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Bioremediation and Bioreduction of Dissolved U(VI) by Microbial Mat Consortium Supported on Silica Gel Particles

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The goal of this research was to simulate natural attenuation processes for uranium (U) by constructing a bioremediation system based on microbial mats. Ancient microbial consortia, such as microbial mats, have evolved the capacity to manage hostile environments and potentially bioremediate metal-contaminated water. To facilitate the engineering applications of microbial mats, the constituent microbial groups of the mat consortium were immobilized with required nutrients in silica gel. Resulting silica mat particles (SMP) were tested for efficiency in removing and, subsequently, reducing U(VI) to U(IV). Uranium-containing groundwaters from a Superfund Site (0.2 mg U(VI) L^{-1}, 293 mg HCO_3 L^{-1} at pH 8.2) and synthetic waters (∼0.05−2 mg U(VI) L^{-1}, pH 8−9) were assessed with this system. Over 80% of the dissolved U(VI), present as mostly U(VI)−carbonate (Superfund) or U(VI)−hydrolysis species (synthetic), was removed by the SMP within 15 min of treatment. X-ray absorption near-edge structure spectroscopy studies showed that the U sequestered in the SMP was reduced to U(IV) within 24 h of exposure. Effective sequential batch treatments and maintenance of a low redox environment by nutrient additions demonstrated the potential for long-term durability and capacity for continuous use of this system. Drying the SMP produced a hard compact product (1% of original weight). Capacity for on-site generation of SMP, relative low-cost constituent materials, the simplicity of management, and the formation of a stable compact disposal product indicate this system has great potential for the field remediation of U-contaminated waters.

Introduction

Natural attenuation of environmental contamination has demonstrated the capacity of living systems to remediate metal-contaminated water, including highly soluble radioactive metals such as uranium (U). Although single strain bacterial cultures can mediate the bioremediation of metal-containing waters (*1*−*6*), successful bioremediation has also been observed in ecosystems with a heterogeneous consortium of species that function in concert (*7*). These multispecies community structures and consortial activities provide important models for engineered remediation systems. This research was designed to simulate the heterogeneous character of natural ecosystems in the selection of a multifunctional consortium of microbes used in the treatment bioreactor. The source of the microbial consortium was a microbial mat, which historically has survived in extremely hostile conditions (*8*) and has been shown to be effective in the removal of toxic metals (*7*). This project explored the capacity of three constituent microbial groups of the mat to remove dissolved U, a common contaminant at U.S. Department of Energy (USDOE) and U.S. military sites.

The biological treatment of U-contaminated water requires an understanding of the U chemistry and the specific biological activity that affect its aqueous behavior. In nature, U exists as U(IV), U(V), and U(VI). Uranium(VI) is highly soluble and exists in solution as the ion group UO_2^{2+}, while U(IV) is sparingly soluble. Uranium(V) is of minor importance to remediation technologies because it is not expected to dominate U speciation in natural waters (*9*). Uranium(VI) forms soluble, negatively charged complexes with CO_3^{2-} such as $UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$ (*9*), which dominate U(VI) speciation in oxic waters with elevated CO_3^{2-} alkalinities. To examine the oxidation states of U in environmental samples such as soil and microbial materials, noninvasive and highly sensitive techniques such as X-ray absorption near-edge structure (XANES) spectroscopy using X-ray fluorescence (XRF) techniques have been successfully applied (*10*−*14*). XANES studies have shown that some U(IV) species are stable with U(VI) species in oxidized soils and sediments (*11*−*14*). The XRF microprobe can be used to obtain semiquantitative information on elemental constituents (*11*).

Studies with dissimilatory metal-reducing bacteria (DMRB) and dissimilatory sulfate-reducing bacteria (SRB) such as *Desulfovibrio (D.) desulfuricans* and *D. vulgaris*, which can use U(VI) as a sole electron acceptor, show that U(VI) is enzymatically reduced to U(IV) and removed from water (*1*−*3*). Mechanisms for the microbially mediated removal of U(VI) have been defined such as the surface sorption of U(VI) on cells, the abiotic reduction of U(VI) by SRB-produced H_2S, and the enzymatic bioreduction of U(VI) with U(VI) acting as a terminal electron acceptor (*1*−*5*, *10*, *11*).

