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Biotransformation of Radioactive Waste: Microbial Reduction of Actinides and Fission Products

Jonathan R. Lloyd,*[,a] Joanna C. Renshaw,[a,b] Ian May,[b] Francis R. Livens,[c] Ian T. Burke,[c] Robert J. G. Mortimerc,[c] and Katherine Morris[c]

[a]*The Williamson Research Centre for Molecular Environmental Studies and The School of Earth, Atmospheric and Environmental Sciences, The University of Manchester, Manchester M13 9PL, UK*
[b]*Centre of Radiochemistry Research and The School of Chemistry, The University of Manchester, Manchester M13 9PL, UK*
[c]*School of Earth and Environment, University of Leeds, Leeds, LS2 9JT, UK*

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The microbial reduction of radionuclides has attracted recent interest as these transformations can play crucial roles in controlling the mobility of key redox active actinides and fission products in a range of environments and, if harnessed, may offer the basis of biotechnological processes for the remediation of radioactive waste. This review focuses on recent research on the reduction of radionuclides including U(VI), Np(V), Pu(IV), and Tc(VII). Rapid advances over the last decade have resulted in a detailed understanding of some of these transformations at a molecular level. Where known, the mechanisms of metal reduction are discussed, alongside the environmental impact of such transformations and possible biotechnological applications that could utilise these activities.

1. Introduction

The release of radionuclides from nuclear sites and their subsequent mobility in the environment is a subject of intense public concern. Natural sources of radioactivity include U (present in Earth's crust at concentration of 1.8 ppm), Th, Ra isotopes, and radon, while significant quantities of natural and artificial/manmade radionuclides were also released as a consequence of nuclear weapons testing in the 1950s and 1960s, and via accidental release e.g. from Chernobyl in 1986.[1] The major burden of anthropogenic environmental radioactivity, however, is from the controlled discharge of process effluents produced by industrial activities allied to the generation of nuclear power. The inventory of radionuclides generated during the last 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, ^{137}Cs, several of which (notably key actinides, ^{99}Tc and ^{129}I) are redox active. Wastes containing some or all of these radionuclides are produced at the many steps in 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. All wastes pose a potential threat to the environment and require (1) treatment prior to release, and (2) a much deeper understanding of the biological and chemical factors controlling the mobility of radionuclides in the environment should they be dispersed by accident or as part of a controlled/monitored release e.g. in effluents. The aim of this review is to describe what is known about the interactions of anaerobic metal-reducing with key redox-active radionuclides, and where appropriate to discuss how such reductive biotransformations can impact on the mobility of radionuclides in the environment. In addition, as there is intense interest in harnessing these natural processes for *in situ* and *ex situ* remediation of radioactive waste, the biotechnological applications of radionuclide-microbe interactions are also discussed. Indeed, research programmes aimed at remediating large areas of land contaminated by radionuclides in the US, at so called "super-fund sites", have resulted in significant advances in the understanding of the mechanisms of metal and radionuclide reduction in the subsurface (for examples see www.lbl.gov/NABIR/).

2. Reduction of Fe(III)

Microbial metal reduction has been studied intensively over the last two decades, and the best studied examples of dissimilatory metal-reducing prokaryotes are the wide range of *Archaea* and *Bacteria* that are able to conserve energy through the reduction of Fe(III) (ferric iron) to Fe(II) (ferrous iron). The environmental relevance of Fe(III) reduction have been well documented.[2, 3] Indeed, geochemical and microbiological evidence suggest that the reduction of Fe(III) may have been an early form of respiration on Earth,[4] and is a candidate for the basis of life on other planets.[5] On modern Earth, Fe(III) can be the dominant electron acceptor for microbial respiration in many subsurface environments.[6] As such, Fe(III)-reducing communities can be responsible for the majority of the organic matter oxidized in such environments. Recent studies have shown that a range of important xenobiotics that contaminate aquifers can also be degraded under anaerobic conditions by Fe(III)-reducing microorganisms.[7] In addition to playing an important role in the degradation of organic material, Fe(III)-reducing microorganisms can influence the mineralogy of sediments through the reductive dissolution of insoluble Fe(III) oxides. These processes can result in the release of potentially toxic levels of Fe(II), and also trace metals and radionuclides that were bound by the Fe(III) minerals. Depending on the chemistry of the water, a range of reduced minerals can also be formed including magnetite (Fe_3O_4), siderite ($FeCO_3$) and vivianite ($Fe_3(PO_4)_2 \cdot 8H_2O$), resulting in a change in structure of the sediments. Finally, Fe(III)-reducing microorganisms can also impact directly on the fate of other high valence contaminant metals and radionuclides through direct enzymatic reduction, and also via indirect reduction catalysed by biogenic Fe(II). The bioreduction of U(VI) and other actinides, in addition to fission products such as Tc(VII) by Fe(III)-reducing

