Journal of Industrial Microbiology & Biotechnology (1998) 20, 13–19

Reduction of Cr, Mo, Se and U by *Desulfovibrio desulfuricans* immobilized in polyacrylamide gels

MD Tucker[1], LL Barton[2] and BM Thomson[3]

[1]*Sandia National Laboratories, Albuquerque, NM 87185;* [2]*Department of Biology, University of New Mexico, Albuquerque, NM 87131;* [3]*Department of Civil Engineering, University of New Mexico, Albuquerque, NM 87131, USA*

Intact cells of *Desulfovibrio desulfuricans*, immobilized in polyacrylamide gel, removed Cr, Mo, Se and U from solution by enzymatic-mediated reduction reactions. Lactate or H_2 served as the electron donor and the oxidized Cr(VI), Mo(VI), Se(VI) and U(VI) served as electron acceptors. Reduction of the oxidized metal species resulted in the precipitation of solid phases of the metals. Metal removal efficiencies of 86–96% were achieved for initial concentrations of 1 mM Mo, Se, and U and 0.5 mM Cr. Insoluble metal phases accumulated on both the surface and the interior of the polyacrylamide gel. In column tests conducted for U removal, effluent concentrations less than 20 μg L^{-1} were achieved with initial concentrations of 5 mg L^{-1} and 20 mg L^{-1} U and residence times from 25–37 h. The enzymatic reduction of Cr, Mo, Se, and U by immobilized cells of *D. desulfuricans* may be a practical method for removing these metals from solution in a biological reactor.

Keywords: immobilized cells; *Desulfovibrio*; dissimilatory metal reduction

Introduction

The solubilities of the metal contaminants Cr, Mo, Se, and U are strongly dependent on their chemical oxidation state. Under oxidizing conditions, these contaminants usually exist as highly soluble oxyanions (CrO_4^{2-}, MoO_4^{2-}, SeO_4^{2-}, or SeO_3^{2-}) or uranyl-carbonate complexes [$UO_2CO_3^o$, $UO_2(CO_3)_2^{2-}$ or $UO_2(CO_3)_3^{4-}$] [2]. However, under reducing conditions these metals may precipitate as insoluble oxides [$Cr_2O_{3(s)}$, $MoO_{2(s)}$, and $UO_{2(s)}$], sulfides [$MoS_{2(s)}$], or other solid phases (Se^o) [where (s) designates a solid phase]. Recent investigations have demonstrated that *Desulfovibrio desulfuricans* and other sulfate-reducing bacteria have the ability to reduce and precipitate these metals by enzymatic reactions [5,8–10,17–19]. Under anaerobic conditions, these microorganisms can use metals as terminal-electron acceptors in a manner analogous to O_2 utilization by aerobic organisms and NO_3^- or SO_4^{2-} utilization by anaerobic organisms. This process is known as dissimilatory metal reduction and occurs when electrons from reduced organic compounds or H_2 are transferred to oxidized forms of the metals [6]. Precipitation of metals by dissimilatory metal reduction has been suggested as a potential method for removing soluble metals from water contaminated as a result of environmental remediation activities such as soil washing/chemical extraction or pump and treat operations [14].

Microbial reduction of soluble U(VI) to insoluble U(IV) by *D. desulfuricans* was recently demonstrated [8]. Lactate or H_2 served equally well as the electron donor and there was no U(VI) reduction in the absence of an electron donor. U(IV) was precipitated as the black $UO_{2(s)}$ mineral phase which was identified by energy-dispersive X-ray analysis. It is important to recognize that, although enzymatic metal reduction occurred, the microoganism may not be able to incorporate this phenomenon into its electron transport chain. This was clearly demonstrated for *D. desulfuricans* because no cell growth occurred with U(VI) as the sole electron acceptor.

Enzymatic reduction of highly soluble and toxic (Cr(VI) (as chromate, CrO_4^{2-}) to less soluble and less toxic Cr(III) by *D. vulgaris* has also been demonstrated [10]. To identify the enzyme responsible for Cr(VI) reduction, cells were broken in a French pressure cell and fractionated into soluble and membrane-bound protein fractions through differential centrifugation. Hydrogenase was purified from the soluble protein fraction and, when combined with cytochrome c_3, Cr(VI) reduction occurred. Neither hydrogenase alone nor cytochrome c_3 alone could reduce Cr(VI).

Transformation of selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) to elemental selenium by *D. desulfuricans* has also been demonstrated [17]. The appearance of red, elemental selenium (confirmed by energy-dispersive X-ray analysis) was attributed to cytoplasmic activities and not to chemical reaction in the growth media. Growth did not occur with either selenate [Se(VI)] or selenite [Se(IV)] as the sole electron acceptor.

