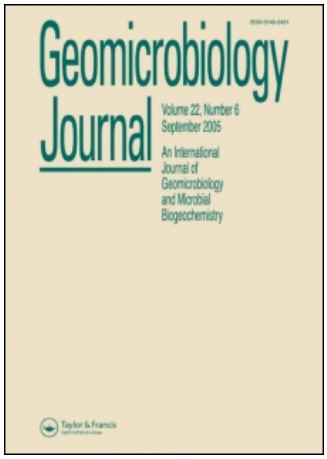

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Technetium Reduction and Reoxidation in Aquifer Sediments

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This study describes the biogeochemical behaviour of the radionuclide technetium (^{99}Tc) in background area sediments from the US Department of Energy Field Research Center (FRC) in Oak Ridge, TN, USA. Microcosm experiments with trace levels of ^{99}Tc(VII) were used to examine Tc reduction and reoxidation. Efficient removal of 0.5 μM Tc(VII) from solution was seen under Fe(III)-reducing conditions, and was attributed to a lower valence insoluble form of the radionuclide. Molecular and cultivation-dependent analysis confirmed the presence of known Fe(III)-reducing bacteria (*Geothrix* and *Geobacter* species) in these sediments. Extended X-ray Absorption Fine Structure (EXAFS) spectroscopic analysis of analogous microcosm experiments, challenged with higher (550 μM) concentrations of Tc(VII), confirmed the presence of reduced insoluble Tc(IV) as hydrous TcO_2 in the Fe(II)-bearing sediments. Reoxidation experiments of pre-reduced microcosms challenged with 0.5 μM ^{99}Tc showed very limited (<3 %) remobilization of the reduced ^{99}Tc with 100 mM nitrate but significant (*ca* 80%) remobilization of ^{99}Tc under air reoxidation conditions. Fe(II) oxidation was, however, significant in all oxidation treatments. EXAFS analyses of Fe(II)-bearing sediments challenged with higher (550 μM) concentrations of Tc(VII) and then reoxidized with 100 mM nitrate contained both Tc(IV) and Tc(VII) immobile phases. These results suggest that under anaerobic oxidation conditions, Tc(IV) will not remobilize rapidly, even in the presence of high concentrations of nitrate. This has implications for the biogeochemical cycling of technetium in contaminated environments, including those where bioreduction has been stimulated to minimize transport of the radionuclide.

Keywords bioremediation, metal reduction, environmental radioactivity, fission product, NABIR, denitrification, pertechnetate

INTRODUCTION

Technetium is a radioactive fission product produced in substantial quantities as a by-product of nuclear activities. Its unique biological and geochemical behaviour presents challenges in waste disposal and remediation. A beta-emitter (E_{max} = 0.29 MeV) with a long half-life (2.1 x 10^5 yr), ^{99}Tc is bioavailable as a sulfate analogue in its oxidized form as the highly mobile pertechnetate ($Tc(VII)O_4^-$) anion. Technetium can rapidly accumulate in both aquatic and terrestrial plants and organisms (Beasley and Lorz 1986; Krijger et al. 1999), leading to concern that this toxic radioactive metal could impact human health via the food chain or contaminated drinking water. Technetium is a priority contaminant at US Department of Energy (DoE) sites such as the Environmental Remediation Sciences Program (ERSP) Field Research Center (FRC) site in Oak Ridge, TN (groundwater concentrations up to 40,000 pCi l^{-1} or 0.02 μM; <http://www.esd.ornl.gov/nabirfrc/>) and the Hanford S-SX tank farm (groundwater concentrations up to 10^8 pCi l^{-1} or 54 μM; Fredrickson et al. 2004a). It is also a contaminant of key concern at sites in the UK (Morris et al. 2000), France (Salbu et al. 2003) and Russia (Aarkrog et al. 1997). The global distribution and impact of ^{99}Tc necessitates a detailed understanding of fundamental ^{99}Tc biogeochemistry to support development of remediation strategies and minimize ^{99}Tc transport in the environment.

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The solubility of pertechnetate (Tc(VII); 11 mol l^{-1}; Boyd 1978) is substantially higher than that of Tc(IV) (10 nmol l^{-1} at neutral pH as hydrous TcO_2; Meyer et al. 1991). Consequently a potentially useful strategy to immobilize Tc(VII) in the environment is to reduce it to Tc(IV). Microorganisms have been demonstrated to reduce many toxic and redox sensitive metals in the environment (reviewed by Lloyd 2003), and are naturally present at many contaminated sites. Thus, accelerated bioremediation by the addition of electron donors or nutrients (biostimulation) is a suitable approach for controlling radionuclide (including ^{99}Tc) solubility in contaminated groundwater (McCullough et al. 2003).

Technetium biogeochemistry has been studied predominantly in laboratory experiments using pure cultures of bacteria at pertechnetate concentrations ranging from 2.5 to 6000 μM (5×10^6 to 12×10^9 pCi l^{-1}) (Lloyd et al. 1997a, 1997b, 1999a, 2000; De Luca et al. 2001; Wildung et al. 2000; Francis et al. 2002). These experiments have identified at least 2 distinct mechanisms of Tc(VII) reduction; enzymatic reduction via microbial hydrogenases (Lloyd et al. 1997a; De Luca et al. 2001;), and indirect reduction via biogenic Fe(II) (Lloyd et al. 2000; Fredrickson et al. 2004b) or sulfide (Lloyd et al. 1998). At the very low concentrations of Tc(VII) present in most contaminated environments, well below the concentrations predicted to be recognized efficiently by microbial hydrogenases (Lloyd et al. 1999b), it is likely that ^{99}Tc solubility is controlled by Fe(III)-reducing organisms that drive the abiotic redox process coupling Fe(II) oxidation to Tc(VII) reduction. Indeed, this process has been studied recently with ^{99}Tc in sediments from both estuarine (Burke et al. 2005, 2006) and freshwater environments (Fredrickson et al. 2004b; Wildung et al. 2004; Abdelouas et al. 2005).

