Chemical stability of ^{99m}Tc -DTPA under aerobic and microbially mediated Fe(III)-reducing conditions in porous media

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Abstract

^{99m}Tc -DTPA has been used as a conservative tracer to quantify water transport through porous media. However, more information on the reactivity of this ^{99m}Tc compound under varying geochemical conditions is desirable to better understand its potential uses. We measured the speciation of Tc following amendment of ^{99m}Tc -DTPA to batch systems spanning a range of controlled biogeochemical conditions. Our results suggest that ^{99m}Tc -DTPA is stable under the reducing conditions tested. However, freshly precipitated Al-ferrihydrite may displace Tc(IV) from DTPA in the absence of Fe(III)-reducing conditions.

1. Introduction

In the earth sciences, achieving a quantitative understanding of physical, chemical, and biological processes and their interaction in porous media is required to predict terrestrial subsurface contaminant transport and carbon transformation. The utility of model laboratory systems to elucidate key aspects of natural systems depends on the extent to which the interaction of these processes is understood. Conservative tracers such as bromide allow inference of flow properties of a whole porous medium, but quantifying variations in water movement within a medium in real-time has been challenging (Werth et al., 2010). With currently available imaging technologies, radioactive molecules commonly used in the medical

field offer one of the few minimally perturbing means of quantifying water transport through porous media. For example, single photon emission computed tomography (SPECT) has been used to dynamically measure the 2D and 3D distribution of $^{99m}\text{TcO}_4^-$ and $^{99m}\text{Tc-DTPA}$ in porous media (Boutchko et al., 2012; Lear et al., 2010), while positron emission tomography (PET) has been used similarly with $^{18}\text{F}^-$ (Kinsella et al., 2012). In a natural sediment subjected to iron and sulfate reduction, Vandehey et al. (2012) found that $^{99m}\text{Tc-DTPA}$ remained soluble while $^{99m}\text{TcO}_4^-$ precipitated. While these early trials demonstrate the utility of radiotracer compounds under some conditions, knowing more about the reactivity of these compounds would enable better selection of radiotracer compounds for a given system.

In this work, we focus on ^{99m}Tc , as it is the most commonly used medical radioisotope and is readily available commercially. With a six-hour half-life, it is well suited for experiments with timescales of hours to a few days. To image water transport through porous media using a ^{99m}Tc -labeled molecule, its chemical form (speciation) must be minimally reactive and stable as a dissolved species. Similarly, to use ^{99m}Tc to image the distribution of specific geochemical conditions, its reactivity under natural conditions must be well understood. Chemically, technetium can often be found in one of three states; oxidized, reduced-hydrolyzed, or reduced-chelated, each of which will behave differently depending on biogeochemical conditions. In its oxidized state (+7), Tc preferentially forms the pertechnetate ion, Tc(VII)O_4^- . While pertechnetate is highly water soluble and does not appreciably sorb to naturally occurring solids, it can be reduced either abiotically or enzymatically by metal-reducing bacteria to Tc(IV) . (Fredrickson et al., 2004; Liu et al., 2002; Marshall et al., 2008; Plymale et al., 2011; Wildung et al., 2000). When reduced—for example, by electron exchange with Fe(II) or by bacterial metabolism— Tc(IV) tends to hydrolyze quickly, forming $\text{TcO}_2 \cdot n\text{H}_2\text{O}$ that is stable under reducing conditions. This reduced-hydrolyzed Tc is poorly suited for imaging flow, as it will likely sorb to solids. In radiochemical syntheses, Tc is often reduced by SnCl_2 in the presence of a chelator.

Following chelation, the chemical properties are largely determined by the properties of the chelator rather than the metal (Jurisson et al., 2004). An example complex that has been reported as suitable as a flow tracer in reduce sediment is ^{99m}Tc -DTPA (Vandehey 2012). It is synthesized by complexing Tc(IV) with the multidentate ligand diethylenetriamine pentaacetic acid (DTPA) to form a soluble aqueous complex:

The thermodynamic equilibrium constant ($\log K$) of equation 1 has been determined to be 26.3 (Levin et al., 1980).

DTPA is a strong chelator of metal cations; as an example, it is added to paper pulp liquors before bleaching with peroxide to prevent iron oxidation (Holzbecher et al., 2005). Due to DTPA's ability to bind cations, Tc(IV) is potentially vulnerable to competition for DTPA from other metals that are more abundant than the pico-to-nanomolar concentrations of ^{99m}Tc used in radiotracer experiments. For instance, DTPA has a stronger thermodynamic stability with Fe^{3+} ($\log K = 28.6$) than it does with $\text{TcO}(\text{OH})^+$ (Vandegaer et al., 1959):

If $^{99m}\text{Tc}(\text{IV})$ is displaced from the DTPA complex by Fe, it will likely transform into slow-moving or immobile particulate $\text{TcO}_2 \cdot 2\text{H}_2\text{O}$ or sorbed species depending on whether the specific conditions exceed the solubility of $\text{TcO}_2 \cdot 2\text{H}_2\text{O}$ ($\sim 4 \times 10^{-9}$ M (Rard et al., 1999), thereby decoupling the tracer from the transport of water. Equations 1 and 2 suggest that DTPA would preferentially bind to Fe(III) if available, but while Fe(III) is present in most soil, sediment, and aquatic systems, the activity of aqueous Fe^{3+} is typically low due to hydrolysis and precipitation. Under aerobic conditions and circumneutral pH, Fe(III) is dominated by (hydr)oxides with $\text{FeOH}_x^{(x-1)+}$ surface functional groups (Filius et al., 2000). By analogy to equation 2, DTPA could adsorb to surface sites on Fe(III) (hydr)oxides:

(≡Fe represents a surface bound Fe site).

