

CHAPTER ELEVEN

The microbial ecology of land and water contaminated with radioactive waste: towards the development of bioremediation options for the nuclear industry

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Introduction

The release of radionuclides from nuclear and mining sites and their subsequent mobility in the environment is a subject of intense public concern and has promoted much recent research into the environmental fate of radioactive waste (Lloyd & Renshaw 2005b). Naturally occurring radionuclides can input significant quantities of radioactivity into the environment while both natural and artificial/manmade radionuclides have also been released as a consequence of nuclear weapons testing in the 1950s and 1960s, and via accidental release, e.g., from Chernobyl in 1986. The major burden of anthropogenic environmental radioactivity, however, is from the nuclear facilities themselves and includes the continuing controlled discharge of process effluents produced by industrial activities allied to the generation of nuclear power.

Wastes containing 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 (Lloyd & Renshaw 2005b). The stewardship of these contaminated waste-streams needs a much deeper understanding of the biological and chemical factors controlling the mobility of radionuclides in the environment. Indeed, this is highly relevant on a global stage as anthropogenic radionuclides have been dispersed to the environment both by accident and as part of a controlled/monitored release, e.g., in effluents. Micro-organisms have adapted

to live even in radioactively contaminated environments (Selenska-Pobell 2002; Lloyd 2003; Fredrickson *et al.* 2004; Ruggiero *et al.* 2005; Akob *et al.* 2007), and they can affect radionuclide speciation via a number of mechanisms which are potentially useful for scalable, cost-effective bioremediation of sediments and waters impacted by nuclear waste (Keith-Roach & Livens 2002; Lloyd 2003; Suzuki *et al.* 2003; Lloyd & Renshaw 2005a; Brodie *et al.* 2006; Fomina *et al.* 2007).

The aim of this chapter is to give an overview of known interactions of micro-organisms with key radionuclides, focusing on potential roles in controlling radionuclide mobility in the subsurface. We will then discuss the influence of microbial processes on the immobilisation of radionuclides described in recent laboratory studies from our groups, focusing on two priority contaminants: technetium and uranium. The former studies address the impact of bioreduction strategies on solubility and include work on stimulated bioreduction (achieved via added organic electron donor) on technetium solubility, while the latter studies demonstrate the immobilisation of uranium in sediments from a uranium mining waste pile without the addition of a carbon source. Finally, due to the widespread use of nitric acid in the nuclear sector, the multiple influences of nitrate and nitrate-reducing bacteria on the solubility of radionuclides will be discussed, especially where the activities of these organisms impact on the biogeochemistry of uranium and technetium.

Microbial interactions with radionuclides

The environmental fate of a radionuclide is governed by the interplay between the background matrix of the radioactive material, the often complex chemistry of the radionuclide in question and a broad range of chemical factors associated with the environment that has been impacted by the radioactive material in question (Lloyd & Renshaw 2005b). In addition, microbial activity will have a profound effect on the solubility of radionuclides via a complex range of often overlapping mechanisms including biosorption, bioaccumulation, biotransformation, biomineralisation and microbially enhanced chemisorption of heavy metals (Fig. 11.1). For a more extensive discussion of these microbe–radionuclide interactions, the reader is directed to the following reviews (Lloyd & Lovley 2001; Keith-Roach & Livens 2002; Pedersen 2005; Renshaw *et al.* 2007). A brief synopsis, focusing on interactions with subsurface micro-organisms, is given below.