Other recent studies have focused on the interactions between Tc(VII) and FRC sediments in situ, using "push-pull" techniques (Istok et al. 2004; Peacock et al. 2004), and examined the impact of nitrate on Tc(VII) reduction. Nitrate (from nitric acid used in the nuclear fuel cycle) may be present at very high (> 100 mM) concentrations at sites where nuclear waste is stored (Senko et al. 2005), and is known to inhibit enzymatic Tc(VII) reduction (Lloyd et al. 1997a, 1999b). Recent in situ push-pull studies at the FRC site found that the rate of Tc(VII) reduction increased in monitoring wells when electron donors were added, even when high concentrations of nitrate (100 mM and greater) were also added to the wells (Istok et al. 2004). These interesting observations contrast with findings from several laboratory-based experiments where nitrate inhibited Tc(VII) reduction in sediment microcosms (Abdelouas et al. 2005; Burke et al. 2005).

This objective of this study was to explore the effect of the presence and absence of added nitrate and electron donor on the progression of terminal electron accepting processes (TEAPs) and ^{99}Tc immobilization in microcosms prepared from FRC background (uncontaminated) sediments. Microcosm experiments were also used to investigate the reoxidation and resolubilization behaviour of immobilized ^{99}Tc in reduced background area sediments exposed to nitrate and air. Finally, Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy was used to determine the coordination environment of ^{99}Tc in the reduced and reoxidized sediments.

MATERIALS AND METHODS

Soils and Groundwater

The FRC background area sediments are composed of an unconsolidated clay-rich saprolite weathered from bedrock of the Dismal Gap Formation and Nolichucky Shale of the Cambrian Conasauga Group. The saprolite is composed of interbedded shale, siltstone, and limestone. Groundwater in this shallow, unconfined aquifer is described on the FRC website <http://www.esd.ornl.gov/nabirfrc/>. Briefly, groundwaters are carbonate buffered with a pH between 7 and 8; aqueous Fe(II) concentrations are below detection, NO_3^- ranges from 0 to 4 mg l^{-1}, SO_4^{2-} from 6 to 7 mg l^{-1}; total organic carbon is between 20 to 30 mg l^{-1} with non-purgeable organic carbon at approximately 2 mg l^{-1}; and total inorganic carbon is between 25 to 60 mg l^{-1}. The background area is located approximately 2 km from the contaminated area of the FRC (see map of FRC and borehole locations at <http://www.esd.ornl.gov/nabirfrc/>).

FRC background area sediments were sampled from borehole FB610 (near FW301) and groundwater was sampled from FW300. Samples were drilled and stored using aseptic methods (D, Watson, personal communication). The Geoprobe drilling rig (which is steam cleaned between uses) was used to drill the cores, and core liners were sterilized by either steam cleaning or rinsing with isopropyl alcohol. Cores were cut to length, capped with sterile caps, and refrigerated prior to shipping on blue ice to Manchester where they were stored at 10°C in darkness until use.

Microcosm Experiments

Groundwater (34 ml) was added to 16 g sediment and sealed in sterile 100 ml glass bottles (Burke et al. 2005) under an N_2 atmosphere. Triplicate samples were spiked with 0.5 μM ^{99}Tc (as pertechnetate) and incubated at 20°C in the dark. Sterile and unspiked controls were also prepared.

Geochemical Analyses

Samples of sediment and groundwater slurry were taken under anaerobic conditions and centrifuged (M-24 centrifuge, Boeco) for 4 min at a relative centrifugal force (RCF) of 15,700 *g*. Measurements of pH and Eh (Basic Bio pH meter, Denver Instruments; O13 and P13 NMR electrodes, Sentek) were taken using sample supernatant. Acid extractable Fe(II) was measured by digestion of the sample pellet in 2.5 ml of 0.5 N HCl for 1 hour, followed by ferrozine assay analysis for Fe(II) concentrations (Lovley and Phillips 1987; Stookey 1970). Total bioavailable iron was estimated by adding 100 μl of 6.25 N hydroxylamine

to the acid extraction mixture, digesting for 1 hour, and assaying the extractant using the ferrozine assay.

Technetium concentration was measured using liquid scintillation counting (Tri-Carb 1900 TR Scintillation Counter, Packard; Optiphase HiSafe3 Liquid Scintillant, Perkin Elmer) for 1 min with a detection limit of 30 cpm (1×10^{-11} M Tc or 0.6 Bq). Samples containing trace ^{99}Tc (post-reduction samples) were analysed using a Quantulus ultra low level liquid scintillation spectrometer (Perkin Elmer). Samples were counted for 200 min, with a detection limit of approximately 6 cpm (5×10^{-12} M Tc or 0.3 Bq). Anion concentrations from microcosm experiments and groundwaters were analysed by Ion Chromatography (IC; Dionex DX600 with Dionex CD20 Conductivity Detector, Dionex AS9-HC column, AG9-HC guard column, mobile phase isocratic 9 mM Na_2CO_3, detection limit 0.05 mg l^{-1} for sulfate, nitrite, and nitrate). Samples were filtered to < 0.22 μm and diluted into the range of 0–50 mg l^{-1} (nitrate and sulfate) and 0–5 mg l^{-1} (nitrite) prior to analysis. Statistical analyses of geochemical data are shown as error bars on diagrams, representing 1 $\times$ standard error (3 replicates).

