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# **Performance Indicators for Uranium Bioremediation in the Subsurface: Basis and Assessment**

**Letter Report to the  
Nuclear Regulatory Commission**

**December 15, 2006**

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Natural Resources Division  
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## List of Acronyms

<b>Organizational Acronyms</b>	
BER	Office of Biological and Environmental Research in DOE/SC
DOE	U.S. Department of Energy
ERSD	Environmental Remediation Sciences Division of DOE/SC/BER
ERSP	Environmental Remediation Sciences Program administered by DOE/SC/BER/ERSD
NABIR	Natural and Accelerated Bioremediation Research; program superseded by Environmental Remediation Sciences Program
PNNL	Pacific Northwest National Laboratory
SC	Office of Science in the DOE
UMTRA	Uranium Mill Tailings Remedial Action program out of U.S. DOE
<b>Terminology</b>	
16S rRNA clone library	technique to identify microorganism phylogeny by comparison to known gene sequences
Ca	calcium
DGGE	denaturing gradient gel electrophoresis; gene sequence comparison technique
DNA	deoxyribonucleic acid
DO	dissolved oxygen
Fe	iron
Fe(II)	iron in the +2 oxidation state
Fe(III)	iron in the +3 oxidation state
ISL	in situ leaching
MCL	maximum contaminant level; drinking water standard established by the U.S. Environmental Protection Agency
Mn	manganese
Mn(II)	manganese in the +2 oxidation state
Mn(IV)	manganese in the +4 oxidation state
mRNA	messenger ribonucleic acid
ORP	oxidation reduction potential
pH	negative log <sub>10</sub> of the hydrogen ion activity
PLFA	phospholipid fatty acid; analysis for microbial community structure using fatty acid biomarkers
<u>rDNA</u>	ribosomal DNA
<u>RNA</u>	ribonucleic acid
rRNA	ribosomal RNA
S	sulfur
<i>Sp.</i>	species
TEAP	terminal electron accepting process
U	uranium
U(IV)	uranium in the +4 oxidation state
U(VI)	uranium in the +6 oxidation state

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# ***Performance Indicators for Uranium Bioremediation in the Subsurface: Basis and Assessment***

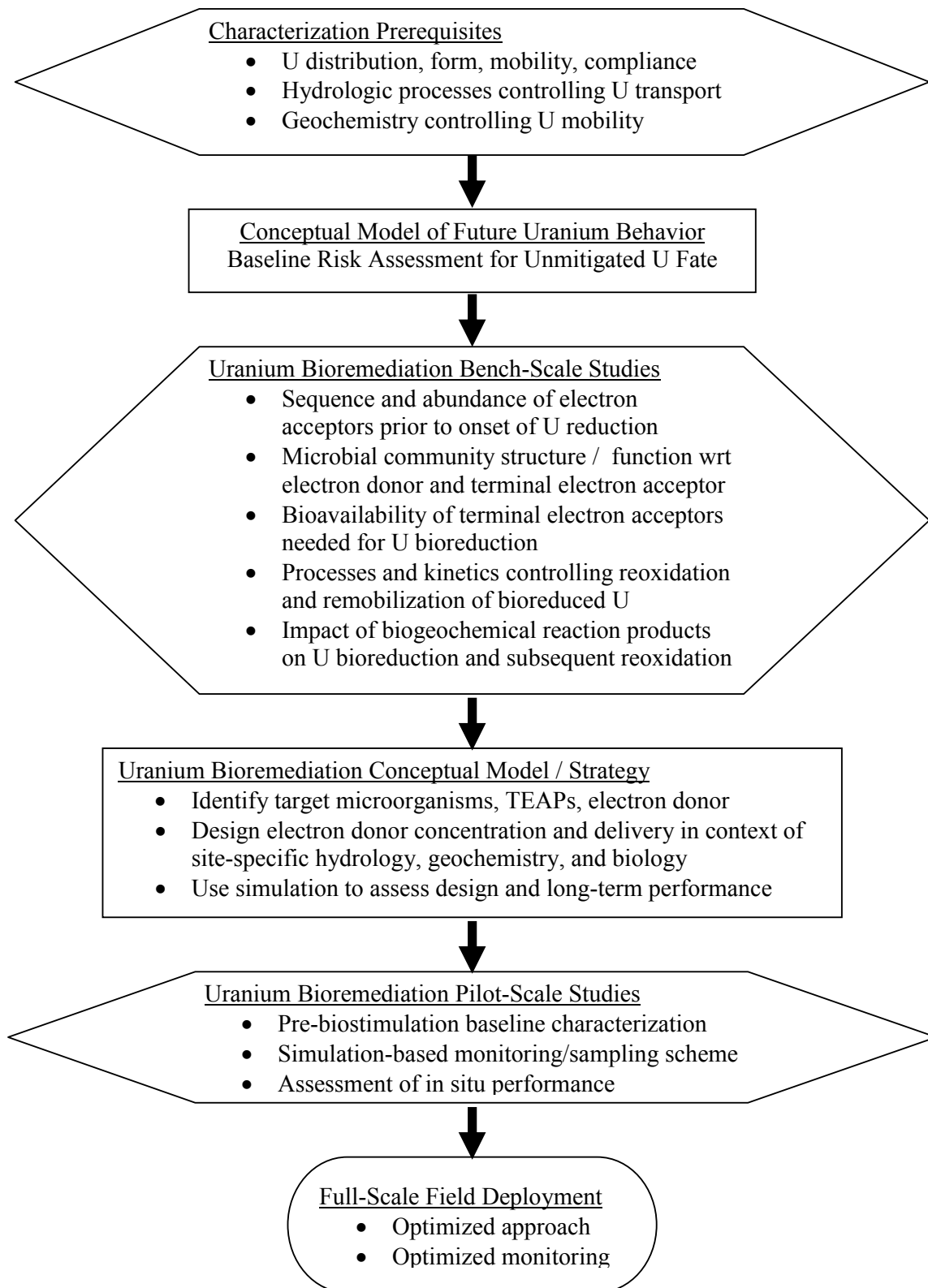
## **1 Introduction**

The purpose of this letter report is to identify performance indicators for *in situ* engineered bioremediation of subsurface uranium (U) contamination. This report focuses on in situ treatment of groundwater by biostimulation of extant in situ microbial populations (see

[http://128.3.7.51/NABIR/generalinfo/primers\\_guides/03\\_NABIR\\_primer.pdf](http://128.3.7.51/NABIR/generalinfo/primers_guides/03_NABIR_primer.pdf) for background information on bioremediation of metals and radionuclides). The treatment process involves amendment of the subsurface with an electron donor such as acetate, lactate, ethanol or other organic compound such that in situ microorganisms mediate the reduction of U(VI) to U(IV). U(VI) precipitates as uraninite or other insoluble U phase. Uranium is thus immobilized in place by such processes and is subject to reoxidation that may remobilize the reduced uranium. Related processes include augmenting the extant subsurface microbial populations, addition of electron acceptors, and introduction of chemically reducing materials such as zero-valent Fe. While metrics for such processes may be similar to those for in situ biostimulation, these related processes are not directly in the scope of this letter report.

The field of subsurface bioremediation has many facets and is rapidly evolving. This report, however, is limited to an overview of the underlying scientific basis for immobilization of U(VI) in groundwater via in situ biostimulation and recommendations for the monitoring of performance indicators based primarily on the authors' ongoing research in this field.

The organization of this report is based on a staged approach to full-scale field deployment that begins with a pre-existing characterization of the processes controlling uranium mobility and transport that leads to a credible conceptual model for future uranium behavior. Bench-scale studies are used to establish the proof-of-principle viability of uranium bioremediation and support the design of a field deployment strategy. A pilot-scale implementation of the uranium bioremediation strategy is used to test understanding and address uncertainties in the field conditions and behaviors that cannot adequately be addressed at the bench-scale. The elements of the approach are summarized in the following flow chart.





## **2 Prerequisites for In Situ Bioremediation of U(VI)**

### **2.1 Characterization of Uranium Contamination**

Uranium contamination must be sufficiently characterized in terms of source, mobility, and compliance to support selection of a remedial strategy. Furthermore, and more importantly, the attributes of this characterization must be reasonably well understood to lead to the identification of bioremediation as a cost-effective solution.

#### **2.1.1 Uranium Inventory**

The expectation is that the total uranium inventory is well-characterized in the sense that the contamination history and characterization activities have identified both current and potential future sources. Extent and concentration of uranium source are critical considerations for the bioremediation deployment strategy. Our experience has been that many groundwater uranium contamination problems have vadose zone sources. In this case, the vadose zone sources may be most active during hydrologic transients: episodic infiltration events; diurnal, seasonal, and episodic water table events. Western arid recharge rates in conjunction with uranium retardation may preclude significant contribution from recharge-driven vadose zone uranium transport. Moreover, cyclical water level fluctuations of relatively small magnitude tend to deplete a stable subsurface uranium source in the saturated zone over time frames of decades or more. However, episodic events of extended duration and/or extreme magnitude can liberate previously unleached contaminated sediments. For this reason, it might be necessary to consider augmenting bioremediation with technologies (e.g., surface barriers) that limit longer-term, recharge-driven vadose zone uranium transport.

#### **2.1.2 Uranium Form, Concentration, and Mobility**

Uranium has a broad range of mobility that is dependent on the ambient water chemistry and the surface reactivity of the sediments. We assume here that the hexavalent (+6) oxidation state [U(VI)] is the nominal valence for the bulk of the aqueous uranium species. While the hexavalent form of aqueous uranium is considered to have the most potential for transport, sorption processes for U(VI) are particularly sensitive to pH, carbonate complexation, and aqueous uranium concentration (Figure 1).

Typically, the characterization of uranium geochemistry is done in laboratory studies with contaminated and uncontaminated site sediments. Extraction procedures attempt to classify the uranium pools on sediments in terms of leachability with different extractants (e.g., labile versus nonlabile using carbonate and acid extraction). A critical issue is to differentiate the labile uranium from the rest of the sediment-associated uranium. Non-labile uranium may be in the form of 1) sparingly soluble uranium and uranium-incorporated minerals, or 2) uranium that is not easily desorbed under ambient or engineered chemical conditions. Under circum-neutral pH and calcite-controlled carbonate chemistry in western arid sediments, we have seen the (labile) uranium partitioning coefficient,  $K_d$  (ml/g), for the less than 2 mm sediment size fraction range from less than 1 (DOE 1999) to greater than 20 (Qafoku, Zachara et al. 2005).

Another aspect of geochemical control on mobility is the reactive sediment surface environment. Although there is a body of work focused on Fe(III) minerals as the principal surface complexation site cf. (Waite, Davis et al. 1994; Payne, Davis et al. 1996), other researchers cf. (Arai, McBeath et al. 2006) have identified poorly crystalline aluminosilicate mineral as potential primary sorption surfaces. Sorption site density (e.g., surface complexation sites) is an input parameter to mechanistic sorption models that is often related to mineral surface area (Davis and Kent 1990).

