Factors Controlling In Situ Uranium and Technetium Bioreduction at the NABIR Field Research Center

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Research Hypotheses

- Indigenous subsurface microorganisms at the FRC have the capability to reduce complex mixtures of NO$_3^-$, U(VI) and Tc(VII)
- Conditions that favor U(VI) and Tc(VII) reduction can be created by adding electron donors to:
  - Increase biomass and microbial activity
  - Remove competing electron acceptors
- Electron donor additions can be controlled to sustain high reduction rates and maintain the stability of U(IV) and Tc(IV)
Project Organization

Laboratory microbial studies (OU)

Geochemical Characterization Reaction path modeling (PNNL, FRC, NABIR)

Geophysical analyses (FRC, NABIR)

FRC Push-Pull Tests Intermediate-Scale Physical Models (OSU, FRC)

Microbial community analysis (OU, NABIR)

Pore clogging (OSU, NABIR)

Numerical modeling (OSU, PNNL, NABIR)

Humic chemistry (ORNL)
Summary of In Situ Testing

- Desired metabolic capability is widespread and ethanol additions stimulated desired microbial activity in a wide range of subsurface environments in FRC Areas 1 and 2:

<table>
<thead>
<tr>
<th>pH</th>
<th>NO$_3^-$ (mM)</th>
<th>SO$_4^{2-}$ (mM)</th>
<th>U(VI) (µM)</th>
<th>Tc(VII) (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3-3.9</td>
<td>100-140</td>
<td>0-1</td>
<td>5-12</td>
<td>10000-15000</td>
</tr>
<tr>
<td>5.2-5.6</td>
<td>90-100</td>
<td>0-1</td>
<td>5-12</td>
<td>10000-15000</td>
</tr>
<tr>
<td>5.6-7.2</td>
<td>0-6</td>
<td>1-2</td>
<td>1-7</td>
<td>200-1000</td>
</tr>
</tbody>
</table>
### In Situ Microbial Activity Quantified in Extensive Series of “Push-Pull” Tests

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Donor</th>
<th>Donor Conc (mM)</th>
<th>No. of Tests</th>
<th>Donor Conc (mM/hr)</th>
<th>Nitrate (mM/hr)</th>
<th>Sulfate (mM/hr)</th>
<th>U (uM/hr)</th>
<th>Tc (pM/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>~120mM Acetate</td>
<td>300-500</td>
<td>2</td>
<td>0.80-24.00</td>
<td>0.51-0.69</td>
<td>0</td>
<td>9-39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>~120mM Ethanol</td>
<td>300-440</td>
<td>10</td>
<td>0.30-7.60</td>
<td>0.03-3.10</td>
<td>0.00-0.01</td>
<td>4-189</td>
<td></td>
<td></td>
</tr>
<tr>
<td>~120mM Glucose</td>
<td>200</td>
<td>3</td>
<td>0.94-4.30</td>
<td>0.04-3.20</td>
<td>0.02-0.03</td>
<td>45-460</td>
<td></td>
<td></td>
</tr>
<tr>
<td>~120mM None</td>
<td>0</td>
<td>8</td>
<td>0.00-0.10</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>~0.5mM Acetate</td>
<td>50</td>
<td>1</td>
<td>-</td>
<td>&gt;.01</td>
<td>0.021</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>~0.5mM Ethanol</td>
<td>15-80</td>
<td>14</td>
<td>0.02-0.30</td>
<td>0.00-0.06</td>
<td>0.00-0.02</td>
<td>0.00-0.04</td>
<td>0-5</td>
<td></td>
</tr>
<tr>
<td>~0.5mM Glucose</td>
<td>200</td>
<td>1</td>
<td>0.31</td>
<td>&gt;.05</td>
<td>0.034</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>~0.5mM None</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0.00-0.001</td>
<td>0.00-0.01</td>
<td>0-1</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion that donor additions stimulated the growth and activity of metal-reducing organisms supported by:

- Aqueous chemistry (dilution adjusted concentrations of added electron donors, electron acceptors; formation of diagnostic metabolic products; response to inhibitors)
- Microbial characterization of groundwater and sediments (diagnostic organisms)
- Geochemical characterization of sediments (reduced metals)
Recent Field Activities

- High (120 mM) NO$_3^-$ concentrations inhibit U(VI) reduction and promote oxidation of U(IV)
- Geochemical reductants may stabilize U(IV)
- “Sulfate Amendment” Study (In progress)
  - Ten new wells in Area 2
  - Inject GW835 groundwater with added sulfate (20 mM) and ethanol (40 mM) to precipitate sulfides
  - Challenge with added nitrate/nitrite
### Background Geochemistry

