Impacts of Mineralogy and Competing Microbial Respiration Pathways on the Fate of Uranium in Contaminated Groundwater

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Overall Goal

Field-oriented project to understand microbially-mediated mechanisms controlling the biostimulation of U(VI) reduction/immobilization in subsurface at FRC

Objectives

- Analyze Fe minerals, other redox-active constituents which may accelerate or inhibit U(VI) immobilization (Zachara, Stucki, Kostka)
- Characterize anaerobic microbial consortia likely to catalyze U(VI) reduction: enrichment cultures of FeRB and SRB (Kostka, Balkwill)
- Measure rates of carbon oxidation and electron acceptor utilization in anoxic sediment incubations (Kostka)
Outline of Results

- Geochemistry, Fe mineral analysis
- Comparison of cultivatable FeRB cloning/sequencing of 16S rRNA genes, T-RFLP of MPN enrichments
- Nitrate reduction in sediment microcosms
- Comparison of bkgd to contaminated (Area 1), low pH to high pH sediments
**Groundwater chemistry**

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>U(VI) (µg l⁻¹)</th>
<th>NO₃⁻ (mM)</th>
<th>NH₄⁺ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bkgd</td>
<td>300</td>
<td>6.8-7.6</td>
<td>0</td>
<td>0.002-0.003</td>
</tr>
<tr>
<td>Contam.</td>
<td>19</td>
<td>7.0</td>
<td>144-149</td>
<td>0.149-0.155</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5.2</td>
<td>122</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3.7</td>
<td>0</td>
<td>88.4-108</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>5.4</td>
<td>975-1030</td>
<td>11.6-11.9</td>
</tr>
</tbody>
</table>
## Sediment geochemistry

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>$\text{NO}_3$ (umol/cm³)</th>
<th>Fe -HCl (umol/cm³)</th>
<th>Fe-Dithio. (umol/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>302-02</td>
<td>5.4</td>
<td>$1.02 \times 10^{-2}$-$1.03 \times 10^{-2}$</td>
<td>4.36</td>
<td>503.5</td>
</tr>
<tr>
<td>302-05</td>
<td>5.7</td>
<td>$1.84 \times 10^{-5}$-$2.40 \times 10^{-3}$</td>
<td>26.1</td>
<td>302.3</td>
</tr>
<tr>
<td>27</td>
<td>3.6</td>
<td>9.80-54.9</td>
<td>13.5</td>
<td>260.7</td>
</tr>
<tr>
<td>28</td>
<td>3.4</td>
<td>1.37-1.80*10²</td>
<td>11.3</td>
<td>329.6</td>
</tr>
<tr>
<td>30</td>
<td>4.2</td>
<td>22.7-31.5</td>
<td>15.2</td>
<td>414.4</td>
</tr>
<tr>
<td>31</td>
<td>3.8</td>
<td>0.120-91.7</td>
<td>10.8</td>
<td>376.4</td>
</tr>
<tr>
<td>32</td>
<td>4.4</td>
<td>$1.67 \times 10^{-2}$-$17.2$</td>
<td>18.9</td>
<td>407.9</td>
</tr>
<tr>
<td>33</td>
<td>3.6</td>
<td>13.6-295</td>
<td>14.5</td>
<td>467.7</td>
</tr>
<tr>
<td>34</td>
<td>3.8</td>
<td>3.28-70.5</td>
<td>16.3</td>
<td>325.0</td>
</tr>
</tbody>
</table>
Silicate Fe(II)

Peak Area = 4.8%

FWB-302 Background (DOE-B-22)

T = 80 K

Counts (x 10^6)

Velocity (mm/s)

Area (%)

<table>
<thead>
<tr>
<th>Experimental</th>
<th>Theoretical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxide 1</td>
<td>8.0</td>
</tr>
<tr>
<td>Oxide 2</td>
<td>42.0</td>
</tr>
<tr>
<td>Silicate Fe(III)</td>
<td>45.2</td>
</tr>
<tr>
<td>Silicate Fe(II)</td>
<td>4.8</td>
</tr>
</tbody>
</table>
## Iron Content After Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CBD</th>
<th>Oxalate</th>
</tr>
</thead>
<tbody>
<tr>
<td>FWB-027</td>
<td>3.67</td>
<td>5.14</td>
</tr>
<tr>
<td>FWB-031</td>
<td>2.72</td>
<td>3.72</td>
</tr>
<tr>
<td>FWB-032-1</td>
<td>3.09</td>
<td>5.52</td>
</tr>
<tr>
<td>FWB-032-2</td>
<td>2.17</td>
<td>5.36</td>
</tr>
</tbody>
</table>
# Comparison of Mössbauer and Chemical Analysis

<table>
<thead>
<tr>
<th>Iron Oxide Component</th>
<th>Chemical Analysis (wt. %)</th>
<th>Peak Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FWB-027</td>
<td>30.2</td>
<td>38.6</td>
</tr>
<tr>
<td>FWB-031</td>
<td>26.1</td>
<td>29.2</td>
</tr>
<tr>
<td>FWB-032-1</td>
<td>46.0</td>
<td>58.8</td>
</tr>
<tr>
<td>FWB-032-2</td>
<td>58.7</td>
<td>63.1</td>
</tr>
</tbody>
</table>
• 20 MPN tubes were analyzed.