Biosorption describes the metabolism-independent sorption of heavy metals and radionuclides to biomass and encompasses both adsorption and absorption. Multiple studies over the past three decades have confirmed that a wide range of micro-organisms are capable of efficient biosorption of radionuclides (Francis *et al.* 2004; Merroun *et al.* 2005; Ohnuki *et al.* 2005). Both living and dead biomass is capable of biosorption, with ligands involved in metal binding including carboxyl, amine, hydroxyl, phosphate and sulphhydryl groups. Most

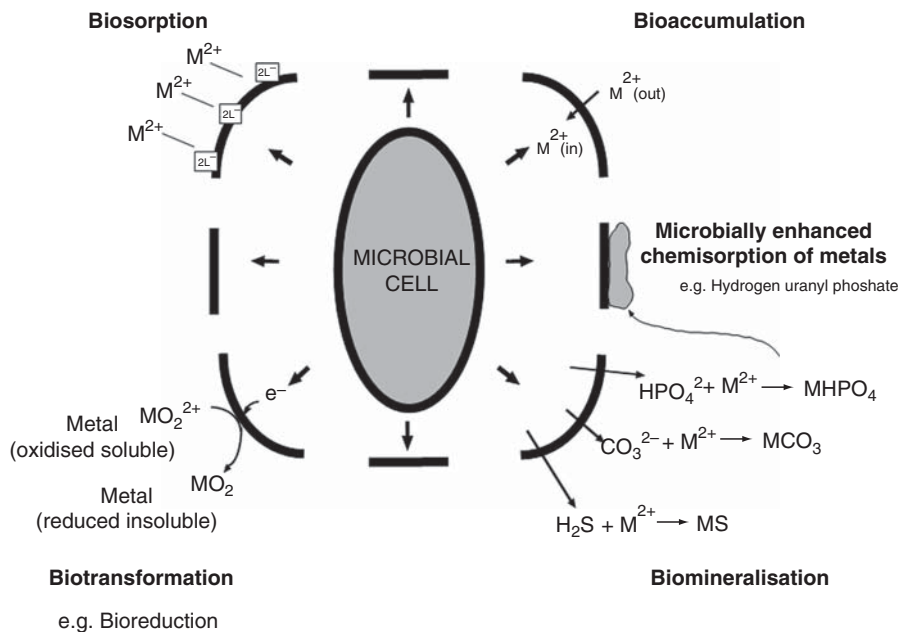


Figure 11.1. Mechanisms of metal/radionuclide-microbe interactions.

studies have investigated sorption of U(VI) (Francis *et al.* 2004; Merroun *et al.* 2005), with far less known about the sorption of other radionuclides, such as Pu (Ohnuki *et al.* 2007). For a more detailed overview of this research area, the reader is referred to Lloyd and Macaskie (2000).

Bioaccumulation, which can be defined as energy-dependent metal uptake, has been demonstrated for most physiologically important metal ions and some radionuclides which can enter the cell as chemical ‘surrogates’ using these transport systems. There have been just a few investigations of the intracellular accumulation of actinides, and almost all of these have concentrated on uranium (Francis *et al.* 2004, Suzuki & Banfield 2004). It has been suggested that intracellular uptake of uranium is metabolism-independent, and results from increased cell-membrane permeability caused, for example, by uranium toxicity (Suzuki & Banfield 1999). In other studies, bioaccumulation has been proposed as a mechanism promoting uranium tolerance in *Arthrobacter* spp., although the final fate of the uranium taken up into the cell remains to be confirmed (Suzuki & Banfield 2004; Martinez *et al.* 2006; Geissler 2007). In contrast, the intracellular uptake of Pu(IV) in *Microbacterium flavescens* was reported to be an active, metabolism-dependent transport process (John *et al.* 2001).

Micro-organisms can also catalyse the direct transformation of toxic metals and metalloids to less soluble or more volatile forms via two distinct enzymatic mechanisms. Bioreduction can result in precipitation of some

metals/metalloids, where the reduced form is less soluble than the high oxidation form, as is the case for enzymatic U(VI) reduction to U(IV) (Lovley 1993), while biomethylation can yield highly volatile derivatives. Both mechanisms can result in a decrease in the concentration of soluble metals in contaminated water. While the microbial enzymatic reduction of radionuclides including U(VI), Tc(VII), Np(V) and Pu(IV) has been demonstrated (Lovley 1993; Lloyd & Macaskie 1996; Lloyd *et al.* 2000a; Boukhalifa *et al.* 2007), biomethylation of radionuclides has received little attention.

