Reoxidation Behavior of Technetium, Iron, and Sulfur in Estuarine Sediments

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Technetium is a redox active radionuclide, which is present as a contaminant at a number of sites where nuclear fuel cycle operations have been carried out. Recent studies suggest that Tc(VII), which is soluble under oxic conditions, will be retained in sediments as Fe(III)-reducing conditions develop, due to reductive scavenging as hydrous TcO_2. However, the behavior of technetium during subsequent reoxidation of sediments remains poorly characterized. Here, we describe a microcosm-based approach to investigate the reoxidation behavior of reduced, technetium-contaminated sediments. In reoxidation experiments, the behavior of Tc was strongly dependent on the nature of the oxidant. With air, reoxidation of Fe(II) and, in sulfate-reducing sediments, sulfide occurred accompanied by ~50% remobilization of Tc to solution as TcO_4^-. With nitrate, reoxidation of Fe(II) and, in sulfate-reducing sediments, sulfide only occurred in microbially active experiments where Fe(II) and sulfide oxidation coupled to nitrate reduction was occurring. Here, Tc was recalcitrant to remobilization with <10% Tc remobilized to solution even when extensive Fe(II) and sulfide reoxidation had occurred. X-ray absorption spectroscopy on reoxidized sediments suggested that 15–50% of Tc bound to sediments was present as Tc(VII). Overall, these results suggest that Tc reoxidation behavior is not directly coupled to Fe or S oxidation and that the extent of Tc remobilization is dependent on the nature of the oxidant.

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Introduction

Technetium is a radioactive contaminant found at nuclear fuel cycle operation sites. It is present in aquifers (*1*, *2*), estuarine and marine environments (*3*, *4*), and rivers (*5*) and is of particular environmental concern because it is formed in high yield in nuclear fission, has a long half-life (2.13 × 10^5 y), and is highly mobile under oxic conditions. Its redox chemistry is a major control on its solubility; under oxic conditions it forms the highly soluble TcO_4^- ion, which is weakly sorbed to mineral surfaces and is among the most mobile of radionuclide species in the environment (*6*). Under reducing conditions, the lower valence forms of technetium, particularly Tc(IV), hydrolyze to form solid phases and, at lower concentrations, sorb to mineral phases (*6*) and, under some circumstances, organic fractions (*7*). Recent work has confirmed that Tc(VII) is reductively precipitated as Tc(IV) as anoxic conditions develop in a range of different environments (*2*, *8*–*10*). This supports observations from the natural environment that Tc is retained over time periods of decades in near shore and riverine sediments, presumably as a result of anoxic scavenging (*3*, *4*, *11*). Although reductive retention of Tc(IV) can occur in sediments, natural systems often undergo redox cycling and the ultimate fate of Tc(IV) is uncertain if conditions switch from reducing to oxidizing. This is important where disturbance of technetium-contaminated anoxic sediments may occur through flooding, dam failure, or during land reclamation (*5*), and is also important where water table fluctuations may occur (*12*, *13*). Finally, bioimmobilization programs, where nutrients are added to the subsurface to stimulate indigenous microbial communities with the potential to remove Tc(VII) reductively from solution by forming Tc(IV), have recently come to the fore as a novel technology to treat in situ contamination in groundwaters (*1*, *2*, *10*, *14*, *15*). Although bioimmobilization has been demonstrated for Tc on addition of electron donor to sediments (*2*, *14*, *15*), the fate of immobilized, reduced Tc on reoxidation of sediments, when electron donor additions cease, remains unclear. Thus, understanding the reoxidation of technetium is relevant to the environmental behavior of technetium in both natural and engineered environments. In these experiments, microcosms containing Humber Estuary sediments with their indigenous microbial populations were spiked with low levels (<5 μM final concentration) of ^{99}Tc (as TcO_4^-) and incubated until Fe(III)- and sulfate-reducing conditions developed. Reductive removal of Tc(VII) to hydrous TcO_2 occurred during Fe(III) reduction in these sediments (*8*). The Tc(IV) radiolabeled Fe(III)- and sulfate-reducing sediments were then reoxidized with both air and nitrate. Air reoxidation will be important on disturbance of anoxic sediments and their subsequent exposure to oxygenated waters. Nitrate reoxidation is of particular concern in the behavior of radionuclides as nitrate is a common co-contaminant at nuclear fuel cycle operation sites (*1*). Theoretical Eh values for relevant redox couples for NO_3^-, Mn(IV), Fe(III), Tc(VII), and SO_4^{2-} are given in the Supporting Information, Table 1. The calculated Tc(VII)/Tc(IV) couple is at ca. +20 mV for pH = 7, very close to the Fe(III)/Fe(II) couple. Recent studies have identified bacteria that can mediate anoxic Fe(II) (*16*), heavy metal (*17*), and uranium oxidation (*15*, *18*) by nitrate in the absence of oxygen. Understanding anoxic oxidation and its impacts on the fate of technetium is important where anoxic, Tc(IV)-labeled sediments are exposed to nitrate-enriched, anoxic groundwaters. To test the hypothesis that reoxidation affects the fate of Tc(IV) in Fe(III)- and sulfate-reducing sediments, we examined the stable element biogeochemistry and remo-