In the natural environment, salt marsh estuaries function as sinks for U, and the presence of organic matter, dissolved Fe, together with reducing bacteria may promote U removal and stabilization in the sediment region (*15*). Although these marsh sediments maintain low redox potentials (Eh) and offer microbial processes for the reduction-stabilization of U(IV), surface sorption in the water column may be more important for achieving the rapid removal rates that are required at remediation sites. Studies from the San Joaquin Valley (SJV), CA describe effective natural processes relevant to the removal of U(VI) from the water column such as biosorption by algae (*11*, *16*). These observations are consistent with laboratory uptake studies (*17*). However, these studies (*17*) observed lower uptake of U(VI) in the presence of elevated levels of dissolved CO_3^{2-} than in its absence, suggesting that U(VI)−carbonate species have low affinities for algal sorption sites. In the SJV system, dispersed algal cells sorb U(VI) on their outer surface, and the cells eventually become deposited in the anoxic sediments, where the sorbed U(VI) can become reduced (when microbial energy sources

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are available) to U(IV) (*11*, *16*). The bioremediation system discussed below was designed to simulate the natural ecosystem conditions detailed above. Surfaces of microbial mats, dominated by filamentous cyanobacteria (*Oscillatoria*), can sequester U(VI) and settle to the bottom, where the biogeochemical conditions exist for reduction of U(VI) to U(IV). The capacity of microbial mats to remove many heavy metals and metalloids (*7*, *18*, *19*) and to degrade recalcitrant organics (*20*) while removing metals (*21*) makes mats an ideal biological material for use in bioremediation. Because the microbial mats are photosynthetic, the Eh can be regulated by shading the environment.

This study uses the natural advantages offered by mat constituent microbes and facilitates the engineering applications by providing a durable and hospitable housing for the microbial community. Live mat microbes were immobilized with required nutrients in silica gel, producing semisolid silica particles covered with mat cells (silica mat particles: SMP). The SMP were then tested for U(VI) removal and reduction to U(IV) in batch systems. The oxidation state speciation of the U associated with the SMP was determined using XANES spectroscopic techniques.

Materials and Methods

Natural microbial mats were collected from a freshwater environment in Florida and cultured in Allen/Arnon media with additions of 7 g L^{-1} ensiled grass clippings (*22*, *23*). Three of the microbial groups that constituted these mats were cyanobacteria (*Oscillatoria* sp), purple non-S photosynthetic bacteria (*Rhodopseudomonas*), and SRB (no specific identifications of this group were made). The three groups were subsequently separated from these initial mats, immobilized separately in 7% silica, and then recombined in 1:1:1 proportions for use as the treatment unit. Separation of the constituent microbial groups was done by selecting each of the three groups from a blended slurry of whole mats. Cultures dominated by *Oscillatoria* were prepared by incubating the mat blend in light and under aerated conditions. *Rhodopseudomonas* was isolated by selecting red colonies from nutrient agar plates that had been incubated in anaerobic jars in the light. Colonies were further characterized as to pigment content, which is characteristic of purple non-S photosynthetic bacteria. The SRB were selected by enrichment in a specific growth medium [0.42 g L^{-1} sodium acetate, 2.1 μM lactate, 3 g L^{-1} beef extract and 5 g L^{-1} peptone (Difco), 1.5 g L^{-1} each of $MgSO_4$ and Na_2SO_4]. The final consortium (called SMP) were prepared by adding 0.25 L of microbial slurry (prepared by blending) per L of microbial mineral medium with 7% silica and gelling the material by adjusting pH to between 8 and 9.5 (*22*, *23*). Before use in the experiments, a growth period of 5−7 days at 22 °C was needed to allow cells to cover the silica particles. Scanning electron microscopy (SEM) was used to verify the populations of microbes distributed over the silica surfaces and to characterize a precipitate that formed after the addition of dissolved U(VI).

The SMP were tested for effectiveness in U removal and reduction in the following experiments, which were performed at SMP concentrations that were 10% of the fluid volume. (1) A high volume experiment was performed outside in 200-L barrels as a single batch with time-series sampling to determine the consistency of high-volume treatment with bench-scale prototypes at 2.5 mg U(VI) L^{-1}. (2) Sequential batch experiments were performed to test the function and durability of the SMP over time and, consequently, the capacity to reuse the SMP for numerous treatments at U levels of 2 mg U(VI) L^{-1}. (3) Batch experiments using lower U(VI) concentrations in synthetic waters without dissolved CO_3^{2-} to test for the potential of the SMP system to remove low levels of U (0.05 mg U L^{-1}). (4) Batch experiments using Superfund groundwater samples with high dissolved CO_3^{2-} (CO_3^{2-} is a potential interference to sorption) and 0.2 mg U L^{-1}. Low U concentrations were used in experiments 3 and 4 to assess ability of SMP to remediate waters to levels below the U.S. recommended drinking water level (DWL) of 0.02 mg U L^{-1}. Batch experiments (2−4) were performed in triplicate with 250-mL volumes of the U-containing solutions. Dissolved U(VI) was added to the synthetic waters in the form of $U(VI)(NO_3)_2 \cdot 6H_2O$ (Aldrich). Dissolved U levels were measured after acidification in 5% HNO_3 by ICP (Inductively-Coupled Argon Plasma Spectrometry, Perkin-Elmer Instruments, detection limit (DL) of 3 μg U L^{-1}) for the 0.25 L batch systems or ICP-Mass Spectrometry (ICP-MS, Perkin-Elmer, DL of 0.1 ng U L^{-1}) for the 200-L barrel treatments. Superfund groundwater samples contained 293 mg HCO_3 L^{-1} and a pH of 8.2. All experiments were performed in the dark to eliminate photosynthesis; pH ranged between 8 and 9. To determine the leachability of U in the disposal product, U-rich SMP were harvested from the batch experiments, dried at 30 °C (to 1% of the initial wet weight), and placed on a shaker (1 g of dried SMP:0.1 L of deionized water) at 100 rpm for 2 h. The leachate (unfiltered) was analyzed for U concentration by ICP. Reducing conditions were assessed by two methods: the presence of extracellular reducing enzymes (reductases as in ref *24*) and Eh measurements in the water column above the SMP (Orion combined Pt redox and Ag/Ag reference electrode). Recovery of Eh as a function of nutrient additions was assessed by adding either ensiled grass clippings (*22*) (at 7 g L^{-1}) or 1.5 g L^{-1} beef extract and 2.5 g L^{-1} peptone (Difco), combined with extra carbon enrichments (lactic and acetic acid at 4 g L^{-1} each). The SMP were dispersed by the slight turbulence of the inflow water in the 200-L systems or by gentle hand-shaking for 10 s prior to each trial in the batch experiments.

