

DOE EPSCoR Final Technical Report

Project Title: Laboratory and *In Situ* Evaluation of Enzyme Activity-Dependent Fluorescent Probes Specific for Chlorinated Solvent Degrading Bacteria

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Results Summary:

When assessing a site for potential bioremediation, it is important to determine whether the native microbial community contains members capable of producing enzymes that can degrade the contaminant of interest. It is also essential to determine whether these genes are actively being expressed, and further, whether the enzymes produced are actively catalyzing the appropriate reactions. Therefore, methods to determine genetic potential, gene expression, as well as direct enzyme activity, are all useful in planning a bioremediation.

Trichloroethylene (TCE) is one of the most common contaminants of groundwater in the US. It is a suspected human carcinogen, and exposure to TCE has been linked to a number of potential human health effects. Several bacterial groups are capable of oxidative, cometabolic degradation of TCE. Bacteria expressing these enzymes are commonly found in soil and groundwater. In addition to TCE, these enzymes degrade a range of aromatic compounds, which themselves are often found as contaminants.

Our lab group has developed enzyme activity-dependent probes for most of the toluene oxygenase pathways which cometabolize TCE. In conjunction with Dr. Frank Roberto and Ms. Amber Miller (INEEL), we have also developed an enzyme activity-dependent probe for soluble methane monooxygenase, which also cometabolizes TCE. These probes are surrogate substrates that are transformed by the enzyme to yield fluorescent and/or colored products. They give a clear, quantifiable signal only when the enzyme of interest is actively functioning. Their pathway specificities have been demonstrated, and response of the probes has been correlated with TCE degradation in microcosm studies. We have also developed PCR primer sets specific for the genes encoding the initial hydrolase for each of the toluene oxidizing pathways. The primers were located within the open reading frame of the gene so that they could be used with extracted DNA to detect the presence of the gene, and with extracted mRNA to detect active transcription.

We have also adapted additional approaches to analyzing bacteria containing the toluene oxygenase pathways. These techniques include fluorescent *in situ* hybridization (FISH) and 5-cyano, 2,3-ditotyl tetrazolium chloride (CTC) reduction. FISH of whole cells using 16S rRNA oligonucleotide probes is a powerful technique for evaluating the identity, abundance, and relative activity of microbial cells. CTC is a monotetrazolium redox dye, which produces a fluorescent formazan crystal when biologically or chemically reduced. CTC provides a direct visualization of actively respiring bacterial cells. These techniques allow us to examine and better understand relationships between microbial community metabolic activity, single cell metabolic activity, and specific enzyme activity in laboratory test cultures.

By applying the entire suite of these techniques, we have assessed the status of microbes containing the desired degradative enzymes in the environment. In order to evaluate the utility of these techniques in an environmental sample, they were applied to groundwater samples from TCE contaminated aquifer at the Test Area North (TAN) site of the Idaho National Engineering and

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Environmental Laboratory (INEEL), and cells were harvested from the samples by sterile filtration of the groundwater. Multiple wells within the plume have been fitted with flute devices, which allow sampling at discrete depths. Samples were also obtained from just outside the plume for comparative purposes. We have completed two full field trials of the enzyme activity probes, along with molecular analysis and other approaches, at the TAN site. The results of these studies, which are being submitted for publication, indicate substantial levels of toluene oxygenase and soluble methane monooxygenase activity. This type of information can play a pivotal role in verification of monitored natural attenuation as well as monitoring of active bioremediation.

Refereed Publications:

- LaViolette, R. , M. E. Watwood, T. R. Finn, and D. L. Stoner. 1999. Spatial disorder and degradation kinetics in intrinsic biodegradation schemes. *Journal of Physical Chemistry A* 103(23): 4480-4484.
- Lee, B., K. Schaller, M. Watwood and W. Apel. 2000. Transition metal catalyst-assisted reductive dechlorination of perchloroethylene by anaerobic aquifer enrichments. *Journal of Bioremediation* 4(2): 97-110.
- O'Connell, S. P., R.D. Lawson, M. Watwood, and R.M. Lehman. 2000. A BASIC Program for reduction of data from community-level physiological profiling using Biolog Plates. *Journal of Microbiological Methods* 40(3): 213-220.
- Miller, A.R., F. Roberto, W. Keener and M. Watwood. 2002. Development of a fluorescence-based assay for soluble methane monooxygenase. *Applied Microbiology and Biotechnology* 58: 183-188.
- Watwood, M.E., Keener, W.K., and Smith, W.A., 2002, Characterization of microbial isolates from the Idaho National Engineering and Environmental Laboratory Test Area North aquifer: Identifying potential enzymatic pathways for toluene oxidation, in Link, P.K., and Mink, L.L., editors, *Geology, Hydrogeology and Environmental Remediation: Idaho National Engineering and Environmental Laboratory, Eastern Snake River Plain, Idaho: Boulder, Colorado, Geological Society of America Special Paper 353*, p. 279-285.
- Kauffman, M.K., W. K. Keener, S. R. Clingenpeel, M. E. Watwood, D. W. Reed, Y. Fujita and R. M. Lehman. 2003. Use of 3-hydroxyphenylacetylene for activity-dependent, fluorescent labeling of bacteria that degrade toluene via 3-methylcatechol. *Journal of Microbiological Methods* 55(3): 541-889
- Keener WK, Schaller KD, Walton MR, Partin JK, Watwood ME, Smith WA, and Clingenpeel SR. Selective Inhibitors and Chromogenic Substrates for Toluene-Degrading Enzymes: Differentiation of Bacterial Groundwater Isolates. *Journal of Microbiological Methods* 46:171-185.
- Colwell, F.S., R.W. Smith, F. Grant Ferris, A.-L. Reysenbach, Yoshiko Fujita, T.L. Tyler, J.L. Taylor, A. Banta, M.E. Delwiche, T. McLing, and M.E. Watwood. Microbially-mediated subsurface calcite precipitation for removal of hazardous divalent cations: Microbial activity, molecular biology, and modeling. ACS Symposium Series: Environmental Management Science Program. American Chemical Society. In review.
- Tyler, T.L., P.P. Sheridan, M.E. Watwood, Y. Fujita and F.S. Colwell. PCR primers for bacterial urease (ureC) genes and comparative phylogenetic analysis of ureC and 16S rRNA gene sequences. *FEMS Microbiology and Ecology*. In review.
- Keener, W.K. and M.E. Watwood. A chromogenic reaction for sensitive chloride detection using 3,3',5,5'-tetramethylbenzidine and chloroperoxidase. *Analytical Biochemistry*. In review.

Clingenpeel, S., W. K. Keener, K. DeJesus, H. Howard-Jones and M.E. Watwood. Activity-dependent fluorescent labeling of bacterial cells expressing the TOL pathway. *Journal of Microbiological Methods*. In review.

Conference Presentations:

- 1999 Miller, A., W. Keener, F. Roberto, M. Watwood, and P. Link. Enzyme Activity-Dependent Probes for Trichloroethylene (TCE) Degradative Pathways for Bacteria. Batelle International Conference on Bioremediation. San Diego, California.
- 1999 O'Connell, S.P., S. Colwell, R.M. Lehman, and M. Watwood. Groundwater microbial species diversity as affected by TCE in microcosm experiments. International Symposium on Subsurface Microbiology, Vail, Colorado.
- 1999 Kauffman, M.E., W.K. Keener and M. Watwood. Activity-dependent enzymatic labeling of bacteria capable of cometabolizing trichloroethylene. Geological Society of America Regional Meeting. Idaho Falls, Idaho.
- 1999 Watwood, M.E. and W. A. Smith. Metabolic and physiological characterization of microbial isolates from Test Area North Aquifer with emphasis on enzymatic pathways for toluene oxidation. Geological Society of America Regional Meeting. Idaho Falls, Idaho.
- 1999 Miller, A., Kauffman, M.W., W. Keener, F. Roberto, M. Watwood, and P. Link. Enzyme Activity-Dependent Probes for Trichloroethylene (TCE) Degradative Pathways for Bacteria. International Symposium on Subsurface Microbiology, Vail, Colorado.
- 1999 Davis, R. and M.E. Watwood. Characterization of TCE degrading microbial consortium via an anaerobic modification of the CLPP analysis. ASM International Microbial Biodiversity Conference. Chicago, Illinois.
- 1999 O'Connell, S. Colwell, F.S., Lehman, R.M. and M.E. Watwood. Groundwater microbial species diversity as affected by trichloroethylene in microcosm experiments. ASM International Microbial Biodiversity Conference. Chicago, Illinois.
- 2000 O'Connell, S. P., M. E. Watwood, R. M. Lehman, and F. S. Colwell. Optimized recovery of microbial community DNA from groundwater. Intermountain Branch Conference of the American Society for Microbiology. Provo, Utah.
- 2000 Keller, C.R., K. De Jesus, and M. Watwood. Preparation of ¹³C-Labeled Aromatic Acetylene Fluorophores. Idaho State University Undergraduate Research Symposium, Pocatello, Idaho.
- 2000 Watwood Mary E., Rebecca A. Davis, Sean P. O'Connell. Dehalogenation in solvent mixtures: Biodegradation kinetics and microbial community characterization. Batelle International Symposium on Chlorinated Solvent Remediation. Monterey, California.