TABLE 1. EXAFS Fits for Reduction and Reoxidation Experiments[a]

experiment	scatterer	N	r (Å)	σ^2 (Å^2)	R
(A) S-R	O	6	1.99	0.007	28.5
	S	1	2.84	0.005	
(B) air (S-R)	O	1.0	1.70	0.003	39.5
	O	4.5	1.99	0.007	
(C) NO_3^- (S-R)	O	1.3	1.69	0.005	38.6
	O	4.1	1.98	0.007	
	Fe	1	3.09	0.003	
(D) TcO_4^- (aq)	O	4	1.72	0.002	30.6
(E) Fe-R	O	6	2.00	0.007	42.9
	Fe	1	2.72	0.005	
(F) air (Fe-R)	O	1.8	1.71	0.004	52.1
	O	3.3	2.00	0.005	
(G) NO_3^- (Fe-R)	O	2.1	1.71	0.004	46.1
	O	2.8	2.00	0.005	
	Fe	1	2.68	0.009	

[a] (A) Sulfate-reducing sediment amended with 1000 μM Tc; (B) sulfate-reducing sediment amended with 250 μM Tc, then reoxidized with air for 26 days; (C) sulfate-reducing sediment amended with 250 μM Tc, then reoxidized with 100 mM nitrate for 30 days; (D) pore−water solution from sulfate-reducing sediment amended with 1000 μM Tc, then reoxidized with air for 42 days; (E) Fe(III)-reducing sediment amended with 1000 μM Tc; (F) Fe(III)-reducing sediments amended with 250 μM Tc, then reoxidized with air for 26 days; (G) Fe(III)-reducing sediments amended with 250 μM Tc, then reoxidized with 100 mM nitrate for 30 days. N is the occupancy (±30 %), r is the interatomic distance (±0.02 Å for the first shell, ±0.05 Å for outer shells), σ^2 is the Debye−Waller factor (±25%), and R is the least squares residual.

bilization of Tc(IV) into solution on air and nitrate reoxidation. In addition, a limited number of experiments were performed at the high concentration of Tc required for X-ray absorption spectroscopic characterization of the fate of Tc in sediments.

Materials and Methods

Safety. ^{99}Tc is a radioactive beta emitter (half-life 2.13×10^5 years; $E_{max} = 294$ keV) and should be handled in a properly equipped radioactive laboratory. The possession and use of radioactive materials is subject to statutory controls.

Characteristics of Sediments. Surface sediments were sampled from the Paull mudflat on the Humber estuary (see ref 8 for sampling location and details) and were transferred directly into sterile polythene containers, transported back to the laboratory, and stored at 4 °C until use. Surface waters were also collected adjacent to the sampling point. Experiments were started within 4 days of sample collection, and sediment manipulation was kept to a minimum prior to incubation. In surface waters, nitrate concentrations were between 120 and 250 μM, and sulfate concentrations were between 14 and 19 mM (Supporting Information, Table 2).

Reoxidation Experiments. To prepare reduced, Tc(IV) labeled samples for reoxidation, sediment slurries (20−30% sediment) were made up with surface water. Sediments were then spiked to a final concentration of 1−5 μM ^{99}Tc (as ammonium pertechnetate) and incubated anaerobically in the dark at 10 °C. Microcosms were then stored until predominantly Fe(III)-reducing conditions (2 months) and predominantly sulfate-reducing conditions (9 months) developed (Supporting Information, Table 2). In previous microcosm experiments with sediments from Paull, TcO_4^- removal from solution was coincident with Fe(II) ingrowth into sediments as a result of Fe(III) reduction and was complete before sulfate reduction began (8). In these experiments, sampling of Fe(III)-reducing sediments at 2 months and sulfate-reducing experiments at 9 months confirmed that >99% of TcO_4^- had been removed from solution at the start of reoxidation. The technetium-labeled Fe(III)- and sulfate-reducing sediments were then reoxidized.

Air reoxidation was carried out in duplicate using diffusion to supply air to 100 mL of radiolabeled sediment slurry in 1000 mL conical flasks. The flasks were capped with a porous bung, incubated in darkness at 10 °C, and kept in constant motion on an orbital shaker at 150 rpm. Nitrate reoxidation was carried out as microcosm experiments in triplicate, and nitrate concentrations were chosen to reflect a range from high natural environmental concentrations (1 mM) to the very high concentrations that may be found at radioactively contaminated sites (100−200 mM (19)). Microcosm bottles containing reduced sediment slurries from both Fe(III)- and sulfate-reducing microcosms were amended with deoxygenated nitrate solutions to produce a final concentration of 22 mM NO_3^- in Fe(III)-reducing sediments, and 1, 15, 100, and 250 mM NO_3^- in sulfate-reducing sediments. Control experiments using autoclaved sediments were also established. All bottles were incubated at 10 °C in the dark.

