Environ. Sci. Technol. **2005**, *39,* 4109−4116

Effects of Progressive Anoxia on the Solubility of Technetium in Sediments

IAN T. BURKE,[†]
CHRISTOPHER BOOTHMAN,[‡]
JONATHON R. LLOYD,[‡]
ROBERT J. G. MORTIMER,[†]
FRANCIS R. LIVENS,[‡,§] AND
KATHERINE MORRIS*,[†]

School of Earth and Environment, University of Leeds, Leeds, LS2 9JT, U.K., Williamson Centre for Molecular Environmental Science, School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Manchester M13 9PL, U.K., and Centre for Radiochemistry Research, and School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Manchester M13 9PL, U.K

Technetium is a significant radioactive contaminant from nuclear fuel cycle operations. It is highly mobile in its oxic form (as $Tc(VII)O_4^-$) but is scavenged to sediments in its reduced forms (predominantly Tc(IV)). Here we examine the behavior of Tc at low concentrations and as microbial anoxia develops in sediment microcosms. A cascade of stable-element terminal-electron-accepting processes developed in microcosms due to indigenous microbial activity. TcO_4^- removal from solution occurred during active microbial Fe(III) reduction, which generated Fe(II) in the sediments and was complete before sulfate reduction began. Microbial community analysis revealed a similar and complex microbial population at all three sample sites. At the intermediate salinity site, Paull, a broad range of NO_3^-, Mn(IV), Fe(III), and SO_4^{2-} reducers were present in sediments including microbes with the potential to reduce Fe(III) to Fe(II), although no differences in the microbial population were discerned as anoxia developed. When sterilized sediments were incubated with pure cultures of NO_3^--, Fe(III)-, and sulfate-reducing bacteria, TcO_4^- removal occurred during active Fe(III) reduction. X-ray absorption spectroscopy confirmed that TcO_4^- removal was due to reduction to hydrous $Tc(IV)O_2$ in Fe(III)- and sulfate-reducing estuarine sediments.

Introduction

Technetium-99 is a long-lived (half life, 2.13×10^5 years), β-emitting radionuclide formed in high yield in nuclear reactors that has been released to the environment in authorized and accidental discharges and is an important component of radioactive wastes. The redox chemistry of technetium is the major control on its environmental solubility. Under oxic conditions, technetium is present as the pertechnetate ion (TcO_4^-), which is only weakly sorbed to mineral surfaces and is one of the most mobile radionuclide species in the environment (*1*, *2*). By contrast, in reducing environments, the lower valence forms of technetium (predominantly Tc(IV)) become associated with solid phases through either hydrolysis or formation of strong surface complexes with Al and Fe oxides and clays (*1*, *2*). Because of the long half life of Tc, and its high environmental mobility, understanding the controls on Tc reduction and scavenging by sediments is essential to informing policy on how to best manage this contaminant. Within the natural environment, Tc is present at ultra-trace concentrations and this, combined with difficulties in its analysis, means that environmental data for this radionuclide are scarce, and little is understood of its solid-phase interactions (*3*−*5*).

One model for the development of reducing environments in sediments is the microbial degradation of organic matter coupled to different terminal-electron-accepting processes (TEAPs). Which TEAP predominates in sediments is a function of both the availability of the terminal acceptors within the sediment and the free-energy yield of the process. Thus, in sediment burial, a succession of different TEAPs with decreasing free-energy yields occur with depth in the following order: oxygen, nitrate, manganese(IV), iron(III), sulfate, and carbon dioxide (methanogenesis) (Table 1). In microcosm experiments, in which sediment is isolated from air and progressive anoxia develops, these processes are separated in time. Here we describe sediment microcosm experiments that examine the changes in solubility and redox speciation of TcO_4^- in the context of the classic biogeochemical evolution of anoxic estuarine sediments.

Previous studies have examined the behavior of Tc(VII) in pure culture experiments with anaerobic microorganisms (*2*, *6*−*10*), and a range of reduced Tc(IV) solid-phase reaction products have been reported. Both direct (enzymatically mediated) and indirect (abiotically mediated) TcO_4^- reduction has been proven for pure culture systems with both Fe(III)- and sulfate-reducing bacteria. In the presence of both Fe(III) and sulfate reducers, with hydrogen present as the electron donor, and with TcO_4^- present as the only electron acceptor, technetium can act as the terminal-electron-accepting species (via a hydrogenase enzyme) to form TcO_2(s) (*2*, *11*). However, at the lower concentrations of Tc seen in the natural environment, indirect mechanisms for Tc(VII) reduction will probably predominate because the kinetics of the enzyme systems that reduce Tc(VII) in whole cells suggest an inability to recognize trace concentrations of the radionuclide (*12*). A broad range of indirect mechanisms have been reported including abiotic, reductive precipitation of TcO_4^- onto Fe(II)-containing minerals in Fe(III)-reducing systems (*2*, *13*) and precipitation of insoluble Tc-sulfides, probably as TcS_2 in sulfate-reducing systems (*8*, *14*). In sediments, the behavior of Tc(VII) as sediment anoxia develops is less clear because studies have focused mainly on the addition of Tc(VII) to already reduced sediments. Although, from pure culture experiments and Gibbs free-energy considerations (Table 1) we expect that TcO_4^- reduction would coincide with Fe(III) reduction. Finally, the role of organic matter in controlling Tc solubility is unclear, with evidence that soluble Tc(IV)-organic matter complexes form in some environments (*5*, *15*) and that Tc(IV) is associated with organic matter in some naturally radiolabeled sediments (*4*). In these experiments, microcosms of Tc-free Humber Estuary surface sediments (with their indigenous microbial populations) were spiked with low levels ($<5\ \mu$M) of TcO_4^- to make the experiments as environmentally relevant as possible. Technetium behavior was then observed as

* Corresponding author phone: +44 113 343 6723; fax: +44 113 343 6716; e-mail: kath@env.leeds.ac.uk.
† University of Leeds.
‡ Williamson Centre for Molecular Environmental Science.
§ Centre for Radiochemistry Research.

10.1021/es048124p CCC: $30.25
Published on Web 04/30/2005

TABLE 1. Depth-Related Sequence of Biogeochemical Zones and Gibbs Free-Energy Calculations

biogeochemical zone	Gibbs free-energy change per mole of acetate consumed as electron donor (kJ mol^{-1})	
oxic respiration[a]	−856	decreasing
nitrate reduction[a]	−806	energy yield
manganese reduction[a]	−569	per mole
technetium reduction[b]	−436	of organic
iron reduction[a]	−361	matter
sulfate reduction[a]	−48	consumed

[a] Data from ref 42. [b] Data from Nuclear Energy Agency Database Project, www.nea.fr, and ref 16.

anoxia developed in the microcosms. Complementary experiments, using single microbial species inoculated into sterile microcosms, have been carried out to define the relationship between TcO_4^- removal and the TEAP sequence, and extended X-ray absorption fine structure (EXAFS) spectroscopy has been used to characterize the chemical form of the reduced Tc.

