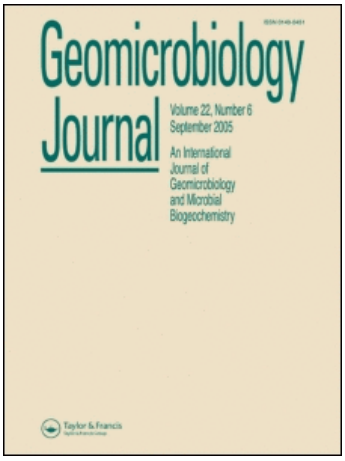

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Bioreduction Behavior of U(VI) Sorbed to Sediments

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It is well known that microbially mediated reduction can result in the removal of U(VI)$_{(aq)}$ from solution by forming poorly soluble U(IV) oxides; however, the fate of U(VI) already associated with mineral surfaces is less clear. Here we describe results from both oxic adsorption and anaerobic microcosm experiments to examine the fate of sorbed U(VI) during microbially mediated bioreduction. The microcosm experiments contained sediment representative of the nuclear facility at Dounreay, UK. In oxic adsorption experiments, uptake of U(VI) was rapid and complete from artificial groundwater and where groundwater was amended with 0.2 mmol l^{-1} ethylenediaminetetraacetic acid (EDTA) a complexing ligand used in nuclear fuel cycle operations. By contrast, uptake of U(VI) was incomplete in groundwaters amended with 10 mmol l^{-1} bicarbonate. Analysis of sediments using X-ray adsorption spectroscopy showed that in these oxic samples, U was present as U(VI). After anaerobic incubation of U(VI) labelled sediments for 120 days, microbially mediated Fe(III)- and SO_4^{2-}- reducing conditions had developed and XAS data showed uranium was reduced to U(IV). Further investigation of the unamended groundwater systems, where oxic systems were dominated by U(VI) sorption, showed that reduction of sorbed U(VI) required an active microbial population and occurred after robust iron- and sulfate- reducing conditions had developed. Microbial community analysis of the bioreduced sediment showed a community shift compared to the oxic sediment with close relatives of *Geobacter* and *Clostridium* species, which are known to facilitate U(VI) reduction, dominating. Overall, efficient U(VI) removal from solution by adsorption under oxic conditions dominated in unamended and EDTA amended systems. In all systems bioreduction resulted in the formation of U(IV) in solids.

Keywords uranium, speciation, bioreduction, sediments, sorbtion

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INTRODUCTION

Uranium is considered a problematic contaminant due to its expected mobility under oxic environmental conditions, toxicity to humans and widespread occurrence throughout the world at sites where nuclear fuel cycle operations have occurred. Thus, there are strong incentives to understand and control the behavior of uranium in contaminated environments. Under environmental conditions, uranium is present in two chemically stable forms; under oxic conditions the uranyl cation ($U(VI)O_2^{2+}$) dominates and under reducing conditions, insoluble $U(IV)O_2$ uraninite (solubility ca 10^{-17} M at pH > 4) dominates (Dozol and Hagemen 1993; Lovley et al. 1991). Under oxic conditions, the behavior of the uranyl cation is complex and dependent on a number of factors. It may interact strongly with sediment components and become sorbed to surfaces (Barnett et al. 2002; Dong et al. 2006; Jeon et al. 2005; Ortiz-Bernad et al. 2004). Alternatively, at circumneutral pH and where carbonate is present, it is likely to form relatively soluble anionic species such as $[UO_2(CO_3)_2]^{2-}$ (Clark et al. 1995).

The microbially mediated development of anoxia in sediments has a significant effect on aqueous U(VI) behavior with U(VI) reduction producing poorly soluble UO_2 that is retained on a wide variety of environmental materials (Gu et al. 2005; Lovley et al. 1991; Wilkins et al. 2007). These observations have led to the development of bioremediation as a treatment for subsurface uranium groundwater contamination. Here, an electron donor is added to the subsurface to promote bioreduction resulting in precipitation of UO_2 on sediments (Anderson et al. 2003; Wu et al. 2006). However, there is a paucity of information on the biogeochemical behavior of U(VI) when it is already adsorbed to sediments. Under certain conditions sediment associated U(VI) is reportedly recalcitrant to bioreduction (Jeon et al. 2005; Ortiz-Bernad et al. 2004) whilst under different conditions, bioreduction may occur (Dong et al. 2006; Kelly et al. 2008). Further complicating the fate of uranium in contaminated environments is its ability to form a range

of stable, soluble complexes with co-contaminant ligands such as ethylenediaminetetraacetic acid (EDTA) and CO_3^{2-}, which may decrease uranium sorption to mineral surfaces (Hass and Northrup 2004; Lloyd and Renshaw 2005).

Here, we investigate the behavior of uranium in the context of a saturated sediment representative of the Dounreay nuclear facility, U.K. To assess the effect of different groundwater compositions on the mobility of uranium under oxic conditions and during the development of microbial anoxia, UO_2^{2+} sorption and redox behavior was examined in sediment microcosms made up with a representative Dounreay groundwater that was: (i) *unamended*; (ii) *carbonate amended* (10 mmol l^{-1}); and (iii) *EDTA amended* (0.2 mmol l^{-1}). The EDTA amendment was representative of EDTA levels at nuclear facilities (Hansen et al. 2001). Throughout, X-ray absorption spectroscopy (XAS) and sequential extraction approaches were used to further characterise solid phase uranium interactions.

MATERIALS AND METHODS

Near surface sediment (~40 cm depth) was collected in the vicinity of the UKAEA Dounreay site (LAT: 58° 34′ 40″ N, LONG: 3° 44′ 50″ W). The sediment was sampled into sterile plastic containers and stored at 4°C prior to use in microcosms.

Uranyl Adsorption under Oxic Conditions

To assess the ability of Dounreay sediments to remove uranium from solution in aerobic environments, time dependent adsorption of U(VI) to Dounreay sediment under oxic conditions was studied in the presence of either: (i) artificial groundwater (*oxic unamended*); (ii) artificial groundwater amended with 10 mmol l^{-1} HCO_3^- (*oxic carbonate amended*); (iii) artificial groundwater amended with 0.2 mmol l^{-1} sodium EDTA (*oxic EDTA amended*) systems.

In these experiments, which were performed in triplicate, the solid solution ratio was maintained at 100 g l^{-1} and experiments were spiked with UO_2^{2+} (as uranyl chloride) to a final concentration of 53 μmol l^{-1}. To maintain oxic conditions, experiments were performed in 250 ml conical flasks with vented stoppers and stirred gently (150 rpm) on an orbital shaker at 21° C in the dark. In previous studies (Begg et al. 2008; Burke et al. 2006; McBeth et al. 2007) these conditions were sufficient to fully reoxidize highly reducing sediments with resultant Eh values above +150 mV. Samples were extracted at time points, centrifuged (16000 *g*, 15 mins) and the supernatant analyzed for pH and Eh by standard electrodes. Aqueous U(VI) was determined by the BromoPADAP method of Johnson and Florence (1971). Briefly, in a 1 ml cuvette 100 μl of supernatant was mixed in order with 80 μl of CyDTA complexing agent, 80 μl buffer solution, 400 μl ethanol, 80 μl Bromo-PADAP indicator and 260 μl deionized water. The absorbance was determined on a CECIL CE 3021 spectrophotometer at 578 nm and compared to matrix matched calibration standards (linear $r^2 = 0.99$ or better). After 7 days equilibration, sequential extractions were performed on solid phases to examine the strength of association of adsorbed U(VI).

