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

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Introduction

Department of Energy (DOE) facilities within the weapons complex face a daunting challenge of remediating huge below inventories of legacy radioactive and toxic metal waste. More often than not, the scope of the problem is massive, particularly in the high recharge, humid regions east of the Mississippi river, where the off-site migration of contaminants continues to plague soil water, groundwater, and surface water sources. As of 2002, contaminated sites are closing rapidly and many remediation strategies have chosen to leave contaminants in-place. In situ barriers, surface caps, and bioremediation are often the remedial strategies of chose. By choosing to leave contaminants in- place, we must accept the fact that the contaminants will continue to interact with subsurface and surface media. Contaminant interactions with the geosphere are complex and investigating long term changes and interactive processes is imperative to verifying risks. We must be able to understand the consequences of our action or inaction.

The focus of this manuscript is to describe recent technical developments for assessing the performance of in situ bioremediation and immobilization of subsurface metals and radionuclides. Research within DOE's NABIR and EMSP programs has been investigating the possibility of using subsurface microorganisms to convert redox sensitive toxic metals and radionuclides (e.g. Cr, U, Tc, Co) into a less soluble, less mobile forms. Much of the research is motivated by the likelihood that subsurface metal-reducing bacteria can be stimulated to effectively alter the redox state of metals and radionuclides so that they are immobilized in situ for long time periods. The approach is difficult, however, since subsurface media and waste constituents are complex with competing electron acceptors and hydrogeological conditions making biostimulation a challenge. Performance assessment of *in situ* biostimulation strategies is also difficult and typically requires detailed monitoring of coupled hydrological, geochemical/geophysical, and microbial processes.

In the following manuscript we will (1) discuss contaminant fate and transport problems in humid regimes, (2) efforts to immobilize metals and radionuclides *in situ* via bioremediation, and (3) state-of – the-art techniques for assessing the performance of *in situ* bioreduction and immobilization of metals and radionuclides. These included (a) in situ solution and solid phase monitoring, (b) in situ and laboratory microbial community analysis, (c) noninvasive geophysical methods, and (d) solid phase speciation via high resolution spectroscopy.

The problem associated with historical waste disposal practices in humid regimes

The disposal of inorganic contaminants at U.S. Department of Energy facilities within the Weapons Complex has historically involved shallow land burial in the vadose zone or liquid disposal in surface impoundments (Figs. 1 a,b). Disposal sites were often without physical or chemical barriers and this allowed for the rapid dissemination of radionuclides and toxic metals through the subsurface environment. In high recharge, humid regions east of the Mississippi river the scope of the problem is massive where the off-site migration of contaminants continues to plague soil water, groundwater, and surface water sources. In these regions subsurface transport processes are driven by large annual rainfall inputs where more than 50% of the infiltrating precipitation results in groundwater and surface water recharge. This condition promotes the formation of massive secondary contaminant sources since storm flow and groundwater interception with trenches and surface impoundments enhances the migration of waste constituents into the surrounding subsurface environment. Typically subsurface media in high recharge regimes is structured, and contaminant migration occurs through a complex continuum of pores (Fig. 2). Highly conductive voids (fractures, macropores) surround a low-permeability, high-porosity soil

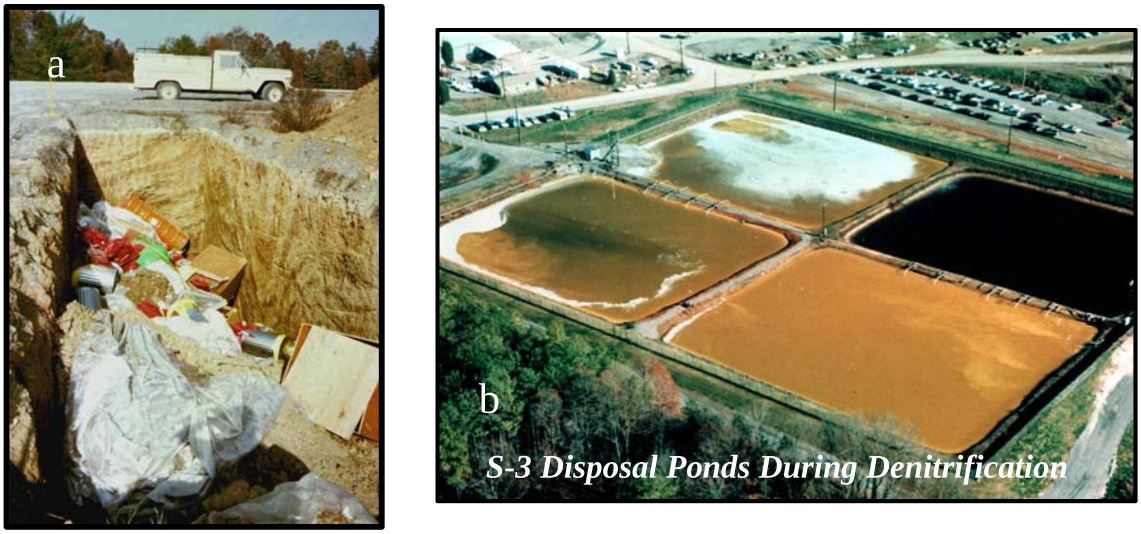


Figure 1: *(a) an example of radionuclide and metal disposal in unlined shallow trenches at ORNL and (b) surface impoundments at the Y-12 complex in Oak Ridge that were used for the disposal of acidic uranium-nitrate liquid waste.*

and bedrock matrix (Jardine et al., 2001). The media is conducive to extreme preferential flow that results in physical, hydraulic, and geochemical nonequilibrium conditions between fast flowing pathways and the surrounding soil matrix (Wilson et al., 1993; Jardine et al., 1999, 2002). In these systems contaminant migration rates can be extremely rapid along preferred flow paths and long-lived due to a significant inventory of the total waste in the soil and bedrock matrixes (secondary sources).

Figure 2: *Typical subsurface media on the Oak Ridge Reservation consisting of interbedded shale – limestone sequences with characteristic dipping bedding planes.*

In these systems, the soil and bedrock matrix (secondary sources) have been exposed to migrating contaminants for many decades, and thus account for a significant inventory of the total waste. A significant limitation in defining remediation needs of the secondary sources results from an insufficient understanding of the transport processes that control contaminant migration. Because of this, the historical remedial approach at many DOE facilities has been to target "hot spots". Stabilization, diversion, and containment of contaminants within the primary source areas have been the major emphasis. Techniques such as barriers, grouting, vitrification and local-scale capping (Fig. 3) have been used on high-risk exit pathways (e.g. preferential flow zones, seeps).

Figure 3: *Recent capping activities on the Oak Ridge Reservation in an effort to impede infiltrating storm water from entering subsurface waste trenches.*

The treatment of contamination in the soil and rock matrix has historically not been emphasized in most remedial endeavors. Three primary reasons are that (1) no feasible removal or immobilization technologies for large volumes of contaminated subsurface saprolite, bedrock, or groundwater are available, (2) remedial costs are often prohibitive, and (3) lacking knowledge on the risk posed by secondary sources. Although huge contaminant inventories reside in the secondary source domain, their low permeability significantly influences and slows contaminant mass-transfer to high-risk pathways.

Many contaminated sites have resorted to capping (Fig. 3) which typically does <u>not</u> completely immobilize contaminants in humid regimes due lateral flowing water and seasonal groundwater fluctuations. For this reason, the Department of Energy (DOE) has invested significant funding into basic science that seeks to understand how subsurface microorganisms can be effectively stimulated to immobilize metals and radionuclides in situ.

Efforts to immobilize toxic metals and radionuclides in situ using bioremediation

In April of 2000, DOE awarded Oak Ridge National Laboratory with a project to establish a field research center where investigators could obtain samples and conduct *in situ* studies that would lead to new insights into the bioremediation of metals and radionuclides and related contaminant fate and transport processes (http://www.esd.ornl.gov/nabirfrc/). They chose the former S-3 ponds at the Y-12 facility where unlined surface impoundments were used to dispose of acidic U, Tc, and NO_3 bearing waste during a period from the early 1950's to the early 1980's (Fig. 1b).

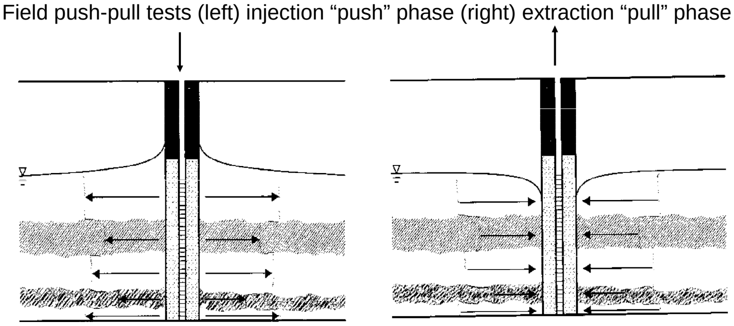
The Waste Disposal Units

The former S-3 Waste Disposal Ponds consisted of four unlined surface impoundments that were constructed in 1951. They received liquid nitric acid and uranium-bearing wastes via a pipeline at a rate of approximately 10 million liters/year until 1983. The Ponds were unlined and approximately 122-m × 122-m in dimension and 5.2-m deep (Fig. 1b). Infiltration was the primary release mechanism to soils and groundwater. In 1984, attempts were made to neutralize and biodenitrify the S3 ponds. The ponds were subsequently closed and capped in 1988. The site is currently a large asphalt parking lot.

