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Competitive Reduction of Pertechnetate ($^{99}\text{TcO}_4^-$) by Dissimilatory Metal Reducing Bacteria and Biogenic Fe(II)

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The fate of pertechnetate ($^{99}\text{Tc(VII)O}_4^-$) during bioreduction was investigated in the presence of 2-line ferrihydrite (Fh) and various dissimilatory metal reducing bacteria (DMRB) (*Geobacter*, *Anaeromyxobacter*, *Shewanella*) in comparison with TcO_4^- bioreduction in the absence of Fh. In the presence of Fh, Tc was present primarily as a fine-grained Tc(IV)/Fe precipitate that was distinct from the $\text{Tc(IV)O}_2 \cdot n\text{H}_2\text{O}$ solids produced by direct biological Tc(VII) reduction. Aqueous Tc concentrations ($<0.2 \mu\text{M}$) in the bioreduced Fh suspensions (1.7 to $3.2 \times 10^{-9} \text{ mol L}^{-1}$) were over 1 order of magnitude lower than when TcO_4^- was biologically reduced in the absence of Fh (4.0×10^{-8} to $1.0 \times 10^{-7} \text{ mol L}^{-1}$). EXAFS analyses of the bioreduced Fh-Tc products were consistent with variable chain length Tc–O octahedra bonded to Fe–O octahedra associated with the surface of the residual or secondary Fe(III) oxide. In contrast, biogenic $\text{TcO}_2 \cdot n\text{H}_2\text{O}$ had significantly more Tc–Tc second neighbors and a distinct long-range order consistent with small particle polymers of TcO_2 . In Fe-rich subsurface sediments, the reduction of Tc(VII) by Fe(II) may predominate over direct microbial pathways, potentially leading to lower concentrations of aqueous $^{99}\text{Tc(IV)}$.

Introduction

Technetium-99 (^{99}Tc) is a long-lived ($t^{1/2} = 2.13 \times 10^5 \text{ y}$) fission product of nuclear production and nuclear fuel reprocessing that is an environmental contaminant (1 and references therein). Environmental contamination by ^{99}Tc is of particular concern at the U.S. Department of Energy's Hanford Site, where subsurface Tc exists as the pertechnetate oxyanion, Tc(VII)O_4^- , which is weakly adsorbed by mineral phases and consequently mobile in vadose-zone water and groundwater (2 and references therein). However, under anoxic conditions, Tc(VII)O_4^- can be reduced to Tc(IV) ,

forming sparingly soluble $\text{Tc(IV)O}_2 \cdot n\text{H}_2\text{O}$ at circumneutral pH and in the absence of strong complexing ligands (3–5). Microbial processes can contribute to Tc(VII) reduction directly, by enzymatic reduction (6–12) or through redox-active organic molecules, such as quinones (8, 13). The terminal reductases for direct enzymatic Tc(VII) reduction by dissimilatory metal reducing bacteria (DMRB) and sulfate reducing bacteria (SRB) include periplasmic hydrogenases (10, 14), outer membrane multiheme c-type cytochromes (10), or, conceivably, periplasmic c-type cytochromes.

Iron oxides and Fe-bearing clay minerals are widespread in the terrestrial subsurface, and ferrous iron (Fe(II)) can be a strong reductant of Tc(VII) when in the sorbed or mineral structural state (15–20). Although reduction of Tc(VII) by aqueous Fe(II) (i.e., homogeneous reduction) is kinetically slow and pH dependent (15, 19), Tc reduction in a system without an initial solid phase can be accelerated by Fe(II) sorption to the insoluble Fe/Tc(IV) redox product resulting from homogeneous reduction of Tc(VII) by Fe(II) (19). However, Fe(II) reactivity toward Tc(VII) depends on the chemical environment and distribution of Fe(II) , as some forms of Fe(II) appear to be less reactive than others (1, 15, 17, 21). Biogenic Fe(II) is similarly reactive toward Tc(VII) , whether the Fe(II) is associated with magnetite (8, 9, 12), mineral surface complexes (1, 21), or fine-grained phyllosilicates (20, 22). In natural sediments that contain reactive Fe(III) , direct and indirect bioreduction pathways of Tc(VII) by dissimilatory metal reducing bacteria (DMRB) may compete, with the predominant pathway being controlled by the fastest reaction rate.

