

# Microbial Fe-Phyllosilicate Redox Metabolism in Hanford 300 Area Sediments

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## Introduction and Objectives

This PNNL-SFA subproject is investigating the potential importance of microbial Fe bearing phyllosilicates metabolism in Hanford 300 Area sediments. Fe is likely to be a major electron donor and/or acceptor in Hanford sediments and may have an important influence on the stability, speciation and reactivity of Hanford risk-driving contaminants U and Tc. The upper, unconfined Hanford 300 Area sediments are comprised of the relatively unweathered, Pleistocene-age Hanford formation sediments dominated by basaltic and granitic fragments with interspersed silt and clay-sized phyllosilicates (chlorites and ferruginous biotites as well as some smectite). The older, Pliocene-age Ringold Formation that underlies the Hanford formation contains more weathered sediments. Oxidic-anoxic transition zones are observed in fine-grained Ringold sediments, which are hypothesized to result from microbiologically-driven processes. While phyllosilicates are becoming more widely recognized as a microbially-utilizable form of Fe in sediments, not much is known about microorganisms involved in electron transfer to and from Fe-bearing phyllosilicates, or about the physiology and mechanisms of electron transfer in such organisms.

The current objectives of this study are:

1. Examine the mineralogy of Ringold formation sediments and their sensitivity to microbial Fe redox metabolism;
2. Enumerate, cultivate, isolate, and identify microorganisms associated with Fe redox cycling in Hanford 300 Area groundwater.

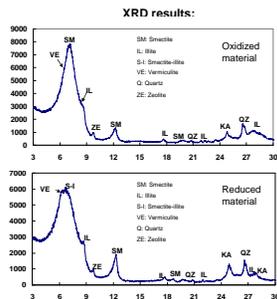
## Characterization of Fine Silt/Clay Size Fraction (< 20 μm) of Ringold Formation Composite Materials

### HF extraction assay:

Oxidized material:  
Total Fe = 1.257 mol/kg = 7.0 wt. %



Reduced material:  
Total Fe = 1.702 mol/kg = 9.5 wt. %



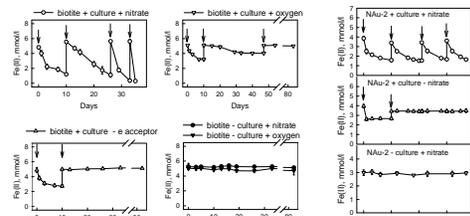
- Oxidized material is dominated by smectite-illite interlayer mineral(s); reduced material is enriched in illite;
- Both oxidized and reduced material contain low Fe(II) (5% and 14%, respectively)

## Ability of phyllosilicates in Ringold formation to serve as a source of electron acceptor or donor for model microorganisms

### Experiments with model microorganisms & minerals

**Model Fe(II)-oxidizing culture:** the lithotrophic nitrate-reducing enrichment culture reported by Straub et al. (AEM, 1996, 62:1458-1460) is capable of oxidizing solid-phase Fe(II)-bearing minerals (siderite and magnetite) and Fe(II) sorbed to mineral surfaces (Weber et al., ES&T, 2001, 35:1644-1650).

Recent SFA-supported studies at UW-Madison have shown that this culture can also repeatedly grow via oxidation of structural Fe(II) in primary (Bancroft biotite) and secondary (reduced NAU-2 smectite) phyllosilicates.

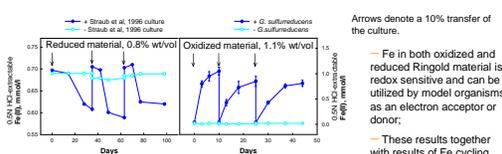


Arrows denote a 10% transfer of the culture. All time course Fe(II) measurements were based on 0.5M HCl extraction.

**Model Fe(III)-reducing culture:** *Geobacter sulfurreducens* was chosen for phyllosilicate Fe-cycling experiments with the lithotrophic nitrate reducing Fe(II) oxidizing Straub et al (1996) culture.

1. *Geobacter sulfurreducens* and limited acetate (0.25 mM) were added to sterile NAU-2 smectite suspension;
2. Once acetate was consumed and Fe(III) reduction stopped, the Straub et al (1996) culture and 2 mM nitrate were added, leading to rapid re-oxidation of reduced smectite.

### Experiments with model organisms and Ringold material

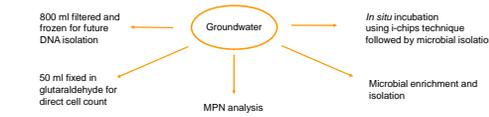


Extent of Fe(II) oxidation: 11-20% of total (HF-extractable) Fe(II)  
Extent of Fe(II) reduction: 15-18% of total (HF-extractable) Fe(III)

The observed ranges of microbial Fe(III) reduction and Fe(II) oxidation agree with findings for model smectites NAU-2 (this study) and SWa-1 (Shelobolina et al., Geomicrobiol J, 2003, 20:143-155) suggest that structural Fe in Ringold material can be cycled by microbial redox metabolism.

## Microbial Enrichments and Isolations from Hanford 300 Groundwater

Groundwater collected on 10/15/09 from Well 399-3-27 was used to determine whether organisms capable of phyllosilicate redox metabolism are present in Hanford sediments.