A restricted set of experiments (solid/solution ratio 50 g l^{-1} and 210 μmol l^{-1} UO_2^{2+}) were undertaken to provide several hundred mg kg^{-1} U samples necessary to allow further characterization of the redox state of uranium by X-ray absorption spectroscopy in *XAS oxic unamended*, *XAS oxic carbonate amended* and *XAS oxic EDTA amended* systems. These experiments were equilibrated under oxic conditions for 7 days, pH, Eh, and aqueous U(VI) monitored, solid phases separated for analysis by centrifugation (16,000 *g*, 15 mins), and samples mounted in air tight, triple contained cells and frozen (−°C) prior to analysis.

Uranium Bioreduction Microcosms

To investigate the behavior of uranium in Dounreay sediment during bioreduction by indigenous sediment microorganisms, microcosms were prepared in sterile, polypropylene tubes in quadruplicate under an argon atmosphere and with a solid solution ratio of 100 g l^{-1} and a UO_2^{2+} spike of 53 μmol l^{-1}. Systems were prepared with 0.2 μm filter sterilized: (i) unamended groundwater (*bioreduction unamended*); (ii) carbonate amended groundwater (*bioreduction carbonate amended*); and (iii) EDTA amended groundwater (*bioreduction EDTA amended*). Sealed tubes were stored in argon purged gas-tight jars to maintain anoxic conditions and incubated at 21°C in the dark. Bioreduction experiments were sampled sacrificially over a 120 day period. Prior to centrifugation, 0.4 ml of sediment slurry was removed for analysis of biogenic Fe(II) by extraction with 0.5 N HCl (Lovley and Phillips 1986). Samples were then centrifuged (10 mins 16,000 g) and the supernatant analyzed for a suite of biogeochemical redox indicators (Eh, pH, NO_3^-, $Fe_{(aq)}$ and SO_4^{2-}) as well as $U(VI)_{(aq)}$ (Lovley and Phillips 1986; Viollier et al. 2000; Johnson and Florence 1971). Sample manipulations and analyses were performed in an anaerobic cabinet as appropriate (Coy Laboratory Products Inc, MI). Finally, sequential extractions were carried out on selected samples to assess if bioreduction resulted in a change in the sediment association of uranium.

To obtain further information about the oxidation state of uranium during bioreduction, a restricted set of sediment microcosms were set up at selected time points for XAS analysis (solid/solution ratio 50 g l^{-1} and 210 μmol l^{-1} UO_2^{2+}). Samples were then incubated under anoxic conditions as described above, analyzed as appropriate for biogeochemical indicators and sampled at 30, 60 and 120 days (*XAS bioreduction unamended)* and 120 days (*XAS bioreduction carbonate amended; XAS bioreduction EDTA amended, XAS heat killed*). To further investigate the influence of both biotic and abiotic processes on the reduction of sorbed U(VI), a series of controls were prepared in the *unamended* groundwater system. Two samples were prereduced for 120 days until the development of sulfate reducing conditions. One of these samples was then sterilized by autoclaving at 120°C for 20 mins whilst the other sample remained

TABLE 1
Sequential Extraction Scheme (Tessier et al. 1979; Keith-Roach et al. 2003)

Leachate Composition	Leach Time
Supernatant	
1 M magnesium chloride (pH 7)	2 h
1 M sodium acetate, adjusted to pH 5 with acetic acid	5 h
0.1 M ammonium oxalate, adjusted to pH 3 with HCl	12 h
30% hydrogen peroxide, adjusted to pH 2–3 with HNO_3, heated (80°C) until dryness. Then leached with 1 M ammonium acetate in 6% (v/v) HNO_3 (pH 2)	24 h/6 h
70% nitric acid heated (80°C) until dryness. Then leached with 1 M ammonium acetate in 6% (v/v) HNO_3 (pH 2)	48 h/6 h

microbially active. The two samples were then exposed to a 210 μmol l^{-1} UO_2^{2+} spike and incubated for a further 30 days. For XAS data collection sediment phases were then separated as described above for oxic samples using an anaerobic glove box.

Sequential Extraction

Sequential extractions were performed using the method developed by Tessier et al. adapted for use with radionuclides in anoxic sediments (Tessier et al. 1979; Keith-Roach et al. 2003). The chemical reagents and extraction methods are given in Table 1. Total uranium concentrations in the leachate were measured by ICP-MS using a Plasmaquad PQ2+ Turbo (Thermo Elemental, Cheshire, UK) and using thorium as an internal standard.

X-ray Absorption Spectroscopy (XAS) Experiments

To assist with data interpretation, a U(VI) compound (synthetic $UO_3 \bullet 6H_2O$; Strem Chemicals, UK) and a U(IV) bearing natural uraninite mineral sample were also prepared by dilution with boron nitride powder and transferred into triple contained cells for analysis. Uranium L_{III}-edge spectra were collected on station 16.5 at the UK CLRC Daresbury SRS operating at 2 GeV with a typical current of 150mA, using a Si(220) double crystal monochromator and unfocused optics. The incident beam intensity was detuned to 80% of maximum for harmonic rejection. Sediment sample data were collected in fluorescence mode with a Canberra 30-element solid state Ge detector. Boron nitride diluted synthetic UO_3 and uraninite data were collected in transmission mode using two ion chambers. Experiments were performed at ambient temperature. X-ray absorption near edge spectroscopy (XANES) spectra were calibrated and background subtracted by use of the Daresbury Laboratory programs EXCALIB and EXBACK, normalized for drift in E_0 and the spectra plotted. After data acquisition, a least squares fitting routine was used to quantify the contributions of the different uranium oxidation states to the observed spectra by fitting between oxic and reducing end members. The accuracy of the valence state determination from the least squares fitting of the XANES data between end member spectra was estimated to be $\pm$ 10%, similar to previous work (Kelly et al. 2008; Morris et al. 2008). Due to sample limitations, only a restricted number of extended X-ray absorption fine structure (EXAFS) spectra were collected and analyzed in EXCURV98 using full curved wave theory (Binsted 1998). Resultant Fourier transforms were then used to fingerprint the diagnostic presence and absence of U(VI) axial U = O at 1.80 Å.

Microbiological Methods

DNA was extracted from sediment taken from start (T = 0) and end (T = 120 day) *XAS bioreduction unamended* microcosms using a Fast DNA spin kit (Powersoil, Soil DNA Isolation Kit, MO BIO Laboratories) and a fragment of the 16S rRNA gene was amplified by PCR (Islam et al. 2004). The broad specificity primers 8f and 519r were used and an approximately 520 b.p. fragment of the 16S rRNA gene was amplified from samples. PCR products were purified using a QIAQuick purification kit (Qiagen) and ligated directly into the cloning vector PCR 2.1 (Invitrogen) before transformation into *Escherichia coli* TOP 10 competent cells. White transformants that grew on LB agar containing ampicillin were screened for an insert using PCR and products purified with a QIAQuick kit. Clones were separated into operational taxonomic units (OTUs) based upon the similarity of restriction fragment length polymorphism (RFLP) profiles, using the restriction enzymes Sau3A and MspI. The nucleotide sequences of each OTU were determined using the dideoxynucleotide method and sequences analyzed against the NCBI (USA) BLAST database, with matching to known 16SrDNA gene sequences.

