Microbial Oxidation of Insoluble Fe(II)-Bearing Minerals Relevant to the Hanford 300 Area and Other Subsurface Environments

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New Pathways and Organisms in Subsurface Fe Redox Metabolism

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Microbial Fe Redox Cycle (Microbial Ferrous Wheel)

- **Fe(II)**
- **Fe(III)**
- CO$_2$, H$_2$O
- Organic Matter
- Reduction (FeRB)
- Oxidation (FeOB)
- O$_2$ + CO$_2$
- NO$_3^-$ + CO$_2$
- Light + CO$_2$
Many Riders on the Ferrous Wheel

Roden et al. (2004), modified from Tebo & He (1999)

Fe(II) Solids
(Silicates Phosphates, Carbonates, Sulfides)

Oxidized S
(SO$_4^{2-}$, S$^0$)

CO$_2$, H$_2$O

Organic Carbon, Contaminants

Reduced S
(HS$^-$, FeS, FeS$_2$)

Sorption/coprecipitation
(metals & rads, organics)

Fe(II)

Solubilized Species
(metal & rads, PO$_4^{3-}$)

O$_2$ + CO$_2$

NO$_3^-$ + CO$_2$

Light + CO$_2$

Organic Matter

Reduced Organics
(RNH$_2$, RH + xCl$^-$)

& Inorganics
(Tc(IV), U(IV), Cr(III), As(III))

Sorption/coprecipitation
(metals & rads, organics)

Fe(III) Solids
(Oxides, Phyllosilicates, Phosphates, Sulfates)

Roden et al. (2004), modified from Tebo & He (1999)
Spatial Scales of Subsurface Fe Redox Cycling

- **m to km (regional) scale**
- **mm to cm (micro) scale**
- **cm to dm (local) scale**

**Surface water** to **Groundwater**

**Fe redox cycling zone**

**Distance (m or km)**

**Conc**

**O₂**

**Fe(II)**

**SO₄**

**O₂**

**Fe/Mn**

**Mass flux**

**Flow path**

**High ϕ +O₂**

**Low ϕ -O₂**
Drilling Further Down: Molecular-to-Field Scale Feedbacks

- Detected genes, transcripts, proteins
- Inferred pathways
- Simulated Metabolism

Advancing a predictive understanding of microbial communities

D. Richardson (next talk)
Both “oxidized” and “reduced” Ringold materials contain large quantities of phyllosilicate Fe [mainly Fe(II)] in the form of interlayered smectite and illite.

Hanford 300 Area Stratigraphy

- Ringold unit represents an advective barrier to vertical contaminant transport in the 300 Area.
- Redox boundary at ~60 ft is a potentially important site-wide determinant of chemical speciation and transport at Hanford.
- U and Tc reduction vary with depth as a function of variations in microbiology & reactive geochemical species [e.g. solid-phase Fe(II) and S(-II)].

Hanford: Catastrophic flood deposits, multiple lithologic transitions. Most-closely coupled to Columbia River (CR) dynamics.

Ringold: Lake and stream deposits with multiple lithologic transitions, including redox boundaries. Less dramatic (but as yet unquantified) coupling to CR dynamics, although historic river channels observed to transmit CR water inland.
Questions

• To what extent are native Fe-bearing solid phases in the vicinity of the redox boundary subject to enzymatic transformation?

• Does the Hanford subsurface harbor populations of solid-phase FeOB?
Oxidized Ringold Material Can be Repeatedly Reduced by *Geobacter sulfurreducens*

Extent of Fe(III) reduction:
~16% of total (HF-extractable) Fe(III)

1.1% (wt/vol) suspension of oxidized Ringold material

Shelobolina et al., in prep
Reduced Ringold Material Can be Repeatedly Oxidized by Straub et al. (1996) Culture

Extent of Fe(II) oxidation:
~15% of total (HF-extractable) Fe(II)

0.8% (wt/vol) suspension of reduced Ringold material

Shelobolina et al., in prep
Fe Redox Cycling in Ringold Materials
(*G. sulfurreducens* & Straub Culture)

Shelobolina et al., in prep

Virtually no Fe in solution at any time
Straub Culture is Dominated by a *Sideroxydans* Relative

Solid-State (Phyllosilicate) Extracellular Electron Cycling


Vaughan & Lloyd, *C.R. Geosci.*, 2011
Biotite and Reduced Smectite Oxidation by the Straub Culture

