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**Redox Cycling Under Nuclear Legacy Conditions**

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<td>16s rRNA</td>
<td>16S Ribosomal Ribonucleic Acid</td>
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<tr>
<td>ADP</td>
<td>Adenosine Diphosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
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<tr>
<td>BIGRAD</td>
<td>Biogeochemical Gradients and Radionuclide Transport</td>
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<td>Bq</td>
<td>Becquerel</td>
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<td>CRR</td>
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<td>DLS</td>
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<td>EXAFS</td>
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Abstract

The University of Manchester
Nicholas Masters-Waage
Doctor of Philosophy
Redox Cycling Under Nuclear Legacy Conditions
2016

Subsurface contamination is common at nuclear sites and it is likely that radioactive wastes will be managed in the long-term via burial in a deep Geological Disposal Facility (GDF). The migration of radionuclides in the geosphere from such sites is a major societal concern. In particular, long-lived, redox-active radionuclides (in the case of this thesis: $^{99}$Tc and Np) can migrate over large distances due to their high solubility under oxic conditions. Bioremediation has been proposed as a mechanism to limit the migration of $^{99}$Tc and Np in the environment. Here, an electron donor is supplied to the subsurface and soluble Tc(VII) and Np(V) are reduced to poorly soluble Tc(IV) and Np(IV), respectively. Reduction occurs via direct microbial action (termed bioreduction) or through radionuclide reaction with the by-products of microbial metabolism (primarily Fe(II)). Given the ubiquity of microorganisms and Fe in the geosphere, similar reactions can be expected in the deep subsurface surrounding a GDF. Once reduced, the long-term stability of the Tc(IV) and Np(IV) phases will significantly impact migration rates. Oxidative dissolution of Tc(IV)- and Np(IV)-bearing solids has been demonstrated in the literature and can be pervasive, thus questioning the efficacy of bioreduction. However, these studies have been conducted over short time-scales and during a single period of oxidation. Given the long half-life of $^{99}$Tc and Np and the ephemeral nature of redox conditions in the subsurface, there is a need to better understand $^{99}$Tc and Np biogeochemistry during longer time-scales and across multiple redox cycles. In this thesis, microcosm experiments have been used to address this knowledge gap. Sediment and groundwater used in the microcosms were representative of the Sellafield Ltd. nuclear site. For Tc, three successive redox cycles (reduction followed by oxidation with $O_2$) over 2 years, gradually reduced the extent of Tc remobilisation during oxidation, and molecular scale characterisation of solids revealed that sediment associated Tc was always present as Tc(IV). Further, over time sequential extractions and EXAFS revealed an increased significance of Tc-Fe bonding in the sediment at the expense of TcO$_2$. Despite this, a small but significant fraction of Tc(IV) was also found to be stable in solution during the experiments and XAS and TEM analysis suggested this was Tc(IV) associated with magnetite colloids. In other experiments completed with higher concentrations of bioavailable Fe (added as ferrihydrite to sediments, and in pure culture experiments with Geobacter sulfurreducens), the significance of Tc-Fe bonding was again highlighted, and potential Tc(IV) incorporation into biogenic magnetite was also documented. In experiments with Np, virtually all of the Np(V) added to oxic groundwater was removed to the sediment commensurate with microbially mediated Fe(III) reduction. Further, in systems with elevated bioavailable Fe, Np removal from solution was more extensive. Taken together, the data for Tc and Np reveals critical links between redox-active radionuclides and Fe cycling in sediments over periods of years and across multiple redox cycles. Furthermore, these processes help to predict the long-term fate of radioactive contamination at the Sellafield Ltd. nuclear site and have implications for contaminated land worldwide.
Declaration

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For Chuck and Karl
Acknowledgements

Most importantly, I extend my gratitude to my supervisors Gareth, Kath and Jon, for their guidance, ‘no-nonsense attitude’ (to pretty much everything I say or write) and much-needed reality checks. Sorry that my speling and grammer bad. I would also like to acknowledge Jacob Law for kidnapping my supervisor for a few, crucial months, really helpful mate. Gareth, I’ll always be your first.

For their analytical assistance and help, I would like to thank Pieter Bots, Katie Law, John Waters, Fred Mosselmans, Steve Parry, Tom Neill, Sam Shaw, Carolyn Pearce, Chris Boothman, Francis Livens, Al Bewsher, Thanos Rizoulis, and Paul Lythgoe.

Further, I acknowledge the EPSRC for the funding for this project.

I would also like to thank the cast of ‘Shewarella’ starring Hannah Roberts as Shewarella, Pieter Bots as The Fairy God Mother, and The Evil Step Mother with her ‘Ugly Cohort’ whose casting shall forever remain a secret (but definitely consists of at least Graham Kenyon, Nick Jones and Adam Fuller). I would like to spread my thanks elsewhere amidst SEAES to my friends from past and present, in particular, Laura Newsome, Helen Downie, Tim Marshall and Adam Williamson, y especialmente, mi Hermana Mexicana Fabiola, apachurro! ;)

My most warm and jovial thanks go to all my friends in the CRR, I can’t list you all because I’ve already assigned the page numbers. But to mention my thanks for a few: The Adams, Langers je t’aime, One pint Fuller I beat you at Risk, Sims it’s your turn on WWF; Nick¹, Nick², and Nick⁴; My crossword partners; The Enviro Crew for deep chats and radio 6, (H)ollie, Hamza, Graham (the deepest of chats), and Dray a founding member of Équipe De Law; The instrument lab devotees, Rosie ‘can I ask you a quick question’ Hibberd, Will ‘obnoxiously reads my whole Ph.D. and makes it better’ Bower, and Kate ‘The Master of Whisperers’ Tucker; and Mark Williams for
many things, but mostly Lab Times and lunches. Moreover, I tip my hat to my MChems, especially Xuan Gu and Connaugh Fallon, I taught you everything you know. Also, I thank Rob Soltysiak and Lawrence Ames for a Jame of Jolf.

I would like to thank the following for their editorial guidance and moral support: Gina Miller, Kody, Syd and Jo, James Turley, Dave from next door, Stevie Wonder, Dr Mor, and my family, also, Ju Jenkinson and Jane Ireland for teaching me to read, and Christine Smyth for teaching me to think. Theo, Alex, Hannah, mum and dad look! Another special award!

And of course, unlimited and perhaps even unprecedented thanks go to my Louise Parke and my Andrew Carson. We’ve come a long way since Redcar, and we finally have something to show for it.
Author

The Author attended The University of Edinburgh graduating with an undergraduate degree in Environmental Geosciences (BSc) in 2012. After which, he joined the Centre for Radiochemistry Research group at the University of Manchester where, to date, he has been conducting the research presented in this thesis.
1. Thesis Content

This chapter provides a rationale for the thesis. It places the work in the context of the UK nuclear legacy and provides a summary of the relevant problems facing the safe handling of radioactive waste. Additionally, it gives an outline of the thesis structure.

1.1 Rationale for Thesis

Nuclear waste is a global problem. In the UK alone, the clean-up and safe storage of our current nuclear legacy is set to cost between £90 and £220 billion over the next 120 years (NDA, 2015). Radioactive materials will be present in the environment for millions of years. Because of this, current and future contamination is of global concern and an understanding of the fate of such contaminants is integral to the future safeguarding of our environment.

Environmental redox chemistry determines the contamination risk of the highly radiotoxic elements neptunium and technetium. To date, no environmentally relevant studies have looked beyond single reduction and oxidation cycles when determining redox-active radionuclide solubility. Despite this, subsurface redox conditions will be highly variable over the timescales ($10^3$ - $10^6$ years) required for the safe disposal of nuclear waste. Furthermore, the potential impacts of climate change on biogeochemical cycling warrant the investigation of transient redox environments (Borch et al., 2010), which could significantly influence the fate of redox-active radionuclides at contaminated sites or in the near- and far-field of a geological disposal facility.

Applying an understanding of redox cycling to the evolution of contaminated land over geological timescales is paramount for the safe disposal and management of nuclear wastes. Thus, this thesis aims to lay the groundwork for understanding how
multiple redox cycles will influence the fate of radionuclides in sediments relevant to the UK nuclear legacy. Specifically, the biogeochemistry of $^{99}$Tc and $^{237}$Np are investigated over multiple redox cycles in conditions relevant to the UK nuclear legacy. Data was collected from microcosm experiments spanning hundreds of days and multiple redox events, across which the systems were monitored for changes in radionuclide solubility, oxidation state and coordination.

1.2 Thesis Structure

This thesis comprises a review of the relevant literature followed by a chapter describing the materials and methods used. Three research chapters (see below) are then presented as papers prepared for publication. A summary chapter follows and, finally, the thesis closes with an appendix of results. Details on the individual chapters are as follows:

**Chapter 2** is a literature review, providing a background to the work presented in this thesis, placing the experiments and discussion in the context of the wider nuclear industry. It provides an explanation of how radioactive waste is produced and its migration into the natural environment. Further, it reviews the current literature in geomicrobiology and environmental radiochemistry, contextualising the relevance of the work. Finally, the aims of this thesis are detailed.

**Chapter 3** is a materials and methods section, covering the experimental techniques used in this thesis and providing the theory behind them.

**Chapter 4** is a reproduction of a paper submitted for publication in Nature Communications entitled “The Biogeochemical Redox Cycling of Technetium”. This
work describes the use of microcosm experiments to investigate the influence of multiple redox cycles on the fate Tc, using sediment representative of the Sellafield site. Contributors: Nicholas Masters-Waage undertook the experimental design, lab work, and data analysis with assistance and guidance from Gareth Law (who provided the initial concept), Kath Morris and Jon Lloyd, who all additionally provided comment for the manuscript. Pieter Bots, Fredrick Mosselmans, and Francis Livens provided assistance and comment on XAS interpretation and modelling. Sam Shaw provided assistance with TEM imaging. Christopher Boothman and Athanasios Rizoulis undertook microbial analysis and statistics.

Chapter 5 is a reproduction of a paper submitted for publication in Environmental Science and Technology entitled “Influences of Iron on Environmental Technetium Redox Behaviour”. In this chapter, Fe was added to batch sediment microcosms to assess its impact on Tc solubility over multiple redox cycles. Further, single mineral/microbe experiments were executed to determine the potential for microbially-mediated incorporation of Tc into any neo-forming Fe phases. Contributors: Nicholas Masters-Waage performed the lab work and data analysis and helped in the experiment design with the assistance of Gareth Law, Kath Morris and Jon Lloyd, who also provided comment on the manuscript. Fredrick Mosselmans and Francis Livens provided assistance in the modelling of the EXAFS data.

Chapter 6 contains a paper draft that will be submitted to Applied Geochemistry entitled “The Biogeochemical Redox Cycling of Neptunium”. In this work, microcosms containing Sellafield sediment and neptunium were taken through two redox cycles and the biogeochemical changes were monitored. Additionally, the
influence of increased Fe concentrations on Np remobilisation, over multiple redox cycles, was investigated. Contributors: Nicholas Masters-Waage undertook the experimental work and analysis of data with the assistance of Kath Morris and Gareth Law, who further provided help with the experimental design and comment for the manuscript. Fred Mosselmans and Francis Livens provided assistance with EXAFS modelling.

Chapter 7 summarises the results and conclusions of this work and includes suggestions for future lines of investigation.
2. Literature Review

2.1 Overview of the UK Civil Nuclear Legacy

The UK has been producing commercial nuclear power since 1956 (Bolton, 2012). Currently, nine nuclear power stations are in operation across the country. The oldest is Wylfa (1971), and the youngest is Sizewell B (1995). Around 18% of the UK’s total electricity comes from nuclear power, a percentage which has increased since 2008 (Figure 2.1). The UK is also at the beginning of a period of new nuclear build (NIA, 2013).

![Electricity supplied from nuclear power stations](image)

Figure 2.1 Electricity supplied by nuclear power in the UK (from Bolton, 2012).

Although nuclear energy has been branded as a green, carbon-neutral energy source, public perception sees it as an unattractive alternative to fossil fuels (Pidgeon et al., 2008). This mostly reflects the large amounts of hazardous waste produced during the nuclear fuel cycle and the potential hazards from nuclear disasters such as Fukushima (2011) and Chernobyl (1987).
As of 2013, the UK was responsible for ~ 4,200,000 m$^3$ of Low-Level Waste (LLW), 290,000 m$^3$ of Intermediate-Level Waste (ILW) and 1100 m$^3$ of High-Level Waste (HLW). High-Level Waste generates heat and this must be taken into account when designing a long-term storage facility. Radioactive waste with > 4 GBq tonne\(^{-1}\) alpha and 12 GBq tonne\(^{-1}\) beta/gamma activity, that is not significantly heat generating, comprises ILW; any waste materials that are radioactive but below the ILW activity threshold are deemed LLW. Despite its small volume, HLW represents approximately 95% of all radioactivity in UK waste (NDA, 2013). The largest contributor to the overall waste legacy is the nuclear power industry, with spent fuel reprocessing accounting for 73% of current waste volumes produced (Figure 2.2).

![Figure 2.2 Nuclear waste volumes created by different industries in the UK (Data from NDA, 2011).](image)

The Nuclear Decommissioning Authority (NDA) has estimated the liabilities of the Nuclear Legacy at between £90 and £220 billion (NDA, 2015). These costs include the decommissioning of current nuclear facilities, the management and disposal of nuclear waste (including geological disposal) and radioactive contaminated land, and the costs of maintenance and general management (NDA, 2011). New nuclear build and the waste that it produces will add to this liability (Morris et al., 2011).
2.2 The Nuclear Fuel Cycle

Mining for uranium (U) ore, from which nuclear fuels are created, is the first step in the nuclear fuel cycle (Figure 2.3). The majority of mining occurs in Canada, Australia, Kazakhstan, Russia, Namibia, and Niger (Sharrad et al., 2011). Conventional open pit and deep shaft mines are becoming less common and are being replaced by in situ leaching. This involves a leaching agent (e.g., H$_2$SO$_4$) being injected into a uranium ore-bearing aquifer or spoil heap, in an effort to dissolve the in situ U. The resultant solution is pumped out via wells (EPA, 2008). At this stage, large amounts of waste are created in the form of mine tailings. Much of the waste materials from the leaching process contain radionuclides that have decayed from $^{238}$U, causing the waste to be radioactive. As a result, these wastes are costly to remediate; for example, the estimate clean-up costs for the Erzgebirge mine (~600 hectares of contaminated land) in eastern Germany is €6 billion (Sharrad et al., 2011).

The next process is milling. The extracted uranium is manufactured into yellowcake (U$_3$O$_8$) and then enriched. The enrichment process can include a conversion step to make UF$_6$, a highly corrosive and reactive compound that makes up much of the depleted uranium inventory throughout the world (Sharrad et al., 2011). Thereafter, the U is enriched in its $^{235}$U content from approximately 0.7% in the naturally occurring material to the 5% required for optimal reactor fuel. Generally, gas diffusion is used for enrichment; here, UF$_6$ is heated to form a gas and diffusion gradients separate the lighter U isotopes from the heavier analogues. Newer technologies also include gas centrifuge processing and laser enrichment (EPA, 2008).
After enrichment, the fuel can be fabricated. Here, it is converted from UF₆ to ceramic UO₂ and, finally, pressed into ceramic pellets. These pellets are loaded into stainless steel or zirconium-alloy tubes that make up part of the fuel assembly used in a reactor (Sharrad et al., 2011). The most common fuel type used is enriched UO₂, however, some reactors can use Mixed Oxide Fuel (MOX). For MOX, a lower percentage of ²³⁵U (a result of recycled spent fuels or the depleted by-product of enrichment) is blended with plutonium (Pu), increasing the efficiency of the plant. Typically, there is a blend of ~ 7 % Pu to ~ 93 % U (Sharrad et al., 2011). The packaged fuel is then used in power plants to generate electricity. This produces spent fuel, which can either be sent directly to waste streams (known as an open fuel cycle) or reprocessed (known as a closed fuel cycle).

In a closed fuel cycle, spent nuclear fuels are reprocessed for their reuse as MOX fuel and for reducing hazardous waste products. In order to facilitate the recycling of

Figure 2.3 The nuclear fuel cycle (from NRC, 2014).
waste, spent fuels must be separated from their waste products using complex technologies. A number of extraction methods exist with solvent extraction being the dominant form. Here, the differing affinities of the actinides for various ligands are exploited. For example, in the UK, the Pu and U extraction process (PUREX) extracts U and Pu into a liquid phase separate from the other fission products and minor actinides (Irish and Reas, 1957). Although this process can successfully remove the majority of U and Pu, a large variety of other waste is produced including some solid wastes (e.g., fuel cladding; filters etc.) and large amounts of liquid waste, ranging from fission product laden solutions with hazardous actinides, to radioactive cooling pond water (Sharrad et al., 2011). Comparatively, an open cycle may not produce as much waste in volume but the waste products are harder to handle (spent fuel) and less energy is produced per kilo of fuel (Sharrad et al., 2011).

2.3 Nuclear Waste Storage & Reprocessing

A number of sites are set up around the UK to reprocess and store waste. Generally, Low-Level Waste is stored at the Drigg LLW repository in Cumbria (NDA, 2010b). Here, suitable waste is super-compacted and stored. Where suitable, very LLW (waste that can be disposed of with municipal, commercial or industrial waste) is disposed of to a range of special landfill sites (NDA, 2010b). In contrast, almost all UK ILW has been stored since its creation. Typically, it is stored in a combination of steel drums and concrete boxes. The amount of water in the waste is often reduced and, where possible, large waste items are super-compacted or cut up into smaller pieces. Storage sites for ILW include Sellafield, Trawsfynydd, and Winfrith (NDA, 2010b). For HLW, after initial cooling of spent fuels in storage ponds, they are reprocessed and the waste liquor is evaporated and vitrified into borosilicate glass. This is stored for a
minimum of 50 years in a modern air-cooled facility. Storage of HLW in the UK only occurs at Sellafield (NDA, 2009).

Ultimately, the long-term goal for UK Higher Activity Wastes (HAW; comprising ILW and HLW) is disposal in a deep Geological Disposal Facility (GDF) that will be able to isolate the waste from the biosphere for the timescales > 1 million years. In the UK, the plan is for HAW to be buried 200-1000 m deep in a suitable rock formation, at a site designated through volunteerism (DECC, 2014). The GDF will utilise a multiple barrier concept which is comprised of (i) the waste form: conditioned for stability; (ii) the waste container: for example ILW encapsulated in steel drums; (iii) buffer materials: designed to provide beneficial functions such as controlled flow conditions; (iv) backfill: such as cements to retard the mobility of radionuclides; (v) a sealing system: a highly impermeable sealing barrier to prevent groundwater ingress throughout the required time period and; (vi) the geosphere: a suitable host rock will provide beneficial physical and chemical conditions for the GDF that will also act to

Figure 2.4 A GDF and the multiple barrier concept (edited from NDA, 2010a).
retard radionuclide migration (Morris et al., 2011). This concept is shown in Figure 2.4.

2.4 Contaminated Land

Contaminated land is a key part of the world’s nuclear legacy. From every step in the handling of nuclear materials contamination can occur from a variety of causes including: mining, warfare, controlled release and, the mishandling of nuclear waste from medical, scientific and, nuclear fuel industries (IAEA, 1998). Reflecting this, since the 1940’s, the production of radioactive materials has resulted in radionuclide-contaminated sites across the globe. At nuclear sites, such as Hanford (USA), Oak Ridge (USA), and Sellafield (UK), on site co-contamination of $^{99}$Tc, $^{90}$Sr, and other radionuclides is present (Kimber et al., 2011; McKenzie et al., 2011; Poston et al., 2011). Additionally, the mining of uranium and thorium has resulted in widespread radioactive mine tailings, a particular problem due to their long half-lives and biotoxicity (IAEA, 2005; Sharrad et al., 2011). Also, processing of U ore has led to site contamination, for example, at the Rifle site (USA) where low levels of U are present in the groundwater (Anderson et al., 2003). Although a less significant source of radionuclide contamination, nuclear disasters such as Fukushima (2011) and Chernobyl (1986) have also been responsible for the release of a range of radionuclides into the environment, including large amounts of $^{137}$Cs and $^{131}$I, and to a lesser extent longer lived actinides (e.g. U, Np, Pu) and fission products ($^{99}$Tc) (UNSCEAR, 2008; Masson et al., 2011; Renshaw et al., 2011).

The reprocessing of nuclear waste in the UK and much of the interim storage of wastes occurs at the Sellafield Nuclear Licensed site, in Cumbria, where subsurface contamination is an on-going problem (McKenzie et al., 2011; Marshall et al., 2014a). Contamination plumes of radionuclides have developed from historical spills and
leaks, with the regional groundwater flow transporting the contaminants towards the site boundary (Figure 2.5). The subsurface radioactive contaminants include: $^{234}\text{U}$, $^{238}\text{U}$, $^{99}\text{Tc}$, $^{90}\text{Sr}$, $^{137}\text{Cs}$, and $^3\text{H}$, of which some, for example, tritium and strontium, exist at concentrations many times greater than that of the WHO drinking water guidelines (Marshall et al., 2014a). To reflect the conditions of this contaminated nuclear site and provide direct relevance, the materials used in this thesis were collected from the same Quaternary unconsolidated alluvial floodplain deposits that underlie the Sellafield Nuclear Licensed site.

Figure 2.5 Conceptual model of contaminated land at the regional level under the Sellafield Licensed Nuclear site (from Marshall et al., 2014a).

2.5 Geochemistry of the Subsurface

When radionuclides are released to the geosphere, their migration is controlled by their speciation, the characteristics of the subsurface (i.e. mineralogy, redox status, pH, geomicrobiology etc.), and the reactions that occur as a result. The solubility of a radionuclide is of great importance for its environmental hazard. In the event of
contamination, more soluble phases will impact greater areas because their migration and distribution will be determined by the regional groundwater flow patterns. Comparatively, the hazards associated with an insoluble radionuclide, whether sorbed to geomedia or precipitated as, or with a mineral phase, are more constrained because exposure requires direct contact with the contaminated area, requiring the reworking of sediment or direct ingestion. Therefore, the key processes that influence radionuclides in the subsurface are sorption, redox reactions, complexation, colloid formations and precipitation (Renshaw, 2011; Brookshaw et al, 2012). With regard to the radionuclides technetium (Tc) and neptunium (Np), particular interest lies in their redox chemistry.

2.6 Redox Reactions

A redox reaction is one that involves the transfer of electrons from one species to another. The species that gains/accepts the electron has been reduced; the species which loses/donates the electron has been oxidized. Ions, atoms, and molecules that readily accept electrons are termed oxidising agents as they promote the oxidation of another species; in turn, the opposite species is termed a reducing agent (Konhauser, 2007).

Redox reactions are of key importance in the movement of radionuclides through the geosphere. This is because the prevalent redox status of an environment can impact the oxidation state of a redox active radionuclide, either encouraging its reduction or oxidation. This, in turn, can impact the radionuclides’ speciation and, hence, solubility. For example, the oxidized form of Tc (Tc(VII)), tends to form pertechnetate (TcO$_4^-$), which is highly soluble and exhibits very poor sorption affinity. In contrast, reduced Tc (Tc(IV)), tends to form hydrous-TcO$_2$ like phases that are relatively insoluble and easily complexed/sorbed (Icenhower et al., 2008). Many other
radionuclides are redox-active, including Np; also examined in this thesis (Figure 2.6 (Lloyd, 2003)).

Figure 2.6 Calculated oxidation state transitions of relevant redox-active radionuclides across an Eh range of 0.8 to -0.4 V at pH 7 and corresponding Eh values for important microbial redox processes (from Lloyd, 2003).

2.7 Microbial Energy Production

In the natural environment, microbial processes will often determine the ambient redox conditions, as microorganisms strive to efficiently metabolise available nutrients. For all energy requiring processes, microorganisms create chemical energy in the form of adenosine triphosphate (ATP), which is used as the energy ‘currency’ in cells. In order to form ATP, oxidative phosphorylation must take place. This is a process that involves moving electrons from an electron donor to an electron acceptor (the electron transport chain). Microorganisms preferentially pair electron donors and acceptors that give the highest energy yield (net free energy). This is dependent on the
difference in electrode potential between the Primary Electron Donor (PED) and the Terminal Electron Acceptor (TEA) (Konhauser, 2007). Different microorganisms can facilitate the use of a range of organic and inorganic substrates as PEDs (e.g., glucose, acetate, and H₂) and a range of compounds/ions as TEAs for chemical energy production. Under oxic conditions, aerobic respiration (using O₂ as a TEA) dominates as it gives the highest free energy yield. Under anaerobic conditions (the absence of O₂), the pool of potential TEAs becomes larger but the free energy yield decreases. Microorganisms will sequentially utilise different TEAs starting with the most energetically beneficial, as discussed above. This gives rise to the redox cascade, which in the subsurface typically takes the order of: O₂ > NO₃⁻ > NO₂⁻ > Mn(IV/III) > Fe(III) > SO₄²⁻, after which methanogenesis occurs (see sections 2.9-2.13 and Konhauser et al., 2002; Konhauser, 2007).

As organic matter becomes progressively more broken down by Terminal Electron Accepting Processes (TEAPs), the subsurface moves towards a more reducing and anoxic environment, as fewer oxidants remain, a process referred to as bioreduction. This is important with regards to the mobility of redox-active radionuclides because under reducing conditions, oxidised radionuclides may be reduced directly by enzymatic processes. For example, U(VI)ₐq reduction to U(IV)ₐq can be facilitated by dissimilatory metal-reducing bacteria (Zhang et al., 2009). In contrast, radionuclide reduction may occur indirectly, for example, Tc(VII)ₐq reduction to Tc(IV)ₐq via abiotic reaction with biogenic Fe(II) (Lloyd et al., 2000b).

The importance of bioreduction at contaminated sites has been highlighted by a number of studies (e.g., Burke et al., 2005; Law et al., 2010; Thorpe et al., 2014) and has been investigated under alkaline conditions relevant to the geological disposal of radioactive wastes (Williamson et al., 2013). An understanding of subsurface
biogeochemistry is important for the monitoring and safekeeping of present and future radionuclide-contaminated sites. Furthermore, the presence of microbes in the subsurface is important with regards to radionuclide fate as microbes are responsible for the biotransformation and biogeochemical cycling of many metals present in the subsurface (Gadd, 2010). Microbes show resilience through variable redox conditions (Pett-Ridge and Firestone, 2005; DeAngelis et al., 2010) and thus will be an important factor in soils over timescales of environmental radioactivity.

2.8 The Redox Cascade in the Natural Environment

The redox cascade is relevant when considering the release of radionuclides into various natural environments. These natural environments will vary greatly with respect to their mineral assemblage and microbial processes, which in turn will determine their redox status. Oxygen availability (often limited by diffusion) and the activity of microbes are the main driving forces behind the redox status of an environment (Konhauser, 2007). The following sections (2.9-2.13) describe the typical redox cascade observed in natural sediments, starting with the highest energy yielding process, aerobic respiration.

2.9 Aerobic Respiration

Microorganisms will use the most energy beneficial TEA at any given time. Therefore, the biotic reduction of a certain TEA is dependent on the concentration of other more preferable TEAs, such as O\textsubscript{2}. The reduction of O\textsubscript{2} coupled to the breakdown of organic matter is termed aerobic respiration and yields the most energy per mole of carbon source oxidised. A number of microorganisms (termed facultative anaerobes) can respire under both aerobic and anaerobic conditions; in contrast, some microorganisms can only respire in the anoxic/oxic zone (obligate anaerobes and obligate aerobes, respectively) (Konhauser, 2007). Therefore, facultative and obligate
aerobic microorganisms dominate aerobic environments. These microbes have a wide range of hydrolytic enzymes that can completely degrade organic substrates to H₂O and CO₂, including complex and refractory organic compounds, such as lignin. An example of the aerobic breakdown of acetate is provided in Equation 1.1 (Konhauser, 2007).

\[
\text{CH}_3\text{COO}^- + 2\text{O}_2 \rightarrow \text{H}_2\text{O} + 2\text{CO}_2 + \text{OH}^- \\
\Delta G^o = -854 \text{ kJ mol}^{-1} \text{ acetate}
\]

(Equation 2.1)

Although microbes show a preference for aerobic respiration, many subsurface environments are dominated by anaerobic respiration. This results from the slow diffusion of oxygen through the ground and high microbial oxygen demands depleting the subsurface concentrations (Konhauser, 2007).