Experimental Section

Sampling. Sediments were collected during August and November of 2002 from intertidal mudflats exposed at low tide at three sites along the Humber Estuary that encompass freshwater to mid-salinity conditions, Boothferry (BF), Paull (PL), and Skeffling (SK) (Figure 1). Care was taken to collect only the top few millimeters of sediment, and river water was also collected adjacent to each site. While wearing face masks and gloves, we collected the sediments into sterile containers. The salinities of BF, PL, and SK were 0.1, 15.5, and 18.2 ‰ respectively. The temperature was between 13 and 14 °C at all of the sites.

Microcosm Experiments. Sediments collected from BF, PL, and SK were homogenized to form a slurry (40−80% sediment w/w). Microcosms containing 10-mL aliquots of sediment slurry and 20 mL of river water were made up using aseptic technique in 30-mL glass serum bottles (Wheaton Scientific, NJ) and sealed with butyl rubber stoppers (Bellco Glass Inc., NJ) and aluminum crimps. Three repeat series of eight time points were amended with a ^{99}Tc spike (UK Atomic Energy Authority, Harwell) (final concentration 5 μM as ammonium pertechnetate) and incubated anaerobically at 10−12 °C. A sterile control series was also established by autoclaving (3 × 20 min at 120 °C) and amending with Tc. Microcosm bottles were then sampled every 3−7 days over a 36 day period. Sampling was done in an anaerobic cabinet (95% N_2, 5% H_2; Coy Laboratory Products Inc, MI), microcosms were shaken, and sediment slurry samples were withdrawn. Aliquots of sediment were stored at 4 °C for most probable number (MPN) work and at −20 °C for microbial characterization work with DNA. Filtered (<0.2 μm) pore water samples were also collected and a range of redox indicators (Eh, NO_3^-, Mn^{2+}, Fe^{2+}, SO_4^{2-}, HS^-), pH, and Tc were analyzed. On Paull sediments, 0.5 M HCl extractable Fe(II) was also determined.

Pure Culture Microcosms. Microcosms were established with 20 mL of PL sediment slurry and 60 mL of river water in 120-mL serum bottles. The sediments were then sterilized by autoclaving (3 × 120 °C for 20 min). Triplicate microcosms were then inoculated with washed pure cultures of bacterial cells to produce a cell concentration of ~10^{3-4} mL^{-1} with the following: a NO_3^--reducing bacteria isolated from PL sediments, *Pseudomonas stutzeri*; an Fe(III)-reducing bacteria, isolated from PL sediments, *Shewanella* sp.; and a reference, sulfate-reducing bacteria, *Desulfovibrio desulfuricans* sp.

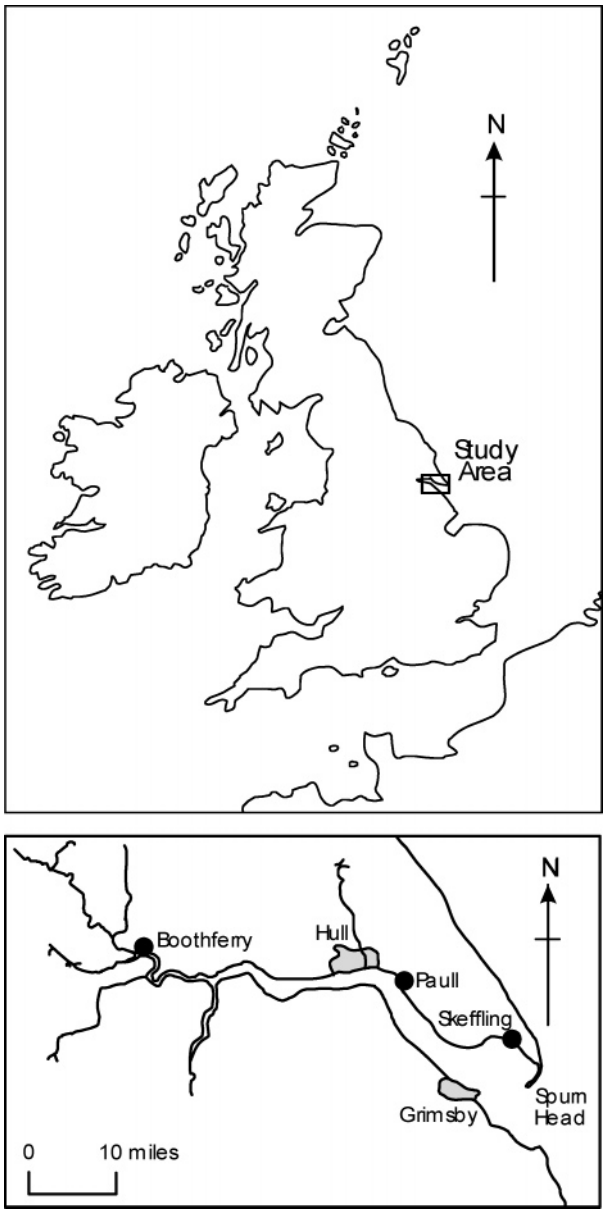

FIGURE 1. Location map of the Humber Estuary, showing the Boothferry, Paull, and Skeffling sampling sites.

Essex. Abiotic controls were also established, and all of the bottles were incubated at room temperature in the dark. After 4 days, sufficient ammonium pertechnetate to give a final concentration of 3 μM was added, and aseptic technique sampling occurred under anaerobic conditions over 56 days. The concentration of the total Tc in solution, SO_4^{2-}, and ingrowth of Fe(II) to sediments was determined at each time point. After 15 days, no geochemical reaction had been observed, so the viability of each culture was checked by inoculating a small subsample from each microcosm into a highly selective medium for growth of the particular strain and then scoring for growth. Following confirmation that the bacteria were viable, an appropriate electron donor for each pure culture was added to the microcosms because it was apparent that the organic matter in the sterile sediments was unavailable for metabolic processes in these pure culture species. This is in contrast to the nonsterile experiments in which an active microbial consortium was able to support microbial reduction, presumably via hydrolysis and fermentation of labile organic carbon to an available electron donor such as acetate (*16*). Acetate (20 mM) was added to the *Pseudomonas stutzeri* microcosms, and 20 mM lactate was

added to the *Shewanella* sp. and *Desulfovibrio desulfuricans* microcosms to act as the electron donor.