SMP samples from the bottom of the batch sample containers were prepared for microprobe-XRF and -XANES spectroscopic analyses. The samples were analyzed in situ using the hard X-ray microprobe beamline ×26A at the National Synchrotron Light Source (NSLS, Brookhaven National Lab., Upton, NY) using a channel cut Si(111) monochromator. All samples were prepared and analyzed in the dark unless noted. Microfocusing optics were used to focus a monochromatic X-ray beam at the U L_3 absorption edge (17 166 eV) to a 15 μm^2 beam (*25*−*27*). Fluorescent X-rays were detected with a Si(Li) detector mounted at 90° to the incident beam and 2−3 cm from the sample. XRF spectra were collected for 120 s. To determine if the samples contained any spatial variability with respect to U, the microprobe X-ray beam was moved across the SMP samples, and the intensity of the fluorescence signal (the U Lα1 emission) was measured. For XANES, 10−35 s per scan point provided adequate counts, and the spectra were acquired at 0.3−2.5 eV step intervals over a 150 eV range. Standards consisted of synthetic $U(IV)O_2$ (uraninite) and $U(VI)O_3 \cdot 2H_2O$. XANES edge energies were defined as the height of the edge-step. Edge energy values were calibrated to be 0 eV with respect to the U(IV) standard and were monitored with UO_2 before and after each sample. An increase with respect to the relative XANES edge energy denotes an increase in the average U oxidation state in the sample or standard of interest and a linear relationship exists between the %U(VI) in the sample and the edge energy (*14*). The thermodynamic equilibrium speciation for U(VI) in the waters was calculated with the computer program MINTEQA2, the Davies Equation and a modified database (*9*, *28*−*30*, as described in ref *30*).

Results and Discussion

Silica and SMP Characterization. The goal in selecting appropriate microbial groups and immobilizing them on silica (Figure 1a) was to provide a system that would rapidly

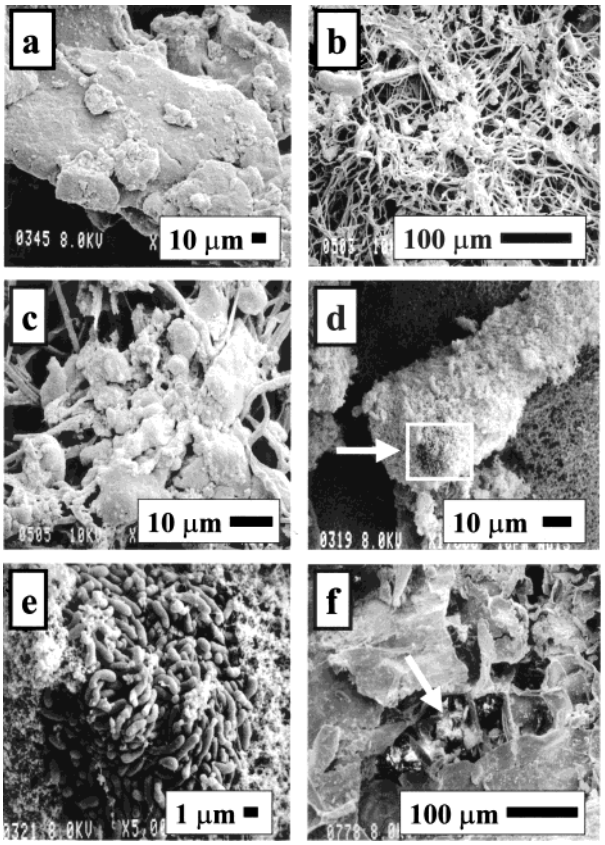

FIGURE 1. SEM of (a) silica particles without attached microbes (silica control), (b) microbial mat: predominantly filamentous (high surface area) cyanobacteria, *Oscillatoria*, with entrapped silica particles with (c) enlargement of central region illustrating cells attached to silica particles, (d) middle left: silica particle with patch of attached *Rhodopseudomonas* (shown with arrow) with (e) middle right: an enlargement of microbial patch embedded in silica from (d) and (e) black granular precipitate (mineral-rich particles shown with arrow) that was formed on the treatment tank bottom after the addition of U(VI). SRB are not shown.

