Savannah River National Laboratory

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Summary
Work performed under the Independent Research and Development (IRD) project 03-007 "Bioelectrochemical Process Development" using *Shewanella oneidensis* MR-1 (henceforth MR-1), a model dissimilitory metal reducing (DMRB) bacteria successfully demonstrates the following:

- MR-1 transfers electrons by hydrogen oxidation to solid phase electrodes
- MR-1 can act as an electron transfer agent when attached to a solid metal electrode surface – a modified electrode has been developed and tested
- MR-1 transfers electrons from solution to a solid electrode

These successful results merit a phase 2 of the project for which additional funding is being sought.

DMRB grow by transferring electrons to insoluble metals. When bacteria oxidize organic compounds or hydrogen this results in electron flow from the bacterial cell for the purpose of cellular energy production. Extracellular electron transfer to solid terminal electron acceptors permits coupling this oxidation process to electrodes. As a result, detection of organic compounds and hydrogen can be accomplished by bacterial contact to an electrode. We have designed a process that incorporates bacterial cells onto electrodes for the purpose of hydrogen detection. In addition, this type of bio-sensor also responds to the presence of Fe(III) thereby providing potential utility as a Fe(III) sensor. Immediate uses of this technology include in-situ detection and quantification of organic compounds and hydrogen in the subsurface that provide energy for growth of indigenous bacteria. This process could allow continuous monitoring over an extended time period and could be useful for assessment on processes such as bioremediation activities, monitored natural attenuation (MNA), and hydrogen detection.

Background
Organic compounds and hydrogen drive bioremediation processes. The detection of these compounds and their bioavailability over extended periods of time can provide useful information regarding microbial activity in the subsurface. The use of bacteria immobilized onto an electrode constitutes a bioelectrochemical probe that detects hydrogen, organics, and/or metals. Bioelectrochemical modified electrodes using MR-1 will have an expected use as a probe that detects bioavailable organics and hydrogen. MR-1 acts as an electron transfer agent to reduce metals in solution. The selectivity of the bacteria for specific metals has potential as a metal specific sensor.

Project Objectives
Some bacteria grow by transferring electrons to insoluble metals. A mechanism for this type of extracellular electron transfer was incorporated with electrochemistry principals in this project. Specifically, bacterial production of quinoid exopolymers provides a means of electron transfer from the bacteria cell to an insoluble electron acceptor (Turick et al. 2002. Appl. Env. Microbiol. 68:2436-2444). Overproduction of these polymers increases external electron transfer rates by as much as 10 times (Turick et al. 2003. FEMS Microbiol. Lett. 220:99-104). Extracellular bacterial electron transfer to glassy carbon electrodes has been demonstrated recently here at SRS with an internally funded

project for accelerated metal and radionuclide reduction/immobilization (PI-C.E.Truick). We exploited this novel phenomenon for potential application in chemical detection and metal, actinide and lanthanide reduction processes.

When bacteria oxidize organic compounds or hydrogen this results in electron flow from the bacterial cell for the purpose of cellular energy production. Extracellular electron transfer permits coupling this oxidation process to electrodes. As a result, detection of organic compounds and hydrogen is accomplished by bacterial contact to an electrode. We designed a process that incorporated bacterial cells onto electrodes for the purpose of chemical detection. Immediate uses of this technology include in-situ detection and quantification of organic compounds and hydrogen in the subsurface that provide energy for growth of indigenous bacteria. This process would allow for continuous monitoring over an extended time period and be useful for assessment on processes such as bioremediation activities and monitored natural attenuation (MNA) as well as hydrogen detection..

Data

Bacterial Modified Electrochemical Cell
For hydrogen detection, both a carbon and platinum working electrode from Bioanalytical Systems (BAS) were used as the electrode substrate. Fe(III) oxide detection was accomplished with an electrode consisting of a carbon paste and ferrihydrite ($Fe^{3+}_2O_3 \cdot 0.5(H_2O)$) mixture. The electrodes were inverted and the working surface of each electrode was covered with a paste of MR-1 in a pH 7 NaCl solution. A 40-microliter sample volume was introduced onto the surface of the electrode and the reference and counter electrodes were contacted with the solution. A Picture of this electrode configuration is shown in Figure 1.

