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Bioremediation of radioactive waste: radionuclide–microbe interactions in laboratory and field-scale studies

Jonathan R Lloyd and Joanna C Renshaw

Given the scale of the contamination associated with 60 years of global nuclear activity, and the inherent high financial and environmental costs associated with invasive physical and chemical clean-up strategies, there is an unparalleled interest in new passive *in situ* bioremediation processes for sites contaminated with nuclear waste. Many of these processes rely on successfully harnessing newly discovered natural biogeochemical cycles for key radionuclides and fission products. Recent advances have been made in understanding the microbial colonization of radioactive environments and the biological basis of microbial transformations of radioactive waste in these settings.

Addresses
Williamson Research Centre for Molecular Environmental Science and School of Earth, Atmospheric and Environmental Sciences, The University of Manchester, Manchester M13 9PL, UK

Corresponding author: Lloyd, Jonathan R (jon.lloyd@manchester.ac.uk)

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Introduction

The release of radionuclides from nuclear sites and their subsequent mobility in the environment is a subject of intense public concern and has prompted much recent research on the environmental fate of key radionuclides [1•]. The major burden of anthropogenic environmental radioactivity is from the controlled discharge of process effluents produced by industrial activities allied to the generation of nuclear power, although significant quantities of natural and artificial radionuclides were also released as a consequence of nuclear weapons testing in the 1950s and 1960s, through accidental release (e.g. from Chernobyl in 1986), and from the ongoing storage of nuclear materials amassed over the past 60 years of nuclear activities. Indeed, the scale of our nuclear legacy is enormous and includes 120 Department of Energy (DOE) sites in the US alone and other facilities in Europe and the former USSR [1•]. In several cases, storage has been compromised, leading to contamination of trillions of gallons of groundwater and millions of cubic metres of contaminated soil and debris. The costs of cleaning up these sites are estimated to be in excess of a trillion dollars in the US and 50 billion pounds sterling in the UK. Given these high costs and the technical limitations of current chemical-based approaches, there has been an unprecedented interest in the interactions of microorganisms with key radionuclides in the hope of developing cost-effective bioremediation approaches for decontamination of sediments and waters impacted by nuclear waste [2]. Indeed, recent reports have suggested that aggressive, invasive treatment strategies can have negative impacts on biodiversity and can even result in increased dispersion of radioactive materials (e.g. wind-aided transport of plutonium-contaminated soil) [3•]. Thus, passive *in situ* biological treatment processes that harness natural biogeochemical cycles for key radionuclides are highly desirable. The aim of this paper is to give a synopsis of the many significant advances in this area over the past 18 months.

Microbial colonization of radioactive environments

The ubiquitous distribution of microbial life in the biosphere is well known, but only recently have we been able to obtain an indication of the level of microbial colonisation of radioactive environments. Some workers have studied uranium mill tailings, comprising uneconomic residues from mining and milling operations, as high-volume, low specific activity radioactive model environments. This work has been reviewed recently [4], focusing on aspects such as the mineral hosts for key radionuclides and the microbial and diagenetic processes that can alter radionuclide mobility. Waste management technologies to limit radionuclide migration were also discussed.

Other environments that have been impacted by intensely radioactive wastes have been studied in less detail. This is almost certainly because of the technical problems associated with sampling such environments and the difficulties of working with highly radioactive environmental samples in the laboratory. However, some notable new studies on such environments have been published recently. For example, the characterization of microorganisms attached to the walls of a pool storing nuclear materials at a Spanish nuclear power plant was reported [5]. Several isolates were obtained, and molecular (16S ribosomal RNA gene) analysis identified six different bacteria (affiliated to β-Proteobacteria, *Actinomycetales*

and the *Bacillus/Staphylococcus* groups), as well as a fungus (*Aspergillus* species).

The microbiology of vadose zone sediments, contaminated with high level nuclear waste and overlaying the water table at the US DOE Hanford Site, Washington State, USA has also been studied [6•]. The sediments were contaminated by a leak from a waste tank containing high concentrations of alkali, nitrate, aluminate, chromate, Cs-137 and Tc-99 in 1962. Viable aerobic heterotrophic bacteria were recovered from 11 of 16 samples, but cell counts were low (maximum of 10^4 colony-forming units g^{-1}). Gram-positive bacteria most closely related to *Arthrobacter* species were the most common isolates among all samples, but other phyla with high G+C contents were also represented, including *Rhodococcus* and *Nocardia*. Interestingly, even the most radioactive sample (>10 μCi of Cs-137 g^{-1}) yielded active bacteria, while two isolates from radioactive samples of >20 μCi of Cs-137 g^{-1} were closely related to *Deinococcus radiodurans* and were able to survive acute doses of ionizing radiation approaching 20 kGy. These organisms are also highly resistant to desiccation, perhaps giving an additional reason for their successful cultivation from these vadose zone samples.

