FINAL REPORT

Mobility of Source Zone Heavy Metals and Radionuclides: The Mixed Roles of Fermentative Activity on Fate and Transport of U and Cr

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A Collaborative Research Project between Montana State University and the Idaho National Laboratory

Submitted to the DOE

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1. Executive Summary

Various U. S. Department of Energy (DOE) low and medium-level radioactive waste sites contain mixtures of heavy metals, radionuclides and assorted organic materials. In addition, there are numerous sites around the world that are contaminated with a mixture of organic and inorganic contaminants. In most sites, over time, water infiltrates the wastes, and releases metals, radionuclides and other contaminants causing transport into the surrounding environment.

We investigated the role of fermentative microorganisms in such sites that may control metal, radionuclide and organics migration from source zones. The project was initiated based on the following overarching hypothesis:

Metals, radionuclides and other contaminants can be mobilized by infiltration of water into waste storage sites. Microbial communities of lignocellulose degrading and fermenting microorganisms present in the subsurface of contaminated DOE sites can significantly impact migration by directly reducing and immobilizing metals and radionuclides while degrading complex organic matter to low molecular weight organic compounds. These low molecular weight organic acids and alcohols can increase metal and radionuclide mobility by chelation (i.e., certain organic acids) or decrease mobility by stimulating respiratory metal reducing microorganisms.

We demonstrated that fermentative organisms capable of affecting the fate of Cr^{6+}, U^{6+} and trinitrotoluene can be isolated from organic-rich low level waste sites as well as from less organic rich subsurface environments. The mechanisms, pathways and extent of contaminant transformation depend on a variety of factors related to the type of organisms present, the aqueous chemistry as well as the geochemistry and mineralogy.

This work provides observations and quantitative data across multiple scales that identify and predict the coupled effects of fermentative carbon and electron flow on the transport of radionuclides, heavy metals and organic contaminants in the subsurface; a primary concern of the DOE Environmental Remediation Science Division (ERSD) and Subsurface Geochemical Research (SBR) Program.

2.0 Background

Project Objectives: Research activities along with processing of nuclear materials have led to the generation of a substantial amount of low-level mixed radioactive wastes that have been disposed in pits, trenches and other waste sites across the DOE complex. DOE has identified more than 10,000 individual waste sites representing 113 facilities spread across 30 states (National Research Council 1999). Sites of particular concern include; the Savannah River Site, Hanford Site, Oak Ridge National Laboratory, Idaho National Laboratory and Rocky Flats. Riley and Zachara (1992) summarized mixtures of contaminants prevalent in sediments and groundwater at 18 DOE facilities and found that radionuclides and metals occurred at more waste sites than any other categories of contaminants listed. The second most prevalent class of contaminants were chlorinated hydrocarbons, followed by a range of other organic contaminants.

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Project Description:
The first task was targeted at understanding the influence of cellulolytic and non-cellulolytic fermenters on Cr and U mobility in simulated LLW. These studies were conducted in batch and small percolating soil columns. Task 2 focused on developing an understanding of the fate of U and Cr in mixed communities as well as the community response to carbon and electron flow through simulated LLW sediments. Microorganisms in simulated low level radioactive waste sediments were identified and isolated. Task 3 was designed to develop a mechanistic understanding of the influence of spatial and temporal variables on metal and radionuclide migration.

Potential Impact: Over 130 sites in 31 states, under the jurisdiction of the U.S. Department of Energy (DOE), comprise over 2 million acres of total contaminated area and approximately 6.4 billion m^3 of contaminated media. Responsibility for management, remediation or monitored natural attenuation, and long-term stewardship of these sites falls on the DOE. The integrated tasks were targeted at performing research to understand microbiological reduction of U(VI) and Cr(VI) by fermentative microbes using high and low molecular weight organics on metal and radionuclide transport. The scientific information produced from this research is highly valuable, identifying the effect of carbon and electron flow through the environment on metal and radionuclide fate would substantially improve predictions of contaminant migration at DOE sites. The proposed research will provide an observational and experimental approach for understanding the coupling of biological, chemical, mineralogical and hydrogeological processes that control fate and transport of U(VI) and Cr(VI) from waste disposal sites at many DOE facilities.

