Uranium Reduction

Judy D. Wall[1] and Lee R. Krumholz[2]

[1]Biochemistry and Molecular Microbiology & Immunology, University of Missouri-Columbia, Columbia, Missouri 65211; email: wallj@missouri.edu

[2]Department of Botany and Microbiology and Institute for Energy and the Environment, University of Oklahoma, Norman, Oklahoma 73019; email: krumholz@ou.edu

Annu. Rev. Microbiol. 2006. 60:149–66

First published online as a Review in Advance on May 10, 2006

The *Annual Review of Microbiology* is online at micro.annualreviews.org

This article's doi:
10.1146/annurev.micro.59.030804.121357

0066-4227/06/1013-0149$20.00

Key Words

c-type cytochromes, nanowires, uraninite, uranium reoxidation, bioremediation

Abstract

The dramatic decrease in solubility accompanying the reduction of U(VI) to U(IV), producing the insoluble mineral uraninite, has been viewed as a potential mechanism for sequestration of environmental uranium contamination. In the past 15 years, it has been firmly established that a variety of bacteria exhibit this reductive capacity. To obtain an understanding of the microbial metal metabolism, to develop a practical approach for the acceleration of in situ bioreduction, and to predict the long-term fate of environmental uranium, several aspects of the microbial process have been experimentally explored. This review briefly addresses the research to identify specific uranium reductases and their cellular location, competition between uranium and other electron acceptors, attempts to stimulate in situ reduction, and mechanisms of reoxidation of reduced uranium minerals.

Contents

INTRODUCTION

Uranium in the environment occurs primarily as 3 of its 17 known isotopes, ^{238}U (99.27%), ^{235}U (0.72%), and ^{234}U (0.005%). All are radioactive; however, it is the chemical toxicity that is of greatest ecological risk (54). Being the forty-ninth most abundant element in the Earth's crust, uranium is not rare. Unfortunately, the anthropogenic use of uranium for nuclear research, fuel production, and weapons manufacturing has resulted in widespread environmental contamination. Additional contamination has resulted from trace amounts of uranium being released from the combustion of coal as well as from the manufacture and application of phosphate fertilizers (54).

In the United States, the end of the Cold War brought about the shutdown of nuclear weapons production and efforts for the decommissioning and reprocessing of weapons material were initiated. With this shift in focus, the U.S. Department of Energy (DOE) began efforts to identify and remediate contaminated areas. This included 120 sites covering 7280 km^2 in 36 states and territories, most of which are contaminated with uranium (64). Seeking to achieve this goal of remediation quickly, with reasonable expense and a minimum of environmental disruption, the DOE has been the driving force for the examination of microbial processes that can be exploited for cleanup.

Bioremediation of metals and radionuclides, including uranium, is distinctly different from the biodegradation of toxic organic substances. With the proper microbe or combination of microbes, the latter generally can be degraded to innocuous compounds or completely to inorganic constituents, CO_2, minerals, and water. Because toxic metals cannot be degraded, their remediation depends on a method of containment that decreases bioavailability and/or biological access. A first step may be to increase contaminant mobility for extraction or the choice may be to immobilize the metal through sequestration, complexation, or changes in speciation that reduce solubility (27). All these capacities are exhibited in the microbial repertoire and should be understood so that practical applications can be intelligently designed and predictions of long-term stewardship can be accurately made.

For bioremediation of uranium-contaminated waters, the chemistry of the element offers an approach that has received much attention in the last 15 years. In oxic waters and soils, uranium is present primarily as soluble salts of the uranyl ion (UO_2^{2+}). When reduced from this U(VI) oxidation state to U(IV), the solubility decreases, resulting in immobilization (44). The list of bacteria known to reduce U(VI) is growing (**Table 1**), yet a complete understanding of the biochemistry of this process in any one bacterium is lacking. Current views of the

DOE: Department of Energy

Table 1 Bacteria shown to reduce U(VI) to U(IV)

Bacterium	Reference(s)	Bacterium	Reference(s)
Anaeromyxobacter dehalogenans strain 2CP-C	78	*Desulfovibrio sulfodismutans* DSM 3696	51
Cellulomonas flaigena ATCC 482[a]	82	*Desulfovibrio vulgaris* Hildenborough ATCC 29579	51
Cellulomonas sp. WS01	82	*Geobacter metallireducens* GS-15	50
Cellulomonas sp. WS18	82	*Geobacter sulfurreducens*	38
Cellulomonas sp. ES5	82	*Pseudomonas putida*	4
Clostridium sp.	22	*Pseudomonas* sp.	4
Clostridium sphenoides ATCC 19403	21	*Pseudomonas* sp. CRB5	57
Deinococcus radiodurans R1	24	*Pyrobaculum islandicum*	39
Desulfomicrobium norvegicum (formerly *Desulfovibrio baculatus*) DSM 1741	51	*Salmonella subterranea* sp. nov. strain FRC1	87
Desulfotomaculum reducens	92	*Shewanella alga* BrY	14, 93
Desulfosporosinus orientis DSM 765	91	*Shewanella oneidensis* MR-1 (formerly *Alteromonas putrefaciens*, then *Shewanella putrefaciens* MR-1)	50
Desulfosporosinus spp. P3	91	*Shewanella putrefaciens* strain 200	11
Desulfovibrio baarsii DSM 2075	51	*Veillonella alcalescens* (formerly *Micrococcus lactilyticus*)	99
Desulfovibrio desulfuricans ATCC 29577	49	*Thermoanaerobacter* sp.	77
Desulfovibrio desulfuricans strain G20 (to be renamed *Desulfovibrio alaskensis*)	68	*Thermus scotoductus*	43
Desulfovibrio sp. UFZ B 490	72, 73	*Thermoterrabacterium ferrireducens*	42

[a]Data for reduction by *Cellulomonas* strains has now been questioned, and evidence for precipitation by phosphate has been obtained (88).

microbial reduction of U(VI) are the topic of this review.

ESTABLISHMENT OF MICROBIAL URANIUM REDUCTION

Microbial reduction of U(VI) was first reported in crude extracts from *Micrococcus lactilyticus* (reclassified as *Veillonella alcalescens*) by assaying the consumption of hydrogen dependent on the presence of U(VI) (99). These experiments were performed with crude extracts with few controls provided. This apparent observation of enzymatic reduction was not pursued for nearly 30 years (50). During the interval, it was generally believed that abiotic processes were responsible for the production of U(IV) in anaerobic or low redox environments, by processes that included reduction by sulfide, Fe(II), or hydrogen.

Because environmental reduction of U(VI) was accepted to be abiotic (40), several lines of evidence were necessary to establish microbial reduction and, subsequently, the potential ecological and geological importance of this process. Work from Lovley and coworkers (29, 49, 50) was pivotal in establishing microbial U(VI) reduction by dissimilatory metal-reducing bacteria (DMRB). In assays with pure cultures of the Fe(III)-reducing bacteria, *Geobacter metallireducens* strain GS-15 and *Alteromonas putrefaciens* (later *Shewanella putrefaciens*), conversion of U(VI) to insoluble U(IV) was followed as a decrease in absorption of U(VI) at 424.2 nm with a directly coupled plasma spectrometer after separation of the two U forms by ion exchange chromatography (50). Subsequent assays based on the differential phosphorescence of the two redox species used a sensitive pulsed nitrogen dye laser and a proprietary complexing

Abiotic: absence of living cells

DMRB: dissimilatory metal-reducing bacteria

E_h: reduction potential

Humic substances: complex organics in soil derived from partial degradation of plant and microbial organic material

agent (Chemchek Instruments, Richland, WA) to determine the decrease in soluble U(VI) (50). It was shown that live cells and an oxidizable substrate were necessary for the U(VI) transformation. During the time course of the experiments, no reduction of U(VI) by Fe(II) in the growth medium could be detected. In addition, cells washed free of Fe(II) reduced U(VI) without a lag when provided a metabolic source of reductant (50).