Enzymatic reduction of Mo(VI) (as molybdate, MoO_4^{2-}) to Mo(IV) by *D. desulfuricans* with both lactate and H_2 as electron donors has also been investigated [19]. Mo(VI) reduction in the presence of sulfide resulted in the extracellular precipitation of the black mineral phase, molybdenite [$MoS_{2(s)}$], which was identified by electron diffraction analysis. Attempts to grow *D. desulfuricans* with Mo(VI) as the sole electron acceptor were unsuccessful.

In the research cited above, freely suspended bacteria cells were used to reduce and precipitate metals from solution in laboratory-scale batch reactors. However, suspended cells will be impractical in full-scale reactors

Correspondence: MD Tucker, Sandia National Laboratories, 1515 Eubank SE, Albuquerque, NM 87123-1139, USA
Received 10 January 1997; revised 18 September 1977; accepted 19 September 1997

because firstly, they must be removed from the effluent solution by filtration, settling, or some other process and secondly, reuse of the cells will be difficult since precipitated metals will be interspersed with the cells. This report investigates what we believe is a more practical method for dissimilatory metal reduction of metals in full-scale reactors. Intact cells of *D. desulfuricans* were immobilized in polyacrylamide gel and used to reduce and precipitate Cr, Mo, Se, and U from solution in batch tests. Column tests were also conducted for U removal. The results suggest that use of immobilized cells of *D. desulfuricans* may be a feasible method for removing soluble metals from contaminated water in a biological reactor.

Materials and methods

Cell culturing

Pure cultures of *D. desulfuricans* (DSM 642) were used throughout this research. The medium used for bacterial growth contained the following: Na-lactate, 2.4 g L^{-1}; yeast extract, 1 g L^{-1}; NH_4Cl, 2 g L^{-1}; K_2HPO_4, 0.5 g L^{-1}; Na_2SO_4, 4 g L^{-1}; $MgSO_4 \cdot 7H_2O$, 2 g L^{-1}; and $FeSO_4 \cdot 7H_2O$, 5 mg L^{-1}, adjusted to pH 7.4 with 20% KOH. Cells were grown for 4 days before immobilization.

Immobilization in polyacrylamide gel

Cells were harvested by centrifugation at $5800 \times g$ for 30 min, washed with sterile, anaerobic bicarbonate buffer (2.5 g L^{-1} $NaHCO_3$) and centrifuged again. The biomass was resuspended in 20 ml sterile, deionized water supplemented with 2.5 g acrylamide monomer and 0.25 g *N,N′*-methylenebisacrylamide as a cross-linking agent. A small aliquot of this solution was collected for protein determination. The polymerizing reaction was initiated by adding 2.5 ml of a 2.5% (w/v) solution of potassium persulfate and accelerated by adding 2.5 ml of a 5% (w/v) solution of 3-dimethylaminopropionitrile. The suspension was stirred gently until the gel began to set and was then refrigerated at 4°C for 1 h. After the gel had set, it was cut into approximately 4-mm cubes and washed with sterile, anaerobic bicarbonate buffer before being placed in a batch or column reactor [15].

Batch tests using immobilized cells

Immobilized cells of *D. desulfuricans* were placed in 10-ml anaerobic serum vials to provide 550–750 mg L^{-1} cell protein in each vial. Anaerobic solutions containing Cr(VI), Mo(VI), Se(VI), or U(VI), with lactate as an electron donor (Table 1) were added to the vials. The vials were capped with rubber septums and aluminum seals and incubated at 37°C in the dark during the test period. For tests that used H_2 as the electron donor instead of lactate, 15-ml serum vials were used. Immobilized cells and anaerobic solutions containing oxidized metals (Table 1) were added to the vials leaving 5 ml of headspace. The vials were capped and the headspace was flushed with purified H_2 for 15 min.

The effect of the absence of an electron donor on metal removal was investigated by adding metal-bearing solutions with no electron donor (Test Solutions 5–8) and immobilized cells to 10-ml serum vials. To determine if viable cells are necessary for metal removal, heat-killed cells were immobilized and added to solutions containing the oxidized metal species and an electron donor (Test Solutions 1–4). Cells were heat-killed by heating the growth medium and cells to boiling for 5 min prior to harvesting. To determine if metal removal occurred as a result of adsorption of the metals to the polyacrylamide gel, metal-bearing solutions (Test Solutions 1–4) were added to 10-ml serum vials which contained only polyacrylamide gel (no cells).