Bacterial Modified Electrode
A bacterial modified electrode was prepared by covering the working electrode surface of the electrode with MR-1 paste. The paste was held on electrode surface using a semi-permiable Nafion membrane. This electrode is shown in figure 2. This configuration was used for potentiometric studies involving hydrogen detection.

Voltammetric Data
Results of cyclic voltammetry experiments and time-based potentiometric experiments are shown in figures 3-6.

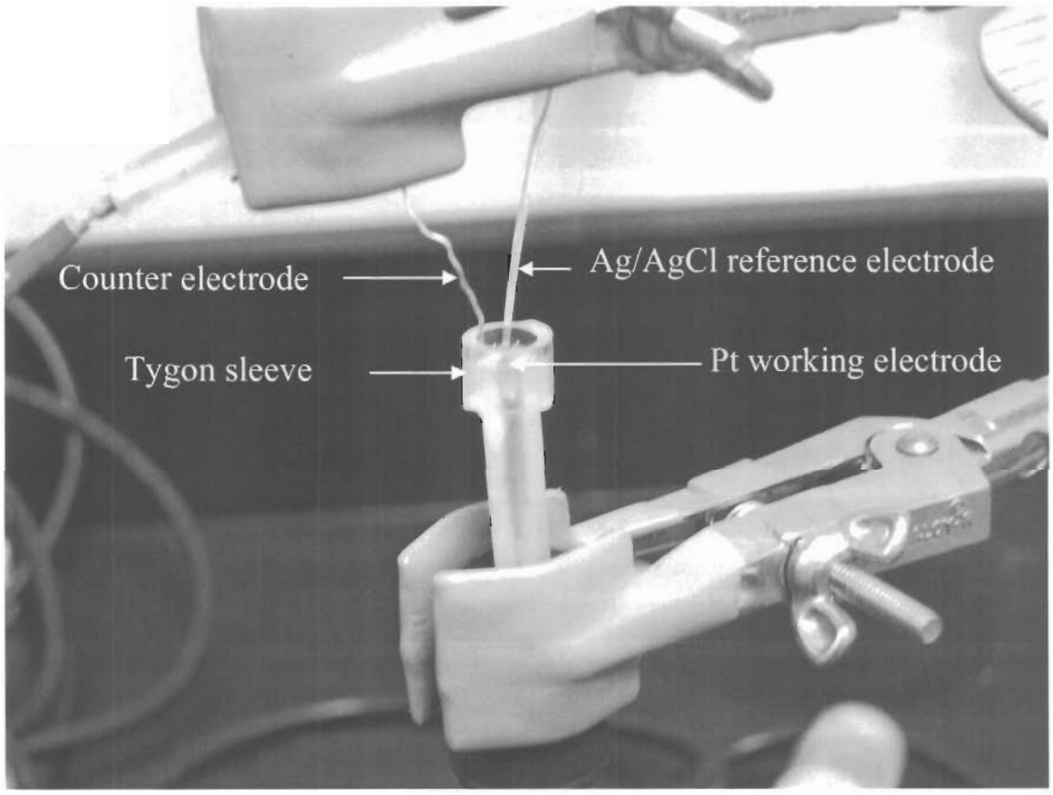

Figure 1. Example of inverted electrode configuration used for voltammetry studies. Bacterial suspensions were placed on the electrode surface inside the tygon sleeve. During anaerobic studies, the electrode was housed in a clear plastic tube and purged continuously with oxygen-free nitrogen.

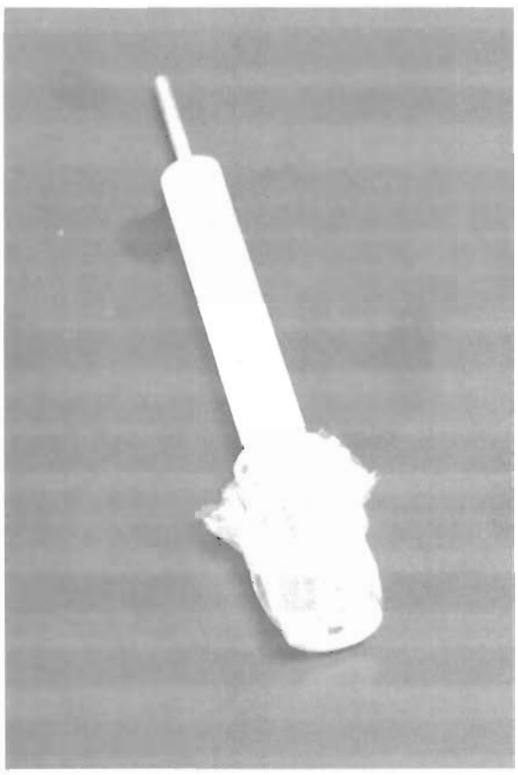

Figure 2. A bacterial modified electrode was prepared by covering the working electrode surface of the electrode with MR-1 paste. The paste was held on electrode surface using a semi-permiable Nafion membrane. This configuration was used for potentiometric studies involving hydrogen detection.