Later experiments demonstrated that the nearly ubiquitous sulfate-reducing bacteria of the genus *Desulfovibrio* were capable of U(VI) biotransformation to U(IV) in bicarbonate buffers (49, 51). Again, live cells provided with an oxidizable substrate were necessary. Controls for abiotic reduction by the metabolic end product sulfide demonstrated that the enzymatic process was far more rapid. Importantly, the temperature profile of the reduction process followed that of cell growth (49). However, it was fortuitous that bicarbonate buffers were used in these experiments, because later research showed that sulfide does rapidly reduce U(VI) in the absence of the stable carbonate complexes (10). **Table 1** shows the variety of bacteria currently reported to be capable of U(VI) reduction. The only common factor evident is the ability of all bacteria to grow anaerobically where a redox potential sufficiently low for U(VI) reduction would be established.

URANIUM REDUCTASES

Bioavailable Uranium Complexes

The identity of the U(VI) reductase and the pathway of electron flow from substrates to the enzyme have been sought in an attempt to understand the enzymatic process, to make an evaluation of the ecological distribution of the potential for uranium reduction, to identify any amendments that might stimulate the reduction, and to determine the possibility of genetic manipulation to increase the amount or activity of the reductase. The accumulated data have not yet identified dedicated reductases. Because uranium is not known to be an essential component of any enzyme or biological structure, the evolutionary pressure for tailoring an efficient system for reduction may not exist. To identify the enzymes/proteins responsible for reduction, it is important to consider what uranium substrates are encountered. Bioavailability and access to reductases are dependent upon the speciation of the metal.

In oxic surface waters, UO_2^{2+} and UO_2OH^+ are present and form stable, soluble complexes with carbonate, phosphate, and humic substances (54). The formation of these complexes is governed by pH, E_h (reduction potential), temperature, and ligand concentration. Because of the complexity of the interactions, a number of modeling programs for predicting speciation based on thermodynamic considerations have been used (e.g., MINTEQA2, PHREEQE, or MINEQL). The equilibrium speciation model HARPHRQ predicted that the U(VI) species in fresh oxic waters (with <40 mg $CaCO_3 \cdot l^{-1}$) would be $(UO_2)_2(OH)_3CO_3^-$ and $UO_2(OH)_3^-$ with pH values ranging from 5.0 to 8.5 (54). In groundwater, higher carbonate concentrations are often present and stable carbonate complexes predominate (29). With a 30-mM bicarbonate buffer and neutral pH, $UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$ account for essentially all the U(VI) present (25, 82). These complexes were still readily reduced by most DMRB (50, 71); however, *Desulfotomaculum reducens* (92) and *Desulfosporosinus orientis* (91) were unable to reduce carbonate complexes of U(VI). Whether that was the result of an inaccessibility of the complex to the reductase or an inhibition of cells by the high ionic strength of the test buffer has yet to be determined. The formation of uranyl complexes with organic ligands also made U(VI) differentially accessible to *Shewanella algae* and *Desulfovibrio desulfuricans* (28). The disparity in access was suggested to reflect differences in location and function of the uranium reductases (28), because *S. algae* can

respire U(VI) for growth, whereas *D. desulfuricans* cannot (49).

More recently it has been recognized that where limestone ($CaCO_3$) contributes to the dissolved minerals, the Ca^{2+} concentration in environmental waters should be included in equilibrium speciation modeling. When the U(VI) species in groundwater from Tuba City, Arizona, were predicted with the inclusion of Ca^{2+}, essentially all the U(VI) was in calcium complexes, $Ca_2UO_2(CO_3)_3$ and $CaUO_2(CO_3)_3^{2-}$, 99.3% and 0.3%, respectively (12). In pure cultures, the addition of increasing concentrations of Ca^{2+} to assays for U(VI) reduction by the DMRB, *S. putrefaciens* CN32, *Geobacter sulfurreducens*, and *D. desulfuricans* slowed reduction dramatically. These results were interpreted to mean that the Ca^{2+} complexes were less effective electron acceptors because their redox potentials were lower (12). The common components of environmental waters, calcium and carbonate, must be considered when the application of natural or accelerated bioremediation is planned.

Enzymatic Reduction by One Electron or Two?

The reduction of U(VI) to U(IV) requires two electrons; however, the mechanism of that microbial electron delivery has not yet been conclusively elucidated for any DMRB. Gorby & Lovley (29) noted that the uncharged species UO_2 would precipitate from solution as the mineral uraninite but that it did not do so immediately upon formation. The cause for this delay remains to be explained, but characteristic nanoparticles of uraninite have been seen to accumulate in the periplasm and around cells reducing U(VI) (55, 90, 91, 101).

The possibility of a one-electron reduction process that would initially generate U(V) was cleverly explored with *G. sulfurreducens* (76). X-ray absorption spectroscopic evidence was obtained for formation of this one-electron intermediate during U(VI) reduction, and complete conversion to U(IV) with time was documented. To generate U(IV), two alternative mechanisms were postulated. The unstable U(V) complexes could disproportionate to form U(IV) and U(VI), or the U(V) species could be a substrate for further enzymatic reduction. Np(V) was used as a proxy for U(V) (since NpO_2^+ does not disproportionate) and the researchers showed that *G. sulfurreducens* did not reduce this pentavalent actinide (76). By extrapolation, they concluded that U(V) was an unlikely substrate for reduction as well. Thus single-electron reduction of U(VI) to U(V) followed by disproportionation was suggested to be the likely mechanism of uranium reduction (76).

Cellular Location of UO_2 Precipitates

Because of the insoluble nature of U(IV) oxide, the site of deposition should give an indication of the location of the reductase. Many researchers have examined DMRB that were reducing U(VI) using unstained transmission electron microscopy (TEM) images and have confirmed uraninite both outside of the cells (46, 47, 49, 101) and accumulated in the periplasm of gram-negative DMRB (46–48, 55). Interestingly, for the gram-positive bacterium *Desulfosporosinus*, uraninite was found in an analogous location, concentrated in the region between the cytoplasmic membrane and the cell wall (91). These results would point to a uranium reductase on the periplasmic (outer) face of the cytoplasmic membrane or in the periplasm itself.

Uraninite deposits within the cytoplasm of a pseudomonad and *D. desulfuricans* strain G20 have also been reported (57, 80). The pseudomonad isolated from a site formerly used for treating wood for preservation removed U(VI) from solution under aerobic or anaerobic conditions. When TEM thin sections of those cells were examined, U(IV) was found inside as well as concentrated at the envelope. Because uranium has no biological function and is toxic, the observation of its precipitation in the cytoplasm was unexpected. McLean & Beveridge (57) speculated

Uraninite: mineral formed from precipitated $UO_{2(s)}$

Periplasm: area between the cytoplasmic membrane and the cell wall of gram-negative bacteria

TEM: transmission electron microscopy

that the polyphosphate granules present in the pseudomonad might protect the cell by forming strong complexes with uranium, thus sequestering it in the cytoplasm.

The internal deposition of uraninite observed in *D. desulfuricans* G20 occurred in cells that had been grown in a medium designed to limit heavy metal precipitation and maximize toxicity (80). In order to prevent the formation of strong complexes, the medium had no specifically added carbonate or phosphate (79). Such a modification could also alter the physiology of the bacterium, stimulating uptake systems that might allow access of the toxic metal to the cytoplasm. Cytoplasmic deposition of U(IV) has not been reported from other studies with *Desulfovibrio* (4, 49, 51), and future analysis on the effects of nutritional stresses on U(VI) reduction may prove interesting.

With the exception of these rare reports of cytoplasmic uraninite, the localized precipitation of insoluble U(IV) in the periplasm and outside of both gram-negative and gram-positive cells suggests that U(VI) complexes do not generally have access to intracellular enzymes. Thus the best candidates for reductases would be electron-carrier proteins or enzymes exposed to the outside of the cytoplasmic membrane, within the periplasm, and/or in the outer membrane.

