Techniques for Assessing the Performance of *In situ* Bioreduction and Immobilization of Metals and Radionuclides in Contaminated Subsurface Environments

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Presentation outline

Contaminant fate and transport problems in humid regimes

Efforts to immobilize metals and radionuclides *in situ* via bioremediation

Techniques for assessing the performance of *in situ* bioreduction and immobilization of metals and radionuclides

- In situ solution and solid phase monitoring
- In situ and laboratory microbial community analysis
- Noninvasive geophysical methods
- Solid phase speciation via high resolution spectroscopy
Subsurface contaminant problems in humid regimes

Research is driven by contaminant transport issues at the m to km scale. Many DOE facilities have literally thousands of unlined pits and trenches filled with low- and high-level radioactive waste.

Large annual rainfall inputs (1400 mm/y) in humid regimes have resulted in a huge secondary contaminant source where radionuclides have been disseminated across vast subsurface environments.

Historical remediation has targeted “hot-spots” with in situ and ex situ treatment of high-risk exit pathways (e.g. preferential flow zones, seeps).
Large volume, long-duration contaminant sources

- Example: S-3 ponds are located at DOE’s NABIR Field Research Center in the Y-12 complex of Oak Ridge, Tennessee.

- Unlined surface impoundments received acidic nitrate and U-bearing waste at a rate of 10 million liters / year for 32 y.

- Attempts were made to neutralize and denitrify the ponds in 1984, followed by subsequent capping as an asphalt parking lot in 1984.

- Long-term infiltration has resulted in a contaminated area of 98 ha. This is a very bad place!
The scope of the problem is massive.
Humid regimes often have highly structured soils which complicates contaminant fate and transport.

Overlying Saprolites

Interbedded fractured weathered shales and limestone at ORNL

Underlying Bedrock
Fractures constitute < 5 –10 % of total porosity, yet are able to carry > 95% of saturated groundwater flux. Serve as conduits for preferential contaminant migration.

Matrix contains > 90-95% of total porosity; however, permeability is orders of magnitude lower than fracture. Serve as high capacity source and sinks for contaminants.
Remediation Dilemma for Secondary Sources

- Large scale *in situ* treatment of contamination in the soil and rock matrix difficult.
  - No feasible removal or immobilization technologies for large volumes of contaminated subsurface saprolites, bedrock, groundwater.
  - Sites have often resorted to capping which typically does **not** immobilize contaminants in humid regimes.
  - Natural processes for immobilizing contaminants.
    - Natural physical attenuation via diffusion into high porosity, low permeability matrix.
    - Natural chemical attenuation via sorption, redox transformation, degradation, dissociation, and precipitation reactions.
    - Bioremediation and immobilization *in situ*.

[Image: Constructing a cap over waste trenches at ORNL]
Push-pull technique for assessing *in situ* bioreduction of metals and radionuclides at ORNL.

Push into formation

Electron donor
Nutrients

Pull and analyze
Biocage designed to intercept metal and radionuclide plumes with *in situ* immobilization via bioreduction at ORNL

Near-source groundwater processing setup

**Biocage**

- Strip volatiles, neutralize acid, precipitate metals
- Fluidized bed reactor for removing nitrate
- Vacuum strippers for removing PCE
- Metal removal with two stage pH adjustment
- Electron donor and nutrient addition for biostimulation

**Near-source groundwater processing setup**

- Electronic donor
- Fluidized bed reactor (FBR)
- Vacuum strippers
**In situ** radionuclide immobilization via bioreduction

Bioreduction of metals and rads. occurs even in the presence of competing electron acceptors (e.g. Fe-oxides)

In situ U(VI) and Tc(VII) reduction following biostimulation with ethanol
Techniques for assessing the performance of *in situ* bioreduction and immobilization of metals and radionuclides
In situ solution / solid phase monitoring
In situ groundwater / solid phase contaminant analysis

Flow-through chemiluminescence sensor for hexavalent chromium

Vo-Dinh, ORNL

Field portable immunoassay biosensor developed for detection of U \textit{in situ}

(Blake of Tulane University)

Nal detector for in situ gamma
Groundwater geochemical monitoring

Continuous dissolved oxygen monitoring

Analysis of redox couples (e.g. Fe(II)/Fe(III), $S^{2-}/SO_4^{2-}$)

Monitor for the intrusion of oxidants and competing electron acceptors (e.g. NO$_3^-$)
In situ solid phase contaminant analysis

Nondestructive In Situ Quantification of Contaminant Immobilization

- Permeable Environmental Leaching Capsules (PELCAPs)
- Nondestructive measurement of the amount of immobilized contaminant in a soil thereby avoiding the necessity for repeated, costly, and destructive soil sampling.
- Direct comparison of several immobilization treatments, including a no-treatment control, under identical field conditions within the same well.
- Internal PELCAP leaching calibration relative to specific reference tracers ($^{233}$U) that have predictable known environmental leaching behaviors. U(VI) would be mobile, U(IV) would be immobile.
- The immobilized contaminant is monitored directly in the soil via a gamma-emitting radioisotope tracer.
- Technique is applicable to many inorganic and radioactive elements (e.g., Cr, Cd, As, Pb, Hg, U, Tc, and Pu).

