STABILITY OF ARSENIC AND SELENIUM IMMOBILIZED BY *IN SITU* MICROBIAL REDUCTION

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ABSTRACT

A promising *in situ* remediation alternative for metal oxyanions such as arsenic, chromium, and selenium is microbial reduction to insoluble oxide, sulfide, or elemental phases. An important question that must be addressed before this technology can be implemented is the stability of these phases. This paper describes an investigation of the stability of reduced arsenic and sulfide precipitates produced by *Desulfovibrio desulfuricans*. The precipitates were first leached by the toxicity characteristic leaching procedure (TCLP), which demonstrated that the metal concentrations in the leachate are below the standards established by RCRA. A series of long-term leaching tests showed moderate release of the immobilized metals over a period of approximately 100 pore volumes, followed by very low leachability for at least 200 additional pore volumes. These results suggest that, although the metals may be slightly leachable, in an *in situ* immobilization process, their concentration would be below federal drinking water standards.

Key words: arsenic, sulfide, TCLP, leachate

INTRODUCTION

Current alternatives for remediating contamination of soils and groundwater by metals, metalloids, and radionuclides are limited and thus present a major challenge to environmental managers. There is much current interest in *in situ* stabilization technologies involving the use of microbial systems to immobilize contaminants, which are present as anions under oxidizing conditions as these are often the most subject to transport through soil and groundwater. These contaminants include metals such as Cr, Mo, and V; the metalloids As and Se; and many radionuclides including Tc, U, Pu, and Am. Much of the work on biological immobilization strategies has focused on the use of microbially mediated reduction in which anaerobic organisms reduce the metals to insoluble precipitates including oxides (e.g. Cr_2O_3, UO_2), sulfides (e.g. FeS, MnS, FeAsS, AsS_2, MoS_2),

and possibly elemental forms (Se). These metals are expected to remain insoluble as long as reducing conditions are maintained in the subsurface formation. However, to date no investigation has considered the stability of these phases over long time periods (i.e., decades to centuries) as geochemical conditions in the formation and groundwater change.

Microbial reduction of metals has been demonstrated for a wide variety of metals and metalloids. Important reviews on this topic have been provided by Lovley and Coates (1997), Chen and Hao (1998), and Nealson and Stahl (1997). Reduction has been demonstrated both for sulfate-reducing bacteria (SRB) (Lovley, 1993a, 1994; El Bayoumy et al., 1999; Tucker et al., 1998) and for dissimilatory metal-reducing bacteria (DMRB) (Frederickson et al., 2000; Lovley 1993a, 1995).
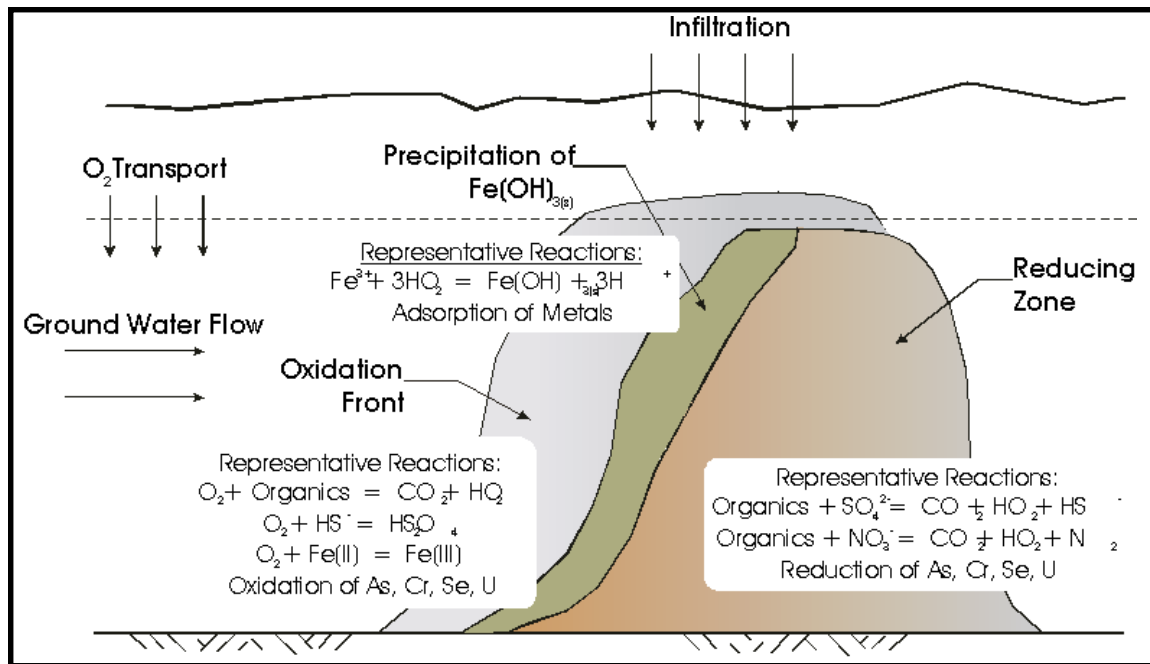
Figure 1. Illustration of representative reactions which may affect stability of metals immobilized by microbial reduction.

