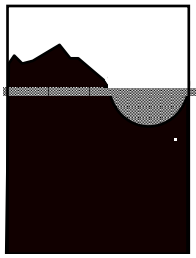

WESTERN REGION
HAZARDOUS SUBSTANCE RESEARCH CENTER

Oregon State University

FINAL REPORT

for the period

October 2001 to August 2007

Supported by

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WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER
FINAL REPORT FOR THE PERIOD 2001-2007

TABLE OF CONTENTS

WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER
FINAL REPORT (2001-2007)

Introduction

The final report for the Western Region Hazardout Substance Research Center provides an overview of the accomplishments of the Center for the period of from October 2001 to August 2007. The Western Region Hazardous Substance Research Center (WRHSRC) was a cooperative activity between Oregon State University and Stanford University and served Regions 9 and 10 of the EPA. The research focus of the Center was to develop innovative technologies for the in situ treatment of volatile organic chemicals (VOCs) in groundwater, especially chlorinated solvents. The Center funded twelve research projects, an outreach center to provide Technical Outreach Services for Communities (TOSC), Technical Assistance to Brownfields Communities (TAB), and technology transfer. The report provides final summary reports for all the Center's projects. Publications that resulted from the Center's research projects are also listed as well as publications that resulted from field demonstrations that were based on basic and applied research of the Center. Also provided is information on Center's administration, such as members of the Science Advisory Board and the Outreach Advisory Board and the funding of the Center.

One of the most import functions of the Center was the education of the next generation of researchers, educators, and practioners in the area of subsurface remediation. As summarized in Table 5, a total of 19 Ph.D. students, five M.S. students, and one post-doctoral student were supported through Center funding.

The Center was very productive in the generation of peer-reviewed journal articles. The journal articles generated are listed with each project summary. Over 60 peer reviewed journal articles resulted from the Center's sponsored research. The articles appeared in many of the top journals in our field, including *Environmental Science and Technology, Water Research, Water Resources Research, Advances in Water Resources, Journal Bacteriology, Langmuir, and the Journal of Hydrology*. Center researchers were also very active in presenting the results of their research at conferences and workshops. Over 60 conference abstracts or conference proceedings are listed in our final report. In addition two software programs related to the adding and mixing of nutrients for subsurface remediation were developed partly with Center funding.

Center researchers were involved in six field demonstration projects that resulted from basic or applied research funded by the Center. These field demonstration projects were funded by other governmental agencies such as Environmental Security Technology Certification Program (ESTCP) and the Strategic Environmental Research and Development Program (SERDP) of the Department of Defense. Several field demonstrations were also supported by Chevron-Texaco Corporation and Textron Corporation. Two of the projects, one funded by ESTCP and another by SERDP, were associated with demonstrations and the development of protocols to assess aerobic cometabolism. Another project ESTCP funded project was centered on using radon-222 as in situ tracer for monitoring the remediation of NAPL contamination. A third ESTCP project

evaluated Pd-catalysts for the in situ treatment of TCE using horizontal wells. Chevron-Texaco and Textron Corporation supported field push-pull tests demonstration using trichlorofluoroethene as a reactive tracer for evaluating TCE remediation. Also reported are the final reports and the peer reviewed journal articles these projects generated.

The report also contains information on our technology transfer program, our Website, and other activities that the Center used to transfer the technology and results of the research. A summary report is also given on the TOSC and TAB programs of the Center.

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The Center at a Glance

The Western Region Hazardous Substance Research Center (WRHSRC) was a cooperative activity between Oregon State University and Stanford University that was established in October 2001. The Center was a continuation of the original Center established in 1989 to address critical hazardous substance problems in EPA Regions 9 and 10. The regions include the states of Alaska, Arizona, California, Hawaii, Idaho, Nevada, Oregon, and Washington, and Guam. The Center received its base financial support from the U.S. Environmental Protection Agency.

The objectives of the Center were

1. To develop innovative technologies for the in situ treatment of volatile organic chemicals (VOCs) in groundwater, especially chlorinated solvents.

2. To increase the number, speed, and efficiency of available treatment options for both high concentration source zones and diffuse contamination plumes.

3. To disseminate the results of research to the industrial and regulatory communities, to foster exchange of information with these communities, and to promote a better understanding of the scientific capability to detect, assess, and mitigate risks associated with hazardous substance usage and disposal.

Groundwater cleanup and site remediation, with a strong emphasis on treatments that used microbes or chemical catalysts to transform VOCs into harmless substances, represented the

major focus of Center activities. Research projects included biological (biotic) and physical and chemical (abiotic) treatment processes, as well as in situ characterization methods for monitoring the progress of both intrinsic and the enhanced remediation. In combination with basic laboratory and field studies, physical and mathematical models were used to study these processes and to provide a bridge between theory and practice. The technology transfer program involved the process of taking new technologies from the laboratory to the field. Center researchers worked with other federal agencies, such as the Department of Defense (DoD) and private industry, in conducting field evaluations of new technologies. Technical Outreach Services for Communities (TOSC) was a technical assistance program designed to aid communities confronted with environmental contamination by hazardous waste sites. TOSC provided interested community groups with technical information and assistance that enabled early and meaningful public participation in decisions that affect health and welfare. The Center's Technical Assistance to Brownfields Communities (TAB) Program provided assistance to communities attempting to address cleanup and redevelopment of properties whose reuse has been prevented by real or perceived contamination. TAB attempted to improve involvement of all affected parties in cleanup and redevelopment process through education and training.

Table 1 lists the 15 OSU and Stanford faculty members who were involved in the Center. They collectively represented an integrated research group of many different disciplines, including biochemistry, chemistry, environmental engineering, environmental chemistry, geosciences, hydrogeology, molecular biology, microbiology, public health, and sociology. Lewis Semprini was the director of the Center and of the research program. Kenneth J. Williamson served as associate director in charge of training, technology transfer and community outreach. Martin Reinhard, the assistant director, was in charge of the Center's quality control program. Garrett Jones was the Center's administrative assistant.

The Center had two major advisory groups to guide its activities. The Science Advisory Committee (SAC) had oversight for all Center research activities and technology transfer activities, and the Outreach Advisory Committee (OAC) oversaw the Center's TOSC and TAB programs. The members of the SAC and OAC are listed in Tables 2 and 3, respectively. They represented federal and state governments, industry, consulting firms, and universities. Experts with a broad range of expertise were included in the SAC and the OAC.

The Center budgets for the 2001 through the 2006 fiscal year are listed by category of support in Table 4. The Center received a total of $3,763,200 of funds from the EPA. The EPA required matching funds at a level of 20%. Oregon State University allocated $405,000 in funds to help meet this required match.

The education of students interested in careers directed toward finding solutions to environmental problems is another important goal. The number of students supported through WRHSRC funds is listed in Table 5. A total of 24 graduate students were supported through Center funding: 19 students received Ph.D. degrees and five received M.S. degrees. One student received post-doctoral experience.

Table 1. Key Personnel at the WRHSRC

Stanford University/Discipline	Oregon State University/Discipline
Craig C. Criddle, Environmental Engineering	Daniel J. Arp, Biochemistry
Peter K. Kitanidis, Hydrogeology	Peter Bottomley, Microbiology
Martin Reinhard, Environmental Chemistry	Linda Ciufetti, Microbiology
Alfred Spormann, Microbiology/Biochemistry	Mark Dolan, Environmental Engineering
	Jennifer Field, Environmental Chemistry
	Anna Harding, Public Health
	James D. Ingle, Chemistry
	Jonathan D. Istok, Hydrogeology
	Denise Lach, Sociology
	Lewis Semprini, Environmental Engineering
	Kenneth J. Williamson, Environmental Engineering

Table 2. Science Advisory Committee

Member	Affiliation	Expertise
Dr. Richelle M. Allen-King (Vice-Chair)	Department of Geology, University at Buffalo, Buffalo, NY	Geochemistry; Hydrogeology
Dr. Harold Ball	U.S. EPA Region 9, San Francisco, CA	Environmental Engineering
Dr. Roseanne Ford	Chemical Engineering Department, University of Virginia, Charlottesville, VA	Microbial Processes; Chemical Engineering
Dr. Joe Hughes (Chair)	Department of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA	Bioremediation; Environmental Engineering
Dr. Gregory D. Sayles	USEPA Office of Research and Development, Cincinnati, OH	Microbial Processes; Bioremediation
Dr. Jim Spain	Department of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA	Microbiology

Table 3. Outreach Advisory Committee

Member	Affiliation	Expertise
Mr. Tim Brincefield	U.S. EPA, Region 10, Seattle, WA	Superfund Cleanup and Brownfields
Mr. Brooks Koenig	Veritas, Vizslas, & Velos, Portland, OR	Policy/law of Environmental Regulations
Ms. Ann Levine	Oregon Department of Environmental Quality, Portland, OR	Policy/law of Environmental Regulations
Mr. Dale Manty	ORD, U.S. EPA, Headquarters	Administration
Mr. Luis Rivera	North Coast Regional Water Quality Board, Santa Rosa, CA	Regulations
Ms. Vicki Rosen	U.S. EPA, Region 9, San Francisco, CA	Superfund community involvement
Mr. Lenny Siegel	Center for Public Environmental Oversight, Mountain View, CA	Policy/guidance for cleanup and reuse
Ms. Kathleen Veit	U.S. EPA, Region 10, Seattle, WA	Community involvement