Microbiological Analyses

Characterization of the microbial communities present in the progressive microcosms before and after incubation was conducted using cultivation-dependent and molecular techniques. Enrichment cultures for nitrate-reducing and Fe(III)-reducing bacteria were prepared by adding 10 ml of freshwater medium (Caccavo et al. 1994) containing 10 mM nitrate or 15 mM amorphous Fe(III) oxyhydroxide (Lovley and Phillips 1986), respectively as the sole electron acceptor, to 0.8 g of sediment and incubated at 20°C in the dark. Most probable number (MPN) dilution series were also prepared from these starting cultures, using 10-fold dilutions into the same media (Fujioka 1997). In addition, cultivation-independent molecular (PCR) techniques were used to assess microbial diversity. Sediment nucleic acids were extracted using a Fast DNA spin kit (UltraClean, Soil DNA Isolation Kit, MO BIO Laboratories INC, CA, USA). Changes in the diversity of the bacterial community, including the unculturable component were determined by denaturing gradient gel electrophoresis (DGGE; Muyzer 1999).

A variable region of the 16S rRNA gene was amplified by PCR using the universal bacterial primers (GC338F and 530R) targeting flanking conserved regions of the genes (van der Gast et al. 2001). Amplification products were loaded onto a 10% (w/v) polyacrylamide gel with a 40–60% denaturing gradient, within a SciPlas denaturing gradient CDC unit (Wolf Laboratories Ltd., York, UK). Electrophoresis was undertaken for 16 hours, at 60°C, using a 0.5% Tris-Acetate-EDTA (TAE; pH 8.0) buffer and the gel imaged under short-wave UV light following staining with 2 mg ml^{-1} Syber Gold (Molecular Probes Inc., Oregon, USA). To identify the groups of bacteria present within the samples, a conserved region of the 16S rRNA gene was amplified by PCR using the bacterial primers 8F and 519R (Lane et al. 1985). PCR products were purified using a QIAQuick Purification kit (Qiagen Ltd., Crawley, UK) and cloned into a PCR 2.1 vector, using a TA Cloning kit (Invitrogen Ltd., Paisley, UK) according to the manufacturers instructions, using competent *Escherichia coli* cells (One Shot TOP10, Invitrogen Ltd., Paisley, UK).

Recombinant clones were selected by antibiotic (ampicillin) resistance (carried within the vector) and blue/white colony screening. The presence of the 16S rRNA gene fragment was then verified by PCR and gel electrophoresis (Sambrook et al. 1989). Clones were separated into Operational Taxonomic Units (OTUs) based upon the similarity of Restriction Fragment Length Polymorphism (RFLP) profiles. For each of the clone libraries, approximately 30 clone sequences were screened. Analysis of saturation indices revealed a low diversity of OTUs, suggesting this relatively small sample size gave a good representation of overall diversity. PCR products were incubated (16 hours, 37°C) with restriction nucleases *SaU 3A1* and *Msp 1* (0.1 μl per reaction; Roche Products Ltd., Welwyn Garden City, UK) and digested fragments imaged following agarose gel electrophoresis staining with ethidium bromide.

The nucleotide sequences of each OTU were determined by the dideoxynucleotide method, using an ABI Prisms Big Dye Terminator Cycle Sequencing Kit, in combination with an ABI Prism 877 Integrated Thermal Cycler and ABI prism 377 DNA Sequencer (Perkin Elmer Applied Biosystems, Warrington, UK). Sequences were analyzed against the NCBI (USA) nucleotide–nucleotide database using the BLAST algorithm (Altschul et al. 1990) and matched to known 16S rRNA sequences. Sequences have been submitted to the NCBI GenBank database (accession numbers EF017373 to EF017382 and EF042297).

Progressive Microcosm ^{99}Tc Reoxidation Experiments

Air and nitrate reoxidation experiments were prepared using sediments that had been pre-reduced for ca. 5 months after the addition of 20 mM acetate. Nitrate reoxidation samples were amended with 100 mM nitrate and incubated at 20°C in the dark. Air reoxidation samples were transferred to 250 ml Erlenmeyer flasks, capped with sterile vented bungs (Whatman Bugstopper; Maidstone, UK), and shaken in the dark at 200 rpm and 20°C. Air reoxidation samples were weighed before sampling and an equivalent mass of distilled, deionized sterile water was added to replace evaporated water.

X-ray Absorption Spectroscopy (XAS) Sample Preparation

Sediment samples for analysis of Tc oxidation state and coordination environment by X-ray Absorption Spectroscopy (XAS) were prepared as follows. Initial XAS experiments focused on the fate of Tc(VII) in Fe(III)-reducing sediments. For detection limitation reasons, it was necessary to use Tc concentrations in the μM range, rather than the nM Tc used in our trace Tc experiments or the nM to sub nM concentrations found in contaminated

areas at the FRC. Pertechnetate (550 μM) was added to sediment samples that had been pre-reduced and contained ca 4 mmol l^{-1} Fe(II) in the sediment slurry, and incubated for 132 days. Samples that had been reoxidized by the addition of nitrate were also prepared for XAS analysis by anaerobically transferring a 5 ml slurry sample of the pre-reduced, ^{99}Tc labeled sediments to a sterile nitrogen-purged 10 ml sealed glass vial. The sample was amended with 100 mM nitrate and shaken periodically over 54 days. Samples of the supernatant were taken regularly over this period to assess Tc reoxidation behaviour. The air reoxidation sample was prepared for XAS analysis by transferring a 5 ml slurry sample to a 10 ml vial and manually aerating twice a week.