For reoxidation experiments, sampling took place over a period of 60 days until nearly complete Fe(II) reoxidation had occurred. Periodically, 3 mL of sediment slurry was withdrawn under a stream of N_2 gas using a sterile syringe and centrifuged (10 min at 14 000 rpm). The supernatant was then analyzed for pH and Eh using calibrated electrodes; total Tc (or pertechnetate; (20)) by liquid scintillation counting; NO_3^- and SO_4^{2-} by ion chromatography (Dionex); NO_2^- and Fe^{2+} by standard UV−vis spectroscopy methods (21, 22); and NH_4^+ by flow injection analysis (23). Sediment bound Fe^{2+} was determined by reaction with ferrozine (24). After geochemical characterization, samples were frozen (−20 °C) and stored for microbiological analysis.

Microbial Characterization of Nitrate Reoxidation Experiments. DNA was extracted from sediments using a Fast DNA spin kit (UltraClean, Soil DNA Isolation Kit, MO BIO Laboratories Inc, Solana Beach, CA) and analyzed using a PCR-based approach (25). Primer sets targeted fragments of the 16S rRNA gene (approximately 520 base pairs (25)), the nitrate reductase gene *narG* (approximately 690 base pairs (26)), and the nitrite reductase gene *nirS* (approximately 890 base pairs (27)) using appropriate PCR protocols. Restriction Fragment Length Polymorphism (RFLP) analysis, using restriction enzymes *Sau3A* and *MspI*, preceded nucleotide sequencing of distinct RFLP types using the dideoxynucleotide method (25). Sequences (typically 700 base pairs in length) were analyzed against the NCBI (USA) database using BLAST program packages and matched to known 16S rRNA, *narG*, and *nirS* gene sequences.

X-ray Absorption Spectroscopy (XAS). A limited number of experiments were carried out at higher Tc concentrations (250−1000 μM) to allow XAS analysis (Supporting Information, section 1). Past XAS work has shown that Tc speciation was identical in sediments allowed to develop progressive anoxia and in sediments where Tc was spiked onto pre-reduced sediments (8). Therefore, small (10 mL) sediment microcosms containing ca. 5 mL of Fe(III)- or sulfate-reducing sediment slurry were spiked with 250−1000 μM ammonium pertechnetate. Under these conditions, >99% TcO_4^- was removed from solution over 2−8 days, and the Tc was retained on both Fe(III)- and sulfate-reducing sediments as TcO_2 (8). Reoxidation was then achieved by either daily injection of air into the headspace of microcosm bottles or by addition of an anaerobic $NaNO_3$ solution to a final concentration of 100 mM NO_3^- in microcosms. Tc K-edge spectra were collected on station 16.5 at the UK CLRC Daresbury SRS operating at 2 GeV with a typical current of 150 mA, using a Si(220) double crystal monochromator and focusing optics as detailed in the Supporting Information, section 1 (8, 28).

Results and Discussion

Air Reoxidation. During air reoxidation, both Fe(III)- and sulfate-reducing sediments produced similar results for the

TABLE 2. Calculated Electron Balance for Nitrate Reoxidation of Sulfate- and Fe(III)-Reducing Sediments Based on Total Electrons Produced and Consumed in Oxidative and Reductive Processes[a]

	oxidative processes			reductive processes					
	$Fe(II) \rightarrow Fe(III) + e^-$	sulfide $\rightarrow$ sulfate $+ 8e^-$	total electrons produced	nitrate added	nitrate utilized	nitrate $+ 2e^- \rightarrow$ nitrite	nitrate $+ 5e^- \rightarrow$ nitrogen	nitrate $+ 8e^- \rightarrow$ ammonia	total electrons consumed
(A) sulfate-reducing 23 days	30.6 ± 2.1	3.5 ± 0.1	(58.8 ± 2.1)	245.3 ± 2.5	19.4 ± 1.9	16.6 ± 1.5		(2.8 ± 2.4)	(55.7 ± 2.8)
(B) sulfate-reducing 60 days	30.6 ± 2.1	5.1 ± 0.3	(71.4 ± 3.2)	102.8 ± 3.4	20.7 ± 3.5	13.8 ± 0.7		6.1 ± 0.1	(76.5 ± 3.5)
(C) Fe(III)-reducing 62 days	8.0 ± 0.9	n.d.	(8.0 ± 0.9)	22.5 ± 1.0	2.0 ± 0.6	0.22 ± 0.0	(1.5 ± 0.9)	0.21 ± 0.0	(7.5 ± 1.9)