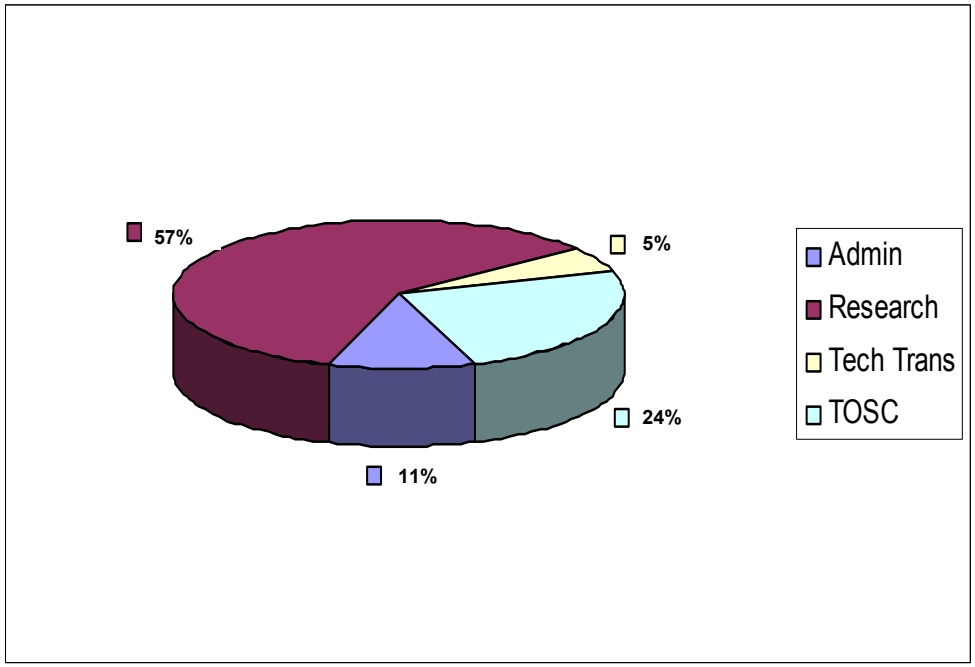
Table 4. Center Funding

Funding Sources	FY 2001	FY 2002	FY 2003	FY 2004	FY 2005*	Total
EPA: Centers Program	$900,000	$885,000	$885,000	$868,160	$225,000	$3,763200
EPA: Brownfields	150,000	150,000	127,000	**	**	427,000
Oregon State University	90,000	90,000	90,000	90,000	45,000	405,000
TOTAL	$ 1,140,000	$1,125,000	$ 1,102,500	$ 958,160	$170,000	$4,595,200

*Sept. 1, 2005- Aug 30, 2006

**Brownfields became a separate grant in FY Sept 2004

The Distribution of the Center's Base EPA Funding



Research Project Summary

The table 5 contains the research projects supported by the WRHSRC from October 2001 through August 2007. A total of twelve projects were funded over the life of the Center. The projects included modeling of bioremediation process, biological processes of aerobic cometabolism to treat dilute plumes, anaerobic processes for treating high concentration source zones, abiotic treatment using catalysis, fundamental studies of sorption onto aquifer materials, abiotic transformation with aquifer materials, and the development of sensors for monitoring redox conditions during anaerobic bioremediation.

Table 6. Research Projects

Project	Title	PI Co-PIs	Project Dates	Total Budget
1-SU-01	Strategies for Cost-Effective In situ Mixing of Contaminants and Additives in Bioremediation	Peter K. Kitanidis, PI; Craig S. Criddle, Co-PI	2001-2003	$150,000
1-OSU-01	Developing and Optimizing Biotransformation Kinetics for the Bio-remediation of Trichloroethylene at NAPL Source Zone Concentrations	Lewis Semprini, PI; Mark E. Dolan, Co-PI	2001-2003	$121,725
1-OSU-02	Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbon Compounds with Butane-Grown Microorganisms	Peter Bottomley, PI Daniel J. Arp Lynda Ciuffetti, Stephen Giovannoni, Lewis Semprini, Ken Williamson, Mark Dolan, Co-PIs	2001-2003	$319,834
1-SU-02	Chemical, Physical and Biological Processes at the Surface of Palladium Catalysts under Groundwater Treatment Conditions	Martin Reinhard, PI; John Westall, Co-PI	2001-2003	$171,297
1-SU-03	Effects of Sorbent Microporosity on Multicomponent Fate and Transport in Contaminated Groundwater Aquifers	Martin Reinhard, PI	2001-2003	$97,355
1-OSU-03	Development of the Push-Pull Test to Monitor Bioaugmentation with Dehalogenating Cultures	Jennifer A. Field, PI; Jonathan D. Istok, Co-PI	2001-2003	$93,283
1-OSU-04	Development and Evaluation of Field Sensors for Monitoring Bioaugmentation with Anaerobic Dehalogenating Cultures for In Situ Treatment of TCE	James D. Ingle, PI	2001-2003	$96,300

Project	Title	PI Co-PIs	Project Dates	Total Budget
2-OSU-05	Aerobic Cometabolism of Chlorinated Ethenes by Microorganisms that Grow on Organic Acids and Alcohols	Peter Bottomley, PI; Daniel Arp, Mark Dolan, Lewis Semprini, Co-PIs, Oregon State University	2004-2007	$294,540
2-OSU-06	Development and Evaluation of Field Sensors for Monitoring Anaerobic Dehalogenation After Bioaugmenatation	James Ingle, PI, Oregon State University	2004-2007	$145,230
2-OSU-07	Continuous-Flow Column Studies of Reductive Dehalogenation with Two Different Enriched Cultures: Kinetics, Inhibition, and Monitoring of Microbial Activity	Lewis Semprini, PI, Oregon State University, Mark Dolan, Co-PI, Oregon State University, Alfred Spormann, Co-PI, Stanford University	2004-2007	$400,850
2-SU-04	Novel Methods for Laboratory Measurement of Transverse Dispersion in Porous Media	Peter K. Kitanidis, PI; Craig Criddle, Stanford Co-PI, Stanford University	2004-2007	$170,000
2-SU-05	Sorption and Hydrolysis of Halogenated Hydrocarbons in Soil Nanopores	Martin Reinhard, PI	2004-2007	$180,000

Research Project Descriptions
from 2001 TO 2007

Following the summary is a description of each project and its goals, rationale, approach, and summary of findings. Also included are the peer reviewed publications, conference abstracts and papers, theses, patents and computer programs that resulted from the project.

Period Covered by the Report: October 2001 – August 2007
Date of Final Report: December 2007
EPA Agreement Number: R828772
Title: Western Region Hazardous Substance Research Center Project 1-SU-01
Strategies for Cost-Effective In Situ Mixing of Contaminants and Additives in Bioremediation
Investigators: Peter Kitanidis, Stanford University; Craig Criddle, Stanford University
Institution: Oregon State University
Research Category: Groundwater, treatment, bioremediation
Project Period: 2001-2003

Goals: (1) To develop and critically evaluate principles and strategies for mixing, using recirculation units, pairs of extraction-injection wells, sparging, biocurtains, combined systems and operations that are sequenced in time and space. (2) To develop methods for cost-effective chemical delivery and mixing, prevention of clogging, and hydraulic control. (3) To define the range of application of these methods and compare them on the same basis in terms of effectiveness and cost. (4) To synthesize available knowledge and previous experience on flow, transport, and biochemical reactions using results from field-scale studies. (5) To advance and test theories for subsurface mixing at field scales through hydrodynamic dispersion, partitioning, fingering, etc. (6) To develop a set of tools and guidelines for the design of cost-effective in situ delivery and mixing systems.

Rationale: Effective mixing and chemical delivery schemes is essential in the success of in situ remediation methods. This is because these methods usually require the injection of growth promoters (in situ bioremediation), chemical additives (e.g., surfactant-enhanced remediation), or cells (bioaugmentation). To achieve successful mixing and chemical delivery at the field-scale, we needed to (1) create a sufficiently large in situ reactor, and (2) regulate residence times.

Approach: In this research, principles of mixing and the performance of mixing schemes were studied, and a broad range of existing and new full-scale mixing and chemical delivery schemes were evaluated through comprehensive mathematical, technical, and economic analysis. Research was guided by case studies.

Summary of Findings The design of an effective chemical delivery and mixing scheme for in situ bioremediation of Uranium (VI) at Oak Ridge National Laboratory (ORNL) was the focus. This was a challenging site, characterized by complex hydrogeology and biogeochemistry. The subsurface material was highly weathered saprolite. In addition to high uranium concentration, the pH was exceptionally low, at about 3.5, and nitrates were exceptionally high, at about 10 g/L. Nitrate needed to be removed and the pH needed to be raised in a controlled fashion, e.g., to prevent clogging of the porous medium from precipitation of aluminum. The speciation of U(VI), and thus its mobility, was controlled strongly by the pH. An elaborate on-site treatment plant was designed and combined with a multi-step in situ treatment experiment. We developed mathematical models of flow, transport and biogeochemistry and are comparing predictions with the results of experiments and field tests. We developed software for the delineation of injection, extraction, and recirculation zones; the efficient determination of breakthrough curves; the application of travel-time methods of modeling transport; and biogeochemical modeling using PHREEQC in conjunction with hydrogeological modeling within the MATLAB computational

environment. These modeling tools were implemented at the ORNL site to extract information from data and assist in the design of new experiments.

