Field scale evaluation of biostimulation in the near source zone of the former S3 ponds at Oak Ridge

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1951-1984: wastes stored in unlined ponds

The Oak Ridge S3 ponds

- Depleted uranium
- Strong acids (HNO₃ and H₂SO₄)
- Halogenated solvents
- Heavy metals

Field research station

A large parking lot now covers the S3 ponds. This is the "source zone".
## Contaminants in groundwater near the S3 ponds

<table>
<thead>
<tr>
<th>Inorganic Constituents</th>
<th>Concentrations</th>
<th>Organic Constituents</th>
<th>Concentrations</th>
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<tr>
<td>TIC</td>
<td>202-401 mg/L</td>
<td>COD</td>
<td>300 mg/L *</td>
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<td>Chloride</td>
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<td>Technetium-99</td>
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<td>Tetrachloroethene</td>
<td>2100-3300 µg/L</td>
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<td>Ni</td>
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<td>Trichloroethene</td>
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<td>Cd</td>
<td>0.45 mg/L</td>
<td>cis-1,2 Dichloroethene</td>
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<td>Al</td>
<td>541±47 mg/L</td>
<td>1,1,2-trichloro-1,2,2-trifluoroethane</td>
<td>1200-1500 µg/L</td>
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<tr>
<td>Ca</td>
<td>931±74 mg/L</td>
<td>Methylene chloride</td>
<td>39-42 µg/L</td>
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<td>Mg</td>
<td>174±11 mg/L</td>
<td>Citric acid</td>
<td>~6 mg/L #</td>
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<tr>
<td>Mn</td>
<td>130±9 mg/L</td>
<td>Acetic acid</td>
<td>~120 mg/L #</td>
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<td>Pb</td>
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<tr>
<td>Se</td>
<td>0.02 mg/L</td>
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</table>

* estimated value: a measurement is needed.  
# values for MLS FW 100, 40' depth.
Where we are

Where the waste is going

Figure A2. Contaminant Migration Pathways at the S-3 Ponds

Historic Bear Creek channel

Density driven

Bear Creek

Pathway 1

Pathway 2

Pathway 3

Nitrate Concentration

- >10,000 mg/L
- 1,000 – 10,000 mg/L
- 100 – 1,000 mg/L

Updip migration of plume into NT-1

Along strike flow of plume > 100 ft deep
Rationale for work near the source zone

The source zone is a reservoir of U(VI) for long-term groundwater and surface water contamination.

About 98% of the U(VI) in the near source zone is sorbed to solids or part of a solid phase.

The remaining 2% of U(VI) is dissolved in the groundwater at highly toxic levels (20-50 mg/L).

Conversion of solid-associated U(VI) into highly insoluble U(IV) will prevent dissolution and desorption, decreasing the time and cost of remediation.
PRIMARY OBJECTIVE

Evaluate the rates and mechanisms of U(VI) reduction by microbial populations

\[ \text{UO}_2(\text{CO}_3) + \text{H}^+ + 2\text{e}^- = \text{UO}_2 + \text{HCO}_3^- \]

A few electrons goes a long ways:

- 119 mg U are reduced for every mmol of electrons transferred to U(VI).
- This is equivalent to 119 mg U reduced/mg H\(_2\)
- It is also equivalent to \( \frac{119}{8} = 14.9 \) mg U reduced/mg COD
Hypotheses

- Biological reduction of U(VI) in the S-3 soils is a multistep process involving desorption/dissolution of U(VI), followed by uptake/reductive mineralization.

- Desorption/dissolution will typically limit the overall reduction rate, with highest rates observed under conditions that favor partitioning of U(VI) into the aqueous phase (i.e., elevated pH and TIC levels).

- Both metal- and sulfate-reducing bacteria will play a role in U reduction, with iron-reducing bacteria acting first followed by sulfate-reducing bacteria.
Chemistry considerations

Low pH (~3.5):
- buffered by Al$^{3+}$ (~20 mM)

High U(VI):
~98% on the soil (~400 mg/kg)
~2% in groundwater (~40 mg/L)

High NO$_3^-$:
130-480 mM in groundwater - NO$_3^-$ and denitrification intermediates inhibit U(VI) reduction (Senko et al., 2001)

High Ca$^{2+}$:
~20 mM in groundwater - Ca$^{2+}$ inhibits U(VI) reduction at 5 mM (Brooks et al., 2003)

\[
\text{UO}_2(\text{CO}_3) + \text{H}^+ + 2e^- = \text{UO}_2 + \text{HCO}_3^- \quad E^{\circ\prime} = +0.105 \text{ V}
\]

\[
\text{Ca}_2\text{UO}_2(\text{CO}_3)_3 + 2e^- = 2\text{Ca}^{2+} + \text{UO}_2 + 3\text{CO}_3^{2-} \quad E^{\circ\prime} = -0.046 \text{ V}
\]
Eh/pH Plot of Uranium Speciation
(300 mg/L TIC, 40 mg/L U)

No Ca

+ 20 mM Ca
Uranium adsorption

Uranium adsorption is concentration dependent. It is also strongly pH dependent.