Geochemical Methods. The bulk mineral and chemical composition of the BF, PL, and SK sediments was determined by XRD on a Philips PW1050 and XRF on a Thermo ARL 9400. Carbon content (both TOC and TIC) of the Boothferry, Paull, and Skeffling sediments was determined on a Carlo Erba EA12. Total Tc activity in pore waters was determined by liquid scintillation counting (LSC) on a Packard Tri-Carb 2100TR (detection limit ~0.4 Bq). In addition to total Tc analyses, an extraction technique was used at appropriate sample points to determine TcO_4^- (*10*). Total Fe, Mn, and HS^- in pore waters were determined by standard UV−vis spectroscopy methods on a Cecil CE 3021 (*17*−*19*). Ingrowth of Fe(II) to sediments was determined by extraction in 0.5 M HCl for 60 min (*20*). This solid-phase Fe(II) extraction has been shown to be specific for microbially produced Fe(II) in sediments (*20*), although small amounts of mineral-derived Fe(II) may also be measured (*21*). Sulfate, nitrate, and chloride were determined by ion chromatography. In samples with high salinity, NO_3^- was determined using a colorimetric method (*22*). Standards were used regularly to check the method quality, and calibration regressions normally produced r-squared values of ≥0.99. Eh and pH readings were taken using an Orion 420A meter and calibrated electrodes.

X-ray Absorption Spectroscopy. For direct spectroscopic analysis, three further samples at higher Tc concentrations (~1000 μM) were prepared. At these higher levels, chemical toxicity of Tc was a concern, so we added TcO_4^- to sediments that had already accumulated biogenic Fe(II) and sulfide as a result of Fe(III) and sulfate reduction. In addition, in a progressive anoxia experiment, TcO_4^- was added at the high concentration (~1000 μM) prior to active Fe(III) and sulfate reduction. Surprisingly, microbial reduction proceeded in this sediment, albeit at a slow rate, and we were able to obtain a further, biotically reduced sediment sample for XAS. Tc K-edge spectra were collected in fluorescence mode using a 30-element solid-state Ge detector on station 16.5 at the UK CLRC Daresbury SRS. Operating conditions, data acquisition, and data analysis procedures were the same as previously reported (*23*) and are described briefly in the Supporting Information.

Microbiological Methods

Sediment was pretreated with sodium oxalate solution (0.3 M), and DNA was extracted using a Fast DNA spin kit (UltraClean, Soil DNA Isolation Kit, MO BIO Laboratories INC, Solana Beach, CA) prior to PCR-based 16S rRNA gene analysis. A fragment of the 16S rRNA gene (approximately 520 bp) was amplified from samples using broad-specificity primers 8F (*24*) and 519r (*25*). Experiments were also conducted using primers 494F and Geo825R, designed to target Fe(III)-reducing members of *Geobacteraceae* (*26*). PCR reactions were performed using a BioRad iCycler (BioRad, Hemel Hempstead, Herts, UK), as described previously for the broad specificity primers (*27*), or using the protocol of Holmes et al. (*26*) for primers 494F and Geo825R. The purity of the amplified product was determined by electrophoresis in tris-borate-EDTA (TBE) gel. Restriction fragment length polymorphism (RFLP) analysis was performed by a previously described method (*27*) with cloning directly into vector pCR 2.1 (Invitrogen, Paisley, UK) prior to transformation into *E. coli* TOP 10 competent cells. The transformants were screened for an insert using PCR, and the resulting PCR products were treated with restriction endonucleases *Sau3A* and *Msp I* prior to separation using an agarose TBE gel. DNA sequencing and phylogenetic analysis was performed as described previously (*27*). Nucleotide sequences (typically 500 base pairs in length) were determined by the dideoxynucleotide method (*28*), analyzed against the NCBI (USA) database using the BLAST program (*29*), and matched to known 16S rRNA gene sequences. Gene sequences were aligned using the ClustalX software package and corrected manually. The TREECON package (*29*) was used for distance analysis using the Jukes and Cantor correction (*30*). A phylogenetic tree was constructed from the distance matrix via neighbor joining (*31*). The nucleotide sequences described in this study are deposited in the GenBank database (AY361914−AY361954).

Intergenic spacer analysis fingerprinting was performed by targeting the 16S-23S intergenic spacer region from the bacterial RNA operon. DNA purified from sediment samples was amplified using PCR with primers S-D-Bact-1522-b-S-20 (eubacterial 16S rRNA small subunit) and L-D-Bact-132-a-A-18 (eubacterial 23S rRNA large subunit) (*32*). S-D-Bact-1522-b-S-20 was labeled at the 5′ end with HEX (6-carboxyhexafluorescein) fluorochrome, and PCR was performed as described previously (*33*). This resulted in amplification of a HEX-labeled gene product containing the intergenic gene sequence and approximately 20 bp of the S-D-Bact-1522-b-S-20 primer and 130 bp of the 23S rRNA gene. The intergenic spacer fragments were resolved on a 5% polyacrylamide gel and run under denaturing conditions for 4.5 h at 3000 V on an ABI Prism 377 DNA sequencer (Perkin-Elmer Applied Biosystems, Warrington, UK). The data were analyzed using GeneScan 3.1 software that converted the fluorescence data into electrophoregrams. Peaks represented fragments of different sizes, with peak heights proportional to the quantity of fragment in the mixed product.

Estimation of Numbers of Fe(III)-reducing Bacteria by MPN Counts and Isolation of Pure Cultures of Fe(III)-reducing Bacteria. A 10-fold dilution series was prepared in triplicate from sediment samples using tubes containing a freshwater medium (*33*) supplemented with 6.66 g L^{-1} NaCl and 1 g L^{-1} $MgCl_2$ to match the brackish conditions. All of the manipulations were made in an anaerobic cabinet. The tubes were incubated at 20 °C and scored on a weekly basis for the reduction of Fe(II) using a ferrozine-based assay (*34*). The numbers of positive tubes were tabulated, and the most probable number of Fe(III)-reducing bacteria was estimated (*35*). Pure cultures of Fe(III)-reducing bacteria were isolated from MPN tubes under anaerobic conditions on a solid medium consisting of the brackish medium above, solidified with 30 g L^{-1} agar.

Results and Discussion

Sediment Properties. An identical suite of minerals (quartz, carbonate minerals (calcite, dolomite), sheet-silicate minerals (kaolinite, clinochlore, muscovite), and feldspars (albite, orthoclase)) was present at all of the sites. BF sediments were relatively rich in Si (60%), which is consistent with sediments richer in quartz. Downstream at PL and SK, sediments had less Si (52 and 45%, respectively) and progressively more Al, Fe, Mg, Na, and K, which are associated with aluminosilicate minerals (Supporting Information Table 1). The total inorganic carbon was 1.9%, 1.7%, and 2%, and the total organic carbon was 2.5%, 2.3%, and 2.8% at BF, PL, and SK, respectively. The main variation between sites was the salinity rather than the physical properties of the sediment.