XAS Analyses

Technetium *K*-edge X-ray Absorption Near Edge Structure (XANES) and Extended X-ray Absorption Fine Structure (EXAFS) spectroscopic analyses were conducted to determine the oxidation state and coordination environment of the Tc in selected sediment samples (Wharton et al. 2000; Burke et al. 2005, 2006). The Synchrotron Radiation Source (SRS) operates at 2 GeV with a typical current of 150 mA. Samples were analyzed on the ultra-dilute spectroscopy beamline (16.5) using focusing optics and a Si-220 double crystal monochromator, calibrated from the *K*-edge of a molybdenum foil. *K*-edge spectra for ^{99}Tc were collected at ambient temperature in fluorescence mode using a 30 element solid state Ge detector. Four to 8 scans were collected and averaged for each sample to improve the signal-to-noise ratio.

Peak shapes and positions in the averaged XANES spectra were compared with previously analyzed data for reduced and reoxidized sediments (Burke et al. 2005). EXAFS data were background subtracted and analyzed using EXCURV98 software using full curved wave theory (Gurman et al. 1984). Phase-shifts were derived from ab initio calculations using Hedin–Lundqvist potentials and von-Barth ground states (Binsted 1998). The data were fitted for each sample by defining a theoretical model and comparing the calculated EXAFS spectrum with experimental data. Shells of backscatterers were added around the central ^{99}Tc atom and by refining an energy correction E_f (the Fermi energy), the absorber-scatterer distance, and the number of atoms in each shell, and a least squares residual (the R-factor; Binsted et al. 1992) was minimized. The Debye-Waller factor ($2\sigma^2$) was fixed at 0.008 Å^2 for the first shell and 0.010 Å^2 for outer shells, values which were obtained from previous analyses of Tc models. Shells were only included if the overall fit (R-factor) improved by $> 5\%$. In addition, XAS samples were analyzed for acid extractable Fe(II), total bioavailable iron, and soluble ^{99}Tc concentrations.

RESULTS AND DISCUSSION

Fe(III) Reduction and Tc(VII) Removal in FRC Sediment Microcosms

Initial experiments focused on the fate of Tc(VII) in FRC sediment microcosms incubated under anoxic conditions, in the presence and absence of added acetate (electron donor) and nitrate (competing electron acceptor; Figure 1). These conditions were chosen to approximate those normally used to stimulate Tc(VII) bioremediation via metal reduction in the subsurface (reviewed by Lloyd and Renshaw 2005). In progressive microcosm experiments, the time required to reach Fe(III)-reducing conditions was variable (data not shown) but generally took 60–90 days. Prior to the establishment of Fe(III)-reducing conditions, there was negligible removal of Tc(VII); however, after Fe(II) began accumulating in the

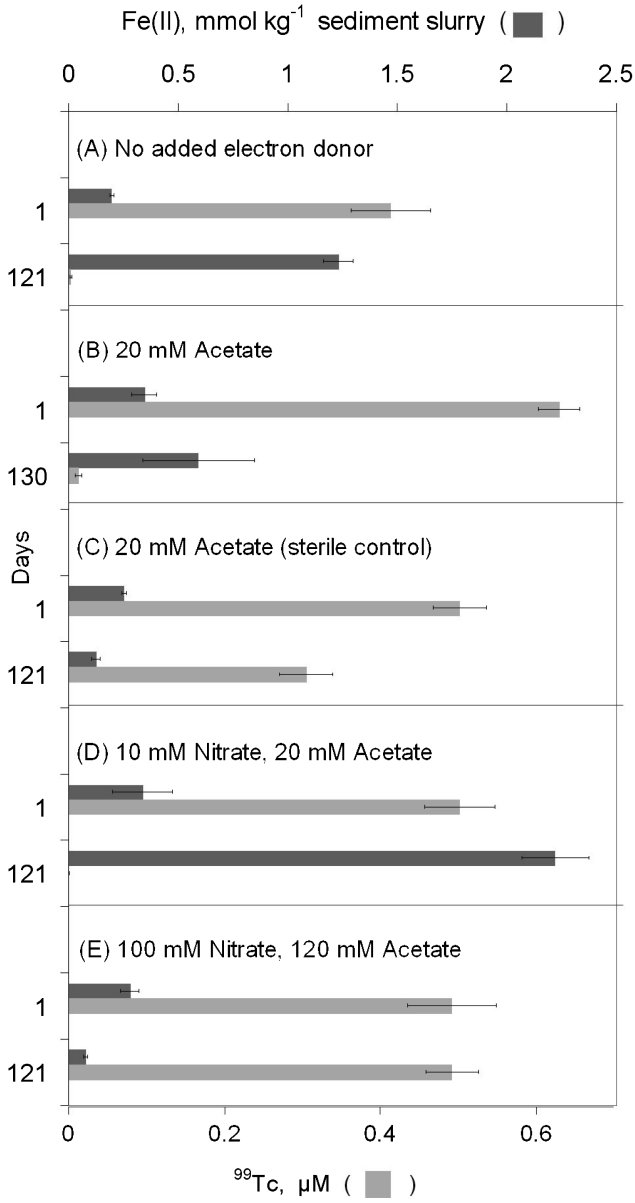

FIG. 1. Fe(II) and ^{99}Tc concentrations in FRC sediment microcosms incubated with and without added electron donor (acetate) and competing electron acceptor (nitrate). Note complete removal of Tc in treatments A, B, and D after 121 days of incubation.

sediments the concentrations of Tc in the sediment porewaters dropped from 0.5 to < 0.1 μM (data not shown).

Figure 1 shows initial and final acid extractable Fe(II) and soluble ^{99}Tc concentrations for each of the sample treatments, after ca. 120-day incubations. Total bioavailable iron in the experiments was 7.6 ± 3 mmol kg^{-1} sediment slurry, and acid extractable Fe(II) concentrations prior to incubation and reduction were ca. 0.25 mmol kg^{-1} sediment slurry (i.e., 3% of the total bioavailable iron). The acid extractable Fe(II) rose following incubation in all microcosms except those containing 100 mM nitrate, which contained less Fe(II) following incubation. The pH of the microcosms remained between 6.7–7.9 over the course of the incubations and the Eh decreased from +265 meV to ca. +100 meV in the microcosms containing no added electron donor or 20 mM acetate (data not shown).