RESULTS AND DISCUSSION

Sediment Characteristics

Full details of sediments have been described previously (Begg et al. 2007). Briefly, the sediment was best described as a sandy loam dominated by quartz, feldspars, chlorite and mica with a pH of 5.3 and a cation exchange capacity of 1.06 $\pm$ 0.07 mol kg^{-1}.

Oxic Adsorption of U(VI)

To characterize the behavior of U(VI) in the presence of Dounreay sediment under oxic conditions, selected adsorption experiments were undertaken. In lower concentration (53 μmol l^{-1} UO_2^{2+}) experiments, U(VI) removal from solution was rapid in both *oxic unamended* and *oxic EDTA amended* systems with $>$ 99% removal occurring within 2 h in both systems and no remobilization observed over 30 days (Figure 1). Controls without sediment showed that U(VI) remained in solution over 7 days

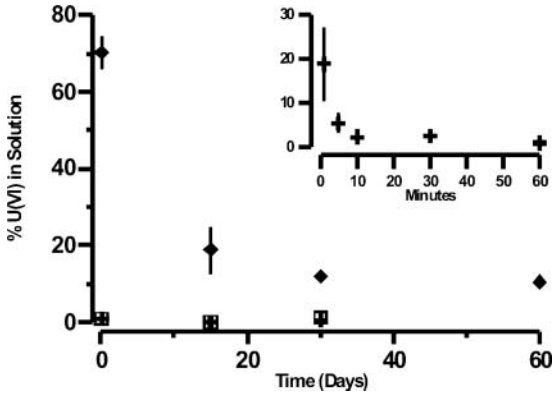

FIG. 1. Time dependent U(VI) removal from solution in lower U(VI) concentration (53 μmol l^{-1}) oxic adsorption experiments: *oxic unamended* (+); *oxic EDTA amended* (■); *oxic carbonate amended* (♦). Inset shows removal of uranium from solution in *oxic unamended* systems over 60 min. Error bars are 1σ of three replicates.

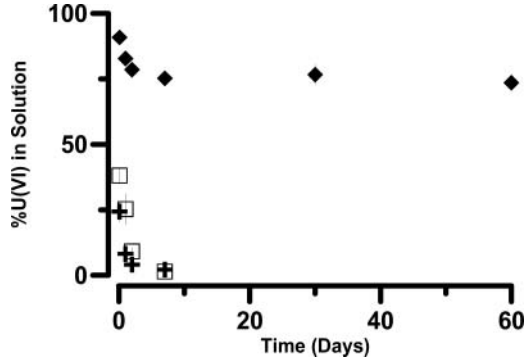

FIG. 2. Time dependent uranium(VI) removal from solution in XAS U(VI) oxic adsorption experiments: *XAS oxic unamended* (+); *XAS oxic EDTA amended* (□); *XAS oxic carbonate amended* (♦). *XAS oxic carbonate amended* showed a plateau in removal of U(VI) from solution. Error bars are 1σ of three replicates.

confirming that U(VI) was not oversaturated in the groundwater system. Eh measurements during oxic sorption typically ranged from +150 mV to +200 mV, indicating oxidizing conditions. The extremely fast sorption of U(VI) in *EDTA amended* experiments confirms that sorption occurred despite the presence of an excess of the complexant EDTA. In the *oxic carbonate amended* experiment, removal from solution was much slower with only 30.0 ± 4.0% removal from solution observed after 2 h and a slow but steady removal occurring over time with 89.5 ± 0.4% sorption in oxic experiments occurring after 60 days (Figure 1). Presumably, incomplete uptake of UO_2^{2+} in these high carbonate systems is due to the formation of neutral or negatively charged U(VI)-carbonate complexes (Clark et al. 1995).

In additional oxic adsorption experiments for XAS analysis, both *XAS oxic unamended* and *XAS oxic EDTA amended* systems showed slower uptake kinetics than the lower uranium concentration experiments (Figure 2). Nonetheless, complete (> 97.8 ± 0.6%) sorption of U(VI) occurred in both experiments by 7 days and these samples were taken for XAS analysis. Fast initial removal of U(VI) followed by slower removal is likely due to rapid adsorption of U(VI) to surface sites followed by slower uptake due to structural arrangement on the solid surface (Cheng et al. 2006; Um et al. 2007; Waite et al. 1994). In the *XAS oxic carbonate amended* systems, 25 ± 1.0% of the initial uranium spike was removed after 7 days and 35.6 ± 0.6% was removed at 120 days. This is a reduced percentage uptake compared with the lower uranium concentration *oxic carbonate amended* experiment. Only the 7-day sediment sample was taken for XAS analysis.

XAS Analysis of Oxic Samples

To assess the speciation of uranium in the oxic sorption experiments, XAS analyses were undertaken on 7-day time point samples from *XAS oxic unamended, carbonate amended* and *EDTA amended* experiments. These samples were challenging to measure due to the relatively low concentrations of U sorbed to sediments (an average of several hundred ppm U on solids). We were able to obtain XANES data for all samples and where feasible we also collected EXAFS data. In all 3 oxic 7-day samples the XANES spectra were characterized by their similarity to the shape of the U(VI) standard and to U(VI) standards reported in the literature (Figure 3A; Boyanov et al. 2007). When compared to the U(VI) and U(IV) standards, linear combination fitting for these 7-day samples showed an ∼ 80% contribution from U(VI) which strongly supports the observed similarity between spectra from sediments, our U(VI) standards and published work showing U(VI) spectra (Boyanov et al. 2007). Thus, as expected in oxic conditions, XAS shows that U(VI) is predominantly found sorbed to the sediment surface. This observation was further supported by EXAFS analysis of the 7 day samples where sediment associated U was characterized by a large peak in the Fourier transforms at ca. 1.80 Å which is diagnostic for the axial U = O bond length in U(VI) (Figure 4, Catalano et al. 2004).

