INTEGRATED FIELD RESEARCH CHALLENGE SITE
Hanford 300 Area

U.S. Department of Energy Office of Science
Environmental Remediation Sciences Program

Biogeochemical Redox Transition with Depth in the Hanford 300 Area IFRC Subsurface

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Background & Motivation

Joint IFRC-SFA research results on the microbial ecology and biogeochemistry of Hanford formation subsurface sediments obtained during the first phase of drilling for well installation at the 300A IFRC revealed a relatively abundant, active, and phylogenetically diverse subsurface microbial community (see Lee et al. and Lin et al. PNNL SFA posters). Molecular analyses, enrichment cultures, and sediment microcosm experiments indicated that community structure and biogeochemical function shifted upon transition from the Hanford formation sand and gravels to the underlying fine-grained Ringold Formation (Unit E). Motivated by these results, a joint IFRC-SFA *in situ* microbial ecology-biogeochemistry experiment was initiated on October 22, 2009. The objective of this experiment was to probe, at finer spatial resolution and in greater detail, the changes in microbial community structure and function as well as Fe redox reactions with various iron-bearing mineral phases and sediments in relation to transitions in geochemical and hydrologic properties across the Hanford-Ringold textural and intra-Ringold redox boundaries.

Approach

Two IFRC wells (3-24 and 3-27) were selected as the screened interval for these extends into the reduced region of the Ringold E unit. Down-hole microcosm MLS units containing site sediments, Fe(III) oxides, basalt coupons, synthetic magnetite, bio-sep beads for microbial capture, an isolation-chip for *in situ* cultivation of Fe(III)-reducing and Fe(II)-oxidizing microorganisms and aqueous and gas phase diffusion cell samplers were deployed at three depths in wells 3-24 and 3-27 (Figure 1). The sampling depths included: i.) within the Hanford formation; ii.) above the redox transition zone in upper Ringold formation; and iii.) below the redox transition zone in the upper Ringold formation. The first set of samples was retrieved from well 3-24 on December 7, 2009 and the second set from well 3-27 on March 1, 2010.

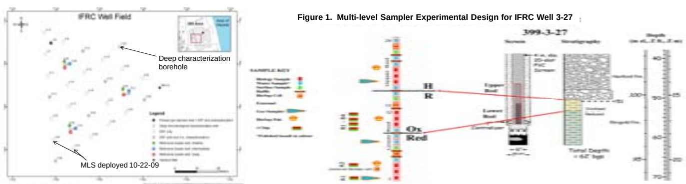


Figure 1. Multi-level Sampler Experimental Design for IFRC Well 3-27

Geochemistry

Groundwater analyses for major ions, pH, and dissolved gases provides a context for biological experiments and test the hypothesis that an aerobic-anaerobic transition zone is imposed across the lower Hanford gravels and the fine-grained upper Ringold Formation. Dissolved gas samplers (air-filled gas syringes) shown in photo on left were shipped to ORNL (B. Spalding& S. Brooks) for analyses immediately upon removal of the MLS. (*McKinley & Resch*).

BioSep Beads

BioSep bead "microbial traps" were deployed in mesh pouches attached to the outside of the support rods. These beads are being used to characterize the activity and phylogenetic composition and metabolic activities of microbial communities inhabiting different regions across the redox transition zone. Beads are also being used as inoculum for enrichment of specific microbial functional groups (Fe- and sulfate-reducing bacteria). Plain beads as well as those pre-imbibed with acetate, nitrate, or ferrihydrite were deployed (*Lin & Konopka; Marshall*).

Isolation-chips

Isolation chips (i-chips) for in situ microbial enrichments were attached by placement in perforated polyethylene centrifuge tubes on the outside of the MLS support rods. The source of microorganisms was groundwater collected from the same well (399-3-27) one week prior to MLS deployment. Before installation, i-chips were incubated in 50 ml Falcon tubes filled with anoxic, filtered groundwater. A1 and A2 (Fig. 1) were placed for cultivation/enrichment of Fe(III)-reducing microorganisms. B1 –B4 were placed in the oxidized Ringold for in situ cultivation/enrichment of Fe(II)-oxidizing microorganisms. Microorganisms were concentrated 10x by filtering groundwater through a 0.2 µm filter and then washing the filter in 1/10 of the initial volume of groundwater, or diluted 1/10 in filtered groundwater. (*Shelobolina & Roden*).

Fe Biogeochemical Transformations

Natural oxidized (RGO) and reduced (RGR) Ringold sediments (~18 g per cell) and ferrihydrite-coated sand (FHS, ~20 g per cell) were aseptically placed into individual MLS cells immediately prior to deployment. Following in situ incubation, materials were analyzed for Fe oxidation state, mineralogy, and redox reactivity. Microbial biomass concentration and population sizes of viable Fe(III)- and sulfate-reducing bacteria are also being determined. (*Lee and Fredrickson*).