Samples with no added electron donor or 20 mM acetate showed similar levels of Fe(III) reduction at the end point of the experiments (1.1 ± 0.1 and 0.8 ± 0.5 mmoles kg^{-1} Fe(II) sediment slurry, Figure 1A and 1B, respectively), and displayed efficient Tc removal ($> 98\%$). These results suggest that there were adequate indigenous electron donors in the groundwater and sediments to sustain efficient reduction of bioavailable Fe(III), and subsequent removal of Tc(VII); however, only ca. 15% of the bioavailable Fe(III) was reduced over the time period of this experiment. This suggests that either Fe(III) reduction was not complete, or that the amount of bioavailable Fe(III) was overestimated using our extraction protocols. Confirmation that Fe(III) reduction and Tc(VII) removal from solution was microbially mediated was obtained in microcosm experiments containing autoclaved sediment, where Tc removal was very much lower than in the parallel, microbially active experiments and Fe(II) in the sediments declined slightly over the 121-day incubation period (Figure 1C).

Surprisingly, the addition of 10 mM nitrate as a competing electron acceptor (with 20 mM acetate as an electron donor) also resulted in very efficient Fe(III) reduction and Tc(VII) removal over the time course of the experiment (Figure 1D). Indeed, in these systems the Eh dropped to $+ 50 \pm 6$ meV by 121 days. Here, 2.2 ± 0.2 mmoles kg^{-1} Fe(II) accumulated in the sediment slurry by the end of the incubation, corresponding to $25 \pm 2\%$ of the bioavailable Fe(III), and $> 99\%$ of the ^{99}Tc was removed from solution by the end point of the experiment (Figure 1D). Although nitrate is known to inhibit the microbial reduction of both Fe(III) and Tc(VII) (reviewed by Lovley 1991, and Lloyd 2003, respectively), in these microcosms all the nitrate had been removed by the end of the experiment.

Additionally, there was no net accumulation of nitrite (data not shown), and this suggested that NO_3^- was being reduced to NH_4^+, N_2 or an intermediate such as N_2O. With the exception of N_2O (Kluber and Conrad 1998), these products of denitrification have not been shown to inhibit metal reduction. When 100 mM nitrate was added to parallel microcosms, Fe(III) reduction and removal of ^{99}Tc from solution was inhibited completely (Figure 1E), despite an Eh drop to $+10 \pm 20$ meV by the end of the incubation. Analysis of the supernatants from these experiments confirmed that approximately 70 mM nitrate remained in the porewaters and ca. 27 mM nitrite had accumulated in the microcosms by the end point. This suggested that the

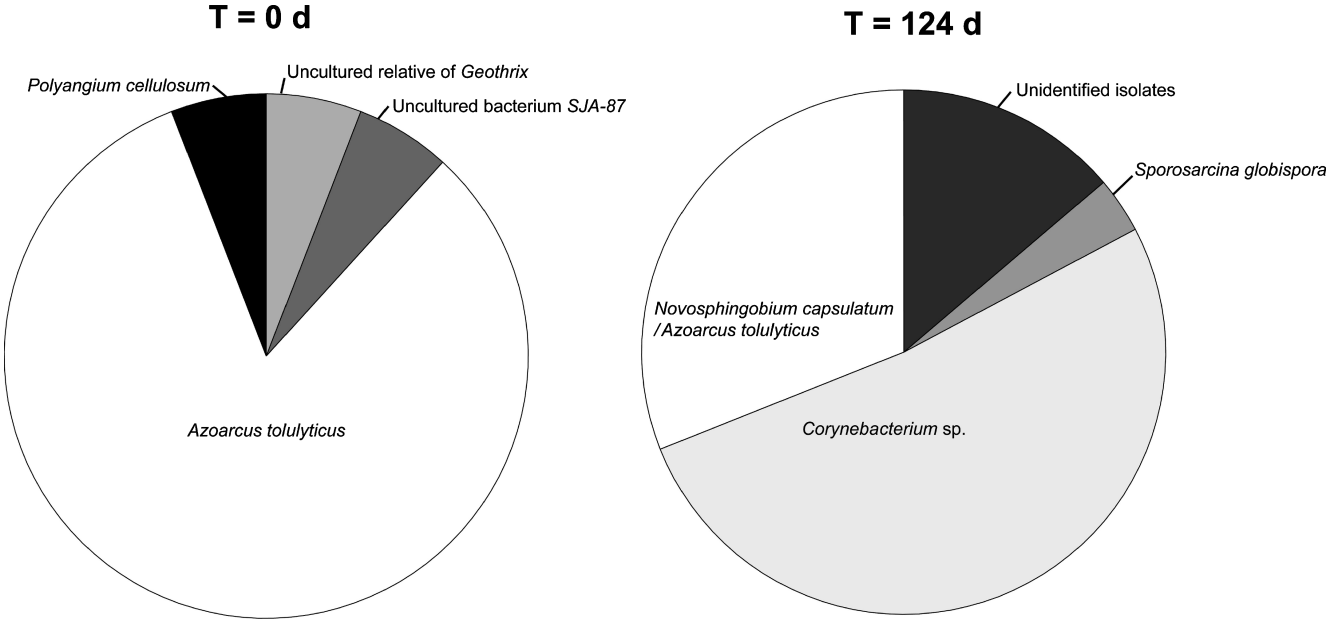

FIG. 2. Sediment microbial community structure (assessed from 16S rRNA gene analysis of clone libraries) for sediments incubated with groundwater and 10 mM nitrate and 20 mM acetate, before incubation (T = 0 d) and after incubation (T = 124 d). Organisms shown on pie chart are closest matching organisms from the NCBI Blast Database. GenBank Accession numbers EF017373 to EF017382.