Desulfovibrio vulgaris Reductase(s)

Two approaches were used to identify U(VI) reductases in DMRB, biochemical (52) and genetic (7, 9, 68, 86, 96). For enzymatic identification, crude extracts of bacteria known to reduce this actinide, such as *G. metallireducens*, *S. putrefaciens*, and *Desulfovibrio vulgaris* Hildenborough, were prepared and tested for activity (52). Only in the *D. vulgaris* cell-free preparation could U(VI) reductase be demonstrated with 95% of the activity present in the soluble fraction. Further fractionation showed that removal of the abundant tetraheme cytochrome c_3 eliminated the activity. Finally it was established that a periplasmic hydrogenase in combination with the cytochrome c_3 was necessary and sufficient for U(VI) reduction by hydrogen (52). The involvement of tetraheme cytochrome c_3 was later confirmed in whole-cell experiments showing that reduced cytochrome c_3 was oxidized during U(VI) reduction, but not during sulfate reduction with H_2 as the donor (19). Further evidence that cytochrome c_3 was biologically important for *Desulfovibrio* U(VI) reduction came from the impairment of this process when a mutation was constructed in a related strain that eliminated the homologous cytochrome (68). U(VI) reduction by this mutant *D. desulfuricans* strain G20 was inhibited by at least 90% with H_2 as the electron donor. However, with organic acids providing reductant, the reduction rates were decreased only 50% to 70%, demonstrating that additional proteins capable of metal reduction were present (68). This alternative reductase(s) remains to be identified. In vitro experiments with purified type 1 cytochrome c_3 from *D. desulfuricans* G20 have shown that the reduced protein can be oxidized by U(VI) very rapidly (M. Pattarkine & J. Wall, unpublished data); however, the in vivo rate of U(VI) reduction is far slower (68). This incongruity might indicate that cellular reductant is limiting or that U(VI) accessibility to the reduced cytochrome is somehow restricted.

Shewanella Reductase(s)

To date, only four strains have been reported to gain sufficient energy from U(VI) respiration to support growth: *S. putrefaciens* (49), *G. metallireducens* (49), *Desulfotomaculum reducens* (87), and *Thermoterrabacterium ferrireducens* (41). For those bacteria able to grow by metal oxide reduction, it was logical to assume that the reductases responsible might also be those functioning to reduce U(VI). Early work with "*Pseudomonas* sp." (now *S. putrefaciens*) showed that cells limited for Fe were unable to use Fe(III) as a terminal electron acceptor (66). These cells also lost their

orange color and reduced-minus-oxidized spectra indicated a major decrease in *c*-type cytochrome content (66). The interpretation of these observations was that cytochromes were involved in the transfer of electrons to the terminal electron acceptor or were the terminal reductases. Subsequently, various cytochromes of *Shewanella* were shown to localize in the periplasm and with either the cytoplasmic or the outer membrane (60).

Mutant analyses of *S. putrefaciens* 200 implicated the nitrite reductase in U(VI) reduction because of the simultaneous loss of U(VI) and NO_2^- reduction in the absence of this reductase (96). Because in the related species *S. oneidensis* this enzyme is a tetraheme *c*-type cytochrome (SO3980) (17), it is a good candidate for this role, although unequivocal evidence is still lacking. Transposon mutagenesis of *S. putrefaciens* identified a decaheme outer membrane *c*-type cytochrome, MtrA, to be necessary for Fe(III) and Mn(IV) reduction (6). Additional mutant studies have implicated other proteins and cytochromes to be involved in metal reduction and a model for electron transfer was proposed (**Figure 1**) (7). The function of these electron carriers for U(VI) reduction was only recently evaluated as a part of the analysis of global transcriptional responses to U(VI) (9).

Genome sequencing of *S. oneidensis* MR-1 revealed the presence of 42 putative *c*-type cytochromes (5, 33). Global transcript analysis of these cytochrome genes during growth on different metal and nonmetal electron acceptors (but not uranium or chromium) showed only one cytochrome, SO3300, to be significantly increased in expression during metal reduction (5). In contrast, when these cells were incubated under nongrowing conditions with 0.1 mM U(VI) or Cr(VI) present, of the 32 genes that increased (≥threefold) in both cultures, 12 were cytochromes, but SO3300 was not among them (9). By assay of mutants, several proteins including one involved in menaquinone biosynthesis (MenC), an outer membrane protein (MtrB), a periplasmic decaheme cytochrome (MtrA), an outer membrane decaheme cytochrome (MtrC, also named OmcB), and a tetraheme cytochrome (CymA) anchored in the cytoplasmic membrane were all shown to be needed for optimal U(VI) reduction (9). Interestingly, CymA was not required for the cytoplasmic reduction of Cr(VI).

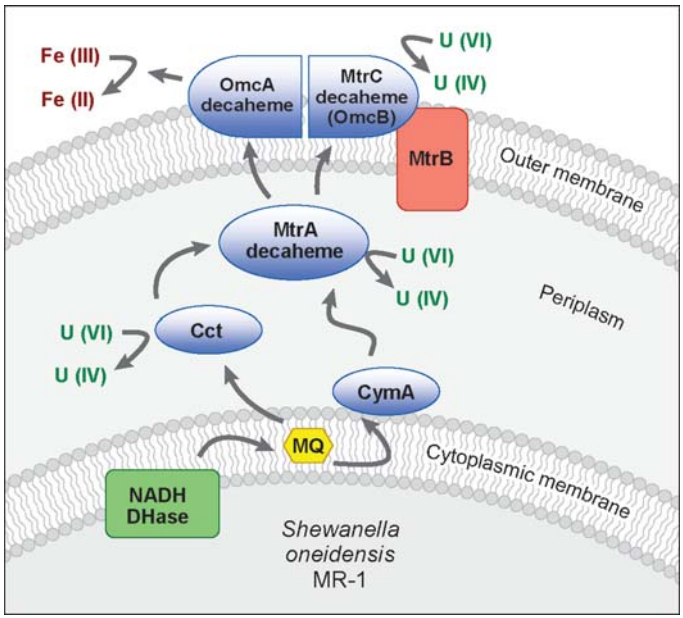

Figure 1

A model (modified from Reference 7) for possible electron transport pathways for U(VI) reduction, emphasizing the possibility of reduction at multiple sites in the periplasm and outer membrane. MQ, menaquinone (62); CymA, tetraheme membrane-bound cytochrome (9); Cct, tetraheme periplasmic cytochrome (30); OmcA, decaheme outer membrane cytochrome (63); MtrA, decaheme periplasmic cytochrome (7); MtrB, outer membrane structural protein (61); MtrC (OmcB), decaheme outer membrane cytochrome (7, 63).

Also of critical importance was the observation that the mutants lacking one or more of these electron transfer components were all still capable of U(VI) reduction with lactate as electron donor (9). Thus multiple pathways for electron delivery to U(VI) are available in *Shewanella*. Comparison of $UO_{2(s)}$ deposition by Δo*mcA* or Δ*mtrC* mutants lacking outer membrane decaheme *c*-type cytochromes showed accumulation predominantly in the periplasm versus the deposition of uraninite external to wild-type

Nanowires (or pili): electrically conductive protein structures extending from microbial cells

cells (41). This result is consistent with the observation that U(VI) reduction is not eliminated by any of the single mutants analyzed and supports the hypothesis that uranium reductases are likely nonspecific, low potential electron donors present in both the periplasm and the outer membrane. It remains to be determined whether the mutants altered for U(VI) reduction are similarly affected in their ability to use U(VI) as terminal electron acceptors for growth.

Geobacter Reductase(s)

Remarkably, the genome sequence of the DMRB *G. sulfurreducens* revealed putative ORFs for 73 multiheme *c*-type cytochromes (58). Mutations have been constructed in a number of the genes for these proteins including *ppcA*, a triheme cytochrome similar to the tetraheme cytochromes c_3 of *Desulfovibrio* (48); *omcB*, which encodes a 12-heme outer membrane cytochrome (45); and *macA*, a diheme periplasmic cytochrome (13). Each of these mutations negatively affected Fe(III) reduction rates with acetate as electron donor. However, analysis of over 15 cytochrome mutants of *G. sulfurreducens* showed that there was not a good correlation between effects on reduction rates of Fe(III) and U(VI) (86). Again mutations that did decrease U(VI) reduction did not eliminate that capacity (86).