One strategy that has been suggested for remediation of sites contaminated with metals is use of permeable barriers in which a zone is created down-gradient from the contaminant plume that will allow groundwater to pass through it but will intercept or degrade the contaminants (Thomson et al., 1991; Scherer et al., 2000; Benner et al., 1999). The barrier zone can be constructed by backfilling a trench with reactive media or through injection of soluble or colloidal materials through injection wells. If the objective is to achieve microbial reduction, the reactive material would most likely be an organic carbon source to stimulate growth of SRB and/or DMRB.

After the organic substrate in a permeable barrier has been consumed, a variety of oxidizing and leaching reactions may occur which will affect the stability of metals immobilized in the barrier. These reactions are illustrated in Figure

1. At the down-gradient end of the system, strongly reducing conditions are present, produced by the microbial oxidation of organic material. Representative reactions are shown for microbial sulfate reduction and denitrification; similar reactions can be written for dissimilatory iron/metal reduction. Soluble metals precipitate from solution in this region as metal sulfides (e.g., CdS, NiS, FeS, HgS, PbS, AsS, and ZnS), elemental phases (e.g. Se^0), or as oxides (e.g., Cr_2O_3 and UO_2). In addition to inorganic precipitates, a large amount of biomass is produced by the microbial growth. At the up-gradient end of the system, oxidizing conditions become established through transport of aerobic groundwater and diffusion of oxygen through the vadose zone. Representative reactions include oxidation of degradable soil organic matter and biomass produced by anaerobic growth, and oxidation of inorganic compounds, including

sulfides and reduced iron (Fe^{2+}) phases. Metal contaminants that are expected to be released in this zone are those that form oxyanions in solution (e.g., As, Cr, Mo, Se, V, and U). At the transition between the up-gradient oxidizing zone and the down-gradient reducing zone, a narrow zone where oxidation of Fe^{2+} would result in the precipitation of Fe^{3+}-bearing mineral phases is expected. Although it is shown as a separate zone in this diagram, due to the rapid oxidation of Fe^{2+} ions in aerobic conditions near neutral pH, it is likely that these phases would be distributed throughout the oxidizing zone. The presence of iron hydroxides is important because they are effective scavengers for metals present in an oxyanionic species, including As, Cr, Mo, Se, V, and U. Thus, even though reduced phases of these metals may be oxidized if oxidizing conditions are re-established in the system, whether or not they become re-mobilized depends on the adsorption and precipitation reactions associated with formation of Fe^{3+}-bearing hydr(oxide) phases.

