

Biogeochemical Activities and Microbial Community Diversity associated with Hanford 300 Area Subsurface Sediments

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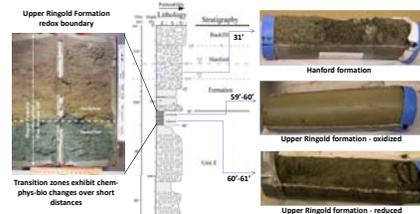
Introduction and Objective

This project is characterizing biogeochemical processes in Hanford 300 Area subsurface sediments with an emphasis on defining redox reactions at the pore scale and across transition zones projected to influence the subsurface fate and transport of U and Tc. In coordination with the Hanford Integrated Field Research Challenge (IFRC), a series of subsurface core samples were collected during July 2008 from the 300A IFRC site as part of site characterization efforts and a series of sediment microcosm experiments initiated to explore biogeochemical processes that may impact contaminant fate and transport at this site.

Motivation

- Tc & U are major risk-driving contaminants at Hanford (and other DOE sites) due to their relatively high mobility in oxic groundwaters and the difficulty in predicting their subsurface behavior. Both contaminants are subject to microbial and geochemical redox and complexation reactions that can alter their solubility and hence transport in the subsurface.
- Microenvironments and transition zones can exert significant control over contaminant behavior, often disproportionate to their mass, and are common features of the Hanford subsurface.
- Nature and distribution of biogeochemical processes in the poorly-understood Hanford subsurface. Potential for native microbes to utilize various terminal electron acceptors (EAs: oxygen; nitrate; ferric iron; sulfate etc.) common to the Hanford unconfined aquifer.

Hanford 300A IFRC Site Subsurface Sediments



Sediment Properties

Sample	Extractable Metals (µmols/g)		
	0.5N HCl extraction	DCB extraction	0.5N HCl extraction (wet)
Hanford formation (<4.75 mm)	Fe: 48.8 ± 3.7 Al: 31.8 ± 0.9 Mn: 1.5 ± 0.2	DCB extraction: 49.1 ± 5.1 51.3 ± 6.4 12.8 ± 0.2	0.5N HCl extraction (wet): 9.7 ± 0.7 33.0 ± 1.0 2.3 ± 0.2
Ringold oxidized (unsieved)	Fe(II): 28.5 ± 3.1 Fe: 34.2 ± 2.8 Al: 55.1 ± 1.8 Mn: 3.0 ± 1.0	53.9 ± 3.7 58.8 ± 5.1 8.8 ± 0.8 1.9 ± 0.1	3.2 ± 0.9 24.7 ± 1.5 40.2 ± 2.2 3.5 ± 0.5
Ringold reduced (unsieved)	Fe(II): 47.4 ± 2.9 Fe: 55.0 ± 2.1 Al: 58.7 ± 1.1 Mn: 0.9 ± 0.0	30.7 ± 0.7 29.9 ± 1.4 7.9 ± 0.2 0.2 ± 0.0	34.7 ± 1.0 40.5 ± 2.5 47.4 ± 1.1 1.3 ± 0.0

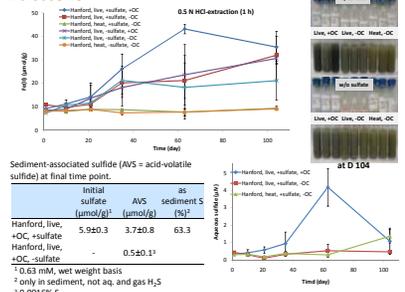
Sample	Sieved	Inorganic C (%)	Organic C (%)	Total Fe (%)	Total Fe (mmol/g)
Hanford	< 0.5 mm	0.03	0.09	7.16	1.279
Ringold oxidized < 0.5 mm	< 0.5 mm	0.06	0.08	3.61	0.645
Ringold reduced < 0.5 mm	< 0.5 mm	<0.02	0.09	3.79	0.677

- All sediments are mineral, <0.1% organic carbon, and high in total iron.
- Weak (0.5 N HCl) acid-extractable Fe(II) amounts: Ringold red > Hanford fines > Ringold oxic.
- Fe(II) from phyllosilicates as implied by the weak acid-extractable Fe and Al at 1 h and 24 h extraction times.