Multiregion flow and transport mechanisms are the norm at the contaminated site which is underlain with fractured weathered saprolite derived from interbedded shale and limestone sequences. High permeability fractures surround low permeability, high porosity matrix blocks on the cm scale, thus the media is not only conducive to significant preferential flow, it is also a source/sink for contaminants (Fig. 2). Contaminants, such as U, Tc, NO_3, PCE, and toxic metals, migrate away from the waste disposal units following both strike and dip, with density effects being quite significant near the source. Near source groundwater concentrations of NO_3 can be as high as 40,000 ppm and U concentrations can be as high as 60 ppm with solid phase concentrations near or above 1000 mg U / kg solid. Elevated nitrate concentrations and significant U have been detected to several hundred feet owing to rapid movement through the saprolite and underlying bedrock.

Examples of In situ Bioremediation Studies at the Oak Ridge FRC

Figure 4a Field push-pull tests (left) injection "push" phase (right) extraction "pull" phase



Example 1: Field research using "push-pull" techniques

A research team headed by Jack Istok (Oregon State University), Lee Krumholz (University of Oklahoma), James McKinley (Pacific Northwest National Laboratory), and Baohua Gu (Oak Ridge National Laboratory) combines subsurface transport, microbiology, and geochemistry expertise to identify the conditions under which bioremediation may be effective for immobilizing uranium and technetium at the Oak Ridge FRC. These researchers want to discover whether naturally occurring microorganisms-in this case, bacteria-with the capability to immobilize uranium and technetium are present at the Y-12 site. Second, they seek to identify the optimum "feeding" conditions to stimulate the microorganisms and immobilize uranium and technetium at the highest possible rate. Finally, they want to determine how long contaminants are immobilized after biostimulation.

The team uses a "push-pull" technique in the field (Fig. 4b) in combination with laboratory analyses. As the name implies, push-pull tests consist of two main parts. The "push" refers to an injection into a well of a solution of site groundwater, tracers, and nutrients whose quantity and composition is known (Fig. 4b). Over time, this solution migrates from the well into the adjacent subsurface environment. Later, the "pull" portion of these tests occurs. The "pull" consists of withdrawing from the same well the injected solution mixed with groundwater. Analyses of these samples can reveal a lot of information about both the microbiological and chemical characteristics of groundwater and directly quantify the microbial activity of the indigenous microorganisms.

The team is working in over 20 wells at two Oak Ridge FRC research sites. Some wells are identified as “controls,” in which no nutrients are injected. Other “treatment” wells receive nutrient additions (acetate, ethanol, and glucose). Multiple wells are tested simultaneously to insure that field experiments are conducted under a wide range of conditions (contaminant levels, acidity, etc.) and therefore are representative of actual site conditions.

To test for microbial activity, site groundwater is mixed with tracers and nutrients in drums and injected into monitoring wells

The team has demonstrated that it is possible to stimulate indigenous bacteria to immobilize uranium and technetium (Fig. 5, Istok et al., 2004). Researchers also found that other bacteria that grow on the high nitrate contamination present at some sites are capable of remobilizing immobilized uranium (but not technetium). Current experiments are attempting to determine if it is possible to remove nitrate and uranium simultaneously, use humic materials to speed reaction rates, and determine if immobilized uranium can be stabilized by the addition of sulfate or other materials.

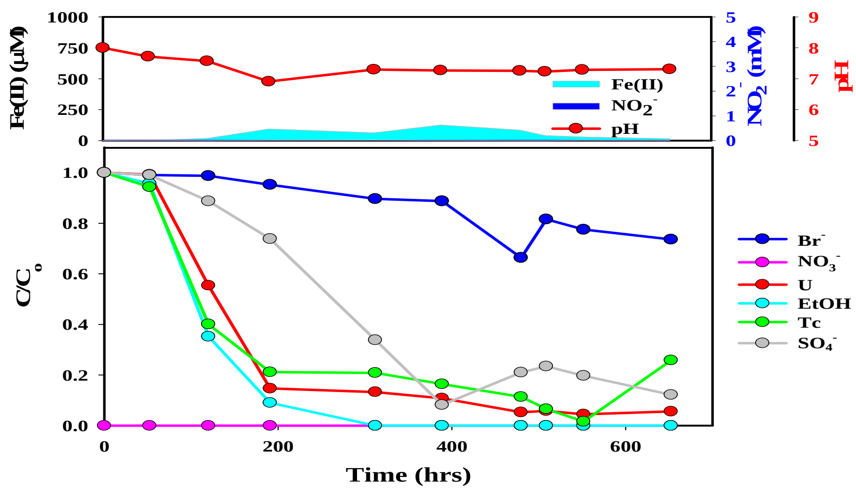


Figure 5: *Simultaneous uranium (U) and technetium (Tc) immobilization in the presence of competing terminal electron acceptor Fe(III)-oxides during field push-pull tests (Istok et al.)*

Example 2: Field research to immobilize uranium using a biostimulated hydraulic cage approach

A research team headed by Craig Criddle (Stanford University) and Philip Jardine (Oak Ridge National Laboratory) is conducting a research project to evaluate rates and mechanisms by which naturally occurring microorganisms transform solution and solid phase uranium VI to uranium IV. Since the research effort is focused near the source where nitrate concentrations are in the thousands of parts per million, the overall plan is to combine above-ground and subsurface activities. Above-ground efforts will remove contaminants that interfere with microbially mediated uranium transformation. Subsurface efforts will focus on immobilizing uranium in groundwater and on the solid phase.

Above ground. In essence, the above-ground activities condition the groundwater in preparation for *in situ* bioremediation of uranium (Fig. 6a). This conditioning consists of a series of processes, including a fluidized bed reactor (Fig. 6b) and pH adjustment that aim to remove aluminum, nitrate, and other groundwater contaminants. Conditioning groundwater in this way allows greater experimental control to assess the impacts of different subsurface characteristics on bioremediation.

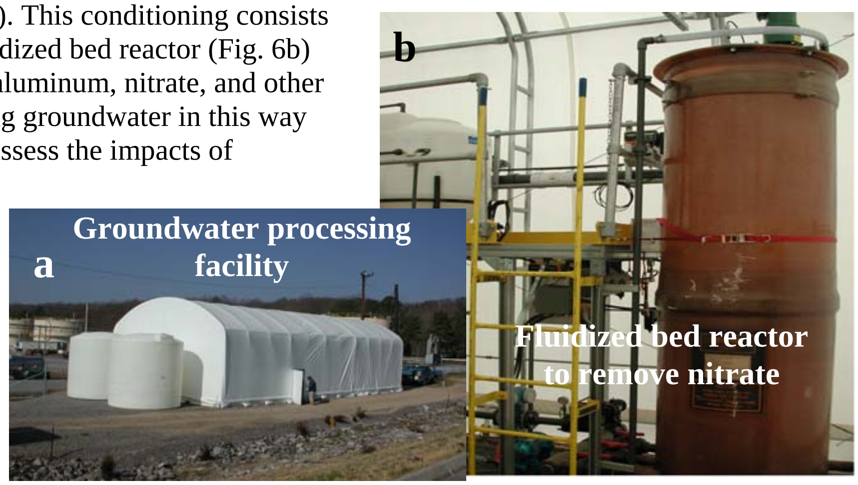
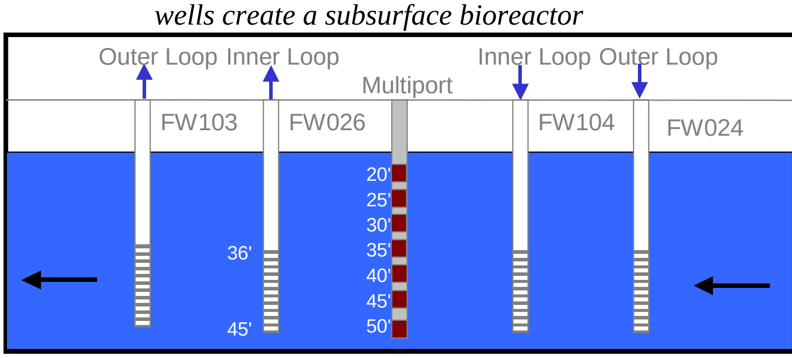


Figure 6

Below ground. In the subsurface, the research team has conducted numerous biostimulation experiments by injecting ethanol and re-circulating this conditioned groundwater in a series of wells (Fig 7 a,b). They have successfully transformed uranium VI into immobile uranium IV by stimulating microorganisms occurring naturally at the research site (Fig. 8). Through the use of X-ray Absorption Spectroscopy at the Advance Photon Source, the researchers could quantify the amount of reduced U(IV) present (Fig. 8).