Publications:

Journal Articles

Fienen, M. N., Luo, J., Kitanidis, P. K. (2005). Semi-Analytical, Homogeneous, Anisotropic Capture Zone Delineation. *Journal of Hydrology.* 312(1-4): 39-50. doi:10.1016/j.jhydrol.2005.02.008.

Fienen, M.N., J. Luo, P. K. Kitanidis (2006). A Bayesian Geostatistical Transfer Function Approach to Tracer Test Analysis. *Water Resources Research*, 42, doi:10.1029/2005WR004576.

Luo, J. and Kitanidis, P. K. (2004). Fluid residence times within a recirculation zone created by an extraction-injection well pair. *J. of Hydrology*, 295(1-4): 149-162.

Luo, J., Cirpka, O.A., Kitanidis, P.K. (2006). Temporal-moment matching for truncated breakthrough curves for step or step-pulse injection. *Adv. Water Resour.*, 29(9), 1306-1313.

Luo, J., Wu, W-M., Fienen, M.N., Jardine, P.M., Mehlhorn, T.L., Watson, D.B., Cirpka, O.A., Criddle, C.S., Kitanidis, P.K. (2006). A nested-cell approach for in situ remediation. *Ground Water*, 44(2), 266-274.

Conferences Proceedings and Presentations

Luo, J., Fienen, M. N. and Kitanidis, P. K. (2002). 3-D Groundwater Flow Modeling for the Oak Ridge Reservation (ORR): Finite-Volume Method on An Unstructured Grid System. *Proceeding of the International Groundwater Symposium,* Berkeley, California, (March 25-28).

Theses

Fienen, M. N. (2006). Inverse Methods for Near-field Hydrogeologic Characterization. PhD, Stanford University.

Luo, J. (2005). Hydraulic Control and Reactive Transport Modeling for In-Situ Bioremediation of Uranium Contaminated Groundwater. PhD, Stanford University.

Software

Fienen, M. N., Luo, J., Kitanidis, P. K. (2005). ComCZAR:Complex Capture Zone Analysis Routine. http://www.talulat.com/mike/software/.

Fienen, M. N., J. Luo, P. K. Kitanidis (2006). A Bayesian Geostatistical Transfer Function Approach to Tracer Test Analysis - Implementation and Examples. http://www.talulat.com/mike/software/lstran.html.

Supplemental Keywords: In situ, remediation, groundwater, technology transfer
Period Covered by the Report: October 2001 – August 2007

Date of Final Report: December 2007
EPA Agreement Number: R828772
Title: Western Region Hazardous Substance Research Center Project 1-OSU-01 Developing and Optimizing Biotransformation Kinetics for the Bioremediation of Trichloroethylene at NAPL Source Zone Concentrations
Investigators: Lewis Semprini and Mark E. Dolan
Institution: Oregon State University
Research Category: Groundwater, TCE, PCE, Vinyl Chloride, DNAPL, Bioremediation
Project Period: January 2002 – December 2005

Objectives: This project aimed to (1) develop a culture with the ability to reductively dechlorinate TCE to ethylene at very high concentrations (above 1,000 □M) and in the presence of DNAPL; (2) characterize microbial growth and measure maximum substrate utilization rates and half velocity coefficients for successive dechlorinations of TCE to ethylene; (3) characterize the microbial consortium by investigating molecular methods to evaluate the diversity of the mixed culture developed in the kinetic studies; (4) provide kinetic information and cultures in support of the Center Projects "Development of the Push-Pull Test to Monitor the Bioaugmentation of Dehalogenating Cultures" and "Development and Evaluation of Field Sensors for Monitoring Bioaugmentation with Anaerobic Dehalogenating Cultures for In-Situ Treatment of TCE."

Rationale: While TCE reductive dechlorination has been demonstrated under a variety of conditions, most laboratory and field projects have been conducted at TCE concentrations of 100 mg/L or less. However, near NAPL sources concentrations of chlorinated aliphatic hydrocarbons approach their solubilities (>1,000 mg/L for TCE and >150 mg/L for PCE). Studies with different enrichment cultures isolated from contaminated sites have shown good potential for treatment of high concentrations of PCE and TCE. The cultures have different dehalogenation kinetic properties, which indicate that a more effective enrichment culture might be obtained by combining cultures. Research is needed to optimize the transformation kinetics for the consortium that has the ability to reductively dechlorinate high concentrations of TCE and PCE to stoichiometric quantities of ethylene. This project will prove useful for the remediation of chlorinated aliphatic compounds in the NAPL source zone.

Summary of Findings: A culture was developed that can rapidly degrade high concentrations of PCE and TCE to ethylene by mixing two enrichment cultures. The Point Mugu enrichment (PM) rapidly transforms TCE to VC, and slowly transforms VC to ethylene at very high PCE and TCE concentrations. The Evanite enrichment (EV) rapidly transforms PCE to cis-DCE, and vinyl chloride to ethylene. By mixing both cultures rapid transformation of PCE and TCE to ethylene was achieved. We used batch reactor studies to determine transformation kinetics for both cultures, and then when both cultures were combined. Inhibition among the CAHs will also be evaluated. Models were constructed to simulate the results of the sequential transformations over a broad range of concentrations up to the solubility limit of PCE and 50% of the solubility limit of TCE.

Kinetic studies were conducted with two mixed cultures and a binary culture (a mixture of the two cultures) to describe the reductive dechlorination of chlorinated ethylenes. Inhibition of the CAHs was also studied. The EV culture and the PM obtained from different contaminated sites

showed different patterns of reductive dechlorination. The simple batch kinetic method was developed that was easy to implement and produced very reproducible kinetic values. The k_{max} (based on the total protein content of the culture) for *c*-DCE of the EV culture was about two times lower than that of the PM culture, reflecting the slower *c*-DCE biotransformation of the EV culture. The k_{max} and K_S values for VC (2.44 ± 0.36 μmol/mg of protein/day and 602 ± 7.06 μM, respectively) of the PM culture were very different from those of the EV culture (8.08 ± 0.94 μmol/mg of protein/day and 62.6 ± 2.37 μM, respectively). Inhibition studies were performed on the inhibition of the CAH on the transformation of each other. Inhibition studies showed the more chlorinated ethylenes inhibit reductive dechlorination of the less chlorinated. PCE inhibited reductive TCE dechlorination, but not *c*-DCE dechlorination, while TCE strongly inhibited *c*-DCE and VC dechlorinations. c-DCE strongly inhibited the transformation of VC. Inhibition constants of each chlorinated ethylene, *KI* (μmol/L), were comparable to their respective half-velocity coefficients, when a competitive inhibition model was applied.

Batch tests to study the sequential transformation of PCE to ETH were also performed over a factor of 30 change in concentration of PCE, up to it solubility limit in water (1128 □M), with the EV, PM, and a 50/50 mixture of both cultures to yield a binary culture (BM). Additional studies were performed with TCE up to a concentration of 4173 □M (550 mg/L), which represents 50% of its solubility limit in water. Simulations of the successive transformations of PCE to ETH, and TCE to ETH using the independently derived kinetic parameters matched well the results of batch kinetic tests for initial PCE concentration up to around 317 □M. The simulations included the growth on the chlorinated solvents, and Monod kinetics including competitive inhibition. Above this concentration simulations deviated from the experimental observations, and predicted more rapid transformation of VC than was observed. The results suggest potential toxicity or inhibition at the higher concentrations of PCE and TCE. Simulations were performed with Halden kinetics incorporated into the transformation models, where high concentrations of a contaminant inhibit its transformation. In order to explain the experimental observations Halden kinetics for TCE transformation were required for both the EV and the PM cultures and for c-DCE and VC transformation by the EV culture. The EV culture appeared to be more inhibited at higher CAH concentrations. TCE concentrations, up to 4173 □M (550 mg/L) were transformed by both the EV and PM culture, with the PM culture more rapid transforming the TCE to VC and ethene. The results indicate less inhibition of the PM culture at higher concentrations. Batch experimental results indicate that the BM culture, which represent a mixture of both cultures, has better transformation abilities that either of the single cultures. Simulations for the BM culture, using individual transformation abilities of each culture, support the experimental observations of more diverse dechlorination ability than either of the single mixed cultures.

Molecular methods analysis using PCR reactions with *Dehalococcoides*-specific primers and *Desulfuromonas*-specific primers found *Dehalococcoides*-like microorganisms in both the cultures, but not *Desulfuromonas*-like microorganisms. The molecular methods could not distinguish between the *Dehalococcoides* species of the EV and the PM cultures.

Publications:

Journal Articles

Pon G. M.R. Hyman, and L. Semprini (2003). Acetylene Inhibition of Trichloroethene and Vinyl Chloride Reductive Dechlorination. *Environmental Science and Technology,* 37 3181-3188.

Pon, G. and L. Semprini (2004). Anaerobic Reductive Dechlorination of 1-chloro-1-fluoroethene to Track the Transformation of Vinyl Chloride. *Environmental Science and Technology,* 38 6803-6808.

Yu, S., and L. Semprini (2002). Comparison of Trichloroethylene Reductive Dehalogenation by Microbial Communities Stimulated on Silicon-based Organic Compounds as Slow-release Anaerobic Substrates. *Water Research* 36(20): 4985-4996.

Yu S. and L.Semprini (2004). Kinetics and Modeling of Reductive Dechlorination at High PCE and TCE Concentrations. *Biotechnology and Bioengineering,* 88 451-464.

