

**2005 ERSD Annual Report
Project #1027697**

Long-term Stewardship of Mixed Wastes: Passive Reactive Barriers for Simultaneous In Situ Remediation of Chlorinated Solvent, Heavy Metal and Radioactive....

Principal Investigator: Gerlach, Robin

Organization: Montana State University

Results To Date

1. MOST RECENT RESULTS TO DATE

This project report addresses one part of a 3-way collaboration between researchers (Drs. Robin Gerlach and Al Cunningham) at Montana State University's (MSU's) Center for Biofilm Engineering (CBE), (Dr. Brent Peyton at the WSU/NSF IGERT Center for Multiphase Environmental Research (CMER) at Washington State University (WSU), and (Drs. William Apel and Frank Roberto at) the Biotechnology Department at the INEEL. Each part of this project is funded under a different contract with the Science Division of the US Department of Energy. The project is designed to evaluate the possibility to develop a subsurface remediation technology for mixed wastes at Department of Energy sites using a group of common soil bacteria of the genus *Cellulomonas*. We are seeking to gain a better understanding of microbial transformation of chromium, uranium, and carbon tetrachloride by *Cellulomonas* spp. in simulated subsurface environments.

The project team is investigating 1) the influence of electron donors and acceptors on the reductive transformation of mixed contaminants in order to determine the most efficient way to utilize the activity of this group of facultative anaerobes capable of reductive transformation reactions under fermentative conditions. (INEEL lead - Dr. Bill Apel) 2) the long term activity of *Cellulomonas* spp. in the absence of external carbon sources. (MSU lead - Drs. Robin Gerlach and Al Cunningham) 3) the development of nutrient injection strategies that will allow the effective stimulation of *Cellulomonas* spp. or similar populations in subsurface environments. (WSU lead - Dr. Brent Peyton) 4) the presence of *Cellulomonas* spp. at a number of DOE sites. (INEEL lead - Dr. Frank Roberto)

MSU's efforts have mostly focused on observing the ability of strain ES6 (most closely related to *Cellulomonas hominis*) to reduce Cr(VI) in the absence of a supplemental carbon source and how the Cr(VI) reduction activity can be

enhanced by the addition of a carbon source. The carbon source screening identified molasses as a very efficient stimulator for Cr(VI) reduction activity and that the addition of electron shuttles such as humic substances or anthraquinone disulfonic acid can enhance the reduction rates significantly. The presence of iron minerals can also influence the rate and extent of Cr(VI) reduction. While the competition of oxidized iron minerals for the electrons needed for Cr(VI) reduction appears to be minimal, the presence of previously reduced iron minerals increases Cr(VI) reduction rates drastically indicating a chemical reaction between surface-associated ferrous iron and Cr(VI). The results of these studies are currently being summarized in a manuscript intended for peer review journal publication. In order to more closely investigate the mechanisms of Cr(VI) reduction, sucrose was chosen as the carbon source for future investigations so that the metabolic pathways involved in carbon source oxidation and Cr(VI) reduction can be investigated in more detail. The Cr(VI) reduction studies with starved cultures of strain ES6 indicate that the number of viable cells and the Cr(VI) reduction activity is decreasing with increasing starvation time but that the Cr(VI) reduction activity can be restored by the addition of sucrose as a carbon source. The lag time until Cr(VI) reduction is observed also increases with increasing starvation time. The results so far indicate that strain ES6 has some potential for long lasting Cr(VI) reduction activity. Carbon Tetrachloride (CT) reduction by strain ES6 has not been observed. It is possible though that the presence of iron minerals can enable strain ES6 to reduce CT. There are abundant reports in the literature describing the reductive transformation of chlorinated aliphatics by reduced iron minerals. MSU just received a Kinetic Phosphorescence Analyzer (ChemCheck Instruments, Richland, WA) that is now operational and will be used to conduct U(VI) reduction studies. Additionally, in the year 2003, Dr. Gerlach supported in kind and Materials and Supplies support for a Ph.D. student (Thomas Borch) funded by a fellowship from the Inland Northwest Research Alliance (INRA) who investigated the transformation of the explosive 2,4,6-Trinitrotoluene (TNT) by strain ES6 in the presence of electron shuttling compounds and iron minerals. The results of these studies are currently prepared for submission to peer review (see below). Meso-scale (4 ft length) column studies investigating the combined effects of direct and indirect microbial Cr(VI)-reduction were performed in a collaborative effort between MSU, WSU, and the INEEL in 2003. The columns studies ran over a period of several months and the results of these experiments form the basis for a manuscript currently in preparation (see below). The project team met on June 08, 2004 in Idaho Falls in order to discuss and coordinate past and future research efforts and collaborations.

