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Investigation of Technetium Redox Cycling in FRC Background Sediments using EXAFS and Gamma Camera Imaging

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Introduction

Technetium-99 is a priority pollutant at numerous DOE sites, due to its long half-life (2.1 x 105 years), high mobility as Tc(VII) in oxic waters, and bioavailability as a sulfate analogue. ^{99}Tc is far less mobile under anaerobic conditions, forming insoluble Tc(IV) precipitates. As anaerobic microorganisms can reduce soluble Tc(VII) to insoluble Tc(IV), microbial metabolism may have the potential to treat sediments and waters contaminated with Tc. Baseline studies of fundamental mechanisms of Tc(VII) bioreduction and precipitation (reviewed by Lloyd et al., 2005, in press) have generally used pure cultures of metal-reducing bacteria, in order to develop conceptual models for the biogeochemical cycling of ^{99}Tc. There is, however, comparatively little known about interactions of metal-reducing bacteria with environmentally relevant trace concentrations of ^{99}Tc, against a more complex biogeochemical background provided by mixed microbial communities in aquifer sediments.

Aims and Objectives

The objective of this project is to probe the site specific biogeochemical conditions that control the mobility of ^{99}Tc at the US DOE Field Research Center Site (FRC; Oakridge, Tennessee). This information is required for the rational design of in situ bioremediation strategies for technetium-contaminated subsurface environments. We are using a combination of geochemical, mineralogical, microbiological and spectroscopic techniques to determine the solubility and phase associations of ^{99}Tc in FRC sediments, and characterize the underpinning biogeochemical controls.

Hypotheses

1. Tc(VII) will be reduced and precipitated in FRC sediments under anaerobic conditions in batch experiments (progressive microcosms).
2. The presence of added nitrate effects the rate of reduction of both Fe(III) oxyhydroxides and Tc(VII)
3. The mineral form of reduced Tc(VII) can be determined using X-ray spectroscopic techniques (Extended X-ray Absorption Fine Structure spectroscopy, EXAFS)
4. Sediment-bound reduced ^{99}Tc can be solubilized by perturbations including oxidation by nitrate.

Experimental

- Sediment and groundwater from FRC background area, with and without added electron donor (acetate) and added nitrate (at either 10 or 100 mM) were set up in progressive microcosms and columns, under anaerobic conditions, 20°C and in the dark.
- A spike of ^{99}Tc was added to progressive microcosms for an initial concentration of 0.5 μM (20 Bq ml-1).
- Parameters monitored: ^{99}Tc solubility, nitrate, nitrite, Fe(II), sulfate, acetate, pH, Eh, mineral phases, MPN counts for key anaerobic bacteria and culture dependent analysis of microbial communities.
- EXAFS analysis of FRC sediments containing 100 ppm of reduced ^{99}Tc.
- Columns and selected progressive microcosms were amended with 10 MBq of ^{99m}Tc (half-life 6.5 hrs) and imaged hourly with a gamma camera.

Results

Progressive Microcosms

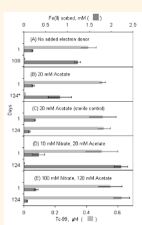


Figure 1. Bar graph showing the change in sorbed Fe(II) and ^{99}Tc concentrations over the course of a 124 day incubation.

^{99}Tc was added to progressive microcosms to ~0.5 μM (20 Bq ml^{-1}) and incubated for 124 days (Figure 1). Under conditions of (A) no added electron acceptor, (B) 20 mM Acetate, (C) 20 mM Acetate (sterile sample), and (D)10 mM Nitrate/20 mM Acetate we observed an increase in sorbed Fe(II) and decrease in aqueous ^{99}Tc concentrations relative to sterile controls (not shown). In (E), 100 mM Nitrate/120 mM Acetate, no significant change was observed in the sorbed Fe(II) concentration or in ^{99}Tc over the period of the experiment. Heat sterilized controls spiked with 0.5 μM ^{99}Tc did not show the same biologically mediated decrease in Tc concentration and increase in Fe(II) concentration with time.