NANOWIRES IN U(VI) REDUCTION

Recently, the DMRB *G. sulfurreducens* was shown to interact with its insoluble terminal electron acceptors, oxides of Fe(III) or Mn(IV), by means of pili produced only on one side of the bacterium (16). Mutant analysis showed that these pili were essential for reduction of insoluble Fe(III) oxides but not for attachment of the bacterium to this substrate (75). When visualized with conducting-probe atomic force microscopy, these pili were highly conductive (75). It has been inferred that electrons flow from the cell to the electron acceptor through these "nanowires" to allow growth. Similar conducting pili have been documented in *S. oneidensis* MR-1 (Y. Gorby & D. Elias, personal communication). The involvement of these nanowires in uranium reduction is implied from the localization of precipitated UO_2. Indeed the possible generality of such nanowires in DMRB has been strengthened by the observation of "needle-like structures" with precipitated uraninite on the surface of cells in biofilms of *D. desulfuricans* G20 (55).

COMPETITION BETWEEN U(VI) AND OTHER ELECTRON ACCEPTORS

In natural environments the E_h of the U(IV)/U(VI) couple should fall in the range of –0.042 to 0.086 V depending on the Ca^{2+} and CO_3^{2-} concentrations (12). On the basis of thermodynamic principles, we would predict that electron acceptors like O_2, Mn(IV), Fe(III), and NO_3^- would be preferentially used as electron acceptors, whereas U(VI) would be a preferred electron acceptor to SO_4^{2-}, S^o, or CO_2.

The competition between β-$MnO_{2(s)}$ (Mn(IV)) as terminal electron acceptor and U(VI) was tested with *S. putrefaciens* CN32 in bicarbonate buffer (26, 47). This interaction was complicated by the ability of β-$MnO_{2(s)}$ to oxidize U(IV) directly. The rate and extent of accumulation of U(IV) were decreased in the presence of β-$MnO_{2(s)}$. Both competition for electrons and reoxidation of U(IV) by the strongly oxidizing β-$MnO_{2(s)}$ slowed the net reduction of U(VI). Predictions of the relative contributions of these two processes were further complicated by the accessibility of $UO_{2(s)}$ to the surface of β-$MnO_{2(s)}$. Both of the minerals have low solubility under natural conditions. TEM observations of CN32 reducing U(VI) in the absence of β-$MnO_{2(s)}$ showed large accumulations of fine-grained precipitates of $UO_{2(s)}$ both outside the cell and apparently in the periplasm (47). When β-$MnO_{2(s)}$ was included, denser

uraninite nodules appeared on the surface, or in the periplasm, but no fine-grained precipitates were visible. Thus the extracellular fine-grained $UO_{2(s)}$ must have been rapidly reoxidized. The presence of U(VI) also stimulated β-$MnO_{2(s)}$ reduction, suggesting that U(VI)/U(IV) formed a shuttle for electrons to the insoluble terminal electron acceptor (47).

Competition between sulfate- and U(VI)-reduction has been explored in three approaches with the sulfate-reducing bacteria. A sulfate-reducing mixed culture and a pure culture of *D. desulfuricans* simultaneously reduced sulfate and U(VI) when provided at equal molar concentrations or equal electron equivalent concentrations (89). In most cases, sulfate was reduced more rapidly and with a different kinetic model when compared with U(VI) reduction, suggesting that the rate-limiting steps were different for the two processes. A similar competition experiment was also carried out with *D. vulgaris* (19), except that Fe(III) was also included. Reactions were discrete, with Fe(III) reduced first, followed by U(VI), and finally sulfate. With H_2 rather than lactate as electron donor, Fe(III) was again reduced first, but U(VI) and sulfate appeared to be simultaneously reduced as was observed in the other reports. Note that studies as described above may not represent events in natural environments, as contaminated aquifers are typically limited for electron donors (37).

In aquifer sediments incubated in the laboratory (1, 18, 20, 83) or the field (37) with multiple electron acceptors, nitrate has been shown to be reduced before U(VI) reduction proceeds, possibly because nitrate is a preferred electron acceptor or perhaps because of its suggested role in U(IV) oxidation (see below). U(VI) reduction is often observed to occur concomitantly with Fe(III) reduction in sediment systems (3, 20) and, because a number of bacterial species that respire Fe(III) also reduce U(VI), it has been inferred that Fe(III)-reducing bacteria are responsible for reduction of U(VI) in subsurface systems (3, 34). Although this seems likely to be the case, it has been difficult to prove because of the co-occurrence of U(VI) with Fe(III) and because the low levels of U(VI) are not generally sufficient to allow significant growth of a particular microorganism.

UMTRA: uranium mill tailing remedial action

BIOREMEDIATION OF ENVIRONMENTAL URANIUM

Approach to Bioremediation

The production of $UO_{2(s)}$, carried out most often by anaerobic bacteria, has been responsible for the economically important accumulation of uranium in several regions including many of the uranium ore deposits in Colorado, Wyoming, and along the Texas gulf coast (2). Presumably these formations occur as soluble U(VI), entrained in oxidized groundwater, encounters an organic-rich region as it moves through permeable sedimentary rock. The organic material, either in the form of partially degraded plant material or petroleum, may then act as a source of electrons for localized U(VI) reduction. The result is a localized U(IV) ore deposit.

Contamination of groundwater with uranium has occurred near sites where uranium was mined or processed over the past half century. In Colorado, New Mexico, and Arizona, documented regional contamination is present at several mining sites now managed by the U.S. Department of Energy Uranium Mill Tailing Remedial Action (UMTRA) program (56). Most of these mining sites currently have very low levels of U(VI) leaching into groundwater and the primary sources of contamination have been contained. Unfortunately, where uranium was processed for the production of weapons and fuel, much higher levels of contamination exist. One of the best studied of these is the Bear Creek Valley site at the Oak Ridge National Laboratory in Tennessee (98).

Microbiologists and geologists have taken the conceptual model for the formation of uranium ore deposits and further refined it

into a model for remediation of U(VI) contamination. This model proposes to apply anaerobic bacteria capable of metal reduction to reduction of U(VI) present in groundwater, forming the sparingly soluble U(IV) minerals. The concept has been tested in the laboratory, with numerous reports showing the feasibility of microbial U(VI) reduction by bacteria in groundwater and in aquifer sediments (1, 18, 32, 67, 83, 94). Such studies have concluded that indigenous microorganisms capable of U(VI) reduction are present in natural aquifers but that aquifer systems are limited by readily available electron donors. To stimulate the bioreduction of U(VI), electron donors are then typically provided in the form of acetate, lactate, or ethanol.

Testing Approach In Situ

The application of the localized reduction model to in situ U(VI) reduction was first tested in an aquifer in Norman, Oklahoma (83), using the push-pull test. The push-pull test involves injecting 50 to 200 liters of a test solution (in this case containing U(VI) and bromide as a tracer) into a well, allowing it to percolate into the surrounding aquifer material. After an incubation period or over a time course, groundwater is withdrawn from the well and compounds of interest are measured (36), along with bromide concentrations to allow for calculation of dilution. In this manner, microbial processes responsible for removal of U(VI) from the aqueous phase can be quantified in situ. In the highly reduced landfill leachate-impacted aquifer, added U(VI) (1.5 μM) was reduced over three days in the absence of an additional electron donor. It is likely that this system contained enough endogenous organic compounds to allow microbial U(VI) reduction. In a subsequent sampling of aquifer sediments located adjacent to the wells used in the study, uranium was present mainly as U(IV). This study presented a straightforward model demonstrating the ability of indigenous aquifer bacteria to reduce U(VI). However, this aquifer is not typical of U(VI)-contaminated aquifers because there was no preexisting contamination and the aquifer had an established low redox potential.