### 2.10 Nitrate Reduction

In the absence of O₂, microorganisms settle for a smaller potential energy gain and will use NO₃⁻ as a TEA. Firstly, select microorganisms (e.g., *Escherichia coli*) react NO₃⁻ with two electrons creating NO₂⁻; select microorganisms (e.g., *Staphylococcus carnosus*) then reduce NO₂⁻ to NH₄ (Neubauer and Götz, 1996). Further, others (e.g., species from the *Pseudomonas* genera) are capable of facilitating complete dissimilatory NO₃⁻ reduction to N₂ (denitrification). This process allows them to maximize the energy yield from NO₃⁻ reduction, using the full 8 e⁻ accepting process as summarised below (Equation 2.2; Konhauser, 2007).

\[
5\text{CH}_3\text{COO}^- + 8\text{NO}_3^- \rightarrow 4\text{N}_2 + 13\text{OH}^- + \text{H}_2\text{O} + 10\text{CO}_2 \\
\Delta G^o = -801 \text{ kJ mol}^{-1} \text{ acetate}
\]

(Equation 2.2)

Aerobic respiration and denitrification can be used to completely oxidise most organic compounds to CO₂. Conversely, other anaerobic microorganisms can only break
down simple substrates such as H₂, ethanol, pyruvate, acetate, and formate (Konhauser, 2007). The production of these simple substrates requires the initial hydrolysis of complex molecules by aerobes or denitrifiers, followed by metabolism by fermentative bacteria. The previous steps, where O₂ and NO₃⁻ are used as TEAs, are key in simplifying the organic substrate for later microbial processes (Konhauser, 2007).

Microbial nitrate reduction is not limited to the oxidation of organic matter. Importantly, freshwater organisms have been identified that can couple the reduction of NO₃⁻ to the oxidation of Fe(II) (Straub et al., 1996; Straub and Buchholz-Cleven, 1998) and denitrifiers have also been evidenced to oxidise HS⁻ (Schulz et al., 1999). Thus, NO₃⁻, a common contaminant at nuclear sites, will play an important role in not only establishing reducing conditions in a sediment site, but also, potentially changing the redox states of reduced, immobilised species, including radionuclides (Istok et al., 2004; Senko et al., 2005; Burke et al., 2006; Law et al., 2010b).

2.11 Manganese Reduction

Metal reduction by dissimilatory processes has been considered a key factor in organic matter decay for a long time (e.g., Canfield et al. 1993, Canfield, 1994). Manganese minerals present in the subsurface consist of those with an average composition of MnO₂, including todorokite, birnessite, and vernadite, and those with lower abundance, including mixed valance (e.g., hausmannite, Mn₃O₄) and trivalent (e.g., manganite (MnOOH) minerals (Konhauser, 2007). Many microorganisms can utilise these manganese oxides as TEAs, reducing Mn(IV/III) to Mn (II), with amorphous phases and minerals with greater surface areas preferentially reduced over more crystalline phases (Burdige et al., 1992). *Pseudomonas, Geobacter, Shewanella,* and *Bacillus* are amongst the genera capable of Mn reduction. Some Mn-reducing
bacteria are facultative, turning to Mn-oxide reduction upon the depletion of O$_2$ and NO$_3^-$ (Nealson and Saffarini, 1994). The manganese reduction process initiates with the oxidation of H$_2$ at the hydrogenase enzyme, as per dissimilatory iron or sulfate reduction (see sections 2.12 and 2.13). The reduction of the terminal electron accepting MnO$_2$ to Mn$^{2+}$ is coupled with H$_2$ oxidation, which commonly originates from an organic source such as acetate (Equation 2.3; Konhauser, 2007).

\[
\text{CH}_3\text{COO}^- + 4\text{MnO}_2 + 3\text{H}_2\text{O} \rightarrow 4\text{Mn}^{2+} + 2\text{HCO}_3^- + 7\text{O}_2^- \\
\Delta G^0 = -558 \text{ kJ mol}^{-1} \text{ acetate}
\]

(Equation 2.3)

Manganese (II) is reasonably soluble and often develops a cycling system in sediments (similar to other redox-active metals e.g., Fe (II)). Here, reduced dissolved metal returns to the surface, by diffusion or a mixing event, and become reoxidized and then sequentially reduced by bacteria (Slomp et al., 1997; Law et al., 2009) (Figure 2.7).

![Figure 2.7 Redox cycling of Mn and Fe (from Konhauser, 2007).](image)

These redox-cycling processes are of particular interest with regard to radionuclide behaviour in the subsurface. Interactions such as these can alter mineralogy and facilitate potential ‘armouring’ of radionuclides (Zachara et al., 2007; Morris et al.,
2008) as well as radionuclide oxidation state changes (*via* both abiotic reduction and oxidation), which impact their mobility. This concept is discussed further in the individual radionuclide sections (see below) and, in terms of reoxidation events, is a key topic of interest.

### 2.12 Iron Reduction

Many microorganisms possess the ability for dissimilatory iron reduction and are responsible for a small but significant amount of organic matter oxidation (Konhauser, 2007). Here, Fe(III) is used as a terminal electron acceptor in a 1e⁻ transfer, creating Fe(II) (Equation 2.4; Konhauser, 2007).

\[
\text{CH}_3\text{COO}^- + 8\text{Fe(OH)}_3 \rightarrow 8\text{Fe}^{2+} + 2\text{HCO}_3^- + 15\text{OH}^- + 5\text{H}_2\text{O}
\]

\[\Delta G^\circ = -337 \text{ kJ mol}^{-1} \text{ acetate} (\text{Equation 2.4})\]

Typically, Fe minerals are the main source of Fe(III) for this reaction, the most reactive being ferric oxy(hydr)oxides (e.g., ferrihydrite (Fe₂O₃.0.5(H₂O))) with a high specific surface area (Konhauser, 2007). Once these relatively easily reducible minerals are depleted, there is a rapid decrease in the rate of Fe(III) reduction (Lovley and Phillips, 1986). Despite this, when ferric hydroxides are lacking, it is found that minerals including hematite (Fe₂O₃), goethite (FeO(OH)), and magnetite (Fe₃O₄) can provide significant sources of Fe(III) (Konhauser, 2007). This process leads to increasing Fe(II)\(_{\text{aq}}\) concentrations until the onset of sulfate reduction, at which point mineralisation occurs. Further, the upward diffusion of aqueous Fe(II) results in its oxidation by MnO₂, creating the clear distinction of an Fe(III)-reducing zone. Iron reducing aquifer zones also have particular significance for redox-active radionuclides (e.g., Tc, U, and Np) as Fe(II) can abiotically reduce mobile Tc(VII), U(VI), and
Np(V) to their insoluble counterparts (Tc(IV), U(IV), and Np(IV), respectively) (Lloyd et al., 2000; Gogolev et al., 2006; Law et al., 2010).

The reduction of Fe(III) by microorganisms results in the production of soluble Fe(II). This, in turn, can sorb to other geomedia or precipitate as discrete minerals that include Fe(II) in their structures. These minerals include, green rust (Fe(OH)$_2$, layered Fe(II)/(III) hydroxides), Fe(II) rich clay minerals, siderite (FeCO$_3$), magnetite (Fe$_3$O$_4$), and vivianite (Fe$_3$(PO$_4$)$_2$.8H$_2$O), amongst others. All of these phases can react with radionuclides (Brookshaw et al., 2012).

2.13 Sulfate Reduction

When organic matter and SO$_2^{4-}$ are available, most anoxic environments will support sulfate reduction. Two metabolic groups of sulfate-reducing bacteria (SRB) work together to fully oxidise many organic compounds to CO$_2$. The first group use compounds such as lactate and formate, oxidising them to acetate, after which another group of microorganisms oxidise acetate to CO$_2$ (Equation 2.5; Konhauser, 2007; Widdel, 1988). There is great diversity in the range of SRB and in their methods of metabolism, these include chemolithoautotrophic growth and disproportionation oxidation reactions. Overall the most widely used process is an 8e$^-$ reduction reaction from SO$_2^{4-}$ to H$_2$S (Equation 2.5; Konhauser, 2007).

\[
\text{CH}_3\text{COO}^- + \text{SO}_4^{2-} + \text{H}_2\text{O} \rightarrow \text{H}_2\text{S} + 2\text{HCO}_3^- + \text{OH}^-
\]

\[\Delta G^0 = -48 \text{ kJ mol}^{-1} \text{ acetate}\]

(Equation 2.5)

2.14 Multiple Redox Cycling

At any point during the redox cascade, sediments have the potential to be reoxidised. This can occur as a result of, for example, microbial ecosystem shifts, glaciation,
flooding, perturbations in the regional groundwater flow, landslides, earthquakes, and co-contamination (Burke et al., 2006; Wu et al., 2007; Charlet et al., 2013). Such processes lead to the oxidation of previously reduced materials (e.g., Fe(II) to Fe(III)) and the resultant changes in the local chemistry causes alteration in the solubility of previously sequestered radionuclides (see sections 2.19 and 2.21). Over the long-term, the redox state of the subsurface will fluctuate, resulting in multiple redox cycles. In light of this, some recent studies have investigated multiple redox cycling in Fe minerals (Coby et al., 2011; Byrne et al., 2015), Fe present in clays (Zhao et al., 2015), and in sediments (Thompson et al., 2006). Zhao et al., (2015) showed that the Fe present in nontronite can be effectively cycled between Fe(II) and Fe(III) in anaerobic conditions, using Fe(III)-reducing bacteria *Shewanella putrefaciens* for reduction and nitrate-dependant Fe(II) oxidising bacteria *Pseudogulbenkiania* for oxidation. Furthermore, studies have reported the biotic, anaerobic cycling of Fe minerals such as goethite (Coby et al., 2011) and magnetite (Byrne et al., 2015), and there is evidence to suggest that redox oscillations lead to more crystalline Fe phases (Thompson et al., 2006). Research has also investigated the influence of redox cycling on Tc(VII) reduction rates; Yang et al., (2012) promoted redox cycling in Fe-rich clay mineral nontronite and investigated its subsequent reactivity to Tc(VII). In their study, Tc(VII) was more rapidly reduced by nontronite that underwent multiple redox cycles. They suggest that Fe(II) dissolution during the reoxidation step results in greater fractions of highly reactive Fe(II) sorbed to the surface of the nontronite, which during the reduction step increases the Tc(VII) reduction rate. Despite such studies, there is a nascent understanding of how redox cycling will influence radionuclide stability in the environment. However, the impact of multiple redox cycles on the mobility and behaviour of various non-radioactive contaminants (such
as arsenic and chromium) has been investigated (Charlet et al., 2013; Parsons et al., 2013) and in one case for the redox-active uranium (U) (Couture et al., 2015). In the latter, a bioreactor system was used to cycle U, and indeed other contaminants, through bioreducing and oxidising (air) conditions. They showed that U was removed from solution under reducing conditions and remobilised during oxidising conditions. Interestingly, a large amount of U was remobilised under oxidising conditions and there was little variation seen between cycles. However, the system may not be completely representative of environmental conditions due to the rapid cycle speed, 14 days each for oxidation / reduction. Further, information on U speciation during the cycles is required for full analysis of the processes present.

2.15 Technetium

Technetium (Tc) is the lightest element to form purely unstable isotopes. Perrier and Sergé first discovered the element in 1937 as a result of deuteron activation of molybdenum metal (Perrier and Sergé, 1937). Apart from the small amount of Tc produced by spontaneous fission of $^{238}\text{U}$, all other Tc results from the anthropogenic fission of $^{235}\text{U}$ and $^{239}\text{Pu}$ or from the neutron/deuteron activation of molybdenum isotopes (Icenhower et al., 2008).

2.16 Sources of Technetium

Of its three dominant isotopes, $^{99}\text{Tc}$ is the favoured U fission product yielding 6.06 %, followed by $^{101}\text{Tc}$ (5.6%) and $^{102}\text{Tc}$ (4.3%) (Till, 1986). Approximately 1 kg of $^{99}\text{Tc}$ is formed per ton of $^{235}\text{U}$-enriched reactor fuel burnt (Chen et al., 2000), filling empty spaces in spent fuel rods as $\varepsilon$-phases (metal aggregates containing molybdenum, platinum, ruthenium, rhodium, and technetium) (Kleykamp, 1985). Technetium can enter waste streams by dissolution from $\varepsilon$-phases, however, in spent fuel rods, it represents a relatively minor constituent of hazardous waste for two reasons. Firstly,
in order to mobilise the Tc, the $\varepsilon$-phases must be exposed and this requires most of the shielding U to be dissolved (Buck et al., 2004), and secondly, studies have found that the dissolution rate of Tc found in metallic waste forms is slow (Johnson et al., 2002). Therefore, the major source of waste Tc is from the reprocessing of spent fuels. For example, in the PUREX method, dissolution of $\varepsilon$-phases creates compounds of Tc that can enter various waste streams, with approximately 20% remaining in the U fraction, the majority of which will eventually find its way into HLW (Till, 1986). This process results in a number of pathways by which Tc can reach the natural environment. These include spillage and leakage due to mishandling of raffinates (radionuclide and metal laden HNO$_3$ liquors) and also the controlled release of Tc (Icenhower et al., 2008).

2.17 Environmental Relevance of Technetium

Technetium-99 is of particular environmental interest due to its long half-life ($2.13 \times 10^5$ years) and high mobility in oxic environments as TcO$_4^-$). Further, $^{99}$Tc is a beta emitter that, as TcO$_4^-$, it is available to biota as a SO$_2^{4-}$ analogue (Cataldo et al., 1989; Harms et al., 1999; Krijger et al., 1999) and is thus susceptible to bioaccumulation (Beasley and Lorz, 1986).

The dominant speciation of Tc in oxic groundwater, over a wide pH range, is TcO$_4^-$ (Lieser and Bauscher, 1987), as shown in Figure 2.8. This is environmentally problematic due to its large size and negative charge, making TcO$_4^-$ highly soluble (11 mol l$^{-1}$ as Tc(VII); Boyd, 1978). Furthermore, TcO$_4^-$ shows no significant absorption to clays or metal oxides (Liu and Fan, 2005; Burke et al., 2005; Lear et al., 2010).
Therefore, there are vast environmental problems associated with Tc(VII), as seen at Sellafield, where a plume of $^{99}$Tc contaminated groundwater is present and mobile in the subsurface (McKenzie et al., 2011), and at the US DOE Hanford site, where groundwater contamination is present (Poston et al., 2011). In contrast, under reducing conditions Tc(IV) is the stable oxidation state with poorly-soluble hydrous TcO$_2$-like phases dominating speciation (Beasley and Lorz, 1986). This reduced counterpart has a much lower solubility (10 nmol l$^{-1}$) as hydrous Tc(IV)O$_2$ at neutral pH (Meyer et al., 1991; Bird and Schwartz, 1997; Lear et al., 2010). These phases
have been found to form discrete precipitates, readily sorb to clays, or associate with metal mineral surfaces, organic matter, sulfides, and carbonates (Bird and Schwartz et al., 1997; Begg et al., 2007; Morris et al., 2008; Peretyazhko et al., 2009). In some cases Tc(IV) can also be incorporated into the structure of select minerals, for example, goethite (Um et al., 2011) and magnetite (Marshall et al., 2014b), and such capture can result in Tc becoming increasingly resistant to mobilisation or reoxidation (Luksic et al., 2015).

2.18 Redox Reactions Involving Technetium

The reduction of soluble Tc(VII) to relatively insoluble Tc(IV) is important to its remediation and containment in the subsurface. Understanding the mechanisms of this redox transition may help decrease technetium’s environmental impact. Two principle mechanisms have been highlighted for the reduction of Tc(VII) in the natural environment: (i) direct enzymatic reduction by microbes, and (ii) indirect abiotic reduction via reaction with Fe(II).

To date, a range of metal- and sulfate-reducing bacteria have been found to facilitate the direct enzymatic reduction of Tc(VII), including microorganisms of the genera *Anaeromyxobacter, Desulfovibrio, Escherichia, Geobacter,* and *Shewanella* (Lloyd et al., 1999a & 2000; Wildung et al., 2000; De Luca et al., 2001; Liu et al., 2002). These microorganisms facilitate electron transfer reactions mediated by enzymes within their cell walls to couple the reduction of Tc(VII) to oxidation of H₂. Further, the literature supports the hypothesis that cytochrome and hydrogenase enzymes catalyse the reduction of Tc(VII) within the periplasm of gram-negative bacteria (Marshall et al., 2008). Strains of microorganisms lacking fully functional periplasmic hydrogenase have been found to be unable to reduce Tc(VII) or show strongly diminished abilities (Lloyd et al., 1999a; De Luca et al., 2001). Microbially reduced
Tc generally aggregates on cell surfaces or within the periplasmic space as a dark precipitate with a structure ranging from amorphous to nanocrystalline (Lloyd et al., 1997, 2000; Wildung et al., 2000). Spectroscopy has confirmed that the composition of these phases is predominately Tc and O (Lloyd et al., 2000; Abdelouas et al., 2002; Marshall et al., 2008) with characteristic Tc-O bond lengths evident in XAS characterisation that suggest the presence of hydrous TcO$_2$ (Burke et al., 2005).

Despite the above, the enzymatic reduction of Tc(VII) is often considered to be ineffective in the subsurface. Low molar concentrations in the natural environment mean that hydrogenase struggles to recognise the radionuclide; additionally, the process is competing with Fe(II)-mediated reduction (a kinetically rapid process) (Lloyd et al., 1999b). In this process, Tc(VII) is reduced abiotically via reaction with Fe(II) which has been formed by the microbial reduction of Fe(III). Zachara et al. (2007) suggest a reaction pathway for this process (Equation 2.6) and Fredrickson et al. (2004) have found that the initial rate of TcO$_4^-$ reduction can be increased with an increase in HCl-extractable Fe(II) concentration.

\[ \text{Tc(VII)O}_4^- + 3\text{Fe(II)}^{2+} + (n + 7)\text{H}_2\text{O} \leftrightarrow \text{Tc(IV)O}_2\cdot n\text{H}_2\text{O}(s) + 3\text{Fe(OH)}_3(s) + 5\text{H}^+ \]

(Equation 2.6)

The process has been found to be thermodynamically feasible even though as a homogenous reaction it is kinetically hindered (Cui and Eriksen, 1996). However, under heterogeneous conditions and as a surface-mediated process, Tc(VII) reduction has been observed to occur rapidly at a rate many orders of magnitude faster than its homogeneous counterpart (Peretyazhko et al., 2009). This occurs in anoxic environments when Fe(II) is sorbed onto iron oxy(hydr)oxides, such as magnetite (Fe$_3$O$_4$), and other mineral surfaces (Zachara et al., 2007; Peretyazhko et al., 2008,
Further, the reaction is highly pH dependent, although, it has been suggested that in anoxic environments, with low environmental Tc concentrations, the process of reduction occurs rapidly at pH > 6 (Peretyazhko et al., 2009). In summary, most studies (e.g., Lloyd et al., 2000; Wildung et al., 2004; Peretyazhko et al., 2009) have found that Fe(III) reduction is the main driver of environmental Tc(VII) reduction, yielding a relatively insoluble Tc(IV)O$_2$•xH$_2$O-like solid phase (e.g., Fredrickson et al., 2004; Maes et al., 2004; Wildung et al., 2004; Burke et al., 2005; McBeth et al., 2007; Morris et al., 2008).

Due to the importance of Tc/Fe interactions in Tc solubility, the nature of Tc-Fe bonding has been explored. Zachara et al., (2007) investigated the abiotic reduction of Tc(VII) by aqueous Fe(II). The precipitated Tc(IV)/Fe phase was analysed using XAS and modelled to include interactions from an Fe shell at a distance similar to that of the Tc-Tc interaction. This fit suggested that Tc could bind to the mineral phases via an edge-sharing Fe octahedron. Fits similar to this have been reproduced in the literature and elude to the nature of the Tc/Fe interaction in reduced sediments and minerals (Morris et al., 2008; Brookshaw et al., 2015). Furthermore, the incorporation of Tc into mineral structures (Luksic et al., 2015) and in particular, Fe-minerals (Um et al., 2011, 2012; Marshall et al., 2014b), has also been explored because they may provide suitable waste forms capable of immobilising Tc (Westsik et al., 2014). Marshall et al., (2014) investigated the abiotic incorporation of Tc into magnetite at high pH conditions relevant to the disposal of radioactive wastes. Here, magnetite was produced via the addition of Fe(II) to ferrihydrite in the presence of Tc(VII). XAS analysis of the mineral revealed Tc was most likely coordinated within the octahedral Fe(III) site of magnetite structure with Tc-Fe bonds at 3-3.5 Å. Technetium within the mineral composite was also recalcitrant to oxidation (see below, section 2.19).
Pertechnetate can also be removed from solution by the production of sulfides. Here, Tc(VII) reacts with sulfide minerals formed during microbially mediated sulfate reduction, likely forming TcO$_2$ and/or TcS$_2$ phases (Abdelouas et al., 2002). Indeed, Lee et al., (2014) showed that in biologically reduced sediment, with artificially elevated concentrations of SO$_4^{2-}$, significant proportions of TcS$_x$-like reduction products formed, alongside Tc(IV)O$_2$-like phases and Tc(IV)-Fe surface clusters. Furthermore, coprecipitation of Tc with mackinawite (FeS) was shown to form TcS$_2$-like phases that are highly resistant to reoxidation (Wharton et al., 2000).

Studies have also elucidated the possible identification of Tc reduction mediated by denitrifying conditions. Istok et al. (2004), found that the removal of Tc in groundwater occurred concurrently with denitrification within an aquifer. These results contrast however with those of other studies (e.g. Abdelouas et al., 2005; Law et al., 2010) where no Tc(VII) removal has been detected during denitrification. In general, the presence of NO$_3^-$ in the subsurface has been found to be problematic for Tc(VII) reduction (Burke et al., 2005; McBeth et al., 2007; Li and Krumholz, 2008). Conversely, Law et al. (2010) found that it can, in select circumstances, aid the reduction of Tc(VII) through pH and micro-ial consortia pre-conditioning but that no Tc(VII) reduction was observed in the actual presence of measureable NO$_3^-$ and NO$_2^-$. Here, it was found that Tc(VII) reduction was inhibited at low pH (< 5), however, under high NO$_3^-$ concentrations (> 10 mM) the starting pH was less relevant. It was found that extensive denitrification (as a result of elevated NO$_3^-$ levels) led to increased pH and augmented the progression of TEAPs. Neutralisation of the pH and Fe(III) reducing conditions allowed for the reduction of Tc(VII), suggesting that NO$_3^-$ could play a role in preconditioning contaminated nuclear site sediments (Law et al., 2010b).
2.19 Reoxidation of Technetium

When considering the long-term removal of Tc from groundwater, it is important to consider the possible reoxidation of Tc(IV). Reoxidation can result in the remobilisation of the radionuclide and because of the long half-life of $^{99}\text{Tc}$, the likelihood of oxidation events in the subsurface are high.

Oxidative events could either occur as a result of exposure to O$_2$ or NO$_3^-$: The latter may be abundant in nuclear sites due to their use in reprocessing. Burke et al., (2006), investigated the behaviour of Tc(IV) during reoxidation in a batch microcosm study. They found that when reduced sediments were reoxidised in air, Tc remobilisation was hindered (~50% of added Tc), despite abundant reoxidation of Fe(II) and sulfide. Reoxidation with NO$_3^-$ was also investigated, again, resulting in Fe(II) and sulfide reoxidation, but very little Tc remobilisation (~10%). Other studies have also investigated Tc remobilisation behaviour, for example, McBeth et al. (2007) found that when reduced sediments containing Tc(IV) were exposed to O$_2$ there was up to 80% remobilisation of the Tc to groundwater, with 20% remaining in the sediments as hydrous TcO$_2$. When NO$_3^-$ reducing, Fe-oxidising, conditions occurred, 50% of the sediment-associated Tc(IV) was oxidised to Tc(VII). Interestingly, XANES analysis revealed a fraction of ‘immobilised’ sediment associated Tc was present as Tc(VII), similar to previous work (Burke et al., 2006). Morris et al. (2008) concur with these findings, documenting both Tc(IV) and Tc(VII) associated with sediments after reoxidation with air and NO$_3^-$; although, Zachara et al., (2007) found that when reoxidised sediments were washed in deoxygenated water prior to XANES analysis, sediment associated Tc was present as Tc(IV) only, suggesting the Tc(VII) signal seen by others may come from porewaters. As with previous studies (e.g., Burke et al., 2006; McBeth et al., 2007, 2011; Fredrickson et al., 2009), Morris et al., (2008)
demonstrated that Tc shows recalcitrance to remobilisation during reoxidation with air and \( \text{NO}_3^- \). This paper also highlighted a possible mechanism for the retardation of Tc(IV) reoxidation. Here, Tc(IV) is armoured by its incorporation into any neo-forming Fe(III) phases. Indeed, it has previously been hypothesised that Tc(IV) resistance to reoxidation results from its veneering by Fe(III) phases formed during reoxidation (Zachara et al., 2007). Furthermore, resistance to remobilisation was observed in Tc abiotically incorporated in magnetite and extractions performed on the reoxidised mineral revealed the formation of Fe ‘rinds’ (Marshall et al., 2014b). Additionally, Jaisi et al. (2009) suggested a number of mechanisms by which a nontronite system could prevent the remobilisation of Tc. Firstly, nontronite physically protects Tc(IV) from oxidants due to the central positioning Tc(IV) within the host structure. Secondly, the presence of residual Fe(II) in nontronite is suggested to act as a ‘buffer’ against Tc(IV) oxidation. Finally, they suggested the mechanism of surface passivation (noted also by Fredrickson et al. 2002). Here, Fe(II) oxidation products prevent further redox reactions by physically blocking oxidant / Tc(IV) interactions and these products could pacify the nontronite surface by changing the surface charge characteristics through complexation with reactive sites.

It is apparent that the sequestering of Tc in the natural environment is a complex process and recent studies have focused on the key processes that determine its mobility. The increased incorporation of Tc within minerals, as summarised by Figure 2.9, undoubtedly results in its reduced mobility and therefore reduced hazard (Luksic et al., 2015). This gives a reason for further study in this area looking at how multiple redox-cycling events will influence the speciation, mineralogy, and mobility of Tc.
Neptunium is an alpha-emitting radionuclide present in nuclear wastes. It represents a relatively small fraction of spent nuclear fuel (approx. 0.03%) and, in the short-term, it is not considered as problematic as its neighbouring actinides (U and Pu). In the long-term, however, ($t > 10,000$ years) $^{237}$Np is one of the most important constituents of nuclear wastes. This is because the fraction of Np in wastes will increase with time through the radioactive decay of $^{241}$Pu ($t_{1/2} = 14.9$) and $^{241}$Am ($t_{1/2} = 432.7$) and as a result of its long half-life ($2.4 \times 10^6$ years) (Runde and Goff, 2011). The problems associated with $^{237}$Np in the environment are further complicated by its high biological toxicity and high mobility when present under oxygenated conditions (Thompson, 1982; Choppin, 2007).
In the natural environment, three oxidation states of Np are relevant: IV, V, and VI (Figure 2.10), with the most important being IV and V (Kaszuba and Runde, 1999). Neptunium’s subsurface mobility in oxic conditions and at near neutral pH is problematic due to the great solubility range of Np(V) compounds, showing the greatest stability of any of the pentavalent actinides (Lieser and Muhlenweg, 1988). In particular, neptunium’s solubility is due to the formation of NpO$_2^+$, with a solubility of up to 10$^{-4}$ M (Choppin, 2007), and negatively charged carbonate complexes (Kim

Figure: 2.10 Eh-pH diagram with the thermodynamically proposed speciation of Neptunium at 25 °C (edited from Lieser and Muhlenweg, 1988).
and Sekine, 1991; Clark et al., 1995; Runde et al., 1996), which sorb poorly to mineral surfaces, resisting hydrolysis and, thus, precipitation (Choppin, 2007). Reflecting this, studies have investigated the potential sorption of Np(V) phases to minerals, sediments, and biomass. Neptunyl can effectively sorb to bacteria, for example, *Shewanella alga* (Deo et al., 2010), and *Pseudomonas fluorescens* (Songkasiri et al., 2002), with some studies noticing a decrease in sorption with increasing ionic strength and pH (Gorman-Lewis et al., 2005, 2013). Effective sorption of Np(V) to Fe minerals has been noted. Combes et al., (1992) demonstrated the sorption of Np(V) onto goethite (α-FeOOH) resulting in ~ 95 % uptake onto the high surface area mineral. Other literature has investigated the sorption of Np(V) and Np(V)-carbonate species onto haematite (Fe₂O₃) (Arai et al., 2007; Müller et al., 2015). Furthermore, Bots et al., (2016) demonstrated the incorporation of Np into Fe minerals (goethite and hematite) during the crystallisation of ferrihydrite at high pH, a potential route for the immobilisation of environmental Np. Significant sorption of Np onto sediments has also been witnessed on sediments at circumneutral (~60 % uptake, Law et al., 2010a) and high pH (pH ~10, ~95 % uptake, Williamson et al., 2015) conditions.

In contrast to sorption reactions, Np(V) reduction to Np(IV) can also control Np solubility. Np(IV) is poorly soluble and prone to hydrolysis, forming oxide and hydroxide solids that readily interact with the surrounding geology (Brookshaw et al., 2012).

### 2.21 Reduction and Oxidation of Neptunium

Much like Tc, neptunium’s fate in the subsurface is largely controlled by ambient redox, and thus, the microbial activity of the natural environment. An understanding
of the behaviour of Np in the environment is key to its safe disposal and containment at nuclear sites.