In spite of this possibility, empirical observations show ^{99m}Tc -DTPA interacts only weakly with Fe (hydr)oxide-containing sediments (Vandehey et al., 2012) under aerobic conditions and non-detectably under reducing conditions. This apparent contradiction is due to the fact that the reactivity of Fe(III) (hydr)oxides can vary greatly. To address these uncertainties, we build on the work of Vandehey et al. (2012) by measuring the speciation of Tc following amendment of ^{99m}Tc -DTPA to batch systems spanning a range of controlled biogeochemical conditions and interpret the speciation results with the aid of ancillary geochemical data and thermodynamic geochemical simulations.

We systematically tested batch systems related to ongoing flow-through experiments aimed at understanding the coupling of water flow, microbially mediated iron transformations, and the transport of chromate. Accordingly, in addition to controls, the batch systems contained some combination of synthetic Al-doped ferrihydrite-coated quartz sand, a single strain of metal-reducing bacteria (*Shewanella putrefaciens* CN32), and chromate. In particular, we determined whether dissolved oxygen abiotically oxidizes Tc(IV)-DTPA to TcO_4^- ; *S. putrefaciens* CN32 degrades DTPA while respiring O_2 or while respiring Fe(III); Al-ferrihydrite sand coatings competitively bind DTPA, releasing Tc(IV) that precipitates as TcO_2 or, if O_2 is present, oxidizes to TcO_4^- ; Cr(VI) outcompetes Tc(IV) for DTPA; Cr(VI) oxidizes Fe(II) such that Fe(III) binds DTPA; and Cr(III) (-Fe) hydroxides outcompete Tc(IV) for DTPA.

Our results suggest that ^{99m}Tc -DTPA is found to be stable under reducing conditions and not degraded by metabolically active *S. putrefaciens* CN32. However, recently precipitated Al-ferrihydrite may displace Tc(IV) from the DTPA complex under aerated (aerobic) conditions, and oxidants such as O_2 and Cr(VI) can partially oxidize the complex. The range of conditions investigated revealed that electrostatic attraction (or lack thereof) between DTPA and Al-ferrihydrite surface sites is likely the mechanism responsible for the dissociation of ^{99m}Tc -DTPA.

2. Materials and methods

2.1 Materials

Artificial groundwater (AGW) was made by dissolving reagent-grade chemicals in deionized water, and included 2.7 mM KCl, 0.58 mM MgSO₄, 7.9 mM NaCl, 0.41 mM CaCl₂, 0.02 mM NH₄Cl, 5 mM sodium lactate, and 10 mM piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES, adjusted to pH 7.12 with NaOH). The ionic strength of this solution is approximately 30 mM.

Two sets of vials (Table 1—systems E6 and E7) were amended with 5 μM potassium chromate.

Aluminum-doped ferrihydrite was synthesized by gradually titrating 0.4 M NaOH into a well-mixed solution of 37.2 mM FeCl₃ and 4.13 mM AlCl₃ (9:1 Fe-to-Al molar ratio) to pH 7.2. The Al-ferrihydrite slurry was desalted three times by centrifugation. Once resuspended in deionized water, the Al-ferrihydrite was mixed by hand with IOTA 6 quartz sand (Unimin Corp.) and allowed to dry at room temperature. Once dried, the Al-ferrihydrite-coated sand was saturated with deionized water and gently agitated to remove loosely bound particles, which were decanted. This washing procedure was performed eight times. The wet sand was then allowed to dry for later use in batch experiments. On the basis of chemical digestion (6 N HCl) and ICP-MS analysis of the finished Al-ferrihydrite-coated sand, the sand contained 0.45% Fe and 0.02% Al on a dry-weight basis, corresponding to a Fe:Al molar ratio of 10.5:1.

A culture of *Shewanella putrefaciens* strain CN32 was grown from a frozen seed culture in tryptic soy broth, washed three times with AGW by centrifugation, and suspended in 100 mL AGW. On the basis optical absorption calibrated to DAPI-enabled epifluorescence microscopy, the stock contained 1.45×10^{10} cells/L.

25.7 mCi of ^{99m}Tc-DTPA received in 0.5 mL of normal saline (Cardinal Health, Richmond, CA) was added to a vial containing 5 mL of N₂-purged AGW. The radiopharmacy reported that the radiochemical purity of the ^{99m}Tc-DTPA exceeded 99.5% (i.e., 99.5% of the total Tc was

^{99m}Tc-DTPA) at time of synthesis, whereas upon receiving the ^{99m}Tc-DTPA (6 h post-synthesis), independent testing indicated 96 ± 1% radiochemical purity ('Cardinal' in Fig. 2) and 96 ± 3% purity of the solution following dilution in anoxic AGW ('AGW' in Fig. 2).

2.2 Batch systems

A variety of batch reactors (10 mL serum vials) was prepared in triplicate for the eight experimental conditions summarized in Table 1, referred to as control (C) and experiments E1-E7. All vials contained 10 mL of AGW, aerated or boiled and purged with UHP N₂ as appropriate. A mass of 0.51 ± 0.02 g Al-ferrihydrite-coated sand was loaded into each vial of E2, E3, E5, and E7; systems intended to exclude O₂ were prepared in an anaerobic chamber. A volume of 69 µL of *S. putrefaciens* stock was added to each vial of E4, E5, and E7 to achieve a starting concentration of approximately 10⁸ cells/mL. All vials were closed by crimp-sealing rubber stoppers to the serum vials. A sterile filtered vent needle was inserted through rubber stoppers of all replicate vials of E4 to allow gases to freely diffuse in or out. Vials were covered with aluminum foil to minimize exposure to ambient light and mixed on an orbital shaker at 100 rpm at room temperature for three days. The slow mixing swirled the water and any suspended material overlying the sand, but did not move the sand itself.