A more detailed and longer-term investigation was recently carried out in the shallow aquifer under the former uranium ore processing facility in Rifle, Colorado (3, 15, 67, 95). These workers used a more traditional bioremediation approach involving injection of acetate as electron donor into a gallery of wells placed in two closely spaced rows that were perpendicular to groundwater flow. Three rows of monitoring wells were installed 3.7, 7.3, and 14.6 m from the injection gallery. Addition of acetate to a final groundwater concentration of 1 to 3 mM (3) over a three-month period resulted in the initial growth of the family *Geobactereaceae* (e.g., *Geobacter* sp.), members of which are known for their ability to couple acetate oxidation to U(VI) reduction. After about two months during which U(VI) concentrations were decreased below the treatment goal, the U(VI) concentration began to increase. Presumably this increase was the result of either desorption from sediments, transport from the upgradient source, and/or U(IV) reoxidation by biotic or abiotic reactions. The microbial community analysis based on phospholipid fatty acids and 16S rRNA clone libraries showed that the change was accompanied by an increase in the numbers of sulfate-reducing bacteria (3). In a second acetate injection at this site, acetate was added at a higher (10 mM) concentration and ^{13}C-acetate was incorporated into microbial sampling devices (Bio-Sep® beads in biotraps) (15) placed in sampling wells down-gradient of the injection (15). The purpose of the study was to use stable isotope probing (74) coupled with 16S rDNA analysis to identify microorganisms actively incorporating acetate into cell material and, by association, identify those bacteria involved in U(VI) reduction. Down-gradient of the acetate injection, a variety of mainly Proteobacteria were shown to have taken up the ^{13}C-labeled acetate. Many of the clones

were related to organisms capable of U(VI) reduction and included relatives of several species of *Geobacter* and *Desulfuromonas*. Additional information on fatty acid and quinone analysis provided further support for the role of δ-Proteobacteria in metal reduction. As well, a number of organisms within groups not thought to be capable of acetate oxidation in the absence of oxygen or nitrate were observed, including relatives of *Pseudomonas putida* and *Dechloromonas agitatus* (15).

Over the past few years, several groups have focused efforts on remediation of U(VI) contamination in the Bear Creek Valley site. Many of the currently studied contaminated zones contain high levels of nitrate (>100 mM) and pH values as low as 3.5. As the presence of nitrate in natural systems has been shown to prevent the reduction of U(VI) (20) or result in U(IV) oxidation (83), it is inferred that nitrate must be reduced first if U(VI) is to be reduced to U(IV). Through the use of push-pull tests, U(VI) reduction could be demonstrated during biostimulation experiments (37). Two groundwater systems were tested, both with 5 to 6 μM U(VI) (37). One system originally at pH 3.3 was neutralized with bicarbonate to pH 5.5 to 6.5 and contained 142 mM nitrate. The second was at pH 6.4 but contained less than 2 mM nitrate. If adequate electron donor were added to the groundwater to reduce all the nitrate, denitrification followed by U(VI) depletion was observed (37). The fraction of ribosomal RNA sequences associated with nitrate-reducing bacteria, as well as *Geobacter* strains, appeared to increase in microbial sampling devices placed in electron donor–treated wells (37, 69). A most-probable-number (MPN)-PCR experiment focusing specifically on two groups of bacteria potentially capable of U(VI) reduction, *Anaeromyxobacter* and *Geobacter*, showed that *Anaeromyxobacter* was most abundant in contaminated sediments prior to biostimulation (70) and both groups were stimulated following the addition of electron donor (65).

A second, more highly engineered system was developed by Criddle and coworkers at the same high nitrate site. The concept involved (*a*) removal of the nitrate in the aquifer with an acidic flush strategy (53), (*b*) removal of nitrate from the flush water in an aboveground ethanol-oxidizing, fluidized bed bioreactor (100), and finally (*c*) reinjection of the treated groundwater into the aquifer in conjunction with the addition of electron donor to stimulate in situ U(VI) reduction. A denitrifying bioreactor biomass clone library was characterized. The sequences revealed that the microbial community was composed largely of β-Proteobacteria with a single dominant group related to *Azoarcus* sp. (35), a genus thought to be involved in denitrification. As the operation of the bioreactor continued, the bacterial community began to diversify with α- and other β-Proteobacteria gaining in importance. This microbial community was shown to be capable of U(VI) reduction as well (100). For part (*c*) of the study, Gu et al. (32) demonstrated the capability of the fluidized bed bioreactor biomass inoculated into a sediment column to reductively remove U(VI) from solution during an ethanol-groundwater flush. The actual field experimental results have not been published as of this writing.

MPN: most-probable-number

Anoxic: absence of oxygen

REOXIDATION OF U(IV)

For effective long-term immobilization of uranium through bioreduction, there should be a low probability of abiotic or biotic reoxidation of the insoluble U(IV). Uraninite is subject to abiotic oxidation by oxygen; however, more than simple maintenance of anoxic conditions appears to be needed for stability. Many experiments with pure cultures or with in situ amendments are beginning to explore possible mechanisms of U(IV) reoxidation (8, 20, 23, 31, 83–85). Nitrate is often found with U(VI) in contaminated sites and can cause U(IV) oxidation (20, 83). Nitrate-grown *G. metallireducens* is capable of directly oxidizing Fe(II) or U(IV) with nitrate as electron

Siderophore: biologically produced iron chelator

Hematite: the mineral Fe_2O_3

acceptor (20). Other experiments showed that rapid oxidation of U(IV) can occur through abiotic interaction with Fe(III), generating U(VI) and Fe(II). This process can be sustained through the coupling of the above reaction to the oxidation of Fe(II) by nitrogen oxides generated during organotrophic bacterial nitrate reduction (83, 84). The relative rate of oxidation was dependent on the mineralogy and the surface area of the Fe(III) mineral that was present (84).

In other work, the presence of humic substances, siderophores, and microbially generated (bi)carbonate have been suggested to stimulate oxidation of $UO_{2(s)}$ by the formation of highly stable U(VI) complexes (23, 31, 97). Studies with pure cultures of *D. desulfuricans* G20 demonstrated U(IV) reoxidation in electron-donor-limited cultures under sulfate-reducing conditions (81). This reoxidation correlated with the amount of Fe(III) present in the system. Microbially generated U(IV) was proposed to be an electron donor for Fe(III) reduction, with hematite being the most effective Fe(III) source (81). Interestingly, the oxidation was dependent on the presence of active bacterial cultures, suggesting that the process was not solely abiotic. Finally, the autotroph *Thiobacillus denitrificans* has the capacity to dissolve U(IV) oxides in a nitrate-dependent respiration at neutral pH (8). Although the picture of $UO_{2(s)}$ as an inert mineral has been modified by these reports, more than 60% of the uranium released in the Chernobyl accident is still present as solid U(IV) phases 14 years later (59). Thus while there are many concerns about the efficacy of bioreduction of U(VI) and the long-term stability of U(IV), the knowledge being gained should allow reasoned predictions for stewardship of this actinide and, by extrapolation, others as well.

SUMMARY POINTS

1. Reductases specific for U(VI) have not been identified and mutations affecting single genes do not eliminate U(VI) reduction, suggesting that multiple low-redox-potential electron carriers in a single bacterium may be capable of reducing uranium.
2. Growth coupled to U(VI) reduction may be restricted to bacteria able to use insoluble minerals as terminal electron acceptors.
3. The slow rate of U(VI) reduction by whole cells relative to cell extracts or purified proteins suggests a major limitation of available reductant at the site of this process.
4. Transport systems for U(VI) have not been reported. This absence may account for reduction sites that are localized in the periplasm, the outer membrane, or both.
5. Studies of the mechanism of U(VI) reduction have revealed the intriguing possibility that cells transfer a single electron to U(VI) and that U(IV) is generated through disproportionation.
6. Microbial U(VI) reduction and subsequent immobilization can be encouraged in aquifers by the addition of organic electron donors, such as acetate or ethanol, and are generally accompanied by growth of Fe(III)-reducing bacteria.
7. Oxidation of immobilized U(IV) can occur if nitrate or sulfate reduction is occurring in the presence in Fe(III) minerals or abiotically in the presence of O_2.

ACKNOWLEDGMENTS

The authors thank Dwayne Elias for critical review of the manuscript. This work was part of the Virtual Institute for Microbial Stress and Survival (**http://vimss.lbl.gov/**) supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics Program:GTL through the Lawrence Berkeley National Laboratory (JDW). This work was also supported by the Natural and Accelerated Bioremediation Research (NABIR) program of the Office of Biological and Environmental Research of the Office of Science, U.S. Department of Energy (LRK & JDW).