• Biomass and DNA concentrations were extremely low.

• All positive clones were screened (total of 708).

• Extraction, amplification, and cloning of sterile water was used as a negative control.
Cultured at pH 4 to 5

Background:

- **Acetate**
  - Contaminated aquifer clone
  - Other
  - (21 clones)

- **Glycerol**
  - Other
  - Desulfosporosinus
  - (18 clones)

- **Lactate**
  - Other
  - Desulfitobacterium
  - (14 clones)

Contaminated:

- **Glucose**
  - Brevibacillus
  - Paenibacillus
  - (86 clones)
Background:

Cultured at pH 7

Acetate
- Burkholderia
- Geobacter/Pelobacter
- Pseudomonas
(50 clones)

Lactate
- Geobacter/Pelobacter
(39 clones)

Glycerol
- Pantoea
- Geobacter/Pelobacter
(49 clones)

Contaminated:

Acetate
- Other
- Anaerovibrio
- Anaeromyxobacter
(152 clones)

Lactate
- Other
- Anaerovibrio
- Anaeromyxobacter
(41 clones)

Glycerol
- Other
- Anaeromyxobacter
- Clostridium
- Paenibacillus
- Desulfitobacterium
(238 clones)
Terminal Restriction Fragment Analysis

A. Contaminated- Acetate MPN

B. Background- Lactate MPN

C. Clones

Lac302-6A (Geobacter)

Ac032-14B (Anaeromyxobacter)

Lac302-3A (Geobacter)
Denitrification Potential (acetylene block technique)
FWB 18, pH = 6.4 to 6.6
Denitrification Potential
Area 1

- FWB 18, at pH of 6 to 7, rates relatively high, ~ 100 nmol N g\(^{-1}\) d\(^{-1}\)
- FWB 27 to 32, at pH of 3 to 3.5, no denitrification detected
Nitrate depletion
FWB 30, pH = 3 to 3.5
Conclusions

- Fe minerals in FRC subsurface mostly aluminosilicates and Al-substituted goethite, mineralogy does not change across pH gradient.
- Diversity of culturable FeRB largely dependent upon pH.
- Culturable FeRB at low pH predominated by Gram positives and organisms most closely related to *Anaeromyxobacter*.
- Members of previously cultured FeRB groups only observed at high pH in bkgd sediments.
- Denitrification potential dependent upon sediment pH with much higher rates measured at neutral pH sites within Area 1.
Implications

- Contaminated FRC subsurface is a heterogeneous “extreme environment” where the metabolism of Fe-reducing bacteria is likely to be controlled by low pH and high NO$_3^-$.
- New model Fe(III)-reducing organisms are needed for FRC where diversity different from past subsurface studies.
- Results point to NO$_3^-$ removal and neutralization of pH for establishment of conditions conducive to U(VI) reduction/immobilization by FeRB.
- Neutralization necessary prior to nitrate removal if denitrification is targeted pathway.
Future Work

- Purify FeRB from Area 1 for use as model organisms
- Determine pathways/controls of nitrate reduction at low pH
- Use more definitive molecular methods (real-time PCR) to study FeRB communities
- Combine approaches (mineral characterization, rate measurements, microbial community analysis) to determine interactions between Fe mineral transformation and U(VI) solubility
Green Non Sulfur

Low G+C
Gram Positives

High G+C
Gram Positives

Acidobacterium

FCB

Planctomycetes

Uncertain
Affiliation
Summary - FeRB MPNs

- ~1800 tubes tested under a range of culture conditions (pH, [NO$_3^-$], C substrates, reductant)
- Growth detected in majority of bkgd vs. minority of contam. sediment samples
- Growth also limited in low pH MPNs
- Counts similar in background vs. contam. sediment
- Little growth detected in unwashed contaminated sediments, whereas washing had no effect on counts in background sediments
FeRB MPNs- Variety of Sediments

- Surface, rooted sediments in aquatic environments: $10^4$ to $10^7$ cells g$^{-1}$
- Aerated agricultural soils: $10^3$ to $10^6$ cells g$^{-1}$
- Subsurface sediments: $10^2$ to $10^4$ cells g$^{-1}$