The significance of the uranium concentration is also important from a compliance perspective. The drinking water MCL of 30 µg/L may not be the regulatory compliance criteria for sites that are not drinking water sources. For example, the Old Rifle, Colorado UMTRA site cleanup criteria is 44 µg/L. In any case, negotiated compliance standards relative to the existing contamination levels should play a role in the level of cleanup required and thus, the remediation technology selection.

## **2.2 Characterization of Relevant Transport Processes and Properties**

In addition to the characterization of the uranium source term and geochemistry, an equally important prerequisite to the selection and design of a remediation technology is the characterization of the relevant transport processes and properties that are operative at the site for uranium. In this case, the objective is to develop an understanding of the environmental properties and process dynamics on the site that will control/influence uranium fate.

### **2.2.1 Hydrologic System Dynamics**

As pointed out above, a focus on the groundwater system alone may ignore a significant and persistent long-term source from the vadose zone. Even if the vadose zone does not need to be explicitly addressed, seasonal water table fluctuations or influence from surface water systems (e.g., rivers, reservoirs, lakes, impoundments) should be considered in the analysis. A critical issue is the dynamics of the principal transport pathway to compliance points/surfaces, especially in the case of directional changes in the regional and local groundwater flow field.

#### **2.2.1.1 Boundary conditions**

The driving forces for the hydrologic system must be identified and their magnitudes known to be able to identify the various transport pathways and timescales.

Recharge. Spatially and temporally variable (Gee, Ward et al. 2002) recharge may be an important hydrologic component for some aquifer systems but must definitely be addressed for the case of uranium mobilization from contaminated vadose zone sediments. This is especially important when a retarded uranium front has already reached the groundwater. If the surface sites for uranium sorption are fully loaded in the system at this point, aqueous uranium can essentially travel through the vadose zone with the infiltrating water.

Recharge is the net result from a competing set of processes (e.g., precipitation, runoff, evaporation, transpiration) that vary with material properties, climate, and site-specific conditions. An inexpensive technique for an integrated estimate of recharge is chloride mass balance, which is based on concentrations of chloride dissolved in meteoric and pore water, and the volume of meteoric water. Water fluxmeters can be used for point estimates. They work well for sands, while for silts and clays the operational recharge range is above a few hundred mm/yr (Gee, Ward et al. 2002).

Water table fluctuation. Water table fluctuations are important for transferring uranium between the aquifer and vadose zone. In the case where uranium-contaminated sediments are present in the vadose zone just above the average or nominal water table, leaching of uranium by seasonally and/or episodically rising water levels can result in a source to groundwater that can be significantly larger than recharge-driven uranium transport. Furthermore, water table fluctuation combined with groundwater uranium transport can displace uranium in the lower vadose zone to down-gradient locations. This can result in a distributed source of vadose zone uranium that is activated by seasonally and/or episodically high water tables (Figure 2).

Another potential consideration for any remedial action based on lowering and/or maintaining the oxidation-reduction potential (i.e., creating/maintaining chemically reducing conditions) is the presence of a vadose zone oxygen source. This can occur when the gas phase of the vadose zone is in direct communication with atmospheric oxygen at the ground surface. Reoxidation from oxygen diffusing through the water table can inhibit the effectiveness of engineered reducing conditions. This effect can be exacerbated by gas entrapment during water table rise that provides an enhanced pathway for oxygen to be partitioned to the aqueous phase. This can be problematic if a significant part of the uranium inventory is near the water table. Figure 3 shows dissolved oxygen stratification as the result of modeling oxygen diffusion through the water table coupled with regional groundwater flow and biologically-mediated oxygen removal.

Flooding can result in an extreme water table or recharge event that may have major impacts on uranium fate and transport including impacts to natural bioreduction. One example is the Gunnison UMTRA site where flood irrigation is practiced on pastures overlying part of the uranium plume at the site. Flood irrigation appears to either have created a downward gradient that has forced the plume downward or has created a downward flux of soil organic carbon that has accelerated natural bioreduction of uranium. Depending on the interaction of floodwater with vadose zone and soil materials and the resulting geochemical or microbiological changes, flooding events could either decrease or increase uranium concentrations in a plume. If flooding is expected to occur at a site, it is particularly important to understand in advance its likely impact and devise a strategy for assessing and mitigating any anticipated increases in uranium concentration.

Heads. Understanding the driving forces for the groundwater flow field, especially when they are transient in time and spatially complex, is important for predictive purposes. Time series of piezometric heads from an adequate distribution of monitoring wells will usually be necessary to drive a flow model. If such a data collection network does not exist from earlier monitoring, it is relatively easy to equip existing wells with hourly - reading, self-contained water level monitors. In addition, new injection or monitoring wells installed for bioremediation provide opportunities to directly test prior flow models.

#### **2.2.1.2 Vadose zone flow and transport**

If the vadose zone is important as a uranium source, characterization must address the material properties controlling flow rates and directions. Characterized parameters include porosity and constants for relationships between capillary pressure, saturation, and hydraulic conductivity (e.g., van Genuchten, Brooks-Corey). Variably saturated flow modeling with these parameters, in the context of pressure head and recharge boundary conditions, will provide the basis for analyzing and predicting the vadose zone component of uranium migration. Unsaturated permeability anisotropy created by adjacent fine and coarse grained layers are particularly important in creating lateral flow in the vadose zone, possibly causing uranium contamination to appear in the groundwater offset from its location of highest concentration in the vadose zone.

#### **2.2.1.3 Saturated flow field**

The saturated flow field will probably be the principal pathway for uranium transport to a compliance point or surface. Thus, the characterization of hydraulic conductivity and porosity in the context of accurate boundary conditions is critical to the prediction of long-term transport. Key saturated hydrogeologic parameters include hydraulic conductivity, porosity, and dispersivity. Material properties important to uranium transport include grain size distribution, porosity, selected mineral abundances (e.g. clays, Fe(III) minerals) and extractable Fe(II) abundance (also important to sustaining Fe-reducing conditions during bioremediation).

### **2.2.2 Reactive Transport**

#### **2.2.2.1 Mineral Reactions**

Transported uranium is typically attenuated through association with the solid phases of the porous media. This can occur through precipitation of uranium mineral or co-precipitation of uranium during formation of other secondary minerals. For the uranium concentrations and geochemical conditions that we have encountered, ambient geochemical conditions are seldom conducive to the precipitation of uranium minerals. It is typically an exotic waste form (e.g., chemical waste stream), engineered system (e.g., polyphosphate injection and resulting autunite formation), or reducing geochemical condition that results in the precipitation of uranium minerals. However, it is possible that sequestration of uranium could occur during co-precipitation events associated with changes in the oxidation-reduction potential.

#### **2.2.2.2 Sorption Reactions**

The dominant non-biotic uranium attenuation is more likely to be controlled by sorption processes. Uranium partitions between the aqueous and solid phases as a function of the reactivity of surface minerals and water chemistry. Reactivity is typically measured in experimental studies over a range of aqueous chemical conditions with field sediments from the <2 mm size fraction. Particle size distributions and mineralogical analyses of the sediments can be useful when there is significant variability in the sorption behavior.

#### **2.2.2.3 Water Chemistry**

Spatial and temporal variability in field water chemistry should be known a priori to guide the chemical conditions for the experimental studies. Concentrations of major ions [especially those important to 1) uranium sorption: carbonate, calcium; and 2) redox chemistry: dissolved oxygen (DO), nitrate, iron, manganese, sulfate, ammonium] should be complemented by measurements of pH, alkalinity, ORP, specific conductivity, and temperature collected at the same time as samples for the major ions. Dissolved organic carbon concentrations are also needed as an indicator of background electron donor support of natural bioreduction of U(VI). These measurements provide the basis for the characterization of the field water chemistry, but must be augmented by analyses for reagents used and by-products formed during biostimulation.

### **2.3 Conceptual Model of Future Uranium Behavior**

The prerequisite characterization of uranium contamination along with relevant transport processes and properties will form the basis of a conceptual model of future uranium behavior. The extension of this conceptual model to a systematic and quantitative coupled process prediction of unmitigated uranium fate can then be used in a baseline risk assessment. The risk assessment usually drives the decision-making for engineering intervention, regulatory compliance, and rationale for specific remedial options. A key issue is uncertainty in the characterized transport processes due to inadequate sampling and monitoring. Risk of failure from the propagation of estimation errors in the process model parameters can be reduced through well thought out monitoring schemes. Where feasible, these schemes should match the time and space scales of the sampled field processes and properties, and have sufficient flexibility to address episodic or extreme events.

Understanding uranium fate and transport in terms of the uranium extent, magnitude, form, and mobility and the environmental process dynamics is critically important to the reliability of the risk assessment. Attenuation mechanisms, such as sorption, precipitation, and dilution, need to be understood in terms of known variability in hydrologic, geochemical, and biological conditions. Thus, it is important that the monitoring of performance indicators include confirmation of the processes and conditions that form the basis of the conceptual model of future uranium fate.

In summary, pre-remediation monitoring of groundwater parameters is a critical prerequisite for a mechanistically systematic understanding of site-specific uranium behavior. Targeted data should include hydraulic head (hourly water level data), U(VI), alkalinity; dissolved oxygen (DO), nitrate, Fe(II), Mn, sulfate, ammonium, pH, ORP,

specific conductivity, and temperature. These measurements will be most useful if made using event-based sampling, (e.g., low and high water table, after major storm events) as well as on a more regular frequency. Once key behaviors and the presence or absence of constituents (e.g. sulfate) are established, the analyte list can be shortened and sampling frequency reduced to capture known trends and event responses. The monitoring list above presumes the fundamental information for hydraulic properties, boundary conditions, uranium sorption, etc. are known.

### **3 In Situ Bioremediation**

Once the decision is made for engineering intervention to achieve compliance goals, the evaluation of remediation alternatives should be based on the site conceptual model and the attributes of the various remediation technologies. Immobilization is currently the most feasible in situ approach for uranium remediation in environmental systems. In situ approaches are typically preferred over pump and treat systems because of considerations of generated waste, worker safety, and accumulation of cost over very long time horizons. To implement immobilization approaches, biological and/or chemical conditions are manipulated to drive reactions that result in the conversion of mobile aqueous U(VI), typically as uranyl ion  $\text{UO}_2^{++}$  or uranyl complexes, to immobile forms. Bioremediation typically accomplishes this through the microbially-mediated reduction of aqueous U(VI) [+6 oxidation state] in porewater to immobile U(IV) phases [+4 oxidation state]. The most common U(IV) target form is the mineral, uraninite ( $\text{UO}_2$ ), although a number of other minerals can occur (Burns 1999). Bioprecipitated uraninite has been shown to precipitate as nanoparticles (Suzuki, Kelly et al. 2002) and recent column studies (Komlos et al in prep.) suggest that uraninite precipitates may be transported as originally suggested by Suzuki et al (2002). Results from field studies discussed below suggest that at field scales (temporal and spatial) bioprecipitation succeeds in immobilizing U(VI) likely by a combination of aggregation of nanoparticles and attachment to mineral surfaces.