Bioreduction

To simulate the behavior of U(VI) in anoxic subsurface environments, a series of bioreduction microcosm experiments were performed at the lower uranium concentration. Overall bioreduction, indicated by lower Eh values (Figure 5A) occurred over several weeks and at similar rates to those observed in previous work (Begg et al. 2007). Development of Fe(III)-reducing conditions, indicated by increases in 0.5 N HCl extractable Fe(II), was rapid in all systems and was measured at 41 ± 6%, 73 ± 2% and 24 ± 4% respectively in *bioreduction unamended*, *bioreduction carbonate amended* and *bioreduction EDTA amended* experiments at 15 days (Figure 5B). As expected, a significant increase in total Fe in porewaters was also observed at 15 days due to production of soluble Fe(II) as Fe(III)-reduction progressed (Figure 5C). In all microcosms there was a rise in pH as bioreduction proceeded, consistent with production of OH^- and HCO_3^- from oxidation of carbon-based electron donors (Figure 5D; Chang et al. 2005). Sulfate reduction, as indicated by

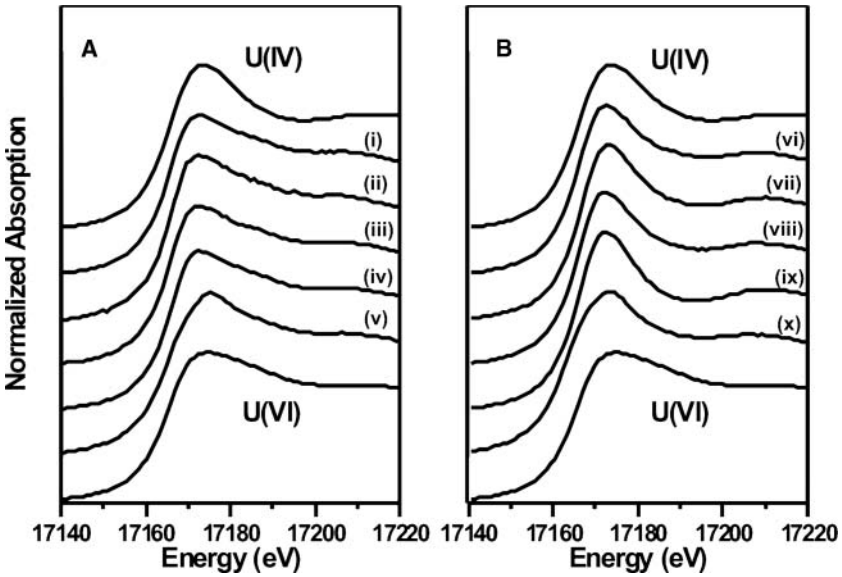

FIG. 3. U L_{III}-edge XANES spectra for uranium associated with Dounreay sediment in (A) Experiments where the predominant oxidation state is U(VI): *XAS oxic unamended* (i); *XAS oxic carbonate amended* (ii); *XAS oxic EDTA amended* (iii); *XAS pre-reduced sterile* (iv); *XAS air reoxidation 1 day* (v) experiments; and (B) experiments where the predominant oxidation state is U(IV): *XAS bioreduction unamended* (vi); *XAS bioreduction carbonate amended* (vii); *XAS bioreduction EDTA amended* (viii); *XAS pre-reduced non-sterile* (ix); *XAS molybdate amended* (dissimilatory sulfate reduction inhibited) experiments (x). U L_{III}-edge XANES spectra for U(IV) and U(VI) standards are also shown for comparison.

a decline in porewater SO_4^{2-}/Cl^- ratio, was observed in all 3 experiments after 60 days (Figure 5E).

In both the *bioreduction unamended* and *EDTA amended* systems >97% of the initial U(VI) spike was removed within 2 h, at a similar rate to that observed under oxic conditions and removal was maintained throughout the 4-month experiment (Figure 5F). Uranium solubility in the lower concentration bioreduction experiments mirrored the behavior observed in the parallel oxic sorption experiments. Similarly, analysis of porewaters after 120 days in the parallel *XAS bioreduction unamended* and *XAS bioreduction EDTA amended* systems confirmed removal of uranium from solution (>98.5%).

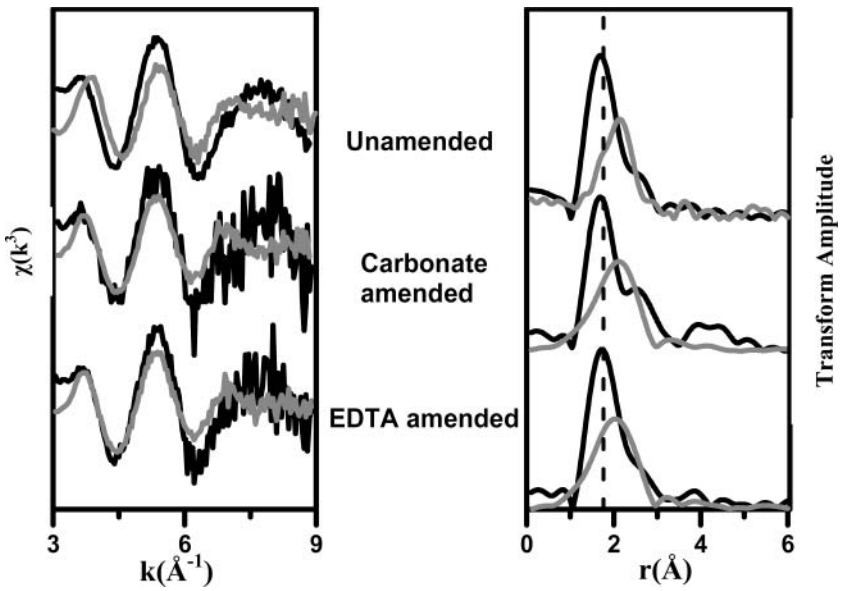

FIG. 4. Background-subtracted, normalised, and k^3-weighted U L_3-edge EXAFS spectra (left) and corresponding Fourier transform (right) obtained for Dounreay sediment samples. Black lines are the experimental data for *XAS oxic unamended* systems (7 d), grey lines are the experimental data for *XAS bioreduction unamended* systems (120 d). Dashed line in Fourier transform plot denotes position of position of principal feature from axial U=O bond at 1.8 Å.