Polished Basalt

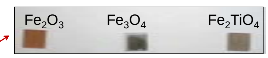
A standard thin section of reference Umtanum basalt was fragmented and distributed in groundwater sampling cells. Surfaces will be examined for weathering and microbial colonization. (*McKinley*).

Mixed Fe, Ti Oxide Thin Films and Particles

Sampling cells containing the following substrates were deployed in each of the three zones (Hanford, Ringold oxidized and Ringold reduced):

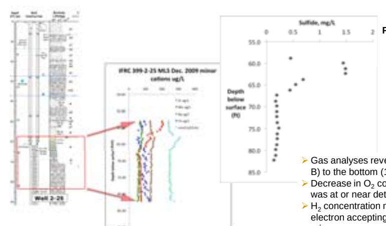
- Thin films (5mm) of Fe_3O_4 (100) and Fe_2TiO_4 (100) on $MgAl_2O_4$ (100) and Fe_2O_3 (0001) on Al_2O_3 (0001) deposited by pulsed laser deposition.
- 41-µm nylon filters containing synthetic $Fe_{3-x}Ti_xO_4$ (x = 0.15, 0.6 and 0.9) and a sample of the magnetic fraction of silty, fine sands from the Hanford 218-E-12B burial ground subsurface.

Thins films will be analyzed by XPS to assess changes in the chemistry and by AFM to image potential microbial activity on the surface. The nylon filters containing mixed Fe, Ti oxide particles will be incubated in 100X concentrated IFRC groundwater to stimulate growth of any microbes associated with the substrate . Changes in the chemistry of the particles will also be assessed. (*Pearce and Rosso*).

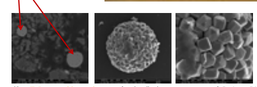


IFRC Geochemical Redox Transition

- MLS water samples from well 2-25 every 6", 1-month equilibration period.
- Redox interface within the fine-grained Ringold Unit E at ~60'.
- Dissolved Mn and Fe peak at/slightly above interface.
- Peak in aqueous sulfide concentration at the redox interface; sulfate 47-52 mg/L over same interval.
- Pyritic sulfur in the reduced region - 0.28% by weight & present as framboids (SEM); below detection in the oxidized region. Total organic C was 0.08% below the RG redox interface and <0.05% above it.

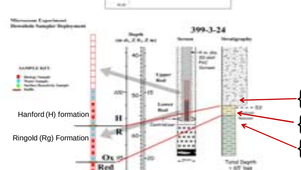


FeS_2 framboids



(See T. Peretyazhko et al. poster for details, images courtesy of B. Arey, PNNL)

- Gas analyses reveal redox transition trending from oxic in the lower Hanford formation (10A, B) to the bottom (1A, B) reduced fine-grained Ringold Unit E.
- Decrease in O_2 concurrent with increases in H_2, CO and CH_4 over the same interval (sulfide was at or near detection).
- H_2 concentration measured in the reduced Ringold consistent with CO_2 as the terminal electron accepting process (i.e., methanogenesis) in systems where organic matter is the primary energy source.
- Similar trends observed in well 3-27 although CH_4 was below detection and sulfide concentrations were 0.9-1.9 µM (0.03 – 0.06 mg/L) at and below the Ringold redox interface.

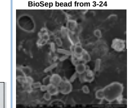


		Equilibrated Gas Phase				Calculated Solution Concentration			
Gas ID	Depth bgs feet	H_2 (ppmv)	O_2 (ppmv)	CO (ppmv)	CH_4 (ppmv)	H_2 nM	O_2 µM	CO nM	CH_4 nM
IFRC-MLS-3-24-10A	52.5	bd	178331.2	bd	bd	bd	266	bd	bd
IFRC-MLS-3-24-10B		bd	190346.4	bd	bd	bd	284	bd	bd
IFRC-MLS-3-24-4A	54.95	bd	1707.4	bd	bd	bd	2.5	bd	bd
IFRC-MLS-3-24-4B		0.52	2077.8	0.39	bd	0.42	3.1	0.4	bd
IFRC-MLS-3-24-1A	56.07	6.14	1861	0.22	326.8	5.01	2.8	0.2	540
IFRC-MLS-3-24-1B		7.49	1210.6	0.55	341.8	6.11	1.8	0.6	560
detection limits		0.08	903	0.13	300	0.11	1.3	0.1	490

* Thanks to Brian Spalding & Scott Brooks of ORNL for gas analyses.