Microbiological Analyses

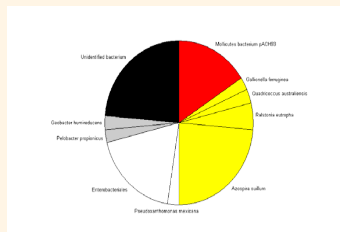


Figure 2. Composition of bacterial communities isolated from background sediment:
( ) β-proteobacteria; (□) γ-proteobacteria; ( ) δ-proteobacteria; (■) molicute; (■) unidentified isolate. Taxonomic units determined using restriction-fragment-length-polymorphism (RFLP) profiles of 38 clones.

Analysis of the sediment microbial community (Figure 2), assessed using clone libraries of the 16S rDNA fragments, revealed a significant population of organisms affiliated with the enterobacteria group (25 %), within background sediments, including uncultured rumen bacterium (clone F24-H06), and other bacteria with enteric associations. Mollicutes also comprised a significant (16 %) portion of the microbial community. Close relatives to the Fe(III)-reducing bacteria *Geobacter humireducens* and Fe(II)-oxidising bacteria *Gallionella ferruginea* were present within the site, as well as the nitrogen-fixing bacterium *Azospira suillum.* The community also included a large number of bacteria which could not be accurately identified to genus level.

Microbiological analyses of the progressive microcosm experiments also showed the presence of an Fe(III)-reducing bacterium known to reduce Fe(III) in axenic culture (*Geothrix* species). Nitrate reducing bacteria were also detected in enrichment cultures (including *Azoarcus* species), and present at higher concentrations than Fe(III)-reducing bacteria in parallel enrichments.

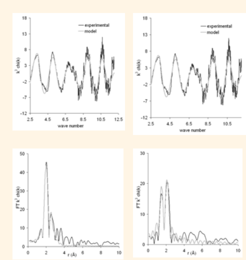
EXAFS Analyses

Table 1. EXAFS fits for reduction and reoxidation experiments. (i) Fe(III)-reducing FRC sediment amended with 550 μM TcO_4^-; (ii) Fe(III)-reducing FRC sediment amended with 550 μM TcO_4^-, then subject to nitrate reoxidation with 100 mM nitrate. N is the occupancy (± 25%), r is the interatomic distance (± 0.02 Å for the first shell, ± 0.05 Å for outer shells), $2\sigma^2$ is the Debye-Waller factor (± 25%), and R is the least squares residual.

Scatterer	N	r (Å)	$2\sigma^2$ (Å^2)	R
(i) O	1	1.732	0.015	38.02
O	5	1.998	0.009	
Tc	1	2.512	0.008	
(ii) O	2	1.709	0.006	68.63
O	4	1.996	0.013	

X-ray absorption spectroscopy was conducted to determine the oxidation state and mineral form of the reduced technetium in our samples. Data for samples of Fe(III)-reducing FRC sediments spiked with 100 ppm ^{99}Tc (Table 1, Figure 3) show that the dominant form of ^{99}Tc in the sediments is TcO_2, with some crystalline pertechnetate present.

Reoxidation of immobilized, reduced ^{99}Tc was assessed using nitrate as an oxidant. Reduced samples containing 20 mM acetate were used for the experiments. 100 mM nitrate oxidation of reduced samples resulted in approximately 30% solubilization of reduced technetium, presumably as reoxidized Tc(VII). Although this implies a level of resistance to remobilization, these results showed more solubilization of ^{99}Tc than in analogous experiments on estuarine sediments (2 % solubilization; Burke et al., submitted). Further characterization is necessary to confirm and delineate the nitrate reoxidation experimental evidence.