bind the U(VI) and, by way of particle density, subsequently deposit the U-rich SMP at the bottom of the treatment tank. Here the dense biological activity of the immobilized microbes would provide ideal conditions for reduction of sequestered U(VI) to the U(IV) species. The filamentous *Oscillatoria* (attached to silica are shown in Figure 1b,c) was incorporated at equal proportions as the SRB (not shown) and as the *Rhodopseudomonas* (attached to silica Figure 1d,e). The three principal microbial groups of microbial mat provide a wide range of mechanisms and many ideal characteristics relevant to the removal of U from solution for the bioreduction of U(VI) (to be discussed).

Maintenance of microbial populations and function is most problematic in systems employing microbes for bioremediation tasks. However, in this system, when nutrients are entrapped with the microbes in the silica gelling process, cell growth continues over the silica particle surfaces, making dense, stable populations that will not wash out into the solution. When particles are grown with vigorous mixing in air, the resulting particles are dense filaments of cells held together by entrapped silica particles. These data (Figure 1b−e) show that the silica with nutrients provide a long-term hospitable environment for the consortium. Identifying the best nutrient supplements and determining the appropriate application strategy is an area of continuing research. In terms of system management, the particle density is central to the successful function of the treatment system. Because the density of the SMP is close to but slightly greater than water, particles were easily dispersed throughout the water column by slight turbulence, yet rapidly settled to the bottom during quiescent periods, leaving a relatively metal-free water column. Water was easily decanted from the area above the bottom deposits of SMP. A granular black precipitate (0.25−0.5 mm in diameter) that formed on the bottom of the batch experimental units by the first day of batch treatment was isolated (Figure 1f). This material, which appears to contain remnants of SMP and a mineral precipitate (see arrow in Figure 1f) was characterized (described below). The quantity of precipitate increased over time during the uptake experiments.

Uranium-Removal Experiments. Figure 2a illustrates the rapid removal of U in the 200-L barrel experiment (88% in 15 min), and after 4 h, this system removed U to levels below the U.S. recommended DWL. The rapid rate with which the SMP removed the U(VI) suggests the initial removal mechanism was surface sorption, not bioreduction. Maintaining the 200-L barrel under dark conditions was effective at decreasing the Eh levels for the duration of the experiment. Laboratory prototypes indicate a continuous trend of decreasing Eh, to levels as low as −100 to −200 mV, sometimes reaching much lower levels for short periods. Although sequential batches were not performed with the 200-L barrel experiment, bench-scale experiments shown in Figure 2b indicated that at least six sequential batch treatments could be done before providing nutrient support to the SMP or replacing the SMP cells. The silica controls were saturated by batch three, with no indication of recovery over time (Figure 2b). Silica (Figure 1a) provides some surface binding capacity of U in the absence of mat microbes, but it does not possess the same surface area or reducing properties and low Eh environment as the SMP. As sequential batches are treated by the SMP, the Eh also rises from −100 mV to +270 mV or more, thereby indicating loss of the low Eh environment, which is conducive to U(VI) reduction (Figure 2b). This may be due to a number of changes within the SMP, in addition to saturation of U-binding sites. Washout of reducing enzymes and loss of nutrients supporting the metabolic activity of the cells can be important to the process of U uptake and reduction. Recovery of the low Eh environment was achieved by adding nutrients (bacterial growth powder or ensiled grass clippings) which lowered the Eh to below −100 mV. Treatment of six batches of 2 mg U L^{-1} (Figure 2b) produced a disposal product containing 1% U (dry wt of SMP). When air-dried the SMP were slightly vitric in appearance and upon strong agitation in deionized water, they did not resolubilize to levels above 3 μg U L^{-1}. Data from low-concentration batch experiments shows that SMP can remove 99% of 0.05 mg U(VI) L^{-1} in 15 min to nondetectable levels (<3 μg U L^{-1}) in 4 h (data not shown). Results from thermodynamic equilibrium calculations (*9, 28−30*) which assumed U(VI) was the dominant oxidation state of U present during the exposure of the U to the SMP predicted that the dominant U species were $UO_2(OH)_3^-$ and $UO_2(OH)_2^o$ in the synthetic low CO_3^{2-} waters at 0.05−2.5 mg U L^{-1} (Figure 2a,b).

