Microbial reduction of uranium(VI) in sediments of different lithologies collected from Sellafield

Laura Newsome a,⇑, Katherine Morris a, Divyesh Trivedi b, Nick Atherton c, Jonathan R. Lloyd a,b

a Research Centre for Radwaste and Decommissioning and Williamson Research Centre for Molecular Environmental Science, School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Manchester M13 9PL, UK
b National Nuclear Laboratory, Chadwick House, Birchwood Park, Warrington WA3 6AE, UK
c Sellafield Ltd., Land Quality, Sellafield, Seascale, Cumbria CA20 1PG, UK

A R T I C L E   I N F O

Article history:
Available online 28 September 2014
Editorial handling by M. Kersten

A B S T R A C T

The presence of uranium in groundwater at nuclear sites can be controlled by microbial processes. Here we describe the results from stimulating microbial reduction of U(VI) in sediment samples obtained from a nuclear-licensed site in the UK. A variety of different lithology sediments were selected to represent the heterogeneity of the subsurface at a site underlain by glacial outwash deposits and sandstone. The natural sediment microbial communities were stimulated via the addition of an acetate/lactate electron donor mix and were monitored for changes in geochemistry and molecular ecology. Most sediments facilitated the removal of 12 ppm U(VI) during the onset of Fe(III)-reducing conditions; this was reflected by an increase in the proportion of known Fe(III)- and U(VI)-reducing species. However U(VI) remained in solution in two sediments and Fe(III)-reducing conditions did not develop. Sequential extractions, addition of an Fe(III)-enrichment culture and most probable number enumerations revealed that a lack of bioavailable iron or low cell numbers of Fe(III)-reducing bacteria may be responsible. These results highlight the potential for stimulation of microbial U(VI)-reduction to be used as a bioremediation strategy at UK nuclear sites, and they emphasise the importance of both site-specific and borehole-specific investigations to be completed prior to implementation.

© 2014 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/3.0/).

1. Introduction

Contamination of groundwater by aqueous uranium is a common problem at sites where mining, milling and reprocessing of uranium for nuclear fuel has taken place. In the UK, Sellafield is a nuclear reprocessing site in the north west of England (Fig. S1), with the largest contaminated land liability in the UK Nuclear Decommissioning Authority’s estate (Cruickshank, 2012). Uranium is known to be present as a contaminant in groundwater underlying the Sellafield site (McKenzie et al., 2011). In oxidising groundwaters uranium is generally present as the mobile uranyl ion (UO2 2+) or uranyl hydroxide complexes below pH 6.5 or as uranyl carbonate complexes at higher pH, whereas under reducing conditions, insoluble U(IV) predominates (Choppin et al., 2002; Newsome et al., 2014). Bioreduction, whereby indigenous soil bacteria are supplied with an electron donor which they oxidise coupled to reduction of aqueous U(VI) to insoluble U(IV), could offer a promising in situ remediation strategy to prevent further migration and dispersal of uranium and other radionuclides in groundwater.

Over twenty years of research has identified a diverse range of relatively common soil bacteria that are able to facilitate the enzymatic reduction of U(VI) to U(IV), mainly, but not limited to Fe(III)- and sulphate-reducing bacteria (Lovley and Phillips, 1992; Lovley et al., 1991; Newsome et al., 2014; Williams et al., 2013). Although abiotic reduction of U(VI) by Fe(II) minerals is possible e.g. Latta et al. (2012), Veeramani et al. (2011), most studies show that under environmental conditions the dominant mechanism is direct enzymatic reduction (Bargar et al., 2013; Law et al., 2011; Singer et al., 2012; Williams et al., 2013, 2011). The form of microbialy reduced U(IV) is often stated to be uraninite e.g. Suzuki et al. (2002), however, more recently another form, commonly termed monomeric U(IV), has been identified e.g. Bernier-Latmani et al. (2010), Kelly et al. (2008). Field trials stimulating microbial reduction of U(VI) in the subsurface have successfully demonstrated uranium immobilisation over periods of up to a year, although the long-term stability of biogenic U(IV) in situ warrants further investigation (Anderson et al., 2003; Bargar et al., 2013; Tang et al., 2013a,b; Williams et al., 2011).

Laboratory microcosm experiments using sediments representative of the Sellafield and Dounraey nuclear facilities, and a sediment sample from the Low Level Waste Repository have
demonstrated the potential for bioreduction of U(VI) as a remediation technique (Begg et al., 2011; Law et al., 2011; Wilkins et al., 2007). To date, there are no data on U(VI) behaviour in sediments from the Sellafield site. The geology underlying Sellafield is complex, with a varying thickness of made ground underlain by a mixture of Quaternary glacial outwash deposits and till, channel sands and gravels (drift deposits), and Sherwood Sandstone (Smith and Cooper, 2004). The Sherwood Sandstone also outcrops at certain locations on site. It is a major aquifer, with the regional groundwater flow southwest towards the Irish Sea. Groundwater in the Sherwood Sandstone is probably in hydraulic continuity with the overlying drift deposits, which have more variable groundwater flow directions. In certain areas of the site there are localised bodies of perched groundwater (Cruickshank, 2012; McKenzie and Armstrong-Pope, 2010). Contaminant flow pathways vary across the site due to the complex heterogeneity of the subsurface (Hunter, 2004).

The aim of this work was to assess the potential for microbial U(VI) reduction within these different lithological units. Here we present the results from biostimulated U(VI)-reduction experiments using unique on-site sediments obtained from a Sellafield site drilling programme and covering a range of different lithologies underlying the site. In brief, sediments were incubated with an artificial groundwater representative of the site, and containing acetate and lactate as electron donors to stimulate microbial U(VI) reduction. Changes in geochemistry were monitored. Generally, the indigenous microbial community could facilitate the removal of 12 ppm (50 L) U(VI) from solution through reduction to U(IV). Some variations in the rate and extent of U(VI) reductive precipitation were observed, and the reasons for this are explored further.

2. Materials and methods
2.1. Sediments

A range of sediments from the 2009 to 2010 Sellafield site drilling programme which were either non-active or had very low levels of radionuclides were shipped to The University of Manchester for analysis. Samples were incubated in triplicate in sterile glass serum bottles with a 1: 10 ratio with sterile anaerobic artificial groundwater representative of the Sellafield area and composed of (g/l) KCl, 0.0066; MgSO4·7H2O, 0.0976; MgCl2·6H2O, 0.081; CaCO3, 0.1672; NaNO3, 0.0275; NaCl, 0.0094; NaHCO3, 0.2424 [Wilkins et al., 2007]. Electron donors were also supplied as 5 mM acetate and 5 mM lactate, while control bottles contained no added electron donor. Uranium as U(VI) in 0.001 M HCl was added to the microcosms to give a final concentration of 12 ppm, representative of elevated concentrations of uranium reported in Sellafield site groundwaters (McKenzie et al., 2011). The bottles were crimp sealed with butyl rubber caps, the headspace purged with N2, and the experiments incubated in the dark at room temperature over three months. In an additional experiment, one replicate set of sediment was stored at 10 °C to represent the average temperature of UK groundwater, in order to investigate the impact of reduced temperature on the rate of microbial U(VI) reduction.