#### **3.1 Microbially-Mediated Processes**

The reduction process occurs as microorganisms consume an electron donor (e.g., organic substrates such as ethanol, acetate, lactate), which are oxidized during metabolism to gain energy and build cellular material, while transferring electrons out of their cells to terminal electron acceptors [e.g., oxygen, nitrate, Fe(III), sulfate, bicarbonate, figure 4]. The sequence of the various terminal electron accepting processes (TEAPs) is related to the energy yield from particular microorganism, electron donor, electron acceptor combinations. For typical microbial consortia, this sequence is similar to the thermodynamic redox “ladder” in aquatic chemistry: oxygen, Mn(IV), nitrate, Fe(III), sulfate, carbonate (Table 1). Uranium, in this context, is typically present in trace quantities when compared to the dominant biogeochemical conditions maintained by the background microbial consortia, major ion chemistry and primary reactive surfaces. At these trace concentrations, uranium bioreduction is not easily differentiable from the concomitant dominant TEAP. At the Rifle site, for example, acetate-oxidizing dissimilatory iron reducing bacteria (i.e., *Geobacter sp.*) are mediating uranium

bioreduction (Anderson, Vrionis et al. 2003). Conversely, ethanol-oxidizing sulfate reducing bacteria appear to be responsible for the primary uranium bioreduction observed at the Oak Ridge FRC (Scheibe, Fang et al. 2006).

### **3.2 Methodology Selection**

The use of any redox-based technology has a number of issues to address including 1) sequence of chemical components that must be reduced before uranium bioreduction can take place, 2) bioavailability of terminal electron acceptors, 3) reoxidation and remobilization, 4) oxidizing sources, and 5) evolving reactivity. The objective is to identify a bioremediation approach that is best suited to the attributes of the uranium inventory and the environmental system.

In addition to oxygen, there are several potential oxidizing agents present in natural systems. Depending on pH, nitrate and minerals with Mn(IV) and/or Fe(III) may be the dominant oxidizer after oxygen. At circumneutral pH, the thermodynamically favored sequence would follow the redox ladder in Table 1: oxygen, nitrate, nitrite, Mn (IV), Fe (III), sulfate, carbonate. Unless there are kinetic limitations, stronger oxidizers must be depleted prior to the availability of weaker oxidizers for reduction. Thus, it may be necessary to include the removal of oxygen and nitrate in the bioremediation methodology. While this may not be a significant issue at low nitrate, suboxic sites like the Rifle site, many sites have high nitrate concentrations (e.g., Hanford, Oak Ridge) that could drastically increase the cost and complexity of bioremediation, especially where there is a continuous influx of oxygen, nitrate, and U(VI) into the treatment zone. Vadose zone sources of these oxidized components could be important if enhanced through high recharge and/or water table fluctuation. Post-biostimulation reoxidation and remobilization of uranium, which have been observed in laboratory settings (Komlos et al in prep), may also be accelerated in the field by the presence of these oxidizers.

Remobilization of sorbed, precipitated, and co-precipitated uranium may be dependent on the dissolution kinetics of the secondary minerals that incorporate or overlie the sequestered contaminants. Furthermore, secondary minerals can contribute to the long-term stability of biogenic uraninite by slowing the reoxidation process. Abdelouas (Abdelouas, Lutze et al. 1999) found that when oxidizing background conditions returned after biostimulation ended, mackinawite (FeS) that precipitated during biotransformation of U(VI) to uraninite, provided an oxygen sink that prevented the oxidation of uraninite. Changes in the structure and function of microbial community dynamics, mineral dissolution and precipitation, and biomass production at bioremediation sites are indicative of the evolving reactivity of the subsurface system and should be monitored in the context of long-term uranium mobility. It is also possible that precipitation of stable secondary phases may serve to isolate previously precipitated sorbed or reduced U, thus removing it from direct contact with pore waters. In this context it is important to note that the goal is to maintain a sufficiently low rate of remobilization such that groundwater standards are maintained.

A key consideration is thus the maintenance of the desired TEAP. At the Rifle site, the principal uranium bioreduction has been attributed to iron-reducing *Geobacter* species.

As bioavailable Fe(III) minerals were depleted near the point of acetate injection at the Rifle site, acetate-oxidizing sulfate reducers succeeded the iron-reducers. The transition to sulfate-reducing conditions was accompanied by an increase in U(VI) concentrations in the groundwater. The inefficiency of U(VI) removal from groundwater when acetate-oxidizing sulfate reducers became dominant was identified by Anderson et al. (2003) as an important consideration in the optimization of a bioremediation strategy based on this approach. Clearly, the selection and implementation of a successful bioremediation strategy requires a reasonably good understanding of the site-specific geochemical conditions and the maintenance of specific microbial populations.

### **3.3 Bench-Scale Testing For Proof-Of-Principle**

The sensitivity of bioremediation efficacy to field attributes (e.g., contaminant inventory, material properties, biogeochemistry, hydrology) is sufficiently high that engineered solutions must be appropriately tailored. From this perspective, a necessary performance indicator is a set of bench scale tests using site materials where possible (e.g., uncontaminated and uranium-contaminated sediments, groundwater) that address proof-of-principle as well as methodology optimization (e.g., electron donor selection and delivery). Bench-scale testing can address:

1. Characterization of unmitigated uranium behavior in terms of variable
  - a. geologic materials
  - b. water chemistry
  - c. geochemistry
2. Electron donor selection; density and composition issues for delivery
3. Achieving onset of principal TEAP associated with uranium bioreduction
4. Maintaining bioreduction efficacy

A significant range of bench scale tests have already been completed and published starting with Lovley's original bottle incubations showing microbial reduction of U(VI) (Lovley, Phillips et al. 1991) and numerous others since (see bibliography). These earlier studies make it possible to perform a minimal set of new tests with site materials to achieve the objectives noted above.

Assessment of proof-of-principle needs to account for the impact of site complexity (geohydrologic, geochemical, and microbiological) on bioremediation performance. For example, low-permeability zones (silt or clay) may not only constrain flow in a system but may also slowly release U(VI) from dead-end pores, maintaining a flux of U(VI) not anticipated from consideration of coarser grain materials (LaBolle and Fogg 2001). Appropriate design of an electron donor system can address these situations (Roden and Scheibe 2005).

In many cases, the understanding of these complexities is qualitative in nature. However, considering their potential impact is still key to effective design of the bioremediation system and helps avoid surprises during implementation. Further, newly developed geophysical monitoring techniques, especially electrical methods (Williams, Kemna et al. 2006), offer promise of inexpensive, minimally invasive approaches sensitive to



geochemical heterogeneity initially and to changes in the distribution of TEAP's during bioremediation.

### **3.4 Conceptual Model for Uranium Bioremediation**

Based on the success of the bench-scale testing and the site baseline analysis, a conceptual model for the engineered bioremediation of a particular site can be formulated. Typically, this knowledge will be incorporated into a quantitative simulation of the dominant processes operative on the site before, during, and after biostimulation. The laboratory-based parameters used in the simulations will likely deviate from those in the field, thus these analyses are preliminary in nature. The value in numerical modeling is being able to identify general responses to variations in design parameters. This will allow the assessment of implementation issues and comparison of bioremediation options in the context of the uranium inventory, environmental transport processes, and the characterized biogeochemical reactions.

### **3.5 Pre-Biostimulation Baseline Characterization**

A key aspect of the monitoring program should be the pre-biostimulation baseline characterization that will be necessary for comparisons with monitoring performed during and after bioremediation or pilot scale tests. It is possible that much of this baseline characterization may have already been a part of earlier characterization performed in support of the risk assessment. One particularly useful consideration is a transport experiment with multiple tracers that have different diffusion characteristics. This will provide important information regarding transport (e.g., effective porosity, preferential flow, transport time scales, multiregion behavior) that will have direct implications for the design of amendment delivery. Key baseline data required include groundwater geochemistry and sediment geochemistry (see discussion above and Table 2 for details).

Point samples from groundwater and sediment may be augmented by geophysical data collection to provide a geophysical baseline prior to conducting pilot scale testing or full-scale bioremediation. Geophysical measurements (complex resistivity, self potential, cross-well radar, and cross-well seismic) provide minimally-invasive monitoring of both initial heterogeneity of redox status and microbially-mediated changes in subsurface mineralogy and redox status. Initial complex resistivity data from the Rifle site indicate that both changes in Fe-bearing mineral coatings on detrital grains and in sulfide mineral abundance can be tracked in 3-D, enhancing our ability to interpret groundwater and sediment geochemical changes and ultimately providing a means for real-time monitoring of uranium bioremediation. While the use of self potential to identify the spatial extent of the electrochemical response to biostimulation is relatively new, the initial results have been very encouraging with excellent consistency and signal-to-noise attributes. At the Rifle site, standard surface electrode deployments are being augmented by copper electrode rings on the outside of PVC well casings to increase spatial resolution afforded by cross-borehole analyses.

### **3.6 Pilot Scale testing**

There are many aspects of field-scale bioremediation that cannot be adequately addressed at the bench scale including: 1) > 2 mm sediment size fraction, 2) structured,

multidimensional, multiscale physical and chemical heterogeneities, 3) microbial microenvironments, 4) density effects, and 5) seasonal and episodic hydrologic transients. The tradeoff with pilot scale testing in the field is the diminished ability to control and observe the engineered reactions. For these reasons and to reduce the risk of failure, pilot-scale field tests are likely to be cost-effective. Such tests provide a bridge to the full-scale deployment of the bioremediation technology that increase understanding of the in situ behavior and allow optimization of the final approach. Monitoring of performance indicators to confirm the field viability of the bioremediation approach is particularly important at the pilot scale, since the results can be used to select the necessary and sufficient performance indicators for full-scale deployment. Again, this is likely to reduce cost of full-scale deployment.

Figure 5 shows the layout of ERSP boreholes for the Old Rifle UMTRA Site at the end of the 2004 experiment, which provides examples of pilot-scale injection and monitoring well configurations. The larger borehole array shows one row of 3 background monitoring wells, one row of 20 injection wells, and three rows of 5 treatment-zone monitoring wells. The overall size of the plot is ~20 m on a side. The smaller borehole array consists of 1 background well, 5 injection wells, and 4 treatment zone monitoring wells. Our experience at this particular site suggests that pilot scale borehole arrays will provide optimal results if they are intermediate in size between the two shown in Figure 5. For the Rifle site, this would mean ~10 m on a side with 3 background monitoring wells, ~10 injection wells, and ~8 down-gradient monitoring wells. This approach assumes that natural gradient pilot scale experiments would be performed. Alternative well designs are possible for both natural and forced gradient experiments.

## **4 Performance Issues and Indicators**

In this section, we describe monitoring of performance indicators that will address the potential issues related to uranium bioremediation. We divide the monitoring approach into three broad groups to assess and confirm: A) uranium removal effectiveness, B) environmental transport conceptual model, and C) microbiological conceptual model. In the following section (Section 5), we provide our best estimate of the priority of the performance indicators.

A general consideration for all three groups is to provide performance indicators with regard to principal issues with engineering uranium behavior. The first is the identification of potentially uncharacterized sources (e.g., vadose zone uranium, dissolved oxygen from water table fluctuation). At the Hanford Site 300 Area for example, the largest uranium plume is associated with the most recently used surface disposal facility that operated until 1994. Recent groundwater measurements, however, have identified a uranium hot spot near a small disposal trench that was decommissioned in 1963. The site conceptual model is also being revised to reflect a chronic source of uranium in the lower part of the vadose zone that is activated during periods of high water table.