Figure 7a: *Below-ground, a series of recirculating wells create a subsurface bioreactor*



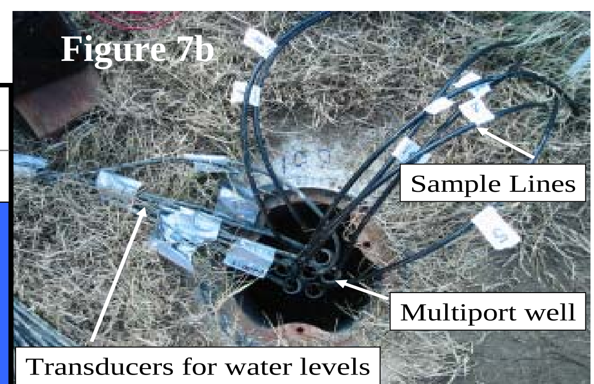
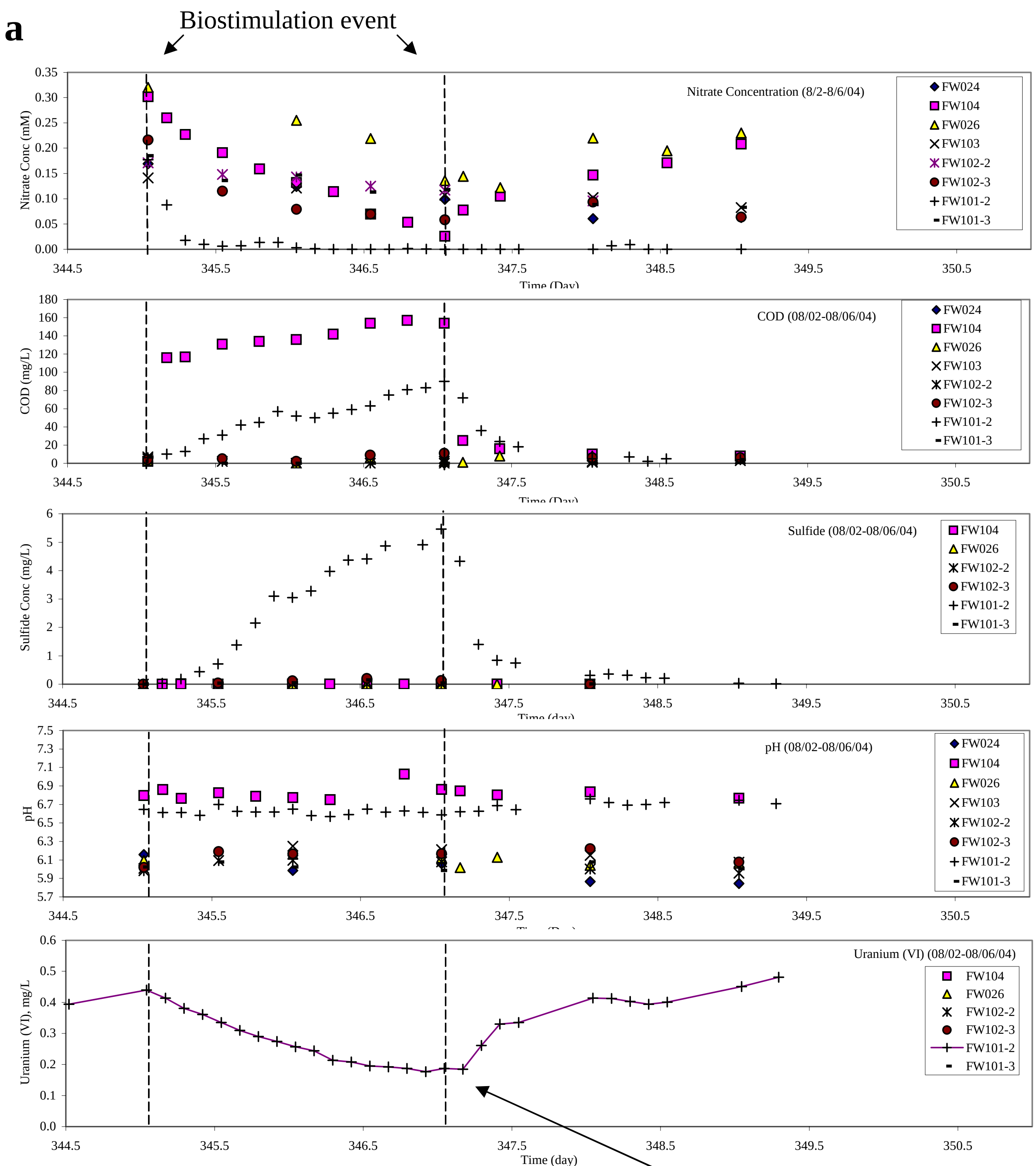


Figure 7b

Together, these studies will strengthen understanding of the microbiology and geochemistry needed to convert uranium VI into uranium IV in situ. Further, researchers hope that the above-ground pre-treatment scheme proves to be a cost-effective means of remediating mixtures of metals, solvents, nitrate, and radionuclides in highly contaminated source zones.



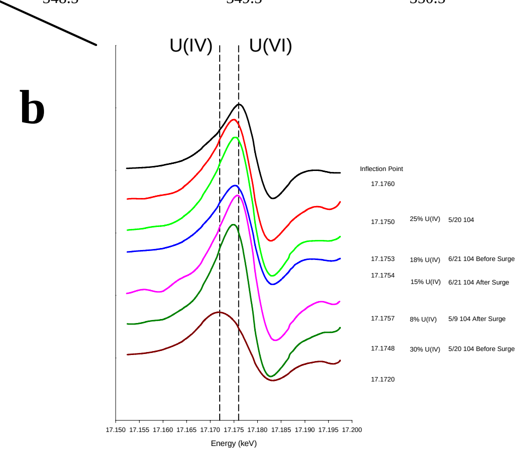


Figure 8: *(a) Field scale biostimulation during dynamic flow. Note the decrease in groundwater* NO_3*, U(VI), and increase in COD and sulfide. (b) XANES analysis of the solid phase confirms the reduction product U(IV) [Criddle, Carley, Wu, Ginder-Vogel, Stanford Univ./ORNL].*

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

Long-term stewardship of contaminated sites will require enhanced in situ and ex situ monitoring of coupled hydrological, geochemical, and microbial processes in order to assess the long term remedial stability / natural attenuation of contaminants. The following section discusses state-of-the-art techniques for assessing the performance of *in situ* bioreduction and immobilization of metals and radionuclides. These included (a) in situ solution and solid phase monitoring, (b) in situ and laboratory microbial community analysis, (c) noninvasive geophysical methods, and (d) solid phase speciation via high resolution spectroscopy.

Geochemical and geophysical techniques for long-term performance monitoring include; (1) in situ geochemical sensors for groundwater contaminants and oxidants, (2) high-resolution surface spectroscopy techniques that quantify the distribution and chemical environment of the solid phase contaminants and competing terminal electron acceptors, (3) cross-well seismic and radar tomography, and (4) seismic and resistivity surface tomography techniques for assessing real time changes in the subsurface geochemical environment. Hydrological monitoring techniques include (1) tracking pressure head dynamic in an effort to prevent biofouling, (2) tracer techniques for tracking changes in flow path dynamics following biostimulation, and (3) storm event monitoring to capture the influence of "new" water within the biostimulated zone. Microbial techniques for long-term performance monitoring of biostimulated subsurface regimes include (1) the use of microarray gene probes to rapidly assess in situ microbial community dynamics, (2) novel groundwater coupons and "bug traps" for microbial colonization and characterization, and (3) various molecular approaches to access phylogenetic diversity of the organisms involved in the biostimulation process. Each of these strategies or techniques is discussed below.