Since U(VI) forms soluble complexes with CO_3^{2-} that may not sorb, tests were performed to determine the capacity of the SMP to remove U(VI) presented in this potentially problematic form. Data in Figure 2c show the treatment capacity of samples taken from a high-CO_3^{2-} alkalinity groundwater containing U(VI) contamination from a Superfund site. The U was removed by SMP (and the silica control initially) at rates similar to that of U(VI)−hydrolysis species removals (Figure 2b). The predicted equilibrium speciation of U(VI) in the CO_3^{2-} waters was dominated by $UO_2(CO_3)_3^{4-}$ (73%) and $UO_2(CO_3)_2^{2-}$ (26%) species. The mechanisms for the removal of U are unknown; however, this phenomenon is observed in other anaerobic microbial systems.

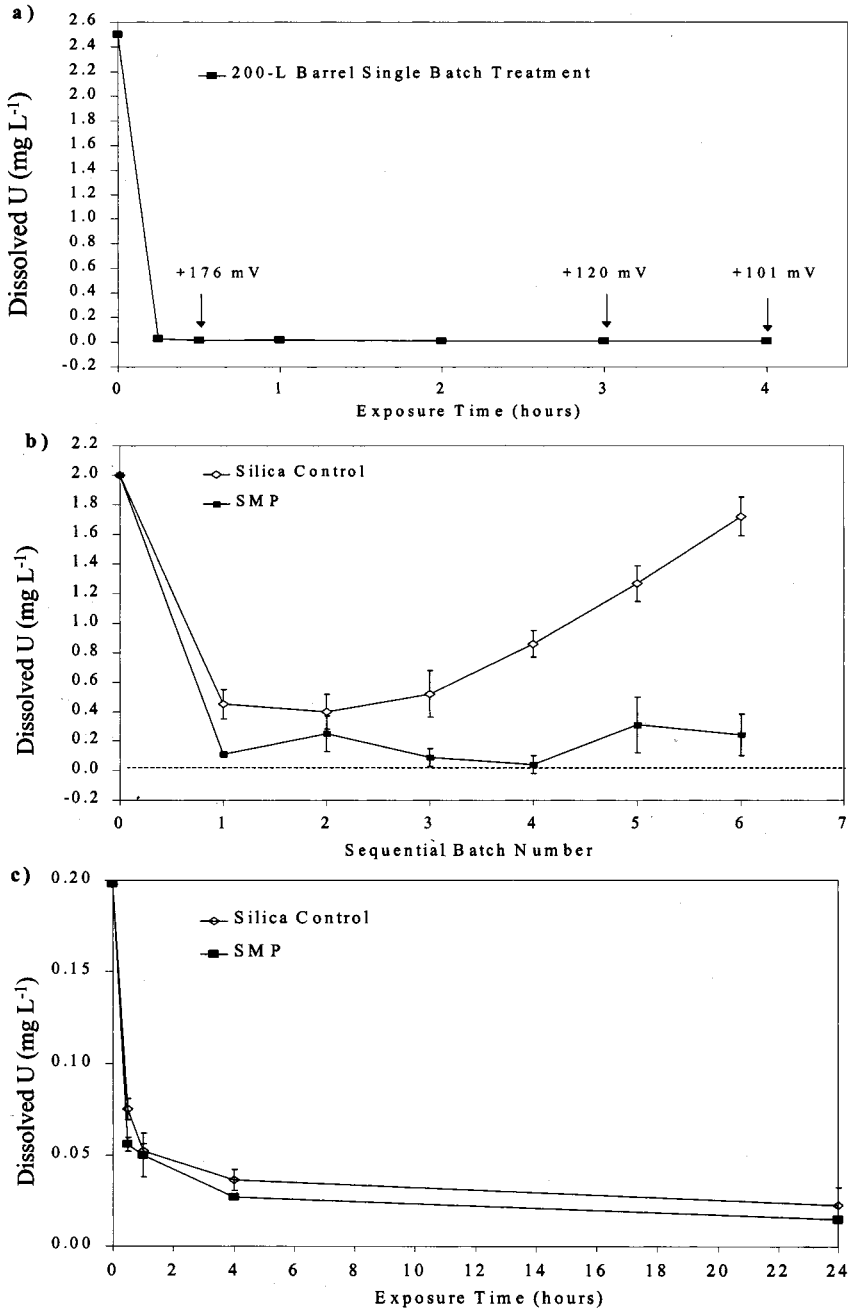

FIGURE 2. Removal of U(VI) from solution for (a) the high-volume treatment in 200-L barrel (with time-series Eh measurements indicated with arrows), (b) the sequential batch treatments, and (c) the batch treatment with the high CO_3^{2-} alkalinity groundwater. The U(VI) in the solutions used in (a) and (b) was predicted (based on thermodynamic calculations) to be present as U(VI)−hydrolysis species. It was expected that the silica controls in (b) would initially show similar removal rates, since the binding sites were not saturated by one treatment batch.

Uranium(VI)−carbonate complexes can be removed from solution and bioreduced to U(IV) by pure cultures SRB and DMRB (*1, 10, 31)*. Calculations of the reduction potentials for the reduction of U(VI)−hydroxide and −carbonate complexes indicate that in the absence of kinetic limitations, U(VI)−hydroxide species [such as $UO_2(OH)_2^o$] are more likely to be reduced than U(VI)−carbonate complexes (*10*).