Neptunium reduction has been shown to occur *via* both biotic and abiotic pathways. A range of studies have investigated biotic Np(V) reduction pathways, for example, Lloyd et al., (2000) showed that Np could be reduced from Np(V) to Np(IV) by two microbes (*Shewanella putrefaciens*, and *Citrobacter sp*) using H₂ as an electron donor. Furthermore, metal-reducing bacteria, such as *Shewanella oneidensis* and anaerobic microbial consortia have been shown to effectively facilitate Np reduction (e.g., Icopini et al., 2007; Rittmann et al., 2002). Interestingly, other studies have shown that the common metal reducers *Geobacter metallireducens* and *Geobacter sulfurreducens* fail to reduce Np(V), despite kinetic favourability (Renshaw et al., 2005; Icopini et al., 2007). In natural environments, Np(V) reduction to insoluble Np(IV) occurs alongside early metal reduction, typically, commensurate with Mn reduction (Law et al., 2010a; Thorpe et al., 2015). Specifically, Law et al., (2010a) demonstrated that microbial communities indigenous to sediment from a nuclear site can reduce Np(V) to Np(IV) when amended with an electron donor, in sediment microcosms. This enhanced the removal of Np from groundwater when compared to that seen in oxic sediments (as a result of sorption), to near complete removal. Further, an abiotic reduction pathway in natural sediments was observed, with the reduction of Np(V) in sterile microcosms in the presence of Fe(II) (Law et al., 2010a). Additionally, a number of studies have noted the abiotic reduction of Np(V) to (IV) during interactions with Fe minerals. For example, interaction of Np(V) with mackinawite (FeS) promoted neptunyl reduction on its sulfide bearing surface, although it proved inefficient at removing environmentally relevant amounts of Np from solution (Moyes et al., 2002). Np sorption to magnetite and hematite has been
investigated under aerobic and anaerobic conditions, with increased sorption occurring at higher pH (pH 8-10) and, under anaerobic conditions, the reduction of Np on the surface of magnetite (Nakata et al., 2000, 2002). It has also been reported that heterogeneous Np reduction on the surface of magnetite is the kinetically preferred process, compared to homogenous reduction with Fe(II) in solution (Nakata et al., 2004). Wylie et al., (2016) demonstrated the near complete reduction of Np(V) on the surface of titanium doped magnetite, under reducing conditions, suggesting that there was preferential binding of Np to Ti-O sites over Fe-O sites. Np(V) reduction has also been shown upon interaction with reduced biotite and chlorite minerals, with the small fraction of ‘reactive Fe(II)’ present in the minerals proposed to be responsible for this reaction (Brookshaw et al., 2015). Further findings include the formation of hydrated NpO$_2$ nanoparticles due to the reaction of Np(V) with green rust (Fe(OH)$_2$), a highly reactive Fe(II)/(III) layered double hydroxide. In this study, Np(V) sorption to green rust was followed by its rapid reduction (minutes) to Np(IV) and, finally, the formation of poorly soluble hydrated NpO$_2$ nanoparticles (Christiansen et al., 2011). This immobilisation, thought to occur at the edge of Green Rust platelets (Bach et al., 2014), might be exploited for the attenuation of Np in subsurface systems; however, lasting attenuation over the timescales of geological disposal may be threatened by reoxidation. Conversely, Christiansen et al., (2011) found that when the reduced Np with Green Rust composite was reoxidised with air only about 50% of the available Np was remobilised to solution. The authors then suggested that remaining solid-associated Np was possibly incorporated in the Green Rust crystal lattice or segregated NpO$_2$ inclusions. Law et al., (2010b) found that air and nitrate reoxidation of microcosm experiments resulted in >20 % and >10 % of Np
remobilisation to groundwater, respectively, despite >90 % reoxidation of labile sediment associated Fe(II).

It is clear from these studies that Np(IV) shows recalcitrance to reoxidation. However, when compared to other radionuclides the knowledge and understanding of Np redox chemistry in the natural environment is poor. Further, there is no literature available that looks past single reduction and oxidation cycles. Considering the long-term environmental problems posed by Np, a greater understanding of its biogeochemistry is vital for its safe containment and disposal in a nuclear waste disposal facility.

2.22 Thesis Aims

The overarching aim of this project is to determine the effects of multiple redox cycles on sediments representative of the UK legacy (relevant to contaminated land and the far-field of a GDF) and the behaviour of two long-lived, risk-driving radionuclides (\(^{99}\)Tc and Np). As such, the results presented help build a picture of the evolution of nuclear waste in the subsurface over longer periods of time as well as providing novel information on potential redox cycling. This work builds on a wide body of evidence that suggests that in single oxidation cycles at least some \(^{99}\)Tc and Np remain recalcitrant to remobilisation by reoxidation. Throughout, microcosm techniques, sequential extractions, and X-ray absorption spectroscopy was used to track radionuclide behaviour.

The hypotheses tested in this thesis are:

- Multiple redox cycling will decrease overall \(^{99}\)Tc and Np solubility in nuclear site sediments treated with electron donors.

- Radionuclide fate will vary from that previously observed in single reduction and
oxidation cycles if further redox cycles occur.

Changes in bioavailable Fe(0) concentrations in complex sediments will have a key influence in determining radionuclide solubility.

Single mineral/microbe experiments will show how $^{99}$Tc recalcitrance to remobilisation arises during multiple redox cycles.
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3. Materials and Methods

This section describes the experimental techniques that have been used in this thesis and the basic theory behind them. As each research chapter is written as a stand-alone research paper, they also contain experimental sections.

3.1 Batch Experiments

In this thesis, batch microcosm experiments were used to replicate the conditions of a natural aquifer system relevant to the UK nuclear legacy. Specifically, they were used to assess the influence of redox cycling on radionuclide behaviour, in a controlled and safe environment. The microcosms consisted of sediment and synthetic groundwater that were spiked with either $^{99}\text{Tc}$ or $^{237}\text{Np}$. In some cases, the microcosms were also amended with added Fe (as 2-line ferrihydrite; Fe(III)$_2$O$_3$•0.5(H$_2$O)) to represent a more Fe-rich aquifer system. Finally, in chapter 5, model Fe mineral systems were used to provide a more constrained understanding of Tc-Fe interactions.

Figure 3.1. Map showing sampling site (red) near Calder Bridge, UK.
3.2 Synthetic Groundwater

All microcosms were prepared with synthetic groundwater (Wilkins et al., 2007), which is considered representative of the Drigg and Sellafield region. The composition of the groundwater is listed in Table 3.1. The groundwater was autoclaved, sparged with Ar gas, and had its pH set to ~ 7.35 prior to use in experiments. In all experiments, acetate was added at a concentration of 10 mM to the groundwater to promote microbial processes.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>gL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>0.0066</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.0976</td>
</tr>
<tr>
<td>MgCl₂·6H₂O</td>
<td>0.081</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>0.1672</td>
</tr>
<tr>
<td>Na₂O₃Si</td>
<td>0.0829</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>0.0275</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.0094</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>0.2424</td>
</tr>
</tbody>
</table>

Table 3.1 Synthetic groundwater composition (Wilkins et al., 2007).

3.3 Sediment

Sediment was collected before each experiment from next to the Calder River, Cumbria (Latitude 54°26′30 N, Longitude 03°28′09 W, Figure 3.1). The site was selected to be representative of the aquifer material that underlies the Sellafield region (e.g., Law et al., 2010; Thorpe et al., 2015). As a consequence, the microbial community and natural minerals would lead to processes characteristic of the Sellafield subsurface. This inference has been confirmed by the work of Newsome, (2015), who used borehole material from the Sellafield site in their research, and
found very similar geochemistry and microbial ecology. The material was collected from next to the river at a depth of 0.5 m. On collection, large items, such as rocks and major plant material, were removed from the sediment and the remaining material was stored in PVC sealable bags. Thereafter, the sediment was stored at 10 °C in the dark until use. The sediment mineralogy was characterised using X-ray diffraction (XRD) on a Philips PW 1050 and was principally composed of quartz and albite (Figure 3.2). Additionally, the major and minor elemental content was analysed using X-ray fluorescence (XRF) (Thermo ARL 9400). XRF analysis of the sediments showed it contained 61.9 % Si, 15.6 % Al, and 8.6 % Fe.

![Figure 3.2 XRD spectra for the Calder River sediment, with superimposed signals from present minerals.](image)

**3.4 Preparation**

Acid washed microcosm bottles were set up at groundwater to sediment ratios of 10:1 (e.g., 100 mL synthetic groundwater with 10 g sediment) and the headspace was sparged with Ar gas. The size of microcosms varied between 25 mL and 1000 mL.
depending on the experiment type. The 25-100 mL microcosms were crimp sealed and sampled aseptically with Ar flushed syringes periodically. Larger microcosms were established in tightly sealed Schott bottles; here, sampling was completed periodically in an anaerobic and sterile environment via a purpose built needle and stopcock device, as shown in Figure 3.3.

![Figure 3.3 2 L microcosm bottles (left) with purpose designed needle and stopcock (right).](image)

### 3.5 Redox Cycles

To promote microbially reducing conditions, experiments were amended with acetate (10 mM, as an electron donor) and incubated in the dark at 21°C. Incubation periods varied depending on experiment and the extent of microbially-mediated reduction was measured using geochemical indicators. Typically, once reducing conditions had been established experiments were oxidised with air (O₂). In order to maintain a neutral pH in the microcosms throughout their lifespan, an air to CO₂ ratio of 80:20 % was required. This was achieved by sparging an analogue system with a mixed flow of CO₂ and air whilst monitoring the pH, and altering the flow of both gases until a neutral pH was consistent for some minutes under gas flow. Then, the experiment headspaces were flushed with this mixed gas, sealed and shaken. The pH of experimental solutions was regularly tested to ensure the accuracy of this method.
During oxidation periods, headspaces were flushed at a periodicity of 2-3 times per week, and the microcosms were rigorously shaken every 1-3 days. Often, after oxidation events, reducing conditions were promoted once more. Here, the microcosm headspaces were flushed with Ar gas and supplemented with an electron donor to a concentration of 5-10 mM acetate. In some cases, for example, in single microbe studies, the systems were amended with mixed electron donors of acetate (5 mM) and lactate (5 mM). Redox cycles consisted of reducing and then oxidising conditions back to back and persisted, in some cases, for three full cycles of reduction and oxidation.

3.6 Colorimetric Methods

Colourimetry can be used to determine the concentration of ions and compounds in solution. When considering a monochromatic wave of light (I₀) passing through a coloured solution, a fraction of the light will be absorbed (absorbance, A) by the sample and a fraction will pass through (I). The transmittance (T) of light is defined by the ratio I/I₀ and the absorbance can be found using Equation 3.1.

\[ A = \log_{10} \frac{I}{T} \]

Equation 3.1

The Beer-Lambert law, shown in Equation 3.2, states that A is determined by the concentration (c, mol L⁻¹) of an absorbing compound, the path length (b, cm) and the extinction coefficient (ε, L mol⁻¹ cm⁻¹), which is a factor of how absorbing the species is.

\[ A = \varepsilon bc \]

Equation 3.2

This linear relationship allows for the back calculation of sample concentrations, although, at higher concentrations the relationship breaks down; this can be amended
using dilution or by reducing the path length. Additionally, non-linearity can result from: sample fluorescence at the same wavelength, sample turbidity resulting in excess light scattering, and in some cases pH (Upstone, 2000). In this thesis, colorimetric methods were used to measure the concentrations of Fe, Mn and NO$_2^-$ in solution on a calibrated Shimadzu UV-1800 spectrophotometer. The regressions from known standards were used ($R^2$ values > 0.99), along with the equation for a straight line ($y = mx + c$), to calculate sample concentration from absorbance. Disposable polystyrene cuvettes were used with a path length of 1 cm. The accuracy of colorimetric methods was tested against certified reference materials (e.g., Fe solution standard, ACROS organics), here, the assays were prepared with standard solutions and compared to calibration curves to determine the methods accuracy.

### 3.7 Determination of Iron Speciation and Concentration

The ferroin group in many molecules can react as a bidentate ligand attaching to metal ions to give a coloured species. Ferrozine, a disodium salt of (3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine, contains this ferroin group and reacts with Fe(II) to form a stable magenta complex. This complex is water soluble and stable between pH 4 and 9, thus, it is suitable for the direct determination of aqueous Fe(II). The absorbance of the aqueous complex is measured at a wavelength of 562 nm (here, $\varepsilon = 27900$), where it obeys the beer-lambert law (Equation 3.1) up to a concentration of 4 mg L$^{-1}$ (Stookey, 1970). Further, it is possible to determine the Fe(II)/Fe(III) ratio of an Fe-containing solution. Total iron in solution is measured via complete reduction of all Fe species in solution. Here, a reducing agent (hydroxylamine hydrochloride) and a buffer (ammonium acetate, to maintain a suitable pH) are added to sample solution (Viollier et al., 2000). Thereafter, % Fe(II) in solution can be calculated as shown in Equation 3.3.
% Fe(II)_{aq} = \frac{\text{Abs Fe(II)}_{aq}}{\text{Abs Fe(II+III)}_{aq}} \times 100

Equation 3.3

Principally, the method was used to calculate the % Fe(II) in sediments after a one-hour acid extraction with 0.5 N HCl. This extraction is considered to be a good estimation of the bioavailable fraction of iron in sediments (Lovley and Phillips, 1987). In this thesis, it provided an indicator for whether the system was in reducing or oxidising conditions (i.e., Fe(II) or Fe(III) dominated), as it has done in previous literature (e.g., Burke et al., 2005; Li and Krumholz, 2008; Wildung et al., 2004; Wilkins et al., 2007). Typically, a sediment pellet of ca. 0.2 g was added to 5 mL of 0.5 N HCl in a polypropylene test tube and shaken for one hour. Subsequently, the sediment and supernatant were centrifuged for 5 minutes at a centrifugal force of 12500 g. The total Fe(II) and Fe(II) & Fe(III) were then measured. For total Fe(II), 0.1 mL of the supernatant was added to a cuvette containing 3 mL of deionised water and 0.3 mL of ferrozine (0.01 M ferrozine in 0.1 M ammonium acetate solution). For total Fe (Fe(II) and Fe(III)), 0.1 mL of the supernatant, 2.1 mL of deionised water, 0.3 mL of ferrozine, 0.3 mL of buffer (10 M ammonium acetate, adjusted to pH 9.5 with ammonium hydroxide, to ensure complete complexation of the sample that requires a range of pH 4-9) and 0.6 mL of reducing agent (1.4 M hydroxylamine hydrochloride prepared in 2 M HCl). For measuring total Fe in groundwater and solutions, 0.1 mL of the sample (centrifuged if necessary, at 12500 g for 5 minutes) was added to 0.9 mL deionised water, 0.1 mL ferrozine, 0.1 mL buffer and 0.2 mL hydroxylamine hydrochloride.

3.7 Determination of Nitrite in Solution

Aqueous NO$_2^-$ concentrations were determined via the formation of a red pink azo dye. The aromatic amine, sulphanilamide (SAN), was added to buffered NO$_2^-$
containing solutions to form a diazoniun compound, after which it is coupled with naphthylethylenediamine dihydrochloride (NED) which forms the azo dye (ε = 46000) (Grasshoff et al., 1983).

Specifically, porewater samples of 0.1 mL (centrifuged at 12500 g for 5 minutes) were added to 0.75 mL deionised water and 0.1 mL buffer (18 mM ammonium chloride adjusted to pH 8.5 with ammonium hydroxide). Subsequently, 25 µL SAN solution (0.25 g SAN, 2.5 mL HCl, 100 µL Brij 35 solution, made up to 100 mL with deionised water) and 25 µL NED solution (0.25 g NED made up to 100 mL with deionised water) were added and after colour formation (~10 minutes) the absorbance was measured at 536.5 nm (Harris and Mortimer, 2002).

3.8 Determination of Manganese (II) in Solution

The multidentate ligand 1-(2-pyridylazo)-2-naphthol (PAN) was used to complex Mn(II) in solution (Goto et al., 1977). The resultant Mn-PAN chelate is red-orange in colour and has a high molar absorptivity (ε = 44000) at the wavelength 562 nm. Porewater samples of 0.25 mL (centrifuged at 12500 g for 5 minutes) were added to 0.75 mL deionised water and 50 µl of ascorbic acid (0.6 M), after which 70 µl of PAN solution (Sigma-Aldrich) and 2 drops of alkaline cyanide solution (HACH) were added. After colour formation (10 minutes,) the absorbance was measured at 562 nm.

3.9 Ion Chromatography

Ion chromatography is a useful technique for measuring the concentration of ions in a solution. Specifically, it was used in this thesis to assess the concentrations of anions not directly measurable by colorimetric methods, such as acetate.

In ion chromatography, the analyte ions are passed through a column containing an ion exchange resin. Once the resin has retained the ions and is at equilibrium, the column is flushed with an eluent. This eluent contains competitive ions that exchange
with the analyte ions. The ability of an ion/compound to remain in the column is
determined by its particular affinity for the exchange resin. Because this will vary
depending on the ion, the various analyte ions will leave the column at different
points as the eluent is changed. For example, by increasing the ionic strength,
temperature, pH, flow rate, or the buffering salt. This will result in distinct and
measurable peaks at different times leaving the column. The concentration of a
particular analyte is found by comparing the time the peak was formed and its size to
known standards (Fritz and Gjerde, 2000). Here, ion chromatography was used to
measure the aqueous concentrations of nitrate, sulphate and acetate in experimental
solutions using a Dionex ICS5000 ion chromatograph. The eluent used was KOH (1-
40 mM) and peaks were assessed using an AS-RS electronic suppressor, with a
detection limit of ~10 ppb. Before analysis samples were centrifuged for 5 minutes at
12,500 g and diluted 100 fold, this reduced the radioactivity of the samples and
reduced the sample concentrations to detectable limits. Standards were run every 10-
15 samples to ensure accuracy.

3.10 pH

The acidity or basicity of a given solution can be determined by its hydrogen ion (H⁺)
concentration. The negative logarithm of this concentration (Equation 3.4) is known
as pH and typically ranges from 1 to 14, known as the pH scale.

$$pH = -log_{10}[H^+]$$

Equation 3.4

All pH measurements were conducted on a calibrated electrode (calibrations used the
following standards: pH 4, pH 7, pH 10) and measured using a Metler Teledo digital
meter. When measuring the pH of synthetic ground waters, the solutions were first
centrifuged (12,500 g, 5 minutes) or left to settle until the solution was transparent.
When necessary, the soil pH was measured in sediment slurries after equilibration in equal parts deionised water for 30 minutes.

3.11 Acid Extraction

Acid extractions were performed to help determine any relationship of Fe and radionuclides in the sediment systems, in particular, at the end-points of reductions and oxidations. Sediments were exposed to increasing HCl concentrations for each extraction step (in order: 0.001, 0.01, 0.1, 1, 2, 3, 4, 6 M HCl), a method modified from Doornbusch et al., (2015). Here, sediments were leached for 30 minutes in 10 mL of HCl and then centrifuged (4000 rpm, 10 minutes), after which the supernatant was removed, analysed, and replaced with the sequential acid. Additionally, there was a 24-hour and 4-day extraction with 6 M HCl and finally, an additional extraction which involved boiling samples in *aqua regia* for 4 hours. Subsequently, the extractants were analysed for Fe concentration using the ferrozine method, and radionuclide concentration using Liquid Scintillation Counting.

An alternate extraction was used to assess the dissolution of Fe and Tc in Fe-mineral experiments. Here, reduced and oxidised Fe-minerals (~0.1 g) were digested in 40 mL of 1 M HCl until complete dissolution of the mineral had occurred as signified by no increase in Fe into solution over a number of time points. This method was selected because it can be performed when a small amount of sample is available. Samples for analysis of Tc (by Liquid Scintillation Counting) and Fe (by the ferrozine method) were taken at specific time intervals. This method has previously been described by Marshall et al., (2014).

3.12 X-ray Absorption Spectroscopy

X-ray absorption spectroscopy (XAS) has many applications ranging from acquiring the average oxidation state of a specific element to probing its average local
coordination environment. In this thesis, XAS was used to determine both the oxidation state and coordination environment of Tc and Np across various experiments.

XAS uses X-rays of a defined energy to excite a targeted electron, of a targeted element in a sample. As the sample is bombarded, the targeted electrons absorb some of the X-rays leading to their excitation and eventually their ejection into continuum. Because each electron of a chosen element has a specific ionisation energy the incident X-ray can be tuned to a particular electron in a particular shell, for example, the Tc K-edge (a 1s electron) at ~21044 eV. If the energy of the incident X-ray is increased from before the ionisation energy (referred to as the pre-edge) to an energy greater than it (the post edge), a spectrum of the resultant absorbance (by the absorbing electron) can be measured, this is shown in Figure 3.4 for an example of Tc(VII). Absorbance spectra can be split into two parts; the X-ray Absorption Near Edge Structure (XANES), which spans the pre-edge to ~50 eV beyond the absorption edge; and the Extended X-ray Absorption Fine Structure (EXAFS), spanning from the XANES region to up to 1000 eV passed the absorption edge. Characteristics in the XANES region allow the determination of the average oxidation state of a specific element in a sample and features in the EXAFS region that can be used to determine the average coordination environment (Calvin, 2013).

3.13 X-ray Absorption Near Edge Structure

As mentioned before, the XANES region includes the pre-edge, absorption edge and ~50 eV of the post-edge (or near edge) of an absorbance spectrum. Structures present in this region can help determine the oxidation state of a sample. This is because the energy of the absorption edge is determined by how many electrons the atom has (i.e., the valence state) and its shape will be influenced by which orbitals, if any, the
targeted electron is promoted to when excited by the incident X-ray (Calvin, 2013). For example, the energy of the absorbance edge will increase with an increase in oxidation state; and when an electron has insufficient energy to be ejected into the continuum it may occupy another electron shell, resulting in pre-edge features (Kirby et al., 1981). A useful example is that of technetium’s environmentally relevant oxidation states, Tc(IV) and Tc(VII). The nucleus of Tc(VII) is less shielded than Tc(IV) and, therefore, has a greater effective charge density and more electrostatic attraction, meaning electrons require more energy to be ejected, hence, the Tc(VII) K-edge is typically found at ~7 eV higher than Tc(IV). However, it is the presence of pre-edge features that easily distinguishes the oxidation states. Electrons promoted from the 1s to 4d orbital during the excitation of Tc(VII) results in the characteristic absorbance spectrum shown in Figure 3.4.

Figure 3.4. XANES spectra for Tc(VII)O$_4^-$ (black line) and Tc(IV)O$_2$ (dotted line) (from Hess et al., 2004).

Fingerprinting of spectra can be used to compare results to ideal standards. Linear combination fitting (LCF) of a collected spectrum between two or more standard
spectra can indicate the quantity of each oxidation state present in a sample (Calvin, 2013). In this thesis, XANES spectra and LCF were used to assess between the valence states of Np(V) and Np(IV) and Tc(VII) and Tc(IV) in reduced and oxidised sediments and minerals.

3.14 Extended X-ray Absorption Fine Structure

The EXAFS region of an absorbance spectra ranges up to 1000 eV after the XANES region, depending on the sample composition and quality of data. Typically, a range of 500 - 700 eV is used. The oscillations visible in this part of the spectra are due to interactions of the outgoing spherical photoelectron wave with neighbouring atoms. As the wave propagates it is elastically scattered by the surrounding atoms, with some back-scattered waves returning to the absorbing atom leading to a modulation in the absorbance probability. These back-scattered waves can affect the outgoing photoelectron wave in two ways, constructively or destructively. Constructive wave interactions lead to an increased electron density and, therefore, an increased probability of absorbance. This is represented by an increased oscillation in the EXAFS data. Conversely, destructive interference would have the opposite effect, decreasing the amplitude of an oscillation. When considering an isolated atom, the photoelectron wave will travel unhindered, resulting in a flat line in the EXAFS. When neighbouring atoms are introduced the number of backscattering waves will increase, which in turn brings structure to the EXAFS. Thus, the structure of the material surrounding the targeted atom determines the EXAFS (Calvin, 2013). For example, in a disordered array of neighbouring atoms, the direct scattering of the photoelectron might produce complex oscillations or, alternately, highly symmetrical systems may see evidence of multiple scattering pathways as the photoelectron scatters off multiple atoms before returning to the absorber. EXAFS is the summation
of these waveforms and, hence, an average (Calvin, 2013). The factors influencing the absorption spectra in this region are summarised in the EXAFS equation (equation 3.5).

$$\chi(k) = S_0^2 \sum_i N_i f_i(k) \frac{2R_i}{kR_i^2} e^{i\theta(k)} e^{-2k^2\sigma^2} \sin (2kR_i + \delta_i(k))$$

Equation 3.5

This equation satisfies the EXAFS function (chi), a sinusoidal function expressed through the photoelectron wave number (k), for all possible scattering paths (i) resulting from the local coordination environment of the absorbing atom. The scattering atoms will influence the probability that elastic scattering will occur, f(k), and the photoelectron phase shift, δ(k). Further, the amplitude reduction factor (S₀²), the number of scatterers in a shell (N), the distance between atoms (R), and the amount of ‘disorder’ or inconsistency in the bond distance (σ²) will all contribute to the EXAFS function. Therefore, the absorption spectra can be modelled with computationally derived scattering paths using the EXAFS equation, to produce values for N, R and σ².

3.15 XAS Sample Preparation, Data Collection, and Analysis

Specific experiments were set up with elevated radionuclide concentrations (to provide ~1000 ppm ⁹⁹Tc or ²³⁷Np on solids) and the samples, of reduced and oxidised sediments and Fe minerals, were analysed on the beamline B18 of the Diamond Light Source using a 9 or 36 element Ge detector. For ⁹⁹Tc, the radioactive material was double sealed in a mini-centrifuge tube within cryo-vial. The samples were prepared under flowing Ar to prevent oxidation and, for oxidised samples, the sediments were washed in deoxygenated water to remove a potential porewater signal from Tc(VII), as performed in previous literature (Fredrickson et al., 2009). ⁹⁹Tc K-edge data were
collected in fluorescence mode at 78 °K using an Oxford Instruments Cryostat. For $^{237}$Np, samples were prepared in specially built Perspex holders and sealed with 2 layers of Kapton tape, then heat sealed in a plastic bag. Again, all sample manipulations were performed in anaerobic conditions. $^{237}$Np L$_{III}$-edge data was collected in fluorescence mode at room temperature. The background subtraction and normalisation of data were achieved using software package Athena and the EXAFS spectra were fitted using Artemis (Ravel and Newville, 2005). When modelling, the significance of fitting additional shells was assessed using an F-test (Downward et al., 2007). Further, relevant standards and information from the literature were used to analyse the data, and where available reference foils were also measured to align the edge energies.

3.16 Transmission Electron Microscopy

Transmission Electron Microscopy (TEM) imaging took place at the Leeds Electron Microscopy and Spectroscopy Centre (LEMAS), University of Leeds. Images were acquired using an FEI Tecnai TF20 field emission gun TEM. Dispersive X-ray spectroscopy (EDX) was used to obtain the elemental composition of the samples with an Oxford Instruments INCA 350 EDX. Briefly, once the beam has excited the sample the electrons will return to their ground state emitting an X-ray, the energies of the X-rays will be characteristic to a certain element and, hence, EDX detects specific elements by their signature energy emissions. Selective area electron diffraction (SAED) was used to analyse the atomic spacing of lattice planes present in the minerals observed. This is achievable because the electron beam is smaller than the typical d-spacing of solid materials, in the region of $10^{-3}$ nm and, therefore, creates a diffraction pattern as the beam passes through the selected area. TEM samples were prepared on carbon-coated copper grids. Here, 5 µl of the sample (ground water
containing particulates) was dropped onto a grid and left to dry in anaerobic conditions, once dry the procedure was repeated. Finally, the grids were washed with deoxygenated water using the same method and dried, before being stored for analysis.

3.17 Microbial analysis

16S rRNA Gene Amplicon Pyrosequencing was undertaken to determine the microbial structure of the sediment used in this study before, during, and after reduction and oxidation events. DNA was isolated from ~ 0.2 g of redox-cycle endpoint sediment samples using the MoBio PowerSoil™ DNA Isolation Kit (MoBio Laboratories Inc., USA). PCR of the V1-V5 hypervariable regions of the bacterial 16S rRNA gene for amplicon pyrosequencing was performed using tagged fusion universal bacterial primers 27F (Lane, 1991) and 907R (Muyzer et al., 1995), synthesised by IDTdna (Integrated DNA Technologies, Belgium). The fusion forward primer

(5’-AGAGTTTGATCMTGGCTCAGNNNNNNNNNAGAGTTGATCMTGGC

-3’) contained the 454 Life Sciences “Lib-L Primer A”, a 4 base “key” sequence (TCAG), a unique ten-base barcode “MID” sequence for each sample (e.g., MID13 CATAGTAGTG, MID14 CGAGAGATAC, or MID16 TCACGTACTA), and bacterial primer 27F. The reverse fusion primer

(5’- CCGTCAATTCMTTRAGTTT -3’) contained the 454 Life Sciences “Lib-L Primer B”, a 4 base “key” sequence (TCAG), and bacterial primer 907R. The pyrosequencing run was completed at The University of Manchester sequencing facility, using a Roche 454 Life Sciences GS Junior system. The 454 pyrosequencing reads were analysed using Qiime 1.8.0 release (Caporaso et al., 2010), and de-noising and chimera removal was performed in Qiime during OTU picking (at 97% sequence
similarity) with usearch (Edgar, 2010). Taxonomic classification of all reads was performed in Qiime using the Ribosomal Database Project (RDP) at 90 % confidence threshold (Cole et al., 2009), while the closest GenBank match for the OTUs that contained the highest number of reads (the representative sequence for each OTU was used) was identified by Blastn nucleotide search. In addition, Qiime was used to show the rarefaction curves.