Solid and Solution Phase Geochemical Monitoring

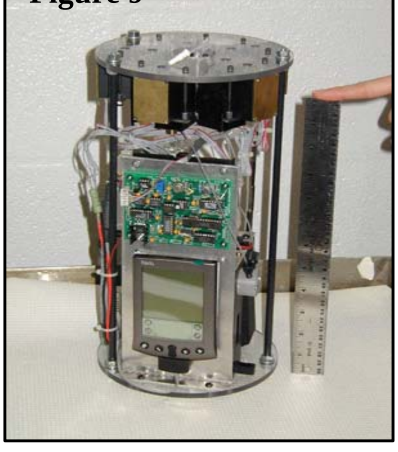
Groundwater contaminant detection and geochemical monitoring

Probably one of the more important items to monitor in the groundwater following a biostimulation experiment is the stability of the contaminant of interest and its long-term groundwater flux and discharge within the environment. If the immobilized contaminant is released into the solution phase, a thorough groundwater monitoring and sampling plan should capture any contaminant release. Ex-situ analytical detection devices are commonplace and are typically the easiest way to track the concentration or activity of the contaminant. However, several in situ contaminant sensing devices have recently been developed for in-line or down hole quantification of U(VI), Cr(VI), ^{99}Tc, gamma activity, and toxic metals.

Field portable immunoassay biosensor developed for detection of U *in situ*

(Blake of Tulane University)

Figure 9



At the Tulane University, Diane Blake and her research group have developed numerous field portable, down hole immunoassay biosensors for the detection of groundwater U(VI) and toxic metals in situ (Fig. 9 and Blake et. al., 2001 a,b). Antibody-based assays offer significant advantages for environmental analysis since immunoassays are quick, inexpensive, and simple to perform. The assays are typically quite sensitive and selective with the antibody-based assays being portable and thus can deliver near real-time data on environmental contamination. Like wise, a field-rugged, flow-through chemiluminescence sensor has recently been developed for hexavalent chromium. Gregory Gillispie of North Dakota State University has developed and built a series of transportable laser systems for field applications. The incorporation of ultraviolet tunable lasers into a rugged field unit is quite unique. Since Cr(VI) is potent carcinogen and highly mobile in soil and groundwater, real time monitoring with the chemiluminescence sensor is an attractive technology for assessing the flux of Cr(VI) from bioremediated sites. At ORNL, Brian Spalding has developed a down hole NaI detector for assessing in situ gamma in both the solution and solid phase. Contaminant profiles of gamma emitters, such as Tc, Th, U, etc, can be easily tracked with time and any changes in activity could be interrupted as a potential disruption to the bioremediate zone. Rapid assessment of the subsurface using this detector could serve as an initial indicator of potential problems at the bioremediated site. At PNNL, Michael Knopf has developed a sensor cell that quantifies the groundwater activity of ^{99}Tc (Fig. 10). By using an integrated preconcentrator / sensor cell design, Knopf have discovered an efficient method for the collection and sensing of ^{99}Tc. Knopf has combined this method with a superior detection and signal processing electronics and has successfully completed several field demonstrations. The method is apparently adaptable to the detection of other radionuclides.

Figure 10:

In situ monitoring of ^{99}Tc in groundwater

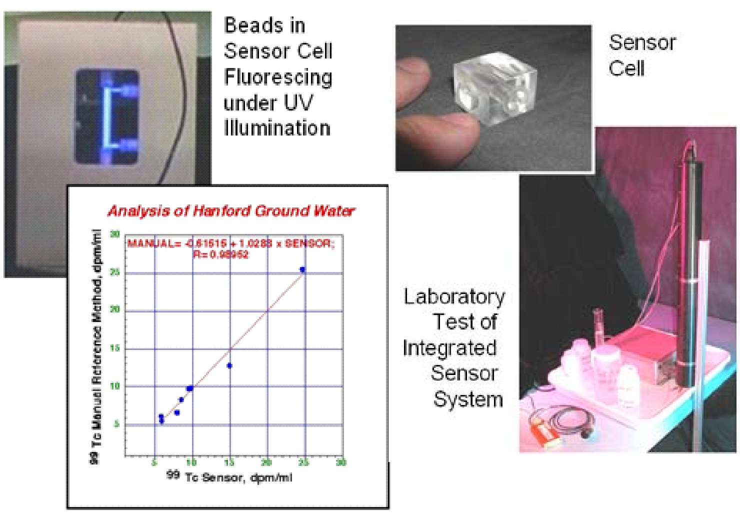


Figure 11: *Field portable X-ray Fluorescence of quantifying metals on soil and sediments*

Field portable X-ray fluorescence instruments are also available for the in situ quantification of toxic metals in soil and sediment cores (Fig. 11). Hand-held, field portable X-ray Fluorescence (XRF) analyzers can be equipped to analyze bulk soil samples or core in the field for elements such as Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, U, Hg, and Pb. Detection limits are often in the tens of ppm (e.g 15 ppm U on the solid phase). Such an instrument would accelerate the screening of cores acquired at contaminated sites when accessing site conditions following bioremediation.

The intrusion of oxidants (e.g. dissolved oxygen) and / or competing terminal electron acceptors (e.g. NO_3^-) are important groundwater constituents to monitor since they can interfere with the stability of bioreduced metals and radionuclides such as U(IV), ^{99}Tc, and As(III). The kinetics of contaminant reoxidation are typically quite rapid, thus a thorough groundwater monitoring and sampling plan should include continuous dissolved oxygen monitoring as well as the analysis of redox couples such as Fe(II)/Fe(III), S^{2-}/SO_4^{2-}, etc. Groundwater redox fluctuations are common-place in humid regimes since large pulses of dissolved oxygen (DO) can enter the groundwater during significant storm events. Such events can significantly altered the groundwater geochemistry and microbiology as well as the speciation of radionuclide and metal contaminants at the site.

<u>In situ solid phase contaminant analysis</u>

Another important item to monitor in the subsurface following a biostimulation experiment is the solid phase speciation and chemical environment of the contaminant of interest. Although more expensive than groundwater analyses, solid phase interrogation methods provide a direct quantitative measure of the stability of the contaminant on the solid phase.

X-ray Absorption Spectroscopy (XAS)

This high-resolution surface spectroscopy technique allows for the elucidation of the chemical and structural environment (valance state and surface configuration) of a contaminant on the sediment or soil solid phase (Fendorf and Sparks, 1996; Kemner et al., 1998; Fendorf, 2000; Bertsch and Hunter, 2001). XAS is one of the few atomic techniques for obtaining molecular level information that can be conducted in unaltered samples, which is crucial for examining the true *in situ* molecular-level speciation of these contaminants. XAS consist of an excitation edge known as the near edge portion of the spectra (XANES) and an extended fine structure portion of the spectra (EXAFS) (Fig. 12). The near edge provides information about the contaminant valence state while EXAFS provides information about the surface configuration and chemical environment of the contaminant (Fig. 13). It is also possible to couple XAS with other microspectroscopic techniques such as Raman spectroscopy and electron microscopy for examining the spatial association of the contaminant with the matrix material. Coupling of the techniques allows one to determine element distributions on undisturbed samples and to elucidate the spatial distribution of contaminant complexes. Another technique known as x-ray fluorescence microtomography is also being used to define the distribution and speciation of contaminants throughout grains observed to sequester the element. Microtomography is a particularly exciting advancement, providing the ability to define the distribution and sequestration mechanisms of contaminants (e.g., Hansel et al., 2001). Tomographic images provide a cross-sectional slice through a grain. These high-resolution X-ray techniques provide quantitative information on the contaminant chemical environment as related to adsorption strength and ultimately migration tendencies which is critical to our conceptual understanding and predictive capability of contaminant migration at bioremediated sites.

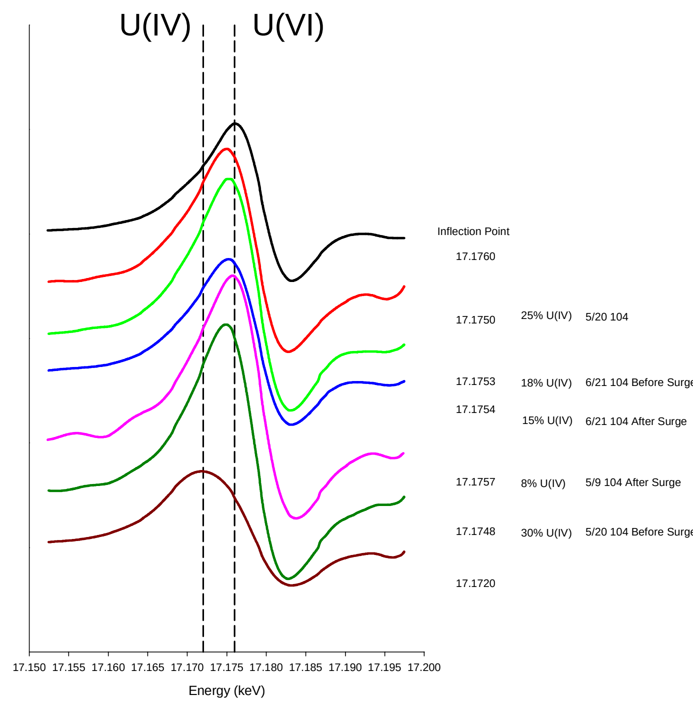


Figure 12: *XAS spectra of contaminated soil from a field site following biostimulation showing reduction of mobile U(VI) to immobile U(IV) [Ginder-Vogel, Fendorf , Stanford University]*

X-ray Absorption Spectroscopy is also useful for quantifying reactive minerals in soils that play a role in the redox properties or sequestration of metals in radionuclides in the subsurface. In particular, XAS is especially useful for quantifying biogenic Fe products and changes in Fe-oxide mineralogy during biostimulation. Uranium sorption and reduction is particular sensitive to Fe-oxide mineralogy since it is this mineral that generally dictates the magnitude of U sorption in humid environments. Also, U speciation is sensitive to the presence of reduced Fe(II) since this species is capable of reducing U(VI) to U(IV). Information on the chemical state of competing electron acceptors is important towards understanding the likelihood of sustained contaminant reduction.