The intent of the current investigation was to assess the nature and distribution of the end products resulting from concurrent bioreduction of 2-line ferrihydrite (Fh) and Tc(VII)O_4^- by DMRB, as a means to determine the relative contributions of direct and indirect pathways under conditions where both are potentially operational. Final aqueous Tc solution concentrations were carefully measured and related to Fe mineralogy, and the nature of Fe–Tc solids was determined by electron microscopy and X-ray absorption spectroscopy.

Experimental Section

Washed late-log-phase cultures ($1 \times 10^8 \text{ cells mL}^{-1}$) of four DMRB (*Shewanella oneidensis* MR-1, *S. putrefaciens* CN-32, *Anaeromyxobacter dehalogenans* 2CP-C, and *Geobacter sulfurreducens* PCA) were incubated with H_2 (80 mL added to 80 mL of N_2 headspace, at overpressure, to give $\sim 4 \text{ mmol L}^{-1} \text{ H}_2$ in solution), 30 mmol L^{-1} 2-line ferrihydrite, and 0.3 mmol L^{-1} ammonium pertechnetate, ($\text{NH}_4^{99}\text{TcO}_4^-$). To prevent the complexation and solubilization of Tc(IV) (11), PIPES (30 mmol L^{-1} , pH 7), which has been shown in our laboratory to be noncomplexing toward Tc(IV) (9, 10), was used.

The DMRB-Fh-Tc samples were incubated under anoxic conditions (50:50 $\text{N}_2:\text{H}_2$ headspace) for 4 days at 50 rpm in the dark. Additionally, a series of biogenic Tc(IV) solids were prepared from incubations of *S. oneidensis*, *A. dehalogenans*, and *G. sulfurreducens* with Tc(VII)O_4^- and H_2 for X-ray absorption spectroscopy (XAS). The *G. sulfurreducens* treatment without Fh was also examined by transmission electron microscopy (TEM). Thin sections prepared from subsamples of the various DMRB-Fh-Tc suspensions were analyzed by TEM and X-ray energy dispersive spectroscopy (EDX) to examine the nature and distribution of Tc in relation to Fe solids and bacterial cells.

Soluble ^{99}Tc was measured by filtering (0.2 μm) and assaying the filtrates by liquid scintillation counting (detec-

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tion limit (DL) of 1.65×10^{-9} mol L⁻¹, according to the method of Currie (23)). Analytical variance (CV) in the experimental electrolyte was found to be 0.16 at twice the DL and 0.096 at 4 times the DL by counting 15 replicates of each concentration. Because biogenic TcO₂·nH₂O exhibits small crystallite size (<4 nm) (9), a test of the efficacy of 0.2 μm filtration was performed with biogenic Tc(IV) precipitate generated by *S. putrefaciens*. The precipitate was equilibrated in PIPES buffer for 1–35 days, and 0.001-μm (10 k MWCO, Pall-Gelman Nanosep) filtration was applied to the 0.2-μm filtrate to define colloidal (0.001 < Tc < 0.2) and soluble (Tc < 0.001) fractions. Soluble Tc(IV) represented from 26 to 65% of the 0.2-μm filtrate, with colloidal Tc comprising the difference (Figure S1 in the Supporting Information (SI)). The fraction of soluble Tc decreased with equilibration time while that of colloidal Tc increased with time.

To further characterize the Fh reduction products generated by these organisms, a separate experiment was conducted where the DMRB were incubated in the absence of TcO₄⁻ but with H₂ as above and with a lower Fh concentration, 15 mmol L⁻¹, in either 30 mM bicarbonate buffer (pH 7) or 25 mmol L⁻¹ PIPES with 5 mmol L⁻¹ bicarbonate (to simulate groundwater HCO₃⁻ concentrations) for 7 days. Samples from both experiments were analyzed by microcapillary X-ray diffraction (XRD). Soluble and weak-acid (0.5 N HCl) extractable Fe(II) were determined by the ferrozine method (19). Additional details on experimental methods are provided in the SI.