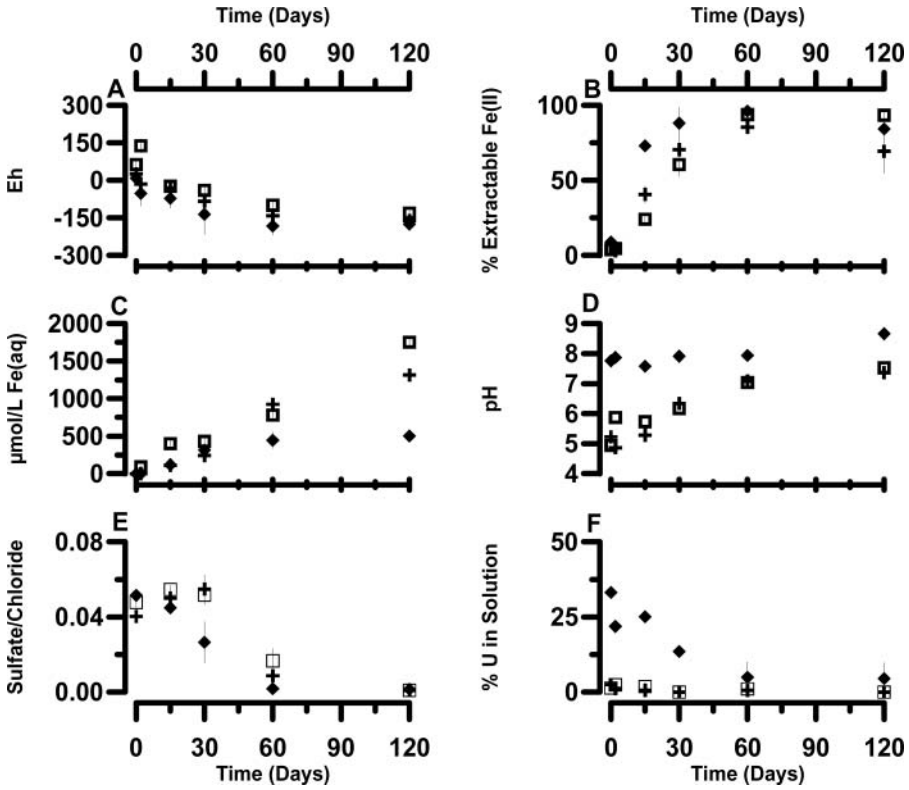

FIG. 5. Microcosm time-series data in low level bioreduction experiments showing: (A) changes in microcosm Eh in *unamended* (+), *carbonate amended* (♦) and *EDTA amended* experiments (□); (B) changes in the percentage of 0.5 N HCl extractable Fe present as Fe(II) in solid phases; (C) changes in total dissolved Fe concentration in solution; (D) changes in pH; (E) changes in porewater sulfate/chloride ratio; and (F) U(VI) removal from solution. Errors are 1σ of four replicates.

In the lower concentration *bioreduction carbonate amended* systems, the sorption behavior was broadly similar to the oxic parallel with $60.8 \pm 0.6\%$ of the U(VI) retained on sediments within the first 2 h and with the pattern of removal to sediments similar throughout the experiment. Interestingly, in the *XAS bioreduction carbonate amended* system, removal of uranium from solution was clearly enhanced by bioreduction with $< 1.5\%$ of the original spike in solution at 120 days compared to the oxic sorption parallel where $64.4 \pm 0.6\%$ U(VI) was in solution at 120 days. Overall, the different bioreduction treatments showed robust development of anoxia and significant (>95%) removal of uranium from solution by 120 days.

XAS Analysis of Bioreduction Samples

XANES analysis showed that, for the three 120-day time point samples from *XAS bioreduction unamended, carbonate amended* and *EDTA amended* systems, the spectra were similar to that of the predominantly U(IV) bearing uraninite spectrum and displayed a higher post amplitude edge peak shape then the U(VI) standard suggesting that U(IV) dominated in these sediments (Figure 3B). Linear combination fitting using XANES spectra from these samples and the U(VI)/U(IV) standards suggested an approximately 90% contribution from U(IV). Additionally, analysis of the Fourier transform from the EXAFS spectra for the *XAS bioreduction unamended* sample showed no amplitude at ~1.80 Å (Figure 4) confirming the absence of the axial U = O bond in these samples (Den Auwer et al. 2003). Thus, both the XANES and EXAFS data confirm that bioreduction of sorbed U(VI) to U(IV) had occurred in these microcosms over 120 days. To quantify the extent of bioreduction with time in microcosms, linear combination fitting was performed between matrix matched end-member spectra. The *XAS oxic unamended* sample was used as the oxic end-member; the *XAS bioreduction unamended* sample which had been incubated for 120 days was used as the bioreduced end-member. The 120 day *XAS bioreduction carbonate amended* and *XAS bioreduction EDTA amended* samples were identical (within error) to the *XAS bioreduction unamended* end member (Table 2).

These results confirm that reduction of sorbed U(VI) occurred over several months as progressive anoxia developed in all 3 amendments. A heat sterilized oxic sediment control showed no significant increase of Fe(II) in sediment and XANES linear combination fitting showed a $100 \pm 10\%$ fit to the U(VI) matrix matched end-member. This confirmed that reduction was only present in microbially active samples (Table 2). Interestingly, in *XAS carbonate amended* experiments bioreduction of U(VI) to U(IV) occurred despite the presence of high levels of both CO_3^{2-} and Ca^{2+} which have been shown to limit the

TABLE 2
XANES analysis of soil spectra as linear combinations of "end member" spectra using the predominately U(VI) *XAS oxic unamended* spectra (A), and the predominately U(IV) *XAS bioreduction unamended* 120 day sample spectra (D). Modeled results are for samples: (B) *XAS bioreduction unamended* 30 day; (C) *XAS bioreduction unamended* 60 day; (E) *XAS bioreduction carbonate amended* 120 day; (F) *XAS bioreduction EDTA amended* 120 day; (G) *heat-sterilized 120 d incubation*; (H) *XAS Pre-reduced sterile*; (I) *XAS Pre-reduced non sterile*; (J) *XAS molybdate amended* (dissimilatory sulfate reduction inhibited); (K) *XAS air reoxidation 1 day;* (L & M) U standards. The fit index of the calculated XANES spectra with experimental XANES spectra is defined as Σ [(Iobs—Icalc)^2].

Sample	% Spec A	% Spec D	Fit
A. Un 7 d	100	0	–
B. Un 30 d	100	0	0.05
C. Un 60 d	81	19	0.04
D. Un 120 d	0	100	–
E. CO_3 120 d	0	100	0.09
F. EDTA 120 d	0	100	0.02
G. Sterile 120 d	100	0	0.12
H. Prered Ste	96	4	0.04
I. Prered NS	0	100	0.17
J. Molybdate	45	55	0.21
K. 1 d Air	100	0	0.12
L. U(VI)O_3 standard	78	22	0.21
M. U(IV)O_2 standard	14	86	0.17

efficiency of UO_2^{2+} bioreduction in some systems (Neiss et al. 2007; Wu et al. 2006). Further, in these carbonate amended systems, the 120- day XANES spectrum confirms that bioreduction to U(IV) causes enhanced removal of uranium from solution compared to the oxic experiment suggesting that U(VI) reduction and removal from solution can occur as bioreduction proceeds even with micromolar concentrations of Ca^{2+} and bicarbonate in these systems.

Timing of U(VI) Reduction

For the *XAS bioreduction unamended* system, a time series of U-XANES data were collected. This provided an opportunity to relate the redox behavior of sorbed U(VI) to the changing biogeochemistry of the system. To characterize the biogeochemistry of these systems, the following operational definitions were used: early Fe(III)-reducing conditions were defined as systems where significant increases (>10%) of solid phase 0.5 N HCl extractable Fe(II) had occurred but where sulfate/chloride pore water ratios remained high; predominantly sulfate reducing conditions were defined as those observed when FeS production was indicated by a formation of black solid-phases and

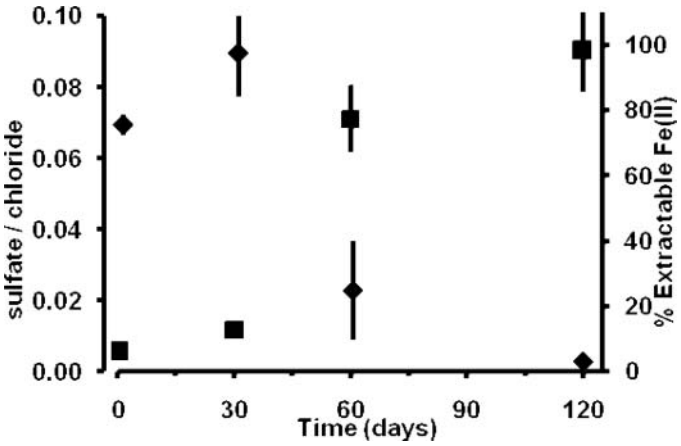

FIG. 6. *XAS bioreduction unamended* time-series showing changes in the percentage of 0.5 N HCl extractable Fe present as Fe(II) in solid phases (■; right hand y-axis) and changes in sulfate/chloride ratio (♦; left -hand y-axis). Error bars are relative standard deviation of three replicates.