SMP and Precipitate Characterization with XRF and XANES Techniques. The micro-XRF analyses indicate the SMP are typically rich in U (inset, Figure 3), and the U distribution was spatially homogeneous on a 15 μm scale. To examine the capabilities of the SMP to reduce U(VI), the average oxidation state of U in the SMP was determined using XANES (Table 1) spectroscopy. The XANES spectra for the standards and the SMP samples from the sequential batch treatments (from Figure 2b) are shown in Figure 3. The XANES data indicate that all of the U(VI) was reduced to U(IV) in the SMP (Figure 3, Table 1). Although these uptake studies were performed in the dark, short periods in light and O_2 (air) produce some oxidation of the U(IV) to U(VI) in the SMP (Table 1). A dark environment eliminates photosynthesis by the cyanobacteria and enhances the heterotrophic functions of the microorganisms, thereby removing residual O_2 and maintaining low Eh. Complete oxidation of the U in the air-dried SMP was observed (Figure 3, Table 1), suggesting the air-drying process promotes the oxidation of U(IV) to U(VI) in air—as shown in other studies (*10*−*12*).

Micro-XRF analyses of the granular black precipitate (Figure 1f) that formed on the bottom of the batch experimental units indicate it was enriched in U, Cl, Ca, Fe, Cu, Zn,

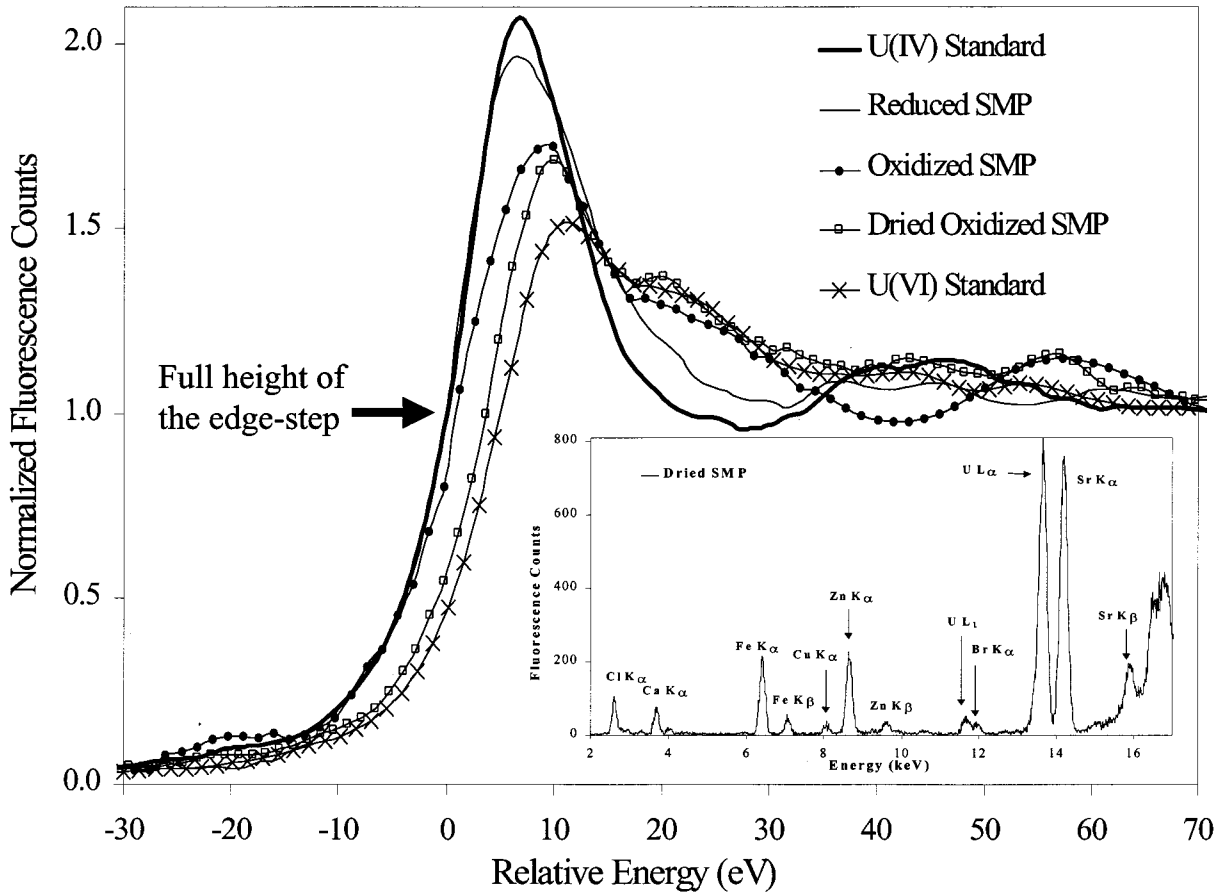

FIGURE 3. Uranium L_3 XANES spectra for the standards [U(IV) and (VI)] and for the U-rich SMP samples plotted with respect to the relative XANES edge energy. A shift in the absorption edge energy with average U oxidation state is noted at the full height of the edge step as labeled with an arrow. The inset contains micro-XRF spectra for the U-rich SMP samples.

