Bioremediation Options for Nuclear Sites a Review of an Emerging Technology

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60+ Years of nuclear power generation has led to a significant legacy of radioactively contaminated land at a number of nuclear licenced "mega sites" around the world. The safe management and remediation of these sites is key to ensuring there environmental stewardship in the long term. Bioremediation utilizes a variety of microbially mediated processes such as, enzymatically driven metal reduction or biominerialisation, to sequester radioactive contaminants from the subsurface limiting their migration through the geosphere. Additionally, some of these process can provide environmentally stable sinks for radioactive contaminants, through formation of highly insoluble mineral phases such as calcium phosphates and carbonates, which can incorporate a range of radionuclides into their structure. Bioremediation options have been considered and deployed in preference to conventional remediation techniques at a number of nuclear "mega" sites. Here, we review the applications of bioremediation technologies at three key nuclear licenced sites; Rifle and Hanford, USA and Sellafield, UK, in the remediation of radioactively contaminated land.

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1. Introduction and Methods for Bioremediation

Concerns surrounding global climate change have led to a dramatic shift in global consciousness towards atmospheric carbon neutrality. This has in turn led to a renewed interest in energy production from nuclear fission, as nuclear power emits considerably less carbon to the atmosphere compared to more traditional electricity-producing practices such as burning fossil fuels [1]. In spite of these benefits compared to power generation from fossil fuels, the management of radioactive wastes generated by the nuclear fuel cycle remains a concern. This challenge includes the significant global legacy of wastes, including radioactively contaminated land, that remain from 60+ years of nuclear power generation and related activities. Clearly, management of this legacy of radioactive wastes must go hand-in-hand with any future nuclear fission power programmes.

Licensed nuclear "mega sites" exist worldwide, many being at various stages of the decommissioning process. In addition to energy production, the release of radionuclides into the hydrosphere has historically occurred through activities related to mining, weapons testing and nuclear accidents. There are many challenges associated with remediating these sites via traditional methods of contaminated land remediation. For example, *ex-situ* treatment methods such as 'dig and dump' and 'pump and treat' are both expensive and carry an inherent risk associated with worker exposure to radioactive materials. The entrenchment of a permeable reactive barrier within radionuclide-impacted aquifers has been viewed as a favourable method of *in-situ* remediation. Although this technology has been successfully applied to treat aqueous uranium and strontium-90 contamination, the clogging of barrier pores with solid precipitates and the need for reactive material refreshment may limit the remediation efficiency over long timescale [2]. In addition to initial barrier emplacement, the potential recovery of treated materials may expose operators to unacceptably high dose rates and again leads to the challenge of waste management

of these materials.

Microbial processes are ubiquitous in the natural subsurface and can impact the solubility of a wide range of radionuclide contaminants [3]. Thus, the targeted enhancement of microbial processes through the stimulation of indigenous soil or sediment bacteria has received widespread attention in the context of remediation technologies in recent decades. Removing aqueous contaminants from solution through microbial metabolism is a form of 'bioremediation', classified into the following four dominant processes (Fig. 1):

1) Bioreduction: specialist anaerobic bacteria couple the oxidation of electron donors, including organic substrates, to the reduction of soluble and inorganic species (i.e. metals) under conditions of anoxia. Redox-active radionuclide contaminants are often soluble under their most oxidised valence states and precipitate as solid biominerals when reduced. In the presence of various electron donors, anaerobic bacteria native to sediments can reduce U(VI) to U(IV) [4], and can facilitate the reduction of Np(V) to Np(IV) [5] and Tc(VII) to Tc(IV) [6] via abiotic reactions. Here, abiotic interactions between oxidised radionuclide contaminants and microbially-generated Fe(II) or sulfide can facilitate radionuclide reduction/precipitation, via 'indirect bioreduction' mechanisms [5, 7-10].

2) Biomineralisation: A broad range of microbial processes can generate soluble ligands that form complexes with radionuclide species, precipitating them from solution. For example, microbial phosphatases cleave organically-bound phosphate in order to obtain for bioavailable phosphorus for phospholipid [11] and nucleic acid [12] synthesis. This microbial mechanism of enzymatic phosphate liberation from organophosphates induces the precipitation of phosphate mineral phases e.g. apatite $(Ca_5(PO_4)_3OH)$ which can sequester radionuclides, and has displayed
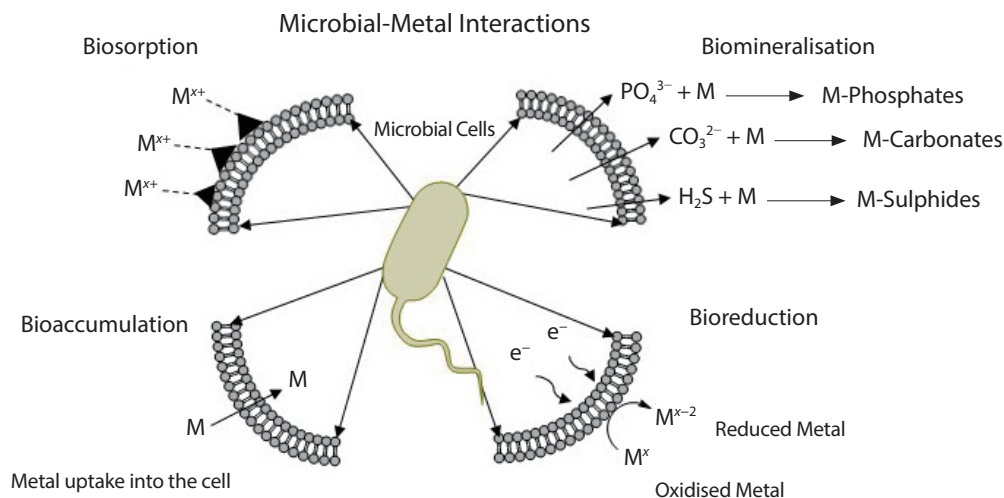
Microbial-Metal Interactions

Fig. 1. Schematic of metal microbe interactions. Adapted from (Lloyd. et. al 2005) [16].

great promise for uranium and strontium remediation [13-15]. Other forms of microbial mineralisation can result in the production of insoluble sulfide and carbonate minerals [16, 17].