Sediment slurry was extracted at set time points using N2 flushed syringes and aseptic technique. An aliquot was immediately added to 0.5 N HCl for analysis of Fe(II) as a fraction of total bioavailable iron (Lovley and Phillips, 1987). Porewaters were then separated via centrifugation (14,800g) and monitored for U(VI) by the bromo-PADAP assay (Johnson and Florence, 1971), nitrite (Harris and Mortimer, 2002), pH and Eh. Surplus porewaters and sediment pellets were frozen at –80 °C for later analysis of nitrate and sulphate via ion chromatography ( Dionex ) or microbial community composition via pyrosequencing (see below).

2.2. Stimulation of microbial U(VI) reduction

Sediments were incubated in triplicate in sterile glass serum bottles with a 1: 10 ratio with sterile anaerobic artificial groundwater representative of the Sellafield area and composed of (g/l) KCl, 0.0066; MgSO4·7H2O, 0.0976; MgCl2·6H2O, 0.081; CaCO3, 0.1672; NaNO3, 0.0275; NaCl, 0.0094; NaHCO3, 0.2424 [Wilkins et al., 2007]. Electron donors were also supplied as 5 mM acetate and 5 mM lactate, while control bottles contained no added electron donor. Uranium as U(VI) in 0.001 M HCl was added to the microcosms to give a final concentration of 12 ppm, representative of elevated concentrations of uranium reported in Sellafield site groundwaters (McKenzie et al., 2011). The bottles were crimp sealed with butyl rubber caps, the headspace purged with N2, and the experiments incubated in the dark at room temperature over three months. In an additional experiment, one replicate set of sediment was stored at 10 °C to represent the average temperature of UK groundwater, in order to investigate the impact of reduced temperature on the rate of microbial U(VI) reduction.

Sediment slurry was extracted at set time points using N2 flushed syringes and aseptic technique. An aliquot was immediately added to 0.5 N HCl for analysis of Fe(II) as a fraction of total bioavailable iron (Lovley and Phillips, 1987). Porewaters were then separated via centrifugation (14,800g) and monitored for U(VI) by the bromo-PADAP assay (Johnson and Florence, 1971), nitrite (Harris and Mortimer, 2002), pH and Eh. Surplus porewaters and sediment pellets were frozen at –80 °C for later analysis of nitrate and sulphate via ion chromatography ( Dionex ) or microbial community composition via pyrosequencing (see below).

2.3. X-ray absorption spectroscopy (XANES)

Uranium speciation in samples of microbially-reduced clay (RB23) and gravelly sand (RB27) frozen back at day 90 was analysed at the DIAMOND Lightsource, Harwell, UK on Beamline IS16. U K-edge spectra were collected in fluorescence mode using a 9 element Ge detector (Dent et al., 2009) with samples loaded in the cryostat. Data were calibrated, background subtracted and nor-

Table 1

| Sediment description, depth and year of excavation | Dark brown SAND and GRAVEL (RB10) | Red brown clayey SANDY SLIT (RB18) | Friable brown gravelly CLAY (RB23) | Brown fine SAND (RB24) | Brown GRAVELLY SAND (RB27) | Brown SANDSTONE (IS16) | Red brown SAND and GRAVEL (IS18) |
|-----------------------------------------------|----------------------------------|-----------------------------------|-------------------------------|------------------------|-------------------------|------------------------|-------------------------------|
| pH                                            | 8.38 ± 0.03                      | 8.36 ± 0.03                       | 5.94 ± 0.03                   | 8.55 ± 0.07            | 8.31 ± 0.03             | 5.05 ± 0.01             | 8.56 ± 0.09                   |
| TOC (%)                                       | 0.35                             | 0.43                              | 2.1 ± 0.045                   | 0.46                   | 0.75 ± 0.11             | 0.27                   | 0.29                         |
| BET (m²/g)                                    | 3.5                              | 5.91                              | 5.43                          | 2.22                   | 5.66                    | 3.51                   | 5.00                         |
| Ca (%)                                        | 1.23                             | 0.377                             | 0.57                          | 0.38                   | 9.72                    | 0.066                  | 0.878                        |
| Mn (%)                                        | 0.117                            | 0.08                              | 0.119                         | 0.075                  | 0.079                   | 0.009                  | 0.121                        |
| Fe (%)                                        | 4.46                             | 2.99                              | 3.42                          | 2.32                   | 2.52                    | 0.666                  | 3.72                         |
| P (%)                                         | 0.073                            | 0.102                             | 0.244                         | 0.065                  | 0.080                   | 0.044                  | 0.153                        |
| U (ppm)                                       | 3.5                              | 3.6                               | 3.4                           | 1.2                    | 3.9                     | 1.9                    | 3.9                          |
| Bioavailable Fe (%)                            | 0.0192 ± 0.003                   | 0.0307 ± 0.003                    | 0.230 ± 0.010                 | 0.0384 ± 0.009         | 0.158 ± 0.010           | 0.0074 ± 0.001           | 0.138 ± 0.022                 |
| Mineralogy (XRD)                              | Quartz Clinochlore               | Muscovite Albite                  | Quartz Clinochlore            | Quartz Clinochlore     | Quartz Clinochlore      | Quartz Calcite           | Quartz Clinochlore           |
|                                               | Muscovite Albite                 | Orthoclase Pyrite                 | Muscovite Albite              | Muscovite Albite       | Muscovite Albite        | Muscovite Albite         | Muscovite Albite            |
malised for drift to a standardised E0 position using ATHENA (Ravel and Newville, 2005). Linear combination fitting was performed on XANES spectra compared to data from the Actinide Reference Database for Spectroscopy (Scheinost et al., 2013) for U(IV) as uraninite (Opel et al., 2007) and U(VI) as a uranyl carbonate complex (Rossberg et al., 2009). EXAFS was not performed due to the low, but environmentally-relevant, concentrations of uranium included in the experiment.