TABLE 1. Redox Potential and Percent U(VI) Data (as Determined by XANES) for the U(IV) and U(VI) Standards from and the SMP Samples from Sequential Batch Studies with Synthetic U-Containing Water[a]

treatment or sample description	Eh (mV)[b]	percent of total U as U(VI) in sample
U(IV) standard	NA[e]	0
U(VI) standard	NA[e]	100
reduced SMP	−68	0[d]
dried oxidized SMP[c]	ND[f]	100[c]
reduced SMP, after 3 h in air and light	ND[f]	35
reduced SMP, 16 h in air and light	ND[f]	20
black precipitate	−170	35
black precipitate after 3 h in air and light	ND[f]	50

[a] The U L_3 XANES edge energies were normalized to 0 eV. XANES energy values were based on the full height of the normalized edge-step of the absorption edge and the end member values for the U(IV) and U(VI) standards were used to calculate the relative proportion of U(IV) and U(VI) in the sample. Error estimates for the average U oxidation state determinations with XANES are near 10%. Uranium concentrations in the silica control were below the detection limit for XANES (3 mg U kg^{-1}). [b] Redox potential values were determined in the water column above the SMP. [c] Average values for two samples. [d] Average values for three samples. [e] NA: not applicable. [f] ND: not determined.

Br, and Sr (data not shown). The distribution measured by micro-XRF of the U-rich precipitate material was heterogeneous on a 30-μm spatial scale—a finding that concurs with the size of the 10−20-μm sized mineral particles in the SEM photomicrograph (see arrow in Figure 1f). XANES studies with this granular material indicate that the predominant form of U is U(IV) (Table 1). After 3 h of exposure to air and light, the black precipitate turned near colorless, and XANES spectra indicate that the U(IV) became oxidized to U(VI) (Table 1). X-ray diffraction and Fourier transform-infrared spectroscopy studies with black and air-oxidized precipitates indicate they are highly amorphous and Si-rich, but no U solid phases were identified (unpublished data, M.C. Duff).

Summary of Mechanisms. A system of immobilized microbes containing a mix of microbial groups rapidly removed U(VI) and reduced it to U(IV). Several likely mechanisms that are offered by this microbial consortium and their possible synergistic effects are discussed below and in Figure 4. The initial reaction is sorption (Figure 4). At neutral to basic pH values, the surfaces of amorphous silica and filamentous *Oscillatoria* carry a negative surface charge (*32*, *33*) and can offer a large surface area for initial sorption of U in the water column. The initial process of surface sorption is central not only for rapid removal of U(VI) but also for anchoring the U(VI) regions of high enzyme activity on the SMP. The rationale for increasing the populations of *Rhodopseudomonas* and SRB above that of their natural occurrence in mats was to benefit from their production of broad spectrum reductases, which might be used for the bioreduction of U(VI) in the system. Enzyme analyses show that these *Rhodopseudomonas* release an extra-cellular reductase, which probably has a broad-spectrum function, since the *Rhodopseudomonas* have the capacity to reduce Se(VI) and Cr(VI) (*34*). After the SMP-bound U(VI) settles, a compact region of dense microbial activity is generated at the bottom of the treatment container (Figure 4), where the concentrations of reductases will be highest and Eh levels

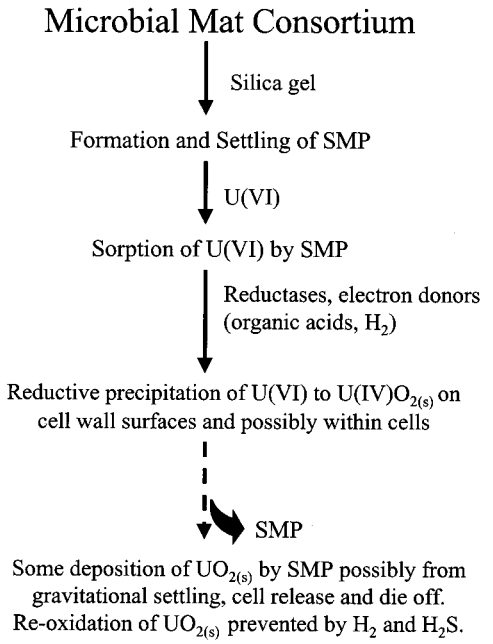

FIGURE 4. Diagram of likely processes that facilitate the removal of dissolved U in the SMP system in the dark.

lowest. Sequestered U(VI) can be reduced by microbial activities, particularly since the SMP provide a surface by which reduction can occur. Natural surfaces are required for the reduction of U as shown in studies with waters associated with anoxic marine sediments in the Black Sea (*35*).