Yu, S., M.E. Dolan, and L. Semprini (2005). Kinetics and Inhibition of Reductive Dechlorination of Chlorinated Ethylenes by Two Different Mixed Cultures. *Environmental Science and Technology,*39 195-205.

Conferences Proceedings and Presentations

Yu S. and L. Semprini (2002). Dechlorination of PCE DNAPL with TBOS Using a Binary Mixed Culture. *The 3rd International Conference on Remediation of Chlorinated and Recalcitrant Compounds*, Monterey, CA (2B-49) (May 20-23).

Yu, S and L. Semprini (2003). Kinetic Studies and Comparison for Reductive Dechlorination by a Binary Mixed Culture. *The Seventh International Symposium* on *In Situ and On-Site Bioremediation*, Orlando FL (June, 2-5).

Thesis

Yu, S. (2004). Kinetic and Modeling Investigations of Anaerobic Reductive Dechlorination of Chlorinated Ethylenes Using Single and Binary Mixed Cultures and Silcon-Based Organic Compounds as Slow-release Substrates. Ph.D., Oregon State University.

Pon. G. (2004). Inhibition, Kinetic and Modeling Studies of Acetylene and 1-chlorofluoroethene on Reductive Dechlorination of TCE and Vinyl Chloride. Ph.D, Oregon State University.

Supplemental Keywords: biotransformation; groundwater; NAPL; VOCs; bioremediation; chlorinated solvent; remediation technologies; in-situ

Period Covered by the Report: October 2001 – August 2007
Date of Final Report: December 2007
EPA Agreement Number: R828772
Title: Western Region Hazardous Substance Research Center Project 1-OSU-02.
Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbon Compounds with Butane-Grown Microorganisms:
Investigators: Peter Bottomley, Dan Arp, Lynda Ciuffetti, Mark Dolan, Lewis Semprini, Kenneth Williamson
Institution: Oregon State University
Research Category: Bioremediation, cometabolism
Project Period: January 2002 – December 2005

Objectives: This project was focused on dealing with evaluating how to maximize the chloroethene (CE) and chloroethane (CA) degrading potential of individual strains and mixed communities of butane degrading bacteria and fungi. Specific objectives included identifying growth conditions that maximize reductant flow to cometabolism, and the mechanisms that sustain monooxygenase enzyme activity and minimize cytotoxic damage to the cells; evaluating the potential for the bioaugmentation of a butane culture for in-situ bioremediation of CE and CAs; and to describe the ability of *Graphium* sp. to degrade a range of CE and CAs volatile organic compounds including chlorinated aliphatic hydrocarbons (CAHs), trichloromethanes and methyl *tertiary*-butyl ether (MTBE). Based on these objectives the project was divided into three subprojects that are each summarized.

Project 1
Investigators: Peter Bottomley and Dan Arp

Objectives: The project aimed to evaluate how to maximize the chloroethene (CE) degrading potential of individual strains of hydrocarbon degrading bacteria. Specific subobjectives included identifying conditions that maximize reductant flow and the cellular mechanisms that minimize the toxic effects of cometabolism and sustain the process.

Rationale: Studies conducted under laboratory and field conditions have shown that hydrocarbon-oxidizing bacteria cometabolize a wide range of CEs. Nonetheless, there is considerable variability in the properties of cometabolism shown by different types of butane oxidizing bacteria both in terms of the range of CEs degraded and in their transformation capacities. More research was carried out to better understand the microbiological reasons for the range of efficiencies observed, with the goal of using this information to improve the biotechnology of bioremediation by cometabolism.

Summary of Findings: We examined the CE degrading properties of several individual strains of butane-oxidizing bacteria that are genotypically distinct from each other and that possess different butane monooxygenases. We examined the impact of cometabolism of different CEs on monooxygenase activity, and assessed the impact on cell viability. While co-oxidation of trichloroethene (TCE) by *Pseudomonas butanovora* and *Nocardioides* CF8 resulted in 96% inactivation of butane monooxygenase, the BMO of *Mycobacterium vaccae* was found to be more resistant to inactivation by TCE and its respiratory activity was unaffected (Halsey et al. 2005).

Based upon these observations it was proposed that situations might be identified where CE degrading bacterial strains with lower rates of degradation might be more appropriate bioremediatory agents than strains showing higher rates of CE degradation when the latter cannot be sustained. Therefore, we genetically engineered strains of *Pseudomonas butanovora* by replacing specific amino acids associated with the BMO hydroxylase alpha subunit (Halsey et al. 2006). We examined the CE degrading properties of these mutants and examined the impact of cometabolism of different CEs on monooxygenase activity, and assessed the effect of cometabolism on cell viability (Halsey et al. 2007). As an example, mutant strain G113N was particularly interesting. This strain had an amino acid substitution in, or close to, the catalytic site of the monooxygenase. This strain oxidized butane primarily to 2-butanol rather than to 1-butanol (as also is the case in *M. vaccae*), but at a slower rate than wild type. Although G113N oxidized TCE and DCEs at slower rates than did wild type, it also liberated less chloride than wild type indicating that the products of oxidation were different from those formed by wild type. In addition, there was no evidence that G113N transformed the epoxide of 1,2 cis DCE that was formed, and, there was no evidence of toxic effects caused by transformation of 1,2 cis-DCE or 1,1DCE in G113N either. The data suggest that a specific amino acid substitution in BMO affected CE turnover-product distribution such that the mutant strain seemed immune to toxicity.

Since aerobic cometabolic biodegradation is often limited by product toxicity in the form of enzyme inactivation or loss of cellular viability, the results obtained in this study indicate that the oxidative pathway favored by mutant strain G113N would promote more sustainable biodegradation of CEs, and that this was likely due to an enzyme mechanism that had been modified to disfavor formation of an unstable epoxide, and also disfavor subsequent attack of the epoxide by BMO. Butane-grown *P. butanovora* co-oxidizes cis-DCE, 1,2, trans-DCE, and 1,1-dichloroethene (1,1-DCE). When *P. butanovora* was exposed to each of the three DCEs, and residual BMO activity measured by ethylene-dependent ethylene oxide formation, BMO activity was reduced in a time-dependent manner that varied with the specific DCE. BMO activity decreased by 50% after 15 min exposure to cis-DCE, after 6 min exposure to trans-DCE, and after 30 sec exposure to 1,1-DCE. In addition, cooxidation of the DCEs had different cytotoxic effects on *P. butanovora*. Although cooxidation of cis-DCE and trans-DCE inactivated the majority of BMO activity, cells retained lactate-dependent O_2 consumption but they were unable to grow normally after removal of the DCEs. In contrast, cooxidation of 1,1-DCE caused a rapid decrease in both BMO activity and lactate-dependent O_2 consumption within three min of exposure, and cells lysed. Treating cells with acetylene to inactivate BMO eliminated the effects of 1,1-DCE, and lactate-grown cells (in which BMO was not expressed) were also unaffected.

Construction of a LacZ/ *BMO* reporter strain allowed us to compare the efficiency of induction of BMO gene expression by DCEs with the induction of BMO activity in the wild type parent by the same compounds (see Table 2). The relative induction characteristics of the three DCEs differed from their substrate properties. Trans-DCE induced BMO activity in both the wild type and in the Lac Z reporter strain, while cis-DCE only induced enzyme activity in the wild type. Enzyme activities in wild type cells were induced to ≤ 25 and 45% of the butane control by cis-DCE and trans-DCE, respectively. LacZ expression was induced to 80% of maximal by trans-DCE in the reporter strain. In the case of trans-DCE, BMO induction could be detected in the reporter strain at lower concentrations (5 to 10 μM) than could be detected by ethylene-dependent ethylene oxide formation in the wild type (30 μM). the ability of 1,2-trans DCE to induce BMO implies that bioremediatory activity might be activated in a polluted plume regardless of the presence of the natural substrate.

Project 2
Investigators: Lewis Semprini and Mark Dolan

Objectives: Evaluate the potential for bioaugmentation of a butane-utilizing culture that is effective in transforming mixtures of 1,1,1-trichloroethane (1,1,1-TCA), 1,1-dichloroethane (1,1-DCA), and 1,1-dichloroethene. Specific objectives were to evaluate the bioaugmentation potential the butane culture in a continuous flow column study; use molecular tools to track the culture upon its addition in the column study; develop kinetic parameters for butane utilization and CAH transformation for use in a modeling analysis of the column tests; model the results of the column experiment with a numerical model that includes kinetic terms for the microbial processes that have been determined independently in the laboratory; and conduct studies on the potential for a butane culture to transform the epoxide of cis-dichloroethene transformation.

Rationale: Laboratory and microcosm studies have shown different abilities of butane-utilizing microorganisms to cometabolize CAHs. Of particular interest is 1,1,1-TCA, 1,1-DCE, and 1,1-DCA. A *Rhodococococcus* sp. culture has been sequenced and obtained in pure culture that effectively transforms these compounds. This culture was bioagumented to the subsurface at the Moffett Field tests zone in a collaborative research grant funded through the DoD SERDP program. The Center project conducted continuous flow column experiments, like those performed in the field, for direct comparison with the field results, and to permit more detailed evaluation of the of processes and conditions that can not be performed in the field. Transformation rate parameters for the *Rhodococococcus* culture, including maximum utilization rate (k_{max}) and half-saturation coefficients (K_s) values were also determined, and compared with previously determined values obtained with the mixed culture from which the pure culture was derived. Modeling studies were then performed to simulate the results of the column experiments. Studies were also conducted to evaluate the ability of the parent mixed culture to transform cis-1,2-dichloroethylene epoxide that is formed during the aerobic cometabolism of cis-dichloroethylene.