Results

Biotransformation of Fh in the Absence of Tc. In the absence of TcO₄⁻, resting cell suspensions incubated with 15 mM Fh and H₂ for 7 days generated variable concentrations of total (0.5 N HCl-extractable) Fe(II), resulting in crystalline end products dominated by goethite (α-FeOOH) and magnetite (Fe₃O₄) (Table S1, Figures S2 and S3). Under these conditions, *S. putrefaciens* and *A. dehalogenans* reduced Fh to the greatest extent and produced the greatest proportion of magnetite relative to goethite. All four organisms reduced more Fe(III) when bicarbonate (30 mmol L⁻¹) rather than PIPES (25 mmol L⁻¹) + bicarbonate (5 mmol L⁻¹) was used as a pH buffer. As expected, much of the resulting Fe(II) was associated with the solid phase, though the concentrations of aqueous Fe(II) were consistently higher in the bicarbonate-only than in the PIPES/bicarbonate buffered suspensions. We attribute this enhanced solubility of Fe(II) to complexation by bicarbonate, although there was no X-ray diffraction or microscopic evidence of siderite (FeCO₃) formation (Figures S2 and S3). *S. oneidensis* and *G. sulfurreducens* reduced significantly less Fe(III) in the PIPES + bicarbonate buffered suspensions, and only goethite diffraction peaks were evident (Table S1, Figures S2 and S3).

Biotransformation of Fh and TcO₄⁻. In a separate experiment, 300 μmol L⁻¹ TcO₄⁻ was incubated in resting cell suspensions of the four DMRB with H₂ in 30 mmol L⁻¹ PIPES (pH 7), without bicarbonate, and with Fh at 30 mmol L⁻¹ for 4 days, to probe the fate of Tc in a system where both direct (enzymatic) and indirect (biogenic Fe(II)) reduction may be operative. Following incubation, HCl-extractable Fe(II) ranged from 1.7 to 3.4 mmol L⁻¹, and the crystalline products of Fh biomineralization included goethite and magnetite (Table 1, Figure S4), similar to results from the non-Tc amended suspensions. However, in the presence of Tc and the absence of bicarbonate, *G. sulfurreducens* formed more magnetite, relative to goethite, than in the experiment with varying amounts of HCO₃⁻ and without Tc. This result is consistent with the well-known goethite-promoting effect of bicarbonate on Fh transformation (24, 25).

Several types of morphologically distinct mineral materials were generated in the incubations (Figure 1). One type of

TABLE 1. Concentrations of Aqueous Tc (<0.2 μm) and 0.5 N HCl-Extractable Fe(II) Following Incubation of the DMRB with Fh and TcO₄⁻ in Comparison with *G. sulfurreducens* Incubated with TcO₄⁻ in the Absence of Fh

organism	Tc _(aq) , mol L ⁻¹	0.5 N HCl-extractable Fe(II), mol L ⁻¹ × 10 ⁻³	goethite/magnetite ratio ^a
<i>S. oneidensis</i>	1.72×10^{-9}	1.69	100:0
<i>S. putrefaciens</i>	2.34×10^{-9}	3.36	75:25
<i>A. dehalogenans</i>	2.11×10^{-9}	2.64	81:19
<i>G. sulfurreducens</i>	3.16×10^{-9}	3.12	75:25
<i>G. sulfurreducens</i> without Fh	1.00×10^{-7}	NA ^a	NA
abiotic control	3.14×10^{-4}	0.37	NA

^a NA = not applicable. ^b Whole-pattern fitting was used to obtain semiquantitative estimates of the relative abundance of goethite and magnetite.