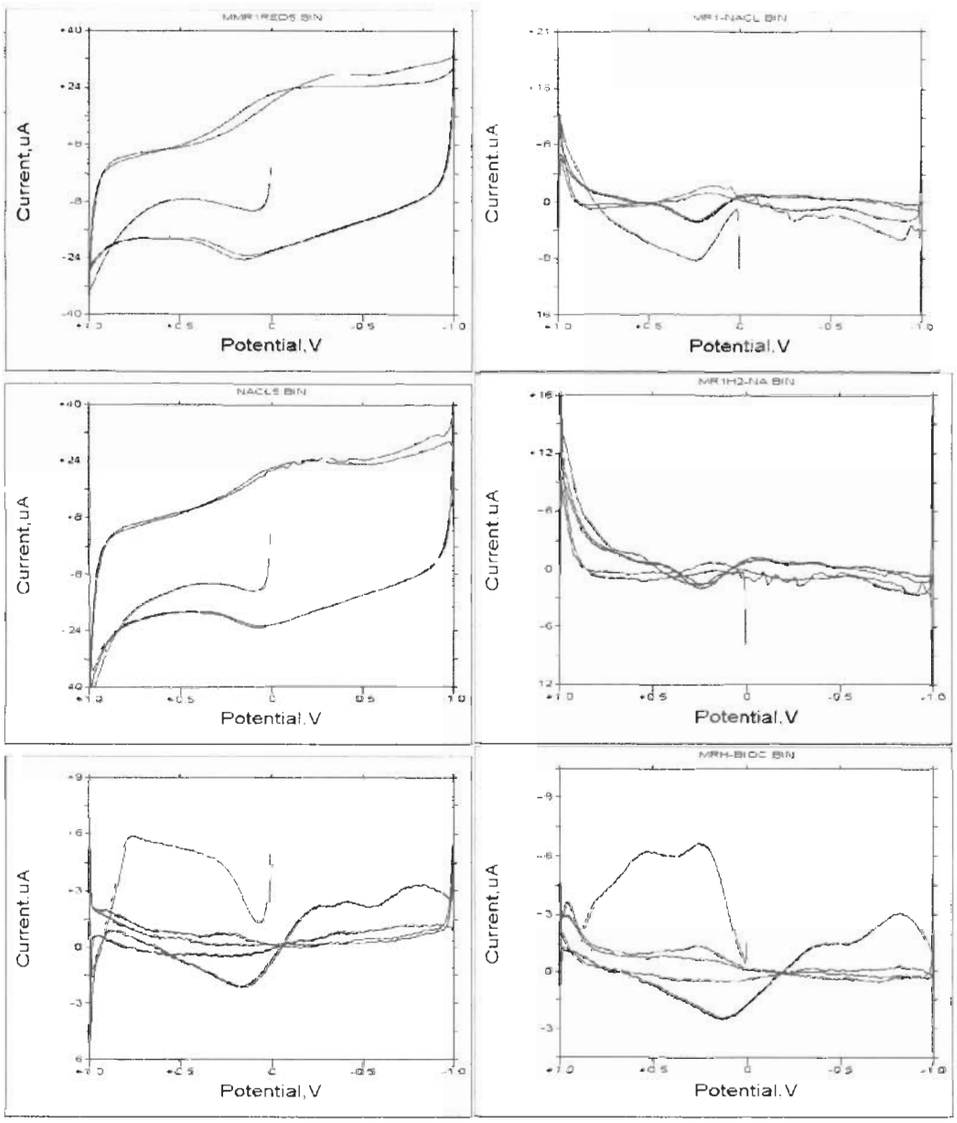

Figure 3. Cyclic voltammograms recorded with glassy carbon working electrode. A pH 7, 10g/L NaCl solution (A-1); 10^9 cells/ ml of *S. oneidensis* MR-1 after H_2 exposure in NaCl solution and with background (NaCl) subtracted (A-2 and A-3 respectively); air and H_2 exposed cells (with background subtracted) (B-1 and B-2 respectively); and the difference between air and H_2 exposed cells (B-3).