This paper describes an investigation to evaluate the long-term stability of selected metals, metalloids, and radionuclides that have been immobilized by microbial reduction. It addresses two questions. First, if the contaminants are immobilized in place, will they meet regulatory criteria which have been established for disposal of hazardous and radioactive materials? The second research question is more fundamental: what is the long-term stability of the immobilized contaminants? There are at least two components to this question: 1) what are the mechanisms which will release the contaminants to solution? and 2) what is the rate of release? This requires a basic understanding of the nature of the immobilized pollutants, knowledge of the transformations they may undergo if the geochemical environment changes, and some information regarding the rates of these transformations. This paper describes work done with (As) and (Se).

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. The experimental system has been described previously (Simonton et al., 2000) and is briefly summarized here.

Experimental system

Microbial reduction was conducted in plastic columns filled with acid-washed silica sand (10-20 mesh size). Large-diameter columns (10 cm) were operated to generate large volumes of media containing immobilized metals for batch leaching tests, while small-diameter columns (1 cm) were operated to generate media for long-term leaching studies. Anaerobic microbial cultures were grown in these columns and contaminants were allowed to precipitate on the sand media. The media fed to the columns was a simulated groundwater at pH 7.5 and is listed in Table 1. Contaminants were added to the solution at 5 mg/L as As(V) ($Na_2AsO_4 \cdot 7H_2O$), Cr(VI) ($KCrO_4$), and

Se(VI) (Na$_2$SeO$_4$). 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.

The columns were inoculated with a sulfate-reducing bacteria (SRB), *Desulfovibrio desulfuricans* (strain Dd 642). Ten mM of lactate were added to the simulated ground-water to serve as the electron donor for microbial metabolism.

Electron microscopy

Samples of the microbial matrix containing the bacteria and immobilized metals were subjected to electron microscopy to determine the phase of the reduced metals. Samples of the sand-biomass material from a microscale column containing SRB and grown in the presence of 5 mg/L of As were shaken to dislodge attached biomass and associated metal precipitates from the sand. The sand was allowed to settle and the lighter biomass was decanted then filtered. The biomass was first subjected to scanning electron microscopy and elemental mapping of the filtered material. An image of the matrix was collected using a JEOL 5800LV scanning electron microscope. Energy dispersive X-ray spectroscopy was used to map the elemental composition of the imaged arc using an Oxford Isis 300 X-ray analyzer.

Leaching studies

Two types of leaching procedures were used to measure the stability of contaminants immobilized by microbial reduction. A toxicity characteristics leaching procedure (TCLP) leach test was conducted using samples from the large columns, while the small columns were subjected to a sequential leaching process by passing simulated groundwater through them with no physical mixing.. 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 decrees of mixing, whereas the sand media in the small columns containing the biomass and immobilized metals were leached in place with no physical distur-bance whatsoever.

The TCLP test procedure (EPA Method 1311, Appendix II, 40CFR Part 261) involves use of an acidic leach solution prepared by

Table 1. Representative reduction reactions for anionic metal contaminants.