Other environments studied recently include the Severnyi repository for low-level liquid radioactive wastes in Eastern Siberia, Russia. Anaerobic denitrifiers, fermenters, sulfate-reducers and methanogens were cultured from water samples collected from a depth of 162–405 m below sea level [7•]. Not only were a wide range of functional groups of microorganisms detected, but cell numbers and rates of a range of respiratory processes were higher in the zone of dispersion of radioactive waste than in background areas. The authors noted that microbial gas production could result in a local increase in pressure in the repository and consequent discharge of wastes, although key microbial activities (e.g. the formation of insoluble metal sulfides from sulfate reduction) could also result in immobilization of some radionuclides. Such processes have been shown recently to occur in microcosms prepared from sediments in two types of freshwater lakes of the Moscow Oblast [8].

These results would seem to suggest that the radioactive burden of several nuclear waste types is not necessarily inhibitory to all microbial life. Indeed, a recent study using pure cultures of bacteria proposed for application in bioremediation programmes addressed the toxicity of actinides, metals and chelators. The model organisms tested include *D. radiodurans, Pseudomonas putida* and *Shewanella putrefaciens* CN32 [9••]. Actinides, including chelated Pu(IV), U(VI) and Np(V), inhibited growth at millimolar concentrations, suggesting that actinide toxicity is primarily chemical (not radiological) and that radiation resistance (e.g. in *Deinococcus*) does not necessarily ensure radionuclide tolerance. This study also suggested, rather surprisingly, that Pu is less toxic than U and that actinides are less toxic than other types of metals under the conditions imposed in these experiments (including the presence of the chelating agents citrate and desferrioxamine B). The authors propose that actinide toxicity will not impede bioremediation using naturally occurring bacteria, although the toxicity of these key radionuclides remains to be determined in sedimentary environments under field conditions.

The inventory of radionuclides

The inventory of radionuclides generated during the past 60 years of operating fission reactors is long and includes ^{237}Np, Pu isotopes, Am, ^{3}H, ^{14}C, ^{85}Kr, ^{90}Sr, ^{99}Tc, ^{129}I and ^{137}Cs in addition to uranium (e.g. ^{235}U) from nuclear fuel. Wastes containing some or all of these radionuclides are produced during the many steps of the nuclear fuel cycle, and vary considerably from low-level, high-volume radioactive effluents produced during uranium mining to the intensely radioactive plant, fuel and liquid wastes produced from reactor operation and fuel reprocessing. However, most studies have addressed two elements that dominate many impact assessments: uranium and the fission product technetium. For a detailed review of microbial interactions with other radionuclides the reader is recommended to read other recent specialist reviews (e.g. [1•]).

Microbe–actinide interactions

There is a large and increasing body of information addressing the biogeochemistry of uranium, although comparatively little work has addressed microbial interactions with other important actinides such as plutonium or neptunium. For over a decade several microbially mediated mechanisms for the removal of uranium from solution have been known, including the enzymatic reduction of soluble U(VI) to insoluble U(IV), precipitation of U(VI) following reaction with inorganic ligands such as phosphate, and the biosorption of U(VI) through complexation with cell-surface groups (reviewed in [1•]). Most recent work has focused on the bioreduction of U(VI), addressing the development of more efficient remediation technologies through *in situ* studies and *ex situ* laboratory-based sediment experiments. Thus, we now have a deeper understanding of the complex interplay between the microorganism, U(VI), other soluble species in the groundwater (e.g. oxyanions such as nitrate and cations such as Ca^{2+}), and the host mineralogy of the sediments. Two types of environment have been targeted in recent studies on the biogeochemistry of uranium, predominantly in the US. Subsurface sediments at the US DOE Field Research Centre (FRC) in Oak Ridge Tennessee (see http://www.esd.ornl.gov/nabirfrc) have given a useful model for aquifers impacted by aqueous wastes from nuclear processing activities, and in some cases these are dominated by high nitrate and relatively

low pH. In addition, several recent papers (see below) have addressed uranium-contaminated mining sites impacted by (e.g. mill tailings) with a contrasting geochemical background (e.g. high sulphate) to that of the FRC site.