Lovley and co-workers were the first to demonstrate the dissimilatory reduction of U(VI) by the Fe(III)-reducing bacteria *Geobacter metallireducens* GS-15 and *Shewanella oneidensis* (formerly *Alteromonas putrefaciens*), a process by which energy is conserved for anaerobic growth of these organisms (Lovley *et al.* 1991; Lovley 1993). Other bacteria able to reduce U(VI) (but without conserving energy), include the sulphate-reducing bacteria *Desulfovibrio desulfuricans* (Lovley & Phillips 1992) and *Desulfosporosinus* sp. (Suzuki *et al.* 2004), in addition to *Clostridium* sp. ATCC 53464 (Francis *et al.* 1994), *Salmonella subterranean* strain FRC1 (Shelobolina *et al.* 2004) and *Anaeromyxobacter dehalogenans* strain 2CP-C (Wu *et al.* 2006).

The mechanism of enzymatic reduction of uranium has been characterised most comprehensively in the sulphate-reducing bacterium *Desulfovibrio vulgaris*, which contains a periplasmic cytochrome c3, identified as the terminal reductase for the reduction of U(VI) (Lovley *et al.* 1993). Similar mechanisms may be important in *Geobacter* spp. (Lloyd *et al.* 2002), but the terminal reductase for U(VI) in this organism remains to be identified unequivocally. Interestingly, it was shown recently that *Geobacter sulfurreducens* cannot reduce NpO_2^+ , even though it reduces UO_2^{2+} efficiently (Renshaw *et al.* 2005). The authors suggested that the enzyme system responsible for uranium reduction is capable of transferring one electron to an actinyl ion, and the instability of the resulting U(V) then generates U(IV) via disproportionation. The reduction of Np(V) is not possible, however, because it appears the enzyme is specific for hexavalent actinides, and cannot transfer an electron to NpO_2^+ (Renshaw *et al.* 2005). In contrast, it has been known for some time that *S. oneidensis* is able to reduce Np(V) (Lloyd *et al.* 2000b), and more recent studies have confirmed that cell suspensions of *S. oneidensis* are able to enzymatically reduce unchelated Np(V) to insoluble Np(IV)(s), but cell suspensions of *G. metallireducens* are unable to reduce Np(V) (Icopini *et al.* 2007), suggesting the factors controlling enzymatic reduction of Np(V) are complex.

These two organisms are also able to reduce highly soluble Tc(VII) to insoluble lower-valence species enzymatically (Lloyd & Macaskie 1996). However, in the subsurface, Fe(III)-reducing bacteria can also indirectly reduce and precipitate Tc(VII) via biogenic Fe(II), and this mechanism is especially efficient when the Fe(II) is associated with mineral phases, e.g., when biogenic magnetite is

present. This was first studied in *G. sulfurreducens*, which used hydrogen as the electron donor for enzymatic reduction of Tc(VII) (presumably via a NiFe hydrogenase identified in other bacteria as the terminal reductase for Tc(VII)) (Lloyd *et al.* 1997; Marshall *et al.* 2008). When acetate was supplied as the electron donor, however, enzymatic reduction of Tc(VII) was not possible, but Tc(VII) reduction was very efficient when Fe(III) oxides were provided with the acetate and reduced to biogenic Fe(II)-bearing magnetite, which could then act as an electron shuttle to Tc(VII) and cause reduction. Thus, Fe(II)-bearing biomagnetite was shown to be an excellent reductant for Tc(VII).