Summary of Findings: The transformation of 1,1,1-trichloroethane (1,1,1-TCA) and 1,1-dichloroethene (1,1-DCE) was evaluated in a continuous flow column reactor after bioaugmentation with the highly enriched *Rhodococococcus* culture The column was packed with aquifer materials and groundwater obtained from the in situ bioremediation test site at Moffett Field, CA and bioaugmented with 0.9 mg of cells on a dry mass basis. While adding only 1,1,1_TCA at 200 μg/La maximum removal of 84% was achieved 10 days after bioaugmentation and remained fairly constant for a period of 20 days. The influent concentration of 1,1,1-TCA was then doubled, while dissolved oxygen and butane addition was maintained constant. The transformation of 1,1,1-TCA during this period fluctuated between 24%-84%. Upon restoring the 1,1,1-TCA concentration back to 200 μg/L the transformation stabilized at 59% removal. In the final phase, 1,1-DCE was injected at 130 ug/L along with 1,1,1-TCA, dissolved butane and oxygen. The butane-utilizing culture transformed 70% of 1,1-DCE; however, the presence of 1,1-DCE inhibited 1,1,1-TCA transformation and approximately 50% of the butane injected was not consumed. The concentration of dissolved oxygen in the column also increased, which also indicated that 1,1-DCE transformation inhibited butane and dissolved oxygen utilization and 1,1,1-TCA transformation. Real-time PCR analysis conducted indicated that during periods of low biotransformation of 1,1,1-TCA, bioaugmented cell densities observed in the column

effluent was high. This corresponded to a period of anoxic conditions, which may have caused cell detachment from the aquifer solids.

The column reactor results were simulated using a combined biotransformation-transport model that uses Monod/Michaelis-Menten kinetics along with first-order sorption kinetics, to predict substrate utilization and chlorinated solvent transformation. The culture parameter values used to simulate biotransformation in the model were obtained from laboratory culture experiments described below. Transport parameters (dispersion coefficient, porosity) were determined from modeling breakthrough test data with the CXTFIT2 transport model prior to bioaugmentation and biostimulation. Simulations of the column data using the transport and biotransformation parameters demonstrated that the model was able to simulate biotransformation of 1,1,1-TCA fairly well. The model also indicated that 1,1-DCE transformation was toxic to the butane-utilizing culture and predicted the decreases in consumption of butane, and dissolved oxygen and in 1,1,1-TCA transformation.

This study showed that column experiments conducted on a small scale in a laboratory were of value in determining the biotransformation capabilities of bioaugmented microorganisms. The results suggest that the butane-utilizing culture could be successfully used in situ for bioremediation, but transformation of mixtures of 1,1-DCE and 1,1,1-TCA could prove difficult. Similar extends of 1,1,1-TCA transformation were observed in the Moffett field tests when only 1,1,1-TCA was added. 1,1-DCE transformation in the field was also observed to be toxic to the butane utilizers and to decrease 1,1,1-TCA transformation.

Single compound tests were performed with the butane grown *Rhodococcus* culture to determine the Monod kinetic parameters. The cells were added to a series of liquid and gas phase microcosms; the maximum degradation rates (k_{max}) and the half saturation coefficients (K_s) of butane, 1,1,1-TCA and 1,1-DCA, and the cell yield (Y) for growth on butane were determined. The purity of the culture was determined by PCR and TRFLP analysis. The transformation capacity of the two CAHs was evaluated through experiments performed both in the presence and in the absence of butane. 1,1,1-TCA was found to have a transformation capacity 50 times lower than 1,1-DCA. The reciprocal degradation inhibition of butane and each of the solvents was also studied. 1,1,1-TCA transformation was found to be more inhibited by the presence of butane than 1,1-DCA. The overall transformation performance of the isolate culture was better than that of the source culture due to the lower degree of inactivation as a result of transformation product toxicity.

Aerobic cometabolism of *cis*-1,2-dichloroethylene (c-DCE) by a butane-grown mixed culture was evaluated in batch kinetic tests. The transformation of c-DCE resulted in the coincident generation of c-DCE epoxide. Chloride release studies showed ~75% oxidative dechlorination of c-DCE. Mass spectrometry confirmed the presence of a compound with mass-to-charge-fragment ratios of 112, 83, 48, and 35. These values are in agreement with the spectra of chemically synthesized c-DCE epoxide. The transformation of c-DCE required O_2, was inhibited by butane and was inactivated by acetylene (a known monooxygenase inactivator), indicating that a butane monooxygenase enzyme was likely involved in the transformation of c-DCE. This study as showed c-DCE epoxide was biologically transformed, likely by a butane monooxygenase enzyme. c-DCE epoxide transformation was inhibited by both acetylene and c-DCE indicating an monooxygenase enzyme was involved. The epoxide transformation was also stopped when mercuric chloride ($HgCl_2$) was added as a biological inhibitor, further support a biological transformation. To our knowledge this is the first report of the biological transform c-DCE epoxide by a butane-grown culture.

Project 3
Investigators: Ken Williamson and Lynda Ciuffetti

Objectives: This project had two main goals. The primary objective of this project was to describe the ability of *Graphium* sp. to degrade a range of volatile organic compounds including chlorinated aliphatic hydrocarbons (CAHs), trichloromethanes and methyl *tertiary*-butyl ether (MBTE). The study also aimed to demonstrate that these reactions are catalyzed by an alkane inducible cytochrome P450 monooxygenase through heterologous expression assays with yeast.

Rationale: Volatile organic compounds including trichloroethylene (TCE), 1,1-dicloroethylene (1,1-DCE), 1,2-dichloroethylene (1,2-DCE), carbon tetrachloride (CT) and chloroform (CF), a trichloromethane, are important soil and groundwater contaminants. The ability of microorganisms to degrade these compounds represents a promising avenue for the attenuation of polluted sites.

Summary of Findings: *Graphium* sp., a filamentous fungus, is one of the few eukaryotes known to grow on gaseous *n*-alkanes. The initial enzymatic step by which *Graphium* sp. oxidizes *n*-alkanes for energy and growth is initiated by a highly nonspecific and alkane-inducible cytochrome P450 monooxygenase. Previous studies have suggested that this enzyme also enables *Graphium* sp. to cometabolically degrade CAHs, trihalomethanes, and PAHs. More specifically, evidence suggests that *Graphium* sp. can degrade numerous CAHs including all 4 trihalomethanes, chloromethane, dichloromethane, chloroethane, 1,2-DCE and 1,1,2,2-tetrachloroethane. This fungus can also reductively dechlorinated CT to CF in the absence of oxygen and then consume CF when aerobic conditions are reestablished. However, neither the substrate range nor the rates of these *Graphium* sp. mediated reactions have been determined. The primary aim of this project was to more quantitatively describe both the substrate range and the rate of these reactions.

Although preliminary evidence suggests that a cytochrome P-450 monooxygenase catalyzes the initial steps of these reactions, the role of this enzyme has not been conclusively established. The study also aimed to demonstrate the role of this enzyme in cometabolic degradation of environmentally significant pollutants.

Graphium sp cultures were grown on a variety of environmentally relevant compounds. These assays indicated that *Graphium* sp. is able to utilize a broader range of alkanes than originally thought and has an alkane substrate range that extends beyond ethane, propane and butane to include isobutane, isopentane, and pentane. Although our results indicated that *Graphium* sp. hydroxylates alkanes at both terminal and subterminal carbons, in the case of isobutane oxidation, *Graphium* sp. is only able to use the immediate downstream intermediates that result from primary oxidation of isobutane. However, it does grow on both the subterminal and the terminal oxidation products of isopentane and straight-chain alkane oxidation. In addition, our investigations demonstrated that *Graphium* sp. utilizes the cyclic ether, tetrahydrofuran, as a growth substrate. Tetrahydrofuran (THF) is metabolized via the pathway that was previously described in some bacteria, including *Rhodococcus ruber* and two *Pseudonocardia* strains. Likewise, *Graphium* sp. grows on the concomitant metabolic products of THF oxidation and a spectrum of related compounds. Although these assays showed that *Graphium* was unable to grow on another cyclic ether, 1,4-dioxane, *Graphium* sp. was able to cometabolize this environmentally relevant ether after growth on propane or THF. We also

observed that cometabolism of MTBE by this fungus results in the incomplete digestion of MTBE oxidation products, and therefore led to an accumulation of the MTBE metabolite, *tert* butyl alcohol. These investigations also determined that *Graphium*-mediated MTBE oxidation is not subject to the regulatory effects produced by MTBE metabolites that have been previously described in an MTBE-utilizing bacterium, *Mycobacterium austroafricanum*.

Throughout studies that investigated the substrate range of *Graphium* sp., trends were observed that indicate that the alkane and ether metabolic pathways are superimposed on each other. At least five separate physiological observations support this hypothesis. First, the oxidation of THF and propane are fully inhibited by the same alkenes and alkynes. Second, both compounds appear to behave as mutually competitive substrates. Third, propane-grown cells oxidize THF without a lag phase or the accumulation of THF metabolic intermediates, indicating an apparent ability of propane-grown mycelia to concurrently consume THF and THF-derived oxidation products. Fourth, the rates of THF oxidation by propane-grown mycelia are equivalent to the rates observed when THF-oxidizing activity is fully induced in PDB-grown mycelia. Last, comparable rates of MTBE and 1,4-dioxane were observed when propane- and THF-grown mycelia were assayed in short-term initial rate experiments. These observations indicate that THF and alkane oxidation are mediated by the same cytochrome P450 hydroxylase and suggest a greater degree of overlap between the remaining enzymes in the alkane and ether oxidation pathways. Because monooxygenase-catalyzed substrate activation is both the first and the rate-determining step of these pathways, the gene encoding the alkane monooxygenase from this *Graphium* sp. was characterized.