Nonmetric multidimensional scaling (NMDS) was performed on Primer v6 (PRIMER-E Ltd, Plymouth Marine Laboratory, Plymouth, UK), based on a Bray-Curtis similarity matrix (Clarke, 1993; Clarke and Gorley, 2006), which was calculated using square root transformed relative (%) abundances of all the identified OTUs of this study.

3.18 Liquid Scintillation Counting

The concentrations of radionuclides in experimental solutions were measured using Liquid Scintillation Counting (LSC). This technique is particularly good for measuring low concentration β and α emitters. LSC works by transferring the energy of an emission into UV light. A sample is mixed with liquid scintillation fluid, which contains a number of compounds (principally fluors). These compounds transfer the energy from radioactive emission in sequence as photons of specific wavelengths. Subsequently, they are converted to electrical pulses of certain amplitude by a photomultiplier. The isotope and its concentration can then be determined from the spectrum of detected amplitudes. In this thesis, solutions (typically 0.1 mL) containing $^{237}$Np or $^{99}$Tc were mixed with scintillation fluid (5 mL), left to equilibrate in the dark (24 hours), and then measured using a Wallac Quantulus 1220. Blank samples and standards were regularly used to test the accuracy of measurement and the limit of detection, equated using blanks, was 0.01 Bq mL$^{-1}$. The accuracy of the
Wallac Quantulus 1220 was determined using certified reference solutions ($^{14}\text{C}$ and $^3\text{H}$, Wallac Packard).

### 3.19 Safety

The supply and manipulation of radioactive materials are subject to statutory controls. Therefore, strict protocols and operating procedures were in place throughout this project. Ionising radiation can be harmful to human health and, hence, safety training is necessary before work. Further, all work must follow the institutions’ guidelines and comply with the Ionising Radiation Regulations 1999, Environmental Permitting Regulations 2010, and the Health and Safety Work Act 1974. All chemicals used in experiments were first risk-assessed in accordance with the Control of Substances Hazardous to Health (COSHH) and based on these assessments appropriate precautions were taken.
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4. Impact of Long-term Redox Cycling on Technetium Mobility

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4.1 Abstract

Long-lived radionuclides released into the environment will likely see numerous changes in their local redox environment over time. Here, we have investigated the fate of technetium in sediments over multiple reduction and oxidation cycles. Technetium spiked microcosms were biogeochemically ‘cycled’ between reducing and oxidising conditions three times and regular monitoring revealed that Tc remobilisation to solution decreased with repeated oxidation cycles. This observation is reinforced by chemical leaching data, where Tc resistance to acid extractions increased with repeated redox cycling. This supports the theory that Tc resistance to reoxidation occurs as a result of association/binding with Fe-oxides and, potentially, redox buffering from residual Fe(II) in oxidation cycles. Furthermore, X-ray Absorption Spectroscopy (XAS) experiments showed that Tc was present as hydrous TcO$_2$-like chains throughout multiple redox cycles and that the hydrous TcO$_2$ chain length shortened with repeated redox cycling. Current literature suggests these chains bind to the edge of Fe phases present in the sediment. Considering this, we present evidence to suggest that Tc resistance to remobilisation on repeated redox cycling results from the increased significance of this Fe interaction. Finally, we confirm the presence of a colloidal Tc(IV) phase in reduced systems at concentrations $>10^3$ times the reported solubility of TcO$_2$.

4.2 Introduction

Technetium-99 ($^{99}$Tc) is a high-yield β-emitting fission product which is a significant contaminant at nuclear sites worldwide (Lloyd and Renshaw, 2005; McKenzie et al., 2011) and is also an important consideration for radioactive waste disposal due to its long half-life ($2.1 \times 10^5$ years). In addition, technetium has a high predicted solubility
(as Tc(VII)), bioavailability, and bioaccumulation potential making it a significant risk-driving radionuclide in environmental systems (Smith et al., 2001; Icenhower et al., 2008). The safe management of Tc in the environment will rely on a thorough understanding of Tc biogeochemistry over extended timescales. The solubility of Tc in the environment is largely governed by its oxidation state. Highly soluble pertechnetate (TcO_4\(^-\); Boyd, 1978) dominates in oxic and nitrate-reducing groundwaters, sorbing weakly to geomedia (Liu and Fan, 2005; Burke et al., 2005; Lear et al., 2010). In contrast, poorly soluble Tc(IV) dominates under anoxic conditions, and Tc(IV) has been shown to strongly sorb to mineral phases at concentrations below the predicted solubility threshold for key Tc(IV) phases such as hydrous TcO_2 (Meyer et al., 1991; Lear et al., 2010; Thorpe et al., 2016). Reduction of Tc(VII) to Tc(IV) has been linked to the development of microbially-mediated Fe(III)- and sulfate-reducing conditions. As a consequence, and in an effort to limit Tc migration at nuclear sites, there has been widespread interest in promoting Tc(VII) reduction in the shallow subsurface. Here, an electron donor (e.g., acetate) is added to groundwater to stimulate microbially-mediated anaerobic processes (Lear et al., 2010; Lee et al., 2014; Thorpe et al., 2014). Electron donor release from waste packages in a geological disposal facility may also lead to stimulation of microbial reduction (Rizoulis et al., 2012) and this “biobarrier” may serve to limit Tc migration through the deep subsurface (Brookshaw et al., 2015).

To date, work in pure culture, sediment-microcosms, flowing columns, and at field scale, has documented a range of Tc(VII) reduction mechanisms and Tc(IV) bioreduction products. Enzymatic Tc(VII) reduction can be facilitated by a range of microorganisms but is thought to be only modestly important in controlling Tc fate under environmental conditions (Lloyd and Macaskie, 1996; Lloyd et al., 1999).
Instead, Tc(VII) reduction is dominated by reaction with the by-products of microbial metabolism (i.e., biogenic Fe(II) and sulfide; Lloyd et al., 1998, 2000; Fredrickson et al., 2004; Burke et al., 2005). This includes abiotic reduction by Fe(II) in a range of minerals (e.g., magnetite, siderite, illite, biotite, and chlorite; Peretyazhko et al., 2009; Marshall et al., 2014; Thorpe et al., 2014; Brookshaw et al., 2015), and sulfide phases (Wharton et al., 2000; Liu et al., 2008; Lee et al., 2014). Under Fe(III)-reducing conditions, reported Tc(IV) reaction products include TcO₆ octahedra / octahedral chains and/or discrete hydrous TcO₂-phases (Lukens et al., 2002; Zachara et al., 2007; Morris et al., 2008; Fredrickson et al., 2009; Peretyazhko et al., 2009; Brookshaw et al., 2015). Tc(IV) can also be incorporated into magnetite (Marshall et al., 2014) and goethite (Um et al., 2011), and TcSₓ-like phases have also been reported (Wharton et al., 2000; Fan et al., 2014; Lee et al., 2014).

Despite the potential for removal of Tc(VII) from groundwater as poorly soluble Tc(IV) phases, it is clear that with such a long half-life, understanding ⁹⁹Tc behaviour over longer timescales is critically important. For example, Tc(IV) oxidation could result in Tc remobilisation and extensive migration. Oxidising conditions in the subsurface can occur via numerous pathways, for example, groundwater perturbations or flooding (Burke et al., 2006; Wu et al., 2007). Work on the durability of Tc(IV) reduction products has to date been relatively sparse with most studies focussing on short duration (weeks) reoxidation studies, typically involving exposure of reduced, Tc(IV)-bearing sediments to air- or nitrate-rich groundwater (Burke et al., 2006; McBeth et al., 2007; Morris et al., 2008; Fredrickson et al., 2009). In this past work, significant (20-80 %) Tc remobilisation to solution has been reported, but in most cases a durable Tc(IV) fraction remains associated with the solid phase (Cui and Eriksen, 1996; Burke et al., 2006; McBeth et al., 2007; Fredrickson et al., 2009;
Geissler et al., 2011; Um et al., 2011; Marshall et al., 2014). Direct spectroscopic
evidence suggests that reoxidised sediments can retain Tc(IV) as hydrous TcO2-like
phases, with the potential for bonding to Fe-oxides in the Tc reoxidation product also
reported (Zachara et al., 2007; Fredrickson et al., 2009). Similar studies with Fe(II)-
mineral systems have also shown that Tc(IV) can be retained in oxidised, Fe(III)
bearing products (Wharton et al., 2000; Um et al., 2011; Marshall et al., 2014). Here,
incorporation of Tc(IV) into mineral lattice sites, physical shielding of Tc(IV) by
Fe(III) oxidation rinds, and/or oxidative buffering by residual Fe(II) have all been
suggested as mechanisms which could protect Tc(IV) from oxidative remobilisation
(Zachara et al., 2007; Morris et al., 2008; Marshall et al., 2014). These single redox
cycle studies provide insights into Tc biogeochemical behaviour in sediment and
mineral systems over short timescales and highlight that irreversible binding of a
fraction of Tc(IV) may be possible. Now, we further explore the environmental
behaviour of Tc over longer (>year) timescales under repeated reduction / oxidation
cycles in natural sediments to reflect that Tc, with its long half-life, will undergo
complex and repeated redox cycling in the natural environment. Our experiments used
acetate as an electron donor to stimulate bioreduction and air (O2) was then used as
the oxidant. We highlight a much increased recalcitrance of sediment-bound Tc(IV) to
oxidative remobilisation after multiple redox cycles. This suggests a pathway to
enhanced irreversible binding of Tc(IV) in sediment systems, a positive prospect for
both contaminated land and radioactive waste geological disposal. In addition, we
also highlight the ingrowth of a Tc(IV) colloidal phase which forms and is persistent
under reducing conditions. This phase may lead to enhanced mobility of 99Tc in the
subsurface and requires further understanding. Overall, these data highlight the
complex behaviour of risk-driving 99Tc under biogeochemical cycling conditions with
both Tc(IV) incorporation and colloid formation impacting on its potential mobility. Furthermore, the processes explored in this research have implications for uranium, plutonium, neptunium, and other risk-driving, long-lived radionuclides as they might display similar chemistry.

4.3 Results and Discussion

4.3.1 Technetium Solubility in Response to Redox Cycling

Large volume, duplicate sediment microcosms were used to explore the fate of Tc during three reduction / oxidation cycles over 1.5 years. The sediment microcosms (100 g : 1 L solid : solution ratio) were amended with an electron donor (10 mM acetate), spiked with Tc(VII) (0.5 µM) and incubated anaerobically until robust Fe(III)- and SO₄²⁻-reducing conditions had developed. Periodically, microcosms were oxidised with air and the re-reduced through amendment with acetate. Microbially-mediated (bio)reduction followed the expected sequence of terminal electron accepting processes (Figure 4.1 and Appendix 1, Figure SII-B). During bioreduction, ⁹⁹Tc was largely scavenged to solids commensurate with Fe(III)-reduction (~94 % removed between days 22 and 57, Figure 4.1). Here, abiotic reduction of Tc(VII) with biogenic Fe(II) likely dominated (Burke et al., 2005; Lloyd et al., 2000). After 119 days, the 0.5 N HCl-extractable Fe(II/III) ratio in solids was 98.3 ± 0.5 % and the Tc concentration in solution stabilised (18 ± 0.6 nM) near the theoretical solubility of hydrous TcO₂ (10 nM L⁻¹; Meyer et al., 1991). At this point, oxidation of the microcosms was initiated via flushing of the headspace with 0.22 µm filtered air every 1-3 days for 60 days. This resulted in the reoxidation of Fe(II), and by 60 days the measured Fe(II/III) ratio in solids stabilised at 10.3 ± 2.9 % (Figure 4.1). Ferrous iron oxidation was accompanied by significant but incomplete Tc remobilisation to
solution with 43.4 ± 15.1 % of the initial Tc spike remobilised in the experimental duplicates by day 179. This level of Fe(II)-reoxidation and Tc remobilisation to solution during air oxidation is in broad agreement with past work (Burke et al., 2006; Fredrickson et al., 2009; Geissler et al., 2011; McBeth et al., 2007; Um et al., 2011).

After the measured sediment Fe(II)/Fe(III) ratio had stabilised at 10.3 ± 2.9 % during oxidation (day 179, Figure 4.1), anaerobic incubation was again stimulated with addition of 5 mM acetate. After acetate addition and anaerobic incubation, Fe(III)-reducing conditions were rapidly re-established concomitant with removal of aqueous Tc. During this second period of bioreduction, the Fe(II/III) ratio in solids reached ~ 100 % within 35 days (Figure 4.1), and the removal of SO$_4^{2-}$ from solution occurred

![Graph](image_url)

Figure 4.1: % Tc in solution (blue) and % Fe(II) in sediments (extractable by 0.5 N HCl for 1 hour; Lovley and Phillips, 1986) (red), for duplicate bottles (Bottle A: triangles, Bottle B: squares). Shaded areas denote ‘reduction events’, where microcosms were amended with acetate and kept anaerobic. Unshaded areas denote ‘oxidation events’, where microcosms were mixed regularly (every 1-3 days) with air.
Interestingly, despite these robust reducing conditions, a greater proportion of Tc remained stable in solution when compared to the first reduction period (11.6 ± 1.4 % compared to 3.8 ± 0.1 %; Figure 4.1). After 229 days, the experiments were again reoxidised through repeated purging of the headspace with air over 92 days. During the second period of reoxidation, less Tc was remobilised into solution (43.4 ± 15.1 % in the first oxidation period vs. 21.3 ± 4.4 % in the second; Figure 4.1). Finally, a third period of reduction (addition of 5 mM acetate and anaerobic incubation for 43 days) and oxidation (controlled exposure to filtered air for 64 days) was completed. After this third period of reduction, 9.4 ± 0.9 % of the added Tc remained in solution whilst, after reoxidation, aqueous Tc increased to 13.1 ± 2.6 % in solution. Overall, during the reduction cycles, a significant increase in residual Tc\textsubscript{(aq)} was observed between the first (3.8 ± 0.1 %) and third reduction periods (9.4 ± 0.8 %). In contrast, during oxidation cycles, the sediment associated Tc became progressively more resistant to remobilisation from solution (43.4 ± 15.1 % remobilised in the first oxidation compared to 13.1 ± 2.6 % in the third). Overall, in these systems, a significant fraction of Tc becomes more strongly resistant to remobilisation with repeated redox cycling whilst at the same time, there is also evidence that, surprisingly, Tc displays enhanced solubility in strongly reducing systems.

4.3.2 Technetium Fate in Sediments

Acid extraction was used to investigate the relationship between Tc and Fe in the sediments from the redox-cycled microcosms, at endpoints from the first and third reoxidation periods (days 179 and 428, Figure 4.2). The two samples showed the same general Tc and Fe dissolution trends in the acid extraction but the Tc in the first reoxidation sample was always more susceptible to leaching than that in the sample.
from the third period of oxidation. In both experiments, after leaches for 30 minutes with between 0.001 – 6 M HCl, the slope of Tc vs. Fe dissolution was > 1 confirming that Tc was unevenly distributed in the samples with enhanced release of Tc compared to Fe. In addition, a significant fraction of the Tc (25 % and 50 % in first and third reoxidation cycles, respectively) was recalcitrant to leaching with 6M HCl for 30 mins (Figure 4.2). These data suggest there is a relative enrichment of Tc on surfaces in the sediment system as ~50 % and ~75 % Tc was leached when only ~20% Fe has been leached in first and third reoxidation samples, respectively. Additionally, it is also clear that the Tc in the reoxidation solids is becoming more recalcitrant to acid leaching with repeated redox cycling (Figure 4.2). After this, in

![Figure 4.2: Cumulative dissolution of total Tc versus total Fe from sediment. Samples are the 1st oxidation (day 179, blue, squares) and 3rd oxidation (day 428, red, circles). Sequentially, the [H\(^+\)] in the 30 minute extraction was increased from 0.001 M to 0.01 M, 0.1 M, 1 M, 2 M, 3 M, 4 M and, 6 M HCl, with the final three extractions 6 M HCl for 24 hours, 6 M HCl for 4 days, and heated in aqua regia for 4 hours. Error bars are the range from duplicate experiments, where error bars are not shown they are within the symbol size.](image)
both samples, as the acid leaches became more extreme, the gradient fell to <1 after
~20 % of the Fe had been extracted (Figure 4.2). This suggested 25 – 50 % of the Tc
in these systems is very strongly bound within Fe-containing minerals; indeed, a full
aqua-regia leach was required for full dissolution of the Tc from the sediments. As
discussed, the first reoxidation sample was routinely more susceptible to leaching than
the third reoxidation sample. This confirms that a shift in the Tc binding in the
samples occurs as a result of repeated redox cycling and that Tc is becoming more
recalcitrant with redox cycling. Indeed, by the third reoxidation cycle, ~40 % of the
Tc was resistant to a 6 M HCl leach for 4 days and required an aqua regia leach to
liberate the residual Tc and Fe suggesting strong Tc binding in these systems. The
increased Tc recalcitrance to oxidative remobilisation with repeated redox cycles
(Figure 4.2, and in the Tc aqueous data, Figure 4.1) may be associated with increased
Fe-crystallinity in the sediments with repeated cycling, as early bioreduction cycles
tend to reduce the poorly crystalline Fe-fraction (Yang et al., 2012; Zhao et al., 2015),
although, the acid leach shows a similar behaviour in Fe extractions, suggesting a
similar environment for Fe after both oxidations. It is possible that this experimental
treatment cannot distinguish the subtleties in Fe-mineral phase change, and that the
system is becoming enriched in more crystalline Fe-oxides. Potentially, some fraction
of the Tc is becoming entrained within these phases. Another possibility is that
increased amounts of residual Fe(II) remaining in the sediments over the second and
third reoxidation periods (0.5 N HCl-extractions of ~25 % Fe(II) in second and third
cycles compared to ~10 % in first cycle; Figure 4.1) acts as a buffer for Tc(IV)
oxidative remobilisation (Heron and Christensen, 1995; Fredrickson et al., 2009).
Finally, Tc(IV) in this system may become physically protected from remobilisation
through armouring by Fe(II)/(III) oxidation products, with the Tc becoming occluded
by the sediment minerals (Zachara et al., 2007; Morris et al., 2008; Marshall et al., 2014).

To further assess changes in the Tc speciation and coordination environment in these complex systems, we undertook X-ray Absorption Spectroscopy on a select group of parallel experiments run at the higher Tc concentrations necessary for XAS. These data were collected at the end of the first, second, and third reduction periods, and at the end of the first and second oxidation periods. All Tc K-edge XANES spectra aligned with a Tc(IV) reference sample (~ 21050 eV), confirming there was no significant Tc(VII) in any of the samples, regardless of whether they were taken from the reduced or reoxidised sediments (Figure 4.3C).

Analysis of the EXAFS Fourier transform data for all samples showed two shells of backscatters were present; the first shell at ~ 2.0 Å and the second shell at ~ 2.5 Å (Figure 4.3A). These data are similar to the model for Tc(IV)O₂•xH₂O chains

Figure 4.3: XAS data for Tc-99 in reduced and oxidised sediment samples Panels: A; Non-phase shifted Fourier transform of k³-weighted EXAFS spectra calculated over a k range of 3.5 to 13.0 Å⁻¹, with their best fit models (including ‘short Fe’ interaction), B; k³-weighted EXAFS spectra (coloured lines) with their best-fit models, and C; Tc K-edge XANES spectra for reduced and oxidised sediment, an aqueous sample from reduction and oxidation end-points, and reference spectra samples.
described by Lukens et al., (2002) and seen in several studies where Tc is present in environmental systems (Morris et al., 2008; Fredrickson et al., 2009; Peretyazhko et al., 2012) and this coordination environment was used as the starting point for our modelling. The data were initially fitted with 6 O backscatterers at 2.0 Å, with the best fit optimising the number of Tc backscatterers at ~ 2.5 Å (see Appendix 1, Table SI1-B). The optimisation process for fitting Tc backscatterers was performed with fixed $S_0^2$ and $\sigma^2$ values (see methods). This approach showed that the average coordination number of Tc decreased with each subsequent redox event, starting at $1.2 \pm 0.1$ Tc for the first reduction and falling to $0.7 \pm 0.1$ Tc by the third reduction (Appendix 1, Table SI1-B). This trend can clearly be seen in the decreasing amplitude of the Fourier transform peak at ~2.5 Å with repeated redox cycles (Figure 4.3A). Interestingly, this decrease in the Tc coordination number at 2.5 Å in the samples corresponds to a shortening of the octahedral chain length in our standard model of Tc(IV)O$_2$.xH$_2$O chains (Lukens et al., 2002). In this “TcO$_2$ chain” model, a Tc coordination number of two represents an infinite hydrous TcO$_2$ chain as each central Tc atom is neighboured by two Tc atoms, a coordination number of one represents a TcO$_2$ dimer, and a coordination number of zero represents a TcO$_2$ monomer. The results of fitting suggest that Tc forms dimers during initial reduction and oxidation, and with repeated redox cycling the speciation and coordination environment favours monomeric TcO$_2$ species. Interestingly, the EXAFS for all samples had features that were not fully resolved with this model alone (between 10.5-12.5 K, Figure 4.3B and Appendix 1, Figure SI1-C) and Tc binding within sediments via an edge-sharing “short Fe” octahedron at ~ 2.6 Å, which have been attributed to attachment of the TcO$_2$ chains at Fe-O surfaces, was also considered relevant (Zachara et al., 2007; Morris et al., 2008; Fredrickson et al., 2009; Liu et al., 2012; Brookshaw et al., 2015).
Reflecting this, the EXAFS spectra were re-fitted to include a contribution from a “short Fe” backscatterer at ~2.6 Å (Table 4.1). As with the fits for the TcO₂ chain model alone, these constrained fits also showed a trend of decreasing Tc coordination number at ~ 2.5 Å with progressive redox cycles, from 1.4 ± 0.2 in the first reduction.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Path Tc-</th>
<th>R (Å)</th>
<th>CN</th>
<th>σ² (Å²)</th>
<th>ΔE₀ (eV)</th>
<th>S₀²</th>
<th>R-factor</th>
<th>Conf (%)</th>
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<td>O</td>
<td>2.02 (1)</td>
<td>6*</td>
<td>0.005</td>
<td>0.80</td>
<td>0.95*</td>
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<tr>
<td></td>
<td>Tc</td>
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<td>1.36 (22)</td>
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<td>0.31</td>
<td>0.95*</td>
<td>0.005</td>
<td>99.8</td>
</tr>
<tr>
<td></td>
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<td>2.62 (4)</td>
<td>0.53 (30)</td>
<td>0.006*</td>
<td>0.73</td>
<td>0.95*</td>
<td>0.011</td>
<td>99.6</td>
</tr>
<tr>
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<td>6*</td>
<td>0.005</td>
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<td>0.95*</td>
<td>0.007</td>
<td>100.0</td>
</tr>
<tr>
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<td>2.01 (1)</td>
<td>6*</td>
<td>0.005</td>
<td>0.80</td>
<td>0.95*</td>
<td>0.006</td>
<td>97.5</td>
</tr>
<tr>
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<td>1.36 (20)</td>
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<td>0.31</td>
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<td>0.006*</td>
<td>0.73</td>
<td>0.95*</td>
<td>0.011</td>
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<td>-0.3</td>
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<td>Fe</td>
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<td>0.69 (27)</td>
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<td>0.73</td>
<td>0.95*</td>
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<td>6*</td>
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<td>6*</td>
<td>0.005</td>
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<td>0.95*</td>
<td>0.006</td>
<td>97.5</td>
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<tr>
<td></td>
<td>Tc</td>
<td>2.55 (1)</td>
<td>0.72 (29)</td>
<td>0.003*</td>
<td>0.31</td>
<td>0.95*</td>
<td>0.010</td>
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<td>0.71 (31)</td>
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<td>6*</td>
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<td>-0.3</td>
<td>0.95*</td>
<td>0.010</td>
<td>99.9</td>
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</table>

Table 4.1: R = atomic distance CN = coordination number; σ² (Å²) = Debye-Waller factor; ΔE₀ (eV) = energy shift from Fermi level; S₀² = amplitude factor; R-factor = normalized least squares residual. To determine change in Tc-Tc CN and Tc-Fe CN their σ² were fixed to 0.003 and 0.006, respectively. The amp (S₀²) for the O shell was fixed. * Denotes fixed parameter, † denotes linear multiple scattering path and their R and σ² values were calculated as multiples of the single scattering path parameters. ▲Confidence level (%) that the improvements in the fits with the ‘Tc-Fe’ shell are significantly different compared to without it, using the F-Test (Downward et al., 2007). Errors are presented in parentheses and are 1 standard deviation of last decimal.
to $0.7 \pm 0.3$ in the third reduction consistent with shortening of the TcO$_2$ chain with repeated cycling. In addition, they show the samples fit best with an increase in the proportion of Tc-Fe bonding at $\sim 2.6$ Å (Table 4.1) as repeated redox cycles occur, and that there is an overall improvement in goodness of fit compared to the TcO$_2$ chain model alone (Table 4.1 and Appendix 1, Figure SII-C). This confirms the samples can be consistently modelled with a relative decrease in the TcO$_2$ chain length, coupled with a proportional increase in Tc-Fe bonding via attachment to surface Fe-O octahedra throughout progressive redox cycles. Finally, statistical analyses for the different fits (F-Test, Downward et al., 2007) show that the confidence in adding this ‘short Fe’ shell increases with the number of oxidation events (Appendix 1, Table SII-B), improving confidence in our model that there is an increase in the role of the edge-sharing FeO as the Tc chain length shortens during repeated redox cycling. Interestingly, the molecular scale changes in Tc(IV) binding observed in these systems directly influence the bulk Tc behaviour in the sediments. For example, it has been postulated that a decrease in oxidative remobilisation of Tc(IV) occurs when TcO$_2$ chains are bound with edge-sharing Fe-O, as the Fe-O “cap” is less susceptible to oxidative remobilisation than the Tc-O ends of the chain and, thus, the binding to Fe-O protects the Tc(IV) from reoxidation (Fredrickson et al., 2009). In summary, repeated redox cycling produces a more robust, shorter TcO$_2$ chain that is bonded to Fe-O in sediments. In turn, and in agreement with the sequential extraction data, this speciation is directly linked to the increased recalcitrance of sediment-associated Tc(IV) over repeated redox cycles.

### 4.3.4 Technetium Fate in the Aqueous Phase

In recent literature, the presence of an aqueous Tc(IV) phase under reducing conditions has been reported, with evidence suggesting that Tc is mobilised as a
colloidal phase (Zakir et al., 2005; Plymale et al., 2011; Lee et al., 2014) or possibly, as Tc(IV) carbonate complexes (Wildung et al., 2000). This is of particular interest as it could represent a previously unrecognised route for the migration of Tc(IV) colloids in reducing aquifers. To examine this, ultrafiltration on the separated (14,400 g) aqueous phase from experiments was used to assess the presence of Tc colloids in these systems. In the lower concentration microcosms (0.5 µM starting concentration of Tc) after an oxidation event, practically all of the Tc in solution (98.2 ± 5.5 %) passed through a 10 kD (~ 3 nm) filter. In contrast, after a reduction event, 94.5 ± 3.0 % passed through a 200 nm filter but only 65.9 ± 0.0 % through a 10 kD filter, suggesting that ~1/3 of the Tc is present as a colloidal fraction of between ~3 - 200 nm, consistent with past work which has proposed elevated Tc(IV) solubility over the theoretical solubility threshold for hydrous TcO₂ (Law et al., 2010; McBeth et al., 2011; Plymale et al., 2011; Lee et al., 2014). The remaining ~2/3 (~30 nM Tc) is presumably present as soluble or complexed Tc(IV), or particles with a size of <3 nm. 