Finally, the mechanism of plutonium reduction by *G. metallireducens* and *S. oneidensis* MR-1 has also been studied recently, and it was demonstrated that both organisms produce very little Pu(III) enzymatically from Pu(IV)(OH)₄, whereas in the presence of ethylenediaminetetraacetic acid (EDTA), most of the Pu(IV)(OH)₄(am) was reduced to Pu(III) (Boukhalfa *et al.* 2007). Inefficient enzymatic production of Pu(III) from Pu(IV)(OH)₄ was also identified in *G. sulfurreducens*, even though the organism is very adept at reducing a broad range of extracellular Fe(III) and Mn(IV) minerals (Renshaw *et al.* 2008).

Bio-mineralisation is the process by which metals and radionuclides can be precipitated with microbially generated ligands, e.g., phosphate, sulphide or carbonate. In these examples, the microbial ligands accumulate to high concentrations around the cell, and the cell surface provides a nucleation site for precipitation, resulting in efficient removal of the radionuclide from solution (Renshaw *et al.* 2007). The process of bio-mineralisation can be induced by secretion of inorganic compounds, such as orthophosphate groups, which can directly bind U(VI) in insoluble polycrystalline uranyl hydrogen phosphate or in meta-autunite-like mineral phases (Macaskie *et al.* 1992; Merroun *et al.* 2006; Beazley *et al.* 2007; Jroundi *et al.* 2007). In addition to direct precipitation by microbially generated ligands, actinide ions can also be removed from solution by chemisorption to biogenic minerals ('microbially enhanced chemisorption') (Macaskie *et al.* 1994).

Biogeochemistry of technetium reduction in sediments

Technetium is a significant, long-lived (⁹⁹Tc half-life = 2.15 × 10⁵ years) radioactive contaminant from nuclear fuel cycle operations. It is highly mobile in its oxidised form (as Tc(VII)O₄⁻) but is scavenged to sediments in its reduced forms (predominantly poorly soluble Tc(IV)). As part of a long-term collaboration between our groups in Manchester and Leeds, we have been studying the biogeochemical behaviour of Tc and its potential environmental mobility, to better inform bioremediation approaches and safety case assessments.

Initial experiments used microcosms constructed from Tc-free Humber Estuary surface sediments (with their indigenous microbial populations), which were spiked with low levels (<5 μM) of TcO₄⁻, and technetium solubility

was then monitored as anoxia developed in the microcosms (Burke *et al.* 2005). As expected, the microcosms progressed through a cascade of terminal electron accepting processes during which time over 99% of the Tc was removed from the pore waters. In sterile controls, Tc remained in solution (presumably as Tc(VII)), indicating that removal to sediments was biologically mediated (Burke *et al.* 2005). A detailed analysis of geochemical indicators demonstrated that Tc removal occurred as Fe(III)-reducing conditions developed after the consumption of most of the nitrate and accumulation of Mn(II) in pore waters.

Pure culture microcosms were established by inoculating sterilised mixtures of sediment slurry and river water with cultures of either a nitrate-reducing bacterium (*Pseudomonas stutzeri*), an Fe(III)-reducing bacterium (*Shewanella* sp.) or a sulphate-reducing bacterium (*Desulfovibrio desulfuricans* sp. Essex), all with the addition of an appropriate electron donor. The generation of Fe(II) and the concomitant removal of Tc occurred only in the presence of *Shewanella* and *Desulfovibrio* spp., which suggested that Tc removal is linked to Fe(II) ingrowth in these sediments (Burke *et al.* 2005). The 16S rRNA gene analysis confirmed the presence of organisms related to known nitrate- (*Rhodobacter capsulatus*), sulphur/metal- (*Pelobacter* sp.) and sulphate-reducing bacteria (*Desulfovibrio senezii*) in the sediments. Additionally, *Geobacteraceae*-specific primers were used to detect Fe(III)-reducing bacteria from this phylogenetic group. Thus, there was a complex range of Fe(III)- and sulphate-reducing bacteria present in the sediments that could have been responsible for production of Fe(II), or potentially sulphide, and the subsequent indirect reduction of Tc(VII) mediated by these reduction products. X-ray absorption spectroscopic analysis from progressive anoxia samples spiked with 1000 μM TcO_4^- confirmed that TcO_4^- removal was due to reduction to hydrous Tc(IV) O_2 in Fe(III)- and sulphate-reducing estuarine sediments (Burke *et al.* 2005).