Reduction of Tc During Progressive Anoxia. Results from the lower concentration progressive anoxia microcosm series are presented in Figure 2. Samples from all of the sites progressed through the cascade of TEAPs as expected. Over 99% of the Tc was removed from pore waters in the BF, PL, and SK experiments during incubation with sediments over 36 days. Technetium speciation extractions on pore waters (*10*) indicated that TcO_4^- was the predominant (normally >96%) soluble Tc species at all of the time points. In sterile controls, Tc remained in solution as TcO_4^-, indicating that

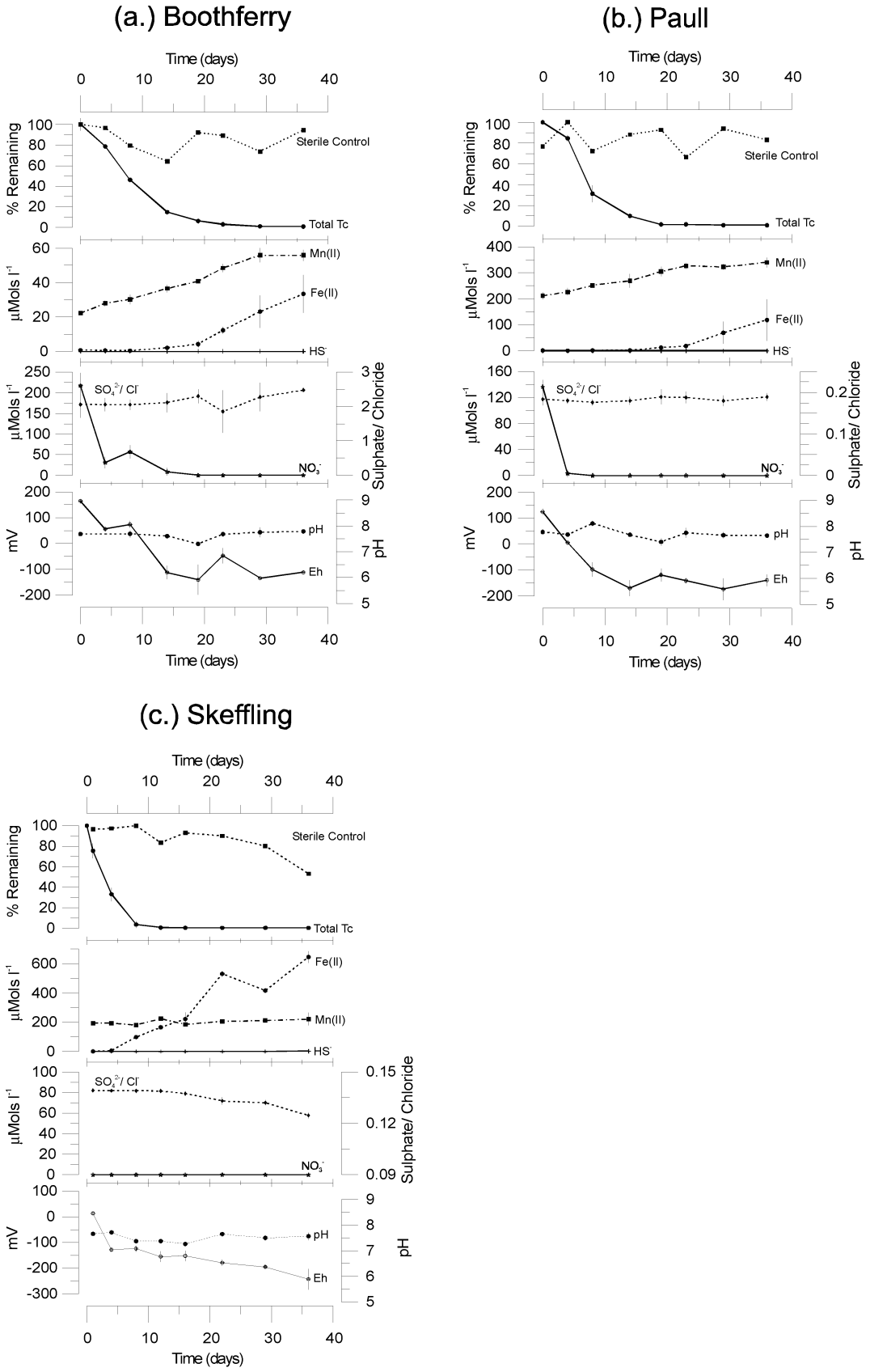

FIGURE 2. Time-series data showing the removal of Tc from solution, and changes in redox indicator species, in incubations with (a) BF, (b) PL, and (c) SK sediments. Error bars are 1 σ of triplicate results (where not shown, errors are within size of symbol).

Tc reduction and removal to sediments was biologically mediated.

In the BF and PL series, Tc removal occurred only when the vast majority (>90%) of nitrate had been consumed in the microcosms and when active accumulation of Mn(II) in pore waters was occurring (Figure 2). In a parallel experiment utilizing PL sediments to investigate Fe(II) ingrowth in sediments, 0.5 M HCl extractable Fe(II) was measurable at

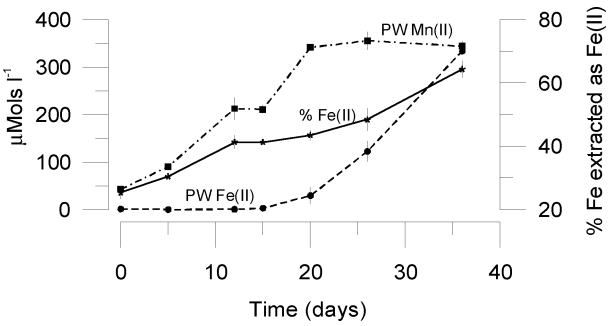

FIGURE 3. Ingrowth of sediment-bound Fe(II) in PL sediments. Error bars are 1 σ of triplicate results (where not shown, errors are within size of symbol).

day 0 (Figure 3), was actively ingrowing at day 5, and had increased by ~20% before Fe(II) was seen in pore waters. This indicates that Fe(III) reduction starts 10−20 days prior to Fe(II) being seen in pore waters in these sediments, in line with observations from other workers (*27*). The rate of Tc removal was relatively fast in all three experiments, was largely complete prior to significant Fe(II) growth into pore waters, and occurred during active sediment Fe(II) ingrowth at PL and by inference at BF and SK. The significantly faster Tc removal in the SK experiment compared to BF and PL most probably reflects the initially more reducing conditions in the SK sediments. For example, NO_3^- was absent at all of the time points in SK microcosms, and Fe(II) started to grow into solution at day 3 in SK compared to days 15−20 at BF and PL. Sulfate reduction (indicated by a decrease in the SO_4^{2-}/Cl^- ratio in pore waters) was observed after 16 days in the SK experiment, but Tc removal was complete before this time.

Microcosm experiments showed that no instantaneous Tc removal occurred at the initial time points. Nitrate was present in solution initially at BF and PL, suggesting that Tc removal does not occur during nitrate reduction, in agreement with past workers (*36*). No removal occurred at the first time point for the SK microcosm even though nitrate removal was complete. Previous workers (*13*) and experiments in our laboratories (unpublished data) have shown that Tc removal does not occur during Mn reduction. This is in agreement with our microcosm results because Mn(II) was present in solution in all three experiments at initial time points without concurrent Tc removal (Figure 2). Sulfate reduction occurred in the SK experiment only after Tc removal was complete. Therefore, the active ingrowth of Fe(II) into sediments as a result of Fe(III) reduction is the most likely controlling mechanism for Tc reduction. Typical concentrations of 0.5 M extractable Fe(II) in these sediments after reduction were 20−30 mM. Therefore, there was a 3 order-of-magnitude excess of Fe(II) (20−30 mM) compared to Tc(VII) (<5 μM) in the experiments, and reaction with sediment-bound microbially produced Fe(II) would certainly be capable of reducing all of the TcO_4^- present in solution.