The redox status of the vials inoculated with *S. putrefaciens* was determined 1 day prior and on the day of ^{99m}Tc injection. One mL of liquid sample was aspirated in a syringe flushed with an ultrahigh purity (UHP) N₂ and injected into a sealed serum vial containing 9 mL of AGW that had been purged with UHP N₂. The sample was quenched with excess MnSO₄ and KOH and then acidified for titration with standardized (0.025 N) sodium thiosulfate according to a modified Winkler method (Granéli and Granéli, 1991). Through the handling and analysis of multiple purged vials, we determined that the detection limit for O₂ was 16 µM. For iron-containing systems, separate 1 mL aliquots were aspirated into UHP N₂-flushed syringes, filtered (0.2 µm),

and acidified (0.05 N HCl) for subsequent analysis of dissolved Fe(II) using the ferrozine assay (Stookey, 1970).

Approximately 500 μCi of $^{99\text{m}}\text{Tc(IV)DTPA}$ in a volume of 150 μL AGW [$\sim 6 \text{ pmol } ^{99\text{m}}\text{Tc-DTPA} + 130 \text{ nmol uncomplexed DTPA}$ (Vandehey et al, 2012)] was injected into each vial through the rubber stoppers using 300 μL syringes. To determine the actual dose of $^{99\text{m}}\text{Tc}$ to each vial, the radioactivity of the syringes was measured before and after injection. Following $^{99\text{m}}\text{Tc}$ injection, the vials were mixed in the dark on an orbital shaker at 100 rpm for approximately 20 h.

Thin layer chromatography (TLC) was used to speciate $^{99\text{m}}\text{Tc}$ into dissolved, particulate, oxidized, and chelated forms (Környei and Zolle, 2007) as shown in Figure 1. Thirty microliters of water was removed from each vial, and one to two drops of each solution spotted 1 cm from the end of two strips of $\frac{1}{4}$ " wide Whatman 1 paper. Aerobic vials were handled under ambient conditions while anaerobic vials were transferred into a glove box filled with 2% H_2 and 98% UHP N_2 equipped with a Pd catalyst. The strips were placed in test tubes containing approximately 200 μL of either 0.9% saline (NaCl) or acetone. The strips were removed from their test tubes when the solvent front had migrated 6 cm (approximately 5 minutes). Each strip was exposed to a phosphor screen for about 14 hours to locate $^{99\text{m}}\text{Tc}$ radioactivity (Knol, 2008). Peaks on line profiles of radioactivity versus position on the strips were baseline-corrected and quantified as percentages of the sum of radioactivity at the origin and solvent front. The percentage of colloidal Tc is determined on a saline eluted strip, where colloidal Tc remains at the origin while TcO_4^- and $^{99\text{m}}\text{Tc-DTPA}$ elute with the solvent front. The percentage of TcO_4^- is determined on an acetone-eluted strip where colloidal Tc and $^{99\text{m}}\text{Tc-DTPA}$ remain at the origin and TcO_4^- elutes with the solvent front. The fraction of $^{99\text{m}}\text{Tc-DTPA}$ is defined as the difference between 100% and the sum of colloidal and TcO_4^- free signal. The purity of $^{99\text{m}}\text{Tc-DTPA}$ as both the starting formulation provided by Cardinal Health and following AGW dilution was similarly evaluated.

The apparent solubility of Fe(III) in AGW with and without PIPES and lactate was determined. Approximately 150 μ M FeCl₃ was dissolved into AGW without pH buffer, with and without lactate, and then titrated with either KOH or PIPES and KOH to pH 7.1. One milliliter samples were taken, filtered a through 0.2 μ m syringe membrane, mixed with 0.25 M hydroxylamine hydrochloride-0.25 M HCl, and assayed with ferrozine (Stookey, 1970).

2.3 Species distribution modeling

The species distribution of DTPA and Tc was calculated using the SpecE8 and React modules of the Geochemist's Workbench. Thermodynamic data were compiled from the default and MINTEQA databases along with surface complexation reaction constants compiled by Dzombak and Morel (1990). Surface complexation was treated using electrical double-layer theory. The database was supplemented with equations 1 and 3. The formation constant of equation 1 was taken as published by Levin et al. (1980). However, the stability constant of equation 3 is uncertain, not only because the reactivity of Fe(III) (hydr)oxides varies depending on mineralogy, but also because the acidity of the systems studied here (pH 7.1) is close to the point of zero charge of Fe(III) (hydr)oxides. On the basis of detailed investigations of the adsorption of EDTA—a compound similar to DTPA—by Nowak and Sigg (1996) and Noren et al. (2009) we suspect that, variables in equations 3 will be $n = 1$ or 2 and $m = 1$. Furthermore, for EDTA and goethite, the log K of equation 3 was found to be strongly dependent on pH: log $K = 13$ at pH 7.7 and log $K = 35$ at pH 6.5, for $n = 2$ (Nowack and Sigg, 1996), since the goethite surface is less positively charged at pH 7.7 compared to pH 6.5. We expect the thermodynamic stability of surface complexes of DTPA and our sorbent (an Al-doped ferrihydrite) to be between that of EDTA-goethite complexes and aqueous Fe³⁺, given that DTPA has an additional electron-donating ligand and that Al-ferrihydrite surfaces are more reactive than those of goethite. Accordingly, the log K of equation 3 is likely between 13 and 26 and the complexation

constant for equation 3 was varied over this range. The basis species were specified according to the composition of the defined AGW medium. Fe(III), Tc(IV), and Cr(VI) basis species were not allowed to undergo redox reactions in the simulations. Non-equilibrium systems were simulated by suppressing the formation of certain species, as described in the results and discussion section.