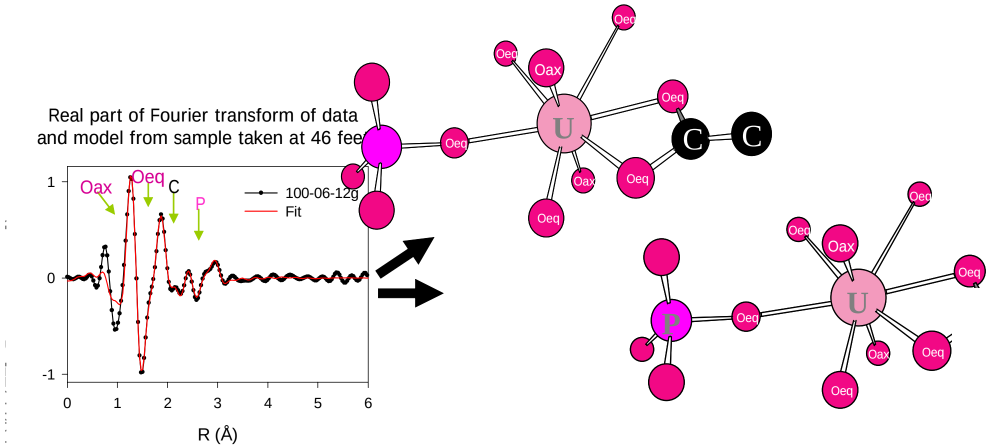


Figure 13: *Fourier transformed EXAFS data for U(VI) bound to heterogeneous humid regime soils at the NABIR FRC showing evidence of U-P and possibly U-CO_3 solid phase species (Kelly and Kemner, ANL).*

Mossbauer spectroscopy

Figure 14

Mossbauer spectroscopy at PNNL

Another technique that is useful for quantifying changes in Fe-oxide mineralogy following biostimulation of contaminated regimes is Mossbauer spectroscopy (Fig. 14). This technique has proven to be an excellent means for determining the oxidation state and coordination of iron in heterogeneous material (see for example Trolard et al., 1997). One can define the valence, mineral phase, and mass percent of different Fe-containing solids. Since ferric (hydr)oxide minerals will have a pronounced influence on contaminant retention it is imperative that the various Fe phase be correctly identified.   Mossbauer spectroscopy in conjunction with XAS and X-ray diffraction will positively identify which reactive Fe phases are present in the subsurface. Such information with help with characterizing the role of biogenic Fe(II) on contaminant bioreduction, quantifying changes in Fe mineralogy following in situ biostimulation using various electron donors, and quantifying the mechanisms of biogenic Fe(II) reactivity with the solid phase and its influence on the rate of contaminant bioreduction.

Permeable Environmental Leaching Capsules (PELCAPs)

An interesting and novel approach for assessing the amount of a contaminant lost from a solid phase over time has been developed by Spalding and Brooks of ORNL. Permeable Environmental Leaching Capsules (PELCAPs) have been developed where soil aggregates are doped with specific reference tracers and trapped within leachable capsules.   They are than lowered into groundwater monitoring wells and retrieved with time. Loss of the reference tracer is monitored with time. Thus a specific reference tracer, say U-233, can be tracked with time using gamma analysis and thus serves as a surrogate for U-238 which has been remediated via microbial reduction processes. The technique is applicable to many inorganic and radioactive elements (e.g., Cr, Cd, As, Pb, Hg, U, Tc, and Pu). The PELCAP technique is a nondestructive measurement of the amount of immobilized contaminant in a soil thereby avoiding the necessity for repeated, costly, and destructive soil sampling. One can also use them for direct comparison of several immobilization treatments, including a no-treatment control, under identical field conditions within the same well.

Non- and semi-invasive geophysical methods

There are several surface based and down hole geophysical methods that have been recently developed that prove useful for monitoring the success or failure of biomanipulated sites. Typically such information is derived from the extrapolation of data determined within expensive point source monitoring wells. Geophysical methods provide a three dimensional, large-scale view of the media structure, the location of contaminant plumes, and the likelihood of a successfully bioremediated site. Geophysical methods also serve as a rapid method for monitoring the re-intrusion of contaminants into a bioremediated site and provide information on where monitoring wells (Fig. 15) should be located in a bioremediated plume.

Electrical Resistivity and Seismic

Electrical resistivity and seismic are two surface-based geophysical tools that serve to map and/or profile contaminant plumes and the structure of the geologic units, respectively (Fig. 16 and Doll et al., 2002; Watson et al., 2004). Both are non-invasive methods that, in the past 5-10 years have been extended from more conventional one-dimensional imaging counterparts to provide two-dimensional profiles of the subsurface. Typically a multielectrode resistivity survey is conducted with 1 m and 2 m electrode separations in both dipole-dipole and Schlumberger configurations. Electrical resistivity is ideal for tracking the ionic strength of contaminant plumes and defining the location of hot-spots and areas to target for bioremediation (Fig. 16). It is also ideal for tracking the intrusion of contaminants into a bioremediated site. Seismic complements electrical resistivity by providing information on media structure based on density contrast. Often the media type and structure dictates the hydrology which in turn determines where the contaminant plume will reside.

Figure 15: *Imagine setting up a well field within this folded fractured shale formation without a good look of the material structure prior to well installation…a total disaster. There is the strong likelihood of complete flow path disconnect on one side of the fold vs. the other.*

Oak Ridge Shale

Electromagnetic Induction Logging

Electromagnetic Induction Logging is a high-resolution geophysical technique that can provide three-dimensional information about biostimulated sites. It is ideal for tracking the ionic strength of contaminant plumes or for tracking changes in the contaminant plume as bioremediation proceeds. It can be used in a time-lapse mode where the spatial and temporal changes to a plume can be quantified during manipulation. It has been shown to complement direct groundwater geochemical tracer measurements and appears to be a powerful method of assessing the bioremediation of metals and radionuclides at large scale and in three-dimension (Beard et al., unpublished data, ORNL).

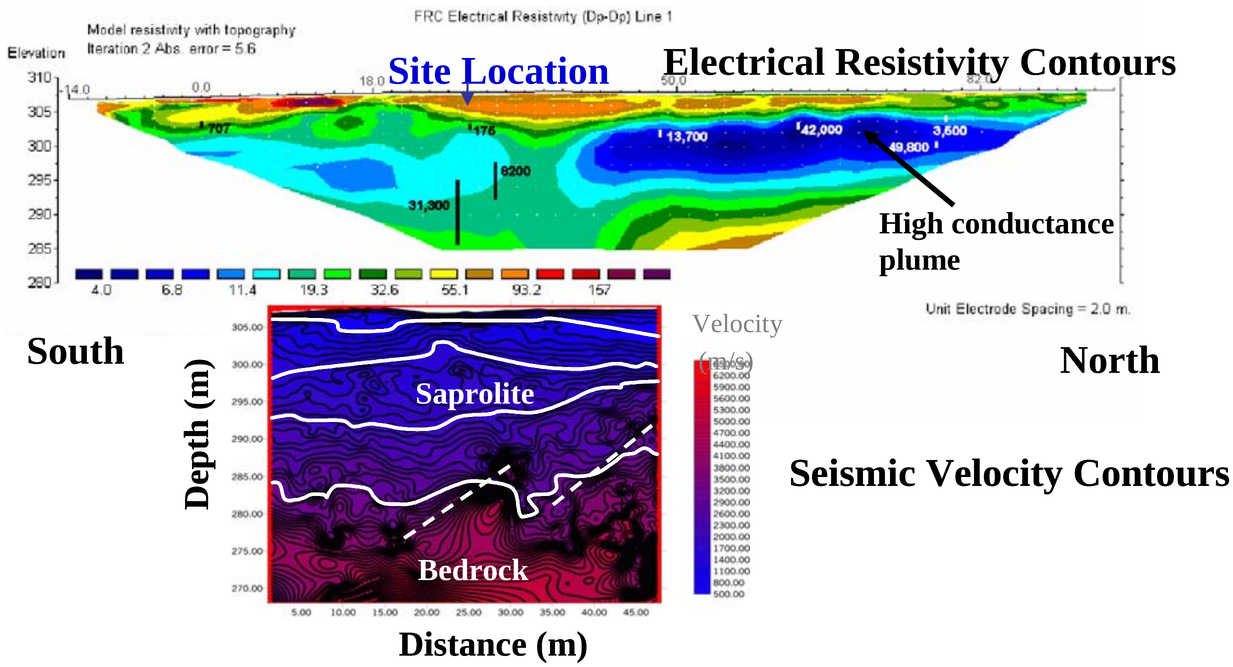


Figure 16: *Electrical resistivity contours of an acidic U – Nitrate plume at the Oak Ridge FRC with accompanying seismic velocity contours showing zones of varying media consolidation (Beard et al., ORNL)*