We used a strategy that was developed in long-chain alkane-utilizing yeasts to identify and clone a cytochrome P450 alkane monooxygenase from *Graphium* sp. This gene was designated *CYP52L1,* which encodes the cytochrome P450 protein GSPALK1. Although *CYP52L1* shares some sequence similarity with other yeast alkane hydroxylases from the CYP52 subfamily, unlike cytochrome P450s from yeast, our analyses indicate that *CYP52L1* is not closely related to any known CYP52 member. Likewise, *CYP52L1* is present in a single copy, whereas alkane hydroxylases from yeast often belong to multi-gene families that encode proteins with overlapping function. The differences between *CYP52L1* and its CYP52 relatives are not surprising given that no other CYP52 member is known to oxidize gaseous n-alkanes.

Initial experiments that attempted to express *CYP52L1* in yeast and in an alternate filamentous fungus were unsuccessful. Unsuccessful forward characterization of *CYP52L1* was most likely due to the lack of a corresponding NADPH oxidioreductase in the heterologous hosts. Therefore, we used a reverse genetics approach to characterize *CYP52L1*. GSPALK1 was functionally characterized by introducing a construct that causes the fungus to express a double-stranded (ds)- *CYP52L1* transcript. Expression of the ds- *CYP52L1* transcript triggered endogenous post-transcriptional gene silencing machinery that degraded the native transcript, and therefore abolished expression of this gene in a sequence specific manner. The diminishment of *CYP52L1* expression was associated with a loss of function phenotype and disabled alkane and ether oxidation. We observed that although the transformed fungi are no longer able to grow on alkanes or ethers, the ability of the transformants to grow on the downstream metabolites of alkane and ether oxidation (propanol, isobutanol or γ-butyrolactone) is not affected by post-transcriptional gene silencing of *CYP52L1*. This observation therefore indicates that only the first step in each of these pathways are affected by *CYP52L1* silencing, and thus correlate alkane and ether oxidation to *CYP52L1* expression.

Another filamentous fungus that is able to grow on gaseous n-alkanes, *Graphium cuneiferum*, also harbors a significantly similar (>99.6% amino acid identity) coding sequence,

indicating that GSPALK1-like sequences are present in other eukaryotic alkanotrophs. During our investigations, a *CYP52L1* silencing vector was produced. This vector provides a tool for post-transcriptional gene silencing of *CYP52L1*-like genes in other fungi. Because the substrate range of these fungi differ from the one characterized here, it would be interesting to compare the primary protein structure of CYP52 members from these fungi to further our understanding of the molecular determinants of both substrate range and cometabolism.

These investigations evidence that extends the substrate range of this fungus to include a spectrum of straight chain and branched alkanes, cyclic ethers, lactones, diols, and acids. They also refined the pathway and the metabolic interactions that were thought to regulate *Graphium*-mediated MTBE cooxidation. We also showed that the oxidation of alkanes and ethers is linked through a common catalyst, a cytochrome P450 alkane monooxygenase that mediates the first step of these pathways. This enzyme, designated GSPALK1, was further characterized through molecular genetic and biochemical analyses. The characterization of GSPALK1 and the gene encoding it, *CYP52L1,* is the first description of a cytochrome P450 involved in the terminal oxidation of gaseous n-alkanes and cyclic ethers as well as the first description of a cytochrome P450 involved in MTBE and 1,4-dioxane cometabolism.

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Supplemental Keywords: Groundwater, remediation, biotransformation, bioremediation

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EPA Agreement Number: R828772
Title: Western Region Hazardous Substance Research Center Project 1-SU-02
Chemical, Physical and Biological Processes at the Surface of Palladium Catalysts under Groundwater Treatment Conditions
Investigators: Martin Reinhard and John Westall
Institution: Stanford University and Oregon State University
Research Category: Groundwater, treatment, chlorinated solvents
Project Period: January 2002- August 2007

Objectives: This project aimed to (1) evaluate the impacts of groundwater on catalyst activity; (2) elucidate the chemical and physical mechanisms responsible for changes in catalyst activity; (3) investigate potential biofouling issues that may result from biological activity expected in long-term treatment applications; (4) develop convenient and economical methods to regenerate catalysts in situ.

Rationale: Batch studies with supported palladium catalysts have demonstrated the potential of the palladium/hydrogen process for treating groundwaters or effluent streams that are contaminated by halogenated compounds. These studies yielded near-complete reductive dehalogenation of chlorinated ethylenes to ethane at room temperature within minutes, with reaction rates that are orders of magnitude higher than zero-valent iron. Other batch studies have shown the ability of palladium to catalyze the reaction of a range of compounds: all six species of chlorinated ethylenes, carbon tetrachloride, chloroform, 1,2-dibromo-3-chloropropane, Freon 113, chlorobenzene, naphthalene and lindane. Laboratory column studies and field tests have indicated that catalyst activity may decline over time because of chemical and biological fouling. This project investigated (1) causes of activity loss, (2) optimization of process operation, and (3) catalyst regeneration under simulated field conditions.

Summary of Findings: A bench-scale column system was developed to that allowed us to observe the rate of TCE transformation in the presence of hydrogen sulfide as a function of time. The column system consisted of pump that supplied water from a tank to a hydrogen contactor. After the contactor, the flow was divided and pumped with three separate pumps to three reactors packed with Pd catalyst. The reactor inflows were connected to auxiliary feed systems that allowed us to augment the reactor feed with TCE, foulant (hydrogen sulfide), or regenerant (bleach, hydrogen peroxide, deionized water) as needed. Catalyst activity was evaluated by measuring comparing the concentrations of TCE at the inlet and outlet of the reactor. The mechanism of catalyst fouling was studied by analyzed using X-ray photoelectron spectroscopy (XPS) for the accumulation of deactivating species at the palladium surface. Observations made at ongoing field project that was executed at Edwards Air Force Base (EAFB), Lancaster, California.

The laboratory portion of the project developed a quantitative model for deactivation kinetics with aqueous sulfide, investigated the effects of pH on a catalyzed dehalogenation reaction and sulfide deactivation, and characterized regeneration with acids, bases, and oxidizing agents. Results obtained with trichloroethylene showed no inherent catalyst deactivation in deionized water. Deactivation increased with sulfide concentration and exposure time. Deactivation was slowly reversible by flushing the catalyst with deionized water at pH 10.4. Treatment with 20

mM sodium hypochlorite quickly and completely regenerated the catalyst, and was significantly more effective than hydroxide, hydrochloric acid, hydrogen peroxide, and air-saturated water. The time required for regeneration increased with increasing sulfide concentrations and exposure times. These results were useful for interpreting reactor behavior and optimizing operating conditions and regeneration procedures at the EAFB field site.

During several prolonged failures of the reactor control system, the catalyst was exposed to high sulfide concentrations for days to weeks. This exposure eliminated catalyst activity nearly 100%. Nearly complete regeneration oft the catalyst was possible by soaking the catalyst in bleach for up to a week. Sustaining catalyst activity was possible by daily bleaching of the reactor for approximately 30 minutes.

X-ray photoelectron spectroscopy (XPS) was performed on supported palladium catalyst and a model catalyst after exposure to EAFB groundwater and sodium hypochlorite. The model catalyst was prepared by depositing palladium onto a polished α-alumina surface. Results from these analyses indicated that organics accumulate on the catalyst surface upon exposure to water, but the accumulation of organic matter did not correlate strongly with catalyst deactivation. The spectroscopic data suggested that sulfide may bind to the Pd surface, and may be oxidized to sulfate with hypochlorite treatment.

Adsorption of trichloroethylene (TCE) on alumina-supported palladium catalysts (Pd/Al_2O_3) was studied in the presence and absence of hydrogen using ^{13}C-solid state NMR. Carbon-13 NMR spectra indicate that at low coverage strongly adsorbed species are formed while at high coverage additional physisorbed species are present. Carbon-13 spin-echo amplitude data measured as a function of pulse separation, τ, was used to determine the ^{13}C-^{13}C intramolecular dipolar coupling and the carbon-carbon bond length of adsorbed species. Results indicate that a substantial fraction of the chemisorbed carbon species had undergone carbon-carbon bond scission forming single-carbon fragments, suggesting that the activation energy for carbon-carbon bond scission is comparable to the heat of adsorption. For the remaining surface species, the double bond is elongated to 1.46 ± 0.03 Å and is suspected to be chemically bonded ethynyl. At room temperature, adding an excess of hydrogen to catalyst that is covered to saturation with TCE precursors produces only in a small amount of ethane, indicating the fraction of surface species that are hydrodehalogenation precursors is small.