To investigate further the mechanisms controlling Tc removal under more constrained biogeochemical conditions, we incubated sterilized samples of PL sediments with pure cultures of nitrate-(*Pseudomonas* sp.), Fe(III)-(*Shewanella* sp.), and sulfate-(*Desulfovibrio desulfuricans* sp. *Essex*) reducing bacteria and then amended with 3 μM TcO_4^- (Figure 4). Both the *Pseudomonas* and *Shewanella* species were isolated from the PL sediments using the appropriate selective media, whereas the *Desulfovibrio* species was a reference strain obtained from a culture collection. As expected from earlier experiments, measurable 0.5 M HCl extractable Fe(II) was present in all of the sediments at the start of the experiment. Removal of Tc(VII) did not occur in the presence of the nitrate-reducing *Pseudomonas* sp. This confirms that

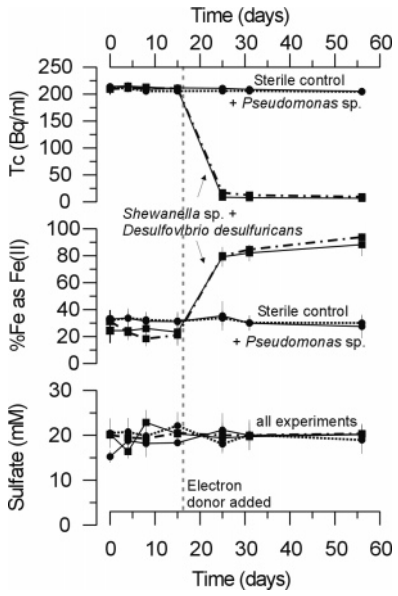

FIGURE 4. Incubation of Tc in sterilized sediment inoculated with pure cultures of NO_3^--, Fe(III)-, and sulfate-reducing bacteria. Error bars are 1 σ of triplicate results (where not shown, errors are within size of symbol).

Tc removal does not occur when nitrate is the sole TEAP and is in agreement with our microcosm results and the results of other workers (*36*). The active generation of 0.5 M HCl extractable Fe(II) in sediments and the concomitant removal of Tc occurred only when *Shewanella* and *Desulfovibrio* growth was stimulated by the addition of acetate or lactate as an electron donor. This suggests that Tc removal is linked to active Fe(II) ingrowth in these sediments and that the 0.5 M HCl extractable Fe(II) present at the start of these experiments is unavailable for reaction with Tc. Furthermore, the requirement for the addition of acetate or lactate in these single-species experiments suggests that a microbial consortium is required to degrade the complex organic matter in the sediments prior to metal reduction using degraded carbon (e.g., short-chain organic acids) as the electron donor. After the addition of an electron donor, the microcosms with *Shewanella* sp. and *D. desulfuricans* showed rapid and almost identical ingrowth of Fe(II) in sediments that was concurrent with Tc removal (Figure 4). Both *Shewanella* and *Desulfovibrio* species are well known to reduce Fe(III), but *Desulfovibrio* sp. are generally not accepted to conserve energy through the reduction of Fe(III) (*37*). In sterile controls and experiments containing the nitrate-reducing bacterium, no Tc removal or Fe(III) reduction occurred, and no sulfate reduction occurred in the experiments with *Desulfovibrio* sp. Overall, these experiments showed that in pure culture experiments TcO_4^- removal occurred only when active Fe(III) reduction was occurring in sediments.

Microbiological Community Analysis. The sediments at all three sites contained complex communities of bacteria, and there were obvious similarities between the banding patterns obtained with intergenic spacer analysis (16S-23 rRNA) from the three sites (Figure 5). Further analysis of the peak distribution confirmed that 76% of the integral peak area was shared between at least two sites, and that 51% of the peak area was common to all three sites. This suggests that there was a very high degree of conservation between the microbial communities at the SK, PL, and BF sites, and is in keeping with the similarities between the mineralogy and geochemical profiles in microcosms from the three sites. Because the microbial communities seemed to be similar on the basis of intergenic spacer signatures, and responded to environmental conditions in a broadly consistent manner,

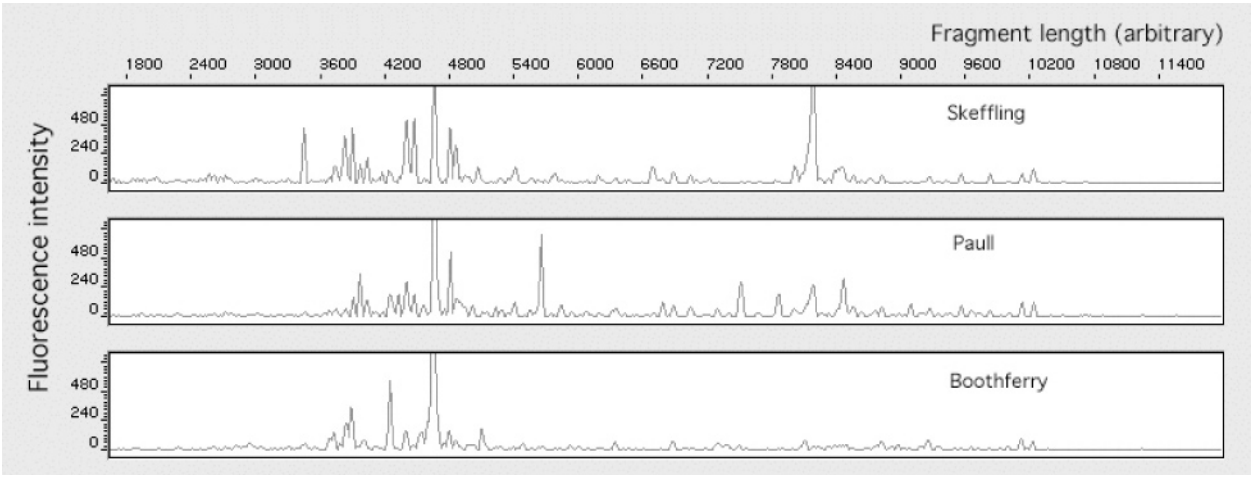

FIGURE 5. ARISA analysis of three sediments in this study (Skeffling, Paull, and Boothferry).