Seismic and Radar Tomography

Seismic and radar tomography are two high-resolution geophysical techniques that also can provide multi-dimensional information about biostimulated sites (Fig. 17). They are cross-borehole methods that serve to map subsurface material heterogeneities and have the potential ability of assessing sustained bioreduction of metals and radionuclides at contaminated sites. They have previously been used to estimate subsurface properties such as water content, hydraulic conductivity, fracture zonation, and sediment geochemistry and lithology (Hubbard et al., 1997 a,b; 2001; Chen et al., 2001; Grote et al., 2003). The methods can be used in a time-lapse mode to quantify the evolution of gas and precipitates during biostimulation. As with surface methods, the cross-borehole methods provide a multi- dimensional, large-scale view of the media structure, the location of contaminant plumes, and the likelihood of a successfully bioremediated site.

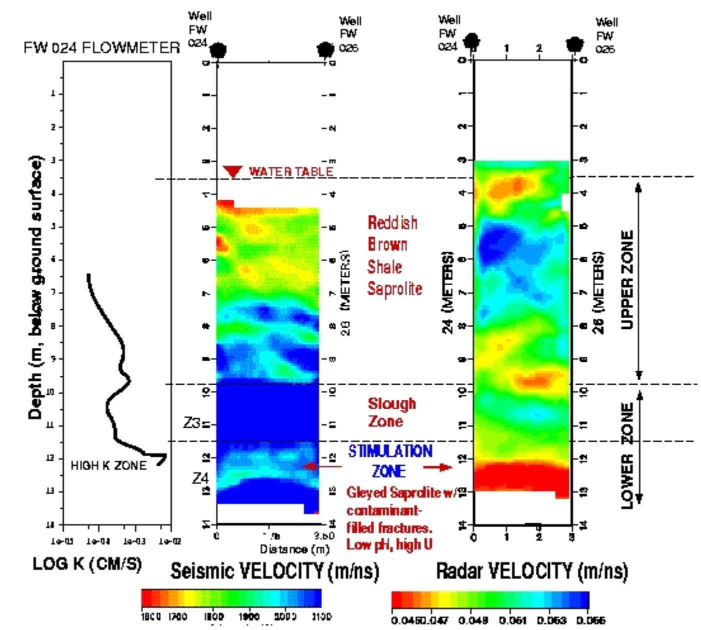


Figure 17: *Seismic velocity and radar velocity profiles of subsurface media at the Oak Ridge FRC showing high resolution estimates of subsurface zonation which correspond nicely to measured hydrologic conductivity measurements (Hubbard / Watson)*

Hydrological monitoring

There are numerous hydrological monitoring techniques that can be used to indirectly assess the performance of in situ bioreduction and immobilization of metals and radionuclides. When combined with geochemical and microbial monitoring approaches, the hydrological information adds further credibility to the success of the bioremediation endeavor. Several techniques include tracking pressure head dynamic in an effort to prevent biofouling, tracer techniques for tracking changes in flow path dynamics following biostimulation, and storm event monitoring to capture the influence of "new" water within the biostimulated zone.

Continuous monitoring of the groundwater level using pressure transducers is a rapid and effective technique for capturing the possibility of biofouling within groundwater injection wells or within the formation during biostimulation. Monitoring the hydraulic head and any shifts in the groundwater hydraulic gradient can assist with the adjustment of feeding rates so as to optimize the biostimulation process. Storm event monitoring is also critical since the interaction of "new" water with the biostimulation zone could adversely influence bioreduction. In humid regimes, the hydraulic gradient through contaminated sites often increases significantly during the winter and spring months due to the influx of new water into the system (e.g. rainfall under conditions of low evapotranspiration. New water generally has a higher dissolved oxygen concentration than the resident groundwater and certain contaminants, such as U(IV), can be easily reoxidized to a more mobile species (e.g. U(VI)).
Thus a thorough spatial and temporal monitoring of groundwater flux and hydraulic gradient at a site are critical for the assessment of bioremediation performance and biofouling.

Changes in flow path dynamics due to biostimulation can also influence the effectiveness of the bioremediation process. Bioclogging and metal or radionuclide precipitates can potentially build to levels that cause groundwater diversion. Numerous groundwater tracers are available for assessing changes in flow path dynamics during bioremediation. Using combinations of tracers often allows one to quantify the rate and direction of groundwater flow with time and the extent of preferential fracture flow vs. matrix diffusion. The use of multiple nonreactive tracers with different diffusion coefficients is an excellent method for quantifying the magnitude of matrix diffusion versus preferential flow (Jardine et al, 1998; 1999; Mayes et al., 2003). Nonreactive tracers such as Br, fluorobenzoates, and dissolved gases He, Ne, Kr, and SF_6 are all useful for this purpose.

Microbial monitoring

There are numerous microbial monitoring techniques that can be used to indirectly assess the performance of in situ bioreduction and immobilization of metals and radionuclides. Microbial techniques for long-term performance monitoring of biostimulated subsurface regimes include (1) the use of microarray gene probes to rapidly assess in situ microbial community dynamics, (2) novel groundwater coupons and "bug traps" for microbial colonization and characterization, and (3) various molecular approaches to access phylogenetic diversity of the organisms involved in the biostimulation process.

Groundwater microbiology

Numerous microbiological monitoring techniques exist for indirectly monitoring the performance of in situ bioreduction and immobilization of metals and radionuclides. Direct cell counts of the groundwater are an easy, cost-effective technique that can be used to monitor microbial biomass as a function of time. More recently stains are available to differentiate live and dead cells, thus providing information about the metabolically-active contingent of the community. Thus, during a healthy bioremediation endeavor, live biomass increases would be expected. Direct cell counts can also be directly correlated with field site geochemical dynamics providing stronger evidence of site performance. Direct cell counts are easier and less-expensive to perform and therefore can be used to monitor the site on a more frequent basis.

In situ microbial community analysis is another technique that determines whether a groundwater system is conducive to bioreduction (Fig. 18). These molecular approaches can be used to assess any changes in the microbial community structure as a function of time during biostimulation (Yan et al., 2003; North et al., 2004). Phylogenetic diversity of samples is characterized using SSU rDNA-based cloning methods where both DNA and mRNA are extracted simultaneously from groundwater samples. The SSU rDNA clonal libraries provide a qualitative assessment of both the cultivable and uncultivable community, but are not directly quantitative. Serial dilutions of groundwater can also be used to establish enrichments of microbial communities with biochemical capacities of interest (e.g., uranium-reduction, TCE-degradation). A medium is typically devised that matches the groundwater geochemistry and possible energy and carbon sources from the site. Serial dilutions allow for the enumeration of predominant, cultivable communities with the biochemical capacity for metal reduction. These approaches are designed to quantify any changes in the cultivable and uncultivable groundwater populations, and what type of biochemical capacity may be present and how it changes over time in relation to the bioremediation endeavor.