3) Biosorption: The high affinity of radionuclides for microbial biomass, including cell walls and surfaces, can result in the removal of dissolved species through adsorption [18].

4) Bioaccumulation: The intracellular uptake of radionuclides by microbes [19], with transporter systems such as Kup for Cs^+ given its similar ionic radii to K^+ [20, 21].

Prospective *in-situ* bioremediation strategies for radionuclide-contaminated land and groundwaters may utilise several of the processes described above, and generally involve 'non-invasive' injection of the relevant biostimulation reagents to promote microbial growth and metabolism through boreholes [22]. If microbial abundance is low at the site requiring remediation, "bioaugmentation" with microbial inocula may be warranted. However, this review details the research conducted over the past couple of decades investigating the potential for indigenous soil bacteria to remediate contaminants of particular concern at

three radioactively contaminated sites, and discusses future research directions.

2. Case Study 1-Rifle, USA

The Rifle site is a former uranium ore processing facility located in the west of Colorado, USA. Leaching of uranium mill tailings produced as a by-product of ore processing has resulted in U- and U-series radionuclide contamination of the subsurface and groundwater beneath the site. An unconfined aquifer containing uranium contaminated groundwater is situated within an alluvial deposit beneath the site, with the groundwater following a hydrological gradient and discharging into the Colorado River. Despite removal of the tailings and contaminated topsoil, uranium concentrations within aquifer remain elevated relative to the Uranium Mill Tailings Radiation Control Act (UMTRA) maximum concentration limit of 0.18 µM, ranging from 0.4 µM to 1.4 µM. The presence of reduced U(IV) associated with ferrous and sulfide mineral phases within sediment samples taken from the site, indicated that natural microbial reduction of Fe(III) and sulphate present within the subsurface was occurring and coupled to the reduction of U(VI) to U(IV) [23].

The presence of these reduced U(IV) phases is sustained by the low dissolved oxygen content of the groundwater (limiting aerobic metabolism) coupled with the occurrence of labile organic carbon acting as an electron donor for the microbial respiration of U(VI), Fe(III) and SO_4^{2-} [23-25]. The gradual reoxidation of these reduced U(IV) phases from exposure to oxygen or reaction with mineral phases such as Fe(III), likely occurs concomitantly with ingrowth of uranium provided by the migration of U(VI) contaminated groundwater from up gradient horizons resulting in the sustained elevation in the uranium concentrations in the aquifer sediments [25].

A number of experimental "biostimulation" field trials were conducted to assess the potential for in-situ bioremediation of the uranium plume. Metal-reducing bacteria, which are ubiquitous within the Rifle subsurface, were stimulated with acetate (via bore hole injections), successfully decreasing the U(VI) concentration through biotic reduction to less soluble U(IV) phases [23, 24]. During one trial in 2002, the sustained addition of acetate to the subsurface (over 3 months) led to a decrease in uranium concentration from ~1.2 μM to below 0.18 μM in some injection wells, within 50 days of initial injection [24]. This reduction in U(VI) was coupled to an increase in Fe(II) within groundwater, suggesting this removal of U(VI) coincided with the reduction of Fe(III) mineral phases, and suggested that iron (Fe)-reducing bacteria (FeRB) may be using the U(VI) in groundwaters as a terminal electron acceptor during respiration. Additional evidence for this was provided by analysis of 16S rRNA genes and phospholipidic fatty acid profiles from within the treatment zone. Here the microbial community was enriched in the FeRB *Geobacter* within the initial 50 days of the acetate injection [24]. Beyond the first 50 days of treatment the subsurface biogeochemistry appeared to develop sulfate-reducing conditions, presumably as a result of exhaustion of bioavailable Fe(III)- in sediments, with a stoichiometric decrease in acetate and sulphate concentrations occurring during this longer timeframe [24]. Enabled by the relativity high concentration of sulphate (6–11 mM) present within the Rifle groundwater [26], the onset of sulphate reduction facilitated by sulphate-reducing bacteria (SRB) after 30–50 days of treatment with acetate, appeared to briefly reduce the removal rate of U(VI) from groundwaters [24-26]. The slowing in U(VI) removal was considered, in part, due to the poorer efficiency of SRB to oxidise acetate (for sulfate and U(VI) reduction), when compared to the FeRB (*Geobacter*) dominated U(VI)-reducing microbial community enriched during the first month of acetate amendment [26]. Additionally, the authors suggested that the increase in pH and alkalinity upon sulphate reduction would directly affect the solubility of the U(VI) through the formation of relatively soluble U(VI) carbonate complexes. Indeed, the formation of these stable aqueous complexes was postulated to lead to an increased partitioning of U(VI) into the aqueous phase, decreasing the concentration of U(VI) sorbed to sediment [26]. More recently, abiotic studies have observed transient remobilisation of uranium to solution during sulfidation of U(VI)-sorbed ferrihydrite [27]. Here, the formation of a transient, and relatively soluble U-persulfide species was identified and proposed as a potential mechanism for remobilisation under sulfidic conditions.

During a 2009 field trial at Rifle, acetate injections lead to the formation of biogenic ferrous iron sulfides e.g. mackinawite (FeS) through microbial sulfate reduction [28]. Fe(II)- and sulphide- phases can reduce U(VI) though direct abiotic reaction to form insoluble U(IV) (uraninite) on surfaces of the reduced mineral phases [29]. Additionally, the presence of sulfide phases can act as a redox buffer thus inhibiting future abiotic U(IV) reoxidation [30]. The reduced U(IV) has also been shown to be present in a relatively labile monomeric U(IV) phase, and is often associated with phosphates and carbonates where microbial U(VI) reduction has been stimulated by electron donor additions. A series of column experiments conducted using cored sediments from the Rifle site [28] suggested biotic- and abiotic-mechanisms may be operating. This may explain the differing uranium phases observed in sediments during sulfate