(A) Reduced FRC sediments

(B) 100 mM NO_3^- reoxidized FRC sediments

Figure 3. EXAFS and Fourier Transform for reduced FRC sediment and 100 mM nitrate reoxidized sample

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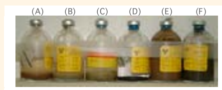
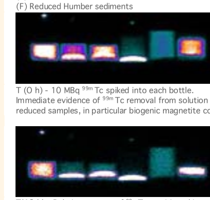
Gamma Camera Imaging



Samples:
(A) Nitrate oxidized background FRC sediments
(B) Reduced background FRC sediments
(C) Reduced background FRC sediments
(D) Biogenic magnetite control
(E) Air oxidized Humber sediments
(F) Reduced Humber sediments



T (0 h) - 10 MBq ^{99m}Tc spiked into each bottle. Immediate evidence of ^{99m}Tc removal from solution in all reduced samples, in particular biogenic magnetite control

T(12 h) - Relative amount of ^{99m}Tc partitioned into sediments has increased in all reduced samples. Oxic controls do not show any partitioning of ^{99m}Tc into sediment phase.

Figure 4. Proof of concept gamma camera experiment.

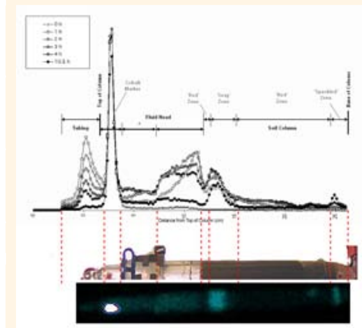


Figure 5. Gamma camera imaging of reduced column of FRC background area sediments. X axis is distance of reading from top of glass column; y axis is magnitude of counts detected (not normalized); * refers to area of column exhibiting lower than expected counts, as a result of shielding of the column by metal clamp. Below counts display is an image of the column and the gamma camera image taken at 10.5 hours.

10 MBq of ^{99m}Tc (a short-lived gamma emitter; half-life 6 hours) was added to each of six progressive microcosm experiments (Figure 4). The samples were shaken, and the partitioning of the ^{99m}Tc between the fluid and sediment phases was observed over a 12 h period. ^{99m}Tc moved into the sediment phase in all samples that containing Fe(III)-reducing sediments, and remained in the fluid phase in oxic controls.

We also conducted column studies (Figure 5) to investigate the behaviour of Tc traveling through spatially separated biogeochemical zones. Mobility of the Tc was monitored in real time using the gamma camera. Counts were taken of ^{99m}Tc decay (14.1 MBq spike) within a glass column containing zoned oxidized and Fe(III)-reducing FRC background area sediment (Flow rate = 7 ml h^{-1}, column internal diameter = 25 mm) hourly over a 10.5 h period. ^{99m}Tc concentrated in regions identified as Fe(III) reducing.

Conclusions

- ^{99}Tc reduction in FRC background area sediments is associated with Fe(III)-reducing conditions.
- The proportion of Fe(III)-reducing bacteria isolated from these sediments is low, and it is unsurprising given this evidence that the rate of iron reduction in these sediments is slow.
- EXAFS analyses of reduced FRC background sediments indicates that pertechnetate is reduced to TcO_2 in Fe(III)-reducing background sediments, and that upon addition of 100 mM nitrate the ^{99}Tc remains partially immobilized as mixed Tc(IV) and Tc(VII) phases.
- Experiments using gamma camera imaging indicate that ^{99m}Tc labelled pertechnetate is reduced and immobilized very rapidly in zones of Fe(III)-reduction in stratified columns. Abiotic reduction of Tc(VII) to Tc(IV) by Fe(II) is implicated.

Future Directions

- We are currently undertaking additional progressive microcosm experiments and EXAFS analyses using sediments and groundwaters from both FRC background and contaminated areas, to address the effect of nitrate on reduction rates in FRC sediments, as well as reoxidation behaviour of ^{99}Tc in FRC sediments. We will use a multidisciplinary approach to identify the biogeochemical controls on ^{99}Tc solubility.
- Further Gamma camera imaging of columns of FRC background and contaminated sediments, including high nitrate low pH sediments. We will characterize the geochemistry, microbiology, and mineralogy of zones where ^{99}Tc is immobilized. We will also explore reoxidation behaviour of reduced columns using this technique.

Acknowledgements

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