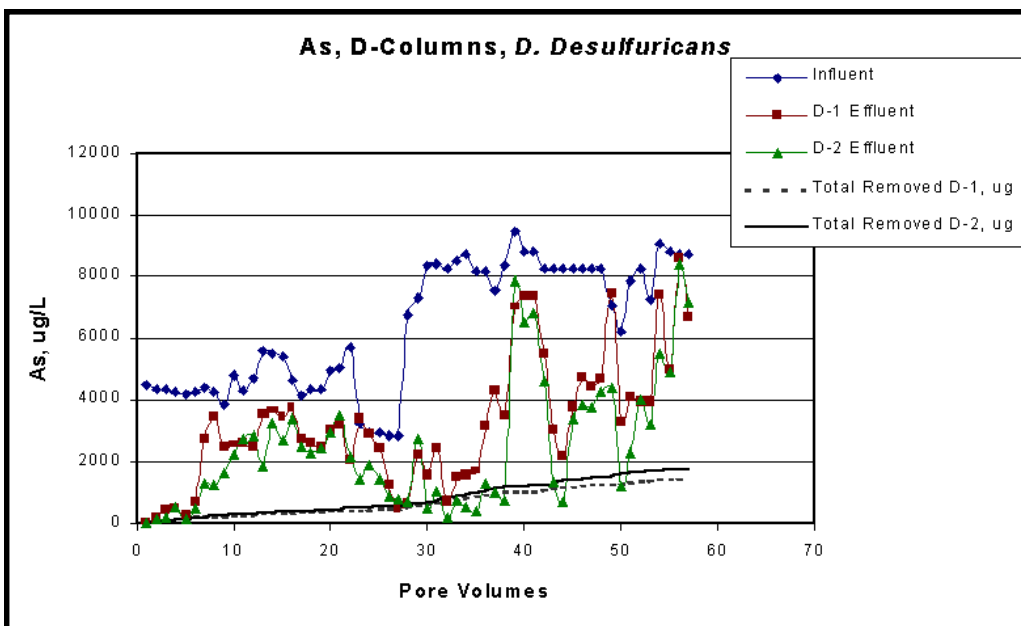| Redox Couple			Representative Reaction	
As(V)	$\rightarrow$	As(III)	$AsO_4^{3-} + 2SO_4^{2-} + 24H^+ + 17e^- \rightarrow$	$AsS_{2(s)} + 12H_2O$
Cr(VI)	$\rightarrow$	Cr(III)	$2CrO_4^{2-} + 10H^+ + 6e^- \qquad\qquad \rightarrow$	$Cr_2O_{3(s)} + 5H_2O$
Se(VI)	$\rightarrow$	Se(0)	$SeO_4^{2-} + 8H^+ + 6e^- \qquad\qquad \rightarrow$	$Se^o + H_2O$
U(VI)	$\rightarrow$	U(IV)	$UO_2(CO_3)_2^{2-} + 2H^+ + 2e^- \qquad \rightarrow$	$UO_{2(s)} + 2HCO_3^-$

Figure 2. Influent and effluent concentrations of As for columns containing SRB grown on lactic acid.

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 is prepared consisting of 10 g of sand media and 200 g of leach solution. This slurry is then mixed for 18 ± 2 hours at 30 rpm. The leachate is filtered and analyzed for contaminants by GFAA spectroscopy.

The long-term stability of the undisturbed sand media in the small columns was investigated by passing simulated groundwater without the metals through the columns and measuring the contaminant concentrations in the leachate. This is intended to simulate passage of uncontaminated groundwater through the contaminants immobilized in place in a subsurface environment.

RESULTS

Results of the As and Se removal by the SRB are presented in Figure 2 and Figure 3. These figures show the influent and effluent

concentrations of each contaminant, and the cumulative mass of each metal removed in the columns. Both columns initially showed high removal of the metals, which is believed to be the result of high microbial growth rate in an unstressed environment. With time, this removal decreased; nevertheless, both columns showed continued metal removal throughout the metal-feeding period.

After the As column had been fed 55 pore volumes of feed solution, samples of the matrix containing the biomass and immobilized metals were collected and subjected to electron microscopy and elemental mapping. Sample results are presented in Figure s 4a - 4d. The electron microscopy shows whisker-like crystals of lengths typically varying from 50 to 100 um. The large size of the crystals relative to a bacterial cell suggests that this phase is formed via an extracellular process. The elemental mapping showed the composition to be limited
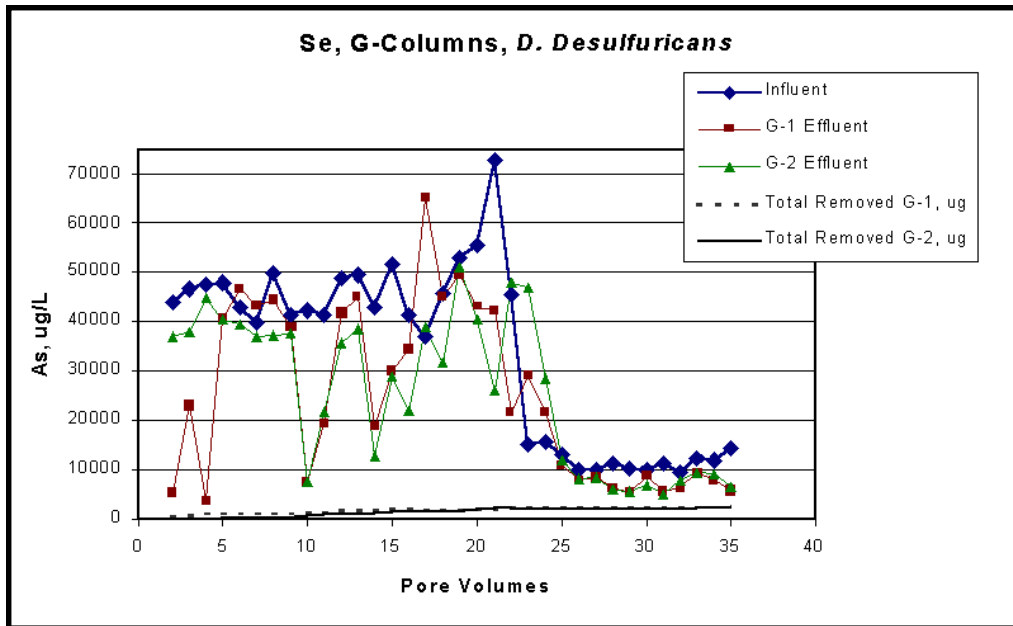
Figure 3. Influent and effluent concentrations of Se for columns containing SRB grown on lactic acid.