The field demonstration results can be demonstrated as follows: Catalytic destruction of TCE in groundwater was demonstrated at Edwards AFB. The site was contaminated with 800 to 1,200 $\mu g\ L^{-1}$ TCE, which was the sole contaminant. A treatment methodology was developed to maintain catalyst activity and keep treated water TCE concentrations at or below the maximum contaminant level (MCL) of 5 $\mu g\ L^{-1}$ without by product formation. The treatment protocol entailed treating 2 gpm in a single catalyst column for 21 h (contact time approximately 1 min) followed by a 3 h bleach cycle to restore and maintain catalyst activity. The maintenance cycle consisted of bleaching of the catalyst for 1 h and flushing with hydrogen-containing groundwater for 2 h. After each maintenance cycle, TCE in the product water was at or below 1 $\mu g\ L^{-1}$ corresponding to 99.9% removal. During a 21 h treatment cycle, effluent TCE concentrations increased slowly to approximately 10-15 $\mu g\ L^{-1}$, corresponding to approximately 99% removal. Daily bleaching maintained catalyst activity by preventing biological fouling with sulfidogenic bacteria (bacteria oxidizing hydrogen and reducing sulfate to hydrogen sulfide). Operational

problems led to episodes of biological sulfide formation and severe catalyst poisoning marked by complete activity loss. Laboratory experiments and field observations demonstrated that the activity of the catalyst is nearly completely recoverable by treating the catalyst with bleach.

Based on data obtained in this demonstration, it is estimated that a capital investment of $572,000 and annual O&M costs of $72,000 (including monitoring & analysis) are sufficient to install and operate a treatment system that creates a barrier approximately 20 m wide in a plume of contaminated groundwater. This estimate applies to sites contaminated with chlorinated ethylenes (PCE, TCE, DCE isomers and vinyl chloride) with a relatively permeable aquifer, shallow water table and low gradient, similar to the Edwards AFB field site. This cost estimate is for a two-well system having a total flow of 2 gpm per treatment well or 4 gpm total. The system operates 87.5% of the time in a daily 21h:3 h treatment:regeneration cycle and remediates a TCE concentration of 1000 μg L^{-1}. The estimate is directly applicable to a full scale system and scalable to multiple sets of two wells. Sites with lower quality water would require more frequent bleaching whereas sites with cleaner (more aerobic) water are expected to require less frequent bleaching. A modification is proposed for continuous (100%) treatment by using two catalytic columns per well whereby one reactor is bleached and reactivated while the other treats the contaminated groundwater.

In the final phase of the project (spring 2007), we focused on improving the design of the field reactor that was used to treat TCE contaminated groundwater installed at the EAFB. We have shown that by recirculating the dilute bleach solution for catalyst regeneration, the regeneration and reactivation cycle could be shortened, the production of TCE containing rinse water was nearly avoided, and the total daily through put could be minimized.

Publications:

Journal Articles

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Goltz, M. N., R.K. Gandhi, S.M. Gorelick, G.D. Hopkins, C. LeBron, P.L. McCarty, and M. Reinhard (2001). Application of Circulating Wells for In Situ Treatment of Contaminated Groundwater. *International Symposium on Soil and Groundwater Contamination Control Strategy,* Kyung Hee University, Seoul, Korea.

Munakata, N., J. Cunningham, R. Ruiz, C. Lebron, and M. Reinhard (2002). Pd-Catalyzed Hydrodechlorination of Chlorinated Organic Pollutants in Groundwater. *Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds*, Monterey, CA; USA (May 20-23).

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Sriwatanapongse, Wataneee (2005). Reductive Dechlorination of Trichloroethylene by Palladium On Alumina Catalyst: a Solid State NMR Study of the Surface Reaction Mechanism. Ph.D., Stanford University.

<u>Supplemental Keywords:</u> groundwater; NAPL; VOCs; chlorinated solvent; remediation technologies; in-situ; technology transfer; Environmental Chemistry

Period Covered by the Report: October 2001 – August 2007
Date of Final Report: December 2007
EPA Agreement Number: R828772
Title: Western Region Hazardous Substance Research Center Project 1-SU-03
Effects of Sorbent Microporosity on Multicomponent Fate and Transport in Contaminated Groundwater Aquifers
Investigators: Martin Reinhard
Institution: Stanford University
Research Category: Groundwater, transport
Project Period: 2001-2003

Goal: The overall goal of this project was to develop a better understanding of organic contaminant sequestration by geosorbents. Specific project goals were to (1) develop a method to characterize microporosity in geological solids in the presence of moisture, (2) determine how micropore hydrophobicity/hydrophilicity and contaminant properties influences contaminant sequestration and desorption, and (3) quantify the interactions among multiple contaminants during uptake in and release from micropores. Information gained from this research allows us to better predict contaminant bioavailability, the rate of natural attenuation processes, and the time scale of contaminant release from natural sorbents.

Rationale: Micropores are pores (less than 2 nm in diameter) that are comparable in size to small organic contaminant molecules, and the sorption potential inside these pores are significantly enhanced due to the proximity of the opposite pore walls. Understanding contaminant sequestration in micropores is essential for predicting the long-term fate of contaminants in groundwater aquifers, and for assessing the significance of natural attenuation processes. Most natural solids contain micropores that form due to weathering, cracking, material imperfections, or turbostratic stacking. Previous work has demonstrated that sorption of hydrophobic organic compounds in micropores can be a significant sequestering process. Sorption in micropores is reversible but rates are very slow and difficult to quantify, especially in the field. Our understanding of geosorbent microporosity and its effect on contaminant sorption is limited because conventional microporosity characterization methods used (vacuum piezometric and gravimetric techniques) employ only a single "model" sorbate and are not sensitive enough to detect the low volumes of micropores typically present in geological solids. Furthermore, our understanding of sorption in micropores is inadequate to predict solid-contaminant interactions based on pore volume and pore size distribution data obtained with these methods.

Approach: The first task was to develop and validate a methodology for measuring slow uptake and release rates of volatile organic compounds (VOCs) by microporous solids. To validate the method, the sorption and desorption of simple sorbates, such as methane, carbon tetrachloride, and trichloroethylene on model sorbents, such as silica gel were studied. Subsequently, the methodology was applied to study contaminant sorption and desorption on natural sorbents and characterize their microporosity under environmentally relevant conditions.

The focus of this investigation was study how contaminant properties (e.g., molecular size, structure, and polarity) and environmental variables (such as relative humidity and temperature) affect micropore sequestration. Sorption and desorption kinetics of model contaminants on

microporous engineered solids and natural solids under different conditions were measured with an apparatus developed as part of this project. The interactions between contaminant molecules and microporous sorbents and the influence of the sorbate and sorbent properties were elucidated by comparing contaminant sorption and desorption kinetics under these conditions.

Summary of Findings: An apparatus has been developed for measuring slow sorption and desorption kinetics of VOCs on solid materials packed in columns. A HP 5890 II GC equipped with FID and ECD detectors is used to analyze gas phase compositions at the inlet and outlet of the column in rapid sequence. Samples of the gas stream that enters and leaves the column are alternatively injected into the GC column (through an valve injector), and the contaminant mixtures are subsequently separated in GC column and detected by both FID and ECD. This design has expanded our investigative capabilities in several ways: data are acquired in real-time with high temporal resolution over the entire contaminant desorption profile; contaminant detection is extremely sensitive (0.1 nmol/L), and sorption and desorption of multiple volatile organic contaminants can be studied. Only a relatively small amount of solid (packed in a column of 3.0 mm i.d. × 304.8 mm length) is required because of the system's high resolution, and gas flows through the column at 2.00 mL/min regulated by a digital mass flow controller. Constant vapor concentrations of organic contaminant and water in the flow line are achieved by bubbling the gas through organic liquid and water reservoirs submerged in a constant temperature water bath. Sorption of TCE on several solid materials as a function of temperature and moisture content were studied.

Data show that high concentrations of water vapor lead to rapid displacement of contaminant molecules sorbed/condensed in mesopores in a natural soil, a process that may be described as "chromatographic elution." By contrast, desorption of contaminants sequestered in micropores is significantly slower. We hypothesized that narrow throats in pores restrict the exchange of background gas molecules with contaminant molecules in the deeper micropores. Data indicated that water molecules have very small effect on the desorption rate of TCE sequestered in micropores in a natural soil, and the kinetics of TCE sorption. In an engineered microporous sorbent (silica gel) desorption was barely influenced by the presence of water molecules. These results suggest that both hydrophilic and hydrophobic micropores exist in these solids, and that sorption and desorption of hydrophobic species (TCE) only occurs in hydrophobic micropores that are not accessible to water molecules. That is, hydrophobic and hydrophilic species are sequestered into two separate micropore-domains (hydrophobic micropores and hydrophilic micropores) independently, and there is no apparent competitive effect between them. This suggests that the affinity of micropores for water, rather than the total volume of the micropores, plays key role in controlling contaminant sequestration and desorption. Also, contaminants sequestered in hydrophobic micropores may not be in contact with water molecules, which prevents it from undergoing chemical and biological transformations (e.g., hydrolysis, biodegradation) during natural attenuation processes. This hypothesis was evaluated in the subsequent phase.

Publications:

Journal Articles

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Thesis

Cheng, H. (2006). Sorption and Hydrolysis of Aliphatic Hydrocarbons in Hydrophobic Micropores. Ph.D, Stanford University.