material present in the *S. oneidensis* suspensions consisted of needle-shaped crystallites (Figure 1A) that contained predominantly Fe with Tc at or below detection (Figure S5A) and exhibited a ring diffraction pattern consistent with goethite (Figure S5B). A second type of material present in the same suspension consisted of clusters of nanoparticulate material (Figure 1B) containing high concentrations of both Fe and Tc (Figure S5C) that was poorly crystalline (Figure S5D). The Fe:Tc ratios (71:29) within different regions of this material were relatively homogeneous at the level of EDX resolution. Thin sections prepared from *A. dehalogenans* suspensions with Fh also contained goethite (not shown) as well as hexagonal-shaped particles (Figure 1C, Figure S6A) that were predominantly Fe (Figure S6B) with a ring diffraction pattern consistent with magnetite (Figure S6C). The crystalline magnetite phase was not a significant Tc host according to EDX analysis. This suspension also contained a nanoparticulate phase (Figure 1D) that was enriched in Tc (Figure S6D) and that was poorly crystalline (Figure S6E). A similar homogeneous Tc-enriched phase was also present in the *S. putrefaciens* suspensions (Figure S7A–C), but this same phase was not apparent in thin sections prepared from *G. sulfurreducens* suspensions (not shown), possibly because this material was more highly dispersed. The Tc-enriched nanoparticle clusters in the suspensions were similar in physical appearance to the unreduced 2-line Fh (compare Figures 1B, D and S7A with Figure 1E). Thin sections prepared from *A. dehalogenans*, *S. putrefaciens*, and *G. sulfurreducens* suspensions with Fh also showed the occasional presence of Tc precipitates that appeared to be confined to the cell periplasm (Figure S9). In all of the biotic treatments, Tc X-ray absorption near-edge structure (XANES) analysis indicated that Tc was present as Tc(IV), whereas in the abiotic Fh control, Tc was present as Tc(VII) (Figure S10).

For comparison with our Tc reduction results in the presence of Fh, and with previous studies of TcO₄⁻ reduction with H₂ in noncomplexing buffers by *Shewanella* (10, 11) and *Anaeromyxobacter* (9), we also incubated *G. sulfurreducens* in PIPES with TcO₄⁻ and H₂ in the absence of Fh. The TcO₄⁻ was reduced (Figure S10), and the resulting Tc(IV) precipitate was associated with the cell envelope, with some deposition evident in the cytoplasm (Figure 1F). This material was predominantly Tc (Figure S8C), presumably TcO₂·nH₂O, and was poorly crystalline (Figure S8D). The material formed primarily in the periplasm and as ~50–100 nm diameter spherical aggregates along the exterior of the outer membrane (Figure S11). This distribution is consistent with the locations of catalytic redox proteins, including hydrogenases (periplasm) and c-type cytochromes (periplasm and outer membrane), implicated in the reduction of pertechnetate (7, 10, 14).

FIGURE 1. Electron micrographs of thin sections from Tc-Fh suspensions incubated with H_2 and *S. oneidensis* MR-1 (A, B) and *A. dehalogenans* 2CP-C (C, D), uninoculated Fh incubated under identical conditions (E), and *G. sulfurreducens* incubated with TcO_4^- and H_2 in the absence of Fh (F).

X-ray Absorption Spectroscopy. Iron XANES of the various Tc-Fh-DMRB suspensions revealed variable amounts of Fe(II) (Figure S12), consistent with measured 0.5 N HCl-extractable Fe(II) concentrations (Table 1). The Fe-edge positions in the suspensions with *S. oneidensis* and *A. dehalogenans* were similar to those for Fh, Fh + Tc(IV), and hematite (Fe_2O_3) standards. The edge positions for the *S. putrefaciens* and *G. sulfurreducens* suspensions were downshifted more closely to the position of the magnetite standard, again consistent with the presence of magnetite, in addition to goethite, in these suspensions (Table 1, Figure S4).

The valence of Tc in all bioreduced Fh suspensions, regardless of the organism, was confirmed to be Tc(IV) by XANES (Figure S10). Tc X-ray absorption fine structure (EXAFS) ($\chi(k)$ data) (Figure S13) and radial transforms for the bioreduced Fh suspensions were similar to a Tc(IV) + Fh standard (made by mixing Fh with Tc(IV) in 2 N HCl and adjusting to pH 7) (19), and were distinct from a $Tc(IV)O_2 \cdot nH_2O$ standard generated by dithionite reduction (Figure 2). These spectra, in turn, were almost identical to those resulting from Tc(VII) reaction with (i) sorbed Fe(II)

on goethite and hematite (17), (ii) fine-grained biomagnetite, and (iii) aqueous Fe(II) (19). The EXAFS spectra for these different Tc(IV)–Fe(III) oxide associations are indistinguishable from one another, and they all can be closely described with a model where variable chain-length Tc–O octahedra ($n = 1-3$) are bonded in an edge-sharing fashion to Fe–O octahedra associated with the Fe oxide (17, 19). In this regard, the Tc(IV) associations with Fh, goethite, and magnetite are indistinguishable from one another. The peak at $\sim 2 \text{ \AA}$ is diagnostic of these particular molecular associations. Given the high levels of Tc associated with the poorly crystalline nanoparticle clusters (Figure 1, Figures S5–S7), we assume that the bulk EXAFS signature is representative of this specific redox product.