The results of the project have been published in a number of peer reviewed journal articles. The abstracts and citations to those articles, given in section 3.0 below, make up the bulk of our final report.

3.0 Publications

3.1 Publication 1

3.1.1. Citation:

Borch, T.; Inskeep W.P.; Harwood, J.A.; R. Gerlach, R. (2005): Impact of Ferrihydrite and Anthraquinone-2,6-Disulphonate on the Reductive Transformation of 2,4,6-Trinitrotoluene by a Gram Positive Fermenting Bacterium. *Environmental Science and Technology*. 39(18):7126-7133

3.1.2. Abstract:

Batch studies were conducted to explore differences in the transformation pathways of 2,4,6-trinitrotoluene (TNT) reduction by a Gram-positive fermenting bacterium (*Cellulomonas* sp. strain ES6) in the presence and absence of ferrihydrite and the electron shuttle anthraquinone-2,6- disulfonate (AQDS). Strain ES6 was capable of TNT and ferrihydrite reduction with increased reduction rates in the presence of AQDS. Hydroxylaminodinitrotoluenes, 2,4- dihydroxylamino-6-nitrotoluene (2,4-DHANT), and tetranitroazoxytoluenes were the major metabolites observed in ferrihydrite- and AQDS-free systems in the presence of pure cell cultures. Ferrihydrite enhanced the production of amino derivatives because of reactions with microbially produced surface-associated Fe(II). The presence of AQDS in the absence of ferrihydrite promoted the fast initial formation of arylhydroxylamines such as 2,4-DHANT. However, unlike in pure cell systems, these arylhydroxylamines were transformed into several unidentified polar products. When both microbially reduced ferrihydrite and AQDS were present simultaneously, the reduction of TNT was more rapid and complete via pathways that would have been difficult to infer solely from single component studies. This study demonstrates the complexity of TNT degradation patterns in model systems where the interactions among bacteria, Fe minerals, and organic matter have a pronounced effect on the degradation pathway of TNT.

3.1.3. Summary of Major Results:

- Demonstration that a *Cellulomonas* sp. isolated from the Pacific Northwest National Laboratory (strain ES6) can reduce not only Cr(VI) but also 2,4,6-trinitrotoluene (TNT).
- The presence of iron minerals and humic-like substances can influence the reduction rate and pathway of nitroaromatic transformation

3.2 Publication 2

3.2.1. Citation:

Cunningham, A.B.; Sharp, R.S.; Caccavo Jr, F.; Gerlach, R. (2007): Effects of Starvation on Bacterial Transport Through Porous Media. *Advances in Water Resources*. 30(6-7):1583-1592.

3.2.2. Abstract:

A major problem preventing widespread implementation of microbial injection strategies for bioremediation and/or microbially enhanced oil recovery is the tendency of bacteria to strongly adhere to surfaces in the immediate vicinity of the injection point. Long term (weeks to months) nutrient starvation of bacteria prior to injection can decrease attachment and enhance transport through porous media. This paper summarizes results of starvation-enhanced transport experiments in sand columns of 30 cm, 3 m, and 16 m in length. The 16 m column experiments compared transport, breakthrough and distribution of adhered cells for starved and vegetative cultures of *Klebsiella oxytoca*, a copious biofilm producer. Results from these experiments were subsequently used to design and construct a field scale biofilm barrier using starved *Pseudomonas fluorescens*. The 30 cm and 3 m sand columns experiments investigated starvation enhanced transport of *Shewanella algae* BrY, a dissimilatory metal-reducing bacterium. In both cases the vegetative cells adsorbed onto the sand in higher numbers than the starved cells, especially near the entrance of the column. These results, taken together with studies cited in the literature, indicate that starved cells penetrate farther (i.e. higher breakthrough concentration) and adsorb more uniformly along the flow path than vegetative cells.

3.2.3. Summary of Major Results:

- Long term (weeks to months) nutrient starvation of bacteria prior to injection can decrease attachment, enhance transport and uniform distribution of injected bacteria in porous media.