Uranium Adsorption
pH~4

U(VI) pH adsorption envelopes on ORNL saprolite

% adsorbed

Model fit
Observed

Uranium in Solution (mg/L)

Uranium Adsorbed (mg/kg)

Sample 101-8
Sample 100-1
Sample 101-7
**Potential clogging agents**

- Aluminum hydroxide forms at pH 5.
- Calcium and magnesium carbonates form at pH 7-9.
- $\text{N}_2$ gas forms during denitrification.
- High levels of biomass are produced during denitrification.

![Solid production from synthetic groundwater](image)

- pH adjusted to 7 with 50% liquid from denitrifying batch cultures
- pH adjusted to 7 with $\text{Na}_2\text{CO}_3$
- pH adjusted to 7 with KOH

2 g/L solids produced
Clogged pump head screen. The white precipitate dissolved in a 2% HCl solution after 1.5 hour. Clogging observed even with <4 mg/L Al.

Clogging happens (and what to do about it)

Surge block for cleaning wells
• Saprolite contains a highly interconnected fracture network with densities of 100-200 fractures/m. Fractures are < 5-10% of the total porosity, but carry >95% of the groundwater flow.

• The fractures surround a high porosity, low permeability matrix that is a source and sink for contaminants.
Core Mineralogical Evaluations

Overlying Gleyed leached flow zone with high U, low pH groundwater

A high U zone was detected in the center of the test cell at a depth of 46'.

XRD results:
Gleyed Zone - Quartz, Vermiculite, Mica, HV, Ca-feldspar
Black Zone - Quartz, Ca-feldspar, Vermiculite, Mica, Goethite

Very fine sands with Fe oxide precipitates
Fe oxide accumulation zone

Phillips/Watson, 2003
Geophysics was used to identify areas of contaminant transport.

S-3 Ponds Cap
Surface Seismic/Electrical Resistivity
(Doll et al., SAGEEP, 2002).

Electrical Resistivity
- Low (~4 Ohm-m)
- High (~150 Ohm-m)

Low Resistivity ~ High Nitrate

Contour Lines: Seismic Velocity (m/s)

- Nitrate Plume
- Poorly Consolidated Zone
- Depth of Auger Refusal-Bedrock
- Nitrate Brine

Nitrate Concentration (mg/L)
Resistivity (Ohm-m)

Low Resistivity ~ High Nitrate

Doll et al., SAGEEP, 2002.
Ex-situ conditioning of water in treatment zone

In-situ reduction of uranium

Adjust pH
Strip volatiles

U(VI) U(IV)

Electron donor

Volatiles
metals

NO₃⁻

Strip volatiles,
neutralize acid,
precipitate metals

FBR

Ex-situ conditioning of water in treatment zone

In-situ reduction of uranium

Adjust pH
Strip volatiles

U(VI) U(IV)

Electron donor

Volatiles
metals

NO₃⁻
The “Big Top” where extracted groundwater is treated to enable metal reduction in-situ

Tanker for chemical sludge disposal

Bag filters for disposal of biomass
Ex-situ conditioning of water in treatment zone

1. Precipitate Al and Ca
2. Remove NO$_3^-$ by denitrification in FBR
3. Vacuum strip to remove VOCs and N$_2$
The aboveground treatment train

Vacuum stripper  Two-step chemical precipitation  Fluidized bed reactor (FBR)

FBR sampling and characterization
  Phylogenetic analyses
  Functional gene microarrays
  Functional monitoring
Two pilot-scale FBRs

Fluidized Bed Reactor

Removes $\text{NO}_3^-$ as $\text{N}_2$
Efficient
Cheap
Raises $\text{pH}$
Demonstrated in two continuous pilot-scale systems (pH 7.4 and 9.2)