reduction, and the abundance of the less stable monomeric U(IV). Stabilisation of monomeric U(IV) through sorption to phosphate groups on biomass, or to the surface sites of mineral phases such as mackinawite was thought to supress the formation of uraninite. Additionally, the sorption of biomass to ferrous sulfides can propagate U(IV)- precipitation by providing additional nucleation sites within the extracellular polymeric substances (EPS) encompassing the sorbed biomass. Both the sorbed biomass and EPS on the surfaces of ferrous sulfide minerals can offer U(IV) binding sites, which can provide additional stabilization and uranium removal through the precipitation of U(IV) phosphate minerals [28]. Indeed, the authors proposed that close association of mackinawite and biomass provides several possible pathways for producing U(IV) phosphate phases during stimulated bioremediation processes. Termination of acetate delivery to the subsurface at Rifle did not lead to an instantaneous rebounding of U(VI) concentrations, suggesting that the removal of U(VI) from sorbed surface sites during biotic reduction to U(IV) produced a source of additional sorption sites for aqueous U(VI) entering the treatment zone from up gradient [26]. Therefore, a gradual increase in U(VI) concentrations was seen after acetate injection stopped, with a number of injection sites returning to their pre-injection levels of U(VI) after cessation of acetate injection. The reoxidation of reduced U(IV) phases through abiotic reactions with any Fe(III) phases present, or upon ingress of oxygenic groundwater, remains an additional possible source of U(VI). Indeed, the reoxidation of reduced U(IV) species remains one of the main challenges associated with bioreduction as a means of large scale bioremediation of uranium contamination [3]. Although the formation of ferrous sulfides can limit reoxidation of U(IV) phases, maintaining reducing conditions, continued addition of organic election donor may be required to ensure reduced phases remain recalcitrant to reoxidation. More recently, studies using materials from other contaminated sites, such as Sellafield, have started to explore more stable endpoints for the bioremediation of groundwaters con-

taminated with uranium and other radionuclides (See Case Study 3).

3. Case Study 2-Hanford, USA

The US Department of Energy (DOE) Hanford Site, lies on the banks of the Columbia River in south central Washington State, USA occupying an area of 1,517 km^2 [31]. Work first began on the site during the early 1940s, were it served as a plutonium production facility for the American nuclear weapons program [31]. During the initial years, the site was subdivided into a number of operative areas including the 100, 200 and 300 Areas. Fuel fabrication, research and development activities occurred within 300 area, with the synthesised fuel being used within one of the nine nuclear reactors that occupied the 100 area of the site. The 200 area was dedicated to a number of separation and purification plants, were irradiated reactor fuel would be de-canned and the plutonium recovered [31]. Although reactor operations were stopped in 1987, decades of operation and fuel reprocessing has left a significant legacy of radioactively contaminated land and groundwater at the site [32]. All three of the main operative areas have significant quantities of contamination within their boundaries. During operational periods, cooling water from reactor 100-N, including the primary coolant circuit, was disposed of into a number of crib and trench liquid waste disposal facilities, where percolation to the subsurface was used as a disposal mechanism. The cooling water disposed to these facilities contained a number of radioactive fission products and reactor generated activation products, including cobalt-60, cesium-137 and strontium-90 [33]. As a result, these radionuclides have percolated into the groundwater at the 100-N area, leading to contamination of the subsurface. The Columbia River, which borders the 100-N area, has received fugitive discharges of a variety of radionuclides including ^{90}Sr, as a result of the migration of the 100-N area contamination plumes toward to river [33]. In situ biominerialisation

has been trialled at the Hanford site to remediate the plumes of ^{90}Sr within the 100-N area. The biominerialisation process that has been assessed relies on the microbially mediated *in situ* reaction between calcium and phosphate, to produce calcium-phosphate and hydroxyapatite (HAP)-like mineral phases. These phases are thought to incorporate ^{90}Sr by initial sorption and subsequent isomorphic substitution for Ca^{2+} sites within the crystal structure [34]. This reduces the ^{90}Sr concentration within the groundwater plumes [35]. Hydroxyapatite is a favourable end state/sink for a number of radionuclides, including ^{90}Sr as it has exceptionally low solubility ($K_{SP} = 10^{-44}$) making it recalcitrant to dissolution, and it is also resistant to radiation damage [36] . In situ formation of calcium phosphate phases can be achieved abiotically via direct injection of calcium and phosphate rich amendment solutions into a contaminated groundwater. In the case of direct injection of phosphate and calcium, instantaneous precipitation of a calcium phosphate phase can result in injection well clogging, and reduce the distribution of the calcium and phosphate amendments within the injected subsurface [37]. Thus, in order to optimise the in situ amendment, complexing calcium with citrate prior to injection allowed subsequent microbial degradation of the calcium citrate complex and thus slow release of $Ca^{2+}_{(aq)}$ into the groundwater. This then reacts with the inorganic phosphate co-injected alongside the calcium-citrate, to form calcium phosphate minerals. The slow release of calcium and the production of calcium-phosphates enables more extensive distribution of the amendment solutions into the subsurface around the injection site, thus increasing the treatment area, and reducing the risk of well clogging [33, 35, 38, 39]. Initially, under the Hanford site conditions, the precipitated calcium phosphate phases are amorphous in nature, and strontium removal is initially dominated by sorption to charged surface sites. However, over time (months) the crystallinity of these phases is thought to increase through mineral dissolution and recrystallization. During these processes, strontium is incorporated into the crystal structure forming strontium substituted HAP [35].

However, under Sellafield representative conditions, laboratory studies have not observed this increase in Sr incorporation over time, suggesting that site specific factors may affect the longer term incorporation rate [15].