(Brooks of ORNL)
In situ and laboratory microbial community analysis

(Making sure that the groundwater conditions are conducive to bioreduction)
Coupons, or “bug traps,” for rapidly assessing *in situ* microbial activity.

Various material such as Fe-oxides and indigenous sediments used for colonization.

Rapid assessment of microbial community dynamics as a function of space and time.

Provides evidence that the correct organisms remain active in the biostimulated zone.

(Cummings / Geesey of INEEL)
“Bio-Traps”

SEM of Bio-Sep® Beads

2-3 mm in diameter
25 % Nomex, 75% PAC
74% porosity
600 m2 of surface area/g
Surrounded by ultrafiltration-like membrane with 1-10 micron holes
Autoclavable
Cleaned of fossil biomarkers by heating to 300 oC

(Peacock and White, Univ. Tenn)

Biofilms Form Rapidly in Bio-Sep® Beads
Down-well “bio-trap” coupons for enhanced microbial monitoring

(Peacock and White, Univ. Tenn)

Rapid and efficient sampling of biofilms

Biofilm community structure is more indicative of *in situ* microbial ecology than samples of planktonic organisms

Rapid and efficient prediction of the effects of amendments on *in situ* microbial ecology

Integrated response over time is better than “grab samples”
Groundwater microbiology

Phylogenetic analysis of groundwater 16S rRNA clonal library. Iron, nitrate, and sulfate reducing organisms are isolated with the later shown to effectively reduce uranium.

![Phylogenetic tree and graph representing microbial activity and uranium reduction](image-url)
DNA Microarrays

Rapid method to assess shifts in microbial community structure via gene detection and expression.

Indirect detection of activity - presence of genes (DNA)

Direct measurement of activity - expression of genes (mRNA)

An increase in the quantity of a given gene (DNA) may indicate an increase in the numbers of the source organism. RNA would be a more direct measurement of activity but is more difficult to extract from environmental samples.
Non- and semi-invasive geophysical methods
Surface based geophysics used to identify probable areas of contaminant transport

- Electrical resistivity: light to dark blue = high ionic strength
- Monitor success or failure of biomanipulation by tracking conductivity of plume (e.g. nitrate reduction).

Doll, ORNL
Electromagnetic Induction Logging

Spatial and temporal plume mapping during manipulation.

Complements direct groundwater geochemical tracer measurements.

Beard/Gamey/Doll, 2003
Seismic and Radar Tomography

Mapping subsurface material heterogeneities using cross-borehole techniques.

Potential use for assessing sustained bioreduction of metals and radionuclides.
Solid phase speciation using high resolution spectroscopy
X-ray absorption spectroscopy

Quantify valance state and chemical environment of contaminant species

Indigenous solid phase is used (no alteration of subsurface media)

Can be coupled with x-ray tomography to assess mechanism of metal reduction
High resolution surface spectroscopy techniques for quantifying contaminant speciation and chemical environment

Example:
- EXAFS used to quantify the chemical environment of solid phase U at the Y-12 site, Oak Ridge.
- U is coordinated by bidentate carbon containing groups and/or monodentate phosphorous containing groups.

Speciation controls rate of contaminant bioreduction

Kelly and Kemner, ANL
Mossbauer spectroscopy

Characterizing the role of biogenic Fe(II) on contaminant bioreduction

Mossbauer used to quantify the types, amounts, and distributions of various Fe-bearing minerals and oxides in heterogeneous FRC background and contaminated samples.

Quantify changes in Fe mineralogy following in situ biostimulation using various electron donors.

Quantify mechanisms of biogenic Fe(II) reactivity with the solid phase and its influence on the rate of contaminant bioreduction.

Zachara, PNNL
Extended X-ray Absorption Fine Structure (XAFS) used to quantify the Fe-oxide mineralogy in heterogeneous samples from the FRC.

Quantifying biogenic Fe products and changes in mineralogy during biostimulation.

Information on chemical state of competing electron acceptors important towards knowing likelihood of sustained contaminant reduction.
X-ray fluorescence microprobe image depicting the distribution of Fe on a Si sand grain after bioreduction. The scale bar (solid white line) represents 40 µm. The transect used for XANES spectra collection (speciation) is illustrated by the dotted white line.

Provides spatial extent of contaminant reduction.

Fendorf, Stanford

XANES spectra obtained along a 300 µm transect. XANES spectra were obtained every 5 µm. The peak at 7120 eV represents the concentration of magnetite relative to goethite/ferrihydrite.
Scanning and Transmission Electron Microscopy

Bacterially populated iron oxide coated sand grain

Scanning electron microscopic image of biogenic green rust.

Transmission electron micrograph of iron oxide clusters showing goethite laths emanating from a ferrihydrite conglomerate.

Fendorf, 2003 / Zachara, 2003
Conclusions

Performance assessment of in situ biostimulation strategies will require detailed monitoring of coupled hydrological, geochemical, and microbial processes.

Knowledge of the processes controlling bioreduction and metal immobilization is critical since competing terminal electrons acceptors and the intrusion of oxidants can impede or reverse the immobilization process.

Knowledge of the contaminant speciation and chemical environment will enhance the opportunity towards maintaining sustained bioreduction and metal immobilization.
Bioindicators

Thanks for having us!

“Radioactive Cats”