3. Results

The system conditions chosen and the results reported below were designed to answer six specific hypotheses:

1. Dissolved O₂ abiotically oxidizes ^{99m}Tc(IV)-DTPA releasing TcO₄⁻
2. *S. putrefaciens* CN32 degrades DTPA if (a) O₂ is available and/or (b) under Fe(III)-reducing conditions.
3. Al-ferrihydrite sand coatings competitively bind DTPA, releasing Tc(IV) that precipitates as TcO₂ or, if O₂ is present, oxidizes to TcO₄⁻
4. Cr(VI) will reactive with ^{99m}Tc-DTPA resulting in release of TcO₄⁻.
5. Metastable dissolved Fe(III)_{aq} and/or Cr(III)-Fe(III) hydroxides (formed via oxidation of Fe(II) by Cr(VI)) will bind with DTPA and displace Tc from the ^{99m}Tc-DTPA complex

3.1 Redox conditions in batch reactors

Oxygen concentrations were measured in the vials of E4, E5, and E7, which contained CN32 and therefore had the potential for microbial consumption of O₂. After nearly three days of incubation, the dissolved O₂ concentrations in the vented vials containing *S. putrefaciens* (290 μM; E4) were similar to that of fully aerated AGW (280 μM; Table 1), indicating that aerobic conditions were maintained with the vent needles. In the closed vials of E5, the bacteria had depleted O₂ in the closed vials to 79 μM and 48 μM dissolved Fe(II) was measured, three days after inoculation. Fe(III) reduction continued throughout the experiment, as dissolved Fe(II)

increased from 48 μM on day 3, when radiotracer was added, to 194 μM on day 4 when the $^{99\text{m}}\text{Tc}$ species TLC measurements were made (Table 1). In E7, with 5 μM Cr(VI) present, O_2 concentrations only decreased to 130 μM on day 3, when radiotracer was added, and Fe(II) concentrations showed a less pronounced increase from 43 μM (day 3) to 61 μM (day 4) (Table 1).

3.2 Measured technetium speciation

For all systems, thin layer chromatography (TLC) indicated no colloidal Tc compounds such as $^{99\text{m}}\text{TcO}_2 \cdot 2\text{H}_2\text{O}$ were suspended in the vials. On the basis of TLC analysis, the $^{99\text{m}}\text{Tc}$ -DTPA solution from the radiopharmacy contained $4 \pm 1\%$ (average ± 1 standard deviation of triplicates) $^{99\text{m}}\text{TcO}_4^-$, Fig. 2.

3.2.1 Impact of O_2 and Cr(VI)

A few minutes after being diluted in anoxic artificial groundwater (AGW) the measured TcO_4^- fraction was $7 \pm 2\%$. After 20 h of mixing in anoxic artificial groundwater (system C), this fraction was statistically indistinguishable at $8 \pm 2\%$ TcO_4^- . By contrast, after 20 h of mixing with aerated AGW (E1), $17 \pm 1\%$ of Tc was TcO_4^- indicating that there is some oxidation of Tc by O_2 , but limited under the time scales employed here and in previous studies (Vandehey et al., 2012). With O_2 and Cr(VI) (E6), the TcO_4^- value was $21 \pm 1\%$, or 5% greater than in the vials containing O_2 alone (E1 and E6; Fig. 2) indicating that there may also be some additional oxidation by Cr(VI). The extent of the impact of Cr(VI) in the absence of O_2 was unfortunately not measured. However, the presence of both O_2 and Cr(VI) represents a worst case scenario with regards to the impact of these two potential oxidants.

3.2.2 Impact of aerobic CN32 metabolic activity

In the presence of O₂ and CN32, only 10 ± 1% of Tc was detected as TcO₄⁻ during aerobic respiration (Fig. 2; E4), less than the aerated and CN32-free system (Fig. 2; E1), which had 17 ± 1% TcO₄⁻, falsifying hypothesis 2a. With anaerobically active CN32 (E5), neither TcO₂ nor TcO₄⁻ were detected, falsifying hypothesis 2b.

3.2.3 Impact of Al-ferrihydrite-coated sand and Fe(III) reduction

After 20 h in an abiotic, anoxic suspension of Al-ferrihydrite-coated sand, all of the Tc was in the form of TcO₄⁻ (Fig 2; E2). In system 4, with Al-ferrihydrite-coated and O₂, 92 ± 8% of the Tc was in the form of TcO₄⁻ (Fig. 2, E3). However, in closed systems containing CN32, reduced Fe(II), and Al-ferrihydrite, Tc remained entirely as ^{99m}Tc(IV)-DTPA, regardless of whether Cr(VI) was present (E5 and E7). These results reject hypothesis 5, while indicating that the validity of hypothesis 3 depends on surface and solution conditions.

3.3 Modeled species distribution of DTPA and technetium

In the presence of hydrous ferric oxide (Fe(OH)₃, (the closest analog to ferrihydrite for which thermodynamic data are available), thermodynamic calculations predict that 9 × 10⁻¹⁰ M of dissolved Fe(III) exists in the pH 7.1 AGW at equilibrium. The model further predicts that this amount of Fe(III) is enough to complex all DTPA in the batch reactors (estimated to be at most 10⁻¹⁰ M), leaving Tc(IV) as TcO₂·2H₂O at equilibrium. In separate batch experiments, the concentration of dissolved Fe(III) in AGW fell below 2 × 10⁻⁶ M within minutes (Fig. 3). The detection limit of the assay was too high to determine whether 9 × 10⁻¹⁰ M dissolved Fe(III) persisted, as predicted by the model.

