Microbial Reduction of Uranium in Mine Leachate by Fermentative and Iron-Reducing Bacteria (Project 6)

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Project Goal

The use of iron-reducing and fermentative bacteria in a treatment strategy to remove uranium(VI) from impacted groundwaters through bioreduction.
U(VI) is highly soluble

Groundwater aquifer
Zone of microbiological activity: PRB

Groundwater aquifer

U(VI)

Immobilize?
Why Uranium?
Primarily a Western U.S. problem

- Uranium mine sites
- Uranium mill sites
- Uranium producing regions
Why Uranium?

• Primarily a Western U.S. problem;

• Numerous abandoned mines sites in the Rocky Mountain region;

• Responsibility for many DOE (UMTRA) sites is being transferred to the states. (0.044 ppm GW protection standard: ~30 pCi/L).
Conventional treatment

- Pump and treat;
- Impermeable barrier emplacement w/ pumping and dilution with municipal waste;
- Ion exchange;
- BaSO$_4$ ppt.;
- Membrane separation.

None given high scores for remediation performance by EPA$^1$.

$^1$Assessment of technologies for the remediation of radioactively-contaminated Superfund Sites, Office of Solid Waste and Emergency Response: 116 (1990)
Novel treatments

- Passive bioreactors using anaerobic microbial sulfate reduction:
  - $\text{H}_2\text{S}$ production; reactor plugging by metal sulfides;
- Constructed wetlands (e.g., *Sphagnum*):
  - Limited capacity;
- Zero Valent Iron (ZVI) Permeable Reactive Barriers (PRBs):
  - Passivation, saturation of surfaces;
Novel treatments, cont’d

- Permeable Reactive Barriers$^1$:  
  - ZVI: 99.9 % removal  
  - Phosphate precipitation: 70 % removal  
  - Sorption onto hydrous ferric oxide (HFO): 70 % removal

Fry Canyon, UT (USEPA, 2000): $U_{med} = 840 \, \mu g \, U \, L^{-1}$
# U(VI) reduction by bacteria

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium sp.</em></td>
<td>Gram pos., Fermenter</td>
<td>Francis <em>et al.</em> (1994, 2002)</td>
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<tr>
<td><em>Desulfovibrio vulgaris</em></td>
<td>Gram neg., SRB</td>
<td>Spear <em>et al.</em> (1999; 2000)</td>
</tr>
<tr>
<td><em>Cellulomonas sp.</em></td>
<td>Gram pos., Cellulose degrader</td>
<td>Peyton <em>et al.</em> 2002</td>
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Example of U(VI) Reduction

Mixed SRB culture; 4 h exposure to 1 mM uranyl acetate

Spear et al. (1999)
Project Objectives (4)

• Optimize processes and promote synergistic reduction of U(VI) with efficient use of a carbon substrate;
  - Demonstrate the precipitation of soluble U(VI) by Clostridium sp. and Shewanella putrefaciens CN32.
  - Demonstrate the basis for synergistic U precipitation by fermentative and iron-reducing bacteria.
Project Objectives, Cont’d

• Reproduce optimum bioreduction conditions in columns to examine U(VI) bioreduction by a microbial consortium under flow conditions.
  - Determine the bioreduction of U(VI) to U(IV) under flow conditions similar to those observed in the field;
  - Determine the efficacy of a PRB based on anaerobic microbial processes for U removal.
**Dissimilatory Iron-Reducing Bacteria (DIRB)**

- **Indirect**
  - Organic acids (Lactate, acetate) + \( \text{H}_2 \) → \( \text{Fe}^{III} \) → \( \text{Fe}^{II} \) → 
  - Organic acids + \( \text{CO}_2 \) → \( \text{U}^{IV} \) → \( \text{U}^{VI} \)

- **Direct**
  - Organic acids + \( \text{CO}_2 \) → \( \text{U}^{IV} \) → \( \text{U}^{VI} \)

\( e^- \)
Fermenting Bacteria

Polysaccharide substrates (Possibly cellulosics)

Glucose

$CO_2$, $H_2$, Alcohols, Organic acids

Lactate, acetate, butyrate: via HPLC
Synergistic Bioreduction

Polysaccharide substrates

Glucose

CO\textsubscript{2}, H\textsubscript{2}, Alcohols, Organic acids

Organic acids + CO\textsubscript{2}

Fermenting

DIRB

Fe\textsuperscript{III} → Fe\textsuperscript{II}

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Effect of organic ligands
Project support

• Project duration: 11/1/02 – 10/30/04
• Funded support level:
  - Year 1: $39,841
  - Year 2: $43,041
• 1 Resident GRA (Angelique Diaz)
Progress to Date

• Efforts began 1/05/03;
• HSRC training:
  - Angelique Diaz (primary GRA)
  - Tiffany Yesavage, Julia Ventker (Figueroa advisees);
• Objective #1 (~ 40 % complete)
  - Demonstrate the precipitation of soluble U(VI) by *Clostridium* sp. and *Shewanella putrefaciens* CN32.
Experimental Methods (1)

Resting Cell Experiments: Bacterial culture (*Shewanella putrefaciens* CN32) was harvested after 24 hours aerobic growth and washed 2x with 0.1M NaCl. Cells were resuspended in O₂-free 0.1M NaCl. Vol. = 10 mL

Growth Experiments: Bacterial culture (*Clostridium* sp.) was inoculated into anaerobic growth media containing glucose and ammonium chloride. Vol. = 50 mL.
Experimental Methods (2)

- **Uranium**: Uranyl nitrate (0.1 mM) was used as well as 1:1 uranyl citrate (0.5 mM) and 1:10 uranyl citrate (0.5 mM). $^{233}$U used as a yield tracer in some cases.