Additionally, microorganisms such as cyanobacteria that contain nitrogenase enzymes can generate H_2 under conditions of $N_{2(g)}$ deprivation and low Eh (*34, 36, 37*). Although H_2 can be reabsorbed into the cell if N_2 again becomes available, in the absence of N_2, H_2 continues to be released as long as a C reductant (such as sugar or similar nutrients) is present in the cell (*34, 36, 37*). The organic acids present in the ensiled grass clippings support the production of H_2 (by the cyanobacteria). Hydrogen can facilitate the reduction of U(VI) by SRB (*3, 5, 6*). This has been shown with pure strains of SRB (such as *D. vulgaris*) via a membrane-bound cytochrome c_3 enzyme when coupled with a hydrogenase enzyme (*3, 5*). Cell membrane surface reduction of U(VI) by indigenous bacteria isolated from a U-contaminated site at Tuba City (AZ) has been observed (*38*). These results indicate membrane-bound reductases may be found in microbial species that have evolved in U-contaminated water, suggesting that the efficacy of the SMP system might be enhanced by increasing the number of active bacterial species in the consortium and by incorporating other microbial groups that are efficient in U(VI) reduction, such as *Shewanella putrefaciens* (a DMRB) (*10*) into the SMP complex.

Biogenic reduced metal precipitates have been observed in studies with SRB, which used H_2 as an electron donor (*6, 10*). *D. desulfuricans* use a periplasmic reductase to reduce the soluble radionuclide species pertechnate [Tc(VII)] to a sparingly soluble Tc(IV)$O_{2(s)}$ precipitate that deposits in the cells and on the cell periphery (*6, 10*). When the U(IV) precipitate is released from binding sites on the SMP (which constantly change in number and type as portions of the consortium grow and die over time) and collect on the vessel bottom (Figures 1f and 4), U(VI) can again be sorbed to the SMP and bioreduced. Additionally, H_2 and H_2S (produced by the SRB), which help to maintain reducing conditions for the active cells (Figure 4), help to preclude oxidation of U(IV) in the precipitate.

As reduction processes followed surface sorption, release of the U(IV) precipitate from the binding sites may have occurred, exposing new sites for subsequent sorption of U(VI) (Figure 4). Over time, as the reducing capacity diminished, the SMP began to lose the ability remove U. Recovery of low Eh was accomplished by nutrient additions. Because the rate of U(VI) reduction is central to this remediation process, the potential biological and chemical mechanisms that are uniquely contributed by each constituent microbial group used in the system must be considered. In summary, the SMP offer major advantages over the silica control because of the reducing properties and consortium activities of the SMP.

Potential of the SMP System in Field Applications. In summary, the results from a barrel experiment demonstrated that a high volume treatment followed the same general dynamic as the bench-scale studies and that a barrel or pond could be used as simple bioreactor. Results of the sequential batch experiment demonstrated the persistence of U removal by the SMP and provided information about the amount of U (1% dry weight) that could be removed by SMP. Results of the Superfund groundwater experiment that tested the potential of the SMP to remove U(VI)−carbonate complexes indicate more research should be done with other U-containing groundwater samples. The XANES analyses provided basic evidence that U(VI) was reduced to a less mobile form.

This microbial material and silica provided an ideal biological community for U(VI) removal. The durability and multifunctional activities of the SMP provided biological stability and a potentially renewable source of mechanisms for continuous U removal by natural reduction processes. Mat immobilization as SMP contributes several field advantages. Live SMP can be easily managed in the field because they withstand seasonal variations and the SMP can be kept alive and functional by nutrition additions. Nutrient costs are minimized because particulate nutrients can be added to the bottom deposits of SMP during quiescent times, eliminating the necessity of supplying nutrients to the entire water column. Nutrient supplement in the form of ensiled grass clippings is an attractive option because it represents a low-cost source of nutrients, readily available on field sites having grass. Removal of U(VI) was observed in waters that promoted the formation of U(VI)−hydrolysis and −carbonate species, which are likely to be found in oxic, neutral to basic surface and subsurface waters (*10*−*12*, *38*). This capability of U removal under these conditions demonstrates the versatility of this system for the bioremediation of natural U-contaminated waters.

The SMP system is relatively easy to produce on site and maintain in a field environment. Photosynthetic processes provide long-term support of the SMP. Preliminary field research showed no deterioration of microbial populations or strain integrity in SMP held in an outside pond for over 6 months (400-L of SMP in a 4000-L water column). By simply storing the SMP in light, the entire consortium is supported through photosynthetic processes of the cyanobacteria. As reducing conditions are required, the SMP can be transferred to an environment of total dark or partial shade. The microbial community will spontaneously generate a reducing environment and shift the microbial populations within the consortium to the desired anaerobic species.

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