to arsenic and sulfide, though the ratio of the two is not known. Two common As-S minerals are realgar (AsS) and orpiment (As$_2$S$_3$), which are consistent with this composition Though it was present in the feed solution, the absence of Fe suggests that an arsenopyrite (FeAsS) phase was not formed. Further geochemical characterization is in progress to confirm the phase of this material. Sulfate-reducing bacteria have previously been shown to be capable of reducing Se to elemental selenium (Tomei et al., 1995).

Samples of the sand containing the biomass and immobilized metals were subjected to

leaching by the TCLP process. The results are summarized in Table 2 and show that, based on the leachability of the metals, the sand containing the immobilized metals would not be classified as a hazardous waste under RCRA. Although the TCLP test involves vigorous mixing of a weak solution of acetic acid, in contrast to many transition metals such as copper and zinc, reduced phases of both As and Se are generally not soluble under these conditions.

As illustrated in Figure 1, one of the principal release mechanisms affecting the long-term stability of metals immobilized by microbial

Table 2. Summary of TCLP leaching results.

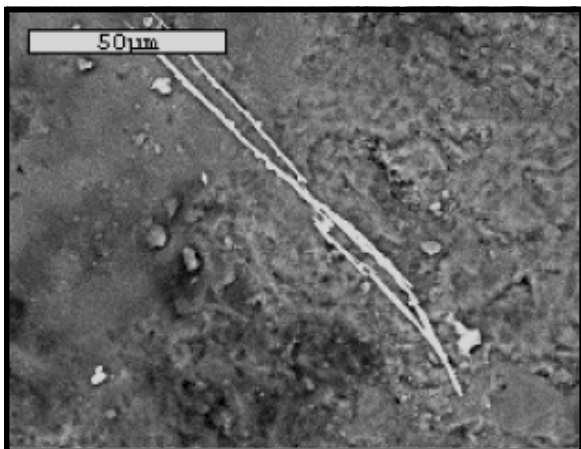| Metal	Total Conc. (mg/Kg)	RCRA Standard (mg/L)	Leachate Conc. (mg/L)	Fraction of Total Leached (%)
		Toxicity Characteristic Leaching Procedure (TCLP)		
Arsenic	9.89	5.0	0.0327	7%
Selenium	1.14	0.16	0.0131	23%

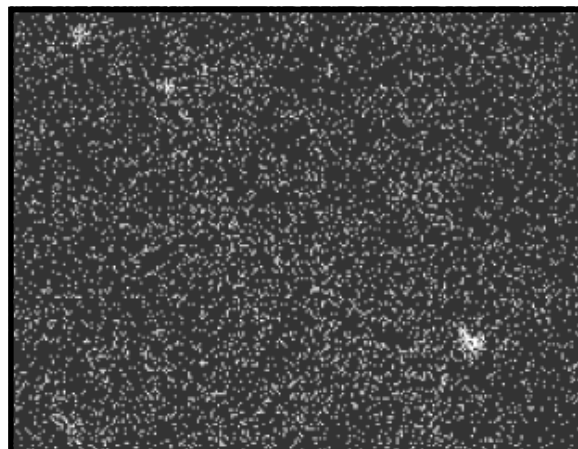**Figure 4a.** SEM image of arsenic sulfide crystals in biomass matrix.