0.5 N HCl extractable Fe(II) was high (>75%) whilst aqueous sulfate/chloride ratio was low. Thus, in the 30-day sample, Fe(III)-reducing conditions had developed, at 60 days both Fe(III)- and sulfate-reduction was occurring while at 120 days sulfate-reducing conditions predominated (Figure 6).

As established XANES and EXAFS techniques showed that the 7-day sample was dominated by U(VI) and the 120-day sample by U(IV). In order to examine the extent of U bioreduction in a fully matrix matched system, linear combination fitting was then employed using the day-7 and day-120 spectra as representative of U(VI) and (U(IV) end members (Table 2). For the 30-day sample, the XANES spectrum also resembled that of the U(VI) standard and linear combination fitting suggesting no contribution from the reduced U(IV) spectra. As the 30-day sample XANES spectrum is indistinguishable from the day-7 spectra by linear combination, this suggests that U(VI) reduction in these experiments did not coincide with early Fe(III)-reducing conditions (Figure 6; Table 2). For the 60 day sample fitting suggested a minor component ($\sim$ 20%) of the spectra was fitted with the bioreduced U(IV) matrix matched end-member, and at this time point both robust Fe(III)-reduction (with 80% Fe(II) in sediments) and the onset of sulfate-reducing conditions had occurred (Figure 6; Table 2). In a separate *XAS bioreduction unamended* experiment where dissimilatory sulfate reduction was inhibited by addition of 20 mM sodium molybdate (Newport and Nedwell 1988), linear combination fitting suggested that at 120 days a significant component ($\sim$50%) of the spectra can be attributed to the presence of bioreduced U(IV). This indicated that U(VI) reduction could occur in sulfate reduction inhibited systems but that the rate of reduction was slower than in the unamended systems (Figure 3B; Table 2).

In parallel experiments containing sterile and microbially active sediments that were pre-reduced (120 days anaerobic incubation) and subsequently reacted with U(VI), Eh values were –33 $\pm$ 6 mV in non sterile samples and –12 $\pm$ 9 mV in sterile

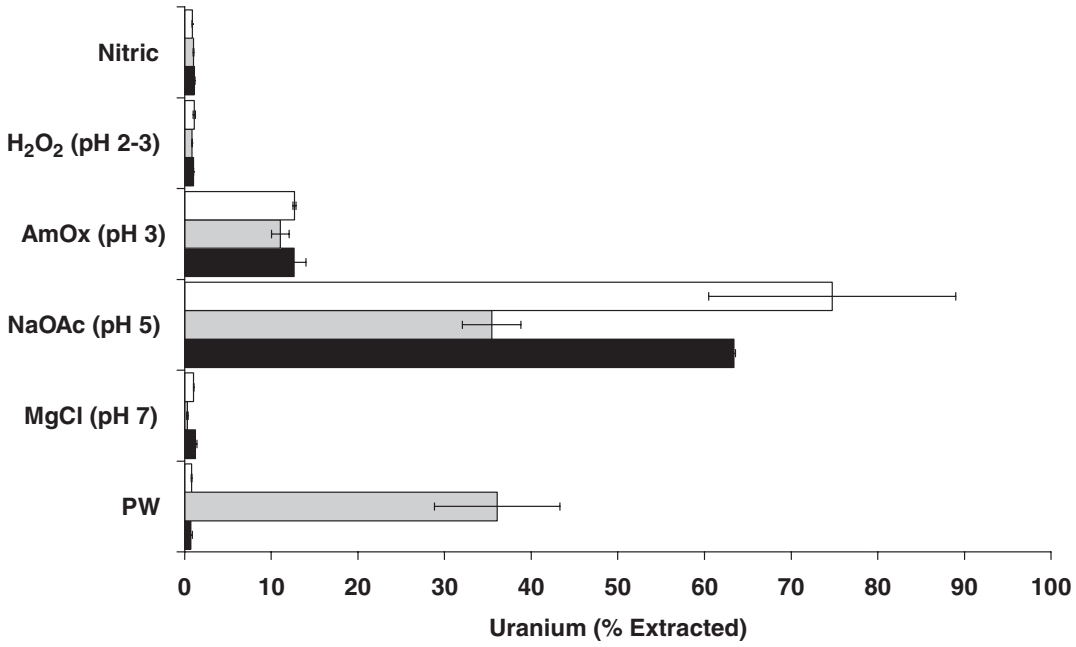

FIG. 7. Sequential extraction data for U(VI) extracted from Dounreay sediment after oxic adsorption in unamended (black bars); carbonate amended (grey bars); and EDTA amended (white bars) systems. Error bars are range of duplicate results. The strength of the lixivant increases from the bottom to the top of the y-axis.

samples after 30 days further incubation. Least squares fitting of U L_{III}-edge XANES spectra from these samples showed that in the sterile sample, the uranium spectrum was virtually identical to the oxic 7-day sample and that in the microbially active control the spectra was virtually identical to the 120-day bioreduced spectrum (Figure 3A; Figure 3B; Table 2). This suggests that in these systems, enzymatic activity is required for reduction of sorbed U(VI) to sediment associated U(IV) and that mineral phases expected to develop during bioreduction are largely ineffective at facilitating electron transfer to adsorbed U(VI) (Finneran et al. 2002; Wilkins et al. 2007).

Fate of U(VI) Following Bioreduction

To assess whether sediment associated uranium was more strongly bound after bioreduction, lower concentration oxic and 120-day bioreduced samples were subjected to a sequential extraction procedure. Sequential extraction on the sediments showed that for *unamended* and *EDTA amended* oxic systems after 7 days, the majority (69.0 $\pm$ 10.5%) of solid phase associated uranium was present in the 1 M sodium acetate extractable fraction, while in the *carbonate amended* experiments, this figure was lower (33–38%) as a significant component of the total uranium remained in porewaters (Figure 7). However, for 120-day bioreduction samples, sequential extractions showed that solid phase associated U(IV) was largely (60.5 $\pm$ 8.4%) leached in the stronger 0.1 M ammonium oxalate fraction (Figure 8). This implies that U(IV) formed after bioreduction of sediments containing adsorbed U(VI) is more strongly bound and thus less prone to remobilization than U(VI) sorbed to oxic sediments.