Deliverables

2. PAPERS AND OTHER PRODUCTS DELIVERED OR IN PREPARATION

2.1. Publication 1: 2.1.1. Citation GERLACH, R.; JENNINGS, L.; CUNNINGHAM, A.; PEYTON, B.M.; APEL, W.A. 2004. Fe(III) Mineral Reduction

and Fe(II)-mediated Cr(VI) reduction by Gram Positive Environmental Isolates from the Hanford Site - Influence of Electron Shuttles. Bioremediation Journal (In preparation)

2.2. Publication 2: 2.2.1. Citation GERLACH, R, JENNINGS, L, PEYTON BM, APEL WA, CUNNINGHAM AB. 2004. "Reduction of Chromate by Microbially Reduced Iron Minerals and Electron Shuttling Compounds - Studies with Bacterial Isolates from Hanford Site". Environmental Science and Technology (In Preparation) 2.3. Publication 3: 2.3.1. Citation VIAMAJALA S, GERLACH R, SIVASWAMY V, PEYTON BM, APEL WA, CUNNINGHAM AB, PETERSEN JN. 2004. "Chromate reduction by Cellulomonas: Multi-scale flow cell experiments" Environmental Science and Technology. (In Preparation)

2.4. Publication 4: 2.4.1. Citation BORCH T.; GERLACH R. & INSKEEP W.P. (2004) The Influence of Fe(Hydr)oxide Phases and the electron shuttle anthraquinone-2,6-disulfonate on the reduction of 2,4,6-Trinitrotoluene by a fermenting Bacterium. Environmental Science and Technology (In preparation).

2.5. Publication 5: 2.5.1. Citation BORCH T.; INSKEEP W.P.; HARWOOD J.A. & GERLACH R. (2004) Biotic and abiotic mechanisms contribute to the degradation of 2,4,6-trinitrotoluene by a Gram-Positive Fermenting Bacterium in the presence of Hydrous Ferric Oxide and Electron Shuttles. Environmental Science and Technology (In preparation)

2.6. Presentation 1: 2.6.1. Citation R. GERLACH; BORCH, T.; CUNNINGHAM, A.B.; VIAMAJALA, S.; PEYTON, B.M.; APEL, W.A. (2003): Influence of Electron Shuttling Compounds on the Reduction of Metals and Organics. Platform Presentation at the 3rd INRA Subsurface Science Symposium, Salt Lake City, UT, October 05-08, 2003

2.7. Presentation 2: 2.7.1. Citation VIAMAJALA, S.; GERLACH, R.; PEYTON, B.M.; APEL, W.A.; CUNNINGHAM, A.B.; PETERSEN, J.N. (2003): Passive Reactive Barrier (PRB) for In Situ Remediation of Cr(VI): Bench- and Meso-Scale Tests using Cellulomonas spp. Platform Presentation at the 3rd INRA Subsurface Science Symposium, Salt Lake City, UT, October 05-08, 2003

2.8. Presentation 3: 2.8.1. Citation T. BORCH; GERLACH, R.; CUNNINGHAM, A.B. (2003): Iron (III) Minerals Can Impact the Microbial Reduction of 2,4,6-Trinitrotoluene. Platform Presentation at the 3rd INRA Subsurface Science Symposium, Salt Lake City, UT, October 05-08, 2003

2.9. Presentation 4: 2.9.1. Citation GERLACH, R.; BORCH, T.; CUNNINGHAM, A.B.; VIAMAJALA, S.; PEYTON, B.M.; APEL, W.A. (2004): Influence of Electron Shuttling Compounds and Iron Minerals on the Reduction of Metals and Organics. Platform presentation. 4th International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, May 24-27, 2004.