Mining and mill tailings sites

Anderson *et al.* [10••] recently investigated microbially mediated removal of uranium from contaminated groundwater at a 'Uranium Mill Tailings Remedial Action' or 'UMTRA' site in Rifle, Colorado. The approach adopted was *in situ* stimulation of dissimilatory metal-reducing bacteria, through the injection of the electron donor acetate into the subsurface, with subsequent groundwater monitoring over a three month period. Soluble U(VI) concentrations began to decrease nine days after acetate injections and had declined to below the UMTRA limit within 50 days in some wells. The decrease in soluble U(VI) was coincident with an increase in Fe(II) in the groundwater and a significant enrichment of *Geobacter* species, known Fe(III)- and U(VI)-reducing bacteria. After 39 days, however, the composition of the microbial community began to change as sulfate-reducing organisms dominated, and soluble U(VI) increased with a decrease in Fe(II), acetate and sulfate and an accumulation of sulfite. Thus, the precise constituents of the microbial communities present in the sediments clearly exert control on U(VI) speciation and require careful optimisation. The geochemistry of the groundwaters should also not be overlooked; for example, Brooks *et al.* [11•] recently showed a pronounced effect of Ca^{2+} on the bacterial reduction of U(VI) in bicarbonate buffer. This is important, as there is evidence that Ca-UO_2-CO_3 complexes may dominate the aqueous speciation of U(VI) in some environments [11•]. The presence of calcium, at millimolar concentrations, caused a significant decrease in the rate and extent of bacterial U(VI) reduction by a range of metal-reducing bacteria, suggesting that U(VI) is a less effective electron acceptor when present as Ca-UO_2-CO_3 complexes.

Experiments have also been conducted using sediments collected from the inactive Midnite open-pit uranium mine in eastern Washington [12]. Hexavalent uranium was removed from solution within one month through reduction to U(IV), after stimulation with organic substrates. Phylogenetic analysis of 16S rRNA genes revealed a shift from microaerophilic Proteobacteria to anaerobic low G+C content Gram-positive spore-forming bacteria during incubation. In keeping with the high-sulfate loadings in the pit water, 45% of the sequences related to sulfate-reducing *Desulfosporosinus* spp., which were implicated in U(VI) reduction and shown to reduce U(VI) in a subsequent study [13]. These organisms were also enriched from highly saline uranium-contaminated sediments at the uranium mine tailings site in Shiprock, New Mexico, which were stimulated for U(VI) reduction by the addition of acetate [14]. This observation suggests a potential role for *Desulfosporosinus* spp. in the bioremediation of a range of uranium mining sites.

US DOE Field Research Centre (Oak Ridge) sediments

Several studies have also addressed microbial transformations of soluble U(VI) in sediments from the US DOE FRC in Oak Ridge, Tennessee. Initial experiments used a series of single-well, push-pull tests to determine the potential for stimulation of indigenous microbial communities to reduce a mixture of U(VI) and Tc(VII) in the presence of high (120 mM) nitrate concentrations [15••]. In the absence of added electron donor, nitrate, Tc(VII) and U(VI) reduction was not detected *in situ*. However, in the presence of added ethanol, glucose or acetate as electron donors, rapid denitrification was observed, concurrent with Tc(VII) reduction. U(VI) reduction was not observed until further additions of electron donor resulted in Fe(III)-reducing conditions. This has been noted in other studies using Shiprock uranium mill tailing site sediments, suggesting that it is important to focus on the reduction of nitrate before the reduction of U(VI) *in situ* [16]. Furthermore, reoxidation/remobilization of U(IV) was observed in tests conducted with high initial nitrate concentrations, suggesting that nitrate-dependent microbial U(IV) oxidation could inhibit or reverse U(VI) reduction and decrease the stability of U(IV) in this environment [15••].

Although some microbial community analyses were reported in this FRC study, an additional publication specifically addressed changes in the microbial communities at this site after biostimulation of metal-reducing bacteria *in situ* [17•]. Examination of the sediment chemistry in cores adjacent to wells treated during push-pull tests revealed that sediment pH increased by 1 to 2 units three and a half months after the addition of electron donor and that nitrate was largely depleted. Retrieved 16S rRNA gene sequences included those of species from the α, β, γ and δ subdivisions of the Proteobacteria, as well as low and high G+C content Gram-positive bacteria. Furthermore, sequences related to previously cultured metal-reducing δ-Proteobacteria increased from 5% to nearly 40% after biostimluation. Quantitative PCR confirmed that *Geobacter*-type 16S rRNA gene sequences increased in biostimulated sediments by one to two orders of magnitude at two of the four sites tested, suggesting that members of the δ-Proteobacteria subdivision, including *Anaeromyxobacter* and *Geobacter* species, may be important metal-reducing organisms in acidic subsurface sediments. This was noteworthy, as *Geobacter* species are normally associated with neutral pH environments.