3.3 Publication 3

3.3.1. Citation:

Viamajala S., Gerlach R., Sivaswamy V., Peyton B.M., Apel W.A., Cunningham A.B., Petersen J.N. (2008): Permeable reactive biobarriers for in-situ Cr(VI) reduction: Bench scale tests using *Cellulomonas* sp. strain ES6. *Biotechnology and Bioengineering*. 101(6):1150-1162. doi:10.1002/bit.22020

3.3.2. Abstract:

Chromate (Cr(VI)) reduction studies were performed in bench scale flow columns using the fermentative subsurface isolate *Cellulomonas* sp. strain ES6. In these tests, columns packed with either quartz sand or hydrous ferric oxide (HFO)-coated quartz sand, were inoculated with strain ES6 and fed nutrients to stimulate growth before nutrient-free Cr(VI) solutions were injected. Results show that in columns containing quartz sand, a continuous inflow of 2 mg/L Cr(VI) was reduced to below detection limits in the effluent for durations of up to 5.7 residence times after nutrient injection was discontinued proving the ability of strain ES6 to reduce chromate in the absence of an external electron donor. In the HFO-containing columns, Cr(VI) reduction was significantly prolonged and effluent Cr(VI) concentrations remained below detectable levels for periods of up to 66 residence times after nutrient injection was discontinued. Fe was detected in the effluent of the HFO containing columns throughout the period of Cr(VI) removal indicating that the insoluble Fe(III) bearing solids were being continuously reduced to form soluble Fe(II) resulting in prolonged abiotic Cr(VI) reduction. Thus, growth of *Cellulomonas* within the soil columns resulted in formation of permeable reactive barriers that could reduce Cr(VI) and Fe(III) for extended periods even in the absence of external electron donors. Other bioremediation systems employing Fe(II)-mediated reactions require a continuous presence of external nutrients to regenerate Fe(II). After depletion of nutrients, contaminant removal within these systems occurs by reaction with surface-associated Fe(II) that can rapidly become inaccessible due to formation of crystalline Fe-minerals or other precipitates. The ability of fermentative organisms like *Cellulomonas* to reduce metals without continuous nutrient supply in the subsurface offers a viable and economical alternative technology for in situ remediation of Cr(VI)-contaminated groundwater through formation of permeable reactive biobarriers (PRBB)

3.3.3. Summary of Major Results:

- Cr(VI) reduction was prolonged in columns containing iron minerals after nutrient injection was discontinued due to the presence of reduced iron minerals that were capable of reducing chromate.
- This demonstrates the potential of permeable reactive barriers capable of reducing Cr(VI) and Fe(III) for extended periods even in the absence of external electron donors, thus potentially reducing the need for re-injecting electron donors (carbon sources) as frequently.

3.4 Publication 4

3.4.1. Citation:

Borch, T., Camper, A.K., Biederman, J.A., Butterfield, P.W., Gerlach, R., Amonette, J.E. (2008): Evaluation of Characterization Techniques for Iron Pipe Corrosion Products and Iron Oxide Thin Films. *Journal of Environmental Engineering*. 134(10):835-844.

3.4.2. Abstract:

A common problem faced by drinking water studies is that of properly characterizing the corrosion products (CP) in iron pipes or synthetic Fe (hydr)oxides used to simulate the iron pipe used in municipal drinking-water systems. The present work compares the relative applicability of a suite of imaging and analytical techniques for the characterization of corrosion products (CP) and synthetic Fe oxide thin films and provide an overview of the type of data that each instrument can provide as well as their limitations to help researchers and consultants choose the best technique for a given task. Crushed CP from a water distribution system and synthetic Fe oxide thin films formed on glass surfaces were chosen as test samples for this evaluation. The CP and synthetic Fe oxide thin films were analyzed by atomic force microscopy (AFM), scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), X-ray powder diffractometry (XRD), grazing incident diffractometry (GID), transmission electron microscopy (TEM), selected area electron diffraction (SAED), X-ray photoelectron spectroscopy (XPS), Fourier transform infrared (FTIR), Mössbauer spectroscopy, BET N_2 adsorption and Fe concentration was determined by the ferrozine method. XRD and GID were found to be the most suitable techniques for identification of the mineralogical composition of CP and synthetic Fe oxide thin films, respectively. AFM and a combined ToF-SIMS – AFM approach proved excellent for roughness and depth profiling analysis of synthetic Fe oxide thin films, respectively. Corrosion products were difficult to study by AFM due to their surface roughness, while synthetic Fe oxide thin films resisted most spectroscopic methods due to their limited thickness (118 nm). XPS analysis is not recommended for mixtures of Fe (hydr)oxides due to their spectral similarities. SEM and TEM provided great detail on mineralogical morphology.