LITERATURE CITED

1. Abdelouas A, Lu Y, Lutze W, Nuttall HE. 1998. Reduction of U(VI) to U(IV) by indigenous bacteria in contaminated ground water. *J. Contam. Hydrol.* 35:217–33
2. Adler HH. 1974. Concepts of uranium-ore formation in reducing environments in sandstones and other sediments. In *Formation of Uranium Ore Deposits*, ed. RD Nininger, pp. 141–68. Proc. Ser. IAEA-SM-183/43. Vienna: IAEA
3. **Anderson RT, Vrionis HA, Ortiz-Bernad I, Resch CT, Long PE, et al. 2003. Stimulating the in situ activity of *Geobacter* species to remove uranium from the groundwater of a uranium-contaminated aquifer. *Appl. Environ. Microbiol.* 69:5884–91**
4. Barton LL, Choudhury K, Thomsom BM, Steenhoudt K, Groffman AR. 1996. Bacterial reduction of soluble uranium: the first step of in situ immobilization of uranium. *Radioact. Waste Manag. Environ. Restor.* 20:141–51
5. Beliaev AS, Klingeman DM, Klappenbach JA, Wu L, Romine MF, et al. 2005. Global transcriptome analysis of *Shewanella oneidensis* MR-1 exposed to different terminal electron acceptors. *J. Bacteriol.* 187:7138–45
6. Beliaev AS, Saffarini DA. 1998. *Shewanella putrefaciens mtrB* encodes an outer membrane protein required for Fe(III) and Mn(IV) reduction. *J. Bacteriol.* 180:6292–97
7. Beliaev AS, Saffarini DA, McLaughlin JL, Hunnicutt D. 2001. MtrC, an outer membrane decahaem *c* cytochrome required for metal reduction in *Shewanella putrefaciens* MR-1. *Mol. Microbiol.* 39:722–30
8. Beller HR. 2005. Anaerobic, nitrate-dependent oxidation of U(IV) oxide minerals by the chemolithoautotrophic bacterium *Thiobacillus denitrificans*. *Appl. Environ. Microbiol.* 71:2170–74
9. Bencheikh-Latmani R, Williams SM, Haucke L, Criddle CS, Wu L, et al. 2005. Global transcriptional profiling of *Shewanella oneidensis* MR-1 during Cr(VI) and U(VI) reduction. *Appl. Environ. Microbiol.* 71:7453–60
10. Beyenal H, Sani RK, Peyton BM, Dohnalkova AC, Amonette JE, Lewandowski Z. 2004. Uranium immobilization by sulfate-reducing biofilms. *Environ. Sci. Technol.* 38:2067–74
11. Blakeney MD, Moulaei T, DiChristina TJ. 2000. Fe(III) reduction activity and cytochrome content of *Shewanella putrefaciens* grown on ten compounds as sole terminal electron acceptor. *Microbiol. Res.* 155:87–94
12. **Brooks SC, Fredrickson JK, Carroll SL, Kennedy DW, Zachara JM, et al. 2003. Inhibition of bacterial U(VI) reduction by calcium. *Environ. Sci. Technol.* 37:1850–58**
13. Butler JE, Kaufmann F, Coppi MV, Nunez C, Lovely DR. 2004. MacA, a diheme *c*-type cytochrome involved in Fe(III) reduction by *Geobacter sulfurreducens*. *J. Bacteriol.* 186:4042–45

3. Describes the in situ biostimulation of a U(VI)-contaminated aquifer coincident with increases in Fe(III)-reducing bacteria.

12. Showed that the presence of Ca^{2+} at mM concentrations dramatically slows U(VI) reduction by pure cultures, likely as a result of a decrease in the redox potential (E_h) of the U(VI)/U(IV) couple.

14. Caccavo FJ, Blakemore R, Lovley D. 1992. A hydrogen-oxidizing, Fe(III)-reducing microorganism from the Great Bay Estuary, New Hampshire. *Appl. Environ. Microbiol.* 58:3211–16
15. Chang YJ, Long PE, Geyer R, Peacock AD, Resch CT, et al. 2005. Microbial incorporation of 13C-labeled acetate at the field scale: detection of microbes responsible for reduction of U(VI). *Environ. Sci. Technol.* 39:9039–48
16. Childers SE, Clufo S, Lovley DR. 2002. *Geobacter metallireducens* accesses insoluble Fe(III) oxide by chemotaxis. *Nature* 63:3589–93
17. Dale JR, Wade RJ, DiChristina TJ. 2003. Uranium reduction by *Shewanella oneidensis* MR-1 requires *nrfA*, a homolog of the *Escherichia coli* nitrite reductase structural gene. *ASM 103rd Gen. Meet.* Q389 (Abstr.)
18. Elias DA, Krumholz LR, Wong D, Long PE, Suflita JM. 2003. Characterization of microbial activities and U reduction in a shallow aquifer contaminated by uranium mill tailings. *Microb. Ecol.* 46:83–91
19. Elias DA, Suflita JM, McInerney MJ, Krumholz LR. 2004. Periplasmic cytochrome c_3 of *Desulfovibrio vulgaris* is directly involved in H_2-mediated metal but not sulfate reduction. *Appl. Environ. Microbiol.* 70:413–20
20. Finneran KT, Housewright ME, Lovley DR. 2002. Multiple influences of nitrate on uranium solubility during bioremediation of uranium-contaminated subsurface sediments. *Environ. Microbiol.* 4:510–16
21. Francis AJ, Dodge CJ, Gillow JB, Halada GP, Honeyman BD. 2004. Reductive precipitation and stabilization of uranium complexed with organic ligands by anaerobic bacteria. *DOE-NABIR PI Workshop* 2004:12 (Abstr.)
22. Francis AJ, Dodge CJ, Lu F, Halada GP, Clayton CR. 1994. XPS and XANES studies of uranium reduction by *Clostridium* sp. *Environ. Sci. Technol.* 28:636–39
23. Frazier SW, Kretzschmar R, Kraemer SM. 2005. Bacterial siderophores promote dissolution of UO_2 under reducing conditions. *Environ. Sci. Technol.* 39:5709–15
24. Fredrickson JK, Kostandarithes HM, Li SW, Plymale AE, Daly MJ. 2000. Reduction of Fe(III), Cr(VI), U(VI), and Tc(VII) by *Deinococcus radiodurans* R1. *Appl. Environ. Microbiol.* 66:2006–11
25. Fredrickson JK, Zachara JM, Kennedy DW, Duff MC, Gorby YA, et al. 2000. Reduction of U(VI) in goethite (α-FeOOH) suspensions by a dissimilatory metal-reducing bacteria. *Geochim. Cosmochim. Acta* 64:3085–98
26. Fredrickson JK, Zachara JM, Kennedy DW, Liu C, Duff MC, et al. 2002. Influence of Mn oxides on the reduction of Uranium(VI) by the metal-reducing bacterium *Shewanella putrefaciens*. *Geochim. Cosmochim. Acta* 66:3247–62
27. Gadd GM. 2004. Microbial influence on metal mobility and application for bioremediation. *Geoderma* 122:109–19
28. Ganesh R, Robinson KG, Reed GD, Sayler GS. 1997. Reduction of hexavalent uranium from organic complexes by sulfate- and iron-reducing bacteria. *Appl. Environ. Microbiol.* 63:4385–91
29. Gorby YA, Lovley DR. 1992. Enzymatic uranium precipitation. *Environ. Sci. Technol.* 26:205–7
30. Gordon E, Pike A, Hill A, Cuthbertson P, Chapman S, Reid G. 2000. Identification and characterization of a novel cytochrome c_3 from *Shewanella frigidimarina* that is involved in Fe(III) respiration. *Biochem. J.* 349:153–58
31. Gu BH, Yan H, Zhou P, Watson DB. 2005. Natural humics impact uranium bioreduction and oxidation. *Environ. Sci. Technol.* 39:5268–75