TABLE 1
Technetium *K*-edge EXAFS parameters for reduced and reoxidized sediments

Scatterer	N	r (Å)	$2\sigma^2$ (Å^2)	R
(i) O	0.7	1.72	0.008	39.8
O	5.3	2.01	0.010	
Tc	1.7	2.52	0.010	
(ii) O	1.6	1.71	0.008	48.0
O	2.6	2.65	0.010	
Tc	1.0	2.54	0.010	
(iii) O	1.9	1.71	0.008	62.4
O	3.2	2.01	0.010	
(iv) O	0.5	1.73	0.008	31.2
O	5.4	2.01	0.010	
Tc	1.8	2.55	0.010	
(v)[a] O	4	1.72	0.007	22.9

(i) Fe(III)-reducing sediment amended with 550 μM TcO_4^-; (ii) Fe(III)-reducing sediment amended with 550 μM TcO_4^-, then subject to reoxidation with 25 mM nitrate; (iii) Fe(III)-reducing sediment amended with 550 μM TcO_4^-, then subject to reoxidation with 100 mM nitrate; (iv) Fe(III)-reducing sediment amended with 550 μM TcO_4^-, then subject to reoxidation with air; (v) $Tc(VII)O_4^{-a}$.

N is the occupancy ($\pm$ 25%), r is the interatomic distance ($\pm$ 0.02 Å for the first shell, $\pm$ 0.05 Å for outer shells), $2\sigma^2$ is the Debye–Waller factor, and *R* is the least squares residual.

[a]Wharton et al. (2000).

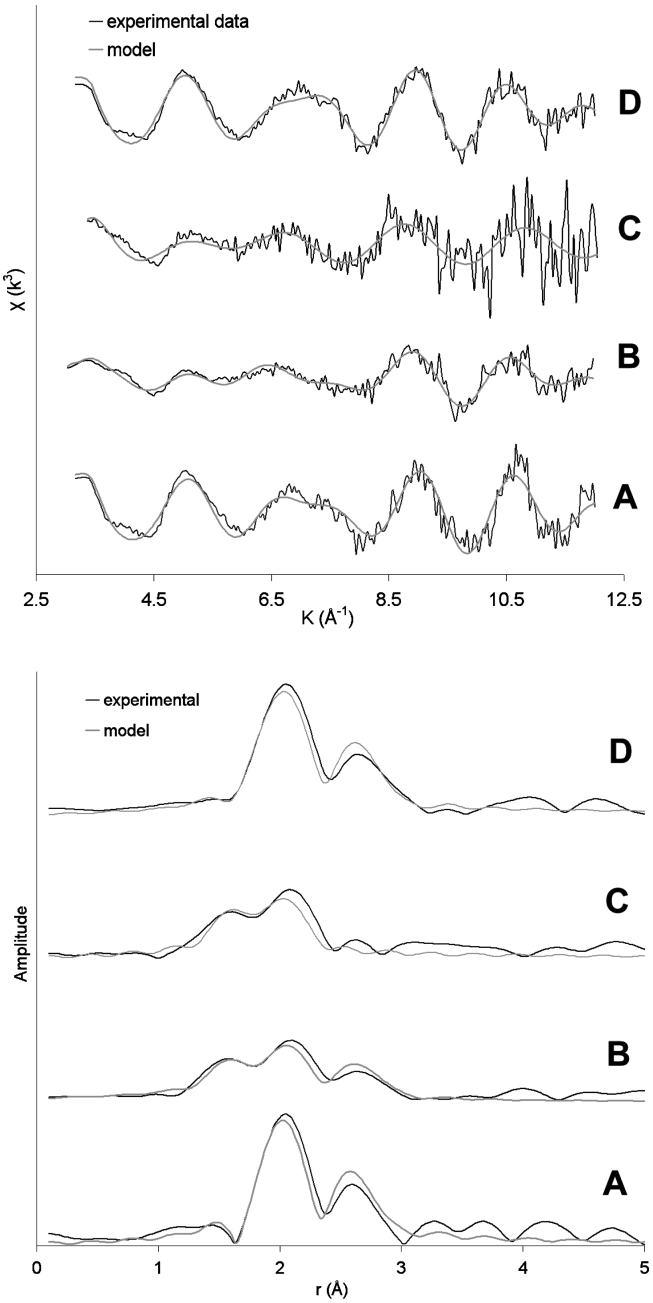

FIG. 3. k^3-weighted ^{99}Tc *K*-edge EXAFS and associated Fourier transforms for (A) Fe(III)-reducing sediment; (B) 25 mM nitrate reoxidized sediment; (C) 100 mM nitrate reoxidized sediment; and (D) air reoxidized sediment

microbial communities in these sediments lacked the capacity to completely denitrify such high concentrations of nitrate under the conditions imposed. These experiments confirm that complete denitrification would be required prior to achieving efficient metal reduction in areas of the FRC site contaminated with high concentrations of nitrate.

Clone library analysis of 16S rRNA genes within the sediment microcosms identified several bacteria (Figure 2, Supporting Information Table SI-1 and SI-2) that were likely to be involved in the key biogeochemical processes highlighted above. In our microcosms, a close relative of the nitrate-reducing microorganism *Azoarcus tolulyticus* was identified as the dominant bacterium in the clone library prior to incubation (Figure 2, t = 0 days, 82.4% of clones; clone JMM3, refer to supplementary information tables SI-1 and SI-2 for more information). This species is common in the contaminated areas of the FRC site (Fields et al. 2005), and these results suggest that this organism is also significant in uncontaminated areas of the site.