The effect of temperature on metal removal was tested by adding metal-bearing solutions and polyacrylamide-immobilized cells to a series of 10-ml serum vials and storing those vials at 10°C, 25°C, 37°C, and 57°C for 24 h (for Cr, Se, and U) and 96 h (for Mo). To test for pH effects, metal-bearing solutions as described above were adjusted to pH values of 4, 5, 6, 7, 8, 9, and 10 with 1 N HCl or 20% KOH. The solutions were added to a series of 10-ml serum vials containing polyacrylamide-immobilized cells and stored for 24 h (for Cr, Se, and U) or 96 h (for Mo) at 37°C.

Three replicates of each test were performed during this research. Data points on all plots represent the mean value of the three replicates. Error bars on plots represent the standard deviation of the replicate values from the mean.

Table 1 Solutions for enzymatic reduction of Cr(VI), Mo(VI), Se(VI), or U(VI) by immobilized cells of *D. desulfuricans* in batch tests

Test solution	Constitutents[a]
1	2.4 g L^{-1} Na-lactate; 0.5 mM K_2CrO_4
2	2.4 g L^{-1} Na-lactate; 1 mM Na_2MoO_4
3	2.4 g L^{-1} Na-lactate; 1 mM Na_2SeO_4
4	2.4 g L^{-1} Na-lactate; 1 mM $UO_2(CH_3COO)_2$
5	5 ml H_2; 0.5 mM K_2CrO_4
6	5 ml H_2; 1 mM Na_2MoO_4
7	5 ml H_2; 1 mM Na_2SeO_4
8	5 ml H_2; 1 mM $UO_2(CH_3COO)_2$

[a]All solutions also contained 1.25 g L^{-1} $NaHCO_3$ and were adjusted to pH 7.0 with 1 N HCl.

Batch tests using suspended cells

Cells of *D. desulfuricans* were harvested and resuspended in anaerobic, metal-bearing solutions to provide 500–700 mg L^{-1} cell protein. The solutions were added to 10-ml serum vials, capped with rubber septum and aluminum seal, and incubated at 37°C in the dark through the test period.

Column tests

Column tests for uranium removal were conducted. All columns, tubing, and flasks were sterilized with a 5% NaOCl solution and rinsed with sterile, distilled water before the start of the tests. *D. desulfuricans* immobilized in polyacrylamide gel were packed into two 2.5-cm × 20-cm liquid chromatography columns (Sigma Chemical Co, St Louis, MO, USA) which were connected in series to give a total fluid volume of approximately 100 ml. All tubing was 1/8″ I.D. × 1/16″ wall thickness (Tygon R-3603, Norton Inc, Akron, OH, USA). Fluid was pumped to the columns by a peristaltic pump. The pump was cycled on and off with an automatic timer to achieve an average flow rate

of approximately 3–4 ml h^{-1}. All vessels consisted of 1-L Erlenmeyer flasks. The head space in each of these flasks and the influent solution were flushed with purified N_2 for 30 min before the start of a test. Vacuum relief and isolation flasks prevented air from entering the column and prevented the creation of a vacuum in the influent flask as media were pumped from the flask. A pressure relief flask prevented a pressure increase in the effluent flask as it received discharge from the columns. The feed solution to the columns contained: uranyl acetate, 0.02 mM or 0.08 mM (5 mg L^{-1} or 20 mg L^{-1} U); Na-lactate, 350 mg L^{-1} or 500 mg L^{-1} (300 mg L^{-1} or 430 mg L^{-1} COD); $NaHCO_3$, 1.25 g L^{-1}; $MgSO_4 \cdot 7H_2O$, 200 mg L^{-1}; NH_4Cl, 50 mg L^{-1}; and KH_2PO_4, 50 mg L^{-1}. The influent solution was sterilized by autoclaving prior to the start of the tests. Tests were run at room temperature (approximately 25°C). The columns were operated for 3 days to achieve steady state conditions before data collection began.

Analytical techniques

Soluble chromium concentration was measured by flame absorption at 357.8 nm using a Perkin-Elmer Model 460 Atomic Absorption Spectrophotometer (Perkin-Elmer Corp, Norwalk, CT, USA). Soluble selenium concentration was measured at 196.0 nm also by flame absorption. Soluble molybdenum concentration was measured by the thiocyanate method [4]. In batch tests, soluble uranium concentration was measured by the hexanol extraction procedure [12]. In column tests, soluble uranium concentration was measured by laser-induced fluorometry (Scintrex UA-3, Scintrex Analytical, Concord, Canada). All test samples were filtered with 0.2-μm syringe filters (Gelman Nylon Acrodisc, Gelman Sciences Inc, Ann Arbor, MI, USA) before analysis for soluble metals. Cell protein was measured using the method of Lowry *et al* [11]. Chemical oxidation demand (COD) was measured by Standard Method 5220 D [1]. Lactate was measured by the acetaldehyde colorimetric method of Neish [13].