If DTPA complexation of dissolved Fe(III) is disallowed, thermodynamic calculations predict DTPA species abundance in the order of CaDTPA³⁻ > MgDTPA³⁻ > H₂DTPA³⁻, while all Tc(IV) is predicted to precipitate as TcO₂·2H₂O in the absence of oxygen or oxidize to TcO₄⁻ in the

presence of 300 μM O_2 . Contrary to model predictions, TLC analysis of $^{99\text{m}}\text{Tc(IV)-DTPA}$ amended anoxic AGW, indicates that 92% of Tc was still $^{99\text{m}}\text{Tc(IV)-DTPA}$ after 20 h, not $\text{TcO}_2 \cdot 2\text{H}_2\text{O}$ (Fig. 2; C),. And while the model predicted full Tc(IV) oxidation in aerated AGW, TLC data indicate that only 17% was oxidized over the same period (E1), and only 10% with aerated AGW in the presence of CN32 (E4).

The DTPA solution complexes listed above are predicted to be more stable than Fe(OH)_3 -DTPA surface complexes, even with a log K of binuclear ($n = 2$ in equation 3) adsorption approaching that of DTPA- Fe^{3+} complexation (equation 2). Surface species abundance is predicted to be $\equiv\text{FeOH} > \equiv\text{FeOH}_2^+ > \equiv\text{FeO}^- > \equiv\text{FeOHCa}^{2+} > \equiv\text{FeOHSO}_4^{2-} >> \equiv\text{Fe}_2\text{HDTPA}^{2-}$ (where $\equiv\text{Fe}$ represents a surface-bound Fe site). The log K of equation 3 needs to be at least 30 for the model to predict appreciable DTPA surface complexation. Not surprisingly, mononuclear adsorption ($n = 1$ in equation 3) simulations predict greater adsorption of DTPA than if the mode of binding is binuclear—complete adsorption if log $K = 30$ and 2% if log $K = 20$. The thermodynamic database did not contain reactions involving more complicated surface complexes such as DTPA co-adsorbing with metals (e.g., $\equiv\text{FeOHCaDTPA}$).

4. Discussion

Near full recovery of the Tc(IV)DTPA in the foil covered aerobic microbial reactors (E4) indicates that aerobically respiring *S. putrefaciens* CN32 did not degrade DTPA. It should be noted, however, that DTPA can degrade over much longer time periods (Means et al., 1980; Sykora et al., 2001), and that degradation can be photolytically accelerated (Metsarinne et al., 2004).

Model predictions indicate that aqueous-phase ligand exchange between $^{99\text{m}}\text{Tc}$ and other metals such as Fe^{3+} , Ca^{2+} , or Mg^{2+} for DTPA could destabilize $^{99\text{m}}\text{Tc-DTPA}$ complex under experimental conditions. However, on the basis of speciation measurements, after 20 h, only

17% of the approximately 5×10^{-11} M Tc(IV) was oxidized to TcO_4^- in oxygenated AGW, leaving 83% of the Tc bound to DTPA. In addition, no $\text{TcO}_2 \cdot 2\text{H}_2\text{O}$ was detected in anoxic AGW, as would be expected if cations displaced Tc(IV) from DTPA. These two findings suggest that use of the Tc-DTPA stability constant of Levin et al. (1980) does not adequately describe the behavior in our system, potentially because of the much lower Tc/DTPA ratios.

Comparison of abiotic systems with and without Al-ferrihydrite-coated sand (C and E3), indicates that Al/Fe hydroxide surfaces can profoundly affect the stability of $^{99\text{m}}\text{Tc}$ -DTPA. The presence of these surfaces caused the release of Tc(IV) from the DTPA complex and subsequent oxidation of Tc(IV). This transformation does not occur under Fe(III)-reducing conditions in the presence of *S. putrefaciens* CN32 (E5 and E7). Like the adsorption of EDTA onto Fe(III) oxyhydroxide (Noren et al., 2009), the adsorption of DTPA is likely impacted by electrostatic forces. At pH 7.1, surface complexation modeling indicates that bare surfaces of the Al-ferrihydrite sand coatings are likely to be neutral or singularly positively charged in AGW. By contrast, bacterial cell surfaces are predominantly negatively charged at this pH (Fein et al., 1997; Sokolov et al., 2001). *S. putrefaciens* CN32 are known to extensively adhere to metal-oxide surfaces (Bonneville et al., 2006; Lower et al., 2001). As a result, Al-ferrihydrite sand coatings covered with microbial biomass are expected to be predominantly negatively charged at pH 7.1, resulting in repulsive electrostatic forces toward $^{99\text{m}}\text{Tc}$ -DTPA. Our prior work (Vandehey et al., 2012) showed that $^{99\text{m}}\text{Tc}$ -DTPA is stable when iron-sulfides form in subsurface sediment due to biological processes, supporting the interpretation of electrostatic governance of DTPA-surface interaction, as iron sulfide surfaces are also negatively charged at pH 7.1 (Rickard and Luther, 2007).

We speculate that adsorption of Ca^{2+} and Mg^{2+} onto Al-ferrihydrite, as suggested by thermodynamic modeling, enriches Al/Fe (hydr)oxide sites with cations that promote DTPA adsorption, in that the surface sites provides a more chemically reactive environment where the

rate of ligand exchange of DTPA from Tc(IV) to Ca^{2+} , Mg^{2+} , or H^+ is much higher than in the bulk solution. This could also be true for Ca^{2+} and Mg^{2+} ions sorbed to the surface of microbial cells.