The ionic strength of a citrate/phosphate amendment solution is an important parameter to consider prior to injection. As strontium attenuation within the subsurface is predominantly governed by weak outer sphere sorption to the surface of mineral phases e.g. iron oxides. In this form Sr^{2+} is highly susceptible to mobilisation through cation ion exchange with mono and divalent cations [40]. As a result, the injection of high ionic strength amendment solutions risks increasing the strontium concentration in the aqueous phase, due to rapid desorption from the surface of mineral phases. Indeed, work carried out at Hanford noted a significant increase in aqueous ^{90}Sr concentrations upon the addition of high ionic strength amendment solutions during series of laboratory and field studies investigating the optimum amendment concentration [39]. One possible solution to this, is to use a calcium deficient amendment solution. The lower calcium concentration reduces the overall ionic strength of the solution, reducing the amount of outer sphere adsorbed Sr^{2+} remobilised from the sediment, whilst enabling enough phosphate release to the subsurface to produce sufficient apatite to effectively sequester ^{90}Sr [33, 35, 39]. A second possible solution is to use a two-stage injection process. Firstly, an injection of a low concentration amendment solution could be delivered, designed to sequester strontium in the immediate vicinity of the injection site via calcium phosphate mineralisation which will sorb strontium, thus preventing widespread desorption and migration further into the aquifer. Secondly, an injection of a higher concentration solution can then occur without the risk of ionic strength related strontium re-mobilization in the immediate area surrounding the injection site [39, 41]. The authors of Hanford studies have experimented with several different amendment solutions during both laboratory and field bioremediation trials, with the aim of optimising the formation of calcium-phosphate phases, whilst

minimising initial ^{90}Sr desorption. For the field conditions at the Hanford site, a solution mixture of 3.6 mM calcium, 9 mM citrate and 40 mM phosphate proved optimal [35]. A 95% decrease in ^{90}Sr concentration in groundwater was reported over 1 year in monitoring wells down gradient of the injection site, compared to the highest baseline level. There was also a long term effect with a significant reduction in ^{90}Sr flux entering the Columbia River for several years after the injection of citrate amendment solutions [35].

4. Case Study 3-Sellafield, UK

The Sellafield nuclear site is located in west Cumbria, UK situated along the Irish Sea coast covering an area of 6 km^2 [42]. Over its 60 year operational history, the site has provided numerous roles, including developing civil power reactors and hosting a number of nuclear fuel reprocessing and storage facilities. As a result of these legacy operations, leaks of radioactive material have occurred, resulting in radioactively contaminated land [43]. Much of the contamination on the site is due to fugitive emissions of radioactively contaminated liquors and/or leaching of radioactive wastes [44]. The migration of radionuclides down gradient from the source of contamination. The rate of migration depends on the contaminant and biogeochemical properties of subsurface environment, but in most cases significant attenuation occurs via interactions with subsurface sediments [43].

Building on the literature, microcosm studies using a range of sediment samples representative of the Sellafield subsurface, displayed varying abilities to support the microbial reduction of mobile U(VI) to sparingly soluble U(IV) concomitantly with Fe(III) reduction [45]. Acetate was the electron donor used in this study. Coupling the oxidation of simple organic acids such as acetate to the reduction of U(VI) typically results in the formation of poorly soluble U(IV), as either nanocrystalline UO$_2$ and/or "monomeric" U(IV). However, this can be susceptible to oxidative dissolution and subsequent U(VI) remobilisation. Consequent to this study, the Sellafield sediment (RB27) was used in a series of microcosms investigating the resistance of bioreduced U(IV) to oxidative remobilisation. The study examined the effects of sediment aging and residual electron donor concentrations on the stability of noncrystalline U(IV) [46]. Dissolved oxygen and nitrate were chosen as the oxidants for the U(IV) bearing bioreduced sediments. Both are realistic oxidants in the radionuclide-impacted/remediated sediment given: i) aquifer recharge with oxygenated groundwater and ii) elevated nitrate concentrations at nuclear facilities resulting from a range of processing operations on site and/or local enhancements of nitrate from e.g. agriculture [43]. Recalcitrance of the U(IV) precipitate to reoxidation did not increase with ageing of the bioreduced sediment prior to reoxidation. Total U(VI) remobilisation was observed approximately 60 days after exposing the bioreduced U(IV)-bearing sediments to oxic conditions. However, the presence of residual electron donors markedly improved the retardation of U(IV) oxidation upon nitrate addition. After 20 days of incubation, the addition of 3 mM nitrate failed to mobilise greater 30% of the bioreduced (IV). In fact, the removal of U(VI) occured over the next 15 days once maximum U(VI) remobilisation was achieved. This is likely due to total nitrate utilisation by the native bacterial community. The addition of 30 mM nitrate was required to remobilise roughly 70% U(VI) after approximately 30 days. Elevated nitrate concentrations partially inhibit microbial U(VI) reduction presumably as bacteria preferentially reduce nitrate over U(VI), given its more favourable energy yield [47].

Other microcosm studies using sediment RB27 documented the metabolic diversity of the indigenous bacteria. Various organic substrates were used to reduce both U(VI) to U(IV) [48] and Tc(VII) to Tc(IV) [49], in order to precipitate biominerals targeted to be recalcitrant to oxidative dissolution. The uranium study showed that the addition of glycerol phosphate successfully stimulated at least two types of indigenous microbial activity. These included the

initial hydrolysis of the C-P bonds resulting in the liberation of inorganic orthophosphate and glycerol to solution, and the subsequent oxidation of the glycerol linked to bioreduction of U(VI) to U(IV). The resultant uranium precipitated was a recalcitrant U(IV)-phosphate species similar to ningyoite [$CaU(PO_4)_2 \cdot 2H_2O$]. Regarding technetium remediation, a series of remediation amendments were added to sediment microcosms aimed at stimulating reducing conditions conducive to causing reductive Tc(IV)-scavenging to treated sediments [49] . For example, adding the commercial metal remediating product Hydrogen Releasing Compound (HRC) to groundwater causes the sustained release of lactate. The microbial oxidation of lactate contributed to the removal of Tc(VII), forming a recalcitrant Tc(IV) endpoint via enzymatic or biogenic Fe(II)-mediated reduction [49].

Other sediment microcosm studies using soil samples obtained from a locality along the Calder River, Cumbria, UK approximately 2 km north of the Sellafield Site, have further demonstrated how various enzymatic processes may be stimulated to remediate radionuclides in groundwaters. Biostimulated denitrification processes in carbonate-buffered, nitrate-amended sediment microcosms enhanced strontium removal through the increased pH in the closed systems and resultant precipitation of strontium-associated carbonate phases [17]. This co-precipitation of Sr^{2+} cations with calcium carbonate phases is often the targeted endpoint to remediation studies [50, 51].