Biotite (~ 20 µm flakes)

- biotite + culture + nitrate
- biotite + culture - nitrate
- biotite - culture + nitrate

Days

Fe(II), mmol/l

Smectite (< 2 µm)

- NAu-2 + culture + nitrate
- NAu-2 + culture - nitrate

Days

Fe(II), mmol/l

Extent of biotite oxidation ~ 5% of total Fe(II)

Shelobolina et al., Appl. Environ. Microbiol., submitted
Microbial Biotite Oxidation Details

- **K release** into solution:
  - + cells: 5.1 µmol/L
  - - cells: 0.0 µmol/L

- **Si release** into solution:
  - + cells: 6.5 µmol/L
  - - cells: 1.6 µmol/L

- **Fe release** into solution:
  - + cells: below detection
  - - cells: below detection

- $\Delta \text{NO}_3^- / \Delta \text{Fe(II)} \approx 0.2$

  $\approx 10^7$ cells/µmol Fe

Shelobolina et al., *Appl. Environ. Microbiol.*, submitted
Enzymatic Alteration of Biotite

**Fig. 2**
- Growth of the Straub 1996 culture with biotite serving as the sole source of the electron donor; the results are the means of triplicate cultures.
- A-D: multiple transfers of the Straub 1996 culture on biotite; arrows designate 10% transfer of the culture into a new medium.
- E and F: growth of the Straub 1996 culture with nitrate serving as the electron acceptor.
- G: Mössbauer Spectroscopy of the control (blue) vs bio-oxidized (red) biotite.

**Fig. 3**
- Differences in the Fe(III) doublet content suggest ~ 5% oxid.
- Slight shift in d<sub>060</sub> due to partial Fe(II) oxid.
- Oxidized: disordered stacking
- Unoxidized: ordered stacking

**Figures**
- Oxidized: disordered stacking
- Unoxidized: ordered stacking

**Tables**
- | HF-extracted Fe(II), mmol/L | HCl-extracted Fe(II), mmol/L |
- | 9 | 2 |
- | 10 | 3 |
- | 11 | 4 |
- | 12 | 5 |
- | 13 | 6 |
- | 14 | 7 |
- | 15 | 8 |

**Graphs**
- Intensity (arb units)
- Intensity (counts)
- Velocity (mm/sec)
- Two-Theta (deg)

**Chemicals**
- Nitrate, mM
  - 5.2
  - 5.4
  - 5.6
  - 5.8
  - 6.0
- Nitrite, mM
  - 0.0
  - 0.2
  - 0.4
  - 0.6
  - 0.8
- Cells/ml x 10
  - 7
  - 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7

**Notes**
Edges of Microbially Oxidized Biotite are Enriched in Fe

Shelobolina et al., *Appl. Environ. Microbiol.*, submitted
New Concept

• New concept: biotite, a stable primary mineral, as a substrate for FeOB
• At neutral pH, biotite is completely insoluble and inert toward abiotic reaction oxygen and nitrate
Enrichment and Isolation of Solid-Phase Fe(II)-Oxidizing Bacteria from Hanford 300 Area Sediments and Groundwater

“i-Chip” enrichments from Hanford formation groundwater

Conventional enrichments from composite reduced Ringold sediments

Fe(II) substrates: reduced Ringold sed, biotite, reduced smectite
In Situ “i-Chip” Incubation Chamber (S. Epstein, Northeastern Univ.)

Nichols et al., *Appl. Environ. Microbiol.*, 2010

Evgenya collecting material from an individual “well”

Agarized suspension of mineral grains (e.g. biotite) in groundwater loaded here

~ 400 cultures initiated from individual wells
Fe(II)-Oxidizer Isolation Strategy

**Media used for enrichment and isolations**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Electron donor</th>
<th>Electron acceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>O₂</td>
</tr>
<tr>
<td>Lithotrophic</td>
<td>Structural Fe(II) in biotite</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Structural Fe(II) in reduced smectite</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Soluble FeCl₂</td>
<td>+</td>
</tr>
<tr>
<td>Heterotrophic</td>
<td>Acetate + YE</td>
<td>+</td>
</tr>
</tbody>
</table>