*Corresponding author. E-mail: jon.lloyd@manchester.ac.uk. FAX: +44-161-275-3947.

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microorganisms will be discussed in detail in this review and can result in immobilization of these potentially mobile radionuclides in sediments.[8]

3. Reduction of actinides and fission products

The metabolic diversity of Fe(III)-reducing bacteria is large, and a wide range of other redox active metals can be reduced in lieu of Fe(III), including other more toxic transition metals such as Cr(VI), Hg(II), Co(III), Pd(II), Au(III), Ag(I), Mo(VI), and V(V), metalloids such as As(V) and Se(VI), and radionuclides including U(VI), Np(V), and Tc(VII).[6] Indeed, because many radionuclides of concern are both redox active and less soluble when reduced, bioreduction offers much promise for controlling the solubility and mobility of target radionuclides in contaminated sediments e.g. the reduction of U(VI) (the uranyl ion; UO_2^{2+}) to U(IV) (uraninite; UO_2)[9] or the reduction of the fission product Tc(VII) (the pertechnetate ion; TcO_4^-) to Tc(IV) (TcO_2).[10]

4. Reduction of U(VI)

The first demonstration of dissimilatory U(VI) reduction was by Lovley and coworkers[9] who reported that the Fe(III)-reducing bacteria *Geobacter metallireducens* (previously designated strain GS-15) and *Shewanella oneidensis (*formerly *Alteromonas putrefaciens* and then *Shewanella putrefaciens)* can conserve energy for anaerobic growth *via* the reduction of U(VI). It should be noted, however, that the ability to reduce U(VI) enzymatically is not restricted to Fe(III)-reducing bacteria. Other organisms including a *Clostridium* sp.[11] and the sulfate-reducing bacteria *Desulfovibrio desulfuricans*[12] and *D. vulgaris*[13] also reduce uranium, but are unable to conserve energy for growth *via* this transformation. To date, *D. vulgaris* remains the only organism in which the enzyme system responsible for U(VI) reduction has been characterised in detail. Purified tetraheme cytochrome c_3 was shown to function as a U(VI) reductase *in vitro*, in combination with hydrogenase, its physiological electron donor.[13] *In vivo* studies using a cytochrome c_3 mutant of the close relative *D. desulfuricans* strain G20 confirmed a role for cytochrome c_3 in hydrogen-dependent U(VI) reduction, but suggested additional pathways from organic electron donors to U(VI), that bypassed the cytochrome.[14]

More recent studies have identified a homologous cytochrome (PpcA), a triheme periplasmic cytochrome c_7 of the Fe(III)-reducing bacterium *Geobacter sulfurreducens* that may also play a role in U(VI) reduction.[15] The protein was able to reduce U(VI) *in vitro,* while a *ppcA* deletion mutant supplied with acetate as an electron donor had lower activity against U(VI). Additional (if indirect) evidence linking the activity of this periplasmic protein with U(VI) reduction *in vitro* included the precipitation of the reduced product U(IV) in the periplasm, and the lack of impact of protease treatment of whole cells on the ability to reduce U(VI).[15] This final result is important, as it implies that U(VI) and Fe(III) may be reduced by different mechanisms in *G. sulfurreducens*. A significant portion of U(VI) would seem to be reduced in the periplasm, while the reduction of insoluble Fe(III) oxides was inhibited dramatically by protease treatment, presumably due to removal of surface bound cytochromes required for reduction of the extracellular electron acceptor. More recent studies from our laboratory have identified U(V) as a key unstable intermediate in the reduction of uranium by *G. sulfurreducens*, and as this organism is unable to reduce stable Np(V), it would seem that the specificity for hexavalent actinides is extremely high in *Geobacter* species.[16] The mechanism of U(VI) reduction by a *Shewanella putrefaciens* strain has also been investigated.[17] A novel screening method was used to identify mutants that were unable to reduce U(VI). Evidence was presented to suggest that the mechanism of U(VI) reduction was distinct from those of Fe(III) and Mn(IV) reduction, but may share components of the nitrite reducing pathway.[17]