- **Uranyl carbonato species**: prepared with uranyl nitrate and sodium bicarbonate.

- **Analyses**: Using sterile and anaerobic techniques, samples were removed over time and analyzed for optical density at 600 nm, pH, organic acids, and uranium.
HPLC

Glove box work

LSC
Uranium Oxidation State

• Determined spectrophotometrically using Bromo-PADAP: forms a complex with U(VI) at pH 7.8 with maximum absorbance at 574 nm.

• Method detects U(VI) only.

• U(IV) determined by difference: U(VI) analyzed first, followed by oxidation of the sample through aeration for 1 hour. Subsequent U(VI) analysis gives “total” U content:

\[
U(IV) = \text{total } U - U(VI)
\]

• Detection of soluble U(IV) species.

• U associated with cells determined after 0.5 N HCl extraction and neutralization.
Removal of uranyl nitrate by *Shewanella putrefaciens* CN 32

Removal -> bioreduction + biosorption

Conditions: *S. putrefaciens* CN32 resting cells; o.d. = 0.8; T = 20 ± 2°C; no shaking.
Removal of 1:1 uranyl citrate by *Shewanella putrefaciens* CN 32

\[ \text{Soluble U(VI) (mM)} \]

~60% of the 1:1 U(VI)-citrate was removed from solution due to biosorption and reduction to U(IV)-citrate

*Conditions: S. putrefaciens* CN32 resting cells; o.d. = 0.8; T = 20 ± 2°C; no shaking.*
Removal of 1:10 uranyl citrate by *Shewanella putrefaciens* CN 32

Lactate promoted reduction of 1:10 U(VI)-citrate; however the majority remained in solution as U(IV)-citrate.

*Conditions: S. putrefaciens* CN32 resting cells; o.d. = 0.8; T = 20 ± 2°C; no shaking.*
U speciation in the Presence of Citric Acid\textsuperscript{1}

\textbf{Oxidized} \hspace{2cm} \textbf{Reduced}

\begin{itemize}
  \item Carbon
  \item Oxygen
\end{itemize}

\textsuperscript{1}Francis et al. (2001)
1:1 U(VI)-citrate is not stable in the presence of cells; 1:10 U(VI)-citrate is not biosorbed and lactate promotes the reduction of U(VI) to the soluble U(IV)-citrate complex. Unaccounted U is most likely U(IV) stable to re-oxidation.
Effect of Ca on U(VI) reduction

Bioreduction using the DIRB *S. putrefaciens*:

Increasing Ca inhibits U(VI) reduction.

Fermenting Bacteria

Polysaccharide substrates (Possibly cellulosics)

Glucose

$CO_2$, $H_2$, Alcohols, Organic acids

$e^-$

Lactate, acetate, butyrate: via HPLC

UVI

UVI
Organic Acid Production by *Clostridium* sp.

**Acetic Acid**

**Butyric Acid**

0.15 mM $\text{UO}_2(\text{CO}_3)_2^{2-}$ or $\text{Ca}_2\text{UO}_2(\text{CO}_3)_2$ does not affect glucose fermentation.
High Performance Liquid Chromatography for Organic Acids

Conditions: Column = Aminex HPX-87H ion-exclusion; 0.008 N H₂SO₄, 0.6 ml minute, Diode-array UV detection at 210 and 254 nm.
Growth of *Clostridium sp.* in the Presence of U

0.15 mM $\text{UO}_2(\text{CO}_3)_2^{2-}$ or $\text{Ca}_2\text{UO}_2(\text{CO}_3)_2$ does not significantly affect growth.
97% (36 ppm) of the added U precipitated at $t = 0$; however, over time there was dissolution of the U precipitate; the dissolved U was removed when cells were present even under sub-optimal conditions for bacterial activity.

Conditions: Growing Clostridium sp., 0.15 mM U(VI), 2mM $\text{HCO}_3^-$, 11 mM $\text{Ca}^{2+}$
Ca-U-CO₃ Precipitate

Ca-U-CO₃ Expt.: U(IV) (green Pellet on left, U(VI) yellow pellet on right

Clostridial biofilm containing U(IV)

Shewanella putrefaciens CN32
Column bioreduction

Reproduce optimum bioreduction conditions in columns to examine U(VI) bioreduction by a microbial consortium under flow conditions.

- Determine the bioreduction of U(VI) to U(IV) under flow conditions similar to those observed in the field;
- Determine the efficacy of a PRB based on anaerobic microbial processes for U removal.
Experimental Setup:
Column Bioreduction Experiments
Synergies

A.J. Francis and Cleve Dodge at Brookhaven National Laboratory + National Light Source