[a] All values are quoted as mmol L^{-1} (mM) change in concentrations between day 0 and day shown. Errors are ±1σ of triplicate measurements. Values shown in normal text are experimental results; values in brackets have been calculated by difference; values in bold brackets are the calculated total production or consumption of electrons; n.d., not detected.
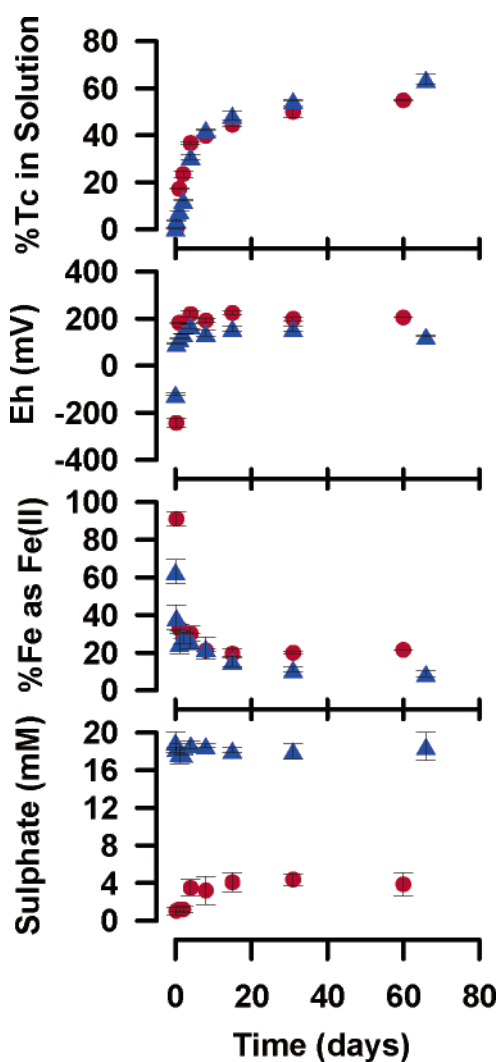

FIGURE 1. Remobilization of Tc to solution and change in redox indicators during the air reoxidation of sulfate-reducing (red ●) and Fe(III)-reducing (blue ▲) sediments. Error bars show the range of duplicate experiments.

remobilization of technetium (Figure 1). In both sediments, there was an initial rapid remobilization of ca. 40% of the bound Tc over the first 8 days rising to 55−64% by 66 days. At the end of the experiment, 36−45% of Tc was retained in the sediments, suggesting that a significant fraction of

sediment bound Tc in these sediments is recalcitrant to remobilization. Both indirect extraction (20) and XAS confirmed that the remobilized Tc was present in solution as Tc(VII). The XANES spectra for the solution from the air reoxidized sulfate-reducing sediment showed the clear pre-edge feature characteristic of TcO_4^- (28) (Supporting Information, Figure 1), while the EXAFS fits for the solution from the sulfate-reducing sediment after air reoxidation are given in Table 1 and illustrated in the Supporting Information, Figure 2. The main peak in the reoxidation solution could be fitted with a shell of four oxygen backscatterers at 1.72 Å, which is identical to the value quoted for a solid tetraphenylphosphonium pertechnetate standard (28) and is diagnostic for TcO_4^-.