5. Reduction of Np(V) and Pu(IV)

Although ^{238}U remains the priority pollutant in most medium and low level radioactive wastes, other actinides including ^{230}Th, ^{237}Np, ^{241}Pu, and ^{241}Am can also be present.[1] Th(IV) and Am(III) are stable across most Eh values encountered in radionuclide-contaminated waters but the potentials for Pu(V)/Pu(IV) and Np(V)/Np(IV), in common with that of U(VI)/U(IV) are more electropositive than the standard redox potential of ferrihydrite/Fe^{2+} (approximately 0 V).[3] Thus, Fe(III)-reducing bacteria have the metabolic potential to reduce these radionuclides enzymatically, or *via* Fe(II) produced from the reduction of Fe(III) oxides. This is significant because the tetravalent actinides are amenable to bioremediation due to their high ligand-complexing abilities,[18] and are also immobilized in sediments containing active biomass.[19] Thus, although it is possible for Fe(III)-reducing bacteria to reduce and precipitate actinides in one step, e.g. the reduction of soluble U(VI) to insoluble U(IV) (see above), some transformations do not result in direct formation of an insoluble mineral phase but in the formation of a cation more amenable to bioprecipitation. This is illustrated when considering highly soluble Np(V) (NpO_2^+), which was reduced to soluble Np (IV) by *Shewanella putrefaciens,* with the Np(IV) removed as an insoluble phosphate biomineral by a phosphate-liberating *Citrobacter* sp.[20] However, not all Fe(III)-reducing bacteria are able to reduce Np(V), as recent work in our laboratory has shown that cells of *Geobacter sulfurreducens* are unable to reduce this actinide, suggesting a surprising degree of specificity for hexavalent actinides such as U(VI).[16] Thus, bioremediation strategies that use *Geobacter* species to treat mixed actinide wastes must be designed and monitored with care. On this note, some studies have suggested that the reduction of Pu(IV) to (III) can be achieved by Fe(III)-reducing bacteria, although the Pu(III) was reported to reoxidize spontaneously.[21] Although this may lead to solublization of sediment-bound Pu(IV), it will yield a trivalent actinide that is also amenable to bioremediation using a range of microbially produced ligands.[18] The biochemical basis of these transformations remain uncharacterised.

6. Reduction of Tc(VII)

The fission product technetium is another long-lived radionuclide that is present in nuclear waste and has attracted considerable recent interest. This is due to a combination of its mobility as the soluble pertechnetate ion (Tc(VII); TcO_4^-), bioavailability as an analog of sulfate and long half-life (2.13×10^5 years).[22] Like Np(V), Tc(VII) has weak ligand complexing capabilities and is difficult to remove from solution using conventional "chemical" approaches. Several reduced forms of the radionuclide are insoluble, however, and metal-reducing microorganisms can reduce Tc(VII) and precipitate the radionuclide as a low valence oxide.

Although microbial metabolism was known to decrease the solubility of Tc from earlier studies,[23, 24] Lloyd and Macaskie were the first to unequivocally demonstrate direct enzymatic reduction of Tc(VII) by microorganisms.[25] In this study, a novel phosphorimager technique was used to confirm reduction of the radionuclide by *Shewanella putrefaciens* and *Geobacter metallireducens,* with similar activities subsequently detected in laboratory cultures of *Rhodobacter sphaeroides, Paracoccus denitrificans,* some Pseudomonads,[26] *Escherichia coli*,[27] and a range of sulfate-reducing bacteria.[28–30] Other workers have used this technique to show that *Thiobacillus ferrooxidans* and