These results were further supported by the observations of Petrie *et al.* [18] who focused specifically on Fe(III)-reducing bacteria indigenous to FRC high uranium, high nitrate, low pH sediments and uncontaminated back-

ground sediments. Phylogenetic analysis of 16S rRNA gene sequences extracted from the highest positive most probable number (MPN) dilutions revealed again that the predominant members of Fe(III)-reducing consortia from background sediments were closely related to members of the *Geobacteraceae* family, while the recently characterized Fe(III)-reducing bacterium *Anaeromyxobacter* sp. and organisms not previously shown to reduce Fe(III) (*Paenibacillus* and *Brevibacillus* spp.) predominated in the Fe(III)-reducing consortia of contaminated sediments.

Finally, similar sediments have also been used in microcosm experiments [19], and again demonstrated effective removal of the U(VI) supplied. Starting at low pH (pH 4), with high (55 mM) concentrations of nitrate, the addition of organic electron donors resulted in slow removal of ~20% of the nitrate over 120 days, with a concurrent increase in pH and uranium precipitation. However, 90% of the precipitated uranium in this study was detected as U(VI), suggesting an alternative mechanism for removal rather than reduction to insoluble U(IV). Interestingly, molecular analysis of the sediment suggested that the addition of electron donors did not stimulate the growth of known metal-reducing bacteria in this study.

Mechanisms of soluble U(VI) reduction

The mechanisms of enzymatic reduction of soluble U(VI) by sulfate-reducing bacteria such as *Desulfovibrio* species remain a topic of interest in several laboratories, following the initial observation over a decade ago that this reduction is mediated by cytochrome c_3. For example, the rate of U(VI) reduction by cell suspensions of *D. desulfuricans* G20 harvested from growth medium containing non-lethal concentrations of uranyl acetate (1 mM) was higher than the rate of U(VI) reduction in cells pre-grown in uranium-free medium [20]. Western blot analysis did not detect cytochrome c_3 in periplasmic extracts from cells grown in the presence of uranium, although it was clearly synthesized as expression of the tetraheme cytochrome was not altered by uranium during growth of the cells (monitored through a translational fusion of the gene encoding cytochrome c_3 to *lacZ*). Instead, the cytochrome was found tightly associated with insoluble U(IV) (uraninite) after the periplasmic contents of cells were harvested by a pH shift.

Elias *et al.* [21] also investigated the role of cytochrome c_3 in Fe(III), U(VI) and sulfate reduction by *Desulfovibrio vulgaris*, using lactate, pyruvate or hydrogen as the electron donor. The study provided further evidence for the role of cytochrome c_3 in metal reduction and suggested a common pathway for metal and sulfate reduction coupled to organic electron donors. However, there appeared to be different H_2-dependent mechanisms for metal and sulfate reduction. Interestingly, *Desulfosporosinus* species do not contain cytochrome c_3 but still reduce U(VI) (see above), suggesting an alternative enzymatic pathway in organisms of this genus [13].

The mechanisms of reduction of actinides and other toxic metals/radionuclides in the specialist metal-reducing bacteria belonging to the genera *Geobacter* and *Shewanella* remain an area of intense activity. Significant advances are anticipated given the availability of the full genome sequences for *Shewanella oneidensis* MR-1 [22] and *Geobacter sulfurreducens* [23].

Solid-phase U(VI) transformations

Although much work has focused on the reduction of soluble U(VI), the fate of sorbed U(VI), which can be appreciable in sediments, has also been addressed. In one study, Ortiz-Bernad *et al.* [24•] investigated the effect of U(VI) adsorption to sediment on microbial reduction of the actinide, using uranium-contaminated sediments from Rifle, Colorado. When sediment was incubated with acetate, U(VI) and Fe(III) reduction was stimulated and the concentration of U(VI) in the groundwater decreased. However, most of the uranium associated with the sediment was U(VI), and this was not reduced, suggesting that sorbed U(VI) was not bioavailable for microbial reduction.