Further analyses of clone library data after 121 days of incubation in the presence of 20 mM acetate and 10 mM nitrate suggested that bacteria closely related to the nitrate-reducing *Novosphingobium capsulatum* (Takeuchi et al. 2001), and *A. tolulyticus* comprised 30% of the total OTUs detected (clone JMM1). This suggests that relatives of *A. tolulyticus* play a role in the denitrification processes described for this system. It should be noted that *A. tolulyticus* has also been found in natural soils low in nitrate at other U.S. contaminated sites (Holmes et al. 2004), grows on a variety of electron donors including acetate, and is capable of aerobic growth, denitrification and nitrogen-fixation (Zhou et al. 1995). Members of the species *Azoarcus* are able to break down organic compounds such as phenol (Van Schie and Young 1998) and toluene (Zhou et al. 1995), suggesting these bacteria may have potential to remove organic co-contaminants at the FRC. Of the remaining clones in this

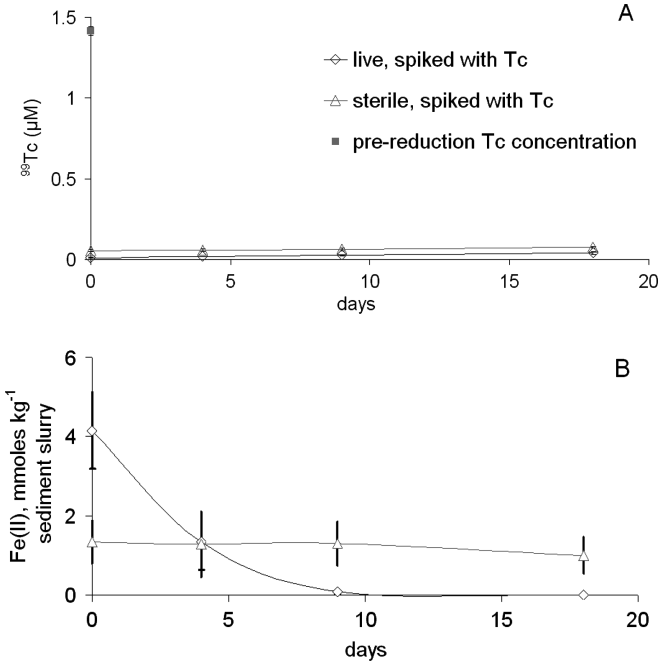

FIG. 4. Reoxidation of pre-reduced Fe(II)/Tc(IV)-bearing FRC sediments with 100 mM nitrate. A: concentration of soluble ^{99}Tc (μM), B: concentration of acid (0.5 N HCl) extractable Fe(II) (mmoles kg^{-1} sediment slurry).

library, 50% (clone JMM2) were closely related to another potential denitrifier, *Corynebacterium* sp. (Watts et al. 2000). This organism was not detected in the sediment at the t = 0 timepoint.

Potential nitrate-reducing bacteria including organisms related to *Azoarcus* species (clone JMM3) were also detected in enrichment cultures, and were present at higher concentrations ($> 10^6$ cells per g, scored by MPN counts with IC analysis of nitrate at 2 weeks, > 80% nitrate removal for positive result) than Fe(III)-reducing bacteria in parallel MPN enrichments (> 10 cells per g, scored by ferrozine at 1.3 year, 30% of available Fe(III) reduced for positive result). A clone (JMM9) related to an Fe(III)-reducing bacterium (uncultured relative of *Geothrix* species, Nedelkova 2005; NCBI Acc. No. AJ583203, 99% sequence homology, 498/499 b.p.) was also detected in the pre-incubation samples. Parallel studies using cultivation-dependent and molecular techniques on materials from the same site have shown the presence of other Fe-cycling bacteria including relatives of the Fe(III)-reducing bacteria *Geobacter* sp. (Clone JMM4; see supplementary information Table SI-3 for more information), *Geobacter bemidjiensis* (Clone JMM6), and Fe(II)-oxidizing bacteria *Gallionella* sp. (Clone JMM5). Other workers (Petrie et al. 2003) have also identified members of the *Geobacteraceae* and other metal-reducing microbes in FRC background area sediments.

Fate of Tc(VII) in Fe(III)-Reducing FRC Sediments

XAS analyses were used to determine the coordination environment and interatomic distances of the ^{99}Tc in Fe(III)-reducing FRC background area sediments. Technetium *K*-edge k^3-weighted EXAFS and associated Fourier transforms are shown in Table 1 and Figure 3. XANES (Supporting Information, Figure SI-1) were consistent with EXAFS data (Table 1). The predominant form (87 ± 22%) of ^{99}Tc in the Fe(III)-reducing sediments (Figure 3a) was Tc(IV) as Tc-O bonds at 2.01 Å are diagnostic for hydrous TcO_2 in environmental matrices (Maes et al. 2004). Additionally, third shell fits were attempted with Tc, Fe and Mn. Tc at 2.52 Å gave the best fit (R = 39.8 for Tc vs. 49.7 and 49.9 for Fe and Mn, respectively). The presence of ^{99}Tc at 2.52 Å is consistent with the formation of discrete hydrous TcO_2 phases (Almahamid et al. 1995; Hess et al. 2004). Similar values for Tc labelling freshwater sediments (Tc-Tc distances of 2.56 Å) were reported by Wildung et al. (2004), and studies of the geomicrobiology of ^{99}Tc in estuarine sediments have also shown evidence for co-reduced Tc existing in a hydrous TcO_2-like environment (Burke et al. 2005).