we focused on the intermediate site (PL) to characterize the microbial community in detail. The overall microbial community profile did not change throughout the 36-day incubation of the sediments, suggesting no dramatic changes in community structure during this time (data not shown). This reflects the targeting of DNA, which is relatively long-lived in the environment and may result in insensitivity to minor changes of the community composition with time. However, MPN counts on the key groups of organisms including Fe(III)- and sulfate-reducing bacteria changed very little as the sediments aged. Counts for the sulfate-reducing bacteria were stable at $\sim 10^6 - 10^7$ cells/g of sediment in all of the experiments. Counts of Fe(III)-reducing bacteria were stable at $\sim 10^2$ cells/g when Fe(III) NTA was used as an electron acceptor in the MPN tubes and at $\sim 10^8$ cells/g when Fe(III) citrate was used. Discrepancies with the different Fe(III) media were probably due to the chelating agents used. Toxicity of the NTA may have resulted in low counts, and reducing equivalents derived from the fermentation of citrate may have caused false positives for dissimilatory Fe(III)-reducing bacteria in the Fe(III)-citrate media. Nevertheless, our experiments suggest that the PL sediments contained a complex microbial community that reduced a range of terminal electron acceptors in sequence without dramatic changes in community structure. It should be noted that in other contexts these same techniques have been shown to detect pronounced changes in the structure of microbial communities (*27*). A more detailed picture of the bacterial community in the PL sediments was obtained by using broad specificity primers combined with PCR to examine an ~500 bp region of the 16S rRNA genes that were cloned and typed using RFLP analysis (*38*). Of the 43 clones analyzed, 27 gave distinct banding patterns, confirming a complex community of bacteria in the sediments. Organisms from the γ-Proteobacteria comprised 60% of the bacteria detected using this approach and included bacteria related to organisms capable of a wide diversity of aerobic and anaerobic processes (Supporting Information Figure 1 and Table 2). Overall, our results are in keeping with the sequence of TEAPs identified in our experiments, with close relatives of known nitrate- (*Rhodobacter capsulatus*), sulfur/metal- (*Pelobacter* sp.), and sulfate-reducing bacteria (*Desulfovibrio senezii*) detected in the clone library. The identification of a close relative of a *Pelobacter* species, of the family *Geobacteraceae,* members of which dominate Fe(III)-reducing sediments (*26*, *39*) is of particular note in the context of this study. Reduction of Fe(III) by sulfate-reducing bacteria (e.g., *Desulfovibrio* species) is also well documented (*37*). Because our geochemical data (and previous work (*2*)) suggested that Tc(VII) reduction may be linked to the reduction of Fe(III), we focused on further characterizing Fe(III)-reducing bacteria in the PL sediments incubated for 8 days, using a combination of molecular and culture-dependent techniques.

PCR-based analysis of portions of the 16S rRNA gene using primers designed to target Fe(III)-reducing bacteria of the family *Geobacteraceae* (*26*) identified further organisms with the potential to reduce Fe(III) in the PL sediment (Supporting Information Table 3). Close relatives of *Geobacter, Pelobacter*, and *Desulfuromonas* species were identified, all of which are documented to reduce Fe(III) (*39*). In addition to molecular-based PCR techniques, organisms were characterized in the lowest-dilution MPN tubes that were scored positive for growth on Fe(III) and resubcultured using an Fe(III)-containing medium. Intergenic spacer analysis suggested that similar organisms were conserved in many of the MPN tubes, targeting Fe(III) reducers (data not shown). Analysis of one of the tubes targeting 16S rDNA with broad-specificity PCR primers showed that these cultures were dominated by γ-Proteobacteria distinct from those detected in the clone library obtained via direct PCR-based analysis of the sediment (Supporting Information Table 4). Approximately 70% of the sequences aligned most closely to *Shewanella* species, which are well-known to respire using Fe(III). This culture was the source of the *Shewanella* strain used in Figure 4. In summary, the microbial communities at our sample sites are complex, and the PL sediments contained many bacteria with the potential to reduce Fe(III), and by inference, Tc(VII). The taxonomic affiliation of organisms identified in the PL sediments that have the potential to reduce Fe(III) are given in Supporting Information Figure 2A and B.

The Fate of Technetium in Sediments. In PL sediments undergoing Fe(III) and sulfate reduction, respectively, which were amended with 1000 μM TcO_4^- under strictly anaerobic conditions, Tc removal occurred within 2 days and was probably due to abiotic reaction with reduced phases produced after initial incubation and prior to the addition of TcO_4^-. By contrast, in the progressive anoxia sample incubated with 1000 μM TcO_4^-, microbially mediated in-growth of extractable Fe(II) was observed over a 6 month incubation period, and >99% Tc was removed from solution. The high Tc radioactivity in this experiment (>62 kBq ml^{-1}) meant that we were unable to characterize the geochemistry of the microcosm fully. However, when sampled for XAS analysis, extractable sediment-bound Fe(II) had increased from 14 to 95% and black spots were observed indicating that a reduced phase such as magnetite, FeS, or even TcO_2 was present. The main features in the EXAFS spectra and Fourier transforms for all three samples are very similar, and modeling produced good fits for all of the samples with an inner shell of six oxygen backscatterers at ca. 2.00 Å

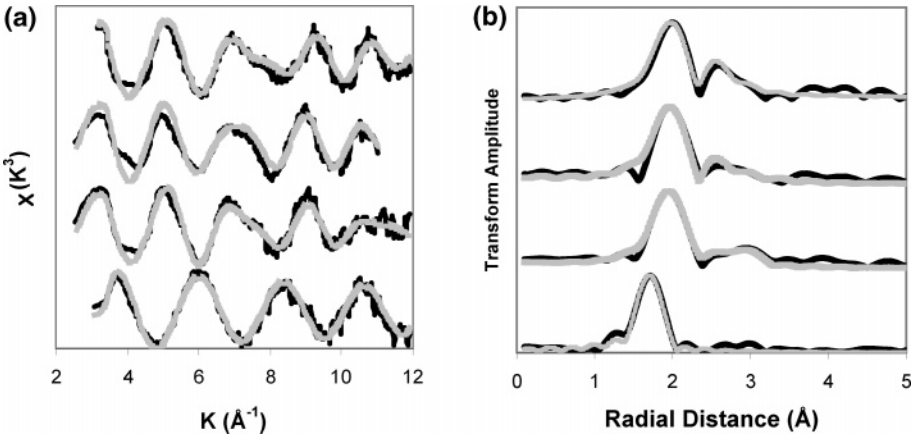

FIGURE 6. (a) k³-weighted Tc K-edge EXAFS spectra and (b) Fourier transforms of the Tc-labeled sediments used in the microcosm experiments of the following (bottom to top): solid pertechnetate standard (23); sulfate-reducing PL sediment amended with 1000 μM Tc; iron-reducing PL sediment amended with 1000 μM Tc; progressive anoxia, biotic PL sediment amended with 1000 μM Tc. The black lines represent experimental data and the gray lines represent the best fit using the parameters listed in Table 5 of the Supporting Information.

(Supporting Information Table 5). This is consistent with the formation of hydrous Tc(IV)O_2-like phases (*23*, *40*, *41*), previously reported as microbial reduction products by other workers (*2*, *5*, *13*) and confirms that the removal of TcO_4^- from solution was accompanied by the formation of hydrous TcO_2 (Figure 6) even under sulfidic conditions.