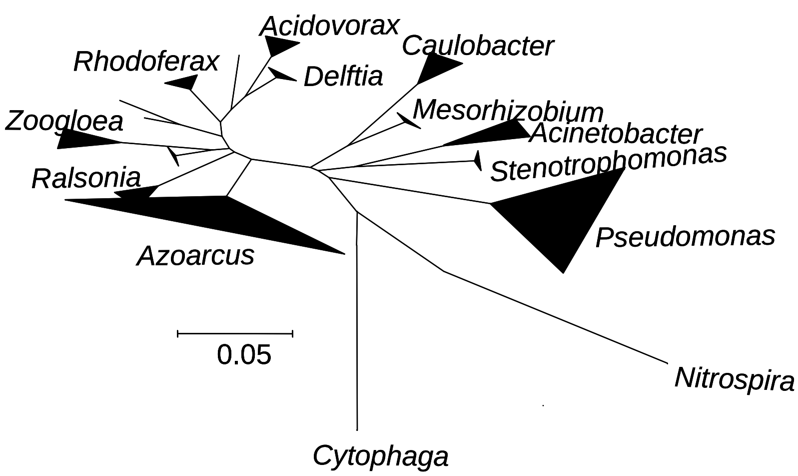
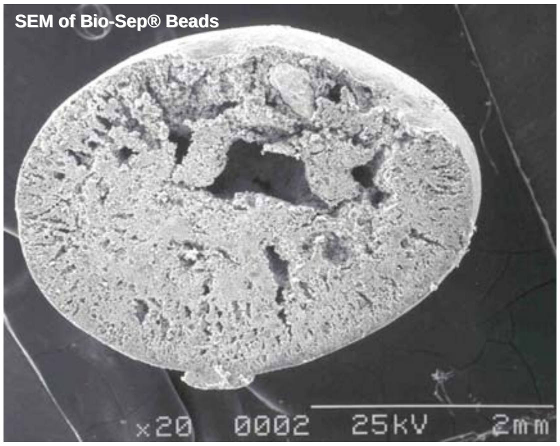


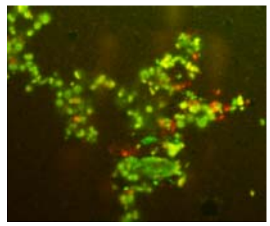
Figure 18: *Phylogenetic analysis of groundwater 16S rRNA clonal library from the Oak Ridge FRC. Iron, nitrate, and sulfate reducing organisms were isolated with the later shown to effectively reduce uranium (Fields et al., University of Miami, Ohio).*

Microbiology captured on Bio-Traps

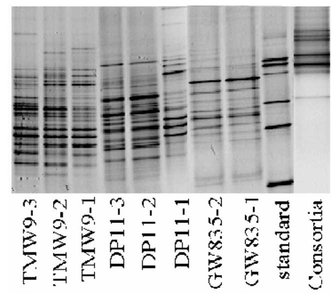
A novel technique that attempts to capture biofilm community structure in the subsurface, has recently been developed by the University of Tennessee (Peacock et al., 2004). David White and Aaron Peacock are deploying Bio-Sep® Beads in groundwater monitoring wells for enhanced microbial monitoring during biostimulation (Fig. 19). The beads are 2-3 mm in diameter, they have a huge porosity of 74%, a huge 600 m^2 surface area/g, and they are autoclavable. Biofilms form rapidly in the Bio-Sep® Beads thereby serving as an efficient cost effective method for sampling biofilms. More importantly the biofilm community structure on the beads is more indicative of *in situ* microbial ecology than samples of planktonic organisms, and the biofilms are an integrated response over time which is better than “grab samples”. Typically phospholipid fatty acid analysis and DGGE analysis of DNA are performed on extracts from the samplers. The beads serve as an excellent technique for assessing the effects of biostimulation on microbial biomass, community composition, and metabolic state.



Colonized Surface



DGGE Profiles of 16S rDNA



SEM of Bio-Sep® Beads,

Figure 19: *An example of a Bio-Sep bead that is used to enhance the formation of bioaggregates for which 16S rDNA can be extracted for microbial characterization (White and Peacock, Univ. Tennessee).*

Microbiology captured on Bug-Traps

In a similar fashion as the Bio-traps, Bug-traps or coupons have also been deployed in groundwater monitoring wells during biostimulation with the intent of rapidly assessing *in situ* microbial activity (Fig 20). Various material such as Fe-oxides and indigenous sediments are used for colonization. As with the Bio-traps, the biofilms generated on the colonized surface are an integrated response over time which is better than “grab samples”. Thus the Bug-Traps provide a rapid assessment of microbial community dynamics as a function of space and time and provides evidence that the correct organisms remain active in the biostimulated zone. Typically clone libraries of 16S rRNA gene sequences are generated from the coupons and microbial community structure and activity during biostimulation is assessed.

“Bug traps”

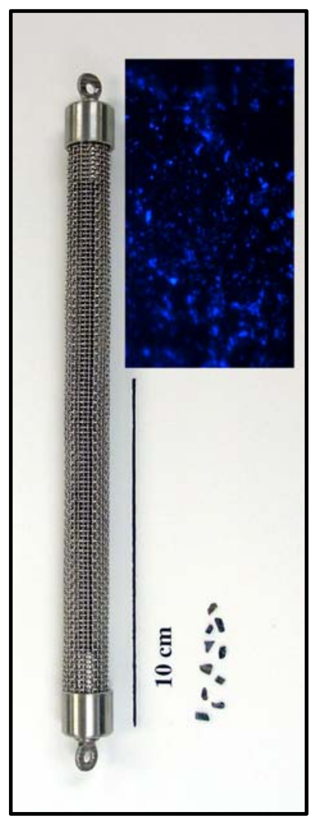
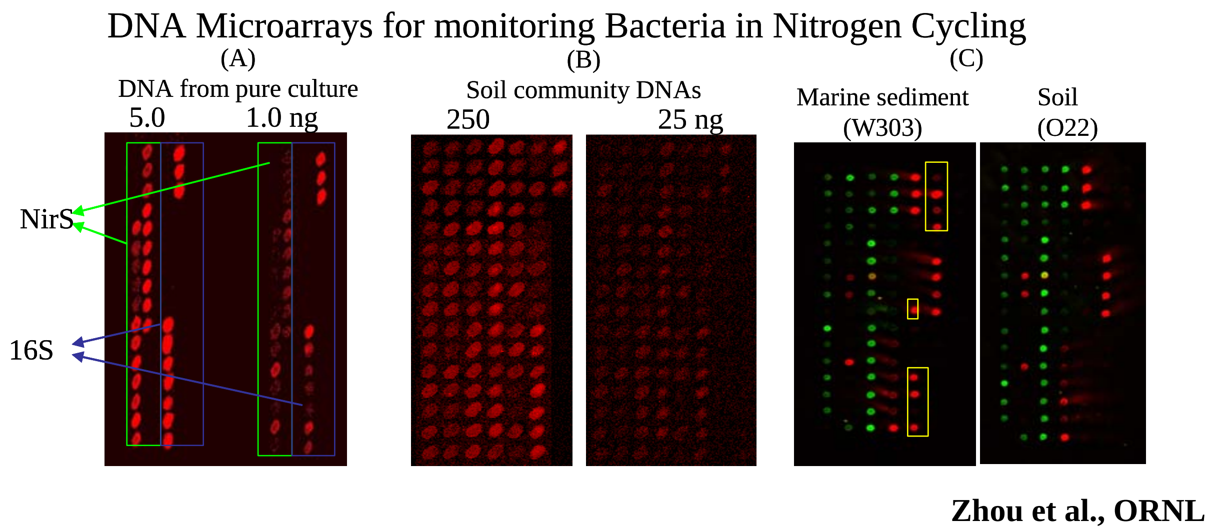


Figure 20: *An example of a bug trap filled with porous media or something similar to enhance the formation of bioaggregates for microbial characterization (Geesey, INNEL)*

DNA Microarrays for Capturing Subsurface Microbiology

Microarrays allow for rapid monitoring of the expression level of genes involved in the biostimulation process (Fig. 21). The DNA microarray technology offers great savings in time, effort, and cost, yet complement traditional molecular microbiology techniques. They provide a rapid method to assess shifts in microbial community structure via gene detection and expression (Wu et al., 2001; Beliaev et al., 2002; Zhou and Thompson, 2002, 2004). The presence of genes (DNA) is an indirect detection of activity while the expression of genes (mRNA) is direct measurement of activity. 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 due to low biomass in most groundwater sources. Gene categories that exist on the arrays monitor for carbon degradation and fixation, metal resistance and reduction, methanogenesis, nitrogen fixation, metabolism, and reduction, and sulfur reduction. Probe numbers vary between category, however, several thousands of probes are present for most categories with a total of 24,000 probes present on the arrays. The information obtained from gene expression and microbial community microarrays combined with the hydrological and geochemical processes obtained at a site will provide a more complete understanding of the processes involved during bioremediation.

Figure 21:



Conclusions

Contaminated sites on U.S. DOE lands are closing rapidly and many remediation strategies have chosen to leave contaminants in-place. In situ barriers, surface caps, and bioremediation are often the remedial strategies of choice. By choosing to leave contaminants in- place, we must accept the fact that the contaminants will continue to interact with subsurface and surface media. Long-term stewardship of contaminated sites will require enhanced in situ and ex situ monitoring of coupled processes in order to assess the long term remedial stability / natural attenuation of contaminants. Performance assessment of in situ bioremediation strategies are not exempt from this notion and will require detailed monitoring of coupled hydrological, geochemical, and microbial processes. This is particularly true for metal and radionuclide contaminated sites since these constituents do not degrade as do organics. Rather they change valance state or ligand association which in turn changes their solubility or geochemical interaction with the solid phase. Often the changes are reversible and this is why long-term stewardship and monitoring of metal and radionuclide contaminated sites is so important. This chapter has attempted to describe some of the techniques for assessing the performance of in situ bioreduction and immobilization of metals and radionuclides in contaminated subsurface environments. Its intent is to demonstrate that not only should the contaminant flux of interest be monitored, but that competing coupled processes that influence contaminant mobility should be assessed as well. Knowledge of the processes controlling bioreduction and metal immobilization is critical since competing terminal electron acceptors and the intrusion of oxidants can impede or reverse the immobilization process. Knowledge of the contaminant speciation and chemical environment is also important in enhancing the opportunity towards maintaining sustained bioreduction and metal/radionuclide immobilization. The impact of the remedial perturbation on the site hydrology, geochemistry, and microbiology are all linked and thus control the routes with regard to bioaccumulation and bioavailability of contaminants in the environment. Future generations are depending upon today's science to ensure that our decision to allow legacy waste to remain in the subsurface was the correct and necessary action. We owe it to our children and their grandchildren to establish a long-term stewardship program that serves as a watchdog to ensure immobilized contaminants stay in-place.