Potential for Tc(IV) Reoxidation and Remobilization

Technetium reduced and immobilized by metal-reducing prokaryotes in aquifer sediments may be subjected to reoxidation and resolubilization by exposure to oxidants such as air or nitrate over time. A further series of microcosm experiments was conducted to simulate this scenario by introducing (i) nitrate and (ii) air into FRC sediments that had been pre-reduced and had accumulated reduced, insoluble Tc presumably as hydrous TcO_2 (Figures 4 and 5, respectively).

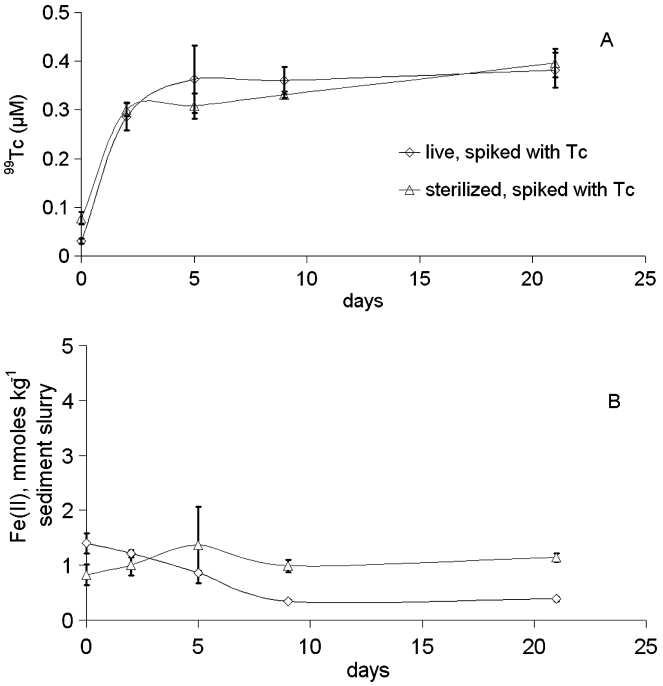

FIG. 5. Reoxidation of pre-reduced Fe(II)/Tc(IV)-bearing FRC sediments with air. A: concentration of soluble ^{99}Tc (μM). B, concentration of acid (0.5 N HCl) extractable Fe(II) (mmoles kg^{-1} sediment slurry).

Addition of 100 mM nitrate (Figure 4) had little effect on ^{99}Tc solubility in Fe(III)-reducing progressive microcosms containing immobilized ^{99}Tc at approximately 1 ± 2 nmol g^{-1} of sediment. Fe(II) concentrations on sediments prior to nitrate reduction coupled to Fe(II) reoxidation were 4 ± 1 mmol l^{-1}, and ^{99}Tc concentrations in solution were below detection, presumably due to reduction to hydrous Tc(IV)O_2. Upon addition of the nitrate, there was a significant decrease in the concentration of acid extractable Fe(II) to 0.12 ± 0.05 mmol l^{-1} (Figure 4), as well as increased gas production in samples, suggesting Fe(II) oxidation was coupled to nitrate reduction. However, significant remobilization of ^{99}Tc was not observed in the samples over the course of the 18-day incubation period. In contrast, exposure of pre-reduced sediment slurries to air resulted in both Fe(II) oxidation and significant remobilization of ^{99}Tc within 2 days. By 20 days approximately 80% of ^{99}Tc in the samples had resolubilized (Figure 5).

In similar microcosm experiments with estuarine sediments, XAS measurements showed that Tc resolubilization on air reoxidation was associated with reoxidation of Tc(IV) to Tc(VII) (Burke et al. 2006) and presumably, a similar mechanism is operating here. To identify the oxidation state of the Tc remaining in the sediments after various reoxidation regimes, pre-reduced sediments containing higher concentrations of Tc(IV) (several hundred ppm on solids) were reoxidized with additions of air or nitrate for 54 days, and analyzed using EXAFS. Interestingly, nitrate (25 mM, Figure 3b; 100 mM, Figure 3c) reoxidized samples indicated approximately equivalent proportions of Tc(IV) and Tc(VII) (Table 1) remained in the solid phase, similar to the observations of Burke et al. (2006). In contrast, there was significant remobilization of the radionuclide with air reoxidation, but negligible Tc(VII) was detected in the solid phase, which was predominantly Tc(IV) (86 ± 21%; Figure 3d). Technetium produced a better third shell-fit than Fe or Mn, suggesting that a discrete hydrous TcO_2 phase persisted following reoxidation.

SUMMARY

We have demonstrated that removal of Tc(VII) from solution in microcosms prepared from FRC background sediments can be linked to the activity of Fe(III)-reducing bacteria, and that following the onset of Fe(III)-reducing conditions the form of the immobilized ^{99}Tc in these sediments is a discrete hydrous TcO_2 phase. We have also found that air reoxidation effectively remobilizes much of the ^{99}Tc from the sediments; however, ca. 20% of the ^{99}Tc remains immobilized under the conditions tested in our microcosm experiments, largely as hydrous Tc(IV)O_2. With the onset of denitrifying conditions, ca. 50% of the Tc(IV) was oxidized to Tc(VII) but surprisingly remained immobilized via a mechanisms that remains to be elucidated. At trace (0.5 to 1.5 μM) concentrations the ^{99}Tc was not significantly solubilized even when challenged with very high (100 mM) concentrations of nitrate although measurable Fe(II) reoxidation had occurred. Therefore, the biogeochemical cycling of technetium in contaminated environments is strongly correlated with exposure to oxygen, while immobilized ^{99}Tc is relatively immune to resolubilization by the anoxic oxidant nitrate. Thus, mitigating the risk of aquifer aeration is an important consideration when designing long-term bioreduction remediation strategies at sites with legacy ^{99}Tc contamination.

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