A second performance issue revealed through groundwater monitoring during bioremediation is the presence of U(VI) in the treatment zone that is not immobilized.

While this could be the result of an uncharacterized uranium source, the interest here is for zones that are not sufficiently exposed to electron donor (and associated tracer such as bromide). In early experiments at the Rifle site, a lack of mixing in injection gallery wells resulted in the delivery of electron donor that bypassed the uppermost part of the saturated zone (Figure 6). Injectate density effects apparently played a key role, but preferential flow paths were also evident as the highest acetate and tracer concentrations were found in the *second* row of monitoring wells downgradient from the injection. This situation could be further complicated by the presence of enhanced dissolved oxygen and U(VI) near the water table. A key monitoring consideration that revealed the vertical variability and stratification during biostimulation was depth-specific sampling for both groundwater (multilevel water sampling in and around the treatment zone, Figure 7) and sediments (core samples, Figure 8).

A third performance issue is the potential for changing bioremediation effectiveness. The transition from iron to sulfate reduction described earlier at the Rifle site was shown to significantly decrease the efficacy of U(VI) removal from groundwater. However, the post-biostimulation uranium behavior showed a subsequent evolution to another biogeochemical state where enhanced uranium removal took place only in the zones that experienced significant sulfate reduction. A key concept is sampling frequency based on the time-scales of the process dynamics, which maybe diurnal, seasonal, and/or episodic. Even the seasonal sampling during the spring runoff should be viewed as event-driven because the specific timing can vary from year to year.

#### **4.1 Group 1: Uranium Removal Effectiveness**

The uranium removal effectiveness group (Group 1) provides a general perspective of the uranium distribution in the subsurface. It does not provide sufficient confirmatory information for the baseline conceptual model of uranium transport and fate addressed in Group 2, or the uranium bioremediation conceptual model addressed in Group 3.

Compliance standards are typically based on the aqueous uranium concentrations. Thus, the most direct indication of compliance status will be through monitoring of groundwater for uranium concentrations. Key components of the Group 1 groundwater monitoring indicators are to establish the initial uranium (i.e., pre-biostimulation) for both the background and contaminated aquifer zones, while capturing the transient aqueous uranium entering the treatment zone. This will provide a basis for comparing uranium concentrations monitored during and after the biostimulation, and linking observed changes with the biostimulation. In some cases (e.g., Old Rifle UMTRA site), the treatment zone may be small compared to the extent of uranium contamination such that an uncontaminated up-gradient zone is not sufficiently close to the treatment zone to reasonably compare changes. In these cases, the unmitigated contaminated zone up-gradient of the treatment zone should be monitored to identify the transient water chemistry and the background geochemical condition of the sediments. See Figure 9 for a depiction of the spatial distribution of U(VI) before and during biostimulation.

Two key facets of U(VI) concentrations are 1) the vertical distribution in the aquifer and 2) the temporal changes associated with water table fluctuations per discussions earlier in

this document. In most cases, the amount of data that can be collected in both space and time is constrained by available funds. However, it is important to note that information on the spatial and temporal distribution of U(VI) may actually save remediation costs, if it is discovered that U(VI) concentrations are restricted to the upper part of an aquifer, for example. Furthermore, we have used passive multi-level samplers that only require a single well bore [see Vrionis et al. (2005) and Figure 7] to obtain data on the depth distribution of U(VI) as well as other dissolved constituents in groundwater.

Sampling frequency is a crucial parameter that needs to be adaptively established based on events suspected of impacting U(VI) (e.g. water table rise) or on time frames previously observed for response of U(VI) concentrations to biostimulation (7 to 10 days). We typically conduct sampling at frequencies ranging from two times per week at the outset of electron donor amendment to monthly during post-amendment monitoring. After event impacts are well established, monitoring could occur less frequently. It should also be possible to link sampling to near-continuous geophysical monitoring, possibly limiting sampling to real-time changes in geophysical response (e.g. a decrease in self-potential voltage).

Concentrations of U(VI) in groundwater should be complemented by uranium extractions performed on sediments sampled before and after primary biostimulation. In our experience at Rifle, spatial variability in the extractions performed on cored sediments at the Rifle site can be considerable. In this case, the spatial resolution of the sampling scheme should be guided by knowledge gained from the pilot study and cost considerations. We are currently researching in-well sediment incubators as possible surrogates for in situ sediment samples. Initial results suggest that it may be possible to use such incubators to inexpensively characterize both sorbed uranium and in situ microbial response under background and locally biostimulated conditions.

A key piece of information is the amount of U(VI) in groundwater that has been reduced to U(IV) and become associated with the sediments. In this case, preparation and maintenance of reduced uranium in cored sediments for subsequent analysis is a critically important task. A specialized laboratory experiment (Komlos, in prep.) was recently performed where an entire column of sediment was brought intact to a nearby x-ray synchrotron for analysis of the uranium oxidation states in a biostimulated column. When compared to standard handling approaches where sediment was removed from columns and shipped offsite, this approach resulted in considerably higher measurements of reduced uranium in sediments.

## **4.2 Group 2: Conceptual Model of Uranium Transport and Fate**

We take the view that monitoring uranium levels in groundwater is a necessary but not sufficient performance indicator. The expectation of the Group 2 performance indicators is the assessment and confirmation of the conceptual model for uranium transport and fate. System complexity may affect the delivery of amendments to the subsurface as well as long-term effectiveness. This would include bypass from preferential flow paths, spatially variable depletion of bioavailable terminal electron acceptors that are critical to the maintenance of effective uranium bioremediation, changes in the geochemical

environment (e.g., mineral precipitation) that affect uranium availability and reactivity, and the impact of hydrologic events (e.g., elevated water table, intense rainfall/recharge) on the reoxidation of reduced sediments. Evolving reactivity may also occur over longer time scales needed for permeability reduction due to formation of secondary minerals and post-biostimulation re-equilibration to ambient conditions.

It should be mentioned that the microbially-mediated creation of low oxidation-reduction potential (ORP) conditions [e.g., through Fe(II) and sulfide formation] that thermodynamically favor abiotic uranium reduction have been shown to be quite limited kinetically in natural sediments (Jeon, Dempsey et al. 2005). Thus, the general concern with the maintenance of reducing conditions is with regard to preventing reoxidation of bio-reduced uranium, not necessarily the continued production of U(IV). However, we also note that post-amendment removal of U(VI) at the Rifle site may result from biosorption of U(VI). The longevity of such immobilization is not yet known but appears to last at least 2 years, depending on abundance of sulfide precipitation during sulfate reduction.

Key monitoring quantities are summarized in Table 2. Aqueous conditions that can be automatically and continuously logged with an in situ sonde include water depth, pH, ORP, alkalinity, specific conductivity, temperature. At the Rifle site, we have four sondes that are continuously operated with bimonthly downloading of this data. An attractive alternative is to set these systems up with telemetry that will allow the data to be downloaded remotely. Water levels can be particularly important to the regional flow field as well as chemical conditions near the water table. Standard multilevel samplers are very useful but it may not be feasible to deploy them in every well. We have supplemented our multilevel samplers with various passive samplers that can be used to economically collect depth-dependent distributions of aqueous and particulate components (Figure 7). In many cases, we can visually identify depth-dependent behavior by examining the sequence of the sampling cells.

Standard aqueous sampling should be directed at the transport of the injectate (e.g., tracer, electron donor, possibly electron acceptors), consumption of electron donors (e.g., ethanol, acetate, lactate) and electron acceptors (e.g., oxygen, nitrate, sulfate), and biostimulation reaction products [carbonate, ammonium, sulfide, reduced metals such as Fe(II), Mn(II)] . Most recently, a technique using tubing and syringes suspended in wells (Spalding and Watson 2006) has been used to provide the most accurate measurements of dissolved gases such as hydrogen, oxygen, carbon dioxide, and nitrogen. The adaptation of this technique to a passive depth-dependent sampling of the water column promises to provide key feedback on bioremediation progress (e.g., hydrogen and carbon dioxide byproduct) and reoxidation (e.g., dissolved oxygen and nitrogen).

Where possible, sediment extractions should be used to identify uranium form [e.g., U(IV), U(VI) as mineral, surface complex], iron form [Fe(II), Fe(III) as mineral and surface complex], and other secondary minerals (e.g., carbonate, sulfide). At the Naturita site, sediments were suspended directly in wells to provide cheaper and easier access to reacted sediments ((Curtis, Fox et al. 2004), see also discussion under Group 1).

### **4.3 Group 3: Microbiological Conceptual Model**

The Group 3 performance indicators are designed to assess and confirm the microbiological conceptual model underlying the engineered bioremediation. Key issues are the ability to differentiate, where necessary, the dominant microbial populations associated with major TEAPs in the bioremediation scheme. Although there is a sequential nature to these TEAPs, there is growing evidence from the field that multiple TEAPs (e.g., iron and sulfate reduction) can be operating simultaneously in the same aquifer sediments, although likely in different microniches. Succession to a new TEAP ostensibly occurs when the preceding TEAP can no longer compete effectively for the electron donor and as a result loses its primacy. This is significant only if the succeeding microorganisms are less effective at uranium bioreduction than their predecessors, which appears to be the case with the acetate-oxidizing microbial consortia at Rifle. Specialized sampling procedures are necessary to identify the microbial community structure and measure the abundance and activity of the dominant microorganisms. Sampling techniques typically are focused on 1) groundwater, 2) sediments, or 3) in-well coupons or sediment incubators. For sampling groundwater, concentration of the planktonic microbial community by filtration is typical. The volume of sample that is filtered depends on the specific microbial analysis. 16S DNA sampling, for example can be done by filtering relatively small volumes (1 to 3 liters), but more sophisticated techniques such as rRNA require larger volumes (~20 liters and more care in sample handling). Sediment sampling is the most challenging since drilling is usually required. Sample volumes again depend on the proposed analysis, ranging from 25 grams to 0.5 kg or more. In-well coupons such as Bio-Sep beads (see below) are attractive since no drilling is involved, but may be problematic if the well-bore environment is not representative of the surrounding formation and groundwater conditions. Recently, efforts have been made to develop an in-well sediment incubator that eliminates this issue by fully occupying part of the well bore with sediment from the site (Peacock, personal communication). This approach makes it possible to track in situ sediment microbial population changes without drilling.

Microbial Analysis Techniques. Phospholipid fatty acid profiles (PLFA) provide a broad but reliable picture of microbial community structure with relatively small groundwater or sediment samples required. Furthermore PLFA analyses are commercially available. Standard molecular biology techniques such as 16-S Clone libraries can also be routinely performed. For some microbial techniques, large volumes (~20 L) of groundwater must be filtered to concentrate sufficient biomass to perform analyses (e.g. gene expression or mRNA). However, molecular biology techniques are evolving rapidly and it is now possible to track both gene expression and mRNA during the course of biostimulation (Holmes, Nevin et al. 2004; Holmes, Nevin et al. 2005). DNA chip arrays and bead arrays make it possible to screen samples for both microbial metabolic function and genetic identity. Many of these tools are used primarily for research, but are quickly becoming available to meet the needs of applied bioremediation. Sediment analyses include the use of similar tools, but require extraction of DNA prior to analysis.