2.4. Molecular ecology

To compare changes in the microbial community during development of U(VI) reducing conditions, DNA was extracted from two sediments (clay RB23 and gravely sand RB27) at the start of the experiment and after approximately 90 days incubation. DNA was extracted from soil/sediment samples (200 μl) using a PowerSoil DNA Isolation Kit (MO BIO Laboratories INC, Carlsbad, CA, USA). The 16S-23S rRNA intergenic spacer region from the bacterial RNA operon was amplified using primers ITSf and ITSreub as described previously (Cardinale et al., 2004). The amplified PCR (polymerase chain reaction) products were separated by electrophoresis in Tris–acetate–EDTA gel. DNA was stained with ethidium bromide and viewed under short-wave UV light. Positive microbial community changes identified by RISA (ribosomal intergenic spacer analysis) justified further investigation by 16S rRNA gene sequencing.

A pyrosequencing methodology was then applied to investigate the microbial diversity within the samples. Isolated DNA from each sample was subjected to PCR amplification of the V1–V2 hypervariable region of the bacterial 16S rRNA gene, using universal bacterial primers 27F (Lane, 1991) and 338R (Hamady et al., 2008). The primers were synthesised by IDT dna (Integrated DNA Technologies, BVBA, Leuven, Belgium) and their design was based on Roche’s guidelines for one way amplicon sequencing with the 454 Life Sciences GS Junior system. The fusion forward primer (5’-CCATCTCACTTCCGTGCTCAAGNNNNNNNNNAGACGGTTGATGATGCTACG-3’) contained the 454 Life Sciences “Lib-I Primer A”, a 4 base “key” sequence (TCAG), a unique ten-base multiplex identifier MID sequence for each sample (NNNNNNNNNNN), and bacterial primer 27F. The reverse fusion primer (5’-CCTATCCCATGTGGATCCGACTTCAGNNNNNNNNNAGACGGTTGATGATGCTACG-3’) contained the 454 Life Sciences “Lib-I Primer B”, a 4 base “key” sequence (TCAG), and bacterial primer 338R. The PCR amplification was performed in 50 μl volume reactions using 0.4 μl (2.0 units) FastStart High Fidelity DNA polymerase (Roche Diagnostics GmbH, Mannheim, Germany), 1.8 mM MgCl2, 200 μM of each dNTP, 0.8 μM of each forward and reverse fusion primers, and 2 μl of DNA template (8.6–11.0 ng DNA per reaction). The PCR conditions included an initial denaturing step at 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and a final elongation step at 72 °C for 5 min. PCR products were then loaded into an agarose gel, and following gel electrophoresis, bands of the correct fragment size (approximately 410 base pairs) were excised, cleaned up using a QIAquick gel extraction kit (QIAGEN, GmbH, Hilden, Germany), and eluted in 30 μl of DNAse free H2O. The cleaned up PCR products were quantified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA), and pooled so that the mixture contained equal amounts of DNA from each sample. The emulsion emPCR and the pyrosequencing run were performed at the University of Manchester sequencing facility, using a 454 Life Sciences GS Junior system (Roche).

The 454 pyrosequencing reads were analysed using Qime 1.6.0 release (Caporaso et al., 2010). Low quality reads (mean quality score less than 25) and short sequences (less than 100 base pairs) were discarded, and both forward and reverse primers were removed from further analysis. Denoising and chimera removal was performed during OTU picking (at 97% sequence similarity) with USEARCH (Edgar, 2010) in Qime, and a representative sequence for each OTU was identified. Taxonomic classification of all reads was performed in Qime using the Ribosomal Database Project (RDP) at 80% 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.

2.5. Sequential extractions

Sequential extractions were performed on sediment samples to provide insight into the mineral phases that Fe and microbially-reduced U(IV) were present in. The modified BCR sequential extraction procedure was used (Rauret et al., 1999). Briefly the extraction steps included 0.11 M acetic acid “exchangeable”, 0.5 M hydroxylamine hydrochloride “reducible”, 8.8 M hydrogen peroxide and 1.0 M ammonium acetate “oxidisable”, and aqua regia “residual” fractions. The Fe-extractions were performed on 0.5 g of sediment. For the U-extractions, 0.5 g of microbially-reduced sediment slurry was used, and the first two extractions were performed under anaerobic conditions (Keith-Roach et al., 2003). Extracts were diluted in 2% nitric acid and analysed for uranium via ICP-MS (Agilent 7500CX).

2.6. Enrichment culture

To enrich for Fe(III)-reducing bacteria present in the clay sediment, 1 ml of microbially reduced sediment slurry was inoculated into 100 ml of anaerobic sterile freshwater minimal medium (Lovley et al., 1991; Thorpe et al., 2012) at pH 7, with 5 mM acetate and 5 mM lactate as an electron donor, and 15 mmol per litre ferrihydrite as the electron acceptor (Cornell and Schwertmann, 2006; Schwertmann and Cornell, 2000). The proportion of Fe(II) in the experiment was monitored using the ferrozine assay (Lovley and Phillips, 1987). When the Fe(II) had reached 30% or greater (usually occurring between two and four weeks of incubation), 1% of the enrichment culture was inoculated into the next batch of freshwater minimal medium. This was repeated until the thirteenth enrichment. From each enrichment culture, the DNA was extracted and the microbial diversity assessed using RISA (as above). The microbial diversity of the first, eighth and thirteenth enrichments was investigated by pyrosequencing (as above). The identity of the reduced Fe(II) mineral was characterised using XRD and ESEM.

To investigate whether sediments IS16 (sandstone) and IS18 (sand and gravel) contained sufficient bioavailable Fe to support a robust population of Fe(III)-reducing bacteria, an aliquot of the Fe(III)-reducing enrichment culture (1%) was added to sediment microcosms with no added U(VI). These were then monitored for changes in Fe(II)/Fe(III) over 100 days. Due to the low, but environmentally-relevant, concentrations of uranium included in the experiment, EXAFS was not performed due to the low, but environmentally-relevant, concentrations of uranium included in the experiment.

2.7. Most probable number (MPN) enumerations

Most probable number (MPN) enumerations were used to assess the abundance of Fe(III)-reducing bacteria in sediments RB23 (clay), RB27 (gravely sand), IS16 (sandstone) and IS18 (sand and gravel); Here, 1 g of sediment was added to 10 ml anaerobic
sterile freshwater minimal medium at pH 7 in triplicate, with 3.9 mM nitrilotriacetic acid (as the trisodium salt), 2 mM acetate as the electron donor and approximately 4 mmol per litre ferricyanide as the electron acceptor. Serial dilutions were performed by adding 1 ml of the slurry to 9 ml of the medium, until a dilution factor of 10⁻⁷ was reached. Fe(III) reduction was monitored using the ferrozine assay (Lovley and Phillips, 1987) after 7 and 11 weeks incubation, parallel to reduction times seen in sediment incubations. MPN estimates were then made using published MPN tables (Man, 1983).