In addition to working with estuarine sediments that initially contained no background radioactivity, we have also worked extensively with sediments from (or representative of) several 'nuclear' sites. For example, microcosm experiments containing soil samples representative of the UKAEA site at Dounreay have been performed with unamended sediments, carbonate buffered sediments and microcosms amended with EDTA, a complexing ligand used in nuclear fuel cycle operations (Begg *et al.* 2007). During the development of anoxia mediated by indigenous microbial populations, Tc(VII) O_4^- was again removed from solution, during periods of microbial Fe(III) reduction when Fe(II) was growing into the microcosms. A pivotal role for biogenic Fe(II) in Tc(VII) reduction and precipitation (Fig. 11.2) was confirmed in microcosms which had been prerduced to the point that Fe(III) reduction dominated, and then sterilised by autoclaving. In these sterile Fe(III)-reducing sediments, the Tc-spike was removed from solution to below the liquid scintillation counting detection limit (>98%) over 21 days.

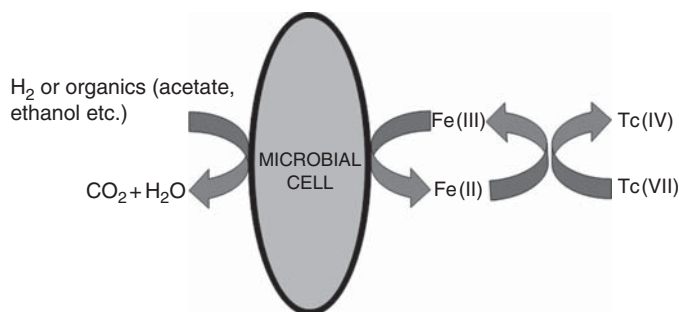


Figure 11.2. Indirect reduction of Tc(VII) mediated by Fe(II).

Similar trends were also noted in sediments collected from the Drigg low-level radioactive waste storage site (Wilkins *et al.* 2007), confirming a generic indirect mechanism for Tc(VII) reduction that is not strongly site specific. Interestingly, in both studies, the reduced insoluble Tc was surprisingly resistant to remobilisation by strong oxidising agents such as nitrate, consistent with data from other parallel studies (Burke *et al.* 2006).

Finally, the interplay between nitrate and the reduction of Tc(VII) was explored in more detail in microcosms prepared from sediments from the US Department of Energy Field Research Center (FRC) in Oak Ridge, Tennessee, USA (McBeth *et al.* 2007). Here the impact of 0, 10 and 100 mM added nitrate on the progression of a range of terminal electron accepting processes and ^{99}Tc immobilisation was assessed. In the nitrate unamended and 10 mM nitrate amended systems, bioreduction proceeded and extractable Fe(II) ingrowth and concomitant Tc(VII) removal was observed. Interestingly, the relatively low (10 mM) addition of nitrate seemed to augment the development of bioreducing conditions. In contrast, in the 100 mM nitrate amended system, Fe(II) ingrowth was limited and no Tc(VII) removal occurred, suggesting strong inhibition of microbial metal reduction at high, but nuclear site relevant concentrations of NO_3^- .

Bacterial community changes induced by uranyl or sodium nitrate treatments and the fate of the added U(VI)

The fate and transport of uranium are governed by the contrasting chemistry of U(IV) and U(VI). U(VI) generally forms soluble, and thus mobile, complexes with carbonate and hydroxide, while U(IV) precipitates as the highly insoluble mineral uraninite. Many studies have focused on *in situ* bioremediative stimulation of native U(VI)-reducing bacteria by the addition of different organic electron donors for aqueous U(VI) reduction such as acetate, lactate, glucose and ethanol to uranium contaminated waters and sediments (Holmes *et al.* 2002; Anderson *et al.* 2003; Suzuki *et al.* 2003; North *et al.* 2004; Brodie *et al.* 2006; Nyman *et al.* 2006).