Enrichments of Fe(III)-reducing bacteria, native to the Calder River sediments were assessed for their potential role in remediating simulated groundwaters contaminated with Sr^{2+} (including ultra-trace level ^{90}Sr) and Tc(VII) [52]. The study compared the impacts of immediate exposure to pre-formed Fe(II) biominerals, versus exposure to progressive Fe(III) bioreduction and more gradual resultant Fe(II)-mineral formation, as well as the pH (either 7 or 9). The reduction of Fe(III) to Fe(II) minerals such as siderite ($FeCO_3$) and vivianite ($Fe_3(PO_4)_2 \cdot 8H_2O$) at both starting pH values coincided with approximately 80% Tc(VII) removal

in both Fe(II) enrichment systems to form poorly soluble Tc(IV). In the case of ultra-trace-level ^{90}Sr levels, progressive Fe(III) reduction at both starting pH values resulted in slightly more ^{90}Sr removal compared with introducing Fe(II) biominerals at the start. A significant and immediate increase in biogenic Fe(II) production coincided with a rapid decrease in aqueous ^{90}Sr, however, further ^{90}Sr removal was not sustained despite increasing Fe(II) generation. Nonetheless, the faster rate of initial ^{90}Sr removal with progressive Fe(III) bioreduction compared with adding Fe(II) minerals at the start, suggests that sorption to Fe(II) biominerals was the mechanism responsible for ^{90}Sr removal. The failure to enhance Sr^{2+} removal in either Fe(II)-bearing system at a starting pH of 7 was notable especially in the context of the Sellafield sub-surface pH which is circumneutral.

Denitrification experiments using low nitrate concentrations stimulated the electron accepting cascade NO_3^- > Mn(IV)/Fe(III) > SO_4^{2-}, suggesting microbial NO_3^- reduction may have a positive impact on sediment conditioning to bioreducing conditions [53]. Under bioreducing conditions, concomitant NO_3^- and Mn(IV) reduction (in the presence of added Mn(IV)) resulted in approximately 90% Np(V) removal prior to Fe(III) reduction, and near total removal by the onset of SO_4^{2-} reduction [54]. Adding Mn(IV) and NO_3^- helped establish a diverse anaerobic microbial community that developed reducing conditions and Np(V) removal continued during initial stages of Fe(III) reduction, likely through abiotic reduction of Np(V) to Np(IV) by surface-associated Fe(II).

The impact of microbial NO_3^- and Fe(III) reduction on the remediation of groundwater co-contaminated with strontium and Tc(VII) was later assessed in a series of flow-through column experiments containing the Calder River sediment [55]. Column experiments represent an important progression from batch sediment microcosms as they attempt to bridge between static experiments and the dynamic conditions in the subsurface. Separate columns were saturated with groundwater amended with $^{99m}Tc(VII)$, Sr^{2+}

and NO_3^-, which was pumped upwards through the column. The influent was then switched to groundwater containing acetate and 12 μM strontium. Use of the gamma-emitting ^{99m}Tc(VII) (half-life = 6 hours) also allowed for the migration of Tc(VII) to be imaged using a gamma-camera, visualising the location of ultra-trace-level ^{99m}Tc(VII) within the sediment core and its real-time reduction to Tc(IV) and immobilization [56]. Maintaining acetate ingress over 120 days resulted in the precipitation of ^{99m}Tc(IV) near the base of the column. Pulsing the introductions of acetate after 60 days of continual influx produced a more even distribution of ^{99m}Tc(IV) throughout the column. Given the successful bioremediation of aqueous U(VI) using glycerol phosphate [48] the same mechanism was investigated in a microcosm study with groundwater co-contaminated with strontium and Tc(VII) [15]. The microbial response to glycerol phosphate amendments selected for anaerobic communities featuring the phosphate-liberating *Chryseobacterium* sp. and an Fe(III)-reducing *Serratia* sp. Strontium was partially removed as both sorbed to calcium-phosphate and with some evidence for incorporated strontium -phosphate. Tc(VII) was reduced to hydrous Tc(IV)O_2 like phases in the study, likely through a combination of the microbial coupling of glycerol oxidation to Fe(III) reduction, with indirect Tc(VII) reduction by surface-associated biogenic Fe(II). There was no evidence for Tc association with the amorphous hydroxyapatite-like phase and the bioreduction endpoint remained relatively susceptible to the oxidative remobilisation of Tc(VII). A previous study investigating the abiotic reduction of Tc(VII) using Sn(II)-doped hydroxyapatite suggested that Tc(IV) was immobilised through incorporation into the hydroxyapatite phase [57]. The substitution of PO_4^{3-} for Tc(IV) within hydroxyapatite lattices was the proposed removal mechanism, describing a Tc(IV) endpoint more resilient to reoxidation than hydrous TcO$_2$-like phases [57, 58].

Research in these systems continues with development of groundwater compositions and selection of different sediment lithologies as well as movement to more dynamic column and ultimately field scale experiments an obvious, if challenging, next steps.

5. Conclusions

This review gives an overview of bioremediation options for the nuclear sector, focusing on three case studies that have explored the in situ (bio)stimulation of radionuclide mineralisation in sediment systems. In some examples (e.g. Rifle and Hanford) field trials have been conducted. The Sellafield example gives a template for work where in situ studies are more challenging; here batch laboratory microcosm studies, followed by column then field trial experiments offers a robust procedure to assess and optimise bioremediation options for land contaminated by radionuclides prior to implementation on site. For all scenarios, it should be noted that although radionuclide bioremediation offers a potentially low-cost alternative to conventional approaches, the long-term effectiveness remains to be assessed and is still a priority for future in situ studies. Key topics to consider in future studies therefore include rigorous assessments of the long-term stability of any radionuclide-containing biominerals formed in situ, taking into account the expected biogeochemical evolution of the sites concerned. Works could also focus on the applicability of bioremediation technologies in the co-treatment of a variety of radionuclides and/or investigate the use of multi (bio)remediation technologies and how these may interact and effect radionuclide sequestration. Additionally, future studies should focus on advancing technologies along the technology readiness level (TRL) scale to address the need for scaling up of technologies from laboratory to field scale whilst also addressing site-specific factors in the implementation of these technologies. This could include the adaptation (or augmentation as appropriate) of indigenous microbial community and manipulations to the subsurface geochemistry to optimise remediation endpoints.