3.4.3. Summary of Major Results:

- This work compares the applicability of different imaging and analytical techniques for the characterization of iron minerals including corrosion products and synthetic Fe oxide thin films
- The work is designed to provide guidance to researchers and consultants in choosing the best technique for a given task

3.5 Publication 5

3.5.1. Citation:

VanEngelen, M.R.; Field, E.K.; Gerlach R., Lee, B.D.; Apel W.A.; Peyton B.M. (2010): UO_2^{2+} speciation: impact on toxicity and bioaccumulation in an environmental *Pseudomonas* sp. isolate. *Environmental Toxicology and Chemistry*. 29(4):763-769. doi:10.1002/etc.126

3.5.2. Abstract:

In the present study, experiments were performed to investigate how representative cellulosic breakdown products, when serving as growth substrates under aerobic conditions, affect hexavalent uranyl cation (UO_2^{2+}) toxicity and bioaccumulation within a *Pseudomonas* sp. isolate (designated isolate A). Isolate A taken from the Cold Test Pit South (CTPS) region of the Idaho National Laboratory (INL), Idaho Falls, ID, USA. The INL houses low-level uranium-contaminated cellulosic material and understanding how this material, and specifically its breakdown products, affect U-bacterial interactions is important for understanding UO_2^{2+} fate and mobility. Toxicity was modeled using a generalized Monod expression. Butyrate, dextrose, ethanol, and lactate served as growth substrates. The potential contribution of bicarbonate species present in high concentrations was also investigated and compared with toxicity and bioaccumulation patterns seen in low-bicarbonate conditions. Isolate A was significantly more sensitive to UO_2^{2+} and accumulated significantly more UO_2^{2+} in low-bicarbonate concentrations. In addition, UO_2^{2+} growth inhibition and bioaccumulation varied depending on the growth substrate. In the presence of high bicarbonate concentrations, sensitivity to UO_2^{2+} inhibition was greatly mitigated, and did not vary between the four substrates tested. The extent of UO_2^{2+} accumulation was also diminished. The observed patterns were related to UO_2^{2+} aqueous complexation, as predicted by MINTEQ (ver. 2.52) (Easton, PA, USA). In the low- bicarbonate medium, the presence of positively charged and unstable UO_2^{2+} hydroxide complexes explained both the greater sensitivity of isolate A to UO_2^{2+}, and the ability of isolate A to accumulate significant amounts of UO_2^{2+}. The exclusive presence of negatively charged and stable UO_2^{2+}-carbonate complexes in the high bi-carbonate medium explained the diminished sensitivity of isolate A to UO_2^{2+} toxicity, and limited ability of isolate A to accumulate UO_2^{2+}.

3.5.3. Summary of Major Results:

- A *Pseudomonas* sp. isolate from the Cold Test Pit South (CTPS) at the Idaho National Laboratory (INL), was significantly more sensitive to UO_2^{2+} and accumulated significantly more UO_2^{2+} in low-bicarbonate concentrations.
- In addition, UO_2^{2+} growth inhibition and bioaccumulation varied depending on the growth substrate.
- In the presence of high bicarbonate concentrations, sensitivity to UO_2^{2+} inhibition was greatly mitigated, and did not vary between the four substrates tested.