Although not the focus of such intensive recent research, the fate of uranium in complex natural systems without the addition of organic substances is also of great environmental importance, in order to predict the potential risks of uranium migration within piles, tailings and depository sites and to prevent their spread via groundwater flow. Thus, different microcosm experiments were performed in order to investigate the influence of uranyl or sodium nitrate on the natural bacterial community of a uranium mining waste site in Germany under acidic and oligotrophic conditions (Geissler & Selenska-Pobell 2005; Geissler 2007; Selenska-Pobell *et al.* 2008).

One of the most important observations obtained from the analyses of the uranyl and sodium nitrate treated subsamples was the extremely high diversity of the indigenous bacterial community and the strong changes in community structure noted by increasing the uranyl or sodium nitrate concentrations, as well as a strict dependence on aeration conditions (Geissler & Selenska-Pobell 2005; Geissler 2007; Selenska-Pobell *et al.* 2008). After longer incubations, even with higher U(VI) concentrations, uranium sensitive populations were established under aerobic as well as under anaerobic conditions. This indicated that U(VI) was no longer bioavailable in these long-term experiments (Geissler & Selenska-Pobell 2005; Geissler 2007; Selenska-Pobell *et al.* 2008). Surprisingly, no U(VI) reduction was observed under anaerobic oligotrophic conditions even after longer incubations, when all the available nitrate was depleted (Geissler 2007; Selenska-Pobell *et al.* 2008). Time-Resolved Laser-induced Fluorescence Spectroscopic (TRLFS) analysis demonstrated that, in the uranyl nitrate treated sample incubated for 14 weeks under reducing conditions, most of the added U(VI) was bound by phosphate phases of biotic origin. U(VI) added to this sample was bound in mixed organic and inorganic phosphate compounds, suggesting that, at this site, U(VI)-phosphate chemistry may have a major role in controlling uranium fate.

Recent publications have also shown that representatives of *Rahnella* spp. recovered from uranium contaminated samples are able to immobilise U(VI) via the secretion of orthophosphate (Martinez *et al.* 2007) leading to the precipitation of meta-autunite-like mineral phases under laboratory conditions (Beazley *et al.* 2007). Moreover, it was demonstrated that various bacteria, phylogenetically unrelated to *Rahnella* spp., are able to protect themselves against potentially toxic U(VI) at acidic pH by secretion of inorganic phosphate groups which are involved in precipitation of uranyl hydrophosphate-like phases (Macaskie *et al.* 1992) or of meta-autunite-like compounds (Merroun *et al.* 2006; Jroundi *et al.* 2007; Martinez *et al.* 2007) when studied in laboratory conditions.

The results of the TRLFS spectra of the uranyl nitrate treated soil samples differed significantly from those of the meta-autunite-like phases produced by different bacterial strains in the presence of U(VI) observed in fully defined and relatively simple microbiological media under laboratory conditions

(Merroun *et al.* 2003; Beazley *et al.* 2007). The organic uranyl phosphate complexes detected in the field-based study are the product of interactions with phosphorylated biopolymers supplied by both dead or live bacteria which, along with the orthophosphate released by components of microbial populations such as *Rahnella* spp., can contribute to U(VI) immobilisation.

Mössbauer spectroscopic analyses revealed increased Fe(III) reduction during incubation from 4 to 14 weeks in the uranyl nitrate treated samples incubated under anaerobic conditions (Geissler 2007; Selenska-Pobell *et al.* 2008). It is suggested that after the reduction of the added nitrate, microbially mediated Fe(III) reduction took place even without bioaugmentation via addition of external carbon sources. However, no previously characterised dissimilatory Fe(III) reducers, for example, *Geobacter*, *Shewanella* or *Geothrix* spp., were identified in the uranyl nitrate treated microcosms. It is possible, therefore, that the *Rahnella* spp. or other components of the microbial community detected in the microcosms were able to respire Fe(III). These could include the betaproteobacterial populations detected, by analogy to other Fe(III)-reducing betaproteobacterial species such as *Ferribacterium limneticum* (Cummings *et al.* 1999) or *Rhodoferax ferrireducens* (Finneran *et al.* 2003).