Well TPB16 enrichment

Inoculum

Full scale FBR

Inoculum
Denitrifying biofilms growing on granular activated carbon in pilot scale FBR at Stanford. Some of the bacterial genera found in this community include Zoogloea, Xanthomonas, Dechloromonas, Dechlorosoma, and Sporumosa.
FBR: Nitrate removal

Nitrate Concentration, mM

Time, days
Strip volatiles, neutralize acid, precipitate metals

Ex-situ conditioning of water in treatment zone

In-situ reduction of uranium

Volatiles, metals, NO₃⁻, U(VI)

Electron donor

FBR

NO₃⁻

Electron donor

U(VI) U(IV)

Remove N₂, Control pH & TIC

A B MLS wells C D
Well layout

before plumbing

after plumbing

Skid with pumps and meters for wells inside Big Top

MLS well locations
Cross-sectional view of the injection/extraction wells and the MLS wells.

Screened Interval = 38-45°
Chemical profiles with depth at the MLS wells

![Graphs showing chemical profiles with depth at MLS wells for Ca, Na, K, Mg, Fe, and Mn.](image)
Chemical profiles with depth at the MLS wells (cont)

- Ca (mg/L)
- Na (mg/L)
- K (mg/L)
- Mg (mg/L)
- Fe (mg/L)
- Mn (mg/L)

FW100
FW101
FW102
Strip volatiles, neutralize acid, precipitate metals

Electron donor

Volatiles
metals
$\text{NO}_3^-$
$\text{U}^{(VI)}$

$\text{FBR}$

$\text{NO}_3^-$

Electron donor

$\text{N}_2$

$\text{Ex-situ conditioning of water in treatment zone}$

Remove $\text{N}_2$
Control pH & TIC

Electron donor

$\text{In-situ reduction of uranium}$

$\text{AQ}^0 + \text{AQ}^-$

Volatiles
metals
$\text{NO}_3^-$
$\text{U}^{(VI)}$

$\text{Q}^0$

$\text{AQ}^0$

$\text{AQ}^+$

$\text{AQ}^{-}$

$\text{A}$

$\text{B}$

MLS wells

$\text{C}$

$\text{D}$
Seismic and Radar Tomography

Mapping subsurface material heterogeneities using cross-borehole techniques.
Regions of the subsurface

- Protective outer loop
- Extraction
- Injection
- U reduction zone
1. Perform a tracer study to determine connectivity of wells and residence time distribution. Obtain desorption rates from the rebound.

2. Flush outer and inner cell with clean water at pH 4 to remove Al, Ca, and most of the nitrate. Follow with flush at pH 5-6 to prepare for denitrification.

3. Stimulate denitrification in-situ and vacuum strip N₂ to remove residual nitrate.

4. Increase pH of inner cell to mobilize U(VI) and add electron donor to inner cell to reduce U(VI).
Tracer study

A dual dipole tracer injection-withdraw test was conducted using CaBr$_2$ and CaCl$_2$ in an effort to create an inner and outer hydraulic cell.

Results confirmed location and transport features of preferential flow regimes and slow flowing matrix regimes.

Experimental data was numerically simulated and the model used to design the in situ U bioreduction system.
Tracer study simulations
Seismic tomography data complements tracer measurements.

Hubbard et al., 2003
Mehlhom et al., 2003
Effect of tracer clean water flush on nitrate in MLS wells

- Mid-depths were flushed well
- Bottom depth was poorly flushed
- All depths were flushed
Natural gradient site recovery solute breakthrough

Natural gradient contaminant transport monitored during site recovery.

Quantification of solute residence times, direction of groundwater flow, and strike vs. dip interactions.
Diffusion from matrix
1. Perform a tracer study to determine connectivity of wells and residence time distribution. Obtain desorption rates from the rebound.

2. Flush outer and inner cell with clean water at pH 4 to remove Al, Ca, and most of the nitrate. Follow with flush at pH 5-6 to prepare for denitrification.

3. Stimulate denitrification in-situ and vacuum strip N\textsubscript{2} to remove residual nitrate.

4. Increase pH of inner cell to mobilize U(VI) and add electron donor to inner cell to reduce U(VI).
pH increase in inner and outer loop extraction well

Flush with pH 4.0 water
Day 9-68

Flush with pH 6.0 water
Day 69-136

1st Run
Biostimulation
Day 137-142

2nd Run
Biostimulation
Day 163-167

Time, days

Δ FW026  ○ FW103
• **Kinetic Model**

**Assumptions**

• Kinetically controlled sorption/desorption

• Kinetic mass transfer between two regions
The half-life of nitrate in the second immobile region is about 3 months. To deplete the second immobile zone would take about one year.