Jeon *et al.* [25] also investigated the effect of synthetic Fe(III) oxides and natural Fe(III) oxide-containing solids on reduction of U(VI) by pure cultures of *G. sulfurreducens*. In most cases, more than 95% of added U(VI) was sorbed to the solid fraction. The presence of the synthetic Fe(III) oxides had no effect on the rate or extent of U(VI) reduction, although U(VI) reduction was generally slower and less extensive with natural Fe(III) oxide-containing solids. The addition of the electron shuttle anthraquinone-2,6-disulfonate (AQDS) stimulated reduction of both Fe(III) and U(VI) with the natural solids, leading the authors to propose that incomplete reduction of U(VI) in the presence of the natural material resulted from the sorption of U(VI) to 'enzymatically inaccessible' surface sites, but AQDS facilitated electron transfer to these sites.

Another study focused on microbial growth and the reduction of both sorbed and soluble phase U(VI) under sulfate-reducing conditions [26•]. Several surfaces were used, including redox-sensitive hematite, goethite and ferrihydrite in addition to redox-insensitive quartz. Growth of *Desulfovibrio desulfuricans* G20 was accompanied by reduction of U(VI) and sulfate, leading to uraninite formation through either enzymatic reduction or reaction with biogenic sulfides. However, after depletion of lactate some of the uranium that had been removed from solution was resolubilized by Fe(III) remaining in the hematite and goethite. Other studies have looked at *D. desulfuricans* attached to hematite surfaces to quantify surface-associated U(VI) complexation, transformation and mobility in flow-through systems [27]. The characteristics of the U(VI) complex(es) formed at the hematite

surface were influenced by the composition of the bulk aqueous phase flowing across the surface and by the presence of surface-associated bacteria. A companion study also quantified U(VI) removal by this organism grown in flat-plate continuous-flow reactors over a 32 week period [28].

New organisms for uranium precipitation

Several new organisms have been isolated recently that remove uranium from solution. Heterotrophic bacteria were isolated from an acidic uranium-contaminated sediment at the Midnite mine [29]. Five isolates and one reference strain, *D. radiodurans*, were studied for uranium accumulation and resistance to the actinide. One Gram-positive isolate of high G+C content, closely related to *Arthrobacter ilicis*, accumulated uranium intracellularly as precipitates associated with polyphosphate granules. The authors suggested this association as a possible detoxification mechanism in this organism.

In another study, a facultatively anaerobic acid-resistant bacterium, designated strain FRCI, was isolated from a low-pH, nitrate- and U(VI)-contaminated subsurface sediment from the US DOE FRC in Oak Ridge, Tennessee [30]. The U(VI)-reducing isolate, designated *Salmonella subterranea* sp. nov., was enriched at pH 4.5 in minimal medium with nitrate as the electron acceptor, hydrogen as the electron donor, and acetate as the carbon source.

Fission products: the reduction of Tc(VII)

Another important radionuclide that has been studied intensively is ^{99}Tc, a long-lived (half-life 2.1×10^5 years) fission product of ^{235}U. Under oxic conditions it is normally present as Tc(VII) (e.g. the pertechnetate anion TcO_4^-), which is both highly soluble and mobile in the environment. However, microbial reduction (enzymatic or mediated by either sulfide or Fe(II)) can lead to the formation of insoluble Tc(IV) [1•]. Recent studies have focused on the redox cycling of Tc in a wide range of environments, including organic matter rich soils, paddy field surface waters, and aquifer sediments.

The behaviour of ^{99}Tc has been studied in organic matter rich soils, colonized with axenic and mixed cultures of bacteria [31]. In the presence of nitrate-reducing bacteria, the solubility of Tc(VII) was not affected until denitrification was complete. At this point, the redox potential dropped, accompanied by Fe(III) reduction and Tc(VII) removal. Significant Tc immobilization was also noted under sulfate-reducing conditions. Sequential chemical extraction of precipitated Tc suggested association with organic matter in the soils, which the authors propose to play a role in the stability of the reduced Tc. Tc(VII) (as $^{95m}TcO_4^-$) immobilization was also noted in surface water covering paddy fields [32] and was compared to measurements of the solubility of other trace elements (^{46}Sc, ^{58}Co, ^{65}Zn, ^{75}Se, ^{83}Rb, ^{85}Sr, ^{88}Y, ^{95}Nb, ^{139}Ce, ^{143}Pm, ^{153}Gd, ^{173}Lu, ^{175}Hf and ^{183}Re). Among the 14 other elements investigated, Nb was immobilised by microbial action, with the amounts of insoluble Nb positively correlated with those of Tc, suggesting some similarities in the biogeochemical cycles of these two elements.