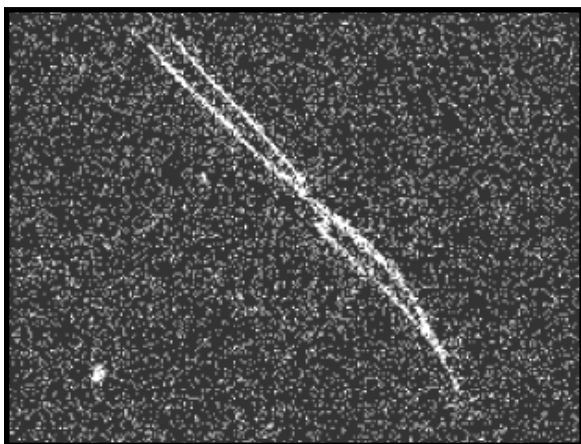**Figure 4b.** Elemental map of Fe in biomass matrix.

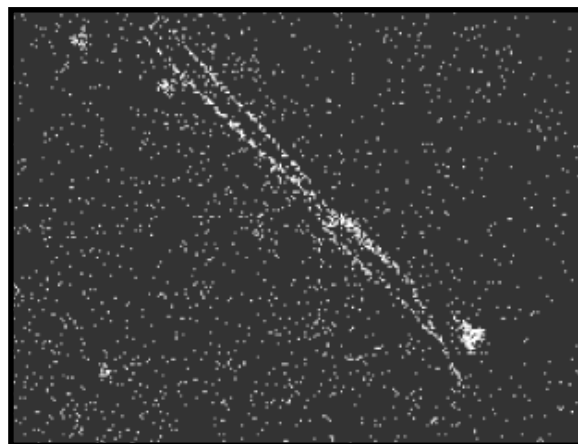**Figure 4c.** Elemental map of As in biomass matrix.

Figure 4d. Elemental map of S in biomass matrix.

reduction is oxidation and dissolution. This was investigated by passing simulated groundwater through each column (Figure 5). In contrast to the TCLP procedure, the columns were leached with no physical disturbance of the sand-biomass matrix to simulate the absence of physical mixing that would be experienced in a subsurface system.

The results show a moderate decrease in the effluent contaminant concentration for about 100 pore volumes of leachate for both metals. This was followed by an apparent steady state leachate containing low but consistently measurable concentrations of both metals. Based on