A novel bio-trap technique that captures microbial community structure in the subsurface is Bio-Sep® Beads (Peacock, Chang et al. 2004). The beads are 2-3 mm in diameter, with high porosity and surface area. Biofilms form rapidly in the Bio-Sep® Beads and the biofilm community structure on the beads is more indicative of *in situ* microbial ecology than samples of planktonic organisms. Standard phospholipid fatty acid analysis (PLFA) 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. Microbial analysis of these beads are commercially available.

Fe-reduction and sulfate reduction TEAPs are also amenable to indirect assessment using geophysical monitoring via electrical methods (Williams, Kemna et al. 2006). Recent unpublished results from the Rifle site using electrical resistivity tomography indicate that iron reducing and sulfate reducing conditions are readily distinguished in adjacent electron donor amendment plots one of which was driven to sulfate reduction and the other maintained in Fe reduction. These results are preliminary but indicate the likely value of electrical methods for minimally invasive means of assessing microbial processes in the subsurface.

## **5 Summary and Prioritization of Performance Indicators**

Table 2 summarizes the performance indicators. Priorities are designated in three categories, mandatory, desirable, and optional. In addition, the temporal dimension of the monitoring regime is provided as a guideline for monitoring frequency. Mandatory performance indicators are those indicators judged to be the minimum set of data needed to validate a uranium bioremediation project. Desirable performance indicators are those, which, if included, could be used to make a stronger case for the success and effectiveness of uranium bioremediation. Optional performance indicators are the lowest priority, but may provide valuable information in selected cases or enhance the understanding of system processes. This prioritization scheme was developed to minimize the cost of system assessment by focusing on low cost measurements in the mandatory category while still ensuring that critical data are obtained. Site specific conceptual or numerical models may indicate different priorities or the need for emphasis on specific measurements.

It is recognized that uranium contamination in groundwater occurs in a variety of hydrogeologic settings and that some settings may be amenable to different priorities. For example, reclamation and clean up of sites where uranium has been mined by In Situ Leaching (ISL) may have a different set of issues and drivers than alluvial aquifers contaminated during uranium milling operations. ISL sites are commonly deeper than milling tailings sites, but have the advantage that existing wells and infrastructure can be used for cleanup and monitoring using geophysical techniques. ISL cleanup is commonly done abiotically by sweeping clean water through the system to dilute uranium concentrations followed by reduction by  $H_2S$  and additional pumping to remove residuals. Bioremediation could decrease cost and increase the effectiveness of

treatment. Geophysical and biogeochemical monitoring is likely critical in such cases to demonstrate effective spatial distribution of treatment and achievement of desired results over appropriate time periods.

The most complete set of performance indicators will not be useful unless effectively analyzed and communicated to problem holders, regulators, and stakeholders. Visualization of the spatial and temporal changes in performance indicators is a particularly useful analysis and communication tool. Such visualization can be as simple as performance indicator versus time plots posted on a borehole map. Figure 9 shows the concentration of U(VI) in space for the Rifle site. A series of such plots are used to animate the visual evidence for U(VI) removal over a 3 month period for the 2002 Rifle field experiment. The animation illustrates key points about the change in U(VI) reduction at the onset of sulfate reduction.

Performance indicators are typically also used to update numerical modeling of the system. For example, if groundwater elevations indicate that permeability of the treatment zone is changing, different values of permeability can be input to the numerical model to assess the extent of change that is consistent with water table elevations. The updated model can then be used to assess the effectiveness of treatment and degree of rerouting of groundwater flow that may be occurring. Such assessments can also be used to estimate the value of additional monitoring points or to indicate that some monitoring point may no longer be cost effective to sample.

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## Tables

Table 1. Environmentally important redox reactions in order of decreasing energy yield.

### Environmentally Important Redox Reactions

Reaction	E <sub>h</sub> (V)	ΔG
Reduction of O <sub>2</sub>		
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$	+0.812	-29.9
Reduction of NO <sub>3</sub> <sup>-</sup>		
$2NO_3^- + 6H^+ + 6e^- \rightarrow N_2 + 3H_2O$	+0.747	-28.4
Reduction of Mn <sup>4+</sup>		
$MnO_2 + 4H^+ + 2e^- \rightarrow Mn^{2+} + 2H_2O$	+0.526	-23.3
Reduction of Fe <sup>3+</sup>		
$Fe(OH)_3 + 3H^+ + e^- \rightarrow Fe^{2+} + 3H_2O$	-0.047	-10.1
Reduction of SO <sub>4</sub> <sup>2-</sup>		
$SO_4^{2-} + 10H^+ + 8e^- \rightarrow H_2S + 4H_2O$	-0.221	-5.9
Reduction of CO <sub>2</sub>		
$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$	-0.244	-5.6



**Table 2. Prioritized Information and Monitoring Parameters for Assessment of Bioremediation of U(VI) in Groundwater**

***Mandatory Uranium Distribution, Magnitude, Form, and Mobility***

<i>Information area/parameter</i>	<i>Desired Range</i>	<i>Comments</i>
Site conceptual model for uranium source term	NA	Consideration of alternative conceptual models critical
Spatial extent of contamination zone (plume geometry)	±20% of estimate	Differentiate between vadose zone and aquifer concentrations; aqueous and sediment associated uranium; geometry drive layout of bioremediation system
Form and mobility/labability	± 30% of estimate	Mobility based on uranium form, potential for future mobility based on labile fraction

***Mandatory Hydrologic and Geologic Site Information***

<i>Information area/parameter</i>	<i>Desired Range</i>	<i>Comments</i>
Site conceptual model for subsurface (vadose zone and groundwater) flow and contaminant transport	NA	Consideration of alternative conceptual models critical
Temporal recharge	±20%	Seasonal and episodic impact to unsaturated flow, extreme recharge event and impact must be considered if flooding probable at the site
Vadose zone hydrogeology: porosity, water retention function parameters	±20% of estimate	Seasonal and episodic impact to flow direction critical
Groundwater flow velocity (Darcy flux) and direction	±30% of estimate	Seasonal and episodic impact critical
Water table dynamics (use hourly data as event-based geochemical sampling driver)	NA	Relationship between water table and U concentration critical
Site hydrogeology: hydraulic conductivity, porosity, and dispersivity	NA	Fundamental to both site and process conceptual model
Remediation process conceptual model	NA	Fundamental to prioritization of monitoring parameters
Particle size characteristics	NA	Reactive surface area, clays, upscaling lab to field

**Mandatory Geochemical and Microbiological Monitoring Parameters**

<i>Parameter</i>	<i>Desired Range</i>	<i>Comments</i>
Background U(VI) concentration, monthly or bi-monthly and event-base (e.g. high water table)	NA	Number of sampling points based on plume and treatment zone complexity and size
Treatment zone U(VI) concentration	Below MCL	Number of sampling points based on plume and treatment zone complexity and size
DO, ORP, specific conductivity, and pH measured hourly to 4 times daily in background and treatment zone (autonomous multiparameter probes)	DO<0.5, ORP<0, conductivity initial increase, pH ~ steady	Values used as overall dynamic indicator of impact of bioremediation on subsurface geochemistry
DO, ORP, specific conductivity, and pH measured at time of groundwater sampling in background and treatment zone using flow-cell with multiparameter probe	See above	Linkage of U(VI) concentrations with parameter change evidence for bioremediation process conceptual model
Aqueous electron acceptors and reduction byproducts in background and treatment zone: nitrate, nitrite, ammonium, Mn(IV/II), sulfate, sulfide	NA	Significant concentrations of oxygen and/or other electron acceptors above the U TEAP on the redox ladder must be addressed by the bioremediation strategy and their reduction products monitored
Fe(III) mineral abundance	NA	Fe(III) minerals provide sorption sites for Fe(II) & U(VI), terminal electron acceptor for Fe-reducing bacteria, dissolved Fe(II) source
Fe(II), sulfide measured in field at time of sampling for U(VI)	Increasing Fe(II); sulfide indicator of sulfate reduction	Maintaining metal reduction may optimize U(VI) removal from groundwater; sulfate reduction may enhance long-term immobilization in sulfate-rich systems
Electron donor concentration in treatment zone	>0	Evidence of delivery and treatment zone distribution; consumption calculation based on tracer data
Tracer for electron donor	>0 in treatment zone	Typically Br is used for conservative tracer, accurate indication of donor distribution
Alkalinity (measured in the field)	NA	Indicator of carbonate geochemistry

**Desirable Monitoring Parameters**

<i>Parameter</i>	<i>Desired Range</i>	<i>Comments</i>
Depth discrete U(VI) data (upper/mid/lower part of contaminated zone)	MCL	Decreased effectiveness of treatment in the uppermost part of the saturated zone may be problematic
Major cations and anions	NA	Provides additional evidence for dominant geochemical reactions
Impact of treatment process on groundwater flow directions (hourly water level at minimum 4 points)	Dependent on background flow	Helps to provide assurance that groundwater is not rerouted around treatment zone
In situ redox status of U using in situ sediment incubators (ISI's)	Significant U(IV) present	Evidence that precipitation of U(IV) is occurring in situ
Microbiological assessment using coupons or in situ incubators	Shift to metal and/or sulfate reduction	Evidence for desired in situ microbial respiration obtained from deploying coupons or in situ incubators in well bores and periodically measuring microbial parameters (see text for additional discussion)
Depth-discrete sediment sampling/extraction for U, Fe, AVS	NA	Evidence for conversion of terminal electron acceptors
Major dissolved gas components in groundwater	NA	Evidence for key TEAPs and microbial metabolism
GPR Cross-well Measurements	Shift in radar velocity in zone of electron donor	Defines 2-D distribution of electron donor

**Optional Monitoring Parameters**

<i>Parameter</i>	<i>Desired Range</i>	<i>Comments</i>
Depth-discrete data for mandatory geochemical parameters	NA	Characterizes spatial distribution of fundamental biogeochemistry in aquifer
Depth-discrete data for desirable monitoring parameters	NA	Characterizes spatial distribution of desired biogeochemical reactions in aquifer
Impact of treatment process on hydraulic properties	<15% change	Documents possible system clogging of pores
Organic and inorganic carbon analyses	NA	More accurate documentation of carbonate geochemistry
Microbiological assessment performed directly on sampling of treatment zone materials	Shift to metal and/or sulfate reduction	Measurements directly on groundwater filtrates or sediment cores provide "gold standard" assessment of microbial community structure (e.g PLFA, 16S, DNA/RNA chip arrays, or functional chip arrays)
In situ redox status of U by direct sampling of in situ materials		U(IV)/U(VI) measurements on in situ sediments provide "ground truth" for U bioreduction
Electrical Resistivity and Self Potential Tomography	NA	Defines 3-D arrangement of dominant TEAP's
Seismic reflection	NA	Sensitive to gas evolution and secondary mineral precipitation