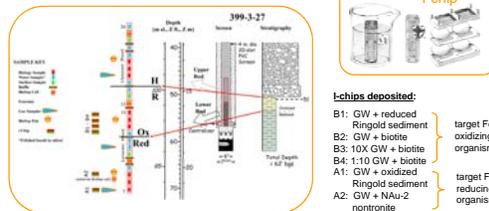


The source of Fe for all microbial analyses and isolations were the model phyllosilicate minerals Bancroft biotite and NAU-2 nontronite.

### Results of Groundwater Direct Microbial Count and Most Probable Number (MPN) Analysis:

Acridine orange stained cells:	(2.22 ± 0.17) × 10 <sup>6</sup> cells/ml
MPN numbers	cells/ml
Aerobic heterotrophs:	2.4 × 10 <sup>4</sup>
NO <sub>3</sub> -dependent Fe(II) oxidizers:	4.3
O <sub>2</sub> -dependent Fe(II) oxidizers:	0.23
Acetate/H <sub>2</sub> -oxidizing Fe(III)-reducers:	0.43

### In situ microbial cultivation at Hanford 300



- The I-chip (developed by S.S. Epstein) is a diffusion chamber-based method of microbial isolation that allows microorganisms to grow in their natural environment.
- An inoculum is sandwiched between semipermeable (0.03-μm-pore-size) membranes of the chamber, which is then returned to the environment. The chamber allows for free exchange of solutes with the external medium by diffusion while restricting the movement of cells.
- In the case of Hanford 300 groundwater where Fe cycling microorganisms are not dominant members of the microbial community (see MPN results), I-chips served as a tool to enrich and secure growth of microorganisms with an ultimate goal of isolating previously unknown microbial agents of solid-phase Fe redox metabolism;
- Fresh groundwater was diluted in agarized medium mixed with clay minerals and loaded into I-chips provided by S.S. Epstein;
- Groundwater was collected on 10/15/09; I-chips were loaded on 10/16/09, and deposited into subsurface on 10/21/09;
- I-chips were recovered on 03/01/10 and processed the same week at UW-Madison;
- All work was done in an anaerobic chamber;
- Each colony was recovered using a sterilized unfolded paper clip (see photo) and transferred into a culture tube containing 2 ml of a defined growth medium containing Fe(II)-phyllosilicates as an electron donor or Fe(III)-phyllosilicates as an electron acceptor.

## Microbial Enrichment and Isolation Results

Active enrichments with clay minerals as the source of electron donor or acceptor were obtained. Roll tube cultivation experiments were used to recover several pure cultures from the enrichments:

- 1) reduced NAU-2 smectite without any organic matter addition, and on
  - 2) Fe(II)-NTA with 0.2 mM acetate as a carbon source.
- Together with the fact that *Acidovorax* spp are not known for lithoautotrophic metabolism, this suggests that the *Acidovorax defluvi* isolate uses Fe(II) as an energy source and small amounts of organic matter in the NAU-2 as a carbon source.
- Geobacter bremensis* (98% similarity) can grow on either nontronite or hydrous ferric oxide as the electron acceptor.

## Summary and Conclusions

- Phyllosilicates have the potential to be an important source of electron donor/acceptor for microbial redox metabolism in Hanford 300 Area sediments;
- The fine silt/clay size fractions of both reduced and oxidized Ringold material are dominated by smectite, with reduced material containing a higher proportion of illite;
- Despite intense gray coloring, the fine silt/clay size fraction of reduced Ringold material contains only 14% Fe(II) as compared to 5% Fe(II) in oxidized Ringold material;
- Oxidized Ringold material can be repeatedly reduced by *Geobacter sulfurreducens* under growth conditions with acetate as the electron donor; the extent of Fe(III) reduction is 15-18% of total HF-extractable Fe(III), which is in the range of microbial Fe(III) reduction in model smectites NAU-2 and SWa-1;
- Reduced Ringold material can be repeatedly oxidized by the Straub et al. (1996) lithotrophic Fe(II)-oxidizing, nitrate-reducing enrichment culture under growth conditions; the extent of Fe(II) oxidation is 11-20% of total HF-extractable Fe(II), which is in the range of microbial oxidation of model chemically reduced smectites NAU-2 and SWa-1;
- Hanford groundwater contains culturable phyllosilicate-Fe reducing and oxidizing microorganisms; enrichment methods resulted in isolation of a Fe(III)-reducing *Geobacter bremensis* strain and a Fe(II)-oxidizing *Acidovorax defluvi* strain; isolation of Fe cycling microorganisms following in situ cultivation (I-chips) at Hanford 300 sediments is in progress.

## Future Research

- Determine whether Fe redox process in Ringold clays are reversible via cycling experiments analogous to studies with model smectite NAU-2; mineralogical changes and solution phase chemistry will be monitored during redox cycling;
- Continue microbial isolations from Hanford groundwater-derived enrichment cultures with emphasis on recovery and identification of biotite-oxidizing organisms;
- Purify and identify cultures derived from I-chips and compare these to conventional and tag-sequencing clone libraries available from SFA collaborators;
- Study the physiology and mechanism of electron transport in isolates of solid-phase Fe(II)-oxidizing microorganisms (collaboration with PNNL and Univ. East Anglia);
- Test ability of Straub et al. (1996) culture and new isolates from enrichment cultures and I-chips to oxidize primary phyllosilicates (Fe(II)-bearing mica and basalt) from Hanford formation sediments;
- Use model organisms (including new isolates) in pore-scale experimental systems to assess impact of microbial Fe redox metabolism on pore scale contaminant transformation/mobility.

## Acknowledgements

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