T. thiooxidans[31] and the hyperthermophile *Pyrobaculum islandicum*[32] are also able to reduce Tc(VII). It should be stressed that Tc(VII) reduction has not been shown to support growth in any of these studies, and seems to be a fortuitous biochemical side reaction in the organisms studied to date. Finally, X-ray absorption spectroscopy studies have recently identified insoluble Tc(IV) as the final oxidation state produced when Tc(VII) is reduced enzymatically by *Geobacter sulfurreducens*,[10] *E. coli* (Lloyd and Sole unpublished), and *Shewanella putrefaciens*.[33] Recent studies have also shown that Tc(VII) can be reduced by indirect microbial processes via, for example, biogenic sulphide,[29] Fe(II)[10] or U(IV).[26] Tc(VII) reduction and precipitation by biogenic Fe(II) is particularly efficient, and may offer a potentially useful mechanism for the remediation of Tc-contaminated sediments containing active concentrations of Fe(III)-reducing bacteria.[10]

The biochemical basis of Tc(VII) reduction has been best studied in *Escherichia coli*. Initial studies demonstrated that anaerobic, but not aerobic, cultures of *E. coli* reduced Tc(VII) with the reduced radionuclide precipitated within the cell.[27] Results obtained from studies conducted with wild type cells and 34 defined mutants defective in the synthesis of regulatory or electron transfer proteins were used to construct a model for Tc(VII) reduction by *E. coli*. The central tenet of this model is that the hydrogenase 3 component of FHL catalyzes the transfer of electrons from dihydrogen to Tc(VII). According to this model, the formate dehydrogenase component (FdhH) is required only if formate, or a precursor, is supplied as an electron donor for Tc(VII) reduction in place of hydrogen. This model has been validated by the observations that a mutant unable to synthesize hydrogenase 3, was unable to reduce Tc(VII) when either hydrogen or formate was supplied as an electron donor.[27]

The identification of hydrogenase 3 of FHL as the Tc(VII) reductase of *E. coli* opened up the way for a program to screen for organisms with naturally enhanced activities against Tc(VII). Several organisms documented to have naturally high activities of FHL or uptake hydrogenase were tested, resulting in the identification of several strains of sulfate-reducing bacteria that were able to couple the oxidation of formate or hydrogen to Tc(VII) reduction.[28] Rates of reduction in some strains were approximately 64 fold greater than those recorded in anaerobic cultures of *E. coli*.[34] *Desulfovibrio desulfuricans*[30] and related strains[21] were also able to utilise formate as an efficient electron donor for Tc(VII) reduction. This is consistent with the existence of a rudimentary FHL complex (consisting of a formate dehydrogenase coupled to a hydrogenase *via* a cytochrome) located in the periplasm of these strains.[35] Accordingly, the site of reduced Tc precipitation was identified as the periplasm in *D. desulfuricans*,[30] and more recent studies have confirmed a role for a periplasmic Ni-Fe hydrogenase in Tc(VII) reduction by a relative in the δ subclass of the *Proteobacteria*, the sulfate-reducing bacterium *Desulfovibrio fructosovorans*.[36] Subsequent studies on the development of a bioprocess to treat Tc(VII)- contaminated water have focused on the use of immobilized cells of sulfate-reducing bacteria such as *D. desulfuricans* which are robust and capable of treating low concentrations of Tc(VII) against a high background of contaminating nitrate ions, which is often noted in nuclear waste.[30, 34] Finally, recent work from our laboratories have suggested that reduced, insoluble Tc(IV) phases are surprisingly resistant to remobilization under oxidizing conditions, e.g. in the presence of high nitrate concentrations.[37] Thus, long-term immobilization of Tc can be achieved in contaminated sediments by *in situ* remediation using indigenous Fe(III)-reducing bacteria.

7. Future directions

Although the environmental relevance of metal reduction processes has only become apparent recently, rapid advances in the understanding of these important biotransformations have been made. However, we still have much to learn about the precise mechanisms involved, and the full impact of such reactions on a range of biogeochemical cycles. Given the availability of genomic sequences for key metal-reducing microorganisms, new post-genomic and proteomic approaches and the possibility of combining these tools with advanced techniques from other branches of science and technology (e.g. isotopic, spectroscopic and computational tools) rapid advances in these areas are predicted.

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