Tc solubility has also been studied using core samples from a shallow sandy aquifer located on the US Atlantic Coastal Plain [33•]. The dominant electron donor in the sediments was (0.5 M HCl extractable) Fe(II), with Tc(IV) hydrous oxide being the major solid-phase reduction product. In buried peats, excess Fe(II) did not result in complete removal of Tc from solution, perhaps because organic complexation of Tc(IV) limited formation of the Tc(IV) hydrous oxide. The authors noted presumptive evidence for direct enzymatic reduction in only a few key sand samples. A new haloalkaliphilic bacterium, isolated from soda-lake environments, was shown to be capable of reducing Tc(VII) enzymatically at pH 10 in carbonate medium [34]. This microorganism should be added to the range of neutraphilic microorganisms capable of enzymatic Tc(VII) reduction.

Returning to the interactions between technetium and organics in sediment systems, the fate of Tc(VII) introduced into a range of humic-rich reducing environments was recently studied using extended X-ray absorption fine structure spectroscopy [35]. The authors reported the formation of small Tc(IV) oxidic polymers, which they argued might aggregate into larger units (colloids) that could precipitate or interact with dissolved/immobile humic substances, offering a potential new model for metal–humate complexation in humic-rich environments.

The potential for biogenic Fe(II)-mediated reduction of Tc(VII) has also been assessed in detail in other studies; for example, using a range of sediments from the US DOE Hanford and Oak Ridge sites [36]. These sediments also contained significant amounts of Mn(III,IV) oxides, which had to be reduced before Fe(III) and Tc(VII) were reduced. The authors concluded that reduction of the Tc(VII) occurred through surface complexes of biogenic Fe(II), based on experiments using pre-reduced, sterilized sediments in combination with chemical extractions and ^{57}Fe Mössbauer spectroscopic analyses of the Fe(III) and Fe(II) phases.

Conclusions

Significant funds are now being spent on bioremediation programmes for environments contaminated with nuclear waste materials, through several large government sponsored programmes (e.g. the US DOE Natural and Accelerated Bioremediation Research program). This high level of investment has resulted in significant advances in both the fundamental understanding of the biogeo-

chemical cycles of priority radionuclides, and also in the science underpinning future remediation efforts to treat contaminated sediments and waters. Remaining challenges include the need to optimise procedures for sustained, effective remediation in the presence of problematic co-contaminants including competing anions, toxic metals, organics and chelating agents. The assessment of suitable bioremediation end points (e.g. reduced, insoluble, sediment-bound radionuclides) remains an important topic. Of particular interest, given the long half-life of many priority radionuclides, is the susceptibility of such 'post-reduction' minerals to reoxidation and remobilisation via microbial metabolism or abiotic mechanisms.

Acknowledgements

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Lloyd JR, Renshaw JC: **Microbial transformations of radionuclides: fundamental mechanisms and biogeochemical implications**. *Met Ions Biol Syst* 2005, **43**: in press.
This recent review discusses the interactions of microorganisms with a wide range of radionuclides, and notes how such interactions can impact on their mobility in the environment. There is also useful information on how these natural processes can be used for *in situ* and *ex situ* remediation of radioactive waste.

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3. • Whicker FW, Hinton TG, MacDonell MM, Pinder JE III, Habegger LJ: **Avoiding destructive remediation at DOE sites**. *Science* 2004, **303**:1615-1616.
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In this paper, the authors surveyed the microbiology of a deep repository injected with liquid waste containing fission products of uranium, other metals and sodium salts (nitrate, acetate, carbonate and sulfate). Sampling at a depth of 162–405 m, a wide range of anaerobic microorganisms were found and in some cases were stimulated by contamination with the radioactive waste.

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Very few studies have looked at the interactions between microorganisms and actinides other than uranium. In this interesting paper, the authors assess the toxicity of uranium, as well as plutonium and neptunium, to a range of bacteria. Plutonium was supplied in two oxidation states (VI and IV) and inhibited growth only at millimolar concentrations under the conditions tested. It is unlikely that these concentrations of soluble Pu would be present in the environment, indicating that toxicity of actinides may not pose a problem for *in situ* bioremediation.

10. •• Anderson RT, Vrionis HA, Ortiz-Bernad I, Resch CT, Long PE, Dayvault R, Karp K, Marutzky S, Metzler DR, Peacock A *et al.*: **Stimulating the *in situ* activity of Geobacter species to remove uranium from the groundwater of a uranium-contaminated aquifer**. *Appl Environ Microbiol* 2003, **69**:5884-5891.
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