Microbial Community Analysis

To identify microorganisms that may be important in the reduction of U(VI) in the unamended experiments, 16S rRNA microbial community analysis was performed on an oxic T = 0 *XAS bioreduction unamended* sample and a sulfate-reducing, 120-day *XAS bioreduction unamended* sample. The initial time point contained a very diverse community of bacteria containing close relatives of novel, uncharacterized microorganisms; 32 of the 58 clones analyzed gave distinct banding patterns and were related to organisms found in aerobic environments (Table 3). Analysis of the 120-day sample showed a less diverse community; 23 of the 56 clones analyzed gave distinct banding patterns (Table 4). Here, organisms closely related to the anaerobic order *Clostridiales* comprised 25% of bacteria detected using the RFLP approach. *Clostridium* spp. cultures have been shown to be able to reduce aqueous U(VI) (Francis et al. 1994). Further, one of the isolates was a close relative (96% over 520 base pairs) of the sulfur-reducing microorganism *Clostridium tunisiense* (Thabet et al. 2004) and has also been found in sediment incubations where reduction of sorbed U(VI) has been observed (Dong et al. 2006). A measurable fraction (7%) of the population was related (92% over 520 bases) to *Geobacter psychrophilus*, a known subsurface Fe(III)-reducing species (Nevin et al. 2005); *Geobacter* spp. are also well known aqueous uranium reducers (Lovley et al. 1991). Interestingly, there was an absence of known dissimilatory sulfate-reducing species in the

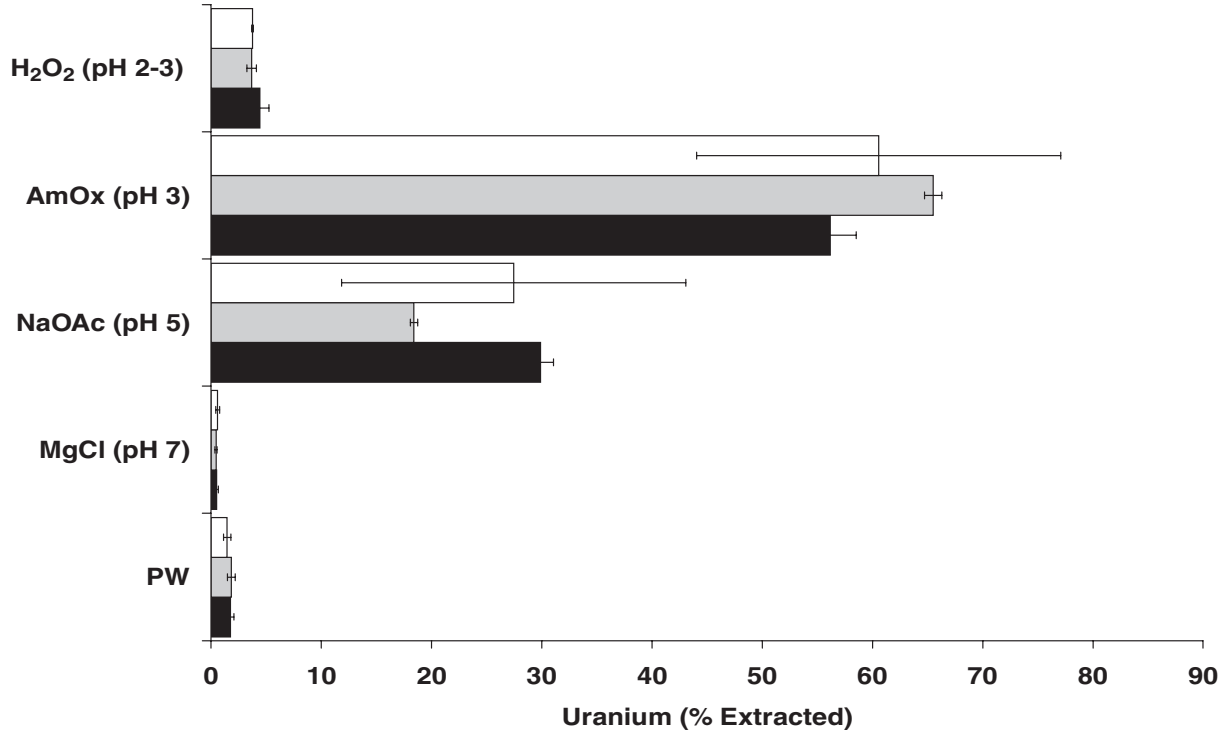

FIG. 8. Sequential extraction data for U(VI) extracted from Dounreay sediment after 120 days bioreduction in unamended (black bars); carbonate amended (grey bars); and EDTA amended (white bars) systems. Error bars are range of duplicate results. The strength of the lixivant increases from the bottom to the top of the y-axis. Data for nitric acid extractions were not collected.

120 day sample despite geochemical evidence for robust sulfate reduction in this sample, and similar to past work at contaminated sites (Fox et al. 2006). Despite similar geochemical behavior in all experimental conditions, the microbes responsible for bioreduction may be different in experiments with carbonate or EDTA present, because of altered pH or mineral solubility resulting in more optimal growth of different species.

CONCLUSIONS

The reduction of $U(VI)_{(aq)}$ to insoluble U(IV) by Fe(III)- and SO_4^{2-}-reducing bacteria in contaminated land scenarios is well reported (Anderson et al. 2003; Dong et al. 2006; Wu et al. 2006). However, the fate of uranium(VI) already associated with solid surfaces is a subject of recent interest (Jeon et al. 2005; Kelly et al. 2008; Liu et al. 2009; Ortiz Bernard et al. 2004). For the systems of study here, XAS analysis indicates that in both *unamended* and *EDTA amended* systems solid-associated U(VI) is largely reduced after development of progressive anoxia during a 120-day anoxic incubation. In *carbonate amended* systems, enhanced removal from solution compared to oxic sorption experiments was observed in bioreduced samples in both lower level and XAS level experiments. Furthermore, reduction of U(VI) was confirmed by XANES and EXAFS analysis. Overall these results demonstrate that in Dounreay sediments immobilization and reduction of U(VI) will proceed in the presence of elevated bicarbonate and Ca^{2+} concentrations. Further, exposure of U(VI) to sterile and non-sterile pre-reduced sediments suggested an enzymatically mediated pathway in these sediments. Assessment of the genetic diversity of cloned 16S rRNA gene fragments showed a substantial shift in the microbial community between the start and end of the incubations. The predominance of clones closely related to *Clostridium* sp. and *Geobacter*, which are known U(VI) reducers, suggest that these species may play a significant role in the reduction of U(VI) in Dounreay sediments. Importantly, it also shows that indigenous microbial populations present in Dounreay-type sediment have the ability to reduce solid-associated uranium(VI). Sequential extraction results indicate that following reduction of sediment associated U(VI), leaching of U(IV) requires more aggressive lixiviant than for adsorbed U(VI). This further confirms that bioreduction of solid-associated U(VI) enhances immobilization of uranium in contaminated environments. Interestingly, even though U(IV) requires a strong chemical leach to remove it from sediments, air reoxidation of U(VI) in these systems is very fast. When a 120 day *XAS bioreduction unamended* system was exposed to air for 1 day and the sediment analyzed by XAS, the resultant XANES spectrum matched the 7-day *XAS oxic unamended* sample, dominated by U(VI) suggesting near-complete reoxidation within 1 day (Figure 3A; Table 2). This is consistent with previous work reporting rapid air reoxidation of U(IV) from sediments (Wilkins et al 2007; Wu et al., 2007)

TABLE 3
Phylogenetic affiliation in oxic sediment of distinct RFLP types detected in a 16S rDNA clone library obtained by PCR amplification using broad-specificity primers. Amplification was from an *XAS bioreduction unamended* T = 0 sample.