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REFERENCES

[1] R.E.H. Sims, H.H. Rogner, and K. Gregory, "Carbon Emission and Mitigation Cost Comparisons Between Fossil Fuel, Nuclear and Renewable Energy Resources for Electricity Generation", Energy Policy, 31(13), 1315-1326 (2003).

[2] B. Gu, D.B. Watson, D.H. Phillips, and L. Liang, "Biogeochemical, Mineralogical, and Hydrological Characteristics of an Iron Reacitve Barrier Used for Treatment of Uranium and Nitrate", in Handbook of Groundwater Remediation Using Permeable Reactive Barriers, D. Naftz, S.J. Morrison, C.C. Fuller, J.A. Davis, eds., 305-342, Elsevier, Cambridge (2002).

[3] L. Newsome, K. Morris, and J.R. Lloyd, "The Biogeochemistry and Bioremediation of Uranium and Other Priority Radionuclides", Chem. Geol., 363, 164-184 (2014).

[4] K.T. Finneran, R.T. Anderson, K.P. Nevin, and D.R. Lovley, "Potential for Bioremediation of Uranium-Contaminated Aquifers With Microbial U (VI) Reduction", Soil Sediment Contam. An Int. J., 11(3), 339-357 (2002).

[5] G.T.W. Law, A. Geissler, J.R. Lloyd, F.R. Livens, C. Boothman, J.D.C. Begg, M.A. Denecke, J. Rothe, K. Dardenne, I.T. Burke, J.M. Charnock, and K. Morris, "Geomicrobiological Redox Cycling of the Transuranic Element Neptunium", Environ. Sci. Technol., 44(23), 8924-8929 (2010).

[6] R.E. Wildung, S.W. Li, C.J. Murray, K.M. Krupka, Y. Xie, N.J. Hess, and E.E. Roden, "Technetium Reduction in Sediments of a Shallow Aquifer Exhibiting Dissimilatory Iron Reduction Potential", FEMS Microbiol. Ecol., 49(1), 151-162 (2004).

[7] J.M. McBeth, G. Lear, J.R. Lloyd, F.R. Livens, K. Morris, and I.T. Burke, "Technetium Reduction and Reoxidation in Aquifer Sediments", Geomicrobiol. J., 24(3-4), 189-197 (2007).

[8] D.E. Latta, M.I. Boyanov, K.M. Kemner, E.J. O'Loughlin, and M.M. Scherer, "Abiotic Reduction of Uranium by Fe(II) in Soil", Appl. Geochemistry, 27(8), 15121524 (2012).

[9] D.R. Brookshaw, R.A.D. Pattrick, P. Bots, G.T.W. Law, J.R. Lloyd, J.F.W. Mosselmans, D.J. Vaughan, K. Dardenne, and K. Morris, "Redox Interactions of Tc(VII), U(VI), and Np(V) With Microbially Reduced Biotite and Chlorite", Environ. Sci. Technol., 49(22), 13139-13148 (2015).

[10] I.T. Burke, C. Boothman, J.R. Lloyd, R.J.G. Mortimer, F.R. Livens, and K. Morris, "Effects of Progressive Anoxia on the Solubility of Technetium in Sediments", Environ. Sci. Technol., 39(11), 4109-4116 (2005).

[11] W.H. Ko and F.K. Hora, "Production of Phospholipases by Soil Microorganisms", Soil Sci., 110(5), 355-358 (1970).

[12] W. Siuda and R. J. Chróst, "Utilization of Selected Dissolved Organic Phosphorus Compounds by Bacteria in Lake Water Under non-Limiting Orthophosphate Conditions", Pol. J. Environ. Stud., 10(6), 475-483 (2001).

[13] L.E. Macaskie, R.M. Empson, A.K. Cheetham, C.P. Grey, and A.J. Skarnulis, "Uranium Bioaccumulation by a Citrobacter sp. as a Result of Enzymically Mediated Growth of Polycrystalline HUO_2PO_4", Science, 257(5071), 782-784 (1992).

[14] W. Gao and A.J. Francis, "Reduction of Uranium(VI) to Uranium(IV) by Clostridia", Appl. Environ. Microbiol., 74(14), 4580-4584 (2008).

[15] A. Cleary, J.R. Lloyd, L. Newsome, S. Shaw, C.

Boothman, G. Boshoff, N. Atherton, and K. Morris, "Bioremediation of Strontium and Technetium Contaminated Groundwater Using Glycerol Phosphate", Chem. Geol., 509, 213-222 (2019).

[16] J.R. Lloyd, R.T. Anderson, and L.E. Macaskie, "Bioremediation of Metals and Radionuclides", in Bioremediation: Applied Microbial Solutions for Real-World Environmental Cleanup, 2nd ed., R.M. Atlas and J.C. Philp, eds., 293-317, ASM Press, Washington (2014).

[17] C.L. Thorpe, J.R. Lloyd, G.T.W. Law, I.T. Burke, S. Shaw, N.D. Bryan, and K. Morris, "Strontium Sorption and Precipitation Behaviour During Bioreduction in Nitrate Impacted Sediments", Chem. Geol., 306-307, 114-122 (2012).

[18] G.M. Gadd, "Biosorption: Critical Review of Scientific Rationale, Environmental Importance and Significance for Pollution Treatment", J. Chem. Technol. Biotechnol., 84(1), 13-28 (2009).

[19] L.E. Macaskie, "The Application of Biotechnology to the Treatment of Wastes Produced From the Nuclear Fuel Cycle: Biodegradation and Bioaccumulation as a Means of Treating Radionuclide-Containing Streams", Crit. Rev. Biotechnol., 11(1), 41-112 (1991).

[20] S.V. Avery, "Caesium Accumulation by Microorganisms: Uptake Mechanisms, Cation Competition, Compartmentalization and Toxicity", J. Ind. Microbiol., 14(2), 76-84 (1995).

[21] S.V. Avery, "Microbial Interactions With Caesium—Implications for Biotechnology", J. Chem. Technol. Biotechnol., 62(1), 3-16 (1995).

[22] K.H. Williams, J.R. Bargar, J.R. Lloyd, and D.R. Lovley, "Bioremediation of Uranium-Contaminated Groundwater: A Systems Approach to Subsurface Biogeochemistry", Curr. Opin. Biotechnol., 24(3), 489-497 (2013).