3. Results

3.1. Characteristics of Sellafield sediment samples

Prior to assessing the potential for microbial U(VI) reduction, the sediment samples were characterised using a range of techniques. The seven sediment samples comprised mainly silicate minerals, namely quartz, mica, chlorite and feldspar (Table 1). Two sediments contained calcite, and three contained pyrite. Lithology ranged from clay and silt, to sand and gravels, broadly representative of glacial till and the buried channel and fluvio-glacial units. One sample (IS16) was of the upper sandstone bedrock. The mean (±1 SD) composition of the seven soils determined by XRF was: Ca 1.89% (3.5), Mn 0.086% (0.04), Fe 2.87% (1.2), P 0.11% (0.07), U 3.06 ppm (1.1), by total organic carbon analysis was 0.84% (0.74), total bioavailable Fe by ferrozine assay was 0.11% (0.07), U 3.2. Microbial U(VI) reduction

To determine whether the range of sediments and their extant microbial communities underlying Sellafield were able to support microbial reduction of U(VI), samples of different lithology were incubated with added electron donors for approximately 90 days. In five of the seven incubations, U(VI) was removed from solution, and Fe(II) produced (Fig. 1). Sediments were observed to darken in colour and geochemical indicators demonstrated the development of progressively reducing conditions. An initial peak of nitrite was detected up to around 300 μM, representing reduction of the nitrate present in the artificial groundwater. Ion chromatographic analyses confirmed nitrate removal, and subsequent sulphate removal (Fig. S2). All microcosms remained around circumneutral pH throughout the incubation period. Final Eh measurements ranged from −79 to −153 mV. For the additional experiment incubated at 10 °C (RB27), a slight time lag before U(VI) reduction occurred compared to the 21 °C incubation (Fig. 1), but the overall extent of U(VI) and Fe(III) reduction was the same after 90 days. Control samples with no added electron donor generally did not exhibit U(VI) removal or Fe(III) reduction; nitrate and sulphate remained in solution and final Eh values were between +102 and +188 mV. The exception was the clay (RB23) control, in which after 90 days U(VI) nitrate and sulphate were completely removed from solution, Fe(II) was generated, and the Eh was −116 mV. In contrast U(VI) remained in solution in two of the electron donor stimulated sediment incubations over the 90 day incubation period; these were the sandstone (IS16) and the sand and gravel (IS18) (Fig. S3). The colour remained orange-brown and the final Eh measurements of +89 and +143 mV indicated that bioreducing conditions did not develop. Possible reasons for this were investigated further using sequential extractions and adding an inoculum of the Fe(III)-reducing Sellafield enrichment culture, to determine if Fe(III) reduction was feasible in these sediments (see below).

XANES spectra were collected for two sediments to identify uranium speciation after 90 days incubation. Both the clay (RB23) and gravelly sand (RB27) samples had a very similar shape to the U(IV) standard, with the same edge position and clearly lacking the post-edge “shoulder” of the U(VI) standard (Fig. 2). Linear combination fitting of these spectra indicated that 100% of the uranium in each sample was present as U(IV).

3.3. Molecular ecology

A rapid molecular profiling approach (gel electrophoresis of the PCR amplified products of the bacterial 16S-23S rRNA intergenic
than doubled, as did sequences of genera belonging to the Bacteroidales, Sphingobacteriales (Flavisolibacter), Clostridiales, Rhodospirillales (Azospirillum), Burkholderiales (Limnospirillum, Polaromonas, Rhodoferax), Hydrogenophila, Desulfitomonas (Geobacter) and Sporochlaetales (Sporochlaeta) bacterial orders. Of these, Clostridiales, Burkholderiales and Desulfitomonas contain known U(VI)- or Fe(III)-reducers (Finneran et al., 2003; Gao and Francis, 2008; Lovley et al., 1991).

The gravelly sand sediment (RB27) was characterised by a large increase in sequences belonging to the Pseudomonales order (from 11.6% to 65.3%), with most of these being closely affiliated to Pseudomonas peli strain R-20805 isolated from a nitrifying inoculum (Table 2, Fig. S5). Other bacterial orders containing genera which increased by a factor of two or more were Bacteroidiales, Neisseriales (Vogesella), Desulfitomonas (Geobacter) and Alteromonadales (Shewanella), and within these were sequences affiliated to known U(VI)- and Fe(III)-reducing Geobacter (1.0%) and Shewanella (1.5%).

3.4. Sequential extractions

As the sandstone (IS16) and sand and gravel (IS18) were unable to generate microbial U(VI) reduction, one possible explanation is that they contained insufficient bioavailable Fe(III) to maintain an active Fe(III)- and consequently U(VI)-reducing microbial community. BCR sequential extractions were performed alongside XRF and 0.5 N hydroxylamine hydrochloride extractions to gain insight into the operationally defined sediment associations of Fe in IS16 and IS18 compared to two sediments which successfully removed U(VI) from solution; the clay (RB23) and gravelly sand (RB27). The proportion of Fe in the four sediments, measured via aqua regia digestion, ranged from 0.1% to 1.8%; similar to but slightly lower than the results obtained by XRF, probably due to refractory Fe present in the mineral lattices of clays not dissolving with aqua regia. The results for total bioavailable iron measured using the ferrozine assay were broadly comparable to the sequential extraction reducible fraction, ranging from 0.07 to 2.3 milligrams per gram of sediment. While the sand and gravel (IS18) contained similar concentrations of total Fe and "bioavailable" Fe to the clay (RB23) and gravelly sand (RB27), the concentration in the sandstone (IS16) were much lower (Fig. 4).

An additional set of sequential extractions were performed on the clay (RB23) and gravelly sand (RB27) to investigate which sediment fraction microbially reduced U(IV) was associated with. In the clay, most uranium was found in the reducible (38% ± 2.0%) and oxidisable (50% ± 7.6%) fractions, while in the gravelly sand, most uranium was in the exchangeable (41% ± 3.7%) and reducible (44% ± 19%) fractions (Fig. 5). This suggests that microbially reduced U(IV) may partition to a more recalcitrant fraction in the clay compared to the gravelly sand.

3.5. Enrichment culture

An Fe(III)-reducing enrichment culture was generated from the clay sediment. This enrichment culture reduced more than 30% of bioavailable Fe(III) in the medium within 2–4 weeks, forming a golden coloured platy mineral. This was identified by XRD as the Fe(III)-bearing minerals siderite (FeCO₃) and vivianite (Fe₃(PO₄)₂·8H₂O) (Fig. 6).