To further investigate these colloidal phases, a sample from an elevated concentration bioreducing experiment, containing ~ 1 ppm Tc (separated at 14,400 g), was analysed using both TEM (with a small aliquot of the separated sample dropped onto a TEM grid) and XAS (by analysis of the separated liquid sample). The TEM data showed clear evidence for Tc colloids in the separated sample with Tc clearly showing associations with Fe-rich aggregates of 20-100 nm diameter (Figure 4.4). Further inspection of the TEM images showed the aggregates were comprised of 5-10 nm nanoparticles and analysis of d-spacing and SAED confirmed their identity as magnetite (Figure 4.4). XANES data was obtained for these very low concentration XAS samples (Figure 4.3). Here, the XANES for the reduced sample was clearly Tc(IV) like in character and linear combination fitting between Tc(IV) and Tc(VII)
end-member spectra confirmed Tc(IV) dominated the sample (S.I.). For comparison, the same solution after oxidation clearly resembled Tc(VII) (Figure 4.3). The clear confirmation by ultra-filtration, TEM, and XAS of colloidal, Fe oxide associated Tc(IV) in the reduced solution phases from these experiments has significant implications for the mobility of Tc(IV) under reducing conditions. Indeed, the significantly enhanced solubility of colloidal Tc(IV) in these experiments compared to that expected for Tc(IV)O₂ (as high as ~33 µM in our higher activity experiments compared to an expected solubility of 10 nM; Meyer et al., 1991) highlights the clear

Figure 4.4: TEM images of particulates present in solution after a reduction period, (panels A and B); selected areas with lattice spacings of 2.6 Å are signified by white circles; blue circles correspond to the EDX spectra (panel D); SAED of particle in panel B, light blue lines show measured D-spacings (Å) of the diffraction profile.
need for further work on these phases to define their stability, persistence and importance in environmental systems.

4.3.5 Microbial Community Change During Redox Cycles

16S rRNA gene amplicon pyrosequencing was used to assess the microbial community response to repeated redox cycles, in both microcosms. Analysis was possible in the low Tc concentration experiments for the starting sediment and at the endpoints of each reduction and oxidation event (Figure 4.1; days = 0, 119, 179, 229, 321, 372, 428). Overall, the results show a diverse microbial community in all sequenced samples (Appendix 1, Table SI1-E), with the majority of the sequences related to Betaproteobacteria, Alphaproteobacteria, Acidobacteria and Bacteroidetes (Appendix 1, Figure SI1-D). Although many of the sequences were related to common soil bacterial genera or families, analysis also detected sequences related to known Fe(III)- and Tc(VII)-reducing genera, most notably *Geobacter* (Lloyd et al., 2000) and *Anaeromyxobacter* (Marshall et al., 2009) (Appendix 1, Table SI1-D). Interestingly, in both microcosms, certain bacteria showed preference for oxidising or reducing conditions. For example, *Clostridia* (which are known obligate anaerobes) were more prevalent after each reduction event and less prevalent after every oxidation event (Appendix 1, Table SI1-D). Similarities between samples based on microbial community composition were explored further using non-metric multidimensional scaling (NMDS; Clarke, 1993; Clarke and Gorley, 2006). The NMDS ordination (Appendix 1, Figure SI1-E) showed that although the microbial communities in the two microcosms diverged over time, common trends could be observed between the microcosms as the redox cycling regime occurred. Both microbial communities remained relatively similar to the initial (sediment) community during the first incubation under anaerobic conditions, but the first and the
second oxidation events caused major microbial community shifts (Fig. S2). After the second oxidation event, the communities seemed to stabilise. These observations show that oxidation events had a more profound effect on microbial community structure than reduction events, possibly due to the conditions becoming unsuitable for obligate anaerobic bacteria.

4.3.6 Summary

Technetium mobility is directly influenced by its oxidation state, with high predicted mobility as Tc(VII) under oxidising conditions, therefore, it is clear that long-term immobilisation of Tc is crucial in environmental remediation and radioactive waste management. Here, we have shown for the first time that during repeated redox cycling (of the type that will be relevant to Tc behaviour in the surface critical zone and in the subsurface over geological timescales) Tc(IV) becomes increasingly strongly bound to sediments. Spectroscopic analysis confirmed a direct correlation between Tc(IV) recalcitrance to reoxidation and molecular scale changes in Tc(IV) speciation. Repeated redox cycling prompted a shortening of TcO₂ chains and increase in associations with Fe-O edge sites in the sediments; at the same time, chemical extractions confirmed an increase in Tc(IV) strongly associated with the sediments with successive redox cycles. This confirms that the recalcitrance of solid phase associated Tc(IV) increases with repeated redox cycles and that currently there may be an overestimation of the influence of oxidation events on Tc mobility. By contrast, analysis of the separated aqueous phase from bioreduction cycles showed a significantly enhanced Tc solubility compared to that predicted from thermodynamic calculations. Using multiple approaches, the presence of a Tc(IV) colloidal phase in the bioreduced systems has been demonstrated. Such findings have significant and, as yet, largely unexplored implications for Tc solubility and mobility in the environment.
Overall, this highlights a complex picture for Tc(IV) solubility during redox cycling with a tendency for solid phase associated Tc(IV) to become increasingly strongly bound to sediments, yet with a small but significant fraction of the Tc occurring as mobile Tc(IV) colloidal phases in these sediment systems.

4.4 Materials and Methods

4.4.1 Safety

$^{99}$Technetium is a $\beta$-emitting radionuclide (half life $2.13 \times 10^5$ years; $E_{\text{max}} = 294$ keV). The possession and use of radioactive materials is subject to statutory controls.

4.4.2 Sediment Collection

Sediment was collected from next to the Calder River, Cumbria (Latitude 54°26′30 N, Longitude 03°28′09 W) in November 2013. The collected material is representative of the Quaternary unconsolidated alluvial floodplain deposits that underlie the Sellafield Nuclear Licenced site. After collection, the sediment was stored in a sealed sterile plastic container at 10 °C in the dark until use.

4.4.3 Microcosm Preparation

Using aseptic technique, duplicate large volume microcosms were prepared with sediment (100 ± 1 g) and a regional synthetic groundwater (1 L ± 1 ml) in 2 L bottles (Schott, Duran); the headspace was flushed with Ar gas and sealed with an air-tight stopper. Prior to microcosm setup, the groundwater had been autoclaved, flushed with Ar gas, and adjusted to pH ~ 7.4. Thereafter, 10 mM acetate was added as an electron donor and 0.5 µM (30 Bq ml$^{-1}$) ammonium pertechnetate (CERCA, France) was spiked into the experiment. Microcosms were incubated in the dark at 21 °C with
periodic sampling. Additionally, control microcosms were prepared in triplicate and sterilised by autoclaving prior to the addition of \( \text{TcO}_4^- \).

### 4.4.4 Geochemical Monitoring

Sediment slurry was sampled at each time point and the solids / supernatant were separated by centrifugation at 1.44 x 10^4 g. To assess the extent of Fe(III)-reduction, the ‘bioavailable’ Fe(II/III) ratio in the sediment was determined by extraction of sediment pellets with 0.5 M HCl 1 hour (Lovley and Phillips, 1986). The resultant supernatant Fe(II/III) ratio was measured using the ferrozine assay (Viollier et al., 2000). Total aqueous \(^{99}\text{Tc}\) concentrations were measured using liquid scintillation counting with appropriate standards and background subtraction such that the detection limit was ~0.01 Bq ml\(^{-1}\) (Wallac Quantulus 1220). The microcosms were also sampled at regular intervals for pH using calibrated electrodes and a Metler Toledo digital meter. UV-vis spectroscopy methods were used to measure total aqueous \( \text{NO}_2^- \) (Harris and Mortimer, 2002), Mn (Brewer and Spencer, 1971) and Fe (Stookey, 1970; Viollier et al., 2000) on a Shimadzu UV-1800 UV spectrometer. Throughout, calibrations had typical regressions of \( R^2 > 0.99 \). Samples were also analysed for aqueous acetate, \( \text{SO}_4^{2-} \) and \( \text{NO}_3^- \) using a Dionex DX120 ion chromatograph.

### 4.4.5 Redox Cycling Experiments

After initial reduction (signified by the 0.5 M HCl-extractable Fe(II/III) ratio reaching \(~ 100 \%)\), all microcosms were oxidised with air (with \( \text{O}_2 \) as the oxidant). Here, the headspace was flushed regularly (every 1-3 days) with 1 L of gas mix passed through a 0.45 \( \mu \text{m} \) filter (80 : 20 air : \( \text{CO}_2 \) mix), to maintain a neutral pH. This continued until the 0.5 M HCl-extractable Fe(II/III) ratio in the sediment stabilised. At this point, acetate (5 mM) was added as an electron donor to stimulate anaerobic metabolism.
(e.g. Fe(III) reduction) and the headspace was flushed with Ar. Bottles were then incubated until the 0.5 M HCl-extractable Fe(II/III) ratio was stable at between 95 – 100 %. In total, three full reduction / oxidation events occurred during experimentation.

4.4.6 Acid Extraction of Sediment Samples

Acid extractions were performed on select sediment samples to assess the relationship between iron and technetium in redox-cycled sediments. Here, sediments were sequentially leached with increased molarity HCl for 30 minutes (unless stated), in the order: 0.001, 0.01, 0.1, 1, 2, 3, 4, and 6 M, 6 M for 24 hours, 6 M for 4 days and, finally, in heated aqua regia for 4 hours (method modified from Doornbusch et al., (2015)). The lixivants were analysed for $^{99}$Tc and Fe using liquid scintillation counting and the ferrozine method, respectively.

4.4.7 Ultrafiltration of Groundwater Samples

Ultrafiltration on microcosm fluids during the experiment (days 428 and 592) was performed using Nanosep® Centrifugal Filters (Pall Corp., USA) to explore the colloidal / aqueous distribution of Tc in separated solutions (14,400 g). Two filter sizes were used; 0.2 µm and 10 kD, the latter, having a geometric particle size of ~3 nm for a globular particle (Dreissig et al., 2011). The filters were pre-equilibrated with sample prior to use. Technetium concentrations in the filtrate were measured using liquid scintillation counting.

4.4.8 X-ray Absorption Spectroscopy

Higher activity parallel samples (2.5 g sediment in 25 ml groundwater) were incubated for 222 days at the elevated concentrations required for X-ray Absorption Spectroscopy (XAS) in order to measure $^{99}$Tc speciation in sediments during redox cycling (~1000 ppm Tc on solids). Sediment was sampled at the end of reduction
(days = 266, 325, 380) and oxidation periods (days = 310 and 372). For oxidation time-points, samples were rinsed with deoxygenated water, under anaerobic conditions prior to analysis (Fredrickson et al., 2009). X-ray Absorption Near Edge Structure (XANES) and X-ray Absorption Fine Structure (EXAFS) spectra were collected on the beamline B18 of the Diamond Light Source from the $^{99}$Tc K-edge (21050.47 eV) in fluorescence mode at 78 °K. Spectra were collected with either a 9 or 36 element Ge detector. XANES data underwent linear combination fitting (LCF) between Tc(IV) and Tc(VII) standards to provide an indication of the percentage distribution of Tc(IV) and Tc(VII) in the samples (see Appendix 1, Table SI1-A). EXAFS data were fitted using Athena and Artemis software packages and using relevant standards and information from the literature (Ravel and Newville, 2005). The statistical viability of additional shells was assessed using the F-test (Downward et al., 2007; Marshall et al., 2014). To determine the trend in the Tc-Tc coordination number, the fitting procedure was constrained to fixed values for the passive electron reduction factor ($S_0^2$) and Debye-Waller factor ($\sigma^2$) (Table 4.1).

4.4.9 Transmission Electron Microscopy

Transmission electron microscope (TEM) grids were prepared under anaerobic conditions with the groundwater from high activity microcosms after the final reduction period. Prior to preparation, the groundwater was centrifuged at $1.44 \times 10^4$ and a small sample of the supernatant was then pipetted on the TEM grid, again under anaerobic conditions. Images were taken using a FEI Tecnai TF20 field emission gun TEM and the particle composition on the TEM grids was analysed with an Oxford Instruments INCA 350 EDX.
4.4.10 16S rRNA Gene Amplicon Pyrosequencing and Data Analysis

DNA from both microcosms was isolated from ~ 0.2 g of sample from: the starting sediment (day 0), sediments after reduction events (days = 119, 229, 372,), and sediment after oxidation events (days = 179, 321, 428) using the MoBio PowerSoil™ DNA Isolation Kit (MoBio Laboratories Inc., USA). PCR for amplicon pyrosequencing was performed using tagged fusion bacterial primers 27F (Lane, 1991) and 338R (Hamady et al., 2008), as described previously (Williamson et al., 2013).

The pyrosequencing run was performed at The University of Manchester sequencing facility, using a Roche 454 Life Sciences GS Junior system. The 454 pyrosequencing reads were analysed using Qiime 1.8.0 release (Caporaso et al., 2010), and de-noising and chimera removal was performed in Qiime during operational taxonomic units (OTU) picking (at 97% sequence similarity) with usearch (Edgar, 2010). Taxonomic classification of all reads was performed in Qiime using the uclust method (Edgar, 2010) against the Greengenes database, release 08/2013 (McDonald et al., 2012).

Nonmetric multidimensional scaling (NMDS) was performed on Primer v6 (PRIMER-E Ltd, Plymouth Marine Laboratory, Plymouth, UK), based on a Bray-Curtis similarity matrix (Clarke, 1993; Clarke and Gorley, 2006), that was calculated using square root transformed relative (%) abundances of all the identified OTUs of this study.

Supporting Information

Supporting information includes supplementary methods, tables and figures for control experiments, geochemical monitoring, alternate EXAFS fits and linear combination fitting, and microbial community data.

Acknowledgments
This work was completed as part of the NERC BIGRAD consortium (NE/H007768/1) and the NERC LO-RISE (Long-lived Radionuclides in Surface Environments; NE/L000547/1) consortium which is part of the NERC RATE program (Radioactivity and the Environment). The RATE programme is co-funded by the Environment Agency and Radioactive Waste Management Ltd. Masters-Waage would also like to acknowledge the UK Engineering and Physical Sciences Research Council for PhD funding. The Diamond Light Source is acknowledged for providing access to B18 (SP10163 and SP10163-2). We also thank Katie Law, Alistair Bewsher, Thomas Neill, and Steve Parry for analytical assistance.

4.5 References


Marshall, T. A.; Morris, K.; Law, G. T. W.; Mosselmans, J. F. W.; Bots, P.; Parry, S.


5. The Influence of Iron Biogeochemistry on Technetium Mobility During Redox Cycling

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5.1 Abstract

Technetium poses an environmental threat due to its radiotoxicity, long half-life and high subsurface mobility as Tc(VII)O$_4^-$\textsuperscript{-}. It is predicted that the mobility of Tc will be greatly influenced by Fe redox cycling, whereby Fe(II) species can facilitate Tc(VII)O$_4^-$ reduction to Tc(IV) and removal from solution. Furthermore, research thus far has shown Fe may play an important role in protecting these Tc(IV) products from reoxidation and mobilisation. In this research, we investigate the influence that varying bioavailable Fe concentrations have on Tc mobility during reduction and oxidation. In microcosm experiments, we demonstrate that elevated Fe concentrations, in the form of ferrihydrite, increase Tc removal from solution during reduction and increase its recalcitrance to remobilisation during reoxidation. The fate of Tc over multiple redox cycles in high Fe microcosms is investigated, revealing greatly reduced Tc remobilisation compared to previous studies without added Fe. Additionally, in higher activity experiments, X-ray Absorption Spectroscopy (XAS) elucidates the potential importance of Tc-Fe bonding in the immobilisation of Tc(IV) species. Finally, we demonstrate the multiple redox cycling of Tc during the reduction of ferrihydrite by Geobacter sulfurreducens and oxidation by air, the products of which reveal, for the first time, the biotically driven incorporation of Tc into magnetite.

5.2 Introduction

Technetium is a high-yield, long-lived ($t_{1/2} = 2.1 \times 10^5$) fission product formed in nuclear reactors. It is of particular environmental concern as it is a significant risk driving contaminant at nuclear sites worldwide and is abundant in radioactive wastes destined for geological disposal (Riley and Zachara, 1992; Lloyd and Renshaw,
2005). Under oxidising conditions, Tc has high mobility in natural and engineered environments as the pertechnetate ion (TcO$_4^-$), a weakly sorbing and readily soluble species, whereas, under reducing conditions, poorly soluble Tc(IV) is formed (Wildung et al., 2000; Burke et al., 2005; Corkhill et al., 2013). To date, a number of mechanisms for Tc(VII) reduction have been observed. Direct enzymatic reduction of Tc(VII) can occur in axenic culture experiments with model metal reducing species (Shewanella putrefaciens and Geobacter metallireducens; Lloyd and Macaskie, 1996; Wildung et al., 2000). However, in soils and sediments, enzymatic reduction is not kinetically feasible at the typical environmental concentrations of Tc (Lloyd et al., 2000). Instead, Tc(VII) reduction in these environments seems to be dominated by abiotic reduction with Fe(II) developed during Fe(III)- reduction. Indeed, Tc(VII) reduction in many environments is dominated by a heterogeneous reaction with adsorbed Fe(II) or with biogenically or abiotically formed Fe(II) bearing minerals (Cui and Eriksen, 1996; Lloyd et al., 2000; Fredrickson et al., 2004; Burke et al., 2005, 2006; Law et al., 2010; Lear et al., 2010). Although the homogenous reduction of Tc by aqueous Fe(II) has been shown, it is kinetically hindered compared to Fe(II) sorbed onto or incorporated within mineral phases and therefore, Fe(II) bearing solid phases are the primary reaction centres for Tc(VII) reduction (Zachara et al., 2007; Peretyazhko et al., 2009). Overall, Tc(IV) phases exhibit poorer subsurface mobility (Kunze et al., 1996; Fredrickson et al., 2004; Burke et al., 2005) and much research has been dedicated toward understanding the mechanisms of Tc(VII) reduction and Tc(IV) stability under both reducing and oxidising conditions to define Tc environmental behaviour and potentially to exploit these reactions for waste treatment technologies (Masters-Waage et al., 2016; Thorpe et al., 2016).
The products of Tc(VII) reduction have been explored using X-ray absorption spectroscopy (XAS). In sediment microcosm systems, the removal of Tc from solution has occurred concomitantly with Fe(III)-reduction (Fredrickson et al., 2004; Burke et al., 2005; McBeth et al., 2007; Law et al., 2010), often forming TcO$_2$-like nanoparticles or chains in the sediment (Burke et al., 2005; McBeth et al., 2007; Zachara et al., 2007; Morris et al., 2008; Fredrickson et al., 2009; Law et al., 2010), additionally, a recent study has observed evidence for the formation of sulfide phases over extended reducing periods (Lee et al., 2014). Furthermore, Tc(IV) has been found to be incorporated into magnetite (Marshall et al., 2014) and goethite (Um et al., 2011, 2012), amongst other minerals, such as rutile and tin dioxide, and materials including cements and alloys (Luksic et al., 2015), and reduced TcS$_2$-like phases have been described during Tc coprecipitation with FeS (Wharton et al., 2000).

Understanding the long-term stability of Tc(IV) phases is important as reoxidation and remobilisation as Tc(VII) may pose problems for the safe disposal of Tc(IV) contaminated materials. Indeed, with technetium’s 2.1 x $10^5$-year half-life, it is highly probable that environmental conditions will change and fluctuating redox conditions must be considered in the context of the potential for oxidative remobilisation of Tc (Burke et al., 2006; Wu et al., 2007). For technetium, reoxidation leads to some remobilisation, but in many cases the Tc(IV) reduction products show significant resistance to remobilisation during oxidation events when air or nitrate are used as oxidants (Burke et al., 2006; Fredrickson et al., 2009; Geissler et al., 2011; Um et al., 2011; Marshall et al., 2014; Masters-Waage et al., 2016). It has been shown that the recalcitrance of Tc(IV) to remobilisation arises from the formation of stable, sediment / mineral associated, Tc(IV) phases (Burke et al., 2006; McBeth et al., 2007; Jaisi et al., 2009; Marshall et al., 2014). Interestingly, evidence for the association of hydrous
TcO$_2$ chains with Fe-oxides has been observed in several studies (Zachara et al., 2007; Morris et al., 2008; Fredrickson et al., 2009; Geissler et al., 2011; McBeth et al., 2011; Heald et al., 2012; Liu et al., 2012; Brookshaw et al., 2015). Here, the “capping” of hydrous TcO$_2$ chains by edge-sharing with Fe-oxides has been postulated to increase the durability of Tc(IV) against reoxidation in Fe-rich clay systems (Fredrickson et al., 2009). Additionally, resistance to remobilisation could arise from mechanisms where the Tc(IV) becomes physically protected from reoxidation by a veneer of Fe(III)-oxidation products (Zachara et al., 2007; Morris et al., 2008; Marshall et al., 2014) or possibly by residual Fe(II) that can buffer Tc(IV) from reoxidation (Jaisi et al., 2009). Irrespective of the mechanism, it is clear that Fe plays an intimate role in both the reduction Tc(VII) and the susceptibility of Tc(VII) to remobilisation. Reflecting this, we examine Tc and Fe interactions during reduction and oxidation events. Specifically, we present data detailing Tc reoxidation behaviour in sediment systems with varying concentrations of bioavailable Fe. Overall, we demonstrate how increasing bioavailable Fe(III) in sediments prior to bioreduction increases the recalcitrance of Tc(IV) to oxidative remobilisation. Finally, we report, for the first time, the incorporation of Tc(IV) into biologically precipitated magnetite produced during reduction with *Geobacter sulfurreducens*. These observations have profound implications for Tc behaviour in natural and engineered environments where Fe is present at elevated concentrations and suggest potential new routes to optimising biological reduction processes to retain and incorporate Tc(IV) into bioreduced mineral systems.
5.3 Materials and Methods

5.3.1 Safety

$^{99}$Tc is a $\beta$-emitting radionuclide ($E_{\text{max}} = 294$ keV, $t_{1/2} = 2.13 \times 10^5$ years). The possession and use of $^{99}$Tc is subject to statutory controls.

5.3.2 Sediment Collection

Quaternary alluvial floodplain sediments representative of the Sellafield subsurface (Law et al., 2010) were collected from the near surface (~ 30 cm depth) near the Calder River, Cumbria, in 2013 (Latitude; 54°26′30 N, Longitude; 03°28′09 W). On collection, samples were stored in sterile bags and refrigerated (10 °C), then used within 1 month of collection.

5.3.2 Microcosm Preparation

Aseptic technique was used to prepare triplicate microcosms with 10 ± 0.1 g of sediment and 100 mL ± 1 ml synthetic groundwater (Wilkins et al., 2007). In order to explore the influence of Fe on Tc behaviour in these systems, microcosms were supplemented with varying concentrations of Fe(III) (0, 2, 5, and 10 mM) in the form of ferrihydrite gel (Schwertmann and Cornell, 2000). The microcosms were then amended with 10 mM sodium acetate to promote bioreduction and spiked with 0.5 µM (30 Bq ml$^{-1}$) ammonium pertechnetate (CERCA, France). Finally, the microcosm headspace was flushed with Ar gas and sealed. Additionally, to examine multiple redox cycling in high Fe environments, larger microcosms were prepared with 100 ± 1 g of sediment and 1 L ± 1 ml synthetic groundwater, these systems were supplemented with 20 mM Fe(III) as ferrihydrite and sealed with an airtight stopper and ported cap. All experiments were incubated in the dark at 21 °C and sampled periodically.
In order to examine the fate of Tc in a model mineral system where biologically precipitated magnetite dominated after reduction, a pure culture experiment was prepared. The experiment contained 20 mM ferrihydrite in 100 ml of synthetic groundwater (SO$_4^{2-}$ and NO$_3^-$ were omitted in this case) which was spiked with 5 µM TcO$_4^-$ which we calculated would yield solids of high enough Tc concentration that XAS analysis would be possible. This system was then reduced by flushing the headspace with Ar and inoculating with a pure culture of *G. sulfurreducens* with 10 mM acetate as the electron donor (Cutting et al., 2009). For reoxidation, the bottle was gently agitated and periodically bubbled with an 80 : 20 air : CO$_2$ gas mix to maintain the pH at ~ 7. Samples were collected at the 1$^{st}$ reduction, 1$^{st}$ oxidation, 2$^{nd}$ reduction, and 2$^{nd}$ oxidation time points, analysed for Fe(II)/Fe(total) and aqueous Tc, and anaerobically frozen for XAS analysis (see below).

5.3.3 Sampling

Sediment slurry was removed from microcosms at regular intervals. The solid was separated from solution via centrifugation (1.44 x 10$^4$ g). Technetium solution concentrations were measured using liquid scintillation counting (LSC) of supernatant with appropriate standards and blanks and with a detection limit of ~0.01 Bq ml$^{-1}$ (Wallac Quantulus). The extent of ‘bioavailable’ Fe reduction in microcosms was determined using the ratio of Fe(II)/Fe(total) (Lovley and Phillips, 1988). After extraction in 0.5 N HCl for 1 hour, the Fe(II)/(III) ratio of the extractant solution was determined using the ferrozine method (Viollier et al., 2000). Additionally, the pH (calibrated Metler Teledo probe and meter), total aqueous Fe (ferrozine method Viollier et al., 2000), and aqueous acetate, NO$_3^-$, and SO$_4^{2-}$ were analysed at regular time points using ion chromatography (Dionex DX120 ion chromatograph).
5.3.4 Reoxidation and Redox Cycling

The smaller (100 ml) microcosms supplemented with 0, 2, 5, and 10 mM Fe(III) were reoxidised after 242 days of incubation. During reoxidation, samples were gently agitated and regularly flushed (every 1-3 days) with filtered gas (80 : 20 air : CO₂ mix) with samples taken at regular time intervals.

The large microcosm experiment (1 L, supplemented with 10 mM Fe(III)) was repeatedly cycled between reducing and oxidising conditions. During reduction, the experiments were incubated until either the 0.5 M HCl-extractable Fe(II)/(total) ratio was > 95 % or a stable and elevated Fe(II)/Fe(III) ratio was observed. During oxidation events, endpoints were selected when either all the 0.5 M HCl-extractable Fe(II) had been reoxidised (a 0.5 N HCl-extractable Fe(II)/Fe(total) ratio of 0) or a stable and low Fe(II)/Fe(total) ratio was observed. In this redox cycling experiment, to promote re-reduction after the reoxidation period, acetate (5 mM) was added to the microcosms and the headspace flushed with Ar gas.

A higher activity, parallel 2 mM Fe(III) supplemented sample was prepared (2.5 g sediment in 25 ml synthetic groundwater) and spiked with elevated TcO₄⁻ to yield several hundred ppm on sediment for analysis (~0.4 mM Tc). The experiment then underwent one redox cycle from oxic to reducing. It was then reoxidised and solid samples were taken at the end of reduction (222 days) and reoxidation (393 days) for XAS analysis.

5.3.5 Acid Extraction of Sediment Samples

To further examine the relationship between Fe and Tc in the sediment systems, selected sediment samples (from the smaller (100 ml) microcosms supplemented with 0, 2, 5, and 10 mM Fe(III)) were acid extracted. The biocycled sediments (0.5 – 1 g) were exposed to 10 ml of increasing strength HCl (for 30 minutes, unless stated), in
order each sediment sample was sequentially exposed to: 0.001, 0.01, 0.1, 1, 2, 3, 4, 6 M HCl, 6 M HCl 24 hour, 6 M HCl 4 days and, finally, heated aqua regia 4 hours (method modified from Doornbusch et al., 2015). After each extraction step, the supernatant (separated at 1.44 x 10^4 g) was analysed for both Fe and Tc using the ferrozine method and liquid scintillation counting.

5.3.6 X-ray Absorption Spectroscopy (XAS)
Tc-99 K-edge (21050 eV) XAS spectra were collected from selected samples at the Diamond Light Source, beamline B18. X-ray Absorption Near Edge Structure (XANES) and X-ray Absorption Fine Structure (EXAFS) spectra were collected in fluorescence mode at 78o K on a 36 element Ge detector. Sediment samples were rinsed with deoxygenated water prior to analysis (Fredrickson et al., 2009). Data were reduced and modelled using software packages Athena and Artemis (Ravel and Newville, 2005), and the statistical viability each modelled shell was assessed using the F-test method (Downward et al., 2007).

5.4 Results and Discussion
We explored the influence of initial, bioavailable Fe(III) concentration on Tc biogeochemistry during reduction and reoxidation. Initially, the microcosms were bioreduced with acetate as the electron donor and with added Fe(III) (as ferrihydrite) at 0, 2, 5, 10 and 20 mM for 242 days by which time aqueous NO_3^- concentrations were below detection, robust Fe(III)-reduction had developed in all systems (>75 % acid extractable Fe(II)/Fe(total), Figure 5.1), and SO_4^{2-} removal from solution had occurred (Appendix 2, Figure SI2-B). At this point, the aqueous Tc concentrations varied between microcosms, but there was a clear trend for increasing Tc removal from solution with increasing initial Fe(III) concentration. For example, in the 0 mM
added Fe(III), 8.2 ± 2.3 % Tc remained after bioreduction whilst in the 10 mM Fe(III) experiments, 1.3 ± 0.2 % of the aqueous Tc remained in solution, and in the 20 mM Fe(III) systems Tc concentrations were below the limit of detection (<1 %) after bioreduction (Figure 5.1 and 5.3).