Results

Batch tests using immobilized cells

When intact cells of *D. desulfuricans* were immobilized in polyacrylamide gel with either H_2 or lactate as an electron donor and Cr(VI), Mo(VI), Se(VI), and U(VI) as potential electron acceptors, there was a marked decrease in the concentrations of the metals in solution over time (Figure 1). Significant metal removal did not occur in the absence of an electron donor, in the absence of cells (polyacrylamide gel only), or with heat-killed cells indicating that metal removal was by enzymatic reduction of the oxidized metal species.

Metal removal was dependent on temperature with the highest metal removal in all cases occurring at 37°C (Figure 2a). Metal removal was also dependent on pH with the highest removal occurring at pH 7 (Figure 2b).

Effect of cell immobilization on metal removal rates

Removal of Cr, Se, and U occurred at a slower rate with immobilized cells than with freely suspended cells (Figure 1a, 1c, and 1d). Mo removal occurred at a slightly faster rate with immobilized cells as compared to removal with freely suspended cells (Figure 1b).

Observations of the precipitated metal compounds

Cubes of polyacrylamide gel (with immobilized cells) from selenium reduction batch tests were cut open with a sharp knife and observed with the aid of a magnifying glass (10×). Bands of red elemental selenium occurred on both the surface and in the interior of the gel.

Column tests

Three column tests were conducted for uranium removal. Each test was run for a period of 30 days. Test parameters are summarized in Table 2. Uranium removal efficiencies greater than 99% occurred in all columns (Figure 3). Effluent COD and lactate concentrations (both shown as mg L^{-1} COD) are plotted in Figure 4. Approximately 20% of the influent COD was removed in the columns. In most cases, over half of the effluent COD was due to lactate. No loss of efficiency in the columns (as measured by increased U or COD concentrations) was observed over the 30-day period of each test.

Discussion

The results demonstrate that intact, immobilized cells of *D. desulfuricans* can remove the metals Cr, Mo, Se and U from solution. Results from the batch tests are consistent with previous research and indicate that the likely mechanism for metal removal is dissimilatory metal reduction where soluble Cr(VI), Mo(VI), Se(VI), U(VI) compounds are reduced to insoluble Cr(III), Mo(IV), Se(0), U(IV) by enzymatic reactions. Significant metal removal did not occur in the absence of an electron donor or in the presence of polyacrylamide gel with no cells. Significant metal removal also did not occur with heat-killed cells. The temperature optimum of 37°C for metal removal also suggests enzymatic reduction of the metals (ie, dissimilatory metal reduction). If adsorption of the metals to *D. desulfuricans* cells or to the polyacrylamide gel was the primary removal mechanism, the highest removal rates would be expected to occur at low temperatures. Alternatively, if chemical reduction of the metals (ie, not enzymatic reduction) was the primary removal mechanism, the greatest removal rates would be expected to occur at the higher temperatures. The optimum pH for metal removal (pH 7) is also consistent with enzymatic reduction of the metals where loss of enzymatic activity occurs at low and high pH values.

Comparison of the batch test data shows that Mo(VI) reduction by suspended cells of *D. desulfuricans* is considerably slower than the reduction of Cr(VI), Se(VI) or U(VI). Pseudo first-order rate constants and half-lives were calculated for reduction of the oxidized metal species by the suspended cells (Table 3). Mo(VI) reduction occurred approximately 35 times slower than Cr(VI) reduction, 25 times slower than U(VI) reduction, and 15 times slower than Se(VI) reduction. Thermodynamic calculations (data from Refs [2] and [16], not shown) show that while the potentials for the $acetate^-/lactate^-$ and the H^+/H_2 redox couples are below the potentials for each of the