3.6 Publication 6

3.6.1. Citation:

Field, E.K.; D'Imperio, S.; Miller, A.R.; VanEngelen, M.R.; Gerlach, R.; Lee, B.D.; Apel, W.A.; Peyton, B.M. (2010): Application of Molecular Techniques to Elucidate the Influence of Cellulosic Waste on the Bacterial Community Structure at a Simulated Low-Level Waste Site. *Applied and Environmental Microbiology*. 76(10):3106-3115. doi:10.1128/AEM.01688-09

3.6.2. Abstract:

Low-level-radioactive-waste (low-level-waste) sites, including those at various U.S. Department of Energy sites, frequently contain cellulosic waste in the form of paper towels, cardboard boxes, or wood contaminated with heavy metals and radionuclides such as chromium and uranium. To understand how the soil microbial community is influenced by the presence of cellulosic waste products, multiple soil samples were obtained from a nonradioactive model low-level-waste test pit at the Idaho National Laboratory. Samples were analyzed using 16S rRNA gene clone libraries and 16S rRNA gene microarray (PhyloChip) analyses. Both methods revealed changes in the bacterial community structure with depth. In all samples, the PhyloChip detected significantly more operational taxonomic units, and therefore relative diversity, than the clone libraries. Diversity indices suggest that diversity is lowest in the fill and fill-waste interface (FW) layers and greater in the wood waste and waste-clay interface layers. Principal-coordinate analysis and lineage-specific analysis determined that the *Bacteroidetes* and *Actinobacteria* phyla account for most of the significant differences observed between the layers. The decreased diversity in the FW layer and increased members of families containing known cellulose degrading microorganisms suggest that the FW layer is an enrichment environment for these organisms. These results suggest that the presence of the cellulosic material significantly influences the bacterial community structure in a stratified soil system.

3.6.3. Summary of Major Results:

- PhyloChip analysis detected significantly more operational taxonomic units than clone libraries.
- Diversity indices suggest that diversity is lowest in the fill and fill-waste interface (FW) layers and greater in the wood waste and waste-clay interface layers.
- *Bacteroidetes* and *Actinobacteria* phyla account for most of the significant differences observed between the layers.
- Cellulose degrading microorganisms appear to be enriched for in the wood waste-clay interface layer.

3.7 Publication 7

3.7.1. Citation:

Sivaswamy, V.; Boyanov, M.I.; Peyton, B.M.; Viamajala, S.; Gerlach, R., Apel, W.A.; Sani, R.K.; Dohnalkova, A; Kemner, K.M.; Borch, T. (2011): Multiple Mechanisms of Uranium Immobilization by *Cellulomonas* sp. strain ES6. *Biotechnology and Bioengineering*. 108:264-276. doi: 10.1002/bit.22956

3.7.2. Abstract:

Removal of hexavalent uranium (U(VI)) from aqueous solution was studied using a Gram-positive facultative anaerobe, *Cellulomonas* sp. strain ES6, under anaerobic, non-growth conditions in bicarbonate and PIPES buffers. Inorganic phosphate was released by cells during the experiments providing ligands for formation of insoluble U(VI) phosphates. Phosphate release was most probably the result of anaerobic hydrolysis of intracellular polyphosphates accumulated by ES6 during aerobic growth. Microbial reduction of U(VI) to U(IV) was also observed. However, the relative magnitudes of U(VI) removal by abiotic (phosphate-based) precipitation and microbial reduction depended on the buffer chemistry. In bicarbonate buffer, X-ray absorption fine structure (XAFS) spectroscopy showed that U in the solid phase was present primarily as a non-uraninite U(IV) phase, whereas in PIPES buffer, U precipitates consisted primarily of U(VI)-phosphate. In both bicarbonate and PIPES buffer, net release of cellular phosphate was measured to be lower than that observed in U-free controls suggesting simultaneous precipitation of U and PO_4^{3-}. In PIPES, U(VI) phosphates formed a significant portion of U precipitates and mass balance estimates of U and P along with XAFS data corroborate this hypothesis. High-resolution transmission electron microscopy (HRTEM) and energy dispersive X-ray spectroscopy (EDS) of samples from PIPES treatments indeed showed both extracellular and intracellular accumulation of U solids with nanometer sized lath structures that contained U and P. In bicarbonate, however, more phosphate was removed than required to stoichiometrically balance the U(VI)/U(IV) fraction determined by XAFS, suggesting that U(IV) precipitated together with phosphate in this system. When anthraquinone-2,6-disulfonate (AQDS), a known electron shuttle, was added to the experimental reactors, the dominant removal mechanism in both buffers was reduction to a non-uraninite U(IV) phase. Uranium immobilization by abiotic precipitation or microbial reduction has been extensively reported; however, the present work suggests that strain ES6 can remove U(VI) from solution simultaneously through precipitation with phosphate ligands and microbial reduction, depending on the environmental conditions. *Cellulomonadaceae* are environmentally relevant subsurface bacteria and here, for the first time, the presence of multiple U immobilization mechanisms within one organism is reported using *Cellulomonas* sp. strain ES6.