The microcosms were then reoxidised with air to explore the rates of Fe(II) oxidation and Tc remobilisation (Figure 5.1). After 122 days of reoxidation, the 0.5 N HCl-extractable Fe(II)/(total) ratio fell to between 17.2 ± 0.6 % and 8.6 ± 1.3 % in all samples, with greater Fe(II) reoxidation seen in sediments with higher initial Fe(III) (Figure 5.1). Indeed for the 0, 2, 5 and 10 mM systems technetium reoxidation clearly trended downwards with 21.3 ± 2.0 %, 15.9 ± 1.0 %, 6.6 ± 1.5 % and 10.0 ± 2.5 % Tc remobilised during the reoxidation cycle, respectively. This trend was also maintained

Figure 5.1: Sediment reoxidation data. Panel A: % total Tc in solution. Panel B: % ‘bioavailable’ Fe(II) in sediments (0.5 N HCl-extractable; Lovley and Phillips, 1986). Sediment samples: blue squares = 0 mM added ferrihydrite, green diamonds = 2 mM added ferrihydrite, orange triangles = 5 mM added ferrihydrite, red circles = 10 mM added ferrihydrite, magenta inverted triangles = large microcosms with 20 mM added ferrihydrite. Error bars are 1 standard deviation of triplicates, where not shown they are within the symbol size.
in the 20 mM large microcosms where only 7.2 ± 3.8 % Tc was reoxidised during the first reoxidation cycle over a similar amount of time (105 days, Figure 5.1). A relationship between Tc stability on reoxidation and Fe in sediment systems has been observed in previous studies and is likely due to its armouring / veneering by oxidation products, incorporation, and / or changes in iron mineralogy as ferrihydrite concentrations change (Zachara et al., 2007; Morris et al., 2008; Fredrickson et al., 2009; Heald et al., 2012; Liu et al., 2012; Brookshaw et al., 2015; Dippon et al., 2015).

In all experiments, SO₄²⁻ levels in solution increased during reoxidation suggesting that the reoxidation of sulfide bearing minerals was occurring. Past work has suggested that over shorter timescales, sulfidation does not significantly impact on Tc speciation and that hydrous TcO₂ chains are the dominant reduction products (Burke et al., 2006). In this study, varying amounts of solid-associated Tc was released back into solution concurrent with both sulfide and Fe(II) reoxidation. Overall, Tc shows greater durability against oxidative remobilisation in sediments with higher initial bioavailable Fe(III) concentrations. Above 5 mM initial Fe(III) concentrations (~0.1 % sediment weight) reoxidation experiments showed a similar retention of Tc associated with the solid phase, suggesting a threshold response to this effect. Further, sediments supplemented with ferrihydrite were unable to fully retain Tc during oxidation events. Instead, a fraction of Tc always associated with phase(s) susceptible to oxidation as significant remobilisation (>6 %) of Tc occurred in all samples. It is possible that this fraction is loosely sorbed to sediment / Fe surfaces during reduction and is therefore easily targetable to oxidation or that it is bound to the non-attached end of a TcO₂ chain, previously stipulated to be easier to reoxidise (Fredrickson et al., 2009; Masters-Waage et al., 2016).
Subsequently, the behaviour of the 20 mM Fe(III) supplemented system was assessed over multiple redox cycles. As previously stated, in these larger volume systems, aqueous Tc was removed during Fe(III) reduction (days 65 to 91) as expected, and at 216 days, the Fe(II)/Fe(total) was 97.3 ± 4.4 % and aqueous Tc concentrations were below the limit of detection (Figure 5.2). Subsequently, the microcosms were reoxidised with air for 105 days which resulted in Fe(II) oxidation and remobilisation of Tc to solution. The amount of Tc remobilisation in these high iron systems varied (7.2 ± 3.8 %) but, in all cases, was significantly lower than that observed in other experiments in this study and than values for oxidative remobilisation of Tc from sediments in other studies (Burke et al., 2006; McBeth et al., 2007; Masters-Waage et al., 2016).

After this initial redox cycle, the microcosms were re-reduced with 5 mM acetate. Fe(III) reduction quickly re-established (Figure 5.2) with the extent of Fe(III) reduction

![Figure 5.2](image-url)  

Figure 5.2: % total Tc in solution (blue) and % ‘bioavailable’ Fe(II) in sediments (0.5 N HCl-extractable; Lovley and Phillips, 1986) (red), for triplicate bottles (Bottle A: squares Bottle B: triangles, Bottle C: circles). Unshaded areas denote ‘Reduction periods’. Areas shaded light blue denote ‘oxidation periods’.

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reduction less than in the first reduction cycle with only $77.9 \pm 3.0 \%$ Fe(II)/Fe(total) after 136 days of reduction (day 457). Aqueous Tc was once again removed from solution to concentrations below the limit of detection. At this point, a second reoxidation cycle was initiated for 150 days by which time the Fe(II)/Fe(total) ratio had dropped to $17.4 \pm 5.6 \%$ (Figure 5.2). During this second cycle, only a small fraction of Tc was remobilised to solution $7.3 \pm 3.8 \%$ (day 607, Figure 5.2), as in the first reoxidation experiment. Overall, the initial addition of Fe(III) resulted in a reduction in Tc solubility over multiple redox cycles to below the limit of detection during reduction and, on reoxidation, remobilisation was less than that seen in similar experiments without added Fe (Masters-Waage et al., 2016).

To further investigate the relationship between Tc and Fe in the samples, an acid extraction (Doornbusch et al., 2015) was performed in triplicate on oxidised sediments from the 0, 2, 5 and 10 mM Fe(III) supplemented experiments (Figure 5.3). Acid leaching was performed on 121 day reoxidation samples with acid extractable Fe(II)/Fe(Total) < 17 % in all samples. In the 0.001 M HCl extraction, the low Fe(III) supplemented systems (0 and 2 mM) showed more Tc leaching (~ 5 – 6 %) than the higher Fe(III) experiments (5 and 10 mM; ~ 2.5 %, Figure 5.3). After the initial “easily extractable” leach, the stronger acid leaching phase (up to 6M HCl for 30 minutes) showed broadly similar behaviour in all systems with a gradient > 1 when Tc vs Fe was plotted and confirming that during this stage, ~50 % of Tc is extracted when only ~18 % of Fe is extracted. This suggests a relative enhancement of Tc leachability compared to Fe in these extractions (Figure 5.3), as seen in pure mineral systems (Marshall et al., 2014). Finally, the very strong extended time acid leaches (6 M 24 h, 6M 4 days) showed different behaviour between the treatments with the 0 and 2 mM Fe(III) supplemented systems showing significantly less leaching of Tc.
throughout this stage compared to the 5 and 10 mM Fe(III) supplemented systems, where $>\sim 93\%$ of Tc was leached. The enhanced durability of the Tc to strong acid leaching in the low Fe systems was confirmed with the strongly oxidising aqua regia leach stage showing the release of $>27\%$ Tc from the 0 mM systems compared to $<8\%$ from the 5 and 10 mM experiments. Interestingly, throughout the extractions, the total Fe leached is consistent across the 0, 2, 5 and 10 mM Fe(III) supplemented systems suggesting a broadly consistent Fe mineralogy in the biocycled experiments but with a dissimilar Tc response to strong acid leaching between Fe and Tc.

Overall, the earlier (less aggressive) extractions showed a subtle trend whereby Tc

Figure 5.3: Total leached Tc versus Fe from sediment. Different sediments are labelled: blue squares = 0 mM added ferrihydrite, green diamonds = 2 mM added ferrihydrite, orange triangles = 5 mM added ferrihydrite, and red circles = 10 mM added ferrihydrite. Extraction steps from left to right = 0.001 M to 0.01 M, 0.1 M, 1 M, 2 M, 3 M, 4 M and, 6 M HCl, with the final three extractions 6 M HCl for 24 hours, 6 M HCl for 4 days, and heated in aqua regia for 4 hours. Inset graph shows enlarged region from 0 – 40 % on the y-axis and 0 - 15 % on the x-axis. Error bars are 1 standard deviation of triplicates, where not shown they are within the symbol size.
was more recalcitrant to dissolution in systems with higher Fe concentrations (Figure 5.3), as seen with oxidative remobilisation (Figure 5.1). Counter to this, Tc became more easily extractable in the most aggressive leaches in systems with higher Fe concentrations (Figure 5.3). One possible explanation is that in the high-Fe systems Tc associates with the products of ferrihydrite reduction that, although not more easily targeted by reoxidation (Figure 5.1), are more easily dissolved by acid. Instead, when no or little added Fe is present, this fraction of Tc potentially bonds to structural Fe phases in the sediment (Fredrickson et al., 2009), which are harder to extract.

A pure culture multiple redox cycling experiment targeted at the bioreduction of ferrihydrite to magnetite was undertaken to assess Fe and Tc interactions in a simplified, microbially active system. Initially, ferrihydrite was incubated with *Geobacter sulfurreducens* and acetate as electron donors to precipitate magnetite (Cutting et al., 2009). After 44 days of incubation, all of the added Tc had been removed from solution and the ferrihydrite had altered to form a magnetic, dark brown mineral, presumably magnetite and consistent with chemical extraction data for the sample (1M HCl, 24 hours) where the Fe(II)/Fe(total) ratio was 31 % (Appendix 2, Table SI2-A). This material was then reoxidised in air for 14 days, resulting in Fe(II) reoxidation (3 % Fe(II)/Fe(total)) and the remobilisation of a significant fraction of Tc to solution (23 %). After which, re-reduction was stimulated by the addition of acetate (10 mM) and incubation for 19 days. Fe(III) reduction occurred as a result of re-reduction, forming a mineral phase with an Fe(II)/Fe(total) ratio of 21 %, less than that seen in the initial reduction. Interestingly, despite Fe(III)-reducting conditions, Tc removal from solution was incomplete, with 6 % of the added Tc remaining soluble. Although no analysis of this phase was undertaken, we have previously documented the occurrence of a mobile Tc phase during re-reduction.
in sediment systems, presenting evidence to suggest that Tc(IV) is mobilised via association with magnetite colloids (Masters-Waage et al., 2016). Finally, re-oxidation in air was performed over 23 days, again leading to Fe(II) oxidation (to 8 % Fe(II)/Fe(total) ratio) and Tc remobilisation (to 13 %), but to a lesser extent than seen in the first oxidation (Appendix 2, Table SI2-A), in line with previous research results in sediment systems (Masters-Waage et al., 2016). Analysis of the XANES spectra for all of these end-point samples showed Tc was present as Tc(IV) in all reduce or reoxidised samples, confirming reductive precipitation (Figure 5.4). The XANES for all the samples also clearly displayed a distinct double peak in the near edge of the spectra which is absent in both the TcO₂ standard and the sediment samples supplemented with 2 mM Fe(III) (Figure 5.4). This feature has previously been identified in systems with Tc and magnetite (Heald et al., 2012; Kobayashi et al., 2013; Marshall et al., 2014). The Fourier transform of the Tc EXAFS revealed a

![Figure 5.4: Technetium-99 k-edge XAS data in reduced and oxidised sediment with 2 mM added ferrihydrite, and in redox cycled ferrihydrite from the first reduction, first oxidation, second reduction, and second oxidation. Panels: A; Non-phase shifted Fourier transform of k³-weighted EXAFS spectra calculated over a k range of 3.5 to 12.5 Å⁻¹, with fit (dashed lines), B; k³-weighted EXAFS spectra with fit (dashed lines), and C; Tc K-edge XANES spectra with reference spectra samples.](image-url)
complex coordination environment for Tc with at least three shells of backscatterers. On comparison with the literature, it was clear these spectra showed characteristic features from two previously published models for Tc coordination in environmental systems. The first feature at ~ 2.5 - 2.6 Å was consistent with a Tc backscatterer in a short chain hydrous TeO\textsubscript{2}-like coordination environment (Lukens et al., 2002; Morris et al., 2008; Fredrickson et al., 2009) and similar to the sediment spectra presented in this study (Figure 5.4). The second feature at between ~3.0 – 3.5 Å was distinct from ‘TeO\textsubscript{2}-like chain’ systems and, instead, consistent with EXAFS data for Tc(IV) incorporated in magnetite, specifically with Tc located in an octahedral coordination environment (Kobayashi et al., 2013; Marshall et al., 2014; Smith et al., 2016).

We investigated these phases further using two approaches. Firstly, we used linear combination fitting of the EXAFS data (Boland et al., 2013) and TeO\textsubscript{2} chain (from this study, Figure 5.4) / Tc(IV) incorporated to magnetite (from Marshall et al., 2014) standards to assess the suitability of a mixed coordination environment and the relative proportion of each fraction in the samples. Here, all of the spectra could be

<table>
<thead>
<tr>
<th>sample</th>
<th>path Tc-</th>
<th>R (Å)</th>
<th>CN</th>
<th>$\sigma^2$ (Å\textsuperscript{2})</th>
<th>$\Delta E^0$ (eV)</th>
<th>$S_0^2$</th>
<th>R-factor</th>
<th>conf.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced</td>
<td>O</td>
<td>2.01 (1)</td>
<td>6*</td>
<td>0.006 (0)</td>
<td>5.52</td>
<td>0.8*</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tc</td>
<td>2.55 (1)</td>
<td>1*</td>
<td>0.003 (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>2.65 (2)</td>
<td>1*</td>
<td>0.011 (4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-Tc-O\textsuperscript{ms}</td>
<td>3.97 (2)</td>
<td>6*</td>
<td>0.007 (1)</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Oxidised</td>
<td>O</td>
<td>2.00 (1)</td>
<td>6*</td>
<td>0.005 (0)</td>
<td>5.39</td>
<td>0.8*</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tc</td>
<td>2.55 (1)</td>
<td>1*</td>
<td>0.002 (1)</td>
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<td></td>
<td></td>
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<td></td>
<td>Fe</td>
<td>2.61 (3)</td>
<td>1*</td>
<td>0.010 (5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-Tc-O\textsuperscript{ms}</td>
<td>3.96 (2)</td>
<td>6*</td>
<td>0.009 (1)</td>
<td></td>
<td></td>
<td></td>
<td>99.2</td>
</tr>
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Table 5.1: Fit parameters for reduced and oxidised sediments with added ferrihydrite (2 mM). R = atomic distance CN = coordination number; $\sigma^2$ = Debye-Waller factor; $\Delta E^0$ (eV) = energy shift from Fermi level; $S_0^2$ = amplitude factor; R-factor = normalized least squares residual. * Denotes fixed parameter, \textsuperscript{ms} denotes linear multiple scattering path. R and $\sigma^2$ values for multiple scattering paths were calculated as multiples of the single scattering path parameters. conf. = confidence level (%) that statistical confidence in added shell calculated using using the F-Test (Downward et al., 2007).
modelled with a \( \sim 35 - 45 \% \) contribution from Tc(IV) incorporated into the mineral phases suggesting a common pool of incorporated Tc(IV) in the bioreduced and biocycled samples (Appendix 2, Table SI2-C). As a second approach, the EXAFS spectra for each sample were fitted between the two coordination environment, hydrous TcO2 chains with ‘short Fe’ interactions (Zachara et al., 2007; Morris et al., 2008; Fredrickson et al., 2009; Brookshaw et al., 2015) and Tc(IV) incorporated into magnetite (Marshall et al., 2014). Modelling performed in Artemis (Ravel and Newville 2005) calculated the best fit for the respective spectra by allowing the proportion of each end member spectrum (short chain TcO2 or incorporated Tc(IV)) to vary between 0 (100 \% Tc incorporated into magnetite) and 1 (100 \% short TcO2 chains) (Appendix 2, Table SI2-C). To constrain the model to reasonable fits, the amplitude factor (\( S_0^2 \)) for all samples was fixed to 1 and the Debye-Waller factor (\( \sigma^2 \)) of the ‘short-Fe’ interaction to 0.01. The best fits for the data were remarkably consistent both with the linear combination analyses and across all samples with a \( \sim 30 \% \) contribution from Tc(IV) incorporated into the redox cycled biomagnetite phases. In summary, we present clear XANES and EXAFS data confirming for the first time that partial incorporation of Tc(IV) can occur in biologically precipitated magnetite and that the incorporated Tc(IV) is resilient across several redox cycles (Figure 5.4 and Appendix 2, Table SI2-A).

Further experiments were undertaken at higher concentrations (suitable for XAS analysis) to investigate the impact of elevated Fe concentrations on the oxidation state and local coordination of Tc during redox cycling in sediment. Reduced and reoxidised samples from a microcosm amended with 2 mM ferrihydrite were analysed by XAS. The XANES spectra confirmed Tc(IV) dominated both the reduction and reoxidation samples (Figure 5.4). EXAFS spectra collected from both samples
showed similarities to spectra of bioreduced Tc(VII) amended sediments as reported in previous studies and in recent work from our group on the sediments used in this study (Morris et al., 2008; Peretyazhko et al., 2012; Masters-Waage et al., 2016). For the two samples studied here, the EXAFS data were initially fitted with a model representative of short hydrous TcO$_2$-chains (Lukens et al., 2002) which has been used as the foundation for fitting the Tc(IV) coordination environment in many environmental systems (Zachara et al., 2007; Morris et al., 2008; Fredrickson et al., 2009; Brookshaw et al., 2015). Fitting both reduced and reoxidised spectra with a Tc-O bond length of ~2.0 Å and coordination number of 6 and a Tc-Tc bond length of ~2.6 Å and coordination number of 1 provided a reasonable fit (Appendix 2, Table SI2-B). It was also apparent that in environmental systems there is growing evidence for a “short Fe” component in many short chain hydrous Tc(IV) spectra where attachment of hydrous TcO$_2$-like chains to surface Fe-O octahedra have been implicated (Zachara et al., 2007; Morris et al., 2008; Fredrickson et al., 2009; Geissler et al., 2011; McBeth et al., 2011; Heald et al., 2012; Liu et al., 2012; Brookshaw et al., 2015). Hence, the EXAFS were refitted to include a varying number of Tc atoms at ~2.6 Å and an Fe backscatterer at ~2.7 Å, with a fixed coordination number of 0.5, realistic of TcO$_2$-like chains tethered to FeO sites (Brookshaw et al., 2015). Refitting with this model improved the fit statistically in both samples suggesting that Fe interactions with short chain hydrous TcO$_2$ were present in both reduced and oxidised samples (Figure 5.4 and Table 5.1). In a separate study, we were able to analyse Tc reduced onto the same sediment without added Fe (Masters-Waage et al., 2016). In this sample, the coordination of Tc was found to be higher after reduction (coordination number = 1.4 ± 0.2) than that with 2 mM added Fe, presented here (coordination number = 0.8 ± 0.4, Table 1). A smaller Tc coordination number
represents shorter TcO$_2$ chains and is proportionally representative of an increase in Tc-Fe interactions over Tc-Tc, which has previously been reported to increase the resistance of Tc to oxidative mobilisation (Fredrickson et al., 2009; Masters-Waage et al., 2016). It is possible that this molecular scale change in Tc coordination causes the decrease in remobilisation seen during oxidation in systems with elevated Fe concentrations (Figures 5.1 and 5.3).

**Environmental Implications.** In this study, we have explored the influence of elevated Fe concentrations on Tc behaviour during redox cycling. In sediments with elevated bioavailable Fe concentrations, bioreduction resulted in Tc removal to concentrations below the limit of detection. This was in contrast to systems supplemented with no, and low (2mM) Fe(III), where a significant fraction of Tc remained soluble. Additionally, systems with high levels of Fe (> 5 mM) showed suppressed Tc(IV) solubility on reoxidation of sediments. Direct speciation analysis using XAS showed clear evidence that in Fe-enriched sediments Tc(IV) was speciated as short chain hydrous TcO$_2$ tethered to Fe-O octahedra. Thus, in high Fe systems, we hypothesise that an increased proportion of the Tc(IV) is “capped” via Fe-O octahedra and that this increases the durability of the Tc(IV) to oxidative remobilisation. Finally, we present unequivocal evidence that during Fe(III)-reduction mediated by the pure culture microorganism *Geobacter sulfurreducens*, Tc(VII) is partially reductively incorporated into the biomagnetite reduction product. This is the first time that biologically mediated incorporation of Tc(IV) into Fe(II)/Fe(III) bearing iron oxides has been reported under ambient environmental conditions. Immobilisation of Tc(IV) as a result of incorporation of Tc(IV) into key environmentally relevant minerals via biologically mediated pathways opens up new routes for the treatment of Tc contamination in natural and engineered environments. Overall, it is clear that a better
understanding of Tc-Fe interactions in the subsurface is vital to understanding Tc’s behaviour over geological timescales and that, when it comes to Tc, sediments with high Fe concentrations may provide a more suitable biogeochemical barrier to migration.
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Thorpe, C. L.; Lloyd, J. R.; Law, G. T. W.; Williams, H. A. J.; Atherton, N.; Cruickshank, J. H.; Morris, K. Retention of 99m Tc at Ultra-Trace Levels in Flowing


6. The Biogeochemical Redox Cycling of Neptunium

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6.1 Abstract

Neptunium is one of the most problematic constituents of radioactive waste due to its long half-life and high radiotoxicity. Environmentally, Np solubility is determined by its valence state and under oxic conditions displays high mobility as the neptunyl ion. However, previous work has demonstrated Np(V) reduction in sediment microcosm systems, resulting in the effective immobilisation of Np as poorly soluble Np(IV) phases, which show greatly reduced solubility. These phases have also shown some resistance to reoxidation. In this study, we describe microcosm experiments that, for the first time, extend over multiple microbially mediated redox cycles in sediment relevant to the UK nuclear legacy. We found that once reduced, Np remained immobilised throughout multiple oxidation and reduction cycles. Furthermore, Np was more effectively removed from solution in systems amended with reactive Fe(III). Chemical extraction revealed Np was associated with more readily exactable Fe-phases, with subtly increased recalcitrance to extraction developing with increased redox cycling. Finally, X-ray absorption spectroscopy revealed Np(V) reduction to Np(IV) in the sediment and, in keeping with previous literature, showed evidence for Np-Fe interactions. Overall we highlight some of the complexities of Np biogeochemistry and provide novel understanding of its long-term fate as Np(IV) species over multiple microbially mediated redox cycles, which will undoubtedly affect the environmental behaviour of Np over the durations relevant to its long half-life.

6.2 Introduction

Neptunium-237 is an alpha-emitting radionuclide and is considered to be one of the most problematic constituents of nuclear wastes (Kaszuba and Runde, 1999). It forms via the decay of Pu-241 ($t_{1/2} = 14.9$ years) and Am-241 ($t_{1/2} = 432.7$ years), and will
thus grow into radioactive wastes destined for geological disposal (Yoshida et al., 2006; Runde and Goff, 2011). Neptunium is also present as a subsurface contaminant at a range of nuclear sites (Morris et al., 2000; Cantrell, 2009; Marshall et al., 2014) and poses environmental problems due to its long half-life ($t_{1/2} = 2.4 \times 10^6$ years) and high potential mobility under oxic subsurface conditions where Np(V) dominates speciation, forming the highly soluble Np(V)O$_2^+$ ion (solubility up to $10^{-4}$, Choppin, 2007) and carbonate complexes (Nitsche et al., 1989; Dozol and Hagemann, 1993; Kaszuba and Runde, 1999; Choppin, 2007; Wu et al., 2009). Sorption reactions may however be important for Np(V) and will play an important site specific role in the control of Np mobility in the subsurface. For example, Np(V) uptake onto goethite and hematite (e.g., Combes et al., 1992; Arai et al., 2007; Müller et al., 2015), cells (e.g., Gorman-Lewis et al., 2005, 2013; Deo et al., 2010; Ams et al., 2013), and sediment (Law et al., 2010; Williamson et al., 2015) have all been documented. Furthermore, recent literature has noted the potential for the incorporation of Np(V) into Fe mineral lattices (hematite and goethite) during their crystallisation under high pH conditions (Bots et al., 2016). Overall, Np solubility in aqueous environmental systems is predominantly controlled by redox chemistry, similar to uranium (Choppin, 2007). Indeed, under reducing conditions it is predicted that Np(IV) forms (Kaszuba and Runde, 1999), which has much lower solubility (e.g., Np(OH)$_4$: 3-20 nM, Nakayama et al., 1996; Neck and Kim, 2001) and forms poorly soluble oxide and hydroxides that readily interact with surfaces (Wu et al., 2009; Law et al., 2010; Brookshaw et al., 2012; Thorpe et al., 2015). For this reason, reducing conditions are considered favourable for the immobilisation of Np and other radionuclides in the environment (Behrends et al., 2012; Rizoulis et al., 2012; Newsome et al., 2013).
To date, Np(V) reduction has been demonstrated to be mediated enzymatically, by select microbes in axenic culture (Lloyd et al., 2000; Icopini et al., 2007; Cherkouk et al., 2015) and in an anaerobic consortium (Rittmann et al., 2002). However, Np(V) reduction can be complex. For example, common metal-reducer *Geobacter sulfurreducens* was not capable of the enzymatic reduction of NpO$_2^{2+}$ at circumneutral pH, despite readily reducing chemically analogous UO$_2^{2+}$ via disproportionation to U(V), and perhaps reflecting the stability of Np(V) (Renshaw et al., 2005). The relatively facile reduction of Np(V) to Np(IV) under environmental conditions has also been demonstrated in natural sediments at ~pH 7 as a result of bioreduction, when supplemented with electron donor (Law et al., 2010; Thorpe et al., 2015), and in high pH conditions (Williamson et al., 2015). Further, these studies have shown that Np reduction occurs alongside the bioreduction of Mn(IV) and Fe(III), and that sterilised Fe(III)-reduced sediments are also capable of facilitating Np(V) reduction abiotically, likely through reaction with biogenic reduction products such as Mn(II) or Fe(II) (Law et al., 2010; Thorpe et al., 2015). Recent studies have also noted similar abiotic reduction of Np(V), in particular, with Fe minerals, including magnetite (Fe$_3$O$_4$) (Nakata et al., 2002, 2004), titanomagnetite (Wylie et al., 2016), mackinawite (FeS) (Moyes et al., 2002), and green rust (Fe(OH)$_2$) (Christiansen et al., 2011; Bach et al., 2014) but, additionally, with biogenically reduced micaceous phyllosilicates (Brookshaw et al., 2015).

Whilst bioreduction offers a mechanism to limit Np(V) solubility in solution, Np mobility over longer timescales will be influenced by the stability of the Np(IV)$_{(s)}$ reductive products to oxidative remobilisation (Law et al., 2010; Christiansen et al., 2011). Indeed, for U(IV)$_{(s)}$, oxidation can cause significant remobilisation into groundwater (e.g., Hee et al., 2007; Komlos et al., 2008) whilst Tc(IV) shows partial
resistance to remobilisation with air and nitrate (e.g., Burke et al., 2006; McBeth et al., 2007). At present there is a paucity of work on the stability of Np(IV) phases to oxidative remobilisation due to the high radiotoxicity of Np but with evidence for significant recalcitrance of Np(IV) to oxidative remobilisation. For example, Christiansen et al., (2011) found that extensive air reoxidation of Np(IV) associated with Green Rust only resulted in ~ 50% remobilisation of the available Np. Furthermore, in sediment systems, Law et al., (2010) demonstrated that reoxidation promoted by air and nitrate amendment resulted in less than 20 % and 10 % of Np remobilisation to solution, respectively, despite >90 % reoxidation of labile Fe(II). Clearly, there is a limited knowledge of Np redox chemistry under environmental conditions and no studies to date explore multiple redox cycling behaviour despite the longevity of Np as a contaminant in both natural and engineered environments. Furthermore, it is expected that Np will interact significantly differently to Tc in previous experiments (Masters-Waage et al., 2016) due to their varied valence states and atomic radii, resulting in different hydrolysis / reduction products and sediment sorption and binding sites. Here, we investigate the behaviour of Np during multiple redox cycles in long-term sediment microcosm studies, using sediments representative of a nuclear licenced site, Sellafield, acetate as an electron donor, and air as an oxidant. Further, in select experiments, ferrihydrite was added to supplement the systems with elevated Fe(III) as metal reduction has been implicated in Np redox cycling (Law et al., 2010; Thorpe et al., 2015), and as Fe(III)-reduction will be crucial in both shallow and deep subsurface environments (Lloyd, 2003; Behrends et al., 2012). Importantly, this work shows resistance of Np(IV) to oxidative remobilisation once bioreduction takes place and confirms that elevated Fe concentrations reduce Np mobility on redox cycling.
6.3 Materials and Methods

6.3.1 Safety

$^{237}$Np is an alpha-emitting radionuclide with beta/gamma-emitting daughter isotopes and its use is subject to statutory controls. The handling of radioactive materials requires trained personnel following risk assessment.

6.3.2 Sediment Collection

The sediment for this study was collected from alluvial floodplain deposits next to the river Calder, ~ 2 km from the Sellafield Ltd. Nuclear site. The materials are representative of the unconsolidated quaternary sediments that underlie this site (Law et al., 2010). On collection, the sediment was homogenised and sealed in plastic bags at stored at 10 °C until use (typically within 1 month of collection).