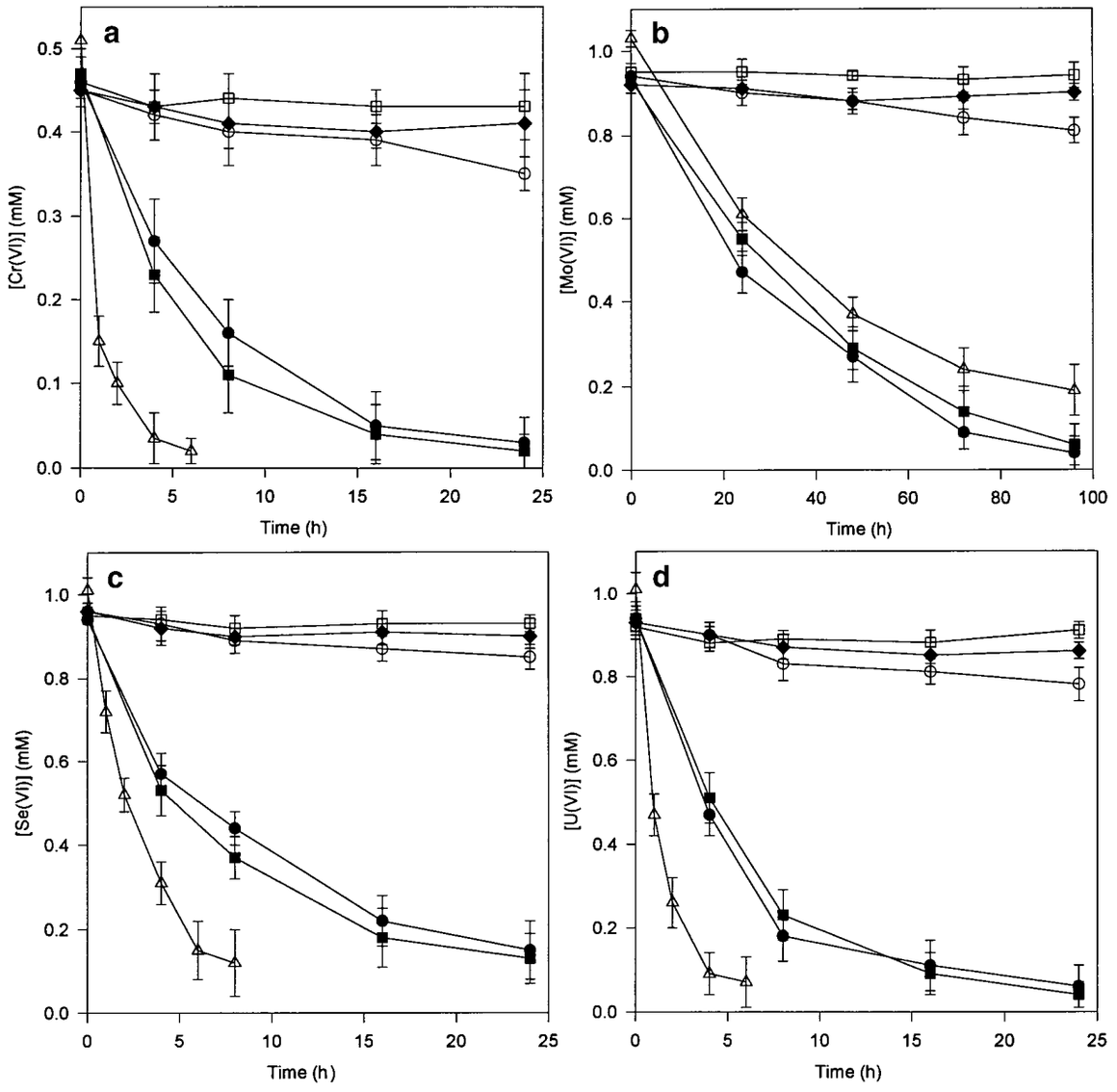

Figure 1 Metal removal by *D. desulfuricans* with (●) immobilized cells and lactate as the electron donor, (■) immobilized cells and H_2 as the electron donor, (◆) immobilized cells and no electron donor, (○) immobilized, heat-killed cells and lactate as the electron donor, and (Δ) freely suspended cells and lactate as the electron donor. Metal removal in (□) cell-free solution with lactate as the electron donor. Average cell protein concentration, 577–689 mg L^{-1}. (a) Cr(VI); (b) Mo(VI); (c) Se(VI); (d) U(VI).

Cr(VI)/Cr(III), Mo(VI)/Mo(IV), Se(VI)/Se(0), and U(VI)/U(IV) redox couples, the Cr(VI), Se(VI), and U(VI) reduction reactions are considerably more thermodynamically favorable than the Mo(VI) reduction reaction which may explain the more rapid reduction of the other three metals.

Pseudo first-order rate constants and half-lives were also calculated for metal removal in batch tests with immobilized cells (Table 3). Cr(VI), Se(VI), and U(VI) were removed by immobilized cells at considerably slower rates than by suspended cells. The slower rates suggest that transport limitations may be controlling the rate of removal of these metals in immobilized cell systems. The presence of solid metal phases [ie Se(0)] in the interior of the gel confirms that diffusion into the gel did, in fact, occur. However, the situation for Mo was quite different. Since the rate of reduction of Mo(VI) by suspended cells was considerably slower than for the other metals, removal by immobilized cells is less likely to be limited by diffusion effects (ie, it is limited by reaction rate). In fact, the removal of Mo(VI) occurred at a slightly faster rate in immobilized cell systems as compared to suspended cell systems perhaps due to the strong reducing conditions which may exist inside of the polyacrylamide gel due to the high concentration of cells. More rapid Mo(VI) reduction in immobilized cell systems *vs* suspended cell systems also indicates that immobilization in acrylamide had little or no deleterious effect on the cells. If the cells had been adversely affected by the immobilization procedure, it would be expected that Mo reduction in the immobilized cell system would have also occurred at a slower rate than in the suspended cell system as it did with the other three metals.