RISA indicated that after eight subcultures, a relatively diverse microbial community remained present (Fig. S4). This was confirmed with the 16S rRNA gene sequencing results, with 582 sequences present in the eighth subculture (compared to 898 in the initial sample). A relatively stable microbial community had developed by the eighth enrichment subculture which remained broadly similar at the thirteenth subculture (Fig. 7 and Table 3).
| Number of clones | % of clones | Closest phylogenetic relative | Accession number | ID similarity (%) | Environment |
|------------------|------------|-----------------------------|------------------|-----------------|-------------|
| RB23 day 0       |            | Uncultured bacterium clone  | QJ384246.2       | 98              | Elevated atmospheric CO₂/warmed Antarctic soils/methane oxidisers |
|                  |            | NC42e10_14934               |                  |                 | Elevated atmospheric CO₂/anaerobic cellulose degraders/rice paddy soil |
|                  |            | Bacterium Ellin643          | DQ075307.1       | 100             | Elevated atmospheric CO₂/activated sludge/drinking water treatment |
|                  |            | Uncultured Rhodospirillaceae bacterium | EF018478.1 | 99 | Cellulosic waste/elevated CO₂ soils/Antarctic soils |
|                  |            | Uncultured bacterium clone WC1.a1 | GQ263698.1 | 98 | Cellulosic waste/elevated CO₂ soils |
| RB23 day 90      |            | Azospirillum sp. 7C         | AF411852.1       | 97              | Nitrogen-fixing bacteria from rhizosphere/fuel contaminated Antarctic soils |
|                  |            | Type strain Pseudoalteromonas caeni strain SB1 | NR_042216.1 | 97 | Activated sludge/anaerobic digester/organics degrader/drinking water treatment |
|                  |            | Flaviscolleibacter ginsengisoli | NR_041500.1 | 97 | Ginseng cultivating soil/organics degrader/rhizosphere |
|                  |            | Uncultured bacterium clone WC2.31 | JF395212.1 | 99 | Microbial community response to U(VI) bioremediation/organics degrader |
| RB27 day 0       |            | Algoriphagus terrigena strain DS-44 | NR_043616.1 | 99 | Korean soil |
|                  |            | Thiobacillus denitrificans ATCC 25259 | NR_047417.1 | 97 | Chemolithotrophic, facultatively anaerobic bacterium. Acetate amended soils/thiosulphate or sulphur oxidiser/acid mine draining/PCB contaminated soil |
|                  |            | Type strain Pseudomonas mobilis | NR_042543.1 | 100 | Degrader of chlorosalicylates or isopimaric acid |
|                  |            | Type strain Polaromonas sp. JS666 | NR_047257.1 | 99 | Microbial community response to U(VI) bioremediation/anaerobic digester/drinkling water treatment/snow/glaciers |
|                  |            | Thiobacillus thiophilus strain D24TN | NR_044555.1 | 97 | Chemolithotrophic, thiosulphate-oxidising bacterium. Acetate amended soils/hydrocarbon bioremediation |
| RB27 day 96      |            | Type strain Pseudomonas peli strain R-20805 | NR_042451.1 | 99 | Nitrifier/organic degrader/nitrate reducer |
|                  |            | Type strain Pseudomonas peli strain R-20805 | NR_042451.1 | 99 | Nitrifier/organic degrader/nitrate reducer |
|                  |            | Type strain Pseudomonas peli mobilis | NR_042451.1 | 100 | Degrader of chlorosalicylates or isopimaric acid |
|                  |            | Type strain Pseudomonas peli mobilis | NR_042451.1 | 96 | Nitrifier/organic degrader/nitrate reducer |
|                  |            | Pseudomonas sagittaria sp. nov. | JQ277453 | 100 | Siderophore producer/oil or herbicide contaminated soil/phenol degrader/magnetite mine drainage |

These represent bacteria assigned to different operational taxonomic units, but each are most closely related to *Pseudomonas peli* strain R-20805.

Fig. 4. Profile of Fe in sediments which were able to facilitate U(VI) bioreduction (RB23, RB27) and were not able to (IS16, IS18), as determined by sequential extraction. Bars represent the average of triplicate samples, error bars are +/-1 standard deviation.

Fig. 5. Profile of bioreduced U in clay and gravelly sand sediments, as determined by sequential extraction. Bars represent the average of triplicate samples, error bars are +/-1 standard deviation.
with 41–46% of sequences closely related to four species of *Thauera*, *Geobacter*, *Sporotalea* (reassigned as *Pelosinus* [Moe et al., 2012]), *Acidovorax* and *Simplicispira*; genera found in anaerobic environments and typically associated with organic degradation or Fe(III)-reduction (Fig. S6).

An alternative explanation for some sediments not being able to support stimulated microbial reduction of U(VI) is that the extant microbial community did not contain viable Fe(III)-reducing bacteria. Results showed that adding an inoculum of enrichment culture to sediment microcosms of the sandstone (IS16) and sand and gravel (IS18) increased the extent of Fe(III)-reduction compared to controls without the added inoculum and Eh data were consistent with this interpretation (Fig. S7). As the sequential extractions demonstrated that the sand and gravel (IS18) contained comparable amounts of bioavailable iron to the sediments which did stimulate U(VI)-reduction, it is possible that this sediment lacks an active Fe(III)-reducing microbial community. Given the low concentration of bioavailable iron in the sandstone (IS16), it is not surprising that when the proportion of sediment was increased and an enrichment culture added, some Fe(III)-reduction could be measured. This indicates that the lack of bioavailable Fe(III) and Fe(III)-reducing bacteria in this sediment might preclude U(VI)-reduction from taking place, unless manipulated through the addition of electron acceptor (Fe(III) or sulphate) and/or microbial inocula.

### 3.6. Most probable number enumerations

After 11 weeks of incubation, approximately $1 \times 10^5$ – $1 \times 10^7$ cells of Fe(III)-reducing bacteria per gram of sediment were measured in the sediments which had previously been shown to reduce U(VI) (Table 4). As expected from biogeochemical measurements, this exceeded the numbers present in the sediments which could not reduce U(VI), with approximately $1 \times 10^4$ cells per gram of sandstone (IS16) and sand and gravel (IS18).

### 4. Discussion

Sellafield site sediments representing a range of different lithologies underlying the site have been characterised in terms of their potential to develop reducing conditions and consequently remove U(VI) from solution. The indigenous microbial community in most of the sediments was able to reduce U(VI) to U(IV) and remove it from solution over approximately 90 days after stimulation with added electron donor, including at 10°C representative of UK groundwater temperatures. The clay sediment (RB23) contained sufficient indigenous and labile organic matter to stimulate microbial U(VI) reduction without addition of an electron donor.