Shelobolina et al., in prep
# Hanford Solid-Phase Fe(II)-Oxidizing Isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cupriavidus necator</em> (formerly <em>Ralstoniaeutropha</em>)</td>
<td>Recently recognized Fe(II)-oxidizing Betaproteobacterial taxa</td>
<td>Swanner et al., <em>Chem. Geol.</em>, 2011</td>
</tr>
<tr>
<td><em>Bradyrhizobium</em> sp. (multiple strains)</td>
<td>Established $\text{H}_2$-oxidizing and/or thiosulfate-oxidizing chemolithoautotrophic Alphaproteobacterial taxa</td>
<td>Hanus et al., <em>PNAS.</em>, 1979; Masuda et al., <em>Appl. Environ. Microbiol.</em>, 2010</td>
</tr>
</tbody>
</table>
Bradyrhizobium in Hanford Sediments

- 16S rRNA gene sequences closely related (99% similarity) to recovered Bradyrhizobium isolates were abundant in clone libraries from 300 Area sediments.
- Multiple pure culture were recovered from i-chip samples...more than mere coincidence?
- Closely related Fe(II)-oxidizing Bradyrhizobium sp. have also recently been isolated from phyllosilicate-rich subsoils in Wisconsin (Shelobolina, *Front. Microbiol.*, 2012)

Lin, et al., unpublished
Aerobic Oxidation of Biotite by *Bradyrhizobium* sp. Strain 22

- Results are from screening of various aqueous Fe(II) (FeCl₂) oxidizing isolates for solid-phase Fe(II) oxidation capacity.

- Extent of Fe(II) oxidation (~ 5%) similar to Straub culture studies.

![Graph showing Fe(II) concentration over time with '+' for cells present and '-' for cells absent.](image-url)
Repeated Growth of *Bradyrhizobium* sp. Strain 22 on Reduced Smectite + NO$_3^-$

Benzine et al., in prep
Oxidation of Soluble Fe(II) by *Bradyrhizobium* sp. strain 22 with NO$_3^-$ as the electron acceptor

Cells were grown previously on Fe(II)-NTA with NO$_3^-$ as the electron acceptor

Benzine et al., in prep
Repeated Growth of *Bradyrhizobium* sp. Strain 22 on FeCl$_2$ + O$_2$

Cell yield ~ 5 × 10$^7$ cells mL$^{-1}$ is comparable to other neutral-pH FeOB

DAPI staining of fixed cells following oxalate dissolution of amorphous Fe(III) oxide

Benzine et al., in prep
Draft Genome of Strain 22

- 7.4 MB
- Automated Annotation by RAST (Argonne National Lab)
- 7003 predicted ORFs
- Complete Calvin-Benson-Bassham CO\textsubscript{2} fixation system (Type I Rubisco)
- Complete denitrification system (Nap)
- Complete N\textsubscript{2} fixation system
- Numerous (> 20) \textit{c}-type cytochromes…but none with homology to proteins known to be associated with Fe(II) oxidation: \textit{mtoAC} (\textit{Sideroxydans}), \textit{pioABC} (\textit{Rhodopseudomonas}), \textit{foxEYZ} (\textit{Rhodobacter}), or \textit{cyc1/cyc2} (\textit{Acidithiobacillus}) (Liang Shi, PNNL)
- No products from amplification of genomic DNA with \textit{mtoAB}-specific primers (Liang Shi, PNNL)
- No hybridization in Southern blot with \textit{mtoAC}-specific probes (Liang Shi, PNNL)
- Something new!
Bradyrhizobium sp. Strain 22: Future and Related Studies

• Potential in situ function of Bradyrhizobium in Hanford 300 Area sediments:
  – Determine capacity to oxidize native sediment Fe(II)-bearing solids.
  – Screen metagenomic/metatranscriptomic libraries from Hanford groundwater for Bradyrhizobium-related Fe(II) oxidation genes.
  – Search for Fe(II) oxidation genes in single cell whole genome amplifications of Bradyrhizobium recovered in situ.
  – Flow-through reactor experiments with Fe(II)-oxidizing Bradyrhizobium pure cultures to replicate in situ redox gradient conditions.

• Determine mechanism of Fe(II) oxidation in strain 22:
  – SDS-PAGE/MS analysis of outer membrane proteins from organotrophic vs. chemolithotrophic Fe(II)-oxidizing cultures (PNNL).
  – Development of genetic system underway (PNNL).

• Determine structure/function of proteins involved in Fe(II) oxidation (U. East Anglia).