In both Fe(III)- and sulfate-reducing systems, sediment bound Fe(II) was reoxidized relatively quickly. Within Fe(III)- and sulfate-reducing sediments, 61 ± 7% and 63 ± 7%, respectively, of the original Fe(II) inventory in the sediments at $t = 0$ was reoxidized by 1 day. This was followed by a slower oxidation of Fe(II) with 8.7 ± 1% and 21.5 ± 0.5% of the total sediment bound iron remaining as Fe(II) in Fe(III)- and sulfate-reducing sediments, respectively, after 66 days (Figure 1). Indeed, after air reoxidation, the sediments were typically more oxidized with respect to the 0.5 M HCl extractable Fe(II) concentration than they were at the point of sampling in the field (Supporting Information, Table 2). In both Fe(III)- and sulfate-reducing sediments, the rate of Tc remobilization to solution was slower than that of Fe(II) reoxidation (Figure 1). For example, in the Fe(III)-reducing sediment after 1 day of air reoxidation, only 8% of the total Tc bound to sediments was remobilized to solution (ca. 0.1 μM), while 61 ± 7% (ca. 6 mmol) of the Fe(II) that was present in the sediments at $t = 0$ was reoxidized within 1 day. Thus, early in the experiment the concentration of Fe(II) that had been reoxidized was in vast molar excess as compared to the Tc remobilized. In sulfate-reducing sediments, the majority of sulfide undergoing oxidative dissolution during the experiment was present as sulfate by 10 days (Figure 1). However, only 28 ± 4% of the sulfide was reoxidized to sulfate after 2 months even though the sediments changed from gray black to orange brown. The presence of refractory, reduced sulfur phases in sediments under oxidizing conditions has been reported previously and is implicated in the stabilization of heavy metals and radionuclides (9, 29−32). However, in our sulfate-reducing experiment, the similar remobilization behavior of Tc during air reoxidation of both sulfate- and Fe(III)-reducing sediments, the lack of direct correlation between sulfide oxidation and Tc remobilization, and the previous XAS results that indicate that reduced Tc sulfides are not important Tc sinks in the reduced sediments
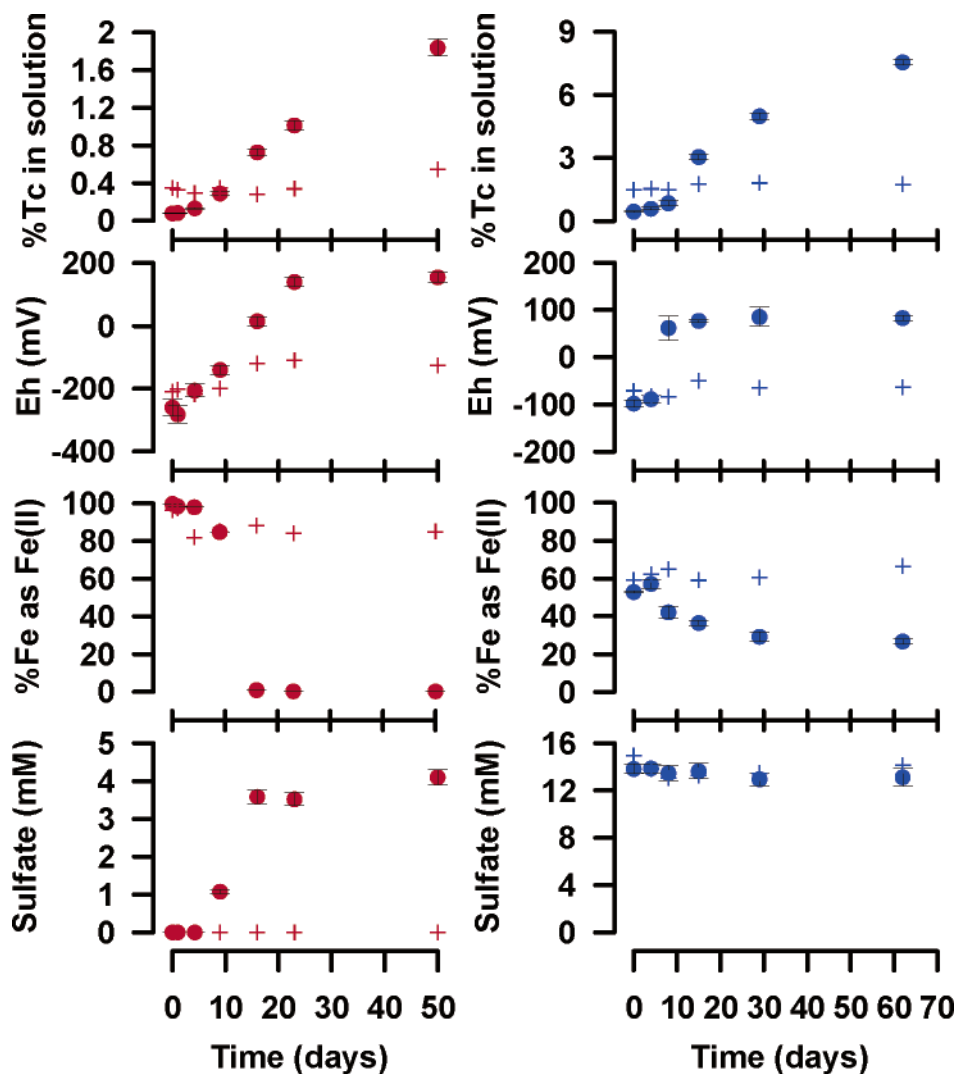
FIGURE 2. Remobilization of Tc(VII) to solution and change in redox indicators during the nitrate reoxidation of sulfate-reducing (red ●) and Fe(III)-reducing (blue ●) sediments, with 250 mM and 22 mM sodium nitrate solutions, respectively. Sterilized control experiments shown as red + and blue +, respectively. Error bars show standard deviation of triplicate experiments.
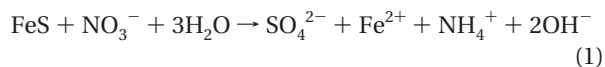
from these sites (*8*) all suggest that the retention of Tc here cannot be directly explained by sulfide stabilizing the sediment-bound Tc(IV).