Sediment Microcosms

- Hanford subsurface sediments (1 gram) were incubated in the synthetic groundwater media (10 mL) (SGW1, pH 7.8; formulated based on 300A groundwater geochemistry) with and without exogenous electron acceptors or donors.
- Incubation conditions: static; 22°C; electron donor mix (0.9 mM acetate, 0.6 mM lactate, and 0.3 mM glucose) or H₂.
- Sterile controls: heat-treated (100°C exposure for 15 min, x3) or autoclaved (121°C for 20 min, x3) on three consecutive days.

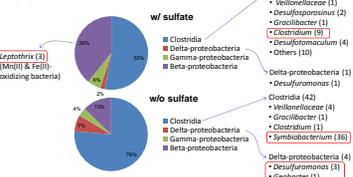
Native Fe & Sulfate Reduction in Hanford Sediment Microcosms



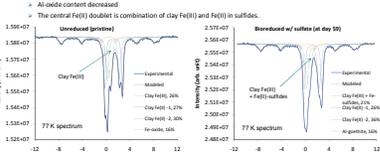
- Fe reduction rate slightly faster with sulfate than without, suggesting Fe(II) reduction facilitated by abiotic reaction with sulfate in addition to direct dissimilation of Fe(II).
- No apparent biological Fe reduction in Ringold oxidized or reduced sediments.
- Sulfate reduction detected by aqueous sulfide production and measurable acid-volatile sulfide (AVS).

- Microbial community analysis of bio-reduced Hanford formation sediments both with and without sulfate (day 104) by 16S rRNA gene library: total 60 colonies from each
 - SRB or sulfur-disproportionating bacteria more abundant and diverse in the tube w/sulfate (*Desulfosporosinus*, *Desulfotomaculum*, *Desulfuromonas* etc.) than in the microcosm w/o sulfate (*Desulfuromonas*)

- Most dominant bacteria (especially *Clostridia*) found in microcosms incubated w/ and w/o sulfate were minor in the 16S clones libraries from DNA extracted from fresh Hanford sediments (see Lin et al. poster).
- Microcosm libraries, however, share many similar sequences with the libraries prepared from fresh Hanford sediments.



- Mössbauer analysis (77-K spectra): Preliminary modeling of the unreduced (pristine) and bio-reduced Hanford formation sediment (w/sulfate; microcosms at day 59) indicate
 - Pristine sediment: Fe-oxides (10%) and Fe(II)/Fe(III)-containing clays (84%) – spectral area ratio ~1/3%
 - At aqueous (pore) is the predominant Fe-oxide (minor oxidant; magnetite and hematite – based on spectra obtained at various temperatures; rigorous modeling is in progress)
 - Two types of clay (Fe): clay Fe(II)/Fe(III) ratio is 2.2
 - Bio-reduced sediment:
 - Clay Fe(II) increased
 - Al-oxide content decreased
 - The central Fe(II) doublet is combination of clay Fe(II) and Fe(II) in sulfide.



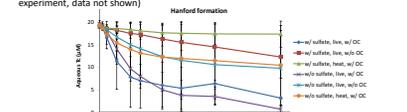
U(VI) Behavior in Sediment Microcosms

- Microbial U(VI) reduction up to 60 µM in Hanford formation sediments amended with electron donor (OC mix) upon extended incubation, with or without sulfate.
- Initial rapid loss of U(VI) from solution in Ringold oxic/red sediments suggests adsorption (data not shown).