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References

Beliaev, A. S., D. K. Thompson, M. Fields, L. Wu, D. P. Lies, K. H. Nealson, and J. Zhou. 2002. Microarray transcription profiling of a Shewanella oneidensis etrA mutant. Journal of Bacteriology 184:4612-4616.

Bertsch PM, Hunter DB . 2001. Applications of synchrotron-based X-ray microprobes. Chemical Reviews, 101 (6): 1809-1842.

Blake, D.A , R.M. Jones, R.C. Blake II, A.R. Pavlov, I.A. Darwish, and H. Yu. 2001. Antibody-based sensors for heavy metal ions, Biosens. Bioelectron., 16:799-809.

Blake, D.A., A.R. Pavlov, H. Yu, M. Khosraviani, H.E. Ensley, and R.C. Blake II. 2001. Antibodies and antibody-based assays for hexavalent uranium, Anal. Chim. Acta, 444:3-11.

Chen, J., S. Hubbard and Y. Rubin. 2001. Estimating Hydraulic Conductivity at the South Oyster Site from Geophysical Tomographic Data using Bayesian Techniques based on the Normal Linear Regression Model. Water Resources Research, 37(6), 1603-1613.

Doll, W.E., J. Ganey, D.B. Watson, and P.M. Jardine, Geophysical profiling in support of a nitrate and uranium groundwater remediation study, SAGEEP extended abstract, EEGS Meeting, Las Vegas, Feb. 2002.

Fendorf, S.E. 2000. Fundamental aspects and applications of x-ray absorption spectroscopy in clay and soil science. In D.G. Schulze and P.M. Bertsch (Eds.) Applications of synchrotron radiation in clay science. CMS Workshop Lectures, Vol. 9. Clay Mineral Society, Ottawa, Canada.

Fendorf, S.E., and D.L. Sparks. 1996. X-ray absorption fine structure spectroscopy. In Methods of Soil Analysis: Chemical Methods. J.M. Bigham (Ed), ASA, Madison, WI. p. 377-416.

Grote, K., S. Hubbard, and Y. Rubin. 2004. Field-Scale Estimation of Water Content using GPR Groundwave Techniques, Water Resources Research. 39(11): 1321.

Hansel, C.M., M.J. La Force, S.E. Sutton, S. Fendorf. 2001. Ecosystem dynamics of zinc and manganese within a mine-waste impacted wetland. Geochemical Society Special Publication, S. Wood and R. Hellmann (Eds.). Crerar Volume.

Hubbard, S.S., J.E. Peterson, E.L. Majer, P.T. Zawislanski, K.H. Williams, J. Roberts, and F. Wobber. 1997a. Estimation of permeable pathways and water content using tomographic radar data, The Leading Edge, 16(11), 1623.

Hubbard, S.S., Y. Rubin, and E. Majer. 1997b. Ground Penetrating Radar-Assisted Saturation and Permeability Estimation in bimodal systems, Water Resources Research, 33(5), 971-990.

Hubbard, S., Chen, J., Peterson, J., Majer, E., Williams, K., Swift, D., B. Mailliox and Y. Rubin. 2001. Hydrogeological Characterization of the D.O.E. Bacterial Transport Site in Oyster Virginia using Geophysical Data, Water Resources Research, 37(10), 2431-2456.

Istok J.D., J.M. Senko, L.R. Krumholz, D. Watson, M.A. Bogle, A. Peacock, Y.J. Chang , and D.C. White. 2004. In situ bioreduction of technetium and uranium in a nitrate-contaminated aquifer. Environ. Sci. Technol. 38 (2): 468-475.

Jardine, P.M., R. O'Brien, Wilson, G.V., and J.P. Gwo. 1998. Experimental techniques for confirming and quantifying physical nonequilibrium processes in soils. p. 243-271. (In) H.M. Selim and L. Ma. Physical Nonequilibrium in Soils: Modeling and Application. Ann Arbor Press, Inc. Chelsea, Michigan.

Jardine, P.M., W.E. Sanford, J.P. Gwo, O.C. Reedy, D.S. Hicks, R.J. Riggs, and W.B. Bailey. 1999. Quantifying diffusive mass transfer in fractured shale bedrock. Water Resour. Res. 35:2015-2030.

Jardine, P.M., G.V. Wilson, R.J. Luxmoore, and J.P. Gwo. 2001. Conceptual Model of Vadose-Zone Transport in Fractured Weathered Shales. (In) Conceptual Models of Flow and Transport in the Fractured Vadose Zone. U.S. National Committee for Rock Mechanics. National Research Council. National Academy Press, Washington D.C. p. 87-114.

Jardine, P.M., T.L. Mehlhorn, I.L. Larsen, W.B. Bailey, S.C. Brooks, Y. Roh, and J.P. Gwo. 2002. Influence of hydrological and geochemical processes on the transport of chelated-metals and chromate in fractured shale bedrock. J. Contamin. Hydrol. 55:137-159.

Kemner, K.M., W. Yun, Z. Cai, B. Lai, H. -R. Lee, D. G. Legnini, W. Rodrigues, J. Jastrow, R. M. Miller, M. A. Schneegurt, C. F. Kulpa Jr., S. T. Pratt, M. A. Schneegurt, C. F. Kulpa, Jr., A. J. M. Smucker. 1998. Using X-ray microprobes for environmental research. (In) X-Ray Microfocusing: Applications and Techniques SPIE 3449, 45-53.

Mayes, M.A., P.M. Jardine, T.L. Mehlhorn, B.N. Bjornstad, J.L. Ladd, and J.M. Zachara. 2003. Hydrologic processes controlling the transport of contaminants in humid region structured soils and semi-arid laminated sediments. J. Hydrol. 275:141-161.

North N.N, S.L. Dollhopf, L. Petrie, J.D. Istok, D.L. Balkwill, J.E. Kostka. 2004. Change in bacterial community structure during in situ Biostimulation of subsurface sediment co-contaminated with uranium and nitrate. Applied Environ. Microbiology 70 (8): 4911-4920.

Peacock A.D., Y.J. Chang, J.D. Istok, L. Krumholz, R. Geyer, B. Kinsall, D. Watson, K.L. Sublette, and D.C. White. 2004. Utilization of microbial biofilms as monitors of bioremediation. Microbial Ecology. 47 (3): 284-292.

Trolard F, J.M.R. Genin, M. Abdelmoula, G. Bourrie, B. Humbert, and A. Herbillon. 1997. Identification of a green rust mineral in a reductomorphic soil by Mossbauer and Raman spectroscopies. Geochimica et Cosmochimica Acta. 61 (5): 1107-1111.

Watson, D.B. W.E. Doll, T.J. Gamey, J.R. Sheehan, and P.M. Jardine. 2004. Use of geophysical profiling to characterize the DOE NABIR Field Research Center. Groundwater (in press).

Wilson, G.V., P.M. Jardine, J.D. O'Dell, and M. Collineau. 1993. Field-scale transport from a buried line source in unsaturated soil. J. Hydrology 145:83-109.

Wu, L., D. K. Thompson, G. Li, R. A. Hurt, J. M. Tiedje, and J. Zhou. 2001. Development and evaluation of functional gene arrays for detection of selected genes in the environment. Applied and Environmental Microbiology 67:5780-5790.

Yan T.F, M.W. Fields, L.Y. Wu, Y.G. Zu , J.M. Tiedje, and J.Z. Zhou. 2003. Molecular diversity and characterization of nitrite reductase gene fragments (nirK and nirS) from nitrate- and uranium-contaminated groundwater. Environ. Microbiology 5 (1): 13-24.

Zhou, J.-Z., and D. K. Thompson. 2002. Microarrays: Applications in Environmental Microbiology, p. 1968-1979. In Britton, G. (ed.), Encyclopedia of Environmental Microbiology, vol. 4. John Wiley & Sons, New York.

Zhou, J. and D. K. Thompson. Microarray Technology and Applications in Environmental Microbiology. 2004. In Sparks, D. L. (ed.), Advances in Agronomy, vol. 82. Elsevier Inc., San Diego, CA (in press).