hour period. The results are shown in Figure 3. These results show a slow release of As from the sand, but there is no significant difference in the release rates for deionized water and acetic acid.

Table 4. Concentration of contaminants immobilized *S. desulfuricans* and by *S. putrefaciens* in leachate from TCLP test (mg/L).

Contaminant	<i>D. desulfuricans</i> Col.	<i>S. Putrefaciens</i> Col.	RCRA Standard
As	2.06 mg/L	-	5.0 mg/L
Cr	0.94 mg/L	5.04 mg/L	5.0 mg/L
Se	1.03 mg/L	0.87 mg/L	1.0 mg/L

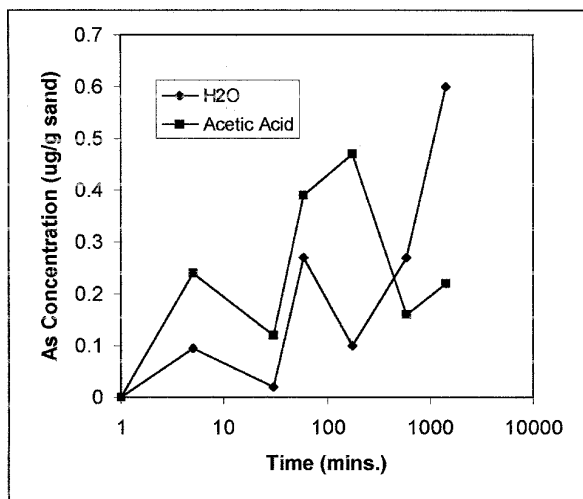


Figure 3. Results of leach test in the presence of mixing using deionized water and acetic acid at pH 4.9.

Small Column Leaching Studies

There are two important limitations associated with the TCLP test: 1) the procedure is a quick test (18 hours), and 2) it involves a high degree of agitation which will destroy the delicate structure of biomass attached to the sand media. In a potential field application of microbial immobilization, the principal environmental scenario which might cause release of the contaminants would consist of long-term leaching of contaminants under quiescent conditions over a very long time period of months or years. This is being simulated in the laboratory by using small columns (1 cm diameter by 20 cm long) in which metals are immobilized by *D. desulfuricans*, as was done for the large columns. Approximately 100 pore volumes of simulated groundwater containing 5 mg/L of As, Cr, and Se were passed through the columns and the metals were reduced and precipitated onto the sand by the anaerobic bacteria. As with the large columns, the feed procedure involved passing one pore volume through the

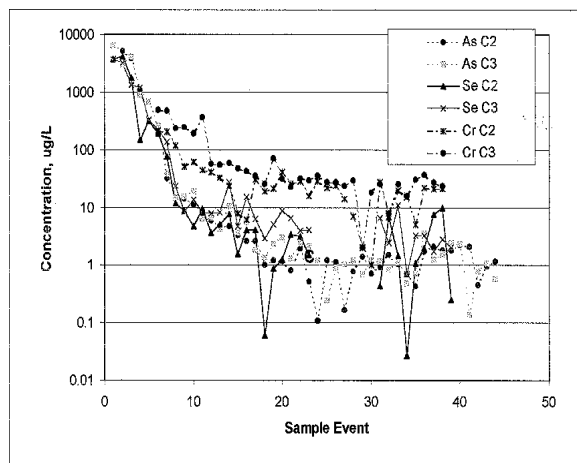


Figure 4. Sequential leaching of 1-cm diameter columns containing metals immobilized by *D. desulfuricans* by aerated, simulated groundwater.

column over a period of 30 minutes each day to rapidly replace the solution in each column. The columns were then subjected to sequential leaching with aerated, simulated groundwater containing no metals at a rate of approximately two pore volumes per day. In contrast to the TCLP extraction, this leaching was accomplished without disturbing the samples. The results for the *D. desulfuricans* columns are shown in Figure 4.

The leaching tests show an asymptotic decrease in the concentration of the three contaminants, which is believed to be primarily due to washing of the unprecipitated metals in the pore fluid from the column; recall that the initial metals concentration in the feed solution was 5 mg/L. It is interesting to note that once the organic substrate was removed from the feed solution, it appears that microbial reduction and precipitation of the metals in the pore fluid immediately stopped. This is evidenced by the fact that the metals concentrations continued to

decline for over 30 pore volumes of leaching. If residual microbial reduction was occurring, the metals concentration in the leachate would be expected to drop to near detection limits more quickly.

At the conclusion of the small-column leach tests, the total residuals metals concentrations were measured by extraction with concentrated HNO₃, followed by measurement with GFAA. The residuals metals concentrations on the sand after the water leach was completed were 4.0, 77.7, and 1.2 mg/kg for As, Cr, and Se, respectively. The fact that high concentrations of contaminants remained on the sand media after roughly 40 pore volumes of leachate suggests there may be two forms of immobilized contaminants: 1) a slightly soluble phase which is slowly released with successive rinsing, and 2) a more stable phase which remains insoluble over the duration of the experiments. More work is needed to determine whether this hypothesis is correct.

CONCLUSIONS

This investigation demonstrated simultaneous microbial reduction and subsequent precipitation of As, Cr, and Se by pure cultures of sulfate-reducing bacteria (*Desulfovibrio desulfuricans*) and by an iron-reducing bacteria (*Shewanella putrefaciens*) on silica sand. These results are consistent with those found by other investigators; however, there are few reports dealing with simultaneous reduction and precipitation of multiple metals (Tucker et al., 1998). The sand containing the biomass and precipitated metals was subjected to a variety of

leaching tests. These tests showed that the metals are relatively stable and that the stabilized mixture will likely pass a TCLP test and therefore be classified as a non-hazardous material.

Long-term leaching studies were conducted using deionized water and aerated, simulated groundwater. These tests were done to simulate conditions that might occur in a subsurface environment where contaminants had been stabilized in place by microbial reduction. These leaching studies were performed without agitation to limit the effects that physical mixing and abrasion might have on the release of metals. Some metals were initially released from the media, yet most of the precipitated contaminants remained stabilized in the mixture of sand and biomass. This suggests that the metals associated with the biomass may be present in two forms. Initially the pollutants were weakly bound to the biomass in a relatively unstable form. They would either be adsorbed or precipitated as fairly soluble phases. With time, it is postulated that the contaminants undergo a chemical transformation to a more stable and less soluble phase, and that this material is more resistant to leaching. Work is continuing to determine if this mechanism is in fact occurring.

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