As expected, over the incubation period a cascade of anaerobic redox processes was observed in most of the sediments, as the available nitrate was depleted quickly (within 1 week) followed by periods of Fe(III)- and sulphate reduction. In addition, within the identified 16S rRNA sequences, there were sequences closely related to known nitrogen-fixing (*Azospirillum*) nitrifying (*Pseudomonas*), and Fe(III)-metal-reducing (*Geobacter, Shewanella*, *Rhodotherax*) genera as well as a number of bacteria closely related to those known to degrade a range of organic compounds. Thus both geochemical and molecular ecology results indicate clearly that the indigenous microbial population in the majority of these Sellafield sediments have the capacity to utilise a wide range of electron donors and acceptors and carry out diverse biogeochemical processes.

Regarding U(VI) reduction, it is noteworthy that during the incubation period the proportion of sequences related to known U(VI)-reducing bacterial genera more than doubled, including *Geobacter* and *Shewanella* (Lovley et al., 1991), albeit they remained in low abundances (1–3%). It is not clear whether U(VI) reduction was carried out by these microorganisms or by more dominant members of the microbial communities, since many of the sequences of this study were not closely related to cultured organisms with known physiological properties. Moreover, in the gravelly sand sediment there was a significant enrichment of sequences closely related to *P. peli* after incubation (Fig. 3 and Table 2). Although *Pseudomonas* is known predominately as a facultative anaerobic denitrifying genus, sequences related to *Pseudomonas stutzeri* dominated similar U(VI) bioreduction experiments established with soil from the nearby Low Level Waste Repository site (Wilkins et al., 2007). Future anaerobic microcosm studies should explore in more detail the potential of these indigenous microbial communities for the reduction of U(VI) and other radionuclides.

Important differences in sediment composition include the clay content, and the amount of total organic carbon and bioavailable Fe(III) present. Although calcium may inhibit microbial U(VI) reduction through the formation of stable Ca-uranyl-carbonate complexes (Brooks et al., 2003), this effect was not observed in these sediments. That is, while calcium was present in the artificial...
extractions indicate that microbially reduced U(IV) partitioned to a tron donor (Fig. 2). Furthermore, results from the sequential phase was present entirely as U(IV) in the sample with added ele-
et al., 2011) and XANES data confirmed that uranium in the solid materials. As other geochemical indicators clearly demonstrated development of reducing conditions (e.g. Fe(II) ingrowth, and nitrate and sulphate reduction) it is likely that the dissolved Fe(III) was also reduced. Indeed, reduction of U(VI) sorbed to soils has been observed previously (Begg et al., 2011; Law et al., 2011) and XANES data confirmed that uranium in the solid phase was present entirely as U(IV) in the sample with added electron donor (Fig. 2). Furthermore, results from the sequential extractions indicate that microbially reduced U(IV) partitioned to more recalcitrant phases of the clay compared to the gravelly sand. Relatively high concentrations of organic matter in the clay sediment (RB23) allowed for bio reducing conditions to be established even when no additional electron donor was supplied. Together this suggests that this clay could act as a natural attenuant of U(VI), through both sorption and, if the organic fraction is bioavail-
Department of Energy, 2011), perhaps suggesting that the influence of calcium on microbial U(VI) reduction is less important in natural soil systems compared to microbial pure cultures. restricting sediments, which may have contributed to their failure to the sandstone (IS16) and sand and gravel (IS18) sediment incu-
Table 3

| Number of clones | % of clones | Closest phylogenetic relative | Accession number | ID similarity (%) | Environment |
|------------------|-------------|-------------------------------|------------------|------------------|-------------|
| **First enrichment culture** | | | | | |
| 349 | 3.5 | Uncultured Acidobacteria bacterium clone 40L1_9 | QG342374.1 | 99 | Biostimulated U mining soil/drinking water treatment/ hydrocarbon contaminated aquifer/bioremediation of U(VI) or TCE/rice paddy soil |
| 281 | 2.8 | Geothrix fermentans | NR_036779.1 | 97 | Fe(III)-reducer/biostimulated U mining soil/rice paddy soil/drinking water treatment |
| 281 | 2.8 | Uncultured bacterium clone B061000128 | AB669001.1 | 99 | Rice paddy soils/acetate amended soils/activated sludge/Arctic sediments |
| 229 | 2.3 | Uncultured bacterium clone ORPFC-FW102–726d-55 | FJ451813.1 | 99 | In situ uranium bioremediation |
| 189 | 1.9 | Flavisolibacter ginsengisoi | NR_041500.1 | 97 | Ginseng cultivating soil/organics degrader/ rhizosphere |

**Eighth enrichment subculture**

| 1878 | 17.9 | Thauera sp. G3DM-88 | EU037291.1 | 99 | Landfill sediments contaminated with Cr/anaerobic digester/activated sludge/iron reducer/organic degrader |
| 1036 | 9.9 | Geobacter benedictensis strain Bem | NR_075007.1 | 99 | Fe(III)-reducer/rice paddy soil/acetate amended soils/wetlands/mining soils |
| 828 | 7.9 | Acidovorax deflavius strain BS411 | NR_026506.1 | 100 | Activated sludge/ammonium rich aquifer/wastewater or drinking water treatment |
| 542 | 5.2 | Simplicispira metamorpha strain DSM 1837 | NR_044941.1 | 99 | Activated sludge/anaerobic digester/water treatment/nitrifying chemostat/ammox |
| 300 | 2.9 | Type strain Pseudomonas peli strain R-20805 | NR_042451.1 | 99 | Nitrifier/organic degrader/nitrate reducer |

**Thirteenth enrichment subculture**

| 2029 | 15.9 | Thauera sp. G3DM-88 | EU037291.1 | 99 | Landfill sediments contaminated with Cr/anaerobic digester/activated sludge/iron reducer/organic degrader |
| 1981 | 15.6 | Uncultured bacterium clone B061000123 J14 (Geobacter) | AB666056.1 | 98 | Rice paddy soil |
| 1137 | 8.9 | Type strain Sporotalea propionica strain TmPN3 | NR_042513.1 | 97 | U(VI) removal by anaerobic microbial communities |
| 1116 | 8.8 | Acidovorax deflavius strain BSB411 | NR_026506.1 | 100 | Activated sludge/ammonium rich aquifer/wastewater or drinking water treatment |
| 783 | 6.2 | Simplicispira metamorpha strain DSM 1837 | NR_044941.1 | 99 | Activated sludge/anaerobic digester/water treatment/nitrifying chemostat/ammox |

* Sporotalea propionica has since been reassigned as Pelosinus propionicus comb. nov. (Moe et al., 2012).