The mobile region definitely responds to flushing and a low average Nitrate concentration can be maintained while removing the Nitrate as it enters the mobile zone.
1. Perform a tracer study to determine connectivity of wells and residence time distribution. Obtain desorption rates from the rebound.

2. Flush outer and inner cell with clean water at pH 4 to remove Al, Ca, and most of the nitrate. Follow with flush at pH 5-6 to prepare for denitrification.

3. Stimulate denitrification *in-situ* and vacuum strip N₂ to remove residual nitrate.

4. Increase pH of inner cell to mobilize U(VI) and add electron donor to inner cell to reduce U(VI).
Next up for the field

- Build up U-reducing biomass
- Increase pH but avoid clogging
- Drilling
- Oxidation experiments
In-line Uranium Immunosensor

This sensor has the ability to autonomously run a standard curve from stock reagents and prepare environmental samples for analysis. The sensor measures U(VI) at concentrations from 4-100 nm (0.6-24 ppb).
Optics, LED, and capillary bed containing particles with immobilized capture ligand.

Fluorescently labeled antibody and environmental contaminant are mixed in a disposable syringe.

Drive motor pushes the antibody-contaminant mixture over the capture ligand.

Handheld Immunosensor
Subsurface Microbiology

Monitoring of succession

Baseline analysis of low pH community

Slides on inner loop sidestream

Small packed columns

Filtered samples from inner loop - collected for microarray analysis and plate counts

U-reducing enrichments - phylogeny, kinetics

Phylogenetic analyses

Kinetic model development
## Genes Included in New Functional Gene Array

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<tr>
<th>Functional Gene</th>
<th># Genes Downloaded</th>
<th># Genes with Unique Probes</th>
<th># Genes with Group Probes</th>
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<td><strong>Denitrification</strong></td>
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<td>Nitrate reductase (narB)</td>
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<td>Nitrate reductase (nasA)</td>
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<td>55</td>
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<td>Nitric oxide reductase (norB)</td>
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<td>Nitrite reductase (nirS)</td>
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<td>Metal resistance/reduction (Cd, Ni, etc.)</td>
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<td>Organic remediation</td>
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<tr>
<td>Sulfur reduction</td>
<td>989</td>
<td>478</td>
<td>345</td>
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<tr>
<td><strong>Grand Total</strong></td>
<td>&gt;16871</td>
<td>&gt;4392</td>
<td>&gt;3341</td>
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Key points

- **Site characteristics**: high acidity, high nitrate, high sulfate, high metals. Organisms identified repeatedly at low pH: Acidovorax, Burkholderia, Pseudomonas, Microbacterium, Clostridia.

- **Aluminum buffers the system** at low pH and precipitates when the pH is increased. Our strategy is to remove it ex-situ by precipitation.

- **Nitrate inhibits U(VI) reduction**. Our strategy is to remove bulk nitrate ex-situ and residual nitrate in-situ (with ex-situ vacuum stripper).

- **Calcium inhibits U(VI) reduction**. Our strategy is to remove it ex-situ by precipitation.
More key points

• A nested recirculation scheme appears to protect the treatment zone from aluminum, nitrate, and acidity.

• Addition of ethanol to the inner loop effectively removes the nitrate.

• We have preliminary evidence for microbial removal of U.
Insights and new tools

• Control of pH/TIC appears to be a useful strategy for increasing reduction, by increasing desorption/dissolution rates.

• The vacuum stripper with pH control will be a useful tool for management of U speciation and control of N₂.

• A two-stage process seems feasible for site remediation. This approach would have coupled ex-situ and in-situ elements.
• **Model - coupled mass transfer and reaction**

**Assumptions**

- Kinetically controlled sorption/desorption
- Kinetic mass transfer between two regions
- Microbial reduction of U(VI) in the mobile zone

\[
\text{Rate of mass transfer} = k_w(U_{aq, eq} - U_{aq})
\]

\[
\text{Rate of reduction} = k'X U_{aq}
\]

\(k_w\) is a lumped parameter accounting for mass transfer. It has units of \(\text{time}^{-1}\). \(U_{aq, eq}\) is the concentration of U in equilibrium with the solid phase concentration. It is a function of pH and TIC. \(X\) is biomass concentration, and \(k'\) is a pseudo second order rate coefficient.
At steady state:

\[ \text{Rate of mass transfer} = k_w(U_{aq,eq} - U_{aq}) = \text{Rate of reduction} = k'X U_{aq} \]