[23] K.M. Campbell, R.K. Kukkadapu, N.P. Qafoku, A.D. Peacock, E. Lesher, K.H. Williams, J.R. Bargar, M.J. Wilkins, L. Figueroa, J. Ranville, J.A. Davis, and P.E. Long, "Geochemical, Mineralogical and Microbiological Characteristics of Sediment From a Naturally Reduced Zone in a Uranium-Contaminated Aquifer", Appl. Geochemistry, 27(8), 1499-1511 (2012).

[24] R.T. Anderson, H.A. Vrionis, I. Ortiz-Bernad, C.T. Resch, P.E. Long, R. Dayvault, K. Karp, S. Marutzky, D.R. Metzler, A. Peacock, D.C. White, M. Lowe, and D.R. Lovley, "Stimulating the In Situ Activity of Geobacter Species to Remove Uranium From the Groundwater of a Uranium-Contaminated Aquifer", Appl. Environ. Microbiol., 69(10), 5884-5891 (2003).

[25] J.M. Zachara, P.E. Long, J. Bargar, J.A. Davis, P. Fox, J.K. Fredrickson, M.D. Freshley, A.E. Konopka, C. Liu, J.P. McKinley, M.L. Rockhold, K.H. Williams, and S.B. Yabusaki, "Persistence of Uranium Groundwater Plumes: Contrasting Mechanisms at two DOE Sites in the Groundwater-River Interaction Zone", J. Contam. Hydrol., 147, 45-72 (2013).

[26] K.H. Williams, P.E. Long, J.A. Davis, M.J. Wilkins, A.L. N'Guessan, C.I. Steefel, L. Yang, D. Newcomer, F.A. Spane, L.J. Kerkhof, L. Mcguinness, R. Dayvault, and D.R. Lovley, "Acetate Availability and its Influence on Sustainable Bioremediation of Uranium-Contaminated Groundwater", Geomicrobiol. J., 28(5-6), 519-539 (2011).

[27] L.T. Townsend, S. Shaw, N.E.R. Ofili, N. Kaltsoyannis, A.S. Walton, J.F.W. Mosselmans, T.S. Neill, J.R. Lloyd, S. Heath, R. Hibberd, and K. Morris, "Formation of a U(VI)-Persulfide Complex During Environmentally Relevant Sulfidation of Iron (Oxyhydr)Oxides", Environ. Sci. Technol., 54(1), 129-136 (2019).

[28] J.R. Bargar, K.H. Williams, K.M. Campbell, P.E. Long, J.E. Stubbs, E.I. Suvorova, J.S. Lezama-Pacheco, D.S. Alessi, M. Stylo, S.M. Webb, J.A. Davis, D.E. Giammar, L.Y. Blue, and R. Bernier-Latmani, "Uranium Redox Transition Pathways in Acetate-Amended Sediments", Proc. Natl. Acad. Sci. U.S.A., 110(12), 4506-4511 (2013).

[29] S.P. Hyun, J.A. Davis, K. Sun, and K.F. Hayes, "Uranium (VI) Reduction by Iron(II) Monosulfide Mackinawite",

Environ. Sci. Technol., 46(6), 3369-3376 (2012).

[30] Y.Bi, S.P. Hyun, R.K. Kukkadapu, and K.F. Hayes, "Oxidative Dissolution of UO_2 in a Simulated Groundwater Containing Synthetic Nanocrystalline Mackinawite", Geochim. Cosmochim. Acta, 102, 175-190 (2013).

[31] D.A. Neitzel, A.L. Bunn, S.D. Cannon, J.P. Duncan, R.A. Fowler, B.G. Fritz, D.W. Harvey, P.L. Hendrickson, D.J. Hoitink, D.G. Horton, G.V. Last, T.M. Poston, E.L. Prendergast-Kennedy, S.P. Reidel, A.C. Rohay, M.R. Sackschewsky, M.J. Scott, and P.D. Thorne. Hanford Site National Environmental Policy Act (NEPA) Characterization Report, Revision 17, Pacific Northwest National Laboratory Repport, PNNL-6415 (2005).

[32] R.E. Gephart, "A Short History of Waste Management at the Hanford Site", Phys. Chem. Earth, 35(6-8), 298-306 (2010).

[33] J.E. Szecsody, M.L. Rockhold, M. Oostrom, R.C. Moore, C.A. Burns, M.D. Williams, L. Zhong, J.S. Fruchter, J.P. McKinley, V.R. Vermeul, M.A. Covert, T.W. Wietsma, A.T. Breshears, and B.J. Garcia. Sequestration of Sr-90 Subsurface Contamination in the Hanford 100-N Area by Surface Infiltration of a Ca-Citrate-Phosphate Solution, Pacific Northwest National Laboratory Repport, PNNL-18303 (2009).

[34] J.F. Rakovan and J. M. Hughes, "Strontium in the Apatite Structure: Strontian Fluorapatite and Belovite-(Ce)", Can. Mineral., 38(4), 839-845 (2000).

[35] V.R. Vermeul, J.E. Szecsody, B.G. Fritz, M.D. Williams, R.C. Moore, and J.S. Fruchter, "An Injectable Apatite Permeable Reactive Barrier for In Situ ^{90}Sr Immobilization", Groundw. Monit. Remediat., 34(2), 28-41 (2014).

[36] J.F. Rakovan and J.D. Pasteris, "A Technological Gem: Materials, Medical, and Environmental Mineralogy of Apatite", Elements, 11(3), 195-200 (2015).

[37] D.M. Wellman, J.P. Icenhower, and A.T. Owen, "Comparative Analysis of Soluble Phosphate Amendments for the Remediation of Heavy Metal Contaminants: Effect on Sediment Hydraulic Conductivity", Environ. Chem., 3(3), 219224 (2006).

[38] R.C. Moore, C. Sanchez, K. Holt, P. Zhang, H. Xu, and G.R. Choppin, "Formation of Hydroxyapatite in Soils Using Calcium Citrate and Sodium Phosphate for Control of Strontium Migration", Radiochim. Acta, 92(9-11), 719-723 (2004).