32. Gu B, Wu WM, Ginder-Vogel MA, Yan H, Fields MW, et al. 2005. Bioreduction of uranium in a contaminated soil column. *Environ. Sci. Technol.* 39:4841–47
33. Heidelberg JF, Paulsen IT, Nelson KE, Gaidos EJ, Nelson WC, et al. 2002. Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis*. *Nat. Biotechnol.* 20:1118–23
34. Holmes DE, Finneran KT, O'Neil RA, Lovley DR. 2002. Enrichment of members of the family *Geobacteraceae* associated with stimulation of dissimilatory metal reduction in uranium-contaminated aquifer sediments. *Appl. Environ. Microbiol.* 68:2300–6
35. Hwang C, Wu WM, Gentry TJ, Carley J, Carroll SL, et al. 2005. Changes in bacterial community structure correlate with initial operating conditions of a field-scale denitrifying fluidized bed reactor. *Appl. Microbiol. Biotechnol.* DOI: 10.1007/s00253-005-0189-1
36. Istok JD, Humphrey MD, Schroth MH, Hyman MR, O'Reilly KT. 1997. Single-well, "push-pull" test for in situ determination of microbial activities. *Ground Wat.* 35:619–31
37. Istok JD, Senko JM, Krumholz LR, Watson D, Bogle MA, et al. 2004. In situ bioreduction of technetium and uranium in a nitrate-contaminated aquifer. *Environ. Sci. Technol.* 38:468–75
38. Jeon BH, Kelly SD, Kemner KM, Barnett MO, Burgos WD, et al. 2004. Microbial reduction of U(VI) at the solid-water interface. *Environ. Sci. Technol.* 38:5649–55
39. Kashefi K, Lovely DR. 2000. Reduction of Fe(III), Mn(IV), and toxic metals at 100°C by *Pyrobaculum islandicum*. *Appl. Environ. Microbiol.* 66:1050–56
40. Kauffman JW, Laughlin WC, Baldwin RA. 1986. Microbiological treatment of uranium mine waters. *Environ. Sci. Technol.* 20:243–48
41. Kennedy DW, Marshall MJ, Dohnalkova AC, Saffarini DA, Culley DE, et al. 2004. Role of *Shewanella oneidensis c*-type cytochromes in uranium reduction and localization. *ASM 105th Gen. Meet.* Q-389 (Abstr.)
42. Khijniak TV, Slobodkin AI, Coker V, Renshaw JC, Livens FR, et al. 2005. Reduction of uranium(VI) phosphate during growth of the thermophilic bacterium *Thermoterrabacterium ferrireducens*. *Appl. Environ. Microbiol.* 71:6423–26
43. Kieft TL, Fredrickson JK, Onstott TC, Gorby YA, Kostandarithes HM, et al. 1999. Dissimilatory reduction of Fe(III) and other electron acceptors by a *Thermus* isolate. *Appl. Environ. Microbiol.* 65:1214–21
44. Langmuir D. 1978. Uranium solution-mineral equilibria at low temperatures with applications to sedimentary ore deposits. *Geochim. Cosmochim. Acta* 42:547–69
45. Leang C, Coppi MV, Lovley DR. 2003. OmcB, a *c*-type polyheme cytochrome, involved in Fe(III) reduction in *Geobacter sulfurreducens*. *J. Bacteriol.* 185:2096–103
46. Liu C, Gorby YA, Zachara JM, Fredrickson JK, Brown CF. 2002. Reduction kinetics of Fe(III), Co(III), U(VI), Cr(VI), and Tc(VII) in cultures of dissimilatory metal-reducing bacteria. *Biotechnol. Bioeng.* 80:637–49
47. Liu C, Zachara JM, Fredrickson JK, Kennedy DW, Dohnalkova A. 2002. Modeling the inhibition of the bacterial reduction of U(VI) by β-MnO_2(s). *Environ. Sci. Technol.* 36:1452–59
48. Lloyd JR, Leang C, Hodges-Myerson AL, Coppi MV, Cuifo S, et al. 2003. Biochemical and genetic characterization of PpcA, a periplasmic *c*-type cytochrome in *Geobacter sulfurreducens*. *Biochem. J.* 369:153–61
49. Lovley DR, Phillips EJP. 1992. Reduction of uranium by *Desulfovibrio desulfuricans*. *Appl. Environ. Microbiol.* 58:850–56
50. Lovley DR, Phillips EJP, Gorby YA, Landa ER. 1991. Microbial reduction of uranium. *Nature* 350:413–16

37. In situ denitrification and U(VI)-reduction were demonstrated through the addition of acetate, glucose or ethanol to a U(VI)- and nitrate-contaminated aquifer.

51. Lovley DR, Roden EE, Phillips EJ, Woodward JC. 1993. Enzymatic iron and uranium reduction by sulfate-reducing bacteria. *Mar. Geol.* 113:41–53
52. Lovley DR, Widman PK, Woodward JC, Phillips EJP. 1993. Reduction of uranium by cytochrome c_3 of *Desulfovibrio vulgaris*. *Appl. Environ. Microbiol.* 59:3572–76
53. Luo J, Cirpka OA, Wu W, Fienen MN, Jardine PM, et al. 2005. Mass-transfer limitations for nitrate removal in a uranium-contaminated aquifer. *Environ. Sci. Technol.* 39:8453–59
54. Markich SJ. 2002. Uranium speciation and bioavailability in aquatic systems: an overview. *Sci. World J.* 2:707–29
55. Marsili E, Beyenal H, Di Palma L, Merli C, Dohnalkova A, et al. 2005. Uranium removal by sulfate reducing biofilms in the presence of carbonates. *Wat. Sci. Technol.* 52:49–55
56. Matthews ML. 1987. The uranium mill tailings remedial actions project: a progress report on DOE's cleanup program. *Waste Manage.* 3:151–55
57. McLean J, Beveridge TJ. 2001. Chromate reduction by a pseudomonad isolated from a site contaminated with chromated copper arsenate. *Appl. Environ. Microbiol.* 67:1076–84
58. Methe BA, Nelson KE, Eisen JA, Paulsen IT, Nelson W, et al. 2003. Genome of *Geobacter sulfurreducens*: metal reduction in subsurface environments. *Science* 302:1967–69
59. Mironov VP, Matusevich JL, Kudrjashov VP, Boulyga SF, Becker JS. 2002. Determination of irradiated reactor uranium in soil samples in Belarus using U-236 as irradiated uranium tracer. *J. Environ. Monit.* 4:997–1002
60. Myers CR, Myers JM. 1992. Localization of cytochromes to the outer membrane of anaerobically grown *Shewanella putrefaciens* MR-1. *J. Bacteriol.* 174:3429–38
61. Myers CR, Myers JM. 2002. MtrB is required for proper incorporation of the cytochromes OmcA and OmcB into the outer membrane of *Shewanella putrefaciens* MR-1. *Appl. Environ. Microbiol.* 68:5585–94
62. Myers CR, Myers JM. 2004. *Shewanella oneidensis* MR-1 restores menaquinone synthesis to a menaquinone-negative mutant. *Appl. Environ. Microbiol.* 70:5415–25
63. Myers JM, Myers CR. 2001. Role for outer membrane cytochromes OmcA and OmcB of *Shewanella putrefaciens* MR-1 in reduction of manganese dioxide. *Appl. Environ. Microbiol.* 67:260–69
64. NABIR. 2003. *Bioremediation of metals and radionuclides. What is it and how it works. Rep. LBNL-42595*
65. North NN, Dollhopf SL, Petrie L, Istok JD, Balkwill DL, Kostka JE. 2004. Change in bacterial community structure during in situ biostimulation of subsurface sediment cocontaminated with uranium and nitrate. *Appl. Environ. Microbiol.* 70:4911–20
66. Obuekwe C, Westlake DW. 1982. Effects of medium composition on cell pigmentation, cytochrome content and ferric iron reduction in a *Pseudomonas* sp. isolated from crude oil. *Can. J. Microbiol.* 28:989–92
67. Ortiz-Bernad I, Anderson RT, Vrionis HA, Lovley DR. 2004. Resistance of solid-phase U(VI) to microbial reduction during in situ bioremediation of uranium-contaminated groundwater. *Appl. Environ. Microbiol.* 70:7558–60
68. Payne RB, Gentry DM, Rapp-Giles BJ, Casalot L, Wall JD. 2002. Uranium reduction by *Desulfovibrio desulfuricans* strain G20 and a cytochrome c_3 mutant. *Appl. Environ. Microbiol.* 68:3129–32
69. Peacock AD, Chang YJ, Istok JD, Krumholz L, Geyer R, et al. 2004. Utilization of microbial biofilms as monitors of bioremediation. *Microb. Ecol.* 47:284–92
70. Petrie L, North NN, Dollhopf SL, Balkwill DL, Kostka JE. 2003. Enumeration and characterization of iron(III)-reducing microbial communities from acidic subsurface sediments contaminated with uranium(VI). *Appl. Environ. Microbiol.* 69:7467–79