<table>
<thead>
<tr>
<th></th>
<th>Cl⁻ (mM)</th>
<th>NO₃⁻ (mM)</th>
<th>SO₄²⁻ (mM)</th>
<th>U (µM)</th>
<th>Tc (pM)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>FW218</td>
<td>1.7</td>
<td>12.9</td>
<td>0.4</td>
<td>0.0</td>
<td>152</td>
<td>7.0</td>
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<tr>
<td>FW219</td>
<td>0.3</td>
<td>0.4</td>
<td>0.6</td>
<td>3.9</td>
<td>508</td>
<td>7.4</td>
</tr>
<tr>
<td>FW220</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>200</td>
<td>7.7</td>
</tr>
<tr>
<td>FW221</td>
<td>0.3</td>
<td>1.2</td>
<td>0.2</td>
<td>0.1</td>
<td>186</td>
<td>7.5</td>
</tr>
<tr>
<td>FW222</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>156</td>
<td>7.8</td>
</tr>
<tr>
<td>FW223</td>
<td>0.1</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
<td>382</td>
<td>8.0</td>
</tr>
<tr>
<td>FW224</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>35</td>
<td>7.7</td>
</tr>
<tr>
<td>FW225</td>
<td>0.3</td>
<td>0.7</td>
<td>0.1</td>
<td>0.2</td>
<td>64</td>
<td>7.5</td>
</tr>
<tr>
<td>FW226</td>
<td>0.4</td>
<td>1.2</td>
<td>1.9</td>
<td>0.2</td>
<td>132</td>
<td>7.2</td>
</tr>
<tr>
<td>FW227</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.1</td>
<td>57</td>
<td>6.6</td>
</tr>
</tbody>
</table>
U(IV) Reoxidation Experiment (In Progress)
Recent Field Activities (cont.)

- Will added humics increase rates of U(VI) reduction?
  - Electron shuttling to solid Fe(III)
  - Complexation of potentially toxic metals
  - Three sets of 10 push-pull tests with and without added humics in Areas 1 and 2
Humic Acid Study

<table>
<thead>
<tr>
<th>Well</th>
<th>Ethanol</th>
<th>Humic Acid (100 mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP15D</td>
<td>80mM</td>
<td>AL-HA</td>
</tr>
<tr>
<td>DP01</td>
<td>80mM</td>
<td>FRC-HA</td>
</tr>
<tr>
<td>DP06</td>
<td>80mM</td>
<td>FRC-HA</td>
</tr>
<tr>
<td>FW002</td>
<td>80mM</td>
<td>FRC-HA</td>
</tr>
<tr>
<td>FW003</td>
<td>None</td>
<td>AL-HA</td>
</tr>
</tbody>
</table>

Experimental Design

GW835 Water

10mM Bicarbonate Tracer
Effect of Added Humic Acid on U(VI) Reduction
Recent Field Activities (cont.)

- Well (and perhaps aquifer) clogging occurs in field tests with low pH groundwater but mechanisms not clearly understood
  - Increased pH precipitates ~2 g/L solids
  - High donor concentrations (>300 mM) produces large amounts of biomass
  - Denitrification produces large quantities of N₂
Area 1
Well Rehabilitation
HCl + Chlorox + Surging

After Biostimulation (Jan 2004)

<table>
<thead>
<tr>
<th>Well</th>
<th>Volume Inj (L)</th>
<th>Time to Inj</th>
<th>Rate (L/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FW28</td>
<td>40-50</td>
<td>14 days</td>
<td>3</td>
</tr>
<tr>
<td>FW29</td>
<td>100</td>
<td>12 days</td>
<td>8</td>
</tr>
<tr>
<td>FW30</td>
<td>100</td>
<td>7 days</td>
<td>14</td>
</tr>
</tbody>
</table>

After Cleaning (May 2004)

<table>
<thead>
<tr>
<th>Well</th>
<th>Volume Inj (L)</th>
<th>Time to Inj</th>
<th>Rate (L/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FW28</td>
<td>50</td>
<td>21 hrs</td>
<td>57</td>
</tr>
<tr>
<td>FW29</td>
<td>50</td>
<td>21 hrs</td>
<td>57</td>
</tr>
<tr>
<td>FW30</td>
<td>50</td>
<td>21 hrs</td>
<td>57</td>
</tr>
</tbody>
</table>
• Multilevel Samplers (MLS)
  – 3 Sets of closely-spaced MLS wells in Areas 1 and 2
  – Each MLS contains 20-30 cells vertically spaced on 10 cm intervals
  – Cells are used to monitor vertical variations in aqueous chemistry and to incubate sediment samples
Vertical Variability in Aqueous Geochemistry

- **U (µM)**
  - Depth (ft)
  - **NO₃⁻ (mM)**
  - **SO₄²⁻ (mM)**

- **Linear (FW064)**
  - $R^2 = 0.78$

Data points include:
- FW062
- FW063
- FW064
Geophysical Investigations

Injection Well

Biostimulated zone

Area of Geophysical Monitoring: Radar, Seismic, Electrical
The Area 1 Physical Model

- Pack: stimulated FRC sediment, Maynardsville Limestone, Bicarbonate
- FW21 water pumped through Q ~ 3 mL/min
- EtOH injection system
- Samples collected thrice weekly
- Measured:
  - Q, Δh, U(VI), Tc(VII), SO$_4^{2-}$, NO$_3^-$, NO$_2^-$, EtOH, pH
Example Area 1 Results

**Nitrate**

- Concentration: NO₃⁻, mM
- Values: 0, 20, 40, 60, 80, 100, 120, 140

**Sulfate**

- Concentration: SO₄²⁻, mM
- Values: 0, 2, 4, 6, 8, 10

**Technetium**

- Concentration: Tc(VII), pM
- Values: 0, 200, 400, 600, 800, 1000, 1200, 1400

**Uranium**

- Concentration: U(VI), µM
- Values: 0, 2, 4, 6, 8
Where does the $N_2$ gas go?

- FRC Background
- Sediment and Maynardsville Limestone
- Denitrifying activity stimulated with ethanol
- Gas and liquid saturations monitored to track fate of $N_2$ gas
Sequential Feeding with 300 mM Ethanol and 100 mM NO$_3^-$