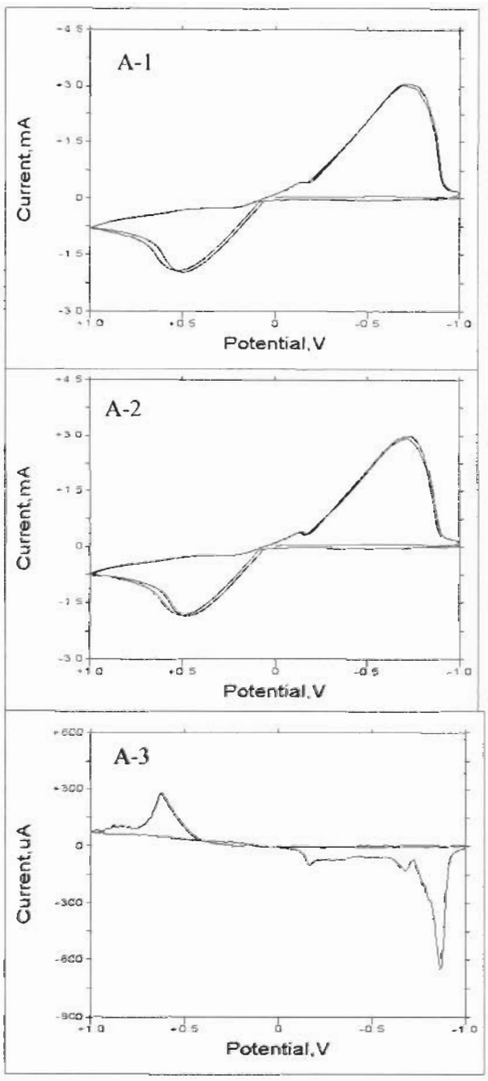

Figure 4. Cyclic voltammograms recorded with a Pt working electrode: a pH 7, 10g/L NaCl solution (A-1); 10^9 cells/ ml of *S. oneidensis* MR-1 after H_2 exposure in NaCl solution (A-2); and with background (NaCl) subtracted (A-3). The current response was over one order of magnitude greater with Pt electrodes then with glassy carbon electrodes (Fig. 3).