3.7.3. Summary of Major Results:

- Phosphate release during anaerobic hydrolysis of intracellular polyphosphates accumulated by ES6 during aerobic growth was responsible for the precipitation UO_2^{2+} as insoluble U(VI) phosphates.

- *Cellulomonas* sp. strain ES6 can remove U(VI) from solution simultaneously through precipitation with phosphate ligands and microbial reduction, depending on the environmental conditions.
- The relative magnitudes of U(VI) removal by abiotic (phosphate-based) precipitation and microbial reduction depended on the solution chemistry.

3.8 Publication 8

3.8.1. Citation:

VanEngelen, M.R.; Szilagyi, R.K.; Gerlach R., Lee, B.D.; Apel W.A.; Peyton B.M. (2011): Uranium exerts acute toxicity by binding to pyrroloquinoline quinone cofactor. *Environmental Science and Technology*. 45(3):937-942. doi: 10.1021/es101754x

***3.8.2. Abstract*:**

Uranium as an environmental contaminant has been shown to be toxic to eukaryotes and prokaryotes; however, no specific mechanisms of uranium toxicity have been proposed so far. Here a combination of *in vivo*, *in vitro*, and *in silico* studies are presented describing direct inhibition of pyrroloquinoline quinone (PQQ)-dependent growth and metabolism by uranyl cations. Electrospray-ionization mass spectroscopy, UV-Vis optical spectroscopy, competitive Ca^{2+}/uranyl binding studies, relevant crystal structures, and molecular modelling unequivocally indicate the preferred binding of uranyl simultaneously to the carboxyl oxygen, pyridine nitrogen, and quinone oxygen of the PQQ molecule. The observed toxicity patterns are consistent with the biotic ligand model of acute metal toxicity. In addition to the environmental implications, this work represents the first proposed molecular mechanism of uranium toxicity in bacteria, and has relevance for uranium toxicity in many living systems.

***3.8.3. Summary of Major Results*:**

- A combination of *in vivo*, *in vitro*, and *in silico* studies demonstrate that the uranyl (UO_2^{2+}) can inhibit the pyrroloquinoline quinone (PQQ) cofactor of the ethanol dehydrogenase.
- This work represents the first proposed molecular mechanism of uranium toxicity in bacteria and has relevance for uranium toxicity in many living systems

3.9 Publication 9

3.9.1. Citation:

Gerlach, R.; Field, E.K..; Viamajala, S; Peyton, B.M.; Apel, W.A.; Cunningham, A.B. (2011) Influence of Carbon Sources and Electron Shuttles on Ferric Iron Reduction by *Cellulomonas* sp. strain ES6. *Biodegradation*. 22(5):983-995. DOI: 10.1007/s10532-011-9457-1.

3.9.2. Abstract:

Microbially reduced iron minerals can reductively transform a variety of contaminants including heavy metals, radionuclides, chlorinated aliphatics, and nitroaromatics. A number of *Cellulomonas* spp. strains, including strain ES6, isolated from aquifer samples obtained at the U.S. Department of Energy's Hanford site in Washington, have been shown to be capable of reducing Cr(VI), TNT, natural organic matter, and soluble ferric iron [Fe(III)]. This research investigated the ability of *Cellulomonas* sp. strain ES6 to reduce solid phase and dissolved Fe(III) utilizing different carbon sources and various electron shuttling compounds. Results suggest that Fe(III) reduction by and growth of strain ES6 was dependent upon the type of electron donor, the form of iron present, and the presence of synthetic or natural organic matter, such as anthraquinone-2,6-disulfonate (AQDS) or humic substances. This research suggests that *Cellulomonas* sp. strain ES6 could play a significant role in metal reduction in the Hanford subsurface and that the choice of carbon source and organic matter addition can allow for independent control of growth and iron reduction activity.