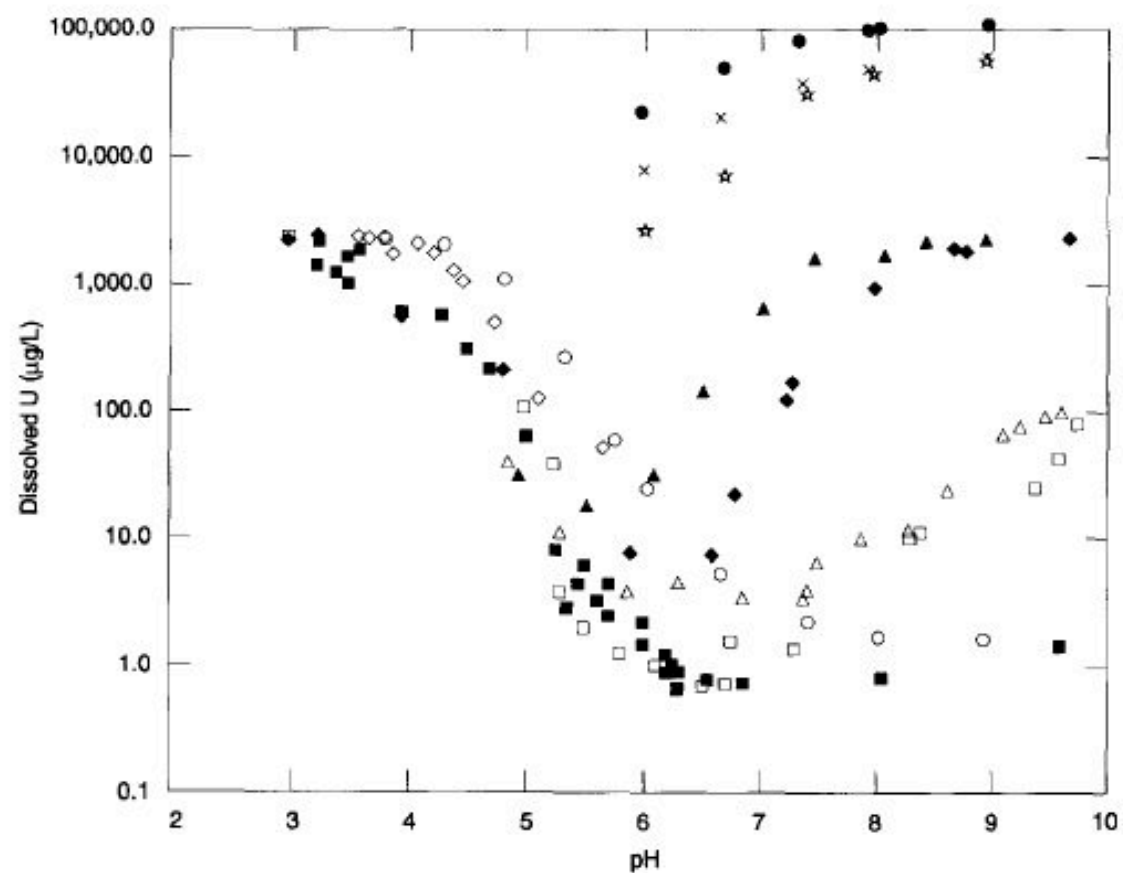
**Temporal Dimension of Monitoring Regime (see also flow chart in text)**

<i>Information area/parameter</i>	<i>Desired Range</i>	<i>Comments</i>
1st Year: monthly or bi-monthly sampling	NA	Sampling during early stage of treatment increases chance of capturing metal reduction phase
2nd and 3rd Year: bi-monthly sampling	NA	Adjustments to sampling frequency needed based on expected life of electron donor or other facets of treatment system
3rd through 10th year: quarterly sampling conditioned on water table behavior	NA	Seasonal and water table fluctuations should drive sampling schedule
Beyond 10 years, adaptive sampling based on prior year results	NA	If a high level of confidence can be demonstrated based on sampling over fewer than 10 years, adaptive sampling strategies could start earlier

## **Figures**



Figure 1. Dissolved uranium as a function of pH, carbonate, nitrate and sulfate (Morrison et al. 1995)



Set		C(IV)	NO <sub>3</sub>	S(VI)	Initial U(VI)	Fe(OH) <sub>3</sub>
		(mol/L)			(µg/L)	(g/L)
1	■	0.0	0.1	0.0	2,380	1.0
2	□	0.001	0.1	0.0	2,380	1.0
3	△	0.001	0.1	0.0	2,380	1.0
4	◆	0.01	0.1	0.0	2,380	1.0
5	◇	0.0	0.1	0.0	2,380	0.15
6	▲	0.0195	0.1	0.0	2,000	0.52
7	●	0.0195	0.0	0.07	100,000	1.0
8	○	0.0	0.0	0.07	2,380	1.0
9	×	0.0195	0.0	0.07	50,000	1.0
10	☆	0.0195	0.0	0.0	50,000	1.0

Figure 2. Changes in dissolved oxygen and U(VI) concentration with water table rise.

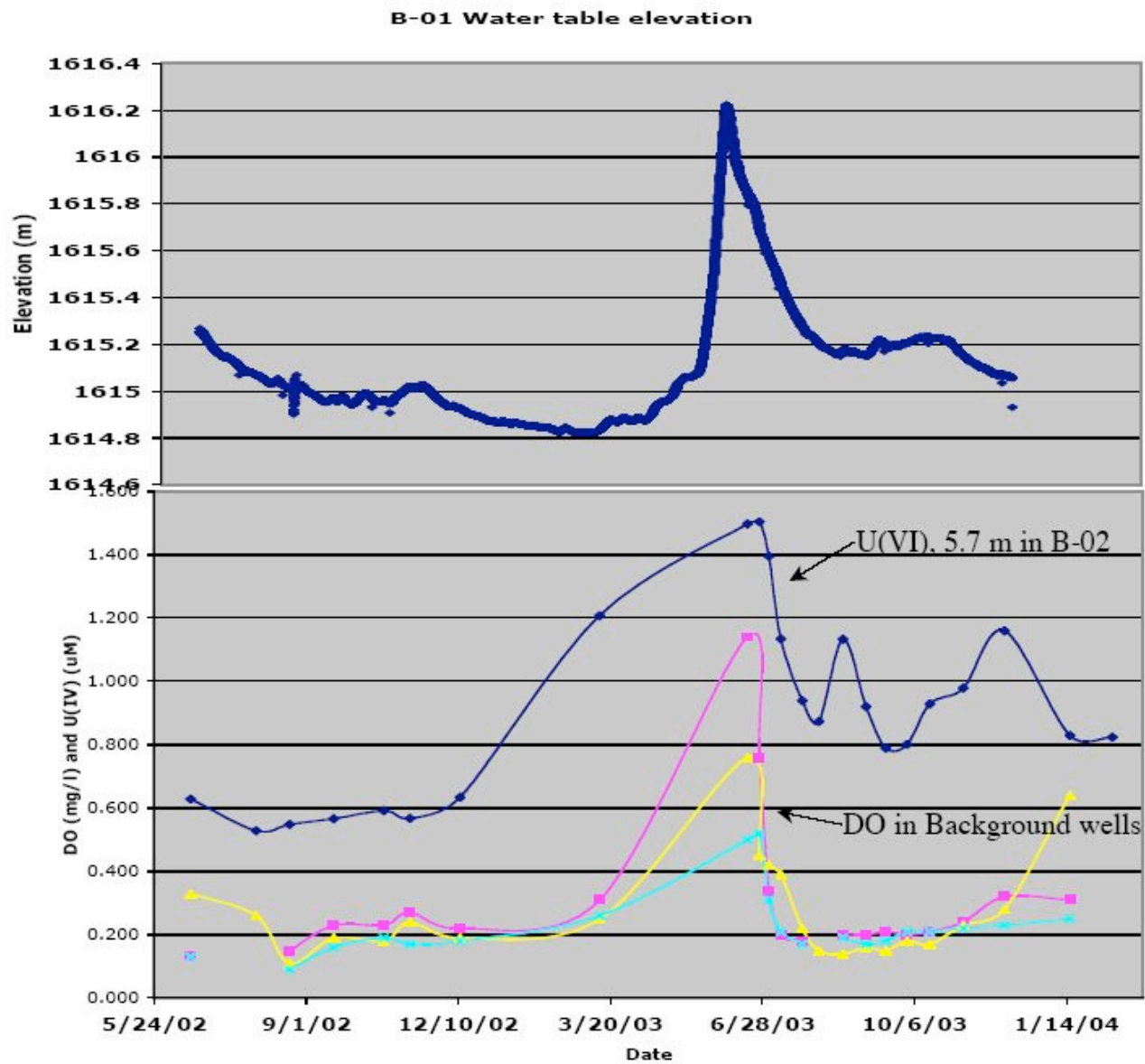
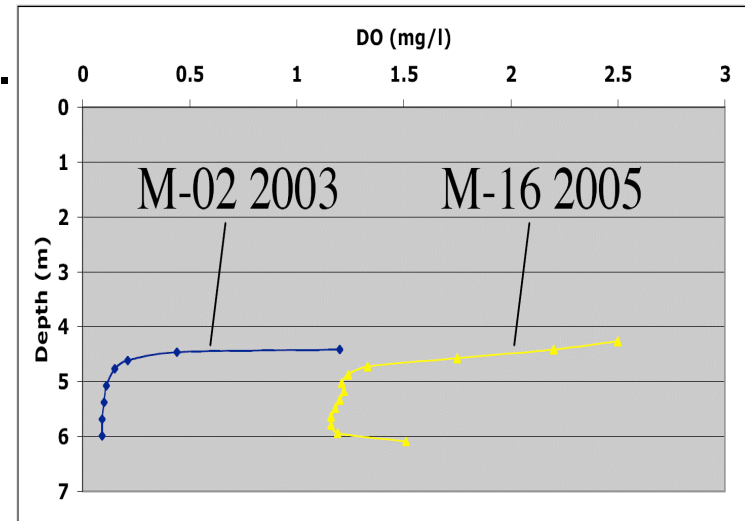


Figure 3. Oxygen stratification observations and modeling.  
 A. Examples of DO stratification. B. Four model cases showing that microbial consumption of oxygen is needed to match observations.

A.



B.