6.3.3 Low Activity Np Redox Cycling Experiments

Microcosms were prepared in triplicate with synthetic groundwater (Wilkins et al., 2006) and Sellafield sediment in a 1: 10 sediment to solution ratio. Additionally, parallel Fe(III) supplemented systems were also set up with addition of 5 mM ferrihydrite to the sediment systems. Prior to experimentation, the groundwater was sterilised (autoclaved at 120 °C for 1 hour) and sparged with 80 : 20 N$_2$ : CO$_2$ to give a final pH ~7. The experimental systems were spiked at a concentration of 1.7 µM (10 Bq mL$^{-1}$) $^{237}$Np(V)O$_2^+$ and, finally, amended with 10 mM sodium acetate prior to incubation. Microcosm bottles were sealed from the air and incubated in the dark at 20 °C, with periodic sampling using aseptic technique. Groundwater was separated from sample slurry via centrifugation (14400 g) and tested for aqueous CH$_3$OO$^-$, NO$_2^-$, NO$_3^-$, and SO$_4^{2-}$ using ion chromatography (Dionex ICS5000). Further, groundwater Fe concentrations were measured using the ferrozine method (Viollier et al., 2000)
and Np concentration was measured with liquid scintillation counting (Wallac Quantulus, limit of detection $\sim 0.1 \text{ Bq mL}^{-1}$). The groundwater pH was measured regularly with a calibrated pH probe (Metler Teledo). An extraction in 0.5 M HCl for 1 hour, was performed on a sediment pellet with the ferrozine method to estimate the bioavailable Fe(II)/Fe(total) in the sediments (Lovley and Phillips, 1987). The microcosms were then taken through two complete cycles of reduction and oxidation. For both reoxidation periods, the microcosms were frequently (typically every 2 days) bubbled with air and CO$_2$ in an 80 : 20 mix. To promote the second period of reduction, the microcosms were flushed with Ar and additional electron donor (5 mM acetate) was added to the system.

6.3.4 Acid Extraction

An extraction was performed on the sediment samples from both oxidation period end points to determine the leachability of Np relative to Fe (Doornbusch et al., 2015). The sediments were sequentially exposed to HCl of increasing molarity (in order, $10^{-3}$, $10^{-2}$, $10^{-1}$, 1, 2, 3, 4, and 6 M) for 30 minutes. Finally, sediments were exposed to 6 M HCl for 24 hours and 4 days, and an extraction with gently heated aqua regia (4 hours) was performed. In all extractant solutions, the concentrations of Fe and Np were determined using the ferrozine method and liquid scintillation counting, respectively.

6.3.5 X-ray Absorption Spectroscopy

Smaller microcosms (1 : 10 sediment to solution; 25 mL volume), were prepared at the elevated concentrations of $^{237}$Np(V)O$_2^{+}$ ($\sim 0.2$ mM) suitable for X-ray Absorption Spectroscopy (XAS) both with and without supplemented Fe(III) at 5 mM. Redox cycling processes were promoted in the microcosms as previously described, and sediment pellets were then taken for XAS analysis from the reduced and reoxidised
end-points. Samples were prepared in purpose built Perspex holders prior to X-ray Absorption Spectroscopy analysis at the Np L\textsubscript{III}-edge at room temperature on beamline B18, at the Diamond Light Source, Harwell, UK. Data reduction and analysis was undertaken with Athena and Artemis (FEFF6) software packages (Ravel and Newville, 2005).

6.4 Results and Discussion

6.4.1 Multiple Redox Cycling of Neptunium

Microcosms were prepared with and without supplemented Fe(III) at 5 mM ferrihydrite to investigate the fate of neptunium over two redox cycles at normal, and elevated Fe concentration in sediments. The microcosms were sealed and incubated with added acetate to promote anaerobic microbial processes and spiked with $^{237}$Np(V) at 10 Bq ml\textsuperscript{-1}. During the first day, significant amounts (~70 - 90 %) of Np sorption had occurred in all of the samples, as previously described by other workers and attributed to sorption of NpO$_2^+$ to the sediments (Combes et al., 1992; Nakata et al., 2002; Arai et al., 2007; Law et al., 2010; Müller et al., 2015; Thorpe et al., 2015). As expected, a greater extent of Np(V) sorption occurred in the systems with added Fe(III) (e.g., Girvin et al., 1991; Williamson et al., 2015) (Figure 6.1, Day 1; 11.7 ± 1.1 % remaining in solution vs. 31.1 ± 1.0 %). Np sorption also occurred in the heat-sterilised control experiments (stabilising at ~65 % Np sorption over 500 days, Appendix 3, Figure SI3-A). By 14 days, in both microbially active systems, bioreducing conditions had established, indicated by total porewater removal of nitrate as a result of denitrification (Appendix 3, Figure SI3-B). At this point, in the system not amended with ferrihydrite, Fe(III) reduction was indicated by increased aqueous Fe (Appendix 3, Figure SI3-B) and the appearance of 0.5 N HCl-extractable
Fe(II) in the sediment. Concurrently, the majority of Np was removed from solution, with only 6.4 ± 2.3 % remaining by day 14 (Figure 6.1). Interestingly, after this, a small increase in Np groundwater concentration was seen from days 27 to 35 (from 6.0 ± 1.6 % to 11.7 ± 2.8 %) and after 71 days of reducing conditions 11.1 ± 2.2 % of Np still remained in solution (Figure 6.1). By comparison, in the system

Figure 6.1: % Np of initially added Np in solution (purple) and % Fe(II) in sediments (extracted in 1 hour by 0.5 N HCl, Lovley and Phillips, 1986) (red), for triplicate microcosms. [A] without added Fe and [B] with 5 mM added Fe. Unshaded sections denote ‘reduction periods’, where microcosms were amended with acetate and kept anaerobic. Light blue shaded areas denote ‘oxidation events’, where microcosms were mixed regularly (every 1-3 days) with air. Error bars are 1 standard deviation from triplicate experiments, where error bars are not shown they are within the symbol size.
supplemented with 5 mM ferrihydrite, metal-reducing conditions were slower to develop with maximum Np removal occurring at day 27, at which point aqueous Np values were below the limit of detection (<1 % of added Np). Similar to the microcosms without added ferrihydrite, a small increase in solution Np concentration was then seen, up to 3.2 ± 1.1 % (day 35) and Np concentrations were then essentially stable at 3.4 ± 0.5 % of Np in solution by the end of the reduction period (day 71, Figure 6.1).

Data collected during the first reduction event indicated that systems with high concentrations of reactive, bioavailable Fe can more effectively remove and retain Np during initially oxidising conditions (day 0, through sorption) and after reduction (day 71), as shown previously (Williamson et al., 2015). Furthermore, a subtle remobilisation of Np in both systems (from day 27 to 35) highlights the complexities of Np biogeochemistry. Similar findings have been previously noted in field studies with uranium, under sulfate reducing conditions (Anderson et al., 2003). Indeed, Np remobilisation occurred around the same time as sulfate-reducing conditions established (Appendix 3, Figure SI3-C), however, the mechanisms responsible for these changes remain an unexplained area of interest.

At the end of the reduction period (day 71, Figure 6.1), oxidising conditions were instigated by reoxidation with air. In the systems without added Fe, sediment Fe(III) reoxidation occurred over a 153-day period, eventually, reaching 11.0 ± 0.1 % 0.5 M HCl- extractable Fe(II) by Day 224 (Figure 6.1). Interestingly, there was a subtle decrease in Np solution concentrations despite concurrent sediment Fe(III) reoxidation. Overall, notwithstanding a small increase in solution Np around day 200, the Np ground water concentrations decreased from 11.1 ± 2.2 % to 6.5 ± 1.1 % over the entire oxidation period. In the systems with added Fe, a similar trend in sediment
Fe(II) oxidation was seen, reaching $7.8 \pm 2.2 \%$ 0.5 M HCl-extractable Fe(II) by day 224. However, the changes in solution Np were negligible during oxidation, with $3.4 \pm 0.5 \%$ of the added Np remaining in solution after reduction (Day 71) and $2.1 \pm 0.4 \%$ after oxidation (day 224) (Figure 6.1). This is in broad agreement with previous studies which have observed only limited amounts of Np remobilisation during the air reoxidation in mineral and sediment phases (Law et al., 2010; Christiansen et al., 2011).

Following the first reoxidation cycle, microcosms were amended with 5 mM acetate and reducing conditions re-established. Forty-four days after acetate addition the 0.5 M HCl-extractable Fe(II) had risen to $>95 \%$ in both systems. During this period, solution Np concentrations remained relatively constant at $7.3 \pm 2.3 \%$ of added Np until day 268. Likewise, over the same period, Np solution concentrations in the added Fe microcosms remained within the error of that recorded at the end of the oxidation treatment ($1.4 \pm 0.3 \%$ of added Np by day 268). After 268 days both systems were oxidised for a final time via treatment with air. Here, after 140 days of reoxidation, the 0.5 M HCl-extractable Fe(II) steadied at $27.0 \pm 2.9 \%$ in the non-added Fe system and no variation in aqueous Np occurred when compared to the previous reduction event. Comparatively, acid extractable Fe(II) reached $29.1 \pm 5.5 \%$ in the added Fe system and was concurrent with a small change in Np solution concentration, from $1.4 \pm 0.3 \%$ to $2.5 \pm 0.5 \%$. The oxidation resistant fraction of 0.5 M HCl-extractable Fe(II) could result from the formation of robust and recalcitrant Fe(II) phases as a result of redox cycling and has been previously noted during redox cycling in microcosms with technetium (Masters-Waage et al., 2016). Interestingly, it was found that the development of such Fe(II)-phases was accompanied by the enhanced retention of technetium by solids over multiple redox
cycles, a process not witnessed for Np in this research. Instead, aqueous Np concentrations stay relatively consistent throughout multiple redox cycling.

In summary, aqueous Np concentrations did not significantly change over multiple microbially mediated reduction and oxidation events. This occurs despite prevalent Fe and SO$_4^{2-}$ redox cycling and is contrary to the behaviour of U in reoxidation experiments, which typically see almost complete remobilisation of reduced species on exposure of O$_2$ (Hee et al., 2007; Komlos et al., 2008; Law et al., 2011). Furthermore, we observe that Np mobility was suppressed in comparison to the no added Fe systems in the Fe(III)-supplemented microcosms throughout redox cycling. It is clear that the nature of Np(IV) binding to the solid phases in these systems prevents easy oxidative remobilisation and that Fe(III) supplementation enhances Np retention in sediments (Law et al., 2010). To further investigate any change in the nature of Np sediment association as a result of redox cycles, extractions were performed on sediments from after the first and second oxidation events.

### 6.4.2 Neptunium and Iron Acid Extractions

To explore the relationship between Fe and Np associations during redox cycling in systems with and without supplemented Fe(III), oxidised sediments were sequentially exposed to hydrochloric acid of increasing strength (extraction modified from Doornbusch et al., 2015). These extractions were performed on sediments samples taken at the end of both oxidation cycles (days 244 and 408). Regardless of initial Fe concentrations in the experimental treatment, the extractions showed similar trends in Np vs. Fe dissolution behaviour (Figure 6.2). After the first extraction point ($10^{-3}$ M HCl, 30 minutes), in all samples, significant Np (~10-20 %) was extracted with comparatively small amounts of Fe, suggesting a loosely bound, potentially surface associated, Np fraction. Following this, there was an increase in Np extracted after the
first oxidation cycle (15.6 ± 1.0 %, without added Fe and 19.0 ± 1.2 %, with added Fe) compared to the second oxidation cycle (10.1 ± 2.4 %, without added Fe and 12.6 ± 0.5 %, with added Fe), suggesting marginally stronger Np binding with repeated redox cycles. Across all samples, then Np extraction remained constant until the strong acid, 6 M HCl, 30 minutes treatment (Figure 6.2, inset). After the 6M HCl, 30 min treatment, the majority of Np (~82%) had been extracted for a smaller fraction of extractable Fe (between 17 and 24 %) in all samples. By this point, extractions dissolved a greater fraction of Fe in sediments with added Fe (21.4 ± 0.6 %, first oxidation and 23.9 ± 1.7 %, second oxidation) than in systems without added Fe (16.9 ± 0.9 %, first oxidation and 18.9 ± 1.0 %, second oxidation), presumably resulting from the added ferrihydrite in these systems. Finally, a smaller, more recalcitrant fraction of Np (~18 %) was dissolved during the final three extraction

Figure 6.2: Cumulative % of total Np dissolution versus total Fe dissolution from sediment. Samples are from microcosms with (triangles) and without (circles) initially added Fe from after the 1st oxidation (blue) and 2nd oxidation (red). Sequentially, the [H+] in the 30 minute extraction was increased from 0.001 M to 0.01 M, 0.1 M, 1 M, 2 M, 3 M, 4 M and, 6 M HCl, with the final three extractions 6 M HCl for 24 hours, 6 M HCl for 4 days and heating in aqua regia for 4 hours. Error bars are 1 standard deviation from triplicate experiments, where error bars are not shown they are within the symbol size.
steps (6 M HCl, 24 hours, 6 days, and boiling aqua regia, 4 hours).

Overall, the results suggest that Np is predominantly leachable by relatively shorter and weaker acid extraction (< 6 M, 30 minute extractions) consistent with more readily extractable Fe, potentially associated with Fe surfaces during Np(V) reduction by Fe-minerals (Nakata et al., 2004). In contrast, ~ 20 % of the Np required a longer and more aggressive acid leach (6 M, >24 hour extractions and heated with aqua regia) to solubilise it and was released with the largest fraction of iron. This suggested a robust association of Np with the more crystalline Fe phases in the sediments which require a stronger acid leach than, for example, “bioavailable Fe”, which is operationally defined by many studies (e.g., Masters-Waage et al.; Law et al., 2010) to be extracted with a 0.5 M HCl (Lovley and Phillips, 1987).

Additionally, there are subtle changes in the extractability of bioreduced Np associated with sediments. After one redox cycle Np is more easily extracted than after the second, suggesting marginally increased resilience during repeated redox cycling (Figure 6.2).

6.4.3 Molecular Fate of Neptunium

Higher activity microcosms (0.2 mM Np(V)), with and without added Fe (5 mM), were prepared to investigate the oxidation state and local coordination of Np in the redox cycled sediments, using X-ray Absorption Spectroscopy (XAS). Np L_{III}-edge XANES and EXAFS data were collected in fluorescence mode on samples following a reduction and subsequent reoxidation period (Appendix 3, Figure SI3-D). Comparison of the L_{III}-edge XANES spectra to known standards of Np(IV) and Np(V)O_{2}^{+} (from Hennig et al., 2009) confirms Np(V) reduction to Np(IV) in both systems. The relative proportions of Np(IV) and Np(V) were assessed using the linear combination fitting (LCF) function in ATHENA (Ravel and Newville, 2005). After
136 days of reducing conditions, LCF for both samples (with and without added Fe) showed essentially complete reduction to Np(IV), whereas in the same samples after 147 days of reoxidation, LCF suggested an increased fraction of Np(V) to ~25% Np(V) in the sample (Appendix 3, Table SI3-A). This type of Np(IV) / Np(V) signal has been seen in the bulk EXAFS from similar reoxidation experiments (Law et al., 2010), and indicates that during oxidation partial reoxidation to Np(V) occurs and that a significant fraction of that remains bound to sediment, potentially via sorption or direct bonding. The EXAFS spectra collected were similar across all samples, displaying a significant peak at ~2.3 – 2.4 Å in the phase-corrected Fourier transformed data (Figure 6.3). The best fit for reduced and reoxidised sediment samples, regardless of whether they were from the added Fe system, included 8 O atms at a distance of ~2.35 Å (Table 1) which is consistent with a Np(IV) like coordination environment in Fe(III)- and Mn-reducing sediments (Thorpe et al., 2015) and on the surface of bio-altered chlorite and biotite (Brookshaw et al., 2015). Our

Figure 6.3: L_{III}\text{-edge} Np-237 XAS data for reduced (light blue and red) and oxidised (dark blue and red) sediment samples, with (red) and without (blue) initially added Fe. Panels: a; Phase shifted Fourier transform of \( k^2 \)-weighted EXAFS spectra calculated over a \( k \) range of 2.0 to 8.0 Å\(^{-1} \), with the best-fit model (dotted line), b; \( k^2 \)-weighted EXAFS spectra with the best-fit model, and c; Np XANES spectra for reduced and oxidised sediment and reference spectra samples for Np(IV) (green) and Np(V)O\(_2^+\) (black).
data, however, lack a significant backscattering interaction at \( \sim 3.80 \) Å in the Fourier transform that has previously been attributed to Np-Np interactions (Brookshaw et al., 2015) in nanocrystalline NpO₂ precipitates.

The fits also shared characteristics with Np(IV) reduced in biologically active sediments (Law et al., 2010) and reduced abiotically by Fe(II) within titanomagnetite (Wylie et al., 2016), in which Np/Fe interactions at \( \sim 3.50 \) Å were proposed. In the data presented here, low amplitude interactions were also noted and, thus, fits were modelled to include this Fe shell. In the Fe(III)-reduced samples, the addition of this shell was only statistically viable (F-Test, Downward et al., 2007) in the sediment with added Fe (Table 1), suggesting a preference for Np-Fe coordination during reduction in this system. Furthermore, after the oxidation of both samples in air, the fitting confidence for including this shell increased (Table 1). This suggests that the molecular scale Np-Fe interaction seen in these fits may suggest a Np(IV) species associated with Fe phases, which is recalcitrant to reoxidation. In direct contrast with

<table>
<thead>
<tr>
<th>Sample</th>
<th>Path Np</th>
<th>( R (\text{Å}) )</th>
<th>( \sigma^2 (\text{Å}^2) )</th>
<th>( \Delta E^0 (\text{eV}) )</th>
<th>( S_0^2 )</th>
<th>R-fact.</th>
<th>Conf.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced Np</td>
<td>O</td>
<td>2.34 (1)</td>
<td>0.010 (1)</td>
<td>4.38</td>
<td>1*</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Reduced Np + Fe</td>
<td>Fe</td>
<td>3.39 (4)</td>
<td>0.008 (6)</td>
<td>5.53</td>
<td>1*</td>
<td>0.009</td>
<td>94.4</td>
</tr>
<tr>
<td>Oxidised Np</td>
<td>O</td>
<td>2.35 (2)</td>
<td>0.012 (1)</td>
<td>3.71</td>
<td>1*</td>
<td>0.01</td>
<td>90.7</td>
</tr>
<tr>
<td>Oxidised Np + Fe</td>
<td>Fe</td>
<td>3.4 (5)</td>
<td>0.009 (8)</td>
<td>2.7</td>
<td>1*</td>
<td>0.008</td>
<td>97.6</td>
</tr>
</tbody>
</table>

Table 6.1: Fit parameters for Np EXAFS samples from reduced and oxidised sediments with and without supplemented Fe(III). \( R (\text{Å}) \) = atomic distance CN = coordination number; \( \sigma^2 (\text{Å}^2) \) = Debye-Waller factor; \( \Delta E^0 (\text{eV}) \) = energy shift from Fermi level; \( S_0^2 \) = amplitude factor; R-factor = normalized least squares residual; conf. = statistical confidence in addition of shell (Downward et al., 2007). * Denotes fixed parameter.
previous studies that noted a change in Np-O coordination after reoxidation, to that expected for Np(V) (Law et al., 2010), the data here shows no such coordination change. Counter to this, the XANES signal suggested the presence of Np(V), however, the error typically associated with LCF is relatively large (±15 %) and the Np(V) EXAFS contribution may be within error of the limit of detection for XANES.

6.5 Summary

Overall, we have demonstrated the importance of reduction processes in the context of Np immobilisation in sediments over multiple microbially induced redox cycles relevant to the long-term environmental fate of this long half-life radionuclide. Interestingly, despite repeated redox cycling, Np remained recalcitrant to significant oxidative remobilisation from the sediments. Furthermore, the data show that Fe(III) supplementation in the sediments caused enhanced removal of Np from groundwater during bioreduction suggesting a direct role for Fe biogeochemistry in controlling Np behaviour in these sediments. In agreement with this molecular scale characterisation, using XAS confirmed that Np-Fe interactions could be modelled in various reaction products and suggests a new route to reduce Np mobilisation in the subsurface. These results stress the significance of Fe(III)-reducing conditions and Fe-rich environments in controlling Np mobility in complex environmental systems and show the durability of Np-sediment associations over multiple redox cycles, a positive message for control of Np contamination in many environments. This work highlights the controlling influence of microbially mediated redox cycling on the behaviour and fate of Np and provides a platform for further investigation into the long-term management of transuranic contamination and wastes.
6.6 References


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7. Conclusions

7.1 Summary and Conclusions
This thesis has explored the impact of redox cycling on $^{99}$Tc and Np in order to further understand their long-term environmental fate. In this chapter, the hypotheses detailed in Chapter 2 are revisited and conclusions are made, alongside discussion of future areas of work.

In Chapter 4, the multiple redox cycling of $^{99}$Tc was investigated in microcosm systems. Here, during three sequential redox cycles (reduction via acetate addition and oxidation via air ($O_2$) addition) $^{99}$Tc became more recalcitrant to oxidative remobilisation, a previously undiscovered process. After a first redox cycle $\sim$45 % of $^{99}$Tc was remobilised as a result of oxidation, compared to only $\sim$15 % in the third and final reoxidation. These findings were supported by chemical extraction data that revealed that $^{99}$Tc was less readily extractable from sediments that had undergone multiple redox cycles. A number of possible controlling factors for this were presented. For example, it is possible that $^{99}$Tc becomes physically protected from oxidants via association with Fe oxidation products, for example, neo forming Fe(III)-oxyhydroxides. Alternatively, the presence of residual Fe(II) in the sediment may buffer Tc(IV) reoxidation. Regardless, EXAFS evidence collected from sediment systems reacted with higher concentrations of $^{99}$Tc revealed changes in $^{99}$Tc behaviour at the molecular scale that may explain the increased recalcitrance. Here, best-fit models of the EXAFS revealed Tc-Fe bonding at the expense of Tc-Tc bonds over repeated cycles. Previously, it has been suggested that Tc-Fe bonds are harder to break than the Tc-Tc bonds present in TcO$_2$-like chains, leading to a more recalcitrant $^{99}$Tc / sediment phase. This chapter also documented a problematic increase in $^{99}$Tc
mobility during reduction periods and here XANES and TEM data revealed that the $^{99}$Tc was present as Tc(IV) that was likely associated with magnetite colloids.

Building on the data presented in Chapter 4, Chapter 5 investigated the influence of increased bioavailable Fe (added as ferrihydrite) concentrations on $^{99}$Tc redox cycling. Here, bioreduction of the Fe-amended sediment resulted in the enhanced retention of $^{99}$Tc in the solid phase over multiple redox cycles. Technetium solubility was greatly reduced in comparison to that seen in Chapter 4, being removed to below the limit of detection during reduction periods and only a small (~7 %) fraction being mobilised as a result of reoxidation. The modelling of EXAFS data revealed more prominent Tc-Fe bonding which had been suggested as a factor that increased Tc(IV) recalcitrance towards oxidative remobilisation in Chapter 4. Finally, redox cycling experiments with ferrihydrite revealed that common Fe-reducing bacteria Geobacter sulfurreducens were capable of the biologically driven incorporation of Tc(IV) within magnetite. This novel discovery presents a new route for the long-term immobilisation of $^{99}$Tc in a phase that shows stability over multiple redox cycling.

In Chapter 6, Np redox cycling was investigated in similar systems to those presented in Chapters 4 and 5. Here, it is demonstrated that Np solubility and speciation, once changed under bioreducing conditions, does not significantly alter over subsequent redox cycles. Furthermore, in systems amended with added ferrihydrite, increased uptake of Np onto the solid phase was observed during initial sorption, reduction, and throughout oxidation. X-ray Absorption Spectroscopy performed on analogue experiments revealed the reduction of Np(V) to Np(IV) and that Np was likely coordinated to the sediment surface through Np-Fe bonds. This highlights the potential benefits for Fe rich subsurface environments when one considers mitigation / management of Np in the environment.
Revisiting the thesis hypothesises, **Hypothesis 1** stated that: *multiple redox cycling will decrease overall \(^{99}\)Tc and Np solubility in nuclear site sediments treated with electron donors.* For \(^{99}\)Tc, this hypothesis was proven to be true. In Chapters 4 and 5, decreased \(^{99}\)Tc solubility was apparent as a result of multiple redox cycling. It is however worth noting, that aqueous Tc(IV) solubility did increase during reduction periods but this was likely due to Tc(IV) association of magnetite colloids. In comparison, the same result was not observed in microcosms containing Np. Instead, in Chapter 6, it was found that once Np(V) is reduced to Np(IV), no significant remobilisation or change in the solubility of Np occurs. **Hypothesis 2** then stated that: *radionuclide fate will vary from that previously observed in single reduction and oxidation cycles if further redox cycles occur.* For \(^{99}\)Tc, this hypothesis holds. For example, the solubility of technetium changed over multiple redox cycles, as detailed in Chapter 2. Additionally, redox cycling directly influenced the bonding of \(^{99}\)Tc to sediments on a molecular scale. EXAFS performed on the sediments suggested the development of shorter TcO\(_2\)-chains and an increased influence of surface binding to Fe as a result of redox cycling. Subtle changes in the recalcitrance of Np were noted in acid extraction and spectroscopy experiments in Chapter 6. Even though changes in Np solubility were not observed, it is possible that over longer timescales they would have a greater impact. Furthermore, previous ‘single cycle’ studies have noted limited remobilisation of Np, but never to the extent presented in this research. Highlighting the importance of reduction processes on the immobilisation of Np.

**Hypothesis 3** stated that: *changes in bioavailable Fe\(_{(s)}\) concentrations in complex sediments will have a key influence in determining radionuclide solubility.* This hypothesis holds for both \(^{99}\)Tc and Np. For \(^{99}\)Tc, in chapter 4, EXAFS data collected throughout the redox cycles revealed a key link between Fe and \(^{99}\)Tc, with \(^{99}\)Tc
directly binding to Fe surfaces at the expense of shortening TcO$_{2(S)}$ chain length. Further, in solution, XANES and TEM analysis revealed that Tc(IV) was associated with magnetite colloids. Then in Chapter 5, when sediment Fe concentrations were increased through the addition of ferrihydrite, $^{99}$Tc concentrations in solution after bioreduction and subsequent oxidation were significantly reduced. For example, after the first reoxidation period approximately six times less $^{99}$Tc was remobilised in the systems with supplemented Fe(III) (~7 % vs. ~45 %). Further, EXAFS revealed an increased significance for Tc-Fe bonding in the sediment. The addition of bioavailable Fe(III) had the same effect on experiments conducted with Np. Here, in Fe(III) supplemented systems, Np reduction resulted in the removal of Np to below detectable limits, whereas systems without added Fe saw only ~90 % removal. Subsequently, Np concentrations in the Fe(III) supplemented systems remained below or near the limit of detection for the entirety of two redox cycles. Furthermore, EXAFS conducted on this system revealed the potential for Np-Fe bonding in reduced sediments with elevated concentrations of Fe. In summary, there is evidence that increased bioavailable Fe alters the mobility and molecular binding of both Np and $^{99}$Tc.

Finally, **Hypothesis 4** stated that: *single mineral / microbe experiments will show how $^{99}$Tc recalcitrance to remobilisation arises during multiple redox cycles.* This hypothesis has been partially proven to be true. In Chapter 5, pure culture experiments were conducted with ferrihydrite supplied as a TEA and acetate as a TED, and with $^{99}$Tc added to solution. Here, after bioreduction, EXAFS revealed the partial incorporation of Tc(IV) into magnetite (~30-40 %) with the remaining fraction fitted as reduced TcO$_2$-like chains, tethered at one end to an FeO octahedron. This was different to Tc(IV) products in sediment reduction, which only showed evidence for
the latter coordination environment, even in systems with supplemented Fe(III). Like in sediment systems, this end product showed increased resistance to reoxidation over multiple redox cycles. Although only subtle changes were discernable over multiple redox cycles, the mechanism of $^{99}$Tc immobilisation supported the findings (in Chapter 4) that showed Tc-Fe bonds promoted greater recalcitrance than Tc-Tc bonds and further suggested that structurally incorporated $^{99}$Tc showed the greatest recalcitrance of all.

This thesis has important implications for the management of contaminated land and geological disposal of radioactive wastes. It has revealed a number of processes that will have both positive and negative effects on the fate of redox-active radionuclides and their safe containment at contaminated sites. For example, the revelation that Tc mobility reduces over multiple redox cycles presents a positive process that will sequester environmental Tc over the long-term and, thus, suggests that long-term Tc remobilisation may have been previously over-predicted. There is also evidence to suggest that the reoxidation of reduced sediments does not influence Np mobility, highlighting the important role that bioreduction / bioremediation could play in radionuclide immobilisation at contaminated sites. Furthermore, evidence presented in this thesis reveals the benefits a high-Fe environment can have on preventing Np and $^{99}$Tc mobilisation, a factor that should be considered in the selection of an appropriate geological disposal site. For instance, this thesis used sediment representative of the Sellafield region, which was capable of retaining significant Tc(IV) on reoxidation, however sites with even higher concentrations of reactive Fe will likely see even greater Tc(IV) recalcitrance. Discouragingly, however, $^{99}$Tc mobility via association with colloids presents a previously unidentified route for contamination, which requires consideration when predicting its environmental movement. Overall, by
taking into account the changeability of the environment over geological timescales, this thesis has identified new and important processes that will help to predict the extent of radionuclide contaminations and aid in mitigating their risks.