XAS data were collected for air-reoxidized, Fe(III)- and sulfate-reducing sediments at the higher Tc concentrations required for XAS detection, and remobilization of Tc into solution was similar to that observed in the lower Tc concentration microcosms (Supporting Information, section 1). The EXAFS spectra were then analyzed using the approach described in Supporting Information, section 2. The spectral features in the data for the sediments subjected to air reoxidation could be fitted with shells of oxygen atoms at ca. 1.70 Å and ca. 2.00 Å (Table 1; Supporting Information, Figure 2). As discussed above, the presence of O atoms at 1.7 Å is diagnostic of the presence of TcO_4^- in the sediment samples. In addition, in hydrous TcO_2, a Tc–O distance around 2.0 Å is characteristic (*8, 33*). Thus, the EXAFS spectra show that, on air reoxidation, the sediments contained a mixture of both hydrous TcO_2 and TcO_4^- species. In the sulfate-reducing, air reoxidation sediment sample, the occupancies of the oxygen shells at 1.7 and 2.0 Å were 1 and 4.5, respectively (Table 1). Based on the Tc coordination numbers in TcO_4^- (4 O atoms) and hydrous TcO_2 (6 O atoms), this suggests ca. 25% of the Tc in the sediment sample was present as Tc(VII) with the remainder as Tc(IV). Similarly, the air reoxidized Fe(III)-reducing sediment gave occupancies of 1.8 oxygen

atoms at ca. 1.7 Å and 3.3 oxygen atoms at 2.0 Å, respectively, suggesting ca. 45% Tc(VII) was present in the sample. It should be noted that these values are subject to significant (±30% relative) uncertainties (Table 1). Additionally, modeling of the XANES data (Supporting Information, section 3 and Table 3; (*36*)) showed that both the sulfate-reducing and the Fe(III)-reducing air reoxidation sediment samples could be modeled with 15% and 30% Tc(VII) respectively, consistent (within error) with the EXAFS analysis. Other studies have shown that reoxidation of Tc in radiolabeled, reduced Fe(II) minerals (*28, 37*) and in naturally radiolabeled sediments (*5*) is very slow, presumably due to kinetic hindrance of Tc(IV) reoxidation. For example, in experiments where naturally radiolabeled, reduced riverine sediments were shaken with seawater, less than 1% of sediment bound Tc remobilized after 2 months, although there was little discussion of the biogeochemical conditions in these experiments (*5*). Here, our results show that vigorous air reoxidation of both Fe(III)- and sulfate-reducing sediments radiolabeled with hydrous TcO_2 leads to a substantial fraction of sediment bound Tc(IV) being remobilized to solution as Tc(VII). Furthermore, analysis of both EXAFS and XANES spectra suggests that the reoxidized sediments contain a mix of Tc(IV) and Tc(VII) with a substantial fraction (15–45%) of Tc(VII) associated with solids. Fe(II) and sulfide were also reoxidized in these sediments in vast molar excess to the Tc present in the

sediments, and, on close inspection, the reoxidation of technetium was also retarded as compared to the reoxidation behavior of either of these stable species.

Nitrate Reoxidation. During nitrate oxidation, the reoxidation of Fe(II), sulfide, and technetium occurred only in microbially active experiments and only when NO_3^- was added in large electron equivalent excess of the concentration of reduced species present. In the Fe(III)-reducing sediments, 22 mM NO_3^- caused substantial reoxidation of Fe(II) (Figure 2); in sulfate-reducing sediments where experiments were performed with ca. 1, 15, 100, and 250 mM NO_3^-, significant reoxidation of Fe(II) and sulfide was only seen in the 100 and 250 mM experiments, where the observed reoxidation behavior was very similar. For experiments with significant oxidation of Fe(II) and sulfide, the remobilization of Tc to solution was extremely limited; for sulfate- and Fe(III)-reducing sediments, respectively, ca. 2% and ca. 8% of total Tc in experiments was present in solution after 60 days of reoxidation. Illustrative data for the 250 mM experiment are provided in Figure 2, and the complete dataset is provided in the Supporting Information, Figure 3. In the 1 mM NO_3^- experiment, there was rapid consumption of NO_3^- and no reoxidation of any species was observed, while in the 15 mM NO_3^- experiment, measurable sulfide reoxidation did occur although no other changes were seen. In the 22, 100, and 250 mM NO_3^- reoxidation experiments, there was an initial lag phase of ca. 10 days and then Fe(II) (and in the sulfate-reducing sediments, sulfide) reoxidation began (Figure 2; Supporting Information, Figure 3). In the sulfate-reducing sediments, Fe(II) reoxidation was more extensive than in the systems reoxidized with air and was complete by 16 days (Figure 2). Here, sulfate increased in solution throughout the experimental run as a result of oxidative dissolution of sulfide, although by 60 days only ca. 27% and 35% of the sulfide had been reoxidized to sulfate in the 250 and 100 mM experiments, respectively. This indicates that there is a substantial refractory reduced sulfur phase in the sediments on their reoxidation with nitrate. By contrast, in the Fe(III)-reducing sediment, there was less reoxidation of the Fe(II) present at the start of the experiment with only 51% of the total Fe(II) inventory in the sediments at $t = 0$ reoxidized by 62 days. For the Fe(III)-reducing system with 22 mM NO_3^- and for both the 100 mM and the 250 mM NO_3^- reoxidation of sulfidic sediments, there was a molar balance for electron transfer calculated during the coupled NO_3^--dependent Fe(II) and sulfide reoxidation (Table 2). Furthermore, this suggested that for sulfate-reducing sediments a two-stage, NO_3^--mediated, bio-oxidation process was occurring:

$$FeS + NO_3^- + 3H_2O \rightarrow SO_4^{2-} + Fe^{2+} + NH_4^+ + 2OH^-$$
(1)

$$2Fe^{2+} + NO_3^- + H_2O \rightarrow 2Fe^{3+} + NO_2^- + 2OH^- \quad (2)$$

In our experiments, the measured mole ratios of $[SO_4^{2-}]/[NH_4^+]$ (1.1 ± 0.3) and $[Fe(II)]/[NO_2^-]$ (2.0 ± 0.2) are consistent with the expected stoichiometric ratios of $[SO_4^{2-}]/[NH_4^+] = 1$ and $[Fe(II)]/[NO_2^-] = 2$ for the oxidation processes (Table 2; eqs 1 and 2). The nitrate-dependent oxidation of sulfide to produce SO_4^{2-} and NH_4^+ has been previously observed in marine sediments (38). This is in contrast to other studies of nitrate-dependent sulfide reoxidation where variously N_2 and SO_4^{2-} (39, 40), NH_4^+ and S^0 (41), and N_2 and S^0 (42) were the reported oxidation products. Additionally, nitrate-dependent Fe(II) oxidation has the potential to reduce NO_3^- to any one of NO_2^-, N_2O, N_2, or NH_4^+ (43), although our reaction stoichiometry suggests that reduction to NO_2^- predominates in these sediments. Interestingly, even though extensive Fe(II) and sulfide reoxidation occurred during NO_3^--mediated oxidation of sulfate-reducing sediments, and

the final Eh of reoxidized experiments (ca. +150 mV; Figure 2) was well above the theoretical Tc(VII)/Tc(IV) couple of ca. +20 mV (Supporting Information, Table 1), Tc remobilization to solution was extremely limited. For example, only ca. 2% remobilization of Tc to solution was measured after 60 days in sulfidic sediments reoxidized with both 100 and 250 mM nitrate.

In reoxidation experiments with Fe(III)-reducing sediments, the anoxic oxidation of Fe(II) is most likely coupled to the formation of gaseous reduced nitrogen phases. For example, the reduction of NO_3^- to N_2 (eq 3) has been proposed by a number of workers for the anoxic oxidation of a range of Fe(II) systems (16, 43), and the formation of $N_2O_{(g)}$ coupled to Fe(II) oxidation has recently been reported (19).

$$10Fe^{2+} + 2NO_3^- + 24H_2O \rightarrow 10Fe(OH)_3 + N_2 + 18H^+$$
(3)
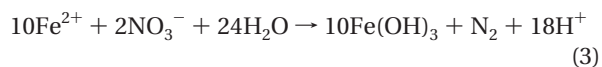
For reduction to N_2, the stoichiometric $[Fe(II)]/[NO_3^-]$ ratio is 5 (eq 3), consistent with our observed ratio of 4.0 ± 1.3 and suggesting that Fe(II) oxidation is coupled to the reduction of NO_3^- to N_2 gas here. Again, even though extensive Fe(II) reoxidation was observed in the Fe(III)-reducing sediments reoxidized with 22 mM NO_3^-, remobilization of Tc was limited with only 8% present in solution after 60 days; a final time point on Tc alone showed only 10% remobilization at 124 days.

To examine the solid-phase association of Tc in these sediments, X-ray absorption analyses of sulfate- and Fe(III)-reducing, microbially active sediments oxidized with 100 mM nitrate for 30 days and with 3−8% of the Tc remobilized to solution (similar to the behavior of the lower, comparable ^{99}Tc concentration microcosms) were also undertaken (Supporting Information, section 1). As with air reoxidation, EXAFS analysis of the NO_3^- oxidized sediments was undertaken. Again, this showed that there was a mixture of TcO_2 and TcO_4^- species in these sediments (Table 1; Supporting Information, Figure 2). The shell occupancies for oxygen in the NO_3^- oxidized sulfate- and Fe(III)-reducing sediment samples at ca. 1.7 Å and ca. 2.0 Å were 1.3 and 4.1, and 2.1 and 1.8, respectively (Table 1). This suggests that on reoxidation with nitrate, ca. 30−50% of the Tc was present as Tc(VII) in sulfate- and Fe(III)-reducing sediments, respectively. These data were again supported by modeling of the XANES data as described earlier, which showed that both the sulfate-reducing and the Fe(III)-reducing nitrate reoxidation sediment samples could be modeled with 18% and 33% Tc(VII), respectively. The origin of the mix of Tc(IV) and Tc(VII) present in the nitrate reoxidation sediments is unclear, although it may be that metal-oxidizing, nitrate-reducing microorganisms indigenous to the sediments have the potential to oxidize Tc(IV) enzymatically. Nonetheless, although extensive microbially mediated oxidation of both Fe(II) and sulfide phases occurred, and measurable Tc(IV) oxidation was indicated by EXAFS analysis, technetium bound to sediments during microbial reduction is extremely recalcitrant to remobilization during nitrate oxidation.