these results, two different release mechanisms are proposed. The moderate rate of decline in effluent concentrations is attributed to release of metals that are not fully reduced and incorporated into macroscopic mineral phases as shown. These contaminants might be associated with biomass, either within the cell structure or adsorbed to cell surfaces. As cells die due to lack of an organic substrate, these metals are released. The accumulation of a fairly dense biomass resulting from several weeks of cell growth provides some substrate which reduces the rate of release.
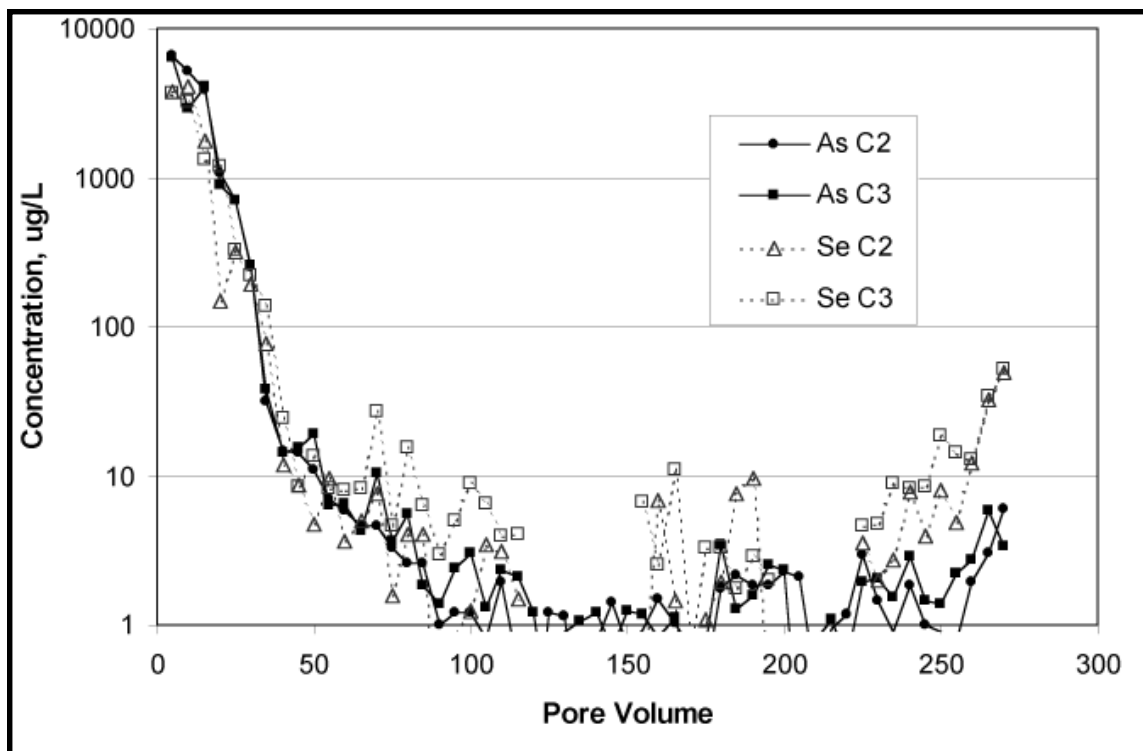
Figure 5. Long-term leaching results for arsenic and selenium.

After about 100 pore volumes, the weakly bound or non-crystalline metals have been completely leached from the system. The continued appearance of low but measurable metal concentrations in the effluent suggests the leaching of less soluble crystalline phases. Although leaching appears to continue indefinitely, it is important to recognize that the effluent concentrations of both As and Se are well below current drinking water standards. This suggests that, unless some type of rapid-release mechanism not postulated in Figure 1 occurs, release of the immobilized metals would not present a significant threat to human health or the environment. Therefore, one potential application of an *in situ* microbial process to achieve remediation of a site contaminated with metals would be rapid removal of the contaminants from solution through microbial reduction

and precipitation. Over a very long period of time, the metals might be released; however, their low solubility would result in a low release rate, and the down-gradient metal concentrations would be below regulatory concern.

CONCLUSIONS

This study has shown that As and Se can be reduced to insoluble phases by *D. desulfuricans*. Arsenic is reduced to a crystalline As-sulfide phase, while previous work has shown that SRB will reduce Se to an elemental form. The reduced phases of As and Se were subjected to a TCLP leach procedure, which produced leachate concentrations of both metals that were much lower than the criteria established for hazardous wastes. A long-term (approximately three-month) leaching procedure found a moderate decrease in both metal

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concentrations to less than 10 ug/L, followed by quasi-steady state leach concentration of 1 to 10 ug/L. It is postulated that the initial moderate decrease was due to release of weakly or incompletely bound metals associated with the biomass. The indefinite leaching of metals at a very low concentration is attributed to release from more stable crystalline phases. The fact that the effluent concentrations are well below current federal drinking water standards suggests that even though the immobilized phases do not appear to be stable forever, the metals concentrations are sufficiently low that they will not limit applicability of *in situ* immobilization techniques.

ACKNOWLEDGMENTS

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