⁹⁹Tc(VII)O₄ Behavior in Sediment Microcosms

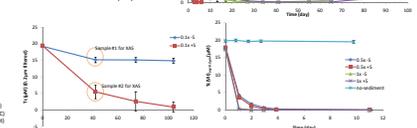
- Tc(VII) is reduced in Hanford formation sediment regardless of OC added, and in the heated sediments (with varying rates) indicating abiotic redox reaction, possibly with native Fe(II).
- Sediment-incubations w/ sulfate a little slower in Tc reduction rather than those w/o sulfate
- No Tc reduction detected in live or autoclaved Ringold oxidized sediments (scoping experiment, data not shown)



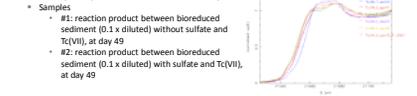
- Tc-XANES & EXAFS
 - Tc-K-edge XANES on the incubated Hanford formation sediments: All similar to the TcO₄⁻ standard regardless of treatment (live vs. autoclave; with vs. without sulfate)
 - Tc-K-edge EXAFS: different from 2nd shells of TcO₄⁻ and Tc-sediment samples but similar with predominantly Tc-Fe bonding, suggesting either mixed Tc-Tc and Tc-Fe bonding in 2nd shell

Abiotic Tc(VII) Reduction by Bio-reduced Hanford fm Sediments w/ and w/o Sulfate

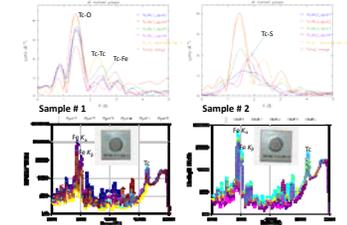
- Bio-reduced Hanford fm sediments (w/ and w/o sulfate) were pasteurized and frozen.
- The sediments were separated into fine fractions, and used with varying concentrations of the slurries to react with Tc(VII).



- Preliminary interpretations of XAS & MCA analyses
 - Sample #1: reaction product between bio-reduced sediment (0.1 x diluted) without sulfate and Tc(VII), at day 49
 - Sample #2: reaction product between bio-reduced sediment (0.1 x diluted) with sulfate and Tc(VII), at day 49



- XANES: Tc-O octahedra from #1, while another phase in varying amounts from #2
- EXAFS: Tc-Tc and Tc-Fe bonding for 2nd peaks from #1, while varying amounts of two different Tc environments for 1st peak (possibly Tc-S) from #2



Summary and Conclusions

- Fe(II), sulfate, and nitrate were reduced in Hanford formation sediments with or without electron donor amendment; potential for native organic substances as carbon and electron sources; Fe-mineral phase alteration – possible reduction of clay-associated Fe(II) (Mössbauer).
- Microbial community analysis from bio-reduced Hanford formation sediments: *Clostridia* and *β-Proteobacteria* commonly detected phylotypes; Sulfate-reducing and sulfur-disproportionating relatively abundant in the sediment incubated with sulfate, while *Symbiobacterium* and *δ-Proteobacteria* (*Geobacter* and *Desulfuromonas*) common in microcosms without sulfate.
- Uranium(VI) loss from solution observed upon extended incubation in Hanford formation sediment; rapid but limited loss of U(VI) from solution in upper Ringold sediments indicative of adsorption (data not shown).
- Tc(VII) reduced in anoxic Hanford formation sediments; abiotic reduction by native Fe(II) involved; Tc(VII) reduction by native Fe(II) also observed in Ringold reduced (see Peretyazhko poster), but not oxidized, sediment.
- Tc(VII) reduced by bio-reduced Hanford fm sediments: Tc-S bonding from preliminary EXAFS of the reaction product between bio-reduced sediment with sulfate and Tc(VII).

Future Research

- Explore in situ biogeochemical reactions and molecular microbiological phylogeny in 300A subsurface (see Fredrickson et al. IFRC poster)
- Bulk XANES/EXAFS analysis for molecular speciation of Tc (S. Heald) and U (K. Kemner/M. Boyanov)
- XMP & SEM microscale analyses of Tc chemistry, element & mineralogical investigations (S. Heald, J. McKinley) to identify reactive Fe(II) phase(s).
- Investigate reactivity of synthetic FeS and framboidal Fe(II) with Tc(VII) and characterization of the products.
- Determine susceptibility of reduced Fe(II) and Tc(VI) to oxidative remobilization by O₂ and nitrate/nitrite.



Research performed through PNNL's Scientific Focus Area (SFA) Supported by DOE Office of Science Office of Biological and Environmental Research (BER) Climate and Environmental Sciences Division (CESD)



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