The EXAFS spectra for biogenic $TcO_2 \cdot nH_2O$ produced by three different organisms (Figure 3) were quite similar to each other. They exhibited spectral features comparable to the Tc(IV) standard with a distinctive long-range order. The biogenic phases, however, had fewer Tc–Tc second neighbors at $2.2 \text{ \AA}$, consistent with their nanometer size (by analogy to biogenic UO_2 26, 27).

FIGURE 2. Tc-EXAFS (Fourier transform radial distribution function) of Tc(IV) coprecipitated with ferrihydrite (Fh), abiotically precipitated $\text{TcO}_2 \cdot n\text{H}_2\text{O}$, Tc reduced by biomagnetite, and Fh-Tc suspensions bioreduced by *S. oneidensis*, *S. putrefaciens*, *G. sulfurreducens*, and *A. dehalogenans*.

FIGURE 3. Tc-EXAFS (Fourier transform radial distribution function) of biogenic Tc(IV) formed via reduction of TcO_4^- by *S. oneidensis* MR-1, *G. sulfurreducens* PCA, and *A. dehalogenans* 2CP-C cells with H_2 as the electron donor.

Discussion

Ferrihydrite Biomineralization. Under the conditions herein, i.e., resting cells with H_2 as the electron donor, the four DMRB all generated Fe(II) from Fh and induced its crystallization to goethite or to a mixture of goethite and magnetite (Tables S1 and 1). These phases were expected under these conditions. However, *S. putrefaciens* and *A. dehalogenans* reduced Fh to a greater extent and generated more magnetite, relative to goethite, than did *S. oneidensis* or *G. sulfurreducens* in the absence of Tc. Although a general consistency of crystalline products (goethite and magnetite) from Fh reductive biomineralization was observed, differences in chemical, physical, and biological variables can result in a range of end products. These factors include the final suspension pH and bicarbonate and phosphate concentrations (24, 25, 28, 29), the presence of complexing ligands or electron shuttling compounds (30–32), advective-flow induced Fe(II) concentration profiles (33), electron donor/acceptor ratio (34), the presence of sorbed ions (28), and Fh aging (28).

The noted differences in the proportion of goethite versus magnetite is likely due to the rate at which Fe(II) is produced by the various organisms. At low Fe(II)/Fe(III) ratios in bicarbonate-containing abiotic systems, Fh rapidly recrystallizes into goethite (24), whereas at higher ratios, magnetite will also form competitively or in parallel (33, 35, 36), or indirectly via goethite (32). We have previously observed the formation of siderite (FeCO_3) from the bioreduction of Fh in bicarbonate medium with lactate as the electron donor and a final pH of 8.1 and a pe of <-4.5 (31). However, in the present study using a medium that does not support cell growth, H_2 as opposed to lactate or acetate as the electron donor, and lower overall alkalinity and higher pe (i.e., lower Fe(II) concentration), magnetite is favored over siderite. An important observation made here is that Tc(IV) does not apparently associate with the primary bioinduced crystalline transformation products of ferrihydrite that may adsorb Fe(II) (e.g., goethite and magnetite), but rather with a poorly crystalline, nanoparticulate phase that bears resemblance

to the redox product of homogeneous Fe(II)–Tc(VII) reaction (19). In previous investigations we have noted the formation of Fe(II)-enriched ferrihydrite approaching magnetite stoichiometry that was slow to recrystallize and that retained the morphologic and size features of Fh (28, 31). We speculate that this was the initial phase to form in the experiments herein and the most reactive toward Tc(VII). Hence, Tc(IV) was largely associated with this material.