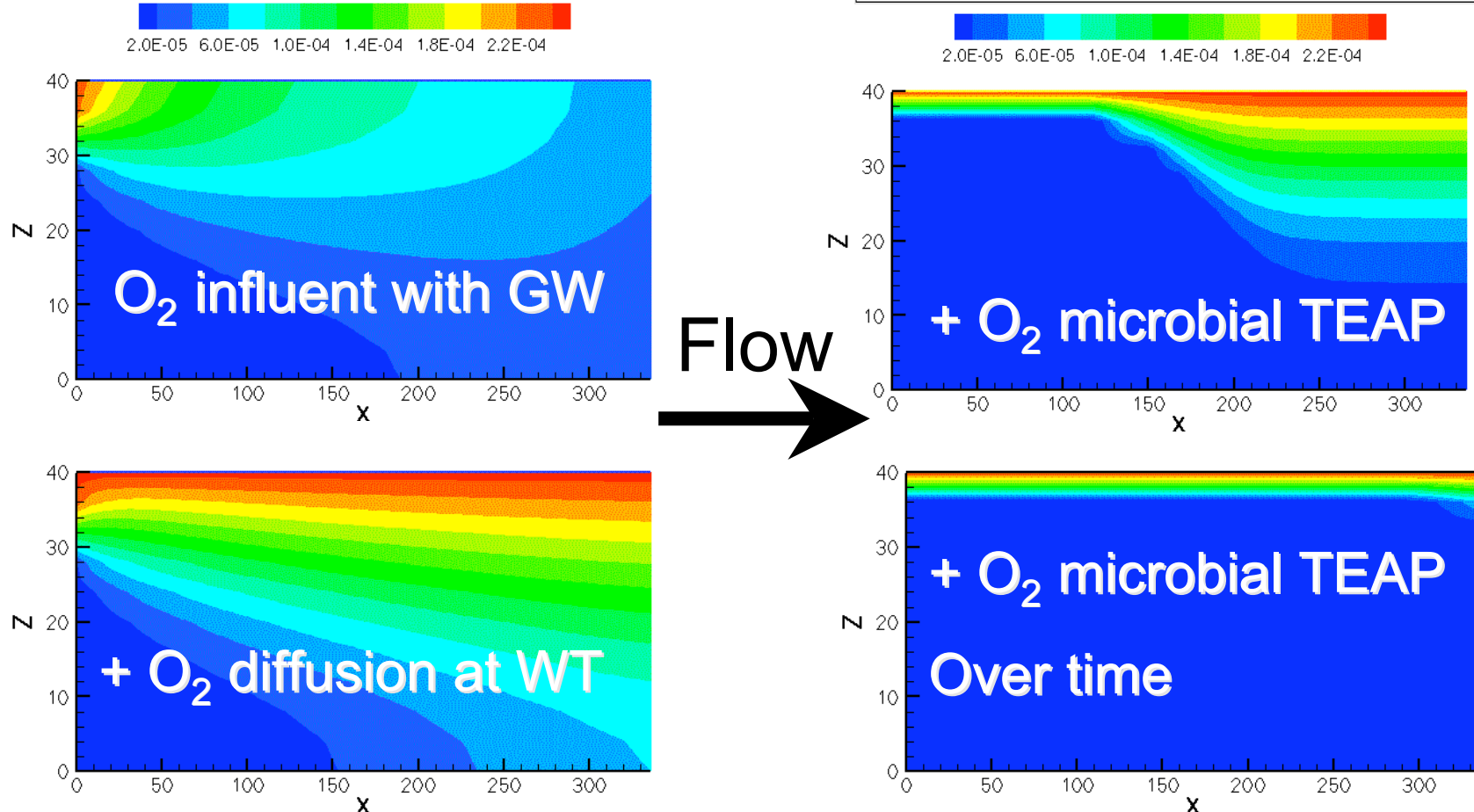


Fig. 4. Microbial mediation of Fe(III) Reduction. U(VI) is the mobile valence state of uranium whereas reduced uranium, U(IV), is insoluble as uraninite. Reduction of U(VI) to U(IV) within aquifers precipitates and immobilizes uranium per lab studies that suggest a simple strategy to promote U(VI) reduction in contaminated aquifers is to add acetate as an electron donor to stimulate dissimilatory metal-reducing microorganisms. U(VI) is reduced concurrently with Fe(III). Original concept from Lovley et al. 1991. Field implementation by Anderson et al 2003.

### Acetate structure

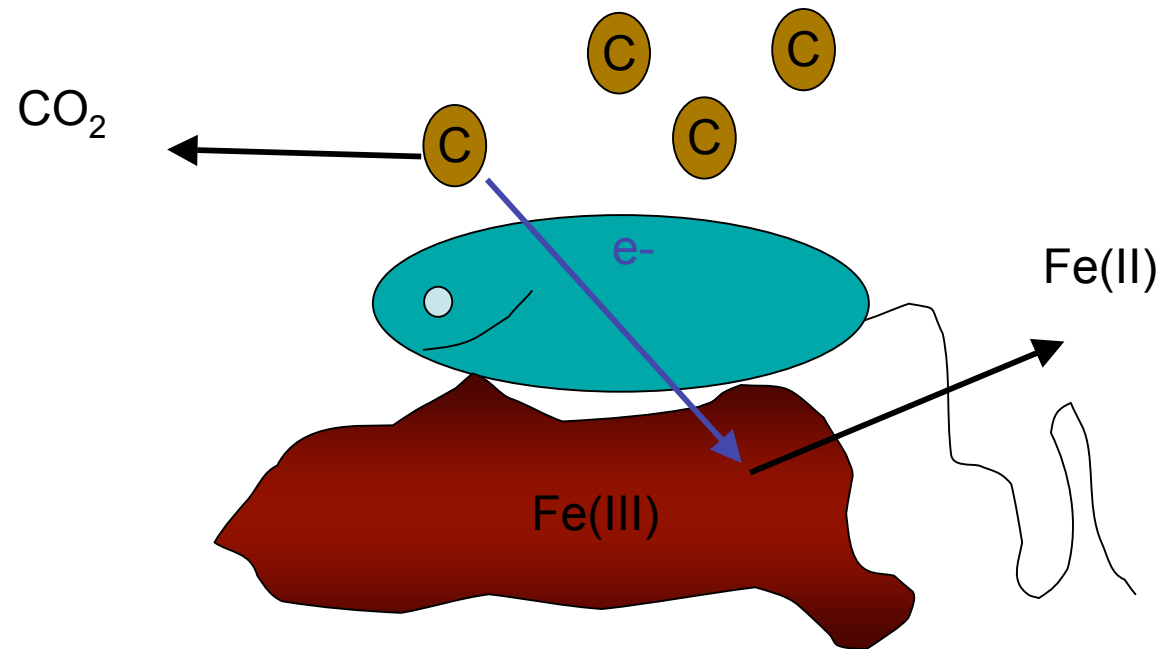
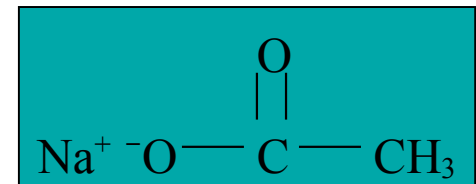
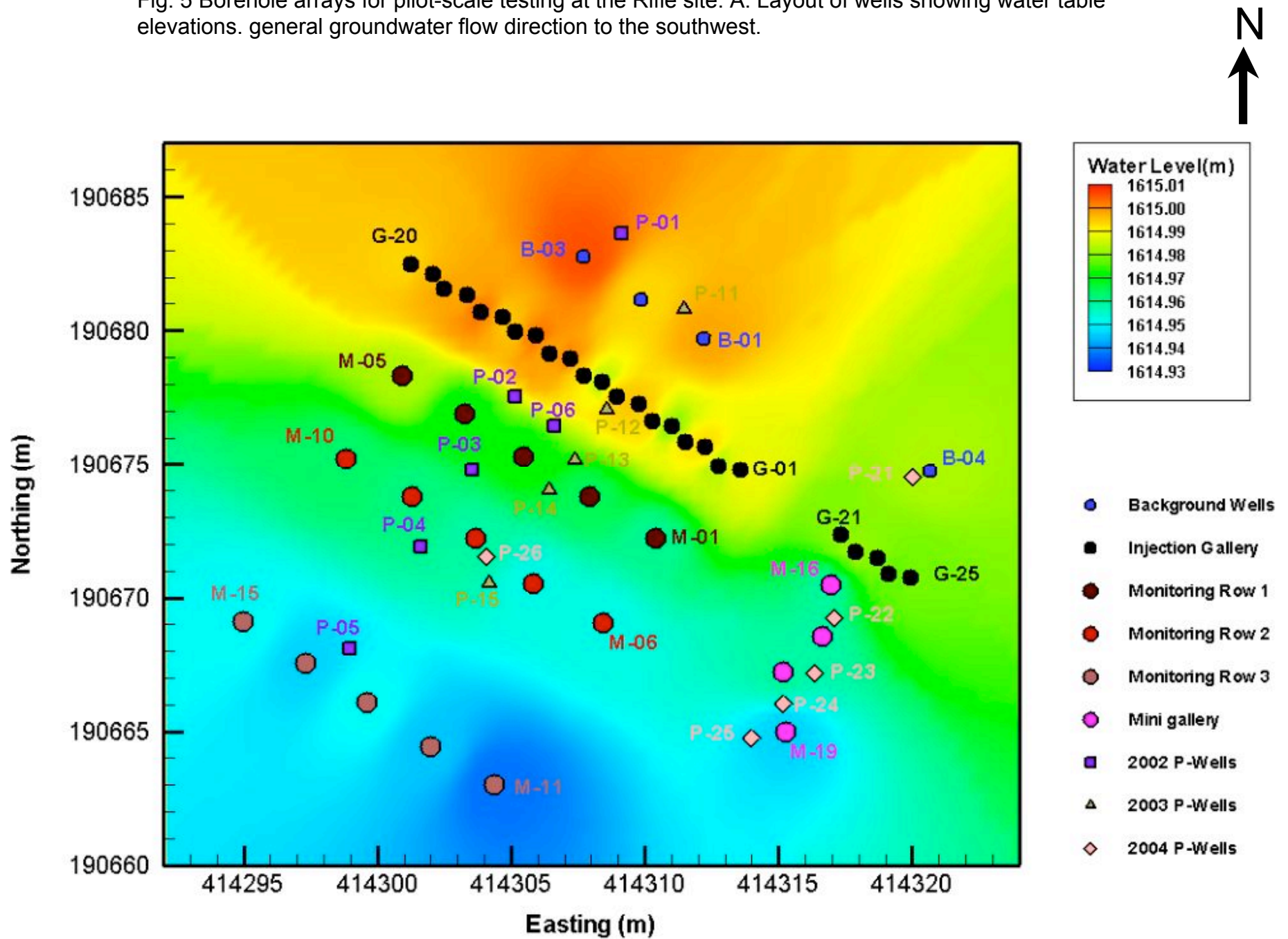


Fig. 5 Borehole arrays for pilot-scale testing at the Rifle site. A. Layout of wells showing water table elevations. general groundwater flow direction to the southwest.