Multiple influences of nitrate and nitrate-reducing bacteria on radionuclide solubility during bioremediation

As noted already, nitrate is a common co-contaminant at sites where nuclear materials have been processed or stored. In these settings, nitrate and nitrate-reducing bacteria have the potential to influence strongly the environmental behaviour of not only uranium but also of other radionuclides. Here we review some important aspects of these influences on U and Tc solubility, highlighted in recent publications.

Due to anthropogenic contamination, e.g., by nitric acid, subsurface nitrate concentrations can often be very high (exceeding 100 mMNO₃⁻; McBeth *et al.* 2007), resulting in a dramatic inhibition of metal reduction (Finneran *et al.* 2002; Senko *et al.* 2002; Istok *et al.* 2004). Typical *in situ* strategies involving the stimulation of metal-reducing bacteria are generally hindered by two factors: the low pH of the environment and the requirement that the nitrate must first be removed via denitrification prior to uranium being utilised as an electron acceptor (Madden *et al.* 2007). Istok and co-workers (2004) have also demonstrated that the addition of electron donors such as ethanol, glucose or acetate can stimulate nitrate reduction, which was apparently followed by a simultaneous bioreduction of U(VI) and Tc(VII) (Istok *et al.* 2004). In contrast, Shelobolina and co-workers (2003) reported that the biological reduction of nitrate, stimulated by acetate amendment, can cause a rise in pH and reduced solubility of UO₂²⁺. Here uranium removal was via hydrolysis and precipitation rather than through bioreduction (Shelobolina *et al.* 2003). The impact of nitrate on

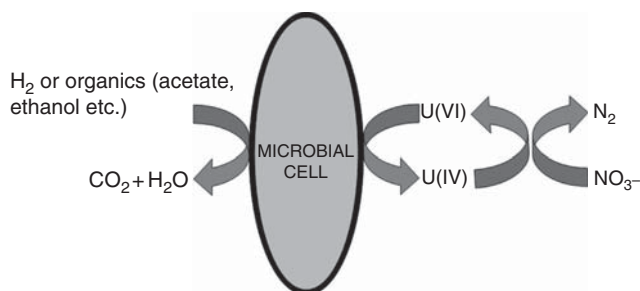


Figure 11.3. Reduction of U(VI) and reoxidation of U(IV) by nitrate.

uranium removal is clearly very complex, and probably site/sediment specific. Indeed, a recent study has even demonstrated that the stimulation of a natural microbial community to immobilise U through bioreduction is possible without the removal of nitrate (Madden *et al.* 2007). Incubations with uranium-contaminated sediments demonstrated nearly complete reduction of U(VI) with very little loss of nitrate from pH 5.7 to 6.2 using methanol or glycerol as a carbon source. The majority of the micro-organisms stimulated by these enrichment conditions consisted of low G + C gram-positive bacteria most closely related to *Clostridium* and *Clostridium*-like organisms (Madden *et al.* 2007).

Finally, it is also obvious from several recent papers that nitrate can not only inhibit U(VI) reduction (as noted in a majority of studies), but it can also have a dramatic impact on pre-reduced sediments containing insoluble U(IV). When added to such systems, nitrate (and oxygen also) can reoxidise the U(IV) (Fig. 11.3), remobilising the uranium as U(VI) (Moon *et al.* 2007). In the case of technetium, reoxidation behaviour is more complex, with remobilisation dependent on the nature of the oxidant (Burke *et al.* 2006; Morris *et al.* 2008).