Direct vs Indirect Microbial Reduction of Tc(VII). In contrast to our previous reports of the rapid reduction of TcO_4^- by biogenic Fe(II) resulting from the microbial reduction of Fh (9) or sediment associated Fe(III) (1, 21), where TcO_4^- was added after Fe(III) reduction and the DMRB were heat killed, the experiments in this study included Tc(VII) at the onset of bacterial reduction, as in previous studies with *G. sulfurreducens* (8, 12). The presence of TcO_4^- and Fh results in the potential for concurrent Tc(VII) reduction via both direct enzymatic reduction and indirect reduction via biogenic Fe(II). However, the collective TEM, Tc solubility, and XAS results suggest that indirect reduction via biogenic Fe(II) was the dominant pathway with all four organism-Fh suspensions. Although qualitative, the relative abundance of cell associated $\text{TcO}_2 \cdot n\text{H}_2\text{O}$ precipitates in the presence of Fh (Figure S9) and in its absence (Figure 1F, Figure S11), in conjunction with the detection of Tc in the fine-grained Fe phase resulting from biological Fh reduction (Figure 1B, D, Figures S5–S7), suggests that the role of direct biotic reduction was minor. Our results indicate that even when the electron donor is H_2 , which promotes rapid and extensive bioreduction of Tc(VII) by DMRB (8–11, 37), the indirect biogenic Fe(II) pathway for Tc(VII) reduction may predominate when poorly crystalline Fe(III) oxides such as Fh are present.

Whereas abundant Tc(IV) deposits were associated with the outer membrane in the *Geobacter* treatment without Fh, as was the case with *Anaeromyxobacter* (9) and, to a lesser extent, *Shewanella* (10) under similar conditions, the cell associated Tc(IV) deposits in the Fh treatments appeared to be contained entirely within the periplasm (Figure S9). These results suggest that in the presence of Fh, outer-membrane cytochromes preferentially reduce Fe(III) rather than Tc(VII), consistent with the results and the kinetic and thermodynamic arguments provided by Marshall et al. (10). In addition, Tc solubility in the DMRB-Fh treatments showing some periplasmic Tc deposition (all treatments except *S. oneidensis* MR-1) was on the order of that measured in experiments where Tc(VII) was abiotically reduced by Fe(II) (Table 1) (17, 19), in contrast with concentrations of aqueous Tc where Tc(VII) was reduced directly by DMRB in the absence of Fe oxides (Table 1, refs 9, 10). Likewise, the EXAFS spectra in the DMRB-Fh-Tc treatments are consistent with the model of variable chain length Tc–O octahedra bonded to Fe–O octahedra associated with Fe(III) oxide (e.g., Fh, goethite, or magnetite; Figure 2). Collectively, these results suggest that cell-reduced Tc(IV) was below XAS detection, as supported by the TEM analyses.

Tc Solubility in Relation to Tc(IV) Products. An important finding from these experiments was that the concentrations of aqueous ($<0.2 \mu\text{m}$) Tc in the Fh-cell suspensions ranged from 1.7 to $3.2 \times 10^{-9} \text{ mol L}^{-1}$ (Table 1), or approximately the literature value for the solubility of $\text{Tc(IV)O}_2 \cdot n\text{H}_2\text{O}$, $3.6 \times 10^{-9} \text{ mol L}^{-1}$ (3–5). In contrast, $\text{Tc}_{(\text{aq})}$ ($<0.2 \mu\text{m}$) concentrations in contact with biogenic $\text{TcO}_2 \cdot n\text{H}_2\text{O}$ were over 1 order of magnitude higher, ranging from 4.0×10^{-8} (Figure S1) to $1.0 \times 10^{-7} \text{ mol L}^{-1}$ (Table 1), consistent with previous studies (9, 10). As noted earlier, the Tc(IV) EXAFS spectra for solids from the bioreduced Fh suspensions were interpreted to result from monomeric, dimeric, and trimeric Tc(IV) octahedral complexes bound to the surface of or within an undefined Fe(III) oxide phase. Likewise, Tc association with

Fe phases has been observed in studies of Tc reduction in natural sediments by XAS (1, 21, 38, 39).