71. Phillips EJP, Landa ER, Lovley DR. 1995. Remediation of uranium contaminated soils with bicarbonate extraction and microbial U(VI) reduction. *J. Ind. Microbiol*. 14:203–7
72. Pietzsch K, Babel W. 2003. A sulfate-reducing bacterium that can detoxify U(VI) and obtain energy via nitrate reduction. *J. Basic Microbiol*. 43:348–61
73. Pietzsch K, Hard BC, Babel W. 1999. A *Desulfovibrio* sp. capable of growing by reducing U(VI). *J. Basic Microbiol*. 39:365–72
74. Radajewski S, McDonald IR, Murrell JC. 2003. Stable isotope probing of nucleic acids: a window to the function of uncultured microorganisms. *Curr. Opin. Biotechnol*. 14:296–302
75. Reguera G, McCarthy KD, Mehta T, Nicoll JS, Tuominen MT, Lovley DR. 2005. Extracellular electron transfer via microbial nanowires. *Nature* 435:1098–101
76. Renshaw JC, Butchins LJC, Livens FR, May I, Charnock JM, Lloyd JR. 2005. Bioreduction of uranium: environmental implications of a pentavalent intermediate. *Environ. Sci. Technol*. 39:5657–60
77. Roh Y, Liu SV, Li G, Huang H, Phelps TJ, Zhou J. 2002. Isolation and characterization of metal-reducing *Thermoanaerobacter* strains from deep subsurface environments of the Piceance Basin, Colorado. *Appl. Environ. Microbiol*. 68:6013–20
78. Sanford RA, Fademacher L, Zhou J, Lundstrom C, Johnson TM. 2004. Uranium reduction and isotope fractionation by *Anaeromyxobacter dehalogenans* strain 2CP-C. *ASM 104th Gen. Meet.* Q-139 (Abstr.)
79. Sani KS, Geesey G, Peyton BM. 2001. Assessment of lead toxicity to *Desulfovibrio desulfuricans* G20: influence of components of lactate C medium. *Adv. Environ. Res*. 5:269–76
80. Sani RK, Peyton BM, Dohnalkova A. 2004. Effect of uranium (VI) on *Desulfovibrio desulfuricans* G20: influence of soil minerals. *ASM 104th Gen. Meet.* Q-007 (Abstr.)
81. Sani RK, Peyton BM, Dohnalkova A, Amonette JE. 2005. Reoxidation of reduced uranium with iron(III) (hydr)oxides under sulfate-reducing conditions. *Environ. Sci. Technol*. 39:2059–66
82. Sani RK, Peyton BM, Smith WA, Apel WA, Petersen JN. 2002. Dissimilatory reduction of Cr(VI), Fe(III), and U(VI) by *Cellulomonas* isolates. *Appl. Microbiol. Biotechnol*. 60:192–99
83. Senko JM, Istok JD, Suflita JM, Krumholz LR. 2002. *In-situ* evidence for uranium immobilization and remobilization. *Environ. Sci. Technol*. 36:1491–96
84. Senko JM, Mohamed Y, Dewers TA, Krumholz LR. 2005. Role for Fe(III) minerals in nitrate-dependent microbial U(IV) oxidation. *Environ. Sci. Technol*. 39:2529–36
85. Senko JM, Suflita JM, Krumholz LR. 2005. Geochemical controls on microbial nitrate-dependent U(IV) oxidation. *Geomicrobiol. J*. 22:371–78
86. Shelobolina ES, Sullivan SA, Coppi MV, Leang C, DiDonato L, et al. 2004. Evaluation of the role of *c*-type cytochromes in U(VI) reduction by *G. sulfurreducens*. *ASM 104th Gen. Meet.* Q-014 (Abstr.)
87. Shelobolina ES, Sullivan SA, O'Neill KR, Nevin KP, Lovley DR. 2004. Isolation, characterization, and U(VI)-reducing potential of a facultatively anaerobic, acid-resistant bacterium from low-pH, nitrate- and U(VI)-contaminated subsurface sediment and description of *Salmonella subterranea* sp. nov. *Appl. Environ. Microbiol*. 70:2959–65
88. Sivaswamy V, Dohnalkova A, Peyton BM, Viamajala S, Gerlach R, Apel WA. 2005. Uranium immobilization by *Cellulomonas*. *ASM 105th Gen. Meet.* Q-388 (Abstr.)
89. Spear JR, Figueroa LA, Honeyman BD. 2000. Modeling reduction of uranium U(VI) under variable sulfate concentrations by sulfate-reducing bacteria. *Appl. Environ. Microbiol*. 66:3711–21
90. Suzuki Y, Kelly SD, Kemner KM, Banfield JF. 2002. Nanometre-size products of uranium bioreduction. *Nature* 419:134

75. Demonstrates a new mechanism for electron transport to extracellular electron acceptors.

76. Suggests that uranium reductases of *Geobacter sulfurreducens* transfer a single electron and that U(IV) is generated by disproportionation of U(V).

81. Shows that the presence of residual Fe(III) oxides in sediments can result in U(IV) oxidation after biostimulation and depletion of the electron donor.

91. Suzuki Y, Kelly SD, Kemner KM, Banfield JF. 2004. Enzymatic U(VI) reduction by *Desulfosporosinus* species. *Radiochim. Acta* 92:11–16
92. Tebo BM, Obraztsova AY. 1998. Sulfate-reducing bacterium grows with Cr(VI), U(VI), Mn(IV), and Fe(III) as electron acceptors. *FEMS Microbiol. Lett*. 162:193–98
93. Truex MJ, Peyton BM, Valentine NB, Gorby YA. 1997. Kinetics of U(VI) reduction by a dissimilatory Fe(III)-reducing bacterium under nongrowth conditions. *Biotechnol. Bioeng*. 55:490–96
94. Uhrie JL, Drever JI, Colberg PJS, Nesbitt CC. 1996. In situ immobilization of heavy metals associated with uranium leach mines by bacterial sulfate reduction. *Hydrometallurgy* 43:231–39
95. Vrionis HA, Anderson RT, Ortiz-Bernad I, O'Neill KR, Resch CT, et al. 2005. Microbiological and geochemical heterogeneity in an in situ uranium bioremediation field site. *Appl. Environ. Microbiol.* 71:6308–18
96. Wade R, DiChristina TJ. 2000. Isolation of U(VI) reduction-deficient mutants of *Shewanella putrefaciens*. *FEMS Microbiol. Lett*. 184:143–48
97. Wan J, Tokunaga TK, Brodie E, Wang Z, Zheng Z, et al. 2005. Reoxidation of bioreduced uranium under reducing conditions. *Environ. Sci. Technol.* 39:6162–69
98. Watson DB. 2002. Natural and accelerated bioremediation research program establishes field research center in Oak Ridge, Tennessee. *Spectrum 2002: Exploring Science-Based Solutions and Technologies, Biennial Int. Conf. Nucl. Hazard. Waste Manag., 9th, Reno, NV, Aug. 4–8, 2002*:1470–75
99. Woolfolk CA, Whiteley HR. 1962. Reduction of inorganic compounds with molecular hydrogen by *Micrococcus lactilyticus*. I. Stoichiometry with compounds of arsenic, selenium, tellurium, transition and other elements. *J. Bacteriol*. 84:647–58
100. Wu WM, Gu B, Fields MW, Gentile M, Ku YK, et al. 2005. Uranium (VI) reduction by denitrifying biomass. *Bioremed. J.* 9:49–61
101. Xu H, Barton LL, Zhang P, Wang Y. 2000. TEM investigation of U^{6+} and Re^{7+} reduction by *Desulfovibrio desulfuricans*, a sulfate-reducing bacterium. *Mat. Res. Soc. Symp. Proc*. 608:299–304