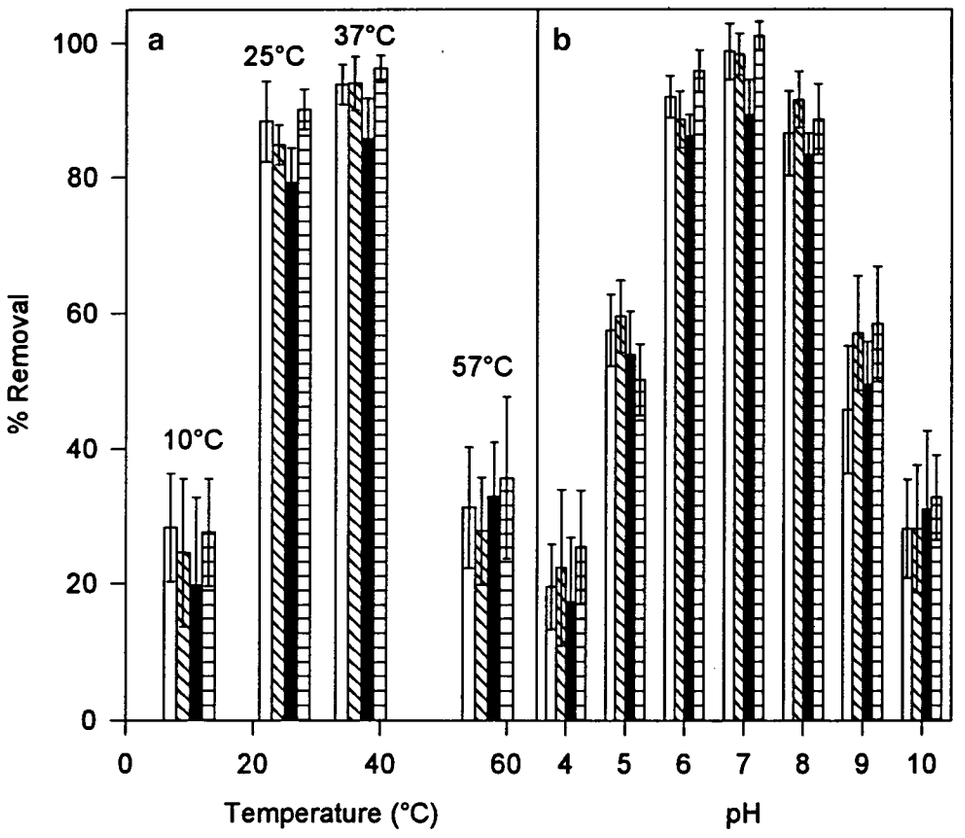

Figure 2 Extent of Cr(VI) (□), Se(VI) (■) and U(VI) (▨) removal after 24 h and Mo(VI) (▤) removal after 96 h at (a) various temperatures and (b) various pH values by polyacrylamide-immobilized cells of *D. desulfuricans* with lactate as the electron donor. Average cell protein concentration, 705 mg L^{-1}.

Table 2 Test parameters for uranium removal column tests

Test No.	Volume (ml)	Average flow rate (ml h^{-1})	Hydraulic residence time (h)	Influent [Na-lactate] (mg L^{-1} COD)	Influent [U] (mg L^{-1})
1	108	4.3	25	430	5
2	98	3.3	30	300	5
3	119	3.2	37	430	20

Tests in column reactors demonstrate that the concentration of uranium in water can be reduced below 20 μg L^{-1} with initial concentrations of 5 or 20 mg L^{-1} U, hydraulic residence times between 25–37 h, and lactate as the electron donor (Figure 3). Although only about 20% of the influent COD was removed in the column (Figure 4), most of the remaining COD in the effluent was due to lactate and, presumably, acetate. Typically, sulfate-reducing bacteria such as *D. desulfuricans* incompletely oxidize lactate to acetate [20]. The theoretical oxygen demand (ThOD) of lactate is 96 mg O_2 $mmol^{-1}$ lactate while the ThOD for acetate is 64 mg O_2 $mmol^{-1}$ acetate. Therefore, oxidation of lactate to acetate in the column results in only a small decrease in the COD of the effluent (Figure 4). This illustrates the need to develop column reactors that utilize electron donors other than reduced organic compounds (eg, H_2) or electron donors that can be more completely oxidized by