The abundance of nitrate in the subsurface environments discussed provides a selective pressure that favours denitrifiers among the soil micro-organisms that can tolerate an acidic, nutrient-starved environment. In agreement with the high nitrate concentrations observed in sediments from the Oak Ridge Field Research Center (ORFRC), 16S rRNA gene sequences related to those of nitrate-reducing bacteria, such as members of the Proteobacteria (including the genera *Sphingomonas*, *Acidovorax*, *Acinetobacter*, *Alcaligenes* and *Ralstonia*), showed a high relative abundance in the total and metabolically active fractions of the microbial community (Akob *et al.* 2007). In a parallel study, the microbial community structure from ethanol-biostimulated sediments of a high-nitrate (>130 mM), low-pH, uranium-contaminated site at the ORFRC, taken at a time point when denitrification was most likely, showed an abundance of betaproteobacterial clones in biostimulated sediments (Spain *et al.* 2007). Multiple lines of evidence suggest the dominance of *Castellaniella* species in these biostimulated sediments and their role in nitrate removal *in situ* (Spain *et al.* 2007).

In a recent study from our laboratories, *Bacillus*-like and *Herbaspirillum*-like bacteria were abundant during denitrification in acetate biostimulated microcosms prepared from sediments representative of the Sellafield nuclear site in North West England and supplemented with 10 mM nitrate and a 5 μ M technetium spike (Law *et al.* 2008). Their abundance was demonstrated by 16S rRNA and functional *narG* (alpha subunit of the membrane bound nitrate reductase) gene analyses of samples taken after 30-day incubation when nitrate depletion was most pronounced but before any metal reduction or Tc(VII) had been observed (Law *et al.* 2008). Similar *narG* gene sequences of *Bacillus* spp. were abundant in sodium nitrate treated samples from uranium mining waste piles in Haberland that had been incubated for 4 weeks under anaerobic conditions (Geissler 2007). The dominance of *Bacillus* spp. in this sample was also demonstrated by 16S rRNA gene analysis (Selenska-Pobell *et al.* 2008). This suggests that in radionuclide contaminated sediments *Bacillus* spp. are also involved in the reduction of nitrate, in agreement with the detection of distinct *Bacillus* spp. in a broad range of heavy-metal and radionuclide contaminated samples (Selenska-Pobell *et al.* 1999; Martinez *et al.* 2006). This is of interest, given their ability to sorb large amounts of uranium and other heavy metals (Selenska-Pobell *et al.* 1999).

Conclusions

It is important that we improve our understanding of the mechanisms underpinning the biogeochemistry and mobility of radionuclides in the environment, to underpin safety case assessments and bioremediation efforts. The studies presented in this chapter are examples where microbiological and geochemical analyses have been combined in the laboratory to obtain a better understanding of the biogeochemical cycle of priority radionuclides. These approaches, applied to field-scale investigations, and operating at environmentally relevant concentrations of radionuclides, are potentially challenging but crucial to drive this area forward and realise the potential of *in situ* bioremediation at nuclear facilities in Europe.

There are also specific questions raised by the studies described in this review. For example, the impact of competing 'direct' enzymatic and 'indirect' (Fe(II)/sulphide-mediated) reductive processes needs clarifying for some radionuclides, most notably Tc(VII). In the case of uranium, more research is clearly needed to understand both the precise mechanisms of U(VI) reduction (and long-term stability of U(IV) phases under field conditions) and also the potential roles that bacteria can play in the *in situ* precipitation of other insoluble phases such as U(VI) phosphate. The tools of molecular ecology can play a role in these investigations, but it is necessary to expand our studies not only to look at the microbial community by targeting long-lived DNA, but also to target more transient mRNA to track the metabolism of the 'active' components of

the bacterial community using functional gene probes. The availability of genome sequences and genetic systems for additional micro-organisms (other than *Geobacter*, *Shewanella* and *Desulfovibrio* spp.) will also help better understand their role in the biogeochemical cycling of radionuclides.

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