- 2000 Miller, Amber R., Mary E. Kauffman, Mary E. Watwood, William K. Keener, Frank F. Roberto, and Paul K. Link. Enzyme Activity-Dependent Probes for Trichloroethylene (TCE) Degradative pathways in bacteria. Intermountain Conference on the Environment. Pocatello, Idaho.
- 2001 Watwood, Mary E. Biodegradation and bioremediation of anthropogenic contaminants. Department of Geology Colloquium. Pocatello, Idaho. (invited)
- 2001 Kauffman, Mary E., William K. Keener, R. Michael Lehman, Mary E. Watwood, Hoi-Ying Holman, and Michael C. Martin. Effects of bacterial attachment on the production of toluene 2-monooxygenase. Batelle International Conference on In situ and On-site Bioremediation. San Diego, California.
- 2001 O'Connell, Seán, Mary E. Watwood, Frederick S. Colwell, and R. Michael Lehman. Molecular and culture-based techniques for monitoring trichloroethylene-exposed microbial communities. Batelle International Symposium on In situ and On-site Bioremediation. San Diego, California.
- 2001 Tyler, T. L., M. E. Watwood, and F. S. Colwell. Development of molecular methods for the detection of microbial urease genes in the Snake River Plain Aquifer. American Society for Microbiology 101st General Meeting. Orlando, Florida.
- 2001 Tyler, T. L., M. E. Watwood, F. S. Colwell. Molecular methods for the detection of microbial urease genes in the Snake River Plain Aquifer. Intermountain Branch of the American Society for Microbiology. Pocatello, Idaho.
- 2001 O'Connell, S.O., M. E. Watwood, R. M. Lehman, and F. S. Colwell. Estimation of groundwater microbial species diversity using denaturing gradient gel electrophoresis and plate count methods. Intermountain Branch of the American Society for Microbiology. Pocatello, Idaho.
- 2001 Tyler, T. L., J. Cook, M. E. Watwood, F. S. Colwell. Primers for PCR detection of microbial urease genes in the Snake River Plain Aquifer. Subsurface Science Symposium of the Inland Northwest Research Alliance. Idaho Falls, Idaho.
- 2001 Keller, Caron, Karl DeJesus, Maribeth Watwood. Synthetic studies on aromatic alkyne fluorophores. Idaho State University Undergraduate Research Symposium, Pocatello, Idaho.
- 2001 Kauffman, M.E., W. K. Keener, M.E. Watwood, R. M. Lehman. Fluorescent probe for the detection of toluene-oxidizing bacteria in groundwater and on mineral surfaces. American Geophysical Union. San Francisco, California (poster won award for M. Kauffman, presenter).
- 2001 Taylor, J.T., Y. Fujita, M. Delwiche, F.S. Colwell, M. Watwood. Urease activity in organisms collected from the Snake River Plain Aquifer. INRA/INEEL Subsurface Science Symposium, Idaho Falls, Idaho.
- 2002 Tyler, T.L., M.E. Watwood, F.S. Colwell. Primers for amplifying genes encoding ureases in subsurface microbial communities. American Society for Microbiology - 102nd General Meeting. Salt Lake City, Utah.

- 2002 Taylor, J.L., M. Delwiche, Y. Fujita, F.S. Colwell, M. Watwood. Evaluation of aquifer urease activities using a single well push-pull test. American Society for Microbiology 102nd General Meeting, Salt Lake City, Utah.
- 2002 Taylor, J.L., Y. Fujita, M. Delwiche, F.S. Colwell, M. Watwood. Evaluation of various nutrient additions for enhancement of urea hydrolysis by Snake River Plain Aquifer microorganisms. Intermountain Conference on the Environment, Pocatello, Idaho.
- 2002 Clingenpeel, S., W.K. Keener, M. Watwood. Development of primers for the detection of toluene oxygenase genes. Batelle Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds. Monterey, California.
- 2002 O'Connell, SP, RM Lehman, ME Watwood, and FS Colwell. Impacts of chemical disturbance on the diversity of subsurface microbial communities. Annual Meeting of the Ecological Society of America, Tucson, Arizona.
- 2002 Watwood, ME, WK Keener, FS Colwell, MH Howard-Jones, S Clingenpeel. Evaluation of enzyme activity probes for the detection of contaminant degrading bacteria. Inland Northwest Research Alliance Annual Subsurface Science Symposium, Boise, Idaho (invited).