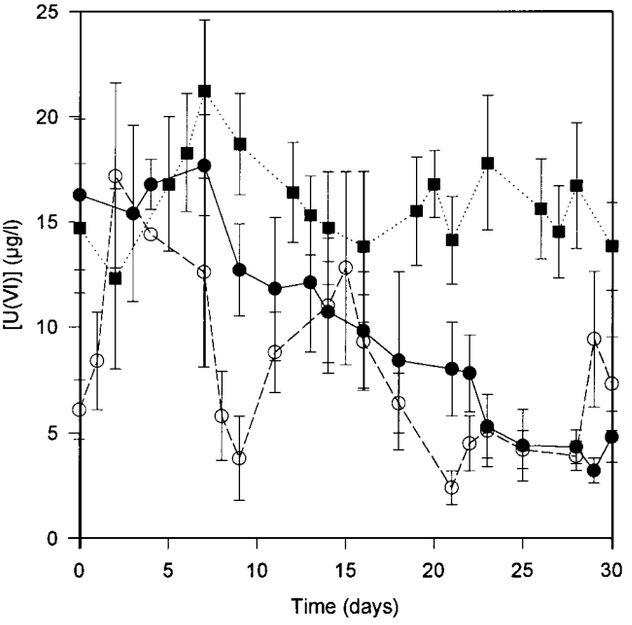

Figure 3 Effluent U(VI) concentration over time in column (●) test 1, (■) test 2, and (○) test 3. *D. desulfuricans* immobilized in polyacrylamide gel. Test parameters shown in Table 2.

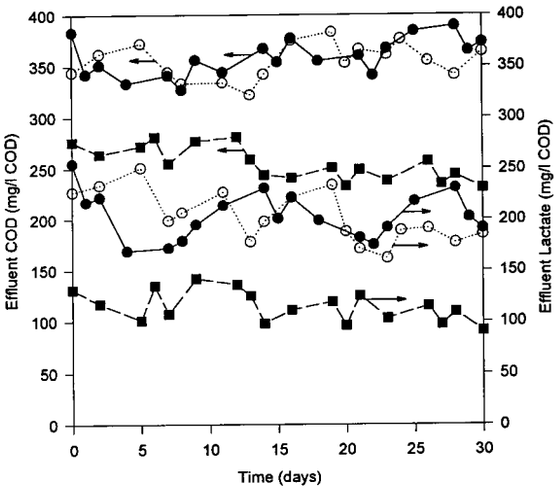

Figure 4 Effluent COD and lactate values over time in column (●) test 1, (■) test 2, and (○) test 3. *D. desulfuricans* immobilized in polyacrylamide gel. Test parameters shown in Table 2. Horizontal arrows indicate scale for data.

Table 3 Pseudo first-order rate constants and half-lives for the removal of Cr(VI), Mo(VI), Se(VI), and U(VI) from solution by suspended and immobilized cells of *D. desulfuricans*

Reduction reaction	Suspended cells		Immobilized cells	
	k (h^{-1})	$t_{1/2}$ (h)	k (h^{-1})	$t_{1/2}$ (h)
Cr(VI)/(III)	0.90	0.8	0.13	5.3
U(VI)/(IV)	0.70	1.0	0.15	4.6
Se(VI)/(0)	0.30	2.3	0.10	6.9
Mo(VI)/(IV)	0.02	33	0.03	23

sulfate-reducing bacteria (eg, formate) during the relatively short residence times in the column. Alternatively, an aerobic biological process could be employed after metal removal to complete the oxidation of lactate and acetate to CO_2.

Systems that utilize immobilized sulfate-reducing bacteria have the potential to play a key role in the remediation of water contaminated with metals as a result of environmental activities. For example, this technology could be utilized in the final stage of a soil washing/chemical extraction system. It has recently been demonstrated that uranium can be extracted from soil with bicarbonate and held in solution in the form of U(VI)-carbonate complexes [3,14]. In a typical soil washing system, uranium is then recovered from the wash solution by a conventional process such as ion exchange allowing the bicarbonate solution to be reused. However, regeneration or disposal of spent ion exchange media is very expensive and the uranium still exists in the highly soluble and mobile U(VI) oxidation state. As an alternative, columns of immobilized sulfate-reducing bacteria could be used to treat water from a soil washing system to precipitate soluble metals such as uranium from solution. Only the addition of small concentrations of nutrients (C, N, P, and S) and an electron donor (lactate or H_2) would be required. And, unlike ion exchange, this process is not too selective but can be used for a spectrum of metals that are often found together (ie, U, Se, and Mo). A system utilizing immobilized sulfate-reducing bacteria could be expected to remain viable for long periods of time provided oxygen is removed from the influent solution.