Our EXAFS results for the Tc-DMRB-Fh suspensions contrast with those of the biogenic Tc solids generated via enzymatic reduction (Figure 3), where the Tc–Tc second neighbors were considerably less numerous than the Tc(IV) standards prepared abiotically by reduction of TcO_4^- with dithionite, as observed in a previous study of TcO_4^- bioreduction by *S. putrefaciens* CN-32 (11). The $\text{Tc}_{(\text{aq})}$ concentration in the *G. sulfurreducens* treatment without Fh, assuming a tetravalent Tc oxidation state, is nearly 2 orders of magnitude above the accepted solubility value for $\text{Tc(IV)O}_2 \cdot n\text{H}_2\text{O}$ (Table 1). The filtration study with biogenic $\text{TcO}_2 \cdot n\text{H}_2\text{O}$ from *S. putrefaciens*, however, revealed that the $0.2\text{-}\mu\text{m}$ filtrate contained significant colloidal Tc that was $>1 \text{ nm}$ in size (Figure S1). Soluble Tc ($<0.001 \mu\text{m}$) was observed to decrease during the 35 day sampling period from 2.75×10^{-8} to $1.5 \times 10^{-8} \text{ mol L}^{-1}$, with the final concentration being a mere 3.8 times the literature solubility value. This soluble Tc was shown to be all Tc(IV) by solvent extraction. However, given the small size of the biogenic $\text{TcO}_2 \cdot n\text{H}_2\text{O}$ precipitates ($2 < x < 4 \text{ nm}$), it is possible that some undetermined fraction of polymeric Tc(IV) species was present in the soluble Tc fraction. Consequently, we conclude that the apparent high solubility of biogenic $\text{TcO}_2 \cdot n\text{H}_2\text{O}$ results from the presence of colloidal Tc(IV) in the $<0.2\text{-}\mu\text{m}$ filtrate, and a possibly slow approach to solubility equilibrium involving polymeric species (>35 days). The presence of these Tc(IV) colloids and polymers (see 18, 40 and references therein) appears inherent to the biotic system and a consequence of the nature of the electron transfer and biogenic $\text{TcO}_2 \cdot n\text{H}_2\text{O}$ precipitation mechanisms.

In a previous study where *G. sulfurreducens* was incubated in bicarbonate buffer with Fh and TcO_4^- with acetate as the electron donor and AQDS as an electron shuttle, Tc(IV) was similarly associated with the Fe solid phase (magnetite), and the Tc solubility ($<5 \times 10^{-9} \text{ mol L}^{-1}$ (8)) was similar to concentrations measured in this study with Fh. Likewise, a *Geobacter* sediment enrichment culture in bicarbonate buffer with acetate as electron donor reduced Fh to magnetite in the absence of AQDS, and again, the aqueous Tc was below the detection limit of $5 \times 10^{-9} \text{ mol L}^{-1}$ (8). Lear et al. (41) provided evidence that biogenic Fe(II) could reduce $^{99\text{m}}\text{Tc(VII)}$ at a solution concentration of $10^{-12} \text{ mol L}^{-1}$, which is 3 orders of magnitude below the literature solubility value for $\text{Tc(IV)O}_2 \cdot n\text{H}_2\text{O}$, but the mechanism for this reduction reaction is as yet undetermined.

The environmental implications of the apparent solubility (e.g., dissolved and colloidal species) of $\text{Tc(IV)O}_2 \cdot n\text{H}_2\text{O}$ generated by direct biogenic reduction in the absence of complexing ligands (i.e., $\sim 10^{-6}$ to $10^{-7} \text{ mol L}^{-1}$, Table 1) being higher than the solubility of Tc(IV) in reduction products generated by biogenic Fe(II) ($\sim 10^{-9} \text{ mol L}^{-1}$) (Table 1) are important, because the EPA maximum concentration level (MCL) for ^{99}Tc in drinking water is 900 pCi L^{-1} , or approximately $10^{-9.4} \text{ mol L}^{-1}$ (42). This solubility enhancement may involve the generation of dispersible, and hence mobile, Tc(IV) colloids, or may simply result from short-term solubility disequilibrium. Whatever the mechanism, Fe(II) facilitated reduction of Tc(VII) may offer a considerable advantage over direct microbial reduction in terms of achieving an aqueous ^{99}Tc concentration that is near or below the MCL. In addition to these solubility considerations, Tc(IV) associated with Fe mineral phases may be much more resistant to reoxidation than biogenic $\text{Tc(IV)O}_2 \cdot n\text{H}_2\text{O}$ (19–21, 43). Such factors may be important considerations for designing in situ remediation strategies or for identifying dominant redox pathways that may be naturally attenuating Tc concentrations in groundwater.

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Supporting Information Available

Additional data as referenced in this article. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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