Supplemental Keywords: groundwater; soil; characterization; chlorinated solvent; VOCs; environmental chemistry

Period Covered by the Report: October 2001 – August 2007
Date of Final Report: December 2007
EPA Agreement Number: R828772
Title: Western Region Hazardous Substance Research Center Project 1-OSU-03
Development of the Push-Pull Test to Monitor Bioaugmentation with Dehalogenating Cultures
Investigator: Mark E. Dolan, Jennifer Field, and Jonathon Istok, Oregon State University
Institution: Oregon State University
Research Category: Bioremediation, groundwater, bioaugmentation.
Project Period: 2001-2003

Goal: The overall goal was to modify the single-well push-pull groundwater test as a means for obtaining quantitative information on in situ dechlorinating activity before and after bioaugmentation. The specific objectives included: 1) modifying TCFE and fumarate assays to determine TCE-transformation potential for use in monitoring bioaugmentation, 2) developing methods for monitoring the transport of dehalogenating cultures during push-pull tests, and 3) evaluating the ability of push-pull tests to monitor changes in TCE-transformation potential resulting from the injection of dehalogenating cultures.

Rationale: Technologies are needed to enhance the in situ remediation of groundwater contaminated by chlorinated aliphatic hydrocarbons (e.g., trichloroethene or TCE). Bioaugmentation of anaerobic dehalogenating consortia may be a viable alternative for remediating TCE source zones. Currently, it is difficult to assess if bioaugmentation has increased in situ dechlorination activity. Single-well "push-pull" tests using TCE, the target compound, can be difficult to interpret due to background TCE concentrations and uncertain flux through the zone. However, single-well "push-pull" tests using the surrogate substrate trichlorofluoroethene, TCFE, can provide quantitative information on in situ biological activity and can be correlated to known culture transformation kinetics for both TCE and TCFE to estimate in situ TCE transformation rates. With modifications, the single-well "push-pull" tests could be used in determining the effectiveness of bioaugmentation.

Approach: Two cultures (Evanite and Pt. Mugu) that transform TCE to ethene will be characterized in collaboration with Dr. Semprini (Developing and Optimizing Biotransformation Kinetics for the Bio-remediation of Trichloroethylene at NAPL Source Zone Concentrations). Transport and transformation tests will be conducted using a physical aquifer model (PAM) shaped like a piece of pie simulating a radial segment around a central injection/extraction well. The transport of the culture(s) will be determined during injection into the PAM that will be maintained under anaerobic conditions. The sediment used to pack the PAM will first be tested for in situ dehalogenation activities towards both TCE and TCFE. Dehalogenating microbial activity will be monitored in the PAM with and without bioaugmentation. Spatial distributions of dechlorinating activity and redox will be determined from a suite of assays conducted at sampling ports and at the injection/extraction location. Push-pull tests will be conducted at the injection/extraction well to assess changes in reductive dechlorination activity resulting from bioaugmentation.

Summary of Findings:

Stimulation of dehalogenation in PAM sediments. The background activity of sediment collected from a site with known indigenous reductive dechlorination activity was characterized with respect to the kinetics of TCE, TCFE, fumarate, and succinate utilization and product formation. These four substrates were proposed for this project as substrates that could be used to assay for reductive dechlorination potential in situ. A microcosm study was used to determine the relationship between TCE and TCFE transformation rates and product speciation when fed fumarate and succinate. Results obtained here will be useful when initiating the assays in PAMs. The microcosms were operated over a period of approximately 250 days. Succinate- and fumarate-fed microcosms produced similar results for lag times, transformation rates and product speciation, with very similar results from triplicate microcosms at each condition. Lag time to the onset of TCE transformation in both fumarate- and succinate-fed microcosms was about two weeks. The corresponding lag times for TCFE transformation under the same conditions was about six weeks and may indicate slower microbial growth using TCFE. Based on a first order model fit after the lag time, TCE transformation rates were from 3.3 times (fumarate-fed) to five times (succinate-fed) faster than microcosms without exogenous electron donor addition. TCFE transformation rates were about 2.4 times faster than control microcosms but about four to five times slower than TCE transformation rates. TCE transformation products were cis-DCE and trans-DCE in approximately a 2:1 ratio and TCFE transformation products were cis-DCFE and trans-DCFE in approximately 2:1 ratio as well. TCE was ultimately reduced to VC, but very little ethene was observed. TCFE was transformed into a mixture of DCFEs and CFEs, with no FE formation. CAH transformation rates were not affected by sulfate addition. From these tests it was determined that succinate was a potential electron donor for further experiments and that TCFE transformation rates would have to assessed in the sediments used in the PAM tests to determine the relationship to TCE rates.

Bioaugmentation dose. A seed culture, obtained from Dr. Semprini's group from their "Evanite" culture reactor, was serially fed butanol and PCE for about two months, and showed complete dehalogenation of PCE to ethene. This culture was used in other tests related to this project. A series of microcosms were prepared with the same sediments that were used to pack the PAM and were used to test the survivability of the bioaugmented culture under different geochemical conditions. The aqueous phase in the microcosms consisted of tap water or tap water amended with 5% media solution used in the culture reactor. Both lactate and butanol were tested as fermentable substrates and bioaugmentation doses of 0.1, 1, and 10 mL of reactor culture, representing culture dilutions of 0.1 to 8%, were tested. Although all of the bioaugmented microcosms survived to exhibit dehalogenation activity, microcosms receiving the highest bioaugmentation dose had dechlorination rates significantly higher than the lower doses in the same time period and microcosms with 5% media addition had faster activity than those without media. Microcosms containing 5% media that received the 10 mL culture dose and were fed butanol were able to completely transform the TCE to ethene within 96 days. This dose was used in the bioaugmentation of the PAMs.

Culture transport. A glass column of 5 cm diameter and 34 cm length was packed with the same sediments used to pack the PAMs, and was used to evaluate the transport characteristics of the bioaugmentation culture. Molecular tests using PCR reactions targeting the 16S rDNA of *Dehalococcoides* sp. showed efficient transport of the bioaugmentation culture through the column. Microscopic direct count of DNA-stained cells was the technique used to assess

transport of the bioaugmented cells in the PAM. Background cell counts were from 1-3 $*10^4$ cells/mL before bioaugmentation and rose to 34 to 57% of the influent cell concentrations ($1*10^7$ cells/mL) throughout the PAM indicating successful transport of the bioaugmentation culture over the 34 cm length of the PAM.

PAM results. The PAM was packed with sediment and saturated with oxygen-free water to produce anoxic conditions for the start of the test. Lactate solution was added to the PAM just prior to bioaugmentation in an effort to assure anaerobic conditions prior to bioaugmentation. The PAM was bioaugmented with a solution containing approximately $1*10^7$ cells/mL and 50 μM TCE. No activity values were obtained with the initial bioaugmentation since all of the TCE was transformed to ethene at all sample ports by the time samples were acquired 7 days later (a longer initial incubation was estimated from microcosm tests). Subsequent injections of solution containing TCE, TCFE or both TCE and TCFE were conducted to evaluate product distribution and transformation rates. Essentially complete transformation of TCE to ethene was accomplished with each injection with the maximum measured transformation of about 250 μM influent TCE and 320 μM TCFE to ethene and fluoroethene respectively within 30 days. Butanol injected as an electron donor was fermented into butyrate, propionate and, eventually, acetate. When injected alone at about 250 μM, TCE transformation rates ranged from 30- 80 μM/d with the highest activity around ports 5 and 6. When injected at the same concentration along with 320 μM TCFE, TCE transformation rates remained high at around 26-36 μM/d. TCFE rates were equally as high with estimates of 29-45 μM/d being transformed in conjunction with the TCE, indicating that inclusion of the surrogate transformational tracer TCFE should enable estimation of attainable TCE transformation rates. Repeated addition of electron donor was required over the 120 day study to provide energy for the dehalogenation reactions. The control PAM operated without bioaugmentation showed limited transformation to cis-DCE and no DCFE production during 70 days of operation.

Push-pull transformation tests. A push-pull test was initiated using the bioaugmented PAM and a solution containing TCFE and bromide as a non-reactive tracer. The solution was injected into the PAM, allowed to reside for 35 hr, and then extracted and analyzed over 25 hr. Using the non-reactive tracer to produce a mass balance on TCFE, a FE production rate of about 60 μM/d was calculated. This is on the order of the TCFE transformation rates found in the initial activity tests in the bioaugmented PAM, but is significantly higher than FE production rates calculated in those tests (~5-8 μM/d). This may in part be due to only capturing 47% of the injected TCFE in transformed products. It is expected that gas pockets trapped during the packing of the PAM provided sinks for the FE produced that were not included in the aqueous sampling and therefore were not recovered here or in the significantly longer initial transformation tests. A reactive model was used incorporating the kinetic characteristics of the bioaugmented Evanite culture to simulate transformation in the PAM. Although the results were consistent with the observed data, estimates of FE production consistently exceeded actual FE concentrations, perhaps due to partitioning of the FE into trapped gas pockets within the PAM. The ability of this dechlorinating culture to transform TCE and TCFE at appreciably similar rates allows estimation of TCE transformation rates based on single-well push pull tests conducted using TCFE as a surrogate substrate. The push-pull tests produced better results due to the shorter time-scale required for the test and the potential for FE losses associated with partitioning into a trapped gas phase.

Publications:

Journal Articles

Field, J. A., J. D. Istok, L. Semprini, P. Bennett, and T. E. Buscheck. (2005). Trichlorofluoroethene: A Reactive Tracer for Evaluating the Effectiveness of In Situ Trichloroethene Remediation, *Ground Water Monitoring Remediation* Vol 25(2):68-77.

Schroth, M.H., J.D. Istok. (2005). Approximate solution for solute transport during spherical-flow push-pull tests, *Ground Water*, 43(2), 