Implications for Tc Behavior in the Natural Environment. We have demonstrated that pertechnetate is removed from solution by reduction to Tc(IV) during the development of anoxic conditions in estuarine sediments, as Fe(III)-reducing conditions develop, and in systems with an extremely heterogeneous microbial community encompassing nitrate-, metal-, and sulfate-reducing bacteria. The development of biogeochemical processes in these estuarine sediments was robust even though no clear change was detected in the microbial communities as terminal-electron-accepting processes developed. This is in contrast to other studies on the microbiology of aquifer systems (*27*). Pure culture experiments, in which an appropriate electron donor was added to support metabolism, demonstrated that Tc removal occurred only when Fe(III)- or sulfate-reducing microbes were actively reducing Fe(III). The microcosm experiments were carried out at low concentrations of Tc so that systems maintained relevance to the natural environment. In progressive anoxia experiments with their indigenous microbial population, bacteria capable of degrading complex organic matter were present and anaerobic respiration proceeded without electron-donor additions. Indeed, the development of progressive anoxia and reductive scavenging of Tc to sediments occurred readily in all of the experiments even though we were unable to discern major changes in the complex microbial community in the sediments over time periods greater than 30 days. In our progressive anoxia experiments, extant Fe(III)-reducing bacteria were stimulated when the reduction of metals represented the most energetically favorable terminal-electron-accepting processes, and Tc(VII) reduction was associated with the active accumulation of microbially generated Fe(II) in sediments as a result of Fe(III) reduction. Furthermore, the majority of Tc was bound to surface sediments because anoxia developed relatively quickly (within 10−20 days) and XAS demonstrated that Tc formed a hydrous TcO_2 phase within these sediments even under sulfidic conditions. This implies that TcO_4^- released to the environment will be scavenged to sediments by reductive precipitation to hydrous TcO_2 when active Fe(III) reduction is occurring or when it is exposed to Fe(III) or sulfate-reducing sediments. Therefore, when TcO_4^- is discharged into oxic environments, Tc reduction is apparently mediated by a potentially wide variety of microbes, and reducing sediments may preferentially accumulate Tc(IV) rather than diluting and dispersing it as TcO_4^-. This may be used to one's advantage in understanding the biogeochemistry of technetium in estuarine environments as well as in remediation of Tc contamination in a range of environments.

Acknowledgments

We thank Lesley Neve and Dave Hatfield (University of Leeds) for carrying out the XRD, XRF, and elemental analyses and Bob Bilsborrow and John Charnock (Daresbury SRS) for their help in acquisition and analysis of XAS data. This research was supported by grant NER/A/S/2001/00652 and NER/A/S/2001/00960 from the UK Natural Environment Research Council and a CLRC beamtime award at Daresbury SRS.

Supporting Information Available

Details of EXAFS methodology. Table detailing the major and minor element composition of sediments. Tables detailing the phylogenetic affiliation of RFLP types for (i) broad specificity primers, (ii) primers designed to target gene sequences in members of the family *Geobacteraceae*, and (iii) MPN results for broad specificity primers. Table detailing the EXAFS fits for sediment samples. Figure showing phylogenetic affiliations for experiments using broad-specificity primers. Figure showing taxonomic affiliations for key experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Bondietti, E. A.; Francis, C. W. Geologic migration potential of Tc-99 and Np-237. *Science* **1979**, *203*, 1337−1340.

(2) Lloyd, J. R.; Solé, V. A.; Van Praagh, C. V. G.; Lovley, D. R. Direct and Fe(II)-mediated reduction of technetium by Fe(III)-reducing bacteria. *Appl. Environ. Microbiol.* **2000**, *66*, 3743−3749.

(3) Morris, K.; Butterworth, J. C.; Livens, F. R. Evidence for the remobilization of Sellafield waste radionuclides in an intertidal salt marsh, West Cumbria, UK. *Estuarine, Coastal Shelf Sci.* **2000**, *51*, 613−625.

(4) Keith-Roach, M. J.; Morris, K.; Dahlgaard, H. An investigation into technetium binding in sediments. *Mar. Chem.* **2003**, *81*, 149−162.

(5) Wildung, R. E.; Li, S. W.; Murray, C. J.; Krupka, K. M.; Xie, Y.; Hess, N. J.; Roden, E. E. Technetium reduction in sediments of a shallow aquifer exhibiting dissimilatory iron reduction potential. *FEMS Microbiol. Ecol.* **2004**, *49*, 151−162.

(6) Lloyd, J. R.; Macaskie, L. E. A novel PhosphorImager-based technique for monitoring the microbial reduction of technetium. *Appl. Environ. Microbiol.* **1996**, *62*, 578−582.