In addition, multidentate ligands like EDTA and DTPA probably form inner-sphere complexes with metal (hydr)oxides; such complexes are simply spectroscopically undetectable at radiotracer levels (Nowack and Sigg, 1996). Inner-sphere complexes could promote dissolution of Fe (hydr)oxides (Johnson et al., 2005; Stumm, 1996), releasing enough dissolved Fe(III) to form $\text{FeH}_x\text{DTPA}^{(2-x)-}$ complexes. Ternary complexes between metal-DTPA adsorbates and Fe (hydr)oxide surfaces (e.g., $>\text{FeO-TcDTPA}$) can form at neutral-to-alkaline pH and higher ionic strength (0.1 M) and precede dissolution of Fe and formation of dissolved Fe-DTPA complexes (Noren et al., 2009; Nowack and Sigg, 1996).

Furthermore, the reduction of reactive Fe(III) surface sites by CN32 and subsequent buildup of Fe(II) may have impacted the reactivity Al-ferrihydrite-coated sand and therefore the degree to which $^{99\text{m}}\text{Tc-DTPA}$ was destabilized. It has been previously shown that ferrihydrite with 6% Al substitution for Fe completely recrystallized to less bio-reactive, more thermodynamically stable lepidocrocite and goethite after six days in contact with 200 μM Fe(II) (Hansel et al., 2011), in accordance with other work (Masue-Slowey et al., 2011). At 13% Al content, 60% ferrihydrite transformed (Hansel et al., 2011). Similar conditions in this study—nearly 3 days of anaerobic Fe respiration by CN32 of 10% Al-substituted ferrihydrite, producing 40 to 200 μM Fe(II)—raises the likelihood that the Al-ferrihydrite-coated sand in E5 and E7 had fewer reactive Fe sites than that in the purely abiotic system (E2). Along with electrostatic forces, microbial diminishing of the chemical reactivity of Fe helps explain why $^{99\text{m}}\text{Tc-DTPA}$ was stable in the presence of Al-ferrihydrite-coated sand under Fe(III)-reducing conditions. These factors are also consistent with observations by Vandehey et al. (2012) that 10% of added $^{99\text{m}}\text{Tc-DTPA}$ associated with Rifle Colorado sediments under aerobic conditions, while none associated under Fe(III)-reducing conditions. Given the extremely low concentrations of $^{99\text{m}}\text{Tc-DTPA}$ in both studies,

only a fraction of the most reactive surface sites appear to be capable of dissociating Tc(IV) from DTPA. Given enough microbial activity, these sites can be readily reduced to Fe(II) or transformed to a lower energy state, eliminating the interaction between ^{99m}Tc -DTPA and Fe (hydr)oxides. The explanation that the ^{99m}Tc -DTPA is only destabilized by the presence of a small number of highly reactive surface sites, not only gives confidence for its performance under other conditions but raises the intriguing possibility that ^{99m}Tc -DTPA could be used to track the quantity of these highly reactive sites under varying geochemical conditions.

5. Conclusion

The hypotheses of this study were designed to better understand the conditions under which ^{99m}Tc -DTPA is suitable as a non-reactive tracer for hydrogeological imaging studies. On the timescale of an experiment determined by the useable lifetime of ^{99m}Tc (up to ~48 h), our findings indicate that oxidative transformation (by O_2 or Cr(VI)) can occur, but to a limited extent (perhaps up to 20%). We found no evidence that DTPA was microbially degraded under either aerobic or Fe(III)-reducing conditions. We provide compelling evidence that fresh Al-ferrihydrite sand coatings displaced the ^{99m}Tc from the ^{99m}Tc -DTPA complex, resulting in the oxidation of Tc(IV) to TcO_4^- , but that surface alteration by bacterial adsorption and solid-phase transformation under Fe(III)-reducing conditions dramatically decreases surface adsorption. There is some evidence that soluble Cr(VI) displaced ^{99m}Tc from the ^{99m}Tc -DTPA, as the addition of K_2CrO_4 resulted in a minor amount of additional oxidation of Tc(IV). While lower dissolved Fe(II) concentrations indicate Fe(II) oxidation by Cr(VI), which results in the formation of Cr(III)-Fe(III) (hydr)oxides, ^{99m}Tc -DTPA did not change. Because of the complexity of ^{99m}Tc -DTPA chemistry in geologic systems, we strongly recommend that the behavior of this radiotracer compound be evaluated before being deployed as a non-reactive tracer. Such evaluation should pay particular attention to redox status and to surface chemistry. Moreover,

the reactivity of ^{99m}Tc -DTPA raises the intriguing possibility of quantifying the most reactive surface sites within solid media at concentrations lower than can be measured in other ways. Finally, additional radiotracer development is recommended to expand the range of non-reactive radiotracers for biogeochemical experiments.