Desorption and reduction of U(VI)

\[ k_w = 0.233/d \]

Reaction Rate (mg U/L-d)

Mass transfer limited region

Aqueous U Conc (mg/L)

U \_aq,eq \_eq \_eq \_eq = 10 mg/L

U \_aq,eq \_eq \_eq \_eq = 7.5 mg/L

U \_aq,eq \_eq \_eq \_eq = 5 mg/L

U \_aq,eq \_eq \_eq \_eq = 2.5 mg/L

U \_aq,eq \_eq \_eq \_eq = 1 mg/L

Preliminary calculations indicate that MT limitation is likely.
Newly added feature:

Vacuum stripper on the inner cell recirculation loop enables control of TIC and N₂
Stage 1 - removal of aluminum, calcium, nitrate

Former S-3 Ponds (now covered with parking lot)

Extraction well gallery

Injection well gallery

Al, Ca sludge for disposal

Al^{3+} \rightarrow Al(OH)_3

Ca^{2+} \rightarrow CaCO_3

N_2 biomass

NO_3^- \rightarrow N_2

Ethanol pH control

U(VI) \rightarrow U(IV)

U(IV) \rightarrow U(IV)
Stage 2 - conversion of $\text{U(VI)}$ to $\text{U(IV)}$

Ethanol
pH control

Injection well gallery

U(VI)$\rightarrow$U(IV)

Extraction well gallery

U(VI)$\rightarrow$U(IV)
Batch microcosm studies: ethanol biostimulation of U reduction in the presence of contaminated sediment.

Variation in pattern of soluble uranium concentration over time:

X-ray absorption spectroscopy shows the reduction of solids-associated uranium in viable, but not control, microcosms.
Column microcosm

- **Source:** A hot spot in our test plot, FWB 104, 38-43 ft.
- **Total U(VI) ~500 mg/kg.**
- **Low pH (3.7), high Al, Ca, Ni, and others.**
- **High phosphate ~1000 mg/kg.**
**Step 1: Acid flush to remove Al and Ca**

Flush with 10 mM KCl, pH ~4.

![Graphs showing the removal of Al, Ca, U(VI), Nitrate, and Sulfate](image)

- **Nitrate** and **Sulfate** concentrations decrease significantly with increasing volume of flush.
- **Al** and **Ca** concentrations also show a decrease, indicating effective removal.
- **U(VI)** concentration remains relatively stable with increasing volume, suggesting less effective removal.

The graphs illustrate the effectiveness of the acid flush in removing Al and Ca, with Nitrate and Sulfate showing a clear drop in concentration.
Step 2: pH adjustment + tracer test

Flush with 30 mM NaHCO₃, 15 mM K₂CO₃, 5 mM K₂SO₄, CO₂ purged, pH ~7.1.
Step 3. Biostimulation

- Strictly anaerobic operation.

- Column was operated in a continuous recirculation mode to simulate field operations (0.2 mL/min).

- Biostimulation was achieved by periodic ethanol addition (~4 mM ethanol spikes, 30 mM NaHCO₃, 30 mM K₂CO₃, 5 mM K₂SO₄, CO₂ purged, pH ~7.1).
No evidence of microbial activity for the first 30 days; pH at ~6.5. U(VI) at 5 mg/L.

FBR biomass was added on day 30. U(VI) begins to increase, along with pH.

Days 30-60: ethanol disappears first, acetate appears then slowly disappears as sulfate drops. Soluble U(VI) increases to 15 mg/L. U(VI) rises to 15 mg/L.
Days 60-130: U(VI) levels fall; pH remains high.
Days 60-120: acetate accumulates as U(VI) falls, no sulfate!

Day 130: sulfate added

Days 130-170: acetate falls, then sulfate

Days 170-220: ethanol added but no acetate formed, sulfate falls, pH increases dramatically, U(VI) starts to increase. Methane detected.

Initially soluble U(VI) levels increased as pH increased
Background levels of denitrifiers, metal-reducers, and sulfate-reducers on sediment (MPN/g)

<table>
<thead>
<tr>
<th></th>
<th>Nitrate reducers</th>
<th>Ferric citrate reducers</th>
<th>Sulfate reducers</th>
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</thead>
<tbody>
<tr>
<td>FW-107 (13.2 m)</td>
<td>3500</td>
<td>46</td>
<td>240</td>
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<tr>
<td>FW-109 (15.4 m)</td>
<td>5400</td>
<td>1700</td>
<td>1100</td>
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</table>