Relatively little is known about the organisms responsible for nitrate-dependent metal oxidation in estuarine sediments, although the process has been reported in marine sediments, aquifers, and lakes (18, 38, 44) and is of relevance to the environmental behavior of radionuclides (18, 19, 45). In our sediments, PCR-based analyses using broad specificity 16S rRNA gene primers of microbial communities supporting maximum oxidation of Fe(II) with nitrate as the electron acceptor revealed a complex bacterial community consistent with other studies using samples from this site (8). A 16S rRNA gene library yielded 26 distinct sequences from 43 clones analyzed, and genes were affiliated predominantly with Gamma Proteobacteria (representing 53% of the clone

library). In addition, Alpha (2.3%) and Beta (4.6%) Proteo-bacteria and Bacteroidetes (13.9%) were also detected. Collectively, close relatives within the phylogenetic groups detected are known to respire using a range of terminal electron acceptors. For example, microbes affiliated with *Paracoccus*, *Burkholderia*, and *Caldothrix* species, which respire using nitrate, each comprised 2% of the clone library. Investigations using PCR to amplify the functional genes required for nitrate reduction coupled to the oxidation of Fe(II), including nitrate reductase (*narG*) (*26*) and nitrite reductase (*nirS*) (*27*) genes, suggest that microorganisms encoding the nitrate- and nitrite-respiring enzymes involved in the oxidation of Fe(II) have not been previously identified as playing a direct role in metal cycling. The sequences retrieved using the *narG* PCR primers yielded 49 clones, of which 70% were most closely related to a nitrate reductase gene sequence from an uncultured bacterium detected in a soil nitrate-reducing community (*46*). Clone libraries obtained using the *nirS* primers were dominated by sequences related to the nitrite reductases of *Pseudomonas* and *Alcaligenes* species (showing an 80–90% match over 100–200 nucleotides). Clone libraries from both sulfate-reducing and Fe(III)-reducing sediments undergoing reoxidation with nitrate, prepared using primers specific for denitrifying enzyme genes, suggested broadly similar microbial communities in the two sediments. For example, approximately 40% of the clone libraries prepared from the *nirS* primers corresponded to those related to *Pseudomonas* and *Alcaligenes* species irrespective of whether the reoxidized sediment was Fe(III)- or sulfate-reducing. Interestingly, these genera have not previously been implicated in the oxidation of Fe(II) coupled to nitrate reduction. Finally, MPN PCR techniques indicated a 10-fold increase in numbers of *nirS* sequences from these organisms on reoxidation of the Fe(III)-reducing sediments with nitrate, further supporting their involvement in metal oxidation coupled to nitrate reduction.

Implications for the Reoxidation Behavior of Technetium. Our findings show that relatively rapid remobilization of 50–60% of Tc(IV) stored in anoxic estuarine sediments occurs if the material is reoxidized in oxygenated waters. By contrast, although microbially mediated nitrate reoxidation led to extensive reoxidation of both reduced Fe(II) and sulfide in sediments, only a small (<10%) amount of technetium was remobilized to solution. The nature of the nitrate-dependent bio-oxidation process is controlled by the redox conditions present prior to reoxidation. In sulfate-reducing sediments, electron balance calculations indicated that Fe(II) and sulfide oxidation were coupled with reduction of nitrate to nitrite and ammonia. In contrast, oxidation of Fe(III)-reducing sediments coupled Fe(II) oxidation with the reduction of nitrate to N_2 gas. EXAFS analysis suggested that reoxidation of sediment bound TcO_2 results in both TcO_2 and TcO_4^- being retained in sediments regardless of the starting geochemical conditions or oxidant used. These results have substantial implications for the management of Tc in the environment, and for the prediction of its mobility.

Supporting Information Available

Sections 1, 2, and 3 with details of XAS methodology. Table 1, redox ladder for key biogeochemical redox couples in sediment microcosms (Eh, mV). Table 2, range of values for geochemical indicators obtained during sediment reduction and reoxidation experiments. Table 3, XANES analysis of sediment spectra as linear combinations of "end-member" spectra. Figure 1, normalized Tc–K edge XANES spectra for relevant samples. Figure 2, k^3-weighted Tc K-edge EXAFS spectra and Fourier transforms for relevant samples. Figure 3, remobilization of Tc(VII) to solution and change in redox indicators during the nitrate reoxidation of sulfate reducing sediments. This material is available free of charge via the Internet at http://pubs.acs.org.

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