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6. References

1. Bonneville, S., Behrends, T., Cappellen, P.V., Hyacinthe, C., R'ling, W.F.M., 2006. Reduction of Fe(III) colloids by *Shewanella putrefaciens*: A kinetic model. *Geochimica et Cosmochimica Acta* 70, 5842-5854.
2. Boutchko, R., Rayz, V.L., Vandehey, N.T., O'Neil, J.P., Budinger, T.F., Nico, P.S., Druhan, J.L., Saloner, D.A., Gullberg, G.T., Moses, W.W., 2012. Imaging and modeling of flow in porous media using clinical nuclear emission tomography systems and computational fluid dynamics. *Journal of Applied Geophysics* 76, 74-81.
3. Dzombak, D.A., Morel, F.M.M., 1990. *Surface Complexation Modeling*. Wiley, New York.
4. Fein, J.B., Daughney, C.J., Yee, N., Davis, T.A., 1997. A chemical equilibrium model for metal adsorption onto bacterial surfaces. *Geochimica et Cosmochimica Acta* 61, 3319-3328.
5. Filius, J.D., Lumsdon, D.G., Meeussen, C.L., Hiemstra, T., Van Riemsdijk, W.H., 2000. Adsorption of fulvic acid on goethite. *Geochimica et Cosmochimica Acta* 64, 51-60.
6. Fredrickson, J.K., Zachara, J.M., Kennedy, D.W., Kukkadapu, R.K., McKinley, J.P., Heald, S.M., Liu, C.X., Plymale, A.E., 2004. Reduction of TcO_4^- by sediment-associated biogenic Fe(II). *Geochimica et Cosmochimica Acta* 68, 3171-3187.
7. Granéli, W., Granéli, E., 1991. Automatic potentiometric determination of dissolved oxygen. *Marine Biology* 108, 341-348.
8. Hansel, C.M., Learman, D.R., Lentini, C.J., Ekstrom, E.B., 2011. Effect of adsorbed and substituted Al on Fe(II)-induced mineralization pathways of ferrihydrite. *Geochimica et Cosmochimica Acta* 75, 4653-4666.

9. Holzbecher, E., Knappe, A., Pekdeger, A., 2005. Identification of degradation characteristics - exemplified by Gd-DTPA in a large experimental column. *Environmental Modeling & Assessment* 10, 1-8.
10. Johnson, S.B., Yoon, T.H., Slowey, A.J., Brown, G.E., Jr., 2005. Adsorption of organic matter at mineral/water interfaces: 3. Implications of surface dissolution for adsorption of oxalate. *Langmuir* 20, 11480-11492.
11. Jurisson, S., Gawenis, J., Landa, E.R., 2004. Sorption of Tc-99m radiopharmaceutical compounds by soils. *Health Phys.* 87, 423-428.
12. Kinsella, K., Schlyer, D.J., Fowler, J.S., Martinez, R.J., Sobecky, P.A., 2012. Evaluation of positron emission tomography as a method to visualize subsurface microbial processes. *Journal of Hazardous materials* 213, 498-501.
13. Knol, 2008. *Journal of Neuroscience Methods* 168, 342-357.
14. Környei, J., Zolle, I., 2007. Technetium-99m Pharmaceuticals, in: Zolle, I. (Ed.). Springer, Berlin, pp. 297-208.
15. Lear, G., McBeth, J.M., Boothman, C., Gunning, D.J., Ellis, B.L., Lawson, R.S., Morris, K., Burke, I.T., Bryan, N.D., Brown, A.P., Livens, F.R., Lloyd, J.R., 2010. Probing the Biogeochemical Behavior of Technetium Using a Novel Nuclear Imaging Approach. *Environmental Science & Technology* 44, 156-162.
16. Levin, V.I., Gracheva, M.A., Ilyushchenko, O.N., 1980. The stability constant of ^{99m}Tc-DTPA complex. *International Journal of Applied Radiation & Isotopes* 31, 382-385.
17. Liu, C.X., Gorby, Y.A., Zachara, J.M., Fredrickson, J.K., Brown, C.F., 2002. Reduction kinetics of Fe(III), Co(III), U(VI) Cr(VI) and Tc(VII) in cultures of dissimilatory metal-reducing bacteria. *Biotechnology and Bioengineering* 80, 637-649.
18. Lower, S., Hochella, M., Beveridge, T., 2001. Bacterial recognition of mineral surfaces: Nanoscale interactions between *Shewanella* and alpha-FeOOH. *Science* 292, 1360-1363.
19. Marshall, M.J., Plymale, A.E., Kennedy, D.W., Shi, L., Wang, Z., Reed, S.B., Dohnalkova, A.C., Simonson, C.J., Liu, C., Saffarini, D.A., Romine, M.F., Zachara, J.M., Beliaev, A.S., Fredrickson, J.K., 2008. Hydrogenase- and outer membrane c-type cytochrome-facilitated reduction of technetium(VII) by *Shewanella oneidensis* MR-1. *Environmental Microbiology* 10, 125-136.
20. Masue-Slowey, Y., Loeppert, R.H., Fendorf, S., 2011. Alteration of ferrihydrite reductive dissolution and transformation by adsorbed As and structural Al: Implications for As retention. *Geochim. Cosmochim. Acta* 75, 870-886.
21. Means, J.L., Kucak, T., Crerar, D.A., 1980. Relative degradation rates of NTA, EDTA and DTPA and environmental implications. *Environmental Pollution Series B-Chemical and Physical* 1, 45-60.
22. Metsarinne, S., Rantanen, P., Aksela, R., Tuhkanen, T., 2004. Biological and photochemical degradation rates of diethylenetriaminepentaacetic acid (DTPA) in the presence and absence of Fe(III). *Chemosphere* 55, 379-388.
23. Noren, K., Loring, J.S., Bargar, J.R., Persson, P., 2009. Adsorption Mechanisms of EDTA at the Water-Iron Oxide Interface: Implications for Dissolution. *Journal of Physical Chemistry C* 113, 7762-7771.
24. Nowack, B., Sigg, L., 1996. Adsorption of EDTA and metal-EDTA complexes onto goethite. *Journal of Colloid and Interface Science* 177, 106-121.
25. Plymale, A.E., Fredrickson, J.K., Zachara, J.M., Dohnalkova, A.C., Heald, S.M., Moore, D.A., Kennedy, D.W., Marshall, M.J., Wang, C., Resch, C.T., Nachimuthu, P., 2011. Competitive Reduction of Pertechetate ((99)TcO(4)(-)) by Dissimilatory Metal Reducing Bacteria and Biogenic Fe(II). *Environmental Science & Technology* 45, 951-957.
26. Rard, J.A., Rand, M.H., Anderegg, G., Wanner, H., 1999. Chemical Thermodynamics of Technetium, in: Sandino, M.C.A., Osthols, E. (Eds.). Elsevier, Amsterdam.
27. Rickard, D., Luther, G.W., 2007. Chemistry of iron sulfides. *Chemical Reviews* 107, 514-562.
28. Sokolov, I., Smith, D.S., Henderson, G.S., Gorby, Y.A., Ferris, F.G., 2001. Cell surface electrochemical heterogeneity of the Fe(III)-reducing bacteria *Shewanella putrefaciens*. *Environmental Science & Technology* 35, 341-347.
29. Stookey, L.L., 1970. Ferrozine-A new spectrophotometric reagent for iron. *Analytical Chemistry* 42, 779-781.
30. Stumm, W., 1996. Reactivity at the mineral-water interface: dissolution and inhibition. *Colloids and Surfaces A: Physicochem. Eng. Aspects* 120, 143-166.