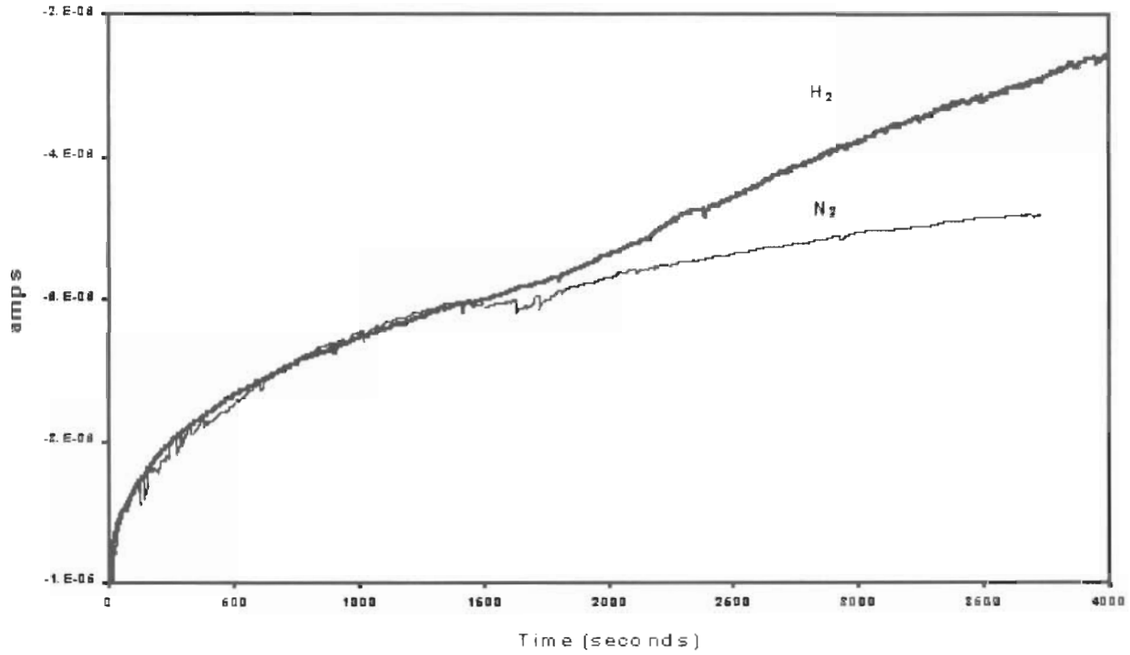

Figure 5. Current response generated by live bacteria (recorded in an undivided cell, in three-electrode configuration). Following an induction period of 1500 s, H_2 oxidation was coupled to reduction at the surface of a Pt electrode (poised at 550 mV vs. RE[Ag]) containing approx. 10^9 cells/mL of *S. oneidensis* MR-1 and resulted in current generation. No current was produced in the control experiment (i.e., during N_2 saturation).