Clone	RFLP Type	Closest Matching Micro Organism (accession number)	Identities (% Match)	% Present	Phylogenetic Division
JBT0-1	1	Uncultured bacterium clone aaa57g07	451/480 (93%)	3.5	Unknown
JBT0-2	2	Uncultured proteobacterium clone GASP-MA4W1_C10	384/395 (97%)	3.5	Unknown
JBT0-3	3	Uncultured bacterium clone AS37	450/455 (98%)	10.3	Unknown
JBT0-6	4	Uncultured beta proteobacterium clone OS-C27	490/499 (98%)	1.7	*Betaproteobacteria*
JBT0-7	5	*Microscilla furvescens*	453/497 (91%)	3.5	*Sphingobacteria*
JBT0-8	6	Uncultured bacterium clone FCPN634	450/483 (93%)	5.2	Unknown
JBT0—9 JBT0-35	7, 19	Uncultured forest soil bacterium clone DUNssu162	467/470 (99%)	7.0	Unknown
JBT0-11	8	Uncultured bacterium clone DOK_CONFYM_clone489	419/492 (85%)	1.7	Unknown
JBT0-12	9	Uncultured Green Bay ferromanganous micronodule bacterium MND8	403/447 (90%)	1.7	Unknown
JBT0-13	10	Uncultured bacterium clone 292c2	451/456 (98%)	3.5	Unknown
JBT0-14	11	Uncultured *Xiphinematobacteriaceae* bacterium clone EB1007	474/518 (91%)	3.5	Unknown
JBT0-15	12	*Burkholderia thailandensis* E264	236/339 (69%)	6.8	*Betaproteobacteria*
JBT0-16	13	*Cytophaga hutchinsonii*	374/418 (89%)	5.2	*Sphingobacteria*
JBT0-18	14	*Fibrobacter succinogenes*	270/304 (88%)	1.7	*Fibrobacterales*
JBT0-20	15	Uncultured *Verrucomicrobia* bacterium clone OS-C04	498/520 (95%)	8.6	Unknown
JBT0-21	16	*Pseudoxanthomonas johnstonii*	465/505 (92%)	3.5	*Gammaproteobacteria*
JBT0-22	17	Uncultured bacterium clone FAC88	389/422 (92%)	3.5	Unknown
JBT0-29	18	*Bacillus fusiformis*	298/301 (99%)	1.7	*Bacillales*
JBT0-36	20	Uncultured bacterium clone P8-GEN-29	39/39 (100%)	1.7	Unknown
JBT0-40	21	Uncultured bacterium clone DOK_NOFERT_clone576	452/462 (97%)	3.5	Unknown
JBT0-42	22	Uncultured bacterium clone NR.1.087	431/472 (91%)	1.7	Unknown
JBT0-43	23	Unidentified bacterium clone FI-2M_B10	472/481 (98%)	1.7	Unknown
JBT0-44	24	uncultured eubacterium clone WR140	442/446 (99%)	1.7	Unknown
JBT0-48	25	Unidentified bacterium clone FI-2M_G10	501/502 (99%)	1.7	Unknown
JBT0-49	26	Uncultured *Verrucomicrobia* bacterium clone Amb_16S_491	490/510 (96%)	1.7	Unknown
JBT0-51	27	Uncultured *Bacteroidetes* bacterium clone 61–01–24c014	453/504 (89%)	1.7	Unknown
JBT0-52	28	*Mortierella verticillata*	414/418 (99%)	1.7	Unknown
JBT0-54	29	Uncultured *Verrucomicrobia* bacterium YNPRH34A	499/519 (96%)	1.7	Unknown
JBT0-55	30	Uncultured *Verrucomicrobia* bacterium clone DOK_BIODYN_clone479	248/339 (73%)	1.7	Unknown
JBT0-57	31	Uncultured alpha proteobacterium clone AI-2M_F03	430/442 (97%)	1.7	*Alphaproteobacteria*
JBT0-58	32	Uncultured candidate division NKB19 bacterium clone BB-1-E10	499/503 (99%)	1.7	Unknown

TABLE 4
Phylogenetic affiliation in reduced sediment of distinct RFLP types detected in a 16S rDNA clone library obtained by PCR amplification using broad-specificity primers. Amplification was from the *XAS bioreduction unamended* 120 d sample.

Clone	RFLP Type	Closest Matching Micro Organism (accession number)	Identities (% Match)	% Present	Phylogenetic Division
JBT120-1 JBT120-16	1, 12	*Clostridium lituseburense*	448/464 (96%)	7.2	*Clostridiales*
JBT120-2	2	Uncultured *Xiphinematobacteriaceae* bacterium clone EB1116	458/462 (99%)	10.6	*Spartobacteria*
JBT120-4	3	Uncultured soil bacterium clone CWT SM03_B11	409/422 (96%)	8.8	Unknown
JBT120-5	4	Uncultured bacterium clone ORS25C_b04	459/482 (95%)	1.8	Unknown
JBT120-6	5	*Clostridium puniceum*	466/470 (99%)	12.5	*Clostridiales*
JBT120-8	6	Uncultured bacterium clone Amb_16S_1261	495/504 (98%)	1.8	Unknown
JBT120-9	7	*Clostridium tunisiense*	383/397 (96%)	1.8	*Clostridiales*
JBT120-11	8	*Candidatus Magnetobacterium bavaricum*	232/277 (83%)	3.6	*Nitrospirales*
JBT120-13	9	*Acetivibrio cellulolyticus*	432/477 (90%)	3.6	*Clostridiales*
JBT120-14	10	*Methylocella palustris*	422/452 (93%)	3.6	*Alpha-proteobacteria*
JBT120-15	11	Uncultured soil bacterium clone HSB NT53_H06	426/463 (92%)	7.1	Unknown
JBT120-18	13	Uncultured bacterium clone ORSFES_f09	435/446 (97%)	1.8	Unknown
JBT120-19	14	*Bacillus longiquaesitum*	483/501 (96%)	7.1	*Bacillales*
JBT120-20	15	*Bacillus litoralis*	497/506 (98%)	3.6	*Bacillales*
JBT120-22	16	Unidentified bacterium clone FI-2M_B10	469/493 (95%)	3.6	Unknown
JBT120-27	17	*Geobacter psychrophilus* strain P35	483/522 (92%)	7.1	*Deltaproteobacteria*
JBT120-28	18	Uncultured bacterium clone aab39a02	499/500 (99%)	3.6	Unknown
JBT120-30	19	*Beijerinckia* sp. TB13	344/379 (90%)	1.8	*Alpha-proteobacteria*
JBT120-33	20	*Methylosinus sporium* strain NR3K	310/338 (91%)	1.8	*Alpha-proteobacteria*
JBT120-34	21	*Bacillus longiquaesitum*	189/216 (87%)	1.8	*Bacillales*
JBT120-45	22	Uncultured alpha proteobacterium clone Fl-1F_C12	282/302 (93%)	3.6	*Alpha-proteobacteria*
JBT120-53	23	Uncultured bacterium clone CON4_C02	282/309 (91%)	1.8	Unknown

and highlights that it is essential to maintain reducing conditions in environments where bioremediation technologies are used to treat U contaminated sites.

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