- 473 31. Sykora, V., Pitter, P., Bittnerova, I., Lederer, T., 2001. Biodegradability of ethylenediamine-based
474 complexing agents. *Water Research* 35, 2010-2016.
- 475 32. Vandegaer, J., Charberek, S., Frost, A.E., 1959. Iron chelates of diethylenetriaminepenta-acetic acid
476 (DTPA). *Journal of Inorganic and Nuclear Chemistry* 11, 210-221.
- 477 33. Vandehey, N.T., O'Neil, J.P., Slowey, A.J., Boutchko, R., Druhan, J.L., Moses, W.W., Nico, P.S.,
478 2012. Monitoring Tc Dynamics in a Bioreduced Sediment: An Investigation with Gamma Camera
479 Imaging of ^{99m}Tc-Pertechnetate and ^{99m}Tc-DTPA. *Environmental Science & Technology* 46,
480 12583-12590.
- 481 34. Werth, C.J., Zhang, C.Y., Brusseau, M.L., Oostrom, M., Baumann, T., 2010. A review of non-invasive
482 imaging methods and applications in contaminant hydrogeology research. *Journal of Contaminant*
483 *Hydrology* 113, 1-24.
- 484 35. Wildung, R.E., Gorby, Y.A., Krupka, K.M., Hess, N.J., Li, S.W., Plymale, A.E., McKinley, J.P.,
485 Fredrickson, J.K., 2000. Effect of electron donor and solution chemistry on products of dissimilatory
486 reduction of technetium by *Shewanella putrefaciens*. *Applied and Environmental Microbiology* 66,
487 2451-2460.
- 488 36.
- 489
- 490

Table 1. Summary of experimental conditions and hypotheses tested and ancillary results. All batch experiments were done in triplicate.

Experiment	C	E1	E2	E3	E4	E5	E6	E7
<i>S. putrefaciens</i> CN32	-	-	-	-	+	+	-	+
O ₂	-	+	-	+	+	-	+	-
Fe-Sand	-	-	+	+	-	+	-	+
Cr(VI)	-	-	-	-	-	-	+	+
Hypothesis tested	control	1	3	3	2a	2b	4	5
Results								
<i>Redox status</i>								
O ₂ (μM) (day4) ^a	(16) ^a	310			290	<16		<16
Fe(II) (μM)								
Before adding Tc (day3)						47.8		42.5
Shortly after TLC (day4)						194		60.7
<i>Phase distribution of Tc</i>								
dissolved (%) ^b			85 ± 13	98 ± 1		89 ± 15		98 ± 1

^aQuantification limit of Winkler assay: 16 μM; fully aerated AGW contained 280 μM O₂

^bdissolved Tc = $\gamma_{\text{liquid}} + \text{rinse 1} + \gamma_{\text{rinse 2}} + \text{rinsed sand} + \gamma_{\text{liquid}} + \text{rinse 1} + \gamma_{\text{rinse 2}}$, where γ is the gamma intensity; due to a small amount of particles in liquid samples, these values likely slightly overestimate the amount of dissolved Tc

Control (C)
9.5% TcO₄⁻
90.5% Tc-DTPA

Exp. 3 (E3)
90% TcO₄⁻
10% Tc-DTPA

TLC Location & Quantification:

	Acetone	Saline	Calculation (x100%)
TcO ₄ ⁻	Front	Front	$A_{SF}/(A_{SF}+A_O)$
Colloidal	Origin	Origin	$S_O/(S_{SF}+S_O)$
DTPA	Origin	Front	100% - (TcO ₄ ⁻ + Colloidal)

Legend:

- ① A_O: Acetone Origin
- ② A_{SF}: Acetone Solvent Front
- ③ S_O: Saline Origin
- ④ S_{SF}: Saline Solvent Front

Figure 1. Representative TLC separations: Control experiment (C) and Experiment E3. Calculation results are shown above images. (Left) Table describing location of tracer with each solvent and calculation used to determine purity percentage. (Right)

Figure 2. Speciation results of ^{99m}Tc on the basis of thin layer chromatography of the unfiltered liquid phase of the batch systems. The horizontal labels indicate system number (see Table 1) and the error bars represent one standard deviation from the mean of three replicate vials.

Figure 3. Apparent solubility of Fe in an FeCl_3 in aerated artificial groundwater at pH 7.1 as a function of time. 10 mM PIPES with and 5 μM lactate present