Conclusion

The studies summarized here demonstrate that dissimilatory metal reduction of Cr(VI), Mo(VI), Se(VI), and U(VI) by *Desulfovibrio desulfuricans* immobilized in polyacrylamide gel may be a practical method for removing these metals from solution in a continuous flow process. Anaerobic bacteria may also reduce and precipitate other metals such as vanadium, copper, gold, and silver, which implies that biological reactors utilizing immobilized anaerobes may have the potential for a wide range of applications [6,7]. More work is needed in the areas of nutrient/substrate requirements and scale-up techniques.

Acknowledgements

This work was supported by the US Department of Energy through the Waste-management Education and Research Consortium and Sandia National Laboratories under Contract DE-AC04-94AL85000.

References

1 American Public Health Association, American Water Works Association, Water Environment Federation. 1992. Method 5220D. Closed reflux colorimetric method. In: Standard Methods for the Examination of Water and Wastewater. pp 5–9, 18th edn, Am Pub Health Assoc, Washington, DC.
2 Brookins D. 1988. Eh-pH Diagrams for Geochemistry. pp 18–19, 102–105, 151–157, Springer-Verlag, New York, NY.
3 Buck E, N Brown and N Dietz. 1996. Contaminant uranium phases and leaching at the Fernald site in Ohio. Environ Sci Technol 30: 81–88.
4 Charlot G. 1964. Molybdenum. In: Colorimetric Determination of Elements: Principles and Methods. pp 299–304. Elsevier Publishing Company, New York, NY.
5 Fude L, B Harris, M Urrutia and T Beveridge. 1994. Reduction of Cr(VI) by a consortium of sulfate-reducing bacteria (SRB III). Appl Environ Microbiol 60: 1525–1531.
6 Lovley D. 1993. Anaerobes into heavy metal: dissimilatory metal reduction in anoxic environments. Trends Ecol Evol 8: 213–217.
7 Lovley D. 1995. Bioremediation of organic and metal contaminants with dissimilatory metal reduction. J Ind Microbiol 14: 85–93.
8 Lovley D and E Phillips. 1992. Bioremediation of uranium contamination with enzymatic uranium reduction. Environ Sci Technol 26: 2228–2234.
9 Lovley D and E Phillips. 1992. Reduction of uranium by *Desulfovibrio desulfuricans*. Appl Environ Microbiol 58: 850–856.
10 Lovley D and E Phillips. 1994. Reduction of chromate by *Desulfovibrio vulgaris* and its c_3 cytochrome. Appl Environ Microbiol 60: 726–728.
11 Lowry O, N Rosebrough, A Farr and R Randall. 1951. Protein measurement with the folin phenol reagent. J Biol Chem 193: 265–275.
12 Meloan C, P Holkeboer and W Brandt. 1960. Spectrophotometric determination of uranium with benzohydroxamic acid in 1-hexanol. Anal Chem 32: 791–793.
13 Neish A. 1952. Procedure 23: Colorimetric determination of lactic acid. In: Analytical Methods for Bacterial Fermentations. pp 42–43,

NRC No. 2952, National Research Council of Canada, Saskatoon, Canada.
14 Phillips E, E Landa and D Lovley. 1995: Remediation of uranium contaminated soils with bicarbonate extraction and microbial U(VI) reduction. J Ind Microbiol 14: 203–207.
15 Pons M and M Fuste. 1993. Uranium uptake by immobilized cells of *Pseudomonas* strain EPS 5028. Appl Microbiol Biotechnol 39: 661–665.
16 Stumm W and J Morgan. 1981: Appendix: thermodynamic properties. In: Aquatic Chemistry: An Introduction Emphasizing Chemical Equilibria in Natural Waters. pp 749–756, 2nd edn, John Wiley & Sons, New York, NY.
17 Tomei F, L Barton, C Lemanski, T Zocco, N Fink and L Sillerud. 1995. Transformation of selenate and selenite to elemental selenium by *Desulfovibrio desulfuricans*. J Ind Microbiol 14: 329–336.
18 Tucker M, L Barton and B. Thomson. 1996. Kinetic coefficients for simultaneous reduction of sulfate and uranium by *Desulfovibrio desulfuricans*. Appl Microbiol Biotechnol 46: 74–77.
19 Tucker M, L Barton and B Thomson. 1997. Reduction and immobilization of molybdenum by *Desulfovibrio desulfuricans*. J Environ Qual 26: 1146–1152.
20 Widdel F and T Hansen. 1992. The dissimilatory sulfate- and sulfur-reducing bacteria. In: The Procaryotes. Vol I (Ballows A, H Truper, M Dworkin, W Harder and K Schleifer, eds), pp 583–624, Springer-Verlag, Berlin.