**Table 4**

| Sediment | 7 weeks | 11 weeks |
|----------|---------|----------|
| Sand (RB10) | 7.4 × 10^4 | 2.4 × 10^6 |
| Clay (RB23) | 1.5 × 10^5 | 7.4 × 10^6 |
| Gravelly sand (RB27) | 1.5 × 10^5 | 9.3 × 10^4 |
| Sandstone (IS16) | 2.3 × 10^3 | 9.3 × 10^3 |
| Sand and gravel (IS18) | 9.2 × 10^2 | 2.4 × 10^4 |

groundwater (1.6 mM) and within the sediments (up to 9.7% Ca measured by XRF (Table 1)), complete U(VI) reduction was observed in most sediments after 90 days incubation. Indeed, microbial reduction of U(VI) as Ca-uranyl-carbonate complexes has also been observed in situ at the US DOE Rifle site (US Department of Energy, 2011), perhaps suggesting that the influence of calcium on microbial U(VI) reduction is less important in natural soil systems compared to microbial pure cultures. Sorption effects were observed in the clay (RB23) sediment incubations; after one hour just over half the added U(VI) had been removed from solution. As other geochemical indicators clearly demonstrated development of reducing conditions (e.g. Fe(II) ingrowth, and nitrate and sulphate reduction) it is likely that the dissolved Fe(III) was also reduced. Indeed, reduction of U(VI) sorbed to soils has been observed previously (Begg et al., 2011; Law et al., 2011) and XANES data confirmed that uranium in the solid phase was present entirely as U(IV) in the sample with added electron donor (Fig. 2). Furthermore, results from the sequential extractions indicate that microbially reduced U(IV) partitioned to more recalcitrant phases of the clay compared to the gravelly sand. Relatively high concentrations of organic matter in the clay sediment (RB23) allowed for bioreducing conditions to be established even when no additional electron donor was supplied. Together this suggests that this clay could act as a natural attenuant of U(VI), through both sorption and, if the organic fraction is bioavailable, microbial reduction. Although groundwater does not flow through clay strata, many soils contain a clay mineral component which may be able to offer some capacity for uranium retention.

In certain sediments, the paucity of bioavailable Fe(III) might preclude an active Fe(III)-reducing microbial community from developing and consequently being able to reduce U(VI), such as in the sandstone IS16. However, the ability to reduce U(VI) is not restricted to just Fe(III)-reducers, and this does not explain the lack of U(VI) reduction in the sand and gravel (IS18) as this sediment contained comparable amounts of bioavailable Fe(III) to the others. The issue of certain amendments working in some locations but not others, or in the laboratory but not in the field, is common and it is often difficult to determine the reasons why, especially in complex heterogeneous systems (Lovley, 2003). Evidence obtained by adding an active Fe(III)-reducing enrichment culture to the sandstone (IS16) and sand and gravel (IS18) sediment incubations, and also from MPN enumerations suggested that these sediments contain fewer Fe(III)-reducing bacteria than the U(VI)-reducing sediments, which may have contributed to their failure to reduce U(VI) in these experiments.

This work highlights the potential for stimulated microbial U(VI) reduction to be a suitable technique for treating uranium contaminated groundwater in situ, both at Sellafield, and given the range of different lithology sediments tested, at other UK nuclear sites. Should remediation of uranium in groundwater...
become a priority, further development of this work such as scale-up to columns prior to field deployment would clearly be warranted.

Acknowledgements

We thank Athanasios Rizoulis and Christopher Boothman (University of Manchester) for assistance with processing and interpretation of pyrosequencing data, Paul Lythgoe, Alastair Bewsher and John Waters (University of Manchester) and David McKendry (Manchester Metropolitan University) for analytical support with XRF, IC, XRD, BET and Leco Truspec, Haydn Haynes (University of Manchester) for help with monitoring the MNP samples and Nicholas Atherton and Julian Cruickshank (Sellafield Ltd) for providing soil samples. Beamtime at beamline B18 was funded by Grant SP8941-2 from the Diamond Light Source. We acknowledge financial support from the Nuclear Decommissioning Authority via a BNFL Fellowship, We also acknowledge financial support from the BIGRAD consortium (NE/H007768/1) and also via a BNFL Fellowship. We also acknowledge financial support from NERC via a CNRA fellowship sponsored by the Nuclear Decommissioning Authority via a BNFL Fellowship. We also acknowledge financial support from the Nuclear Decommissioning Authority via a CNRA fellowship sponsored by the Nuclear Decommissioning Authority via a BNFL Fellowship.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.apgeochem.2014.09.008.

References

Andersen, R.T., Vironis, H.A., Ortiz-Bernad, I., Resch, C.T., Long, P.E., Dayvault, R., Karp, K., Marutzky, S., Metzler, D.R., Peacock, A., White, D.C., Lowe, M., Lovley, D.R., 2003. Stimulating the in situ activity of Geobacter species to remove uranium from the groundwater of a uranium-contaminated aquifer. Appl. Environ. Microbiol. 69, 5884–5891.

ASTM, 2006. D4972-01 Standard test method for pH of soils. In: Annual Book of ASTM Standards. American Society for Testing and Materials, pp. 963–965.

Bargar, J.R., Williams, K.H., Campbell, K.M., Long, P.E., Stubbs, J.E., Suovura, E.I., Lezama-Pacheco, J.S., Alessi, D.S., Stylo, M., Webb, S.M., Davis, A., Gambhir, D.E., Blue, L.V., Bernier-Latmani, R., 2013. Uranium redox transition pathways in acetate-amended sediments. Proc. Natl. Acad. Sci. 110, 4506–4511.

Begg, J.D.C., Burke, I.T., Lloyd, J.R., Boothman, C., Shaw, S., Charnock, J.M., Morris, K., 2011. Bioreduction behavior of U(VI) sorbed to sediments. Geochim. Cosmochim. Acta 78, 160–171.

Bernier-Latmani, R., Veeramani, H., Hunter, J., 2004. SCLS Phase 1 – Conceptual model of Contamination below Ground at Sellafield. British Nuclear Fuels Ltd.

Johnson, D.A., Florence, T.M., 1971. Spectrophotometric determination of uranium(VI) with 2-(5-bromo-2-pyridylazo)-5-dimethylaminophenol. Anal. Chem. Acta 53, 73–79.

Keith-Roach, M.J., Morris, K., Dahlgaard, H., 2003. An investigation into techiniet binding in sediments. Mar. Chem. 81, 149–162.

Keith-Roach, M.J., Morris, K., Dahlgaard, H., 2003. An investigation into technetium binding in sediments. Mar. Chem. 81, 149–162.

Krumpa, N.A., Jones, C.P., Robbins, P.E., 2009. B18: a core XAS spectroscopy laboratory for in situ analysis of complex bacterial communities. J. Synchrotron Radiat. 16, 537–541.