[39] J.E. Szecsody, C.A. Burns, R.C. Moore, J.S. Fruchter, V.R. Vermeul, M.D. Williams, D.C. Girvin, J.P. Mckinley, M.J. Truex, and J.L. Phillips. Hanford 100-N Area Apatite Emplacement: Laboratory Results of Ca-Citrate-PO_4 Solution Injection and Sr-90 Immobilization in 100-N Sediments, Pacific Northwest National Laboratory Technical Report, PNNL-16891 (2007).

[40] S.H. Wallace, S. Shaw, K. Morris, J.S. Small, A.J. Fuller, and I.T. Burke, "Effect of Groundwater pH and Ionic Strength on Strontium Sorption in Aquifer Sediments: Implications for ^{90}Sr Mobility at Contaminated Nuclear Sites", Appl. Geochemistry, 27(8), 1482-1491 (2012).

[41] V.R. Vermeul, B.G. Fritz, J.S. Fruchter, J.E. Szecsody, and M.D. Williams. 100-NR-2 Apatite Treatability Test: High-Concentration Calcium-Citrate- Phosphate Solution Injection for In Situ Strontium-90 Immobilization, Pacific Northwest National Laboratory Technical Report, PNNL-19572 (2010).

[42] Sellafield Ltd. July 31 2017. "Corporate Strategy." GOV UK. Accessed Apr. 4 2022. Available from: https://www.gov.uk/government/publications/sellafield-ltd-corporate-strategy.

[43] Sellafield Ltd. April 1 2015. "Groundwater Monitoring at Sellafield: Annual Data Review 2016." GOV UK. Accessed Apr. 4 2022. Available from: https://www.gov.uk/government/publications/groundwater-monitoring-at-sellafield-2014-data-review.

[44] Sellafield Ltd. June 25 2021. "Leak Prevention or Minimisation." Game Changers-Challenges. Accessed Apr. 4 2022. Available from: https://www.gamech-

angers.technology/challenge/Leak_prevention_or_minimisation.

[45] L. Newsome, K. Morris, D. Trivedi, N. Atherton, and J.R. Lloyd, "Microbial Reduction of Uranium(VI) in Sediments of Different Lithologies Collected From Sellafield", Appl. Geochemistry, 51, 55-64 (2014).

[46] L. Newsome, K. Morris, S. Shaw, D. Trivedi, and J.R. Lloyd, "The Stability of Microbially Reduced U(IV); Impact of Residual Electron Donor and Sediment Ageing", Chem. Geol., 409, 125-135 (2015).

[47] T.J. Dichristina, "New Insights Into the Molecular Mechanism of Microbial Metal Respiration", Geochim. Cosmochim. Acta Suppl., 69(10), A670 (2005).

[48] L. Newsome, K. Morris, D. Trivedi, A. Bewsher, and J.R. Lloyd, "Biostimulation by Glycerol Phosphate to Precipitate Recalcitrant Uranium(IV) Phosphate", Environ. Sci. Technol., 49(18), 11070-11078 (2015).

[49] L. Newsome, A. Cleary, K. Morris, and J.R. Lloyd, "Long-Term Immobilization of Technetium via Bioremediation With Slow-Release Substrates", Environ. Sci. Technol., 51(3), 1595-1604 (2017).

[50] R.D. Shannon and C.T. Prewitt, "Effective Ionic Radii in Oxides and Fluorides", Acta Crystallogr. B. Struct. Sci. Cryst. Eng. Mater., B25, 925-946 (1969).

[51] Y. Fujita, G.D. Redden, J.C. Ingram, M.M. Cortez, F.G. Ferris, and R.W. Smith, "Strontium Incorporation Into Calcite Generated by Bacterial Ureolysis", Geochim. Cosmochim. Acta, 68(15), 3261-3270 (2004).

[52] C.L. Thorpe, C. Boothman, J.R. Lloyd, G.T.W. Law, N.D. Bryan, N. Atherton, F.R. Livens, and K. Morris, "The Interactions of Strontium and Technetium With Fe(II) Bearing Biominerals: Implications for Bioremediation of Radioactively Contaminated Land", Appl. Geochemistry, 40, 135-143 (2014).

[53] C.L. Thorpe, G.T.W. Law, C. Boothman, J.R. Lloyd, I.T. Burke, and K. Morris, "The Synergistic Effects of High Nitrate Concentrations on Sediment Bioreduction", Geomicrobiol. J., 29(5), 484-493 (2012).

[54] C.L. Thorpe, K. Morris, J.R. Lloyd, M.A. Denecke, K.A. Law, K. Dardenne, C. Boothman, P. Bots, and G.T.W. Law, "Neptunium and Manganese Biocycling in Nuclear Legacy Sediment Systems", Appl. Geochemistry, 63, 303-309 (2015).

[55] C.L. Thorpe, G.T.W. Law, J.R. Lloyd, H.A. Williams, N. Atherton, and K. Morris, "Quantifying Technetium and Strontium Bioremediation Potential in Flowing Sediment Columns", Environ. Sci. Technol., 51(21), 12104-12113 (2017).

[56] C.L. Thorpe, J.R. Lloyd, G.T.W. Law, H.A. Williams, N. Atherton, J.H. Cruickshank, and K. Morris, "Retention of ^{99m}Tc at Ultra-trace Levels in Flowing Column Experiments-Insights Into Bioreduction and Biomineralization for Remediation at Nuclear Facilities", Geomicrobiol. J., 33(3-4), 199-205 (2016).

[57] J.B. Duncan, "Reduction and Stabilization (Immobilization) of Pertechnetate to an Immobile Reduced Technetium Species Using Tin (II) Apatite", Sep. Sci. Technol. J., RPP-53855 (2012).

[58] C.I. Pearce, R.C. Moore, J.W. Morad, R.M. Asmussen, S. Chatterjee, A.R. Lawter, T.G. Levitskaia, J.J. Neeway, N.P. Qafoku, M.J. Rigali, S.A. Saslow, J.E. Szecsody, P.K. Thallapally, G. Wang, and V.L. Freedman, "Technetium Immobilization by Materials Through Sorption and Redox-Driven Processes: A Literature Review", Sci. Total Environ., 716, 132849 (2020).