3.9.3. Summary of Major Results:

- *Cellulomonas* sp. strain ES6 is capable of reducing solid phase and dissolved Fe(III) while utilizing different carbon sources and various electron shuttling compounds.
- This research suggests that *Cellulomonas* sp. strain ES6 could play a significant role in metal reduction in the Hanford subsurface and that the choice of carbon source and organic matter addition can allow for independent control of growth and iron reduction activity.

3.10 Publication 10

3.10.1. Citation:

Khilyas, I.V.; Ziganshin, A.M.; Pannier, A.J.; Gerlach, R. (2012): Effect of ferrihydrite on 2,4,6-trinitrotoluene biotransformation by an aerobic yeast. *Biodegradation*. 24(5):631-644. DOI 10.1007/s10532-012-9611-4

3.10.2. Abstract:

This study investigated the impact of ferrihydrite on the pathway and rate of 2,4,6-trinitrotoluene (TNT) transformation by *Yarrowia lipolytica* AN-L15. The presence of ferrihydrite in the culture medium decreased the rate of TNT biotransformation but resulted in the accumulation of the same TNT metabolites as in the absence of ferrihydrite, albeit at slightly different concentrations. Transformation products observed included aromatic ring reduction products, such as hydride-Meisenheimer complexes, and nitro group reduction products, such as hydroxylamino- and amino-dinitrotoluenes. Independently of the presence of ferrihydrite the subsequent degradation of the hydride complex(es) resulted in the release of nitrite followed by its conversion to nitrate and nitric oxide at the low pH values observed during yeast cultivation. Nitric oxide generation was ascertained by electron spin resonance spectroscopy. In addition, increased Fe^{3+}-reduction was observed in the presence of TNT and Y. lipolytica. This study demonstrates that in the presence of yeast cells, TNT hydride complexes were formed at approximately the same level as in the presence of ferrihydrite, opening up the possibility of aromatic ring cleavage, instead of promoting the production of potentially toxic nitro group reduction products in the presence of iron minerals.

3.10.3. Summary of Major Results:

- The presence of ferrihydrite in the culture medium decreased the rate of TNT biotransformation by the common soil yeast, *Yarrowia lipolytica*.
- However TNT transformation occurred and resulted in the accumulation of the same metabolites as in the absence of ferrihydrite, albeit at slightly different concentrations.
- The greater (and unintuitive) UO_2 reoxidation by hematite compared to ferrihydrite can be explained by the exhaustion of hematite from reaction with sulfide.
- Increased Fe^{3+}-reduction was observed in the presence of TNT and Y. lipolytica indicating the possibility of abiotic reduction of Fe^{3+} by reduced TNT metabolites.
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3.11 Publication 11

3.11.1. Citation:

Field, E.K.; Gerlach, R.; Viamajala, S.; Jennings, L.K.; Peyton B.M.; Apel W.A.; Cunningham A.B. (2012): Influence of Carbon Source, Iron Minerals, and Electron Shuttling Compounds on Hexavalent Chromium Reduction by *Cellulomonas* sp. Strain ES6. *Biodegradation*. DOI: 10.1007/s10532-012-9600-7