Microbial Community Structure-Function

SEM image of colonized BioSep bead from 3-24

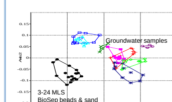


(Image courtesy of A. Dohnalkova)

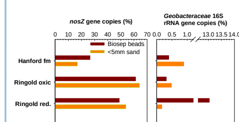
Most probable number (MPN) of Fe(III)- and sulfate-reducing bacteria

Samples	FeRB (MPN per g soil)	SRB (MPN per g soil)
RGR in Hf	7.09×10^2	7.09×10^0
RGO in Hf	8.90×10^2	
FHS in Hf	1.10×10^3	
RGR in Rg-ox	5.09×10^3	5.09×10^0
RGO in Rg-ox	1.18×10^3	
FHS in Rg-ox	1.01×10^4	1.01×10^1
RGR in Rg-red	1.36×10^4	
RGO in Rg-red	8.20×10^3	8.20×10^0
FHS in Rg-red	1.44×10^5	1.44×10^4

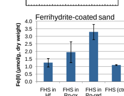
- Multiple materials (see experimental design) incubated in situ using the baffled MLS system in well 3-24 to isolate different regions of the aquifer to assess microbial community structure and biogeochemical function across the hydrological and geochemical transition zone.
- Materials colonized by the extant microbiota - biomass concentrations, determined by ATP assay, Picogreen binding of ds DNA, and qPCR of Bacterial 16S rRNA gene, varied among the different materials and between regions within the aquifer.
- Lowest biomass concentrations observed in the reduced region of the fine-grained Ringold which also contained the highest populations of viable Fe(III)- and sulfate-reducing bacteria.



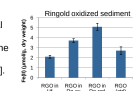
- Non-Metric Multidimensional Scaling (NMS or Bray-Curtis distance) and cluster analysis of TRFLP profiles of DNA revealed that variation in community composition within the different regions were greater than those between the two types of materials incubated within the same zone.
- TRFLP analyses of populations associated with groundwater samples collected from pumped IFRC wells were distinct from those colonizing BioSep beads or sand incubated within the well bore.



- Dissimilatory sulfite reductase (*dsrA*) was below detection in all samples while nitrous oxide reductase (*nosZ*) was relatively abundant in all 3-24 microcosm BioSep and sand samples. Nitrate concentrations in the MLS aqueous samples ranged from 22-28 mg/L.
- Results consistent with microcosm experiments where nitrate reduction was observed in Hanford and Ringold sediments (see Lee et al. poster) and detection of *nosZ* genes in sediment DNA extracts (see Lin et al. poster).
- Geobacteraceae were relatively abundant in the MLS microcosm samples with the highest abundance (13% relative to Bacterial 16S copy number) below the Ringold redox interface.



- Ferrihydrite-coated sand (76 µmol total Fe/g) incubated in situ in 3-24 was reduced to the greatest extent below the Ringold redox interface. [Hf: 51-52 ft.; Rg-ox: 53-54 ft.; Rg-red: 55-56 ft. bgs].



- Ringold sediment from above the redox transition zone (37 µmol/g DCB-extractable Fe) incubated in situ in 3-24 was also reduced to the greatest extent below the Ringold redox interface.

Conclusions

- A well-defined redox transition zone is present in the Hanford 300A subsurface that extends from the base of the Hanford formation, through the upper oxidized Ringold Unit E and into the underlying reduced Ringold.
- Electron acceptors utilized over this interval include O_2, nitrate, possibly Mn(III/IV), Fe(III), sulfate and CO_2.
- H_2 concentrations suggest the fermentation of sedimentary organic carbon as the primary energy source.
- While the areal extent of the reduced fine-grained Ringold Unit E is believed to be confined to the 300A, other similar redox boundaries exist within the Ringold elsewhere on the Hanford Site and could have important implications for the subsurface migration of redox sensitive contaminants.

Future Research

In-well multi-level sampler microcosm experiments can be used to probe community structure and functional responses either to perturbations or natural physical and geochemical gradients at small scales. By extending such analyses and experiments to depths beneath the Hanford formation, into the Ringold formation and down to basalt, there is a longer-term opportunity to understand system behavior of the 300A subsurface in terms of the overall hydraulic and geochemical factors influencing microbial community structure and function as a function of depth. What is (are) the source(s) of electron donor and acceptor that drive the function of this system? Fermentation of buried organic matter? H_2 or CH_4 from the underlying basalt aquifer? What are the hydraulic and physical controls on the transport of electron donors and acceptors within the system?

The Hanford 300A IFRC also provides an excellent opportunity to investigate how the physical environment and hydraulic properties within the Hanford formation influence overall native-state microbial community structure and function. There have been noted and unanticipated order-of-magnitude differences in hydraulic conductivity within the 300A IFRC Hanford formation saturated zone. Using the multi-level well clusters it is possible to sample from these distinctly different regions and apply deep sequencing technologies (e.g., pyrosequencing) to assess community structure as a function of time, where there are variations in groundwater flow velocity and direction as a result of changing Columbia River water levels as well as depth where there are noted differences in hydraulic conductivity. Plans also include using IFRC infrastructure to impose specific changes in the hydraulic gradient and or geochemistry (i.e., subtle manipulation of electron donor or acceptor concentration or nutrient combinations) within the well field to perturb the microbial community and measure its response in terms of structure and function. In addition to multi-well experiments, "push-pull" experiments to probe community response to subtle manipulations may be possible in selected wells & regions of the Hanford formation.

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