Lamontagne, S., Goulet, A., McLean, Y., 2013. U(VI) bioreduction with emulsified vegetable oil as the electron donor. Geobiology 11, 175–185.

Lovelock, C.E., 1991. 16S/23S rRNA sequencing. In: Stackebrant, E., Goodfellow, M. (Eds.), Nucleic Acid Techniques in Bacterial Systematics. John Wiley & Sons Ltd, pp. 479–504.

Lovelock, D.R., 2003. Cleaning up with genomics: applying molecular biology to bioremediation. Nat. Rev. Microbiol. 1, 35–44.

Lovelock, D.R., Phillips, E.J.P., 1987. Rapid assay for microbially reducible ferric iron in aquatic sediments. Appl. Environ. Microbiol. 53, 1536–1540.

Lovelock, D.R., Phillips, E.J.P., 1992. Reduction of uranium by Desulfovibrio desulfuricans. Appl. Environ. Microbiol. 58, 850–856.

Lovelock, D.R., Phillips, E.J.P., Gorby, Y.A., Landa, E.R., 1991. Microbial reduction of uranium in soil. Geochim. Cosmochim. Acta 55, 1555–1564.

Lovley, D.R., 2003. Uranium redox cycling in sediment and biomineral systems. Geochim. Cosmochim. Acta 67, 497–504.

Lovley, D.R., 2003. Uranium redox cycling in sediment and biomineral systems. Geochim. Cosmochim. Acta 67, 497–504.

Man, J.C., 1983. MPN tables, corrected. Eur. J. Appl. Microbiol. Biotechnol. 17, 301–305.

McKenzie, L., Armstrong-Pope, N., 2010. Sellafield Ltd., Groundwater Annual Report 2010. Sellafield Ltd.

McKenzie, H.M., Coughlin, D., Laws, F., Stamper, A., 2011. Uranium redox cycling in sediment and biomineral systems. Geochim. Cosmochim. Acta 79, 497–504.

Moe, W.M., Stebbing, R.E., Kao, J.U., Bowman, K.S., Noreen, M.F., da Costa, M.S., Rabiner, F.A., 2012. Petroleum hydrocarbon sp. nov., isolated from chlorinated solvent contaminated groundwater, emended description of the genus Pelosinus and transfer of Sporarosedicola poppinica to Pelosinus poppinica comb. nov. Int. J. Syst. Evol. Microbiol. 62, 1369–1376.

Newsome, L., Morris, K., Dahlgaard, H., 2004. Comparison of different primer sets for use in automated ribosomal intergenic spacer analysis of complex bacterial communities. J. Microbiol. Methods 5, 235–237.

Opel, K., Weiss, S., Hubener, S., Zanker, H., Bernhard, G., 2007. Study of the solubility of amorphous and crystalline uranium dioxide by combined spectroscopic methods. Radiochim. Acta 95, 143–145.

Rauret, G., López-Sánchez, J.F., Sauquillo, A., Rubio, R., Davidson, C., Ure, A., Quevauviller, P.F., Lopez-Sanchez, J., 1999. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. J. Environ. Monit. 1, 57–61.

Ravel, B., Newville, M., 2005. ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. J. Synchrotron Radiat. 12, 537–541.

Rossberg, A., Ulrich, K.-U., Weiss, S., Tsushima, S., Hiemstra, T., Scheinostt, A.C., 2009. Identification of uranyl surface complexes on ferrihydrite: advanced EXAFS data analysis and CD-MUSIC modeling. Environ. Sci. Technol. 43, 1400–1406.

Scheinost, A.C., Schmeisser, N., Banerjee, D., Denecke, M., Dardenne, K., Rothe, J., Daelem, R., 2013. AXAS – An Active Reference X-ray Absorption Spectroscopy Database [WWW Document]. URL https://www.hzdr.de/acxas.

Schwertmann, U., Cornell, R.M., 2000. Iron Oxides in the Laboratory. second ed. Wiley-VCH Verlag GmbH, Berlin, Germany.

Singer, D.M., Chatman, S.M., Ilton, E.S., Rosso, K.M., Banfield, J.F., Waychunas, G.A., 2012. U(VI) sorption and reduction kinetics on the magnetite (111) surface. Environ. Sci. Technol. 46, 3821–3830.

Smith, N., Cooper, S., 2004. QIIME Phase 1 – Sellafield Geofological Conceptual Model. Sellafield Ltd.

Suzuki, Y., Kelly, S.D., Kenner, K.M., Banfield, J.F., 2002. Uranium redox contamination: nanometre-size products of uranium bioreduction. Nature 419, 134.

Tang, G., Watson, D.B., Wu, W.-M., Schmidt, C.A., Baker, J.C., Brooks, S.C., 2013a. U(VI) bioavailability with emulsified vegetable oil as the electron donor – microcosm tests and model development. Environ. Sci. Technol. 47, 3209–3218.
Thorpe, C.L., Morris, K., Boothman, C., Lloyd, J.R., 2012. Alkaline Fe(III) reduction by a novel alkali-tolerant Serratia sp. isolated from surface sediments close to Sellafield nuclear facility, UK. FEMS Microbiol. Lett. 327, 87–92.

US Department of Energy, 2011. Review of the Natural Flushing Groundwater Remedy at the Old Rifle Legacy Management Site, Rifle, Colorado. LMS/RFO/507263.

Veeramani, H., Alessi, D.S., Suvorova, E.I., Lezama-Pacheco, J.S., Stubbs, J.E., Sharp, J.D., Dippon, U., Kappler, A., Bargar, J.R., Bernier-Latmani, R., 2011. Products of abiotic U(VI) reduction by biogenic magnetite and vivianite. Geochim. Cosmochim. Acta 75, 2512–2528.

Wilkins, M.J., Livens, F.R., Vaughan, D.J., Beadle, I., Lloyd, J.R., 2007. The influence of microbial redox cycling on radionuclide mobility in the subsurface at a low-level radioactive waste storage site. Geobiology 5, 293–301.

Williams, K.H., Long, P.E., Davis, J.A., Wilkins, M.J., Nguesan, A.L., Steefel, C.I., Yang, L., Newcomer, D., Spane, F.A., Kerkhof, L.J., McGuinness, L., Dayvault, R., Lovley, D.R., 2011. Acetate availability and its influence on sustainable bioremediation of uranium-contaminated groundwater. Geomicrobiol. J. 28, 519–539.

Williams, K.H., Bargar, J.R., Lloyd, J.R., Lovley, D.R., 2013. Bioremediation of uranium-contaminated groundwater: a systems approach to subsurface biogeochemistry. Curr. Opin. Biotechnol. 24, 489–497.