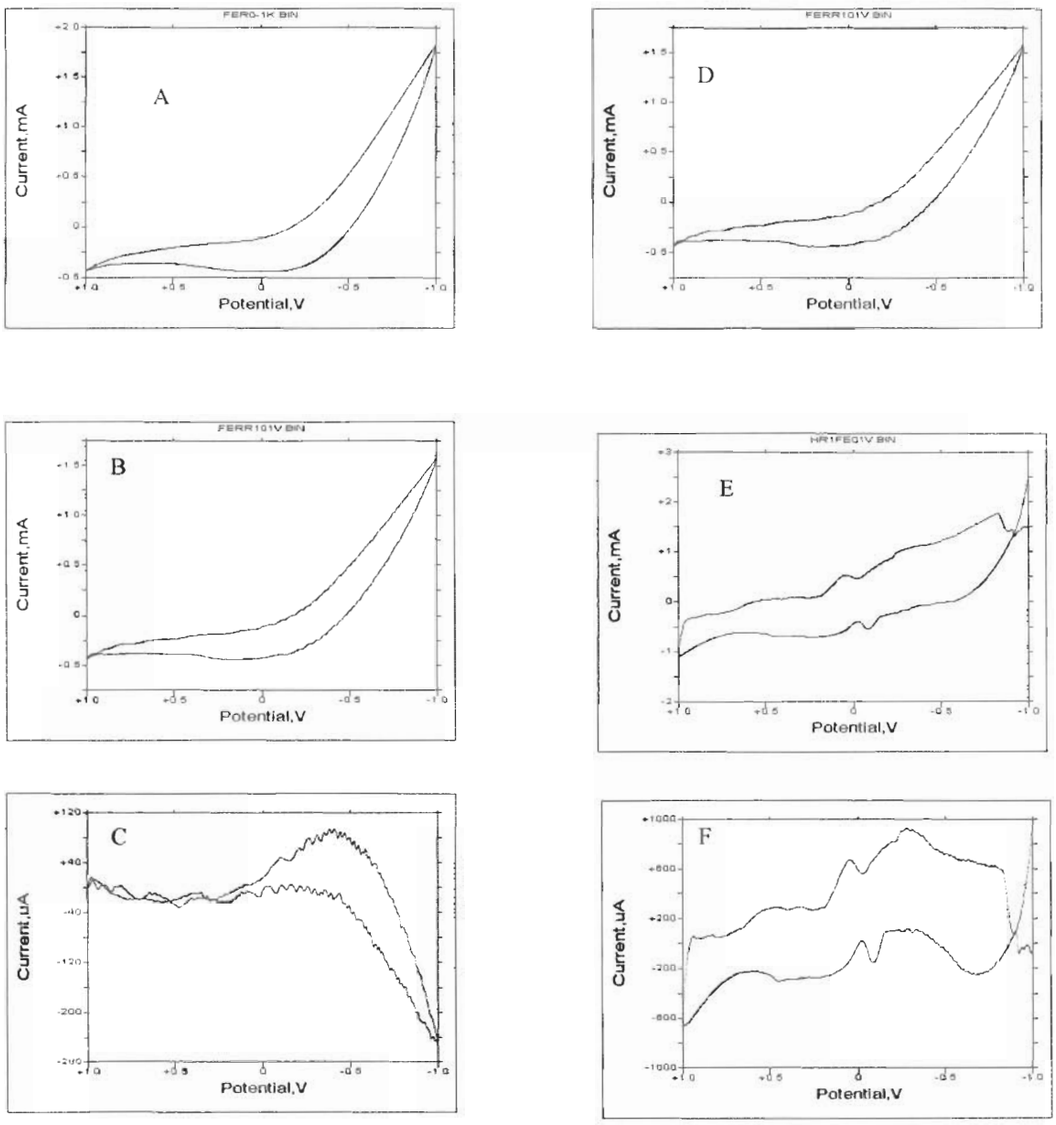

Figure 6. Cyclic voltammograms with Ferrihydrite and carbon paste electrode. LEFT COLUMN: fig A – electrode alone; fig B- electrode and *S. oneidensis* MR-1 (without hydrogen); fig C - graph B minus graph A. RIGHT COLUMN: fig D – same as graph B; fig E – *S. oneidensis* MR-1 with hydrogen; fig F – fig E minus fig D. All voltammograms conducted in pH 7 NaCl (10g/l). Ag/AgCl ref electrode and Pt counter electrode. Sweep rate=100mv/min. initial sweep = -1000mV.

Results

We have demonstrated a proof of concept that exploits a bacterial mechanism for electron transfer between an electrode and bulk solution and relates to ongoing work at SRS.

A prototype of a bioelectrochemical probe with incorporated bacteria capable of electron transfer to a solid electrode was configured and demonstrated. When the bacteria oxidize organics and/or hydrogen, the bacteria transfer electrons to the electrode. In this way an increased current from the electrode demonstrated the presence of hydrogen.

Interaction of the MR-1 with the electrode in an active electrochemical cell and shown in the cyclic voltammetric data acquired during this study. Figures 3 and 4 demonstrate electron transfer by MR-1 from species in solution to electrode, the use of MR-1 as an electron shuttle or transfer agent. A significant increase in electrode current response from MR-1 was detected when dosed with hydrogen (reduced) as compared to oxygen (oxidized aerobic), and nitrogen (anerobic). Steady electron transfer by MR-1 to the electrode is seen during time based potential experiments (figure 5). This indicated that the electron transfer is an active process performed by the MR-1 and not a simple electrochemical redox process. Redox reactions (oxidation or reduction of species at the electrode surface) would be seen by a decrease in current during time based experiments. Electron transfer behavior of the MR-1 is not seen using heat-killed cells (data not shown), only with live bacteria.

Path Forward

Upon further development this proof of concept will have use in detecting subsurface hydrogen or metal concentrations. This would be especially useful for real-time detection of nutrient flow during bioremediation activities. In addition such a probe will be useful in evaluating real-time microbial activity over extended time periods related to monitored natural attenuation. Its usefulness as an analytical tool for understanding electron transfer dynamics from bacteria to solid metals can be applied in the fields of bioremediation research and biocorrosion.

The following areas will be investigated in the continuation of this work:

- Quantify electron transfer
- Determine mechanism and rates of electron transfer
- Test modified electrodes on iron
- Determine bacterial density on electrode surface
- Radiochemical studies to determine mechanism of hydrogen metabolism

Presentations, Patents, Proposals, and Collaborations Initiated from this Work

Presentations

- 8th Annual Green Chemistry Conference, June 29, 2004, National Academy of Sciences, Washington DC.
- Presentation to Mark Frei, Director, EM-21, and to Claire Sink, EM-21, SRNL Visit, July 15, 2004.

Patent disclosures

- Bacteria Modified Electrode Sensor for Hydrocarbons in Groundwater
- Removal of Metals from Groundwater Using Bacterial Modified Electrodes
- Inverted Electrode Configuration for Ultramicrovolume Electrochemistry
- Bacteria Modified Electrode Design
- Bacteria Modified Electrode Design- 2
- Sensor

Proposal

- Electron Transfer Behavior at the Microbe/Mineral Interface. Funding agency: Natural and Accelerated Bioremediation Research (NABIR) Program, Program Announcement LAB 04-06.

Collaborations initiated

- Dr. Daniel Lowy and Dr. Leonard Tender – Naval Research Lab. Arlington VA
- Dr. Derek Lovley – University of Massachusetts

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