3.11.2. Abstract:

The reduction of hexavalent chromium, Cr(VI), to trivalent chromium, Cr(III), can be an important aspect of remediation processes at contaminated sites. *Cellulomonas* species are found at several Cr(VI) contaminated and uncontaminated locations at the Department of Energy site in Hanford, Washington. Members of this genus have demonstrated the ability to effectively reduce Cr(VI) to Cr(III) fermentatively and therefore play a potential role in Cr(VI) remediation at this site. Batch studies were conducted with *Cellulomonas* sp. strain ES6 to assess the influence of various carbon sources, iron minerals, and electron shuttling compounds on Cr(VI) reduction rates as these chemical species are likely to be present in, or added to, the environment during in situ bioremediation. Results indicated that the type of carbon source as well as the type of electron shuttle present influenced Cr(VI) reduction rates. Molasses stimulated Cr(VI) reduction more effectively than pure sucrose, presumably due to presence of more easily utilizable sugars, electron shuttling compounds or compounds with direct Cr(VI) reduction capabilities. Cr(VI) reduction rates increased with increasing concentration of anthraquinone-2,6-disulfonate (AQDS) regardless of the carbon source. The presence of iron minerals and their concentrations did not significantly influence Cr(VI) reduction rates. However, strain ES6 or AQDS could directly reduce surface-associated Fe(III) to Fe(II), which was capable of reducing Cr(VI) at a near instantaneous rate. These results suggest the rate limiting step in these systems was the transfer of electrons from strain ES6 to the intermediate or terminal electron acceptor whether that was Cr(VI), Fe(III), or AQDS.

3.11.3. Summary of Major Results:

- The type of carbon source as well as the type of electron shuttle present influences Cr(VI) reduction by Cellulomonas sp. strain ES6.
- Molasses stimulated Cr(VI) reduction more effectively than pure sucrose, presumably due to presence of more easily utilizable sugars, electron shuttling compounds or compounds with direct Cr(VI) reduction capabilities.
- Cr(VI) reduction rates increased with increasing concentration of anthraquinone-2,6-disulfonate (AQDS) regardless of the carbon source.
- The presence of iron minerals and their concentrations did not significantly influence Cr(VI) reduction rates.

3.12 Publication 12

3.12.1. Information:

Field, E.K.; Blaskovich, J.P.; Peyton B.M.; Gerlach, R. (2014): **Carbon source influences hexavalent chromium toxicity and reduction by an environmental *Arthrobacter* sp. isolate**. *Microbiology*. In preparation.

3.12.2. Abstract:

Arthrobacter spp. are well-known for their Cr(VI)-resistance and reduction capabilities and have been isolated from Department of Energy sites, such as low-level waste sites. These sites typically contain cellulosic waste co-disposed with heavy metals and radionuclides, such as hexavalent chromium. It is possible that as cellulose is degraded, its breakdown products can be utilized by metal-reducing microorganisms as carbon and energy sources. To-date, most of the studies regarding Cr(VI) toxicity and reduction by *Arthrobacter* spp. have solely utilized nutrient rich medium or glucose. The purpose of this study was to determine the influence of environmentally relevant carbon sources including potential cellulose degradation products on the chromate toxicity and reduction capabilities of the environmental *Arthrobacter* sp. isolate EF01. Results indicate that the carbon source present influenced Cr(VI) toxicity and reduction by isolate EF01. When glucose was the sole carbon source in the presence of 50 µM Cr(VI), isolate EF01 had a decreased growth rate and maximum optical cell density (OD_{600}), but no significant different was observed when lactate was present. Compared to lactate, Cr(VI) was found to be at least 30-fold and 10-fold more toxic when ethanol or butyrate was the sole carbon source available, respectively. A decrease in Cr(VI) reduction was only observed when either glucose or lactate were present. The addition of sulfate, a structural analog to chromate, mitigated toxicity somewhat, but not completely. Overall, these results suggest that there is a carbon-mediated toxicity mechanism in *Arthrobacter* sp. isolate EF01 that influences both growth and reduction capabilities. This can have important implications when designing remediation strategies and monitoring microbial chromate reduction in the environment.

3.12.3. Summary of Major Results:

- Determination of the influence of environmentally relevant carbon sources including potential cellulose degradation products on the chromate toxicity and reduction capabilities of the environmental *Arthrobacter* sp. isolate EF01.
- Compared to lactate, Cr(VI) was found to be at least 30-fold and 10-fold more toxic when ethanol or butyrate was the sole carbon source available, respectively.
- The results suggest that there is a carbon-mediated toxicity mechanism in *Arthrobacter* sp. isolate EF01 that influences both growth and reduction capabilities.

Final Report - Mobility of Source Zone Heavy Metals and Radionuclides: The Mixed Roles of Fermentative Activity on Fate and Transport of U and Cr

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