7.2 Directions for Future Research

The experiments presented in this thesis are the first of their kind to investigate the influences of multiple redox cycling under realistic environmental conditions. Therefore, an obvious extension of this work is to conduct similar experiments using other long-lived, redox-active radionuclides, for example, uranium, plutonium, and iodide. This research would provide a basic understanding for how multiple redox cycles influence the most problematic long-lived contaminants in nuclear waste.

All experiments in this thesis were completed in batch microcosms and ultimately such work can only provide a limited understanding of ‘real world’ processes. Thus, it is suggested that there is a need for multiple redox cycling experiments in more complex, realistic scenarios. This may include column experiments or field studies. Furthermore, such studies should be conducted over a wide range of conditions, for example, with varying pH, electron donor type and concentration, in sediments with varying Fe content, and in the presence of co-contaminants (e.g., nitrate, EDTA etc.).

The experiments in this thesis were conducted for nearly 2 years, providing some of the longest continually monitored microcosm data to date. Changes seen over these timescales warrant even longer experiments, which may elucidate slower processes that control radionuclide fate. It is suggested that decadal reoxidation experiments are undertaken for both $^{99}$Tc and Np and that, in the future, the products of such experiments are investigated using improved spectroscopic techniques.

With specific regard to $^{99}$Tc, there are clear lines for investigating the behaviour of colloidal phases. In Chapter 4, it was discovered that Tc(IV) can associate with
magnetite colloids, elevating $^{99}$Tc solubility. There is a vital need to understand the long-term behaviour of such phases, as they present an significant route for radionuclide transport (Kersting et al., 1999). Future research should investigate their mobility, potential for reoxidation, and the sorption kinetics of Tc to these phases. When compared to other radionuclides (e.g., U and $^{99}$Tc), there is a clear lack of research into Np biogeochemistry. Thus, more environmentally relevant studies should be conducted into the processes that will govern Np fate, especially as, with time, it will become one of the most abundant and problematic constituents of nuclear waste. Additionally, studies should focus on the potential of Np-incorporation into mineral phases, as a potential route for immobilisation.

A few hypothesise are proposed for onward research: Complete Tc(IV) reoxidation to Tc(VII) will occur over decadal reoxidation experiments. The mobility of Tc-associated magnetite colloids will result in the significant migration of $^{99}$Tc from contaminated land sites. Np(IV) presents no real migration risk from contaminated sites over decadal timescales of reoxidation.

7.3 References

Thesis Appendices

Appendix 1: Supporting Information for Chapter 4. Impact of Long-term Redox Cycling on Technetium Mobility

Appendix 2: Supporting Information for Chapter 5. The Influence of Iron Biogeochemistry on Technetium Mobility During Redox Cycling

Appendix 3: Supporting Information for 6. The Biogeochemical Redox Cycling of Neptunium
Appendix 1:

Supporting Information for:

4. Impact of Long-term Redox Cycling on Technetium Mobility

Nicholas Masters-Waage¹, Katherine Morris², Jonathan R. Lloyd³, J. Fredrick M. Mosselmans³, Christopher Boothman², Pieter Bots², Sam Shaw², Athanasios Rizoulis², Francis R. Livens¹, Gareth T W Law¹*

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Control Experiment

Three 100 ml control microcosms were prepared with the same sediment solution ratios (1:10) as the microcosms. Prior to incubation, the controls were heat sterilized at 121 °C for 20 minutes. Pore water Tc % and Fe(II) % in sediments did not change over a 429 day period (Figure SI-a).

Figure SI1-A: % of added Tc in solution (blue) and % 0.5 N HCL extractable Fe(II) in sediment (red) for triplicate sterilized control microcosms. Error bars are 1 standard deviation from duplicate experiments, where error bars are not shown they are within the symbol size.
Porewater Analysis

Porewaters were analysed for a range of ions in order to identify geochemical processes occurring in the microcosm systems. The results are shown in Figure SI1-b.

Figure SI1-B: Microcosm incubation data over reduction (shaded) and oxidation (unshaded) events for porewater: A, \( \text{C}_2\text{H}_3\text{O}_2^- \); B, \( \text{NO}_3^- \) (blue) and \( \text{NO}_2^- \) (red); C, porewater Mn; D, porewater Fe; E, porewater \( \text{SO}_4^{2-} \) for duplicate microcosms (bottle A, circles; bottle B, squares); F, pH. Error bars are 1 standard deviation from duplicate experiments, where error bars are not shown they are within the symbol size.
XANES Linear Combination Fitting

Linear combination fitting, using Athena\textsuperscript{8}, was performed on the oxidised and reduced solution Tc XANES samples in normalized $\mu(E)$ space (-30 to +40 eV). The two ‘end-member’ reference standards used were for Tc(IV) (TcO$_2$.xH$_2$O) and Tc(VII) (TcO$_4^-$), from Hess et al., (2004). Fits were restrained to between 0 and 1 for each standard with no combination exceeding 1 and are given in Table SI1-a.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TcO$_2$</th>
<th>TcO$_4^-$</th>
<th>R</th>
<th>$X^2$</th>
<th>$X_v^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced (Aq)</td>
<td>0.83 (0.15)</td>
<td>0.17 (0.15)</td>
<td>3.10E-02</td>
<td>2.56E-01</td>
<td>5.11E-03</td>
</tr>
<tr>
<td>Oxidised (Aq)</td>
<td>0.01 (0.15)</td>
<td>0.99 (0.15)</td>
<td>3.25E-02</td>
<td>6.82E-01</td>
<td>4.87E-3</td>
</tr>
</tbody>
</table>

Table SI1-A: Linear combination fit results for reduced and oxidised solution samples (Reduced (Aq) and Oxidised (Aq)). $R$ = normalized least squares residual; $X^2$ = Chi square value; $X_v^2$ = reduced Chi square value. TcO$_2$ and TcO$_4^-$ reference standards from Hess et al., (2004). An approximate error of 15 % was calculated for the sample.
**EXAFS TcO$_2$ chains**

In the displayed fit in the main text, the Fe surface is represented by a short Tc-Fe bond at ~ 2.6 Å, a model used in previous literature (e.g., Fredrickson et al., 2009; Morris et al., 2008; Zachara et al., 2007). In order to highlight relative changes in the coordination numbers of the Fe and Tc shells the amp ($S_0^2$) and $\sigma^2$ were fixed. The $\sigma^2$ value (0.003) for the ‘Tc-Tc’ was fixed to that used by Lukens et al., (2002) and for ‘Tc-Fe’ the value (0.006) was selected after one iteration, values were kept the same for all samples. We provide an alternative EXAFS fit without a ‘short Fe-shell’, this is displayed in Figure SI1-C and detailed in Table SI1-B. The fit parameters are for a TcO$_2$ chain of varying lengths. These fits were compared in pairs to the fits provided in the main text (Table 1; i.e., with and without the added ‘short Fe-shell’) using the F-Test (Downward et al., 2007), results are shown in (Table 1) and a visual comparison is provided in Figure SI1-C.

![Figure SI1-C: XAS data modelled to TcO$_2$ chains with and without an edge sharing FeO mineral. Panels: A; Non-phase shifted Fourier transform of $k^3$-weighted EXAFS spectra of fit with (smooth lines) and without (dashed lines) ‘short Fe-shell’ calculated over a K range of 3 to 12.5 Å$^{-1}$ and B; $k^3$-weighted EXAFS spectra for fit without ‘short Fe-shell’. Coloured lines are data and black lines are best-fit models.](image-url)
<table>
<thead>
<tr>
<th>sample</th>
<th>Path Tc-</th>
<th>R (Å)</th>
<th>CN</th>
<th>$\sigma^2$ (Å²)</th>
<th>$\Delta E^0$ (eV)</th>
<th>$S_0^2$</th>
<th>R-factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st reduction</td>
<td>O</td>
<td>2.03 (0.01)</td>
<td>6*</td>
<td>0.005</td>
<td>2.3</td>
<td>0.95*</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Tc</td>
<td>2.55 (0.01)</td>
<td>1.18 (0.10)</td>
<td>0.003*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-Tc-O$^{ms}$</td>
<td>4.08 (0.01)</td>
<td>6*</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st oxidation</td>
<td>O</td>
<td>2.02 (0.01)</td>
<td>6*</td>
<td>0.005</td>
<td>2.0</td>
<td>0.95*</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Tc</td>
<td>2.56 (0.01)</td>
<td>1.08 (0.10)</td>
<td>0.003*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-Tc-O$^{ms}$</td>
<td>4.06 (0.01)</td>
<td>6*</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd reduction</td>
<td>O</td>
<td>2.02 (0.01)</td>
<td>6*</td>
<td>0.005</td>
<td>2.4</td>
<td>0.95*</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Tc</td>
<td>2.55 (0.01)</td>
<td>0.99 (0.11)</td>
<td>0.003*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-Tc-O$^{ms}$</td>
<td>4.06 (0.02)</td>
<td>6*</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd oxidation</td>
<td>O</td>
<td>2.02 (0.01)</td>
<td>6*</td>
<td>0.005</td>
<td>1.4</td>
<td>0.95*</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>Tc</td>
<td>2.54 (0.01)</td>
<td>0.80 (0.12)</td>
<td>0.003*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-Tc-O$^{ms}$</td>
<td>4.07 (0.02)</td>
<td>6*</td>
<td>0.011</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd reduction</td>
<td>O</td>
<td>2.02 (0.01)</td>
<td>6*</td>
<td>0.005</td>
<td>1.9</td>
<td>0.95*</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>Tc</td>
<td>2.53 (0.01)</td>
<td>0.74 (0.13)</td>
<td>0.003*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-Tc-O$^{ms}$</td>
<td>4.07 (0.02)</td>
<td>6*</td>
<td>0.011</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table SI1-B: Fit parameters for Tc(IV) chains (Lukens et al., 2002). R = atomic distance CN = coordination number; $\sigma^2$ (Å²) = Debye-Waller factor; $\Delta E^0$ (eV) = energy shift from Fermi level; $S_0^2$ = passive electron reduction factor; R-factor = normalized least squares residual. To determine change in Tc-Tc coordination number, the amplitude factor ($S_0^2$) and Debye Waller factor ($\sigma^2$) were fixed to 0.95 and 0.003, respectively. *Donates fixed parameter, $^{ms}$ denotes linear multiple scattering path and their R and $\sigma^2$ values were calculated as multiples of the single scattering path parameters. Errors are quoted in parentheses and are one standard deviation.
Aqueous Tc in XAS Experiments

A table is provided with the concentrations (%) of added Tc (initial spike: 383 µM TcO₄⁻ / 24 kBq mL⁻¹) remaining in solution after reduction and oxidation events in the high-level XAS microcosm (Table SI-D).

<table>
<thead>
<tr>
<th>Sample</th>
<th>1st reduction</th>
<th>1st oxidation</th>
<th>2nd reduction</th>
<th>2nd oxidation</th>
<th>3rd reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Tc (aq)</td>
<td>4.6</td>
<td>17</td>
<td>7.4</td>
<td>15.2</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Table SI1-C: % of added Tc in solution after reduction and oxidation events for the high-level XAS microcosm.
Figure S11-D: Taxonomic diversity at the phylum level (class level for the Proteobacteria and Firmicutes) of the pyrosequencing reads obtained in this study, for the starting sediment, microcosm A, and microcosms B.
Table SII-D: The lowest taxonomic classifications of the pyrosequencing reads obtained in this study (g = genus, f = family, o = order, c = class), as determined in Qiime. Only taxonomic groups that were represented with more than 5% of the reads in at least one of the sequenced samples are shown. Abundances of more than 10% are highlighted in red, between 5% and 10% in yellow, and between 3% and 5% in grey. (A) Denotes Bottle A and (B) denotes bottle B, R = reduction period and O = oxidation period.
Non-metric multidimensional scaling

Non-metric multidimensional scaling (NMDS) was carried out to find similarities between pyrosequencing sample sets for all reduction and oxidation end points. NMDS plots were created on Primer-7 software using a Bray-Curtis dissimilarity matrix (Clarke and Gorley, 2006). The 2D plots are shown in figure SI-d and have a stress factor of 0.04, a stress factor of <0.1 is generally considered good (Ramette, 2007).

Figure SI1-E: Nonmetric multidimensional ordination of the samples of this study, based on identified OTUs (at 97% sequence similarity) after 16S rRNA gene amplicon pyrosequencing. Sample names for bottle A: 1\textsuperscript{st} reduction; 4R, 1\textsuperscript{st} oxidation; 4RO, 3\textsuperscript{rd} reduction 4ROR etc. For bottle B: 1\textsuperscript{st} reduction; 5R, 1\textsuperscript{st} oxidation; 5RO, 3\textsuperscript{rd} reduction 5ROR etc.
**Pyrosequencing Read Counts**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of reads</th>
<th>Number of reads after chimera check &amp; denoising</th>
<th>Observed OTUs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment</td>
<td>9888</td>
<td>6985</td>
<td>693</td>
</tr>
<tr>
<td>1st Reduction (A)</td>
<td>3304</td>
<td>2481</td>
<td>460</td>
</tr>
<tr>
<td>1st Oxidation (A)</td>
<td>4397</td>
<td>3509</td>
<td>327</td>
</tr>
<tr>
<td>2nd Reduction (A)</td>
<td>13911</td>
<td>10327</td>
<td>536</td>
</tr>
<tr>
<td>2nd Oxidation (A)</td>
<td>10174</td>
<td>7541</td>
<td>365</td>
</tr>
<tr>
<td>3rd Reduction (A)</td>
<td>8130</td>
<td>6453</td>
<td>375</td>
</tr>
<tr>
<td>3rd Oxidation (A)</td>
<td>2146</td>
<td>1748</td>
<td>199</td>
</tr>
<tr>
<td>1st Reduction (B)</td>
<td>6272</td>
<td>5085</td>
<td>751</td>
</tr>
<tr>
<td>1st Oxidation (B)</td>
<td>7283</td>
<td>5952</td>
<td>458</td>
</tr>
<tr>
<td>2nd Reduction (B)</td>
<td>5524</td>
<td>3793</td>
<td>401</td>
</tr>
<tr>
<td>2nd Oxidation (B)</td>
<td>4242</td>
<td>2047</td>
<td>168</td>
</tr>
<tr>
<td>3rd Reduction (B)</td>
<td>6009</td>
<td>3025</td>
<td>224</td>
</tr>
<tr>
<td>3rd Oxidation (B)</td>
<td>5708</td>
<td>2840</td>
<td>208</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>86988</strong></td>
<td><strong>61786</strong></td>
<td><strong>1719</strong></td>
</tr>
</tbody>
</table>

Table SI-E: Number of pyrosequencing reads obtained in this study, reads remaining after denoising and chimera check, and number of observed operational taxonomic units (OTUs) at 97 % similarity level.
Appendix 2:

Supporting Information for:

5. The Influence of Iron Biogeochemistry on Technetium Mobility During Redox Cycling

Nicholas Masters-Waage\textsuperscript{1,2}, Katherine Morris\textsuperscript{2}, Jonathan R. Lloyd\textsuperscript{2}, Francis R. Livens\textsuperscript{1}, J. Fredrick M. Mosselmans\textsuperscript{3}, Gareth T. W. Law\textsuperscript{1*}

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\textsuperscript{2}Research Centre for Radwaste Disposal and Williamson Research Centre, School of Earth, Atmospheric and Environmental Sciences, The University of Manchester, M13 9PL, Manchester, United Kingdom.
\textsuperscript{3}Diamond Light Source Ltd., Diamond House, Harwell Science and Innovation Campus, Didcot Oxfordshire OX11 0DE, United Kingdom.

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Control Experiments

Triplicate 100 ml controls were prepared with sediment solution ratios of (1:10), and 20 mM added ferrihydrite. The controls were heat sterilized at 121 °C for 20 minutes and incubated. There was no significant change in pore water Tc % or Fe(II) % in sediments over a 575 day period (Figure SI2-A).

Figure SI2-A: Triplicate data for heat-sterilised control experiments. Error bars are 1 standard deviation, where not shown they are within the symbol size.
Porewater Analysis

Analysis was undertaken to assess the porewater concentrations of a range of ions in order to identify geochemical processes occurring in the microcosm systems. The results are shown in Figure SI2-B for the microcosms with varying initial Fe(III) concentrations and in Figure SI2-C for the multiple redox cycled experiments (with 20 mM initial ferrihydrite concentration).

Figure SI2-B: Aqueous concentrations of Fe (left), acetate (middle), and sulfate (right) during reoxidation for microcosms with varying concentration of ferrihydrite (see key).
Figure SI2-C: Solution concentrations of acetate (top left), sulfate (top right), Fe (bottom left), and solution pH (bottom right) in large microcosms. Unshaded sections = reduction periods, blue shaded sections = oxidation periods. Error bars are 1 standard deviation of triplicate experiments, where error bars are not shown they are within the symbol size.
Single mineral / microbe experiment

Data for the variation in Fe(II) % in the mineral phase and Tc % in solution is provided in Table SI2-A for the single mineral / microbe experiment, whereby ferrihydrite was reduced by *Geobacter sulfurreducens* to magnetite and subsequently redox cycled.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day</th>
<th>% Fe(II) in mineral</th>
<th>% Tc in solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st reduction</td>
<td>44</td>
<td>31</td>
<td>below detectable limit</td>
</tr>
<tr>
<td>1st oxidation</td>
<td>58</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>2nd reduction</td>
<td>77</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>2nd oxidation</td>
<td>100</td>
<td>8</td>
<td>13</td>
</tr>
</tbody>
</table>

Table SI2-A: % Fe(II) in Fe phase after total Fe extraction (1 M HCl, 24 hours) and total Tc (%) in solution for ferrihydrite redox cycling experiment.
EXAFS fitting

An alternate fit to that presented in Chapter 5 (without a Tc-Fe interaction) for the reduced and oxidised sediments with added ferrihydrite (2 mM) is provided in Table SI2-B. Additionally, the fit parameters for $^{99}$Tc k-edge EXAFS in the ferrihydrite redox cycling experiment are provided in Table SI2-C. Also in Table SIC-C are the linear combination fitting results using two ‘end-member’ reference standards Tc(IV) (TcO$_2$.xH$_2$O) and Tc(VII) (TcO$_4$), from Hess et al., (2004). Here the fits were restrained to between 0 and 1 for each standard and no combination was allowed to exceed 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Path Tc-</th>
<th>R (Å)</th>
<th>CN</th>
<th>$\sigma^2$ (Å$^2$)</th>
<th>$\Delta E^0$ (eV)</th>
<th>$S_0^2$</th>
<th>R-factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced</td>
<td>O</td>
<td>2.02</td>
<td>6*</td>
<td>0.006 (0)</td>
<td>7.72</td>
<td>0.8*</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Tc</td>
<td>2.54</td>
<td>1*</td>
<td>0.003 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-Tc-O$^{ms}$</td>
<td>4.00 (2)</td>
<td>6*</td>
<td>0.0012 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidised</td>
<td>O</td>
<td>2.01</td>
<td>6*</td>
<td>0.005 (1)</td>
<td>7.4</td>
<td>0.8*</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Tc</td>
<td>2.54</td>
<td>1*</td>
<td>0.003 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-Tc-O$^{ms}$</td>
<td>3.98 (2)</td>
<td>6*</td>
<td>0.009 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table SI2-B: Fit parameters for reduced and oxidised sediments with added ferrihydrite (2 mM) without ‘short Fe’ interaction. R = atomic distance; CN = coordination number; $\sigma^2$ = Debye-Waller factor; $\Delta E^0$ (eV) = energy shift from Fermi level; $S_0^2$ = amplitude factor; R-factor = normalized least squares residual. * Denotes fixed parameter, $^{ms}$ denotes linear multiple scattering path. R and $\sigma^2$ values for multiple scattering paths were calculated as multiples of the single scattering path parameters.
Table S12-C: Fit parameters for $^{99}$Tc k-edge EXAFS in ferrihydrite redox cycling experiment. R = atomic distance, CN = coordination number; $\sigma^2$ = Debye-Waller factor; $\Delta E_0^0$ (eV) = energy shift from Fermi level; $S_0^2$ = amplitude factor; R-factor = normalized least squares residual. * Denotes fixed parameter, $^m$ = denotes linear multiple scattering path. R and $\sigma^2$ values for multiple scattering paths were calculated as multiples of the single scattering path parameters. N is the % weighting of each fit, where $^1$ denotes the TcO$_2$-like chains with ‘short-Fe’ interaction fit, and $^2$ denotes the Tc incorporated in magnetite fit. LCF is the result from linear combination fitting between the two alternate fits and R-Factor (LCF) is the normalized least squares residual of these fits.

<table>
<thead>
<tr>
<th>sample</th>
<th>path Tc-</th>
<th>R (Å)</th>
<th>CN</th>
<th>$\sigma^2$ (Å$^2$)</th>
<th>$\Delta E_0^0$ (eV)</th>
<th>$S_0^2$</th>
<th>R-factor</th>
<th>N (%)</th>
<th>LCF</th>
<th>R-factor (LCF)</th>
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<tr>
<td>Tc-FHY first reduction</td>
<td>O$^*$</td>
<td>2.00</td>
<td>6*</td>
<td>0.003 (1)</td>
<td>2.63</td>
<td>1*</td>
<td>0.009</td>
<td>72.0 ± 12.9</td>
<td>0.65 (7)</td>
<td>0.032</td>
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<tr>
<td></td>
<td>Tc$^*$</td>
<td>2.50</td>
<td>1.8*</td>
<td>0.003 (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fe$^*$</td>
<td>2.57</td>
<td>0.8*</td>
<td>0.01*</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>Fe-Te-O$^m_{\text{ref}}$</td>
<td>3.96</td>
<td>6*</td>
<td>0.006 (2)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>O$^*$</td>
<td>1.90</td>
<td>6*</td>
<td>0.026 (12)</td>
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<td></td>
<td>Fe$^*$</td>
<td>3.10</td>
<td>4*</td>
<td>0.005 (4)</td>
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<td></td>
<td>Fe$^2*$</td>
<td>3.55</td>
<td>4*</td>
<td>0.008 (7)</td>
<td></td>
<td></td>
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<tr>
<td>Tc-FHY first oxidation</td>
<td>O$^*$</td>
<td>2.00</td>
<td>6*</td>
<td>0.003 (1)</td>
<td>3.35</td>
<td>1*</td>
<td>0.009</td>
<td>68.0 ± 12.4</td>
<td>0.54 (5)</td>
<td>0.053</td>
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<td></td>
<td>Tc$^*$</td>
<td>2.49</td>
<td>0.8*</td>
<td>0.003 (1)</td>
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<tr>
<td></td>
<td>Fe$^*$</td>
<td>2.53</td>
<td>0.8*</td>
<td>0.01*</td>
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<td></td>
<td>Fe-Te-O$^m_{\text{ref}}$</td>
<td>3.94</td>
<td>6*</td>
<td>0.006 (3)</td>
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<td>6*</td>
<td>0.016 (6)</td>
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<td>3.08</td>
<td>4*</td>
<td>0.004 (3)</td>
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<td>Fe$^2*$</td>
<td>3.53</td>
<td>4*</td>
<td>0.006 (5)</td>
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</tr>
<tr>
<td>Tc-FHY second reduction</td>
<td>O$^*$</td>
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<td>6*</td>
<td>0.004 (1)</td>
<td>3.74</td>
<td>1*</td>
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<td>70.0 ± 12.2</td>
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<td>0.003 (1)</td>
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<tr>
<td></td>
<td>Fe$^*$</td>
<td>2.55</td>
<td>0.8*</td>
<td>0.01*</td>
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<td>Fe-Te-O$^m_{\text{ref}}$</td>
<td>3.95</td>
<td>6*</td>
<td>0.007 (3)</td>
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<td>O$^*$</td>
<td>1.94</td>
<td>6*</td>
<td>0.020 (6)</td>
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<tr>
<td></td>
<td>Fe$^*$</td>
<td>3.09</td>
<td>4*</td>
<td>0.005 (3)</td>
<td></td>
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<tr>
<td></td>
<td>Fe$^2*$</td>
<td>3.54</td>
<td>4*</td>
<td>0.007 (6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Tc-FHY second oxidation</td>
<td>O$^*$</td>
<td>1.99</td>
<td>6*</td>
<td>0.003 (2)</td>
<td>1.77</td>
<td>1*</td>
<td>0.013</td>
<td>65.9 ± 16.4</td>
<td>0.54 (5)</td>
<td>0.057</td>
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<tr>
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<td>Tc$^*$</td>
<td>2.49</td>
<td>0.8*</td>
<td>0.003 (1)</td>
<td></td>
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<tr>
<td></td>
<td>Fe$^*$</td>
<td>2.55</td>
<td>0.8*</td>
<td>0.01*</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Fe-Te-O$^m_{\text{ref}}$</td>
<td>3.95</td>
<td>6*</td>
<td>0.006 (3)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>O$^*$</td>
<td>1.94</td>
<td>6*</td>
<td>0.020 (8)</td>
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<td>Fe$^*$</td>
<td>3.09</td>
<td>4*</td>
<td>0.005 (4)</td>
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</tr>
<tr>
<td></td>
<td>Fe$^2*$</td>
<td>3.53</td>
<td>4*</td>
<td>0.007 (6)</td>
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</tr>
</tbody>
</table>
6. The Biogeochemical Redox Cycling of Neptunium

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Control Experiments

100 mL control experiments were prepared in triplicate with sediment solution ratios of (1:10). The controls were heat sterilized (20 minutes at 121 °C) and incubated. After sorption processes had occurred (removing ~60 % of Np from solution) there were no significant changes in pore water Tc % or Fe(II) % over a 490 day period (Figure SI3-A).

Figure SI3-A: Heat sterilised control experiments. Purple lines = aqueous Np concentrations (%) and red lines = 0.5 N HCl-extractable Fe(II) (%).
Porewater Analysis

The porewater concentrations of a range of ions were measured in the microbially active microcosm systems. Figure S32-B shows the aqueous concentrations of acetate, nitrate, Fe and sulfate, in systems with and without supplemented Fe(III). Enhanced resolution of aqueous sulfate concentrations are provided in figure SI3-C.

Figure S13-B: Aqueous concentrations in microcosm experiments of (top to bottom): acetate (mM), nitrate (mM), Fe (µM), and sulfate (mM). Without added ferrihydrite = left column and with added ferrihydrite = right column. Unshaded areas = reduction periods and shaded blue areas = oxidation periods. Error bars are 1 standard deviation of triplicate microcosms, where not shown they are within symbol size.
Figure SI3-C: Aqueous sulfate (mM, green) and neptunium (%, black) concentrations during first reduction for microcosms without (top) and with (bottom) added ferrihydrite. Error bars are 1 standard deviation of triplicate microcosms, where not shown they are within symbol size.
**High-Activity Redox Cycling**

The aqueous Np concentrations and sediment Fe(II)/Fe(total) for the higher activity redox cycling experiment suitable for XAS analysis are presented in Figure SI3-D, for systems with and without supplemented Fe(III). Additionally, the results from linear combination fitting of these samples to Np(V)O$_2^+$ and Np(IV) standards (Hennig et al., 2009) is displayed in Table SI3-A. These fits were restrained to between 0 and 1 for each standard with no combination exceeding 1.

![Graph](image)

**Figure SI3-D:** Aqueous Np (%, blue) and sediment 0.5 N HCl-extractable Fe(II)/(total) ratio (%, orange) in XAS level microcosms without (triangles) and with (squares) added ferrihydrite (5 mM). Arrows indicate points where samples were taken for XAS.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Np(V)O$_2^{+}$</th>
<th>Np(IV)</th>
<th>R</th>
<th>$X^2$</th>
<th>$X^2_v$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced</td>
<td>10.9</td>
<td>89.1</td>
<td>6.00E-04</td>
<td>1.20E-02</td>
<td>1.00E-04</td>
</tr>
<tr>
<td>Reduced (with added Fe)</td>
<td>9.5</td>
<td>90.5</td>
<td>2.70E-03</td>
<td>5.30E-02</td>
<td>7.60E-04</td>
</tr>
<tr>
<td>Oxidised</td>
<td>25.8</td>
<td>74.2</td>
<td>1.70E-03</td>
<td>6.40E-02</td>
<td>4.60E-04</td>
</tr>
<tr>
<td>Oxidised (with added Fe)</td>
<td>23.1</td>
<td>76.9</td>
<td>2.60E-03</td>
<td>9.70E-02</td>
<td>6.90E-04</td>
</tr>
</tbody>
</table>

Table SI3-A: Linear combination fitting results for XAS samples, with standards Np(V)O$_2^{+}$ and Np(IV) (from Hennig et al., 2009). R = normalized least squares residual; $X^2$ = Chi square value; $X^2_v$ = reduced Chi square value.
Appendices References