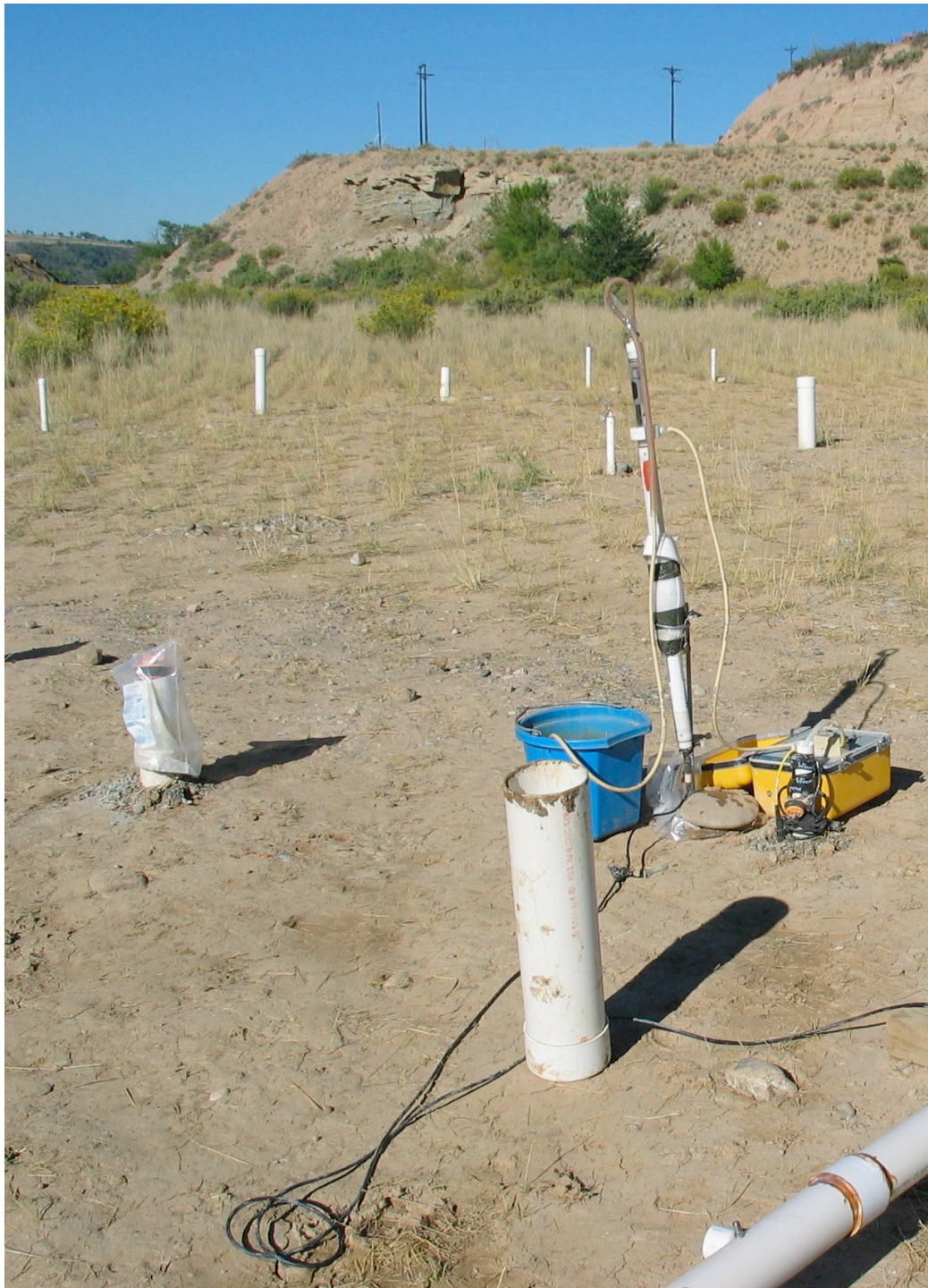


Fig. 5 Borehole arrays for pilot-scale testing at the Rifle site. B. Photo of wells and sampling apparatus.



Figure 6. Distribution of U(VI) as a function of depth during a biostimulation experiment. Note increase in U(VI) concentration near water table on 7/30/2002. Data obtained using passive multilevel samplers.

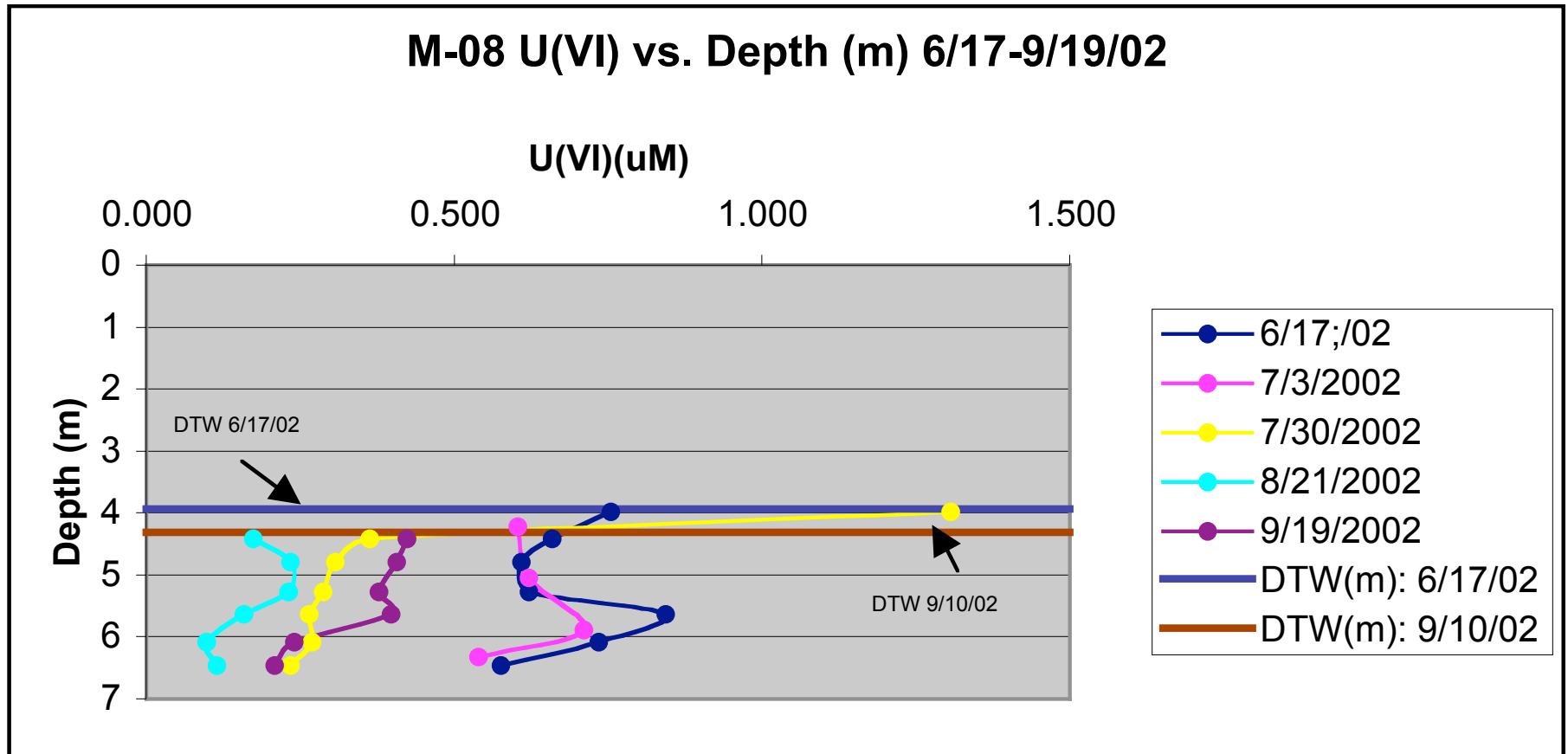
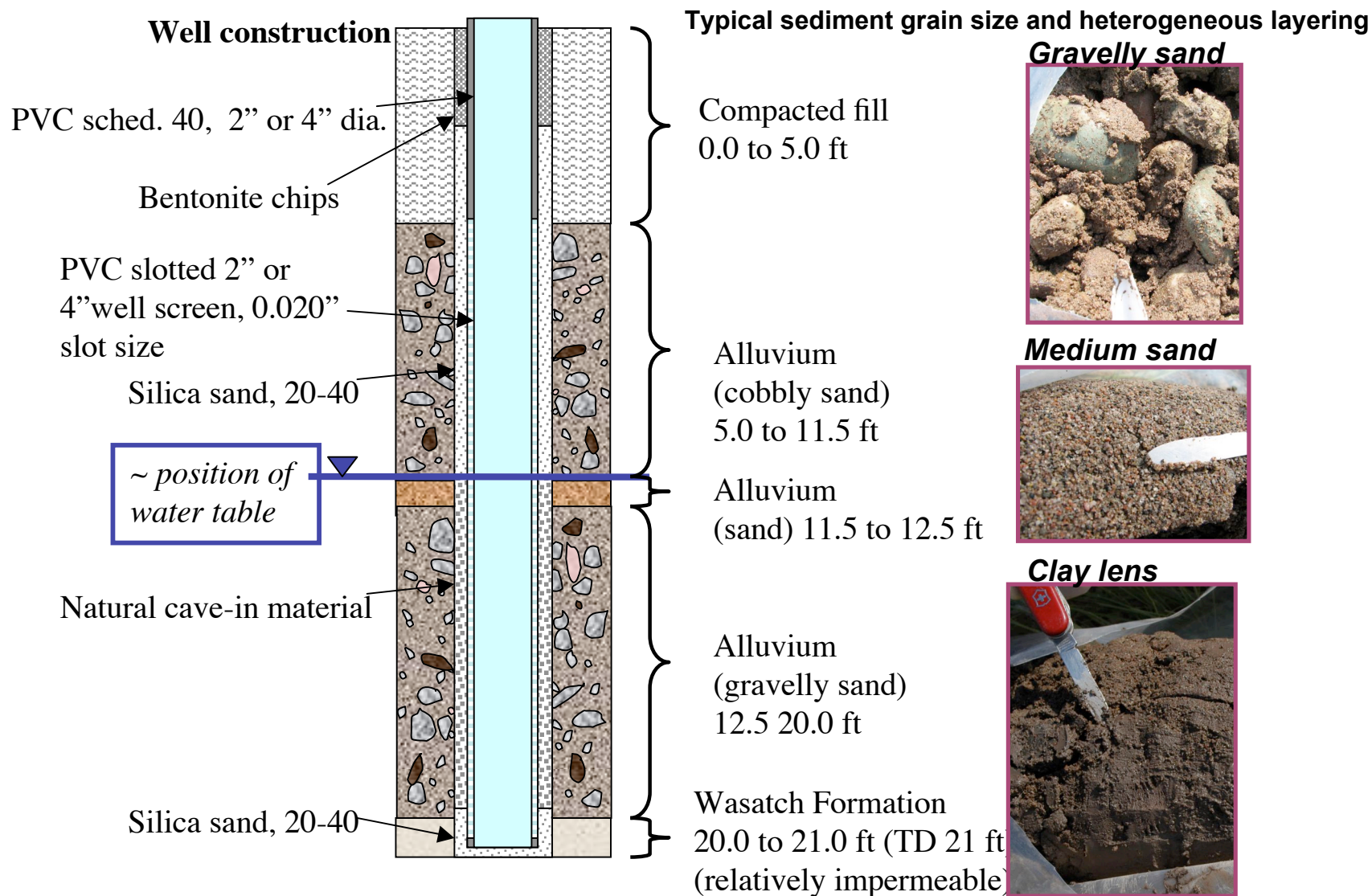


Figure 7. Passive multilevel samplers. A. Cell on support rod being lowered into monitoring well. B. MLS cells from a background well. C. MLS cells from a treatment zone well undergoing sulfate reduction.





**Figure. 8. Example of heterogeneity in alluvial sediment**





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