(7) Lloyd, J. R.; Nolting, H. F.; Solé, V. A.; Bosecker, K.; Macaskie, L. E. Technetium reduction and precipitation by sulfate-reducing bacteria. *Geomicrobiol. J.* **1998**, *15*, 43−56.
(8) Lloyd, J. R.; Ridley, J.; Khizniak, T.; Lyalikova, N. N.; Macaskie, L. E. Reduction of technetium by *Desulfovibrio desulfuricans*: Biocatalyst characterization and use in a flowthrough bioreactor. *Appl. Environ. Microbiol.* **1999**, *65*, 2691−2696.
(9) Kashefi, K.; Lovley, D. R. Reduction of Fe(III), Mn(IV), and toxic metals at 100 degrees C by *Pyrobaculum islandicum*. *Appl. Environ. Microbiol.* **2000**, *66*, 1050−1056.
(10) Wildung, R. E.; Gorby, Y. A.; Krupka, K. M.; Hess, N. J.; Li, S. W.; Plymale, A. E.; McKinley, J. E.; Fredrickson, J. K. Effect of electron donor and solution chemistry on products of dissimilatory reduction of technetium by *Shewanella putrefaciens*. *Appl. Environ. Microbiol.* **2000**, *66*, 2451−2460.
(11) Lloyd, J. R.; Mabbett, A. N.; Williams, D. R.; Macaskie, L. E. Metal reduction by sulphate-reducing bacteria: physiological diversity and metal specificity. *Hydrometallurgy* **2001**, *59*, 327−337.
(12) Lloyd, J. R.; Thomas, G. R.; Finlay, J. A.; Cole, J. A.; Macaskie, L. E. Microbial reduction of technetium by *Escherichia coli* and *Desulfovibrio desulfuricans*: Enhancement via the use of high-activity strains and effect of process parameters. *Biotechnol. Bioeng.* **1999**, *66*, 122−130.
(13) Fredrickson, J. K.; Zachara, J. M.; Kennedy, D. W.; Kukkadapu, R. K.; McKinley, J. P.; Heald, S. M.; Liu, C.; Plymale, A. E. Reduction of TcO_4^- by sediment-associated biogenic Fe(II). *Geochim. Cosmochim. Acta* **2004**, *68*, 3171−3187.
(14) Henrot, J. Bioaccumulation and chemical modification of Tc by soil bacteria. *Health Phys. 57, 239−245.* **1989**, *57*, 239−245.
(15) Maes, A.; Geraedts, K.; Bruggeman, C.; Vancluysen, J.; Rossberg, A.; Hennig, C. Evidence for the interaction of technetium colloids with humic substances by X-ray absorption spectroscopy. *Environ. Sci. Technol.* **2004**, *38*, 2044−2051.
(16) Madigan, M. T.; Martinko, J. M.; Parker, J. *Brock Biology of Microrganisms,* 9th ed.; Prentice Hall: New Jersey, 1999.
(17) Cline, J. D. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol. Oceanogr.* **1969**, *14*, 454−458.
(18) Brewer, P. G.; Spencer, D. W. Colourimetric determination of Mn in anoxic waters. *Limnol. Oceanogr.* **1971**, *16*, 107−110.
(19) Viollier, E.; Inglett, P. W.; Hunter, K.; Roychoudhury, P.; Van Cappellen, P. The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters. *Appl. Geochem.* **2000**, *15*, 785−790.
(20) Lovley, D. R.; Phillips, E. J. P. Availability of ferric iron for microbial reduction in bottom sediments of the freshwater tidal Potomac River. *Appl. Environ. Microbiol.* **1986**, *52*, 751−757.
(21) Tuccillo, M. E.; Cozzarelli, I. M.; Herman, J. S. Iron reduction in the sediments of a hydrocarbon-contaminated aquifer. *Appl. Geochem.* **1999**, *14*, 655−667.
(22) Harris, S. J.; Mortimer, R. J. G. Determination of nitrate in small water samples (100 μL) by the cadmium−copper reduction method: A manual technique with application to the interstitial waters of marine sediments. *Int. J. Environ. Anal. Chem.* **2002**, *82*, 369−376.
(23) Wharton, M. J.; Atkins, B.; Charnock, J. M.; Livens, F. R.; Pattrick, R. A. D.; Collison, D. An X-ray absorption spectroscopy study of the coprecipitation of Tc and Re with mackinawite (FeS). *Appl. Geochem.* **2000**, *15*, 347−354.
(24) Eden, P. E.; Schmidt, T. M.; Blakemore, R. P.; Pace, N. R. Phylogenetic analysis of *Aquaspirillium magnetotacticum* using polymerase chain reaction amplified 16S ribosomal RNA specific DNA. *Int. J. Syst. Bacteriol.* **1991**, *41*, 324−325.
(25) Lane, D. J.; Pace, B.; Olsen, G. J.; Stahl, D. A.; Sogin, M. L.; Pace, N. R. Rapid determination of 16S ribosomal-RNA sequences for phylogenetic analyses. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 6955−6959.
(26) Holmes, D. E.; Finneran, K. T.; O'Neil, R. A.; Lovley, D. R. Enrichment of members of the family *Geobacteraceae* associated with stimulation of dissimilatory metal reduction in uranium-contaminated aquifer sediments. *Appl. Environ. Microbiol.* **2002**, 2300−2306.
(27) Islam, F. S.; Gault, A. G.; Boothman, C.; Polya, D. A.; Charnock, J. M.; Chatterjee, D.; Lloyd, J. R. Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. *Nature* **2004**, *430*, 68−71.
(28) Sanger, F.; Nicklen, S.; Coulsen, A. R. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5463−5467.
(29) Van der Peer, Y.; Wachter, R. D. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput. Appl. Biosci.* **1994**, *10*, 569−570.
(30) Jukes, T. H.; Cantor, C. R. Evolution of protein molecules. In *Mammalian Protein Metabolism.*; Munro, H. N., Ed.; Academic Press Inc: New York, 1969.
(31) Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406−425.
(32) Ranjard, L.; Poly, F.; Lata, J. C.; Mougel, C.; Thioulouse, J.; Nazaret, S. Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: Biological and methodological variability. *Appl. Environ. Microbiol.* **2001**, *67*, 4479−4487.
(33) Ranjard, L.; Poly, F.; Combrisson, J.; Richaume, A.; Gourbiere, F.; Thioulouse, J.; Nazaret, S. Heterogeneous cell density and genetic structure of bacterial pools associated with various soil microenvironments as determined by enumeration and DNA fingerprinting approach (RISA). *Microb. Ecol.* **2000**, *39*, 263−272.
(34) Lovley, D. R.; Goodwin, S. Hydrogen concentrations as an indicator of the predominant terminal electron-accepting reactions in aquatic sediments**.** *Geochim. Cosmochim. Acta* **1988**, *52*, 2993−3003.
(35) Collins, C. H.; Lyne, P. M.; Grange, J. M. *Microbiological Methods*, 7th ed.; Butterworth-Heinemann: Oxford, U.K., 1995.
(36) Abdelouas, A.; Fattahi, M.; Grambow, B.; Vichot, L.; Gautier, E. Precipitation of technetium by subsurface sulfate-reducing bacteria. *Radiochim. Acta* **2002**, *90*, 773−777.
(37) Lloyd, J. R. Microbial reduction of metals and radionuclides. *FEMS Microbiol. Rev.* **2003**, *27*, 411−425.
(38) Schmidt, T. Fingerprinting bacterial genomes using ribosomal RNA genes and operons. *Methods Mol. Cell. Biol.* **1994**, *5*, 3−12.
(39) Snoeyenbos-West, O.; Van Praagh, C. G.; Lovley, D. R. *Trichlorobacter thiogenes* should be renamed as a Geobacter species. *Appl. Environ. Microbiol.* **2001**, *67*, 1020−1022.
(40) Almahamid, I.; Bryan, J. C.; Bucher, J. J.; Burrell, A. K.; Edelstein, N. M.; Hudson, E. A.; Kaltsoyannis, I. N.; Lukens, W. W.; Shuh, D. K.; Nitsche, H.; Reichl, T. Electronic and structural investigations of technetium compounds by X-ray absorption spectroscopy. *Inorg. Chem.* **1995**, *34*, 193−198.
(41) Pepper, S. E.; Bunker, D. J.; Bryan, N. D.; Livens, F. R.; Charnock, J. M.; Pattrick, R. A. D.; Collison, D. Treatment of radioactive wastes: An X-ray absorption spectroscopy study of the reaction of technetium with green rust. *J. Colloid Interface Sci.* **2003**, *268*, 408−412.
(42) Konhauser, K. O.; Mortimer, R. J. G.; Morris, K.; Dunn, V. A. The role of microorganisms during sediment diagenesis: Implications for radionuclide mobility. In *Interactions of Microorganisms with Radionuclides*; Keith-Roach, M. J., Livens, F. R., Eds.; Elsevier: London, 2002.

Received for review November 29, 2004. Revised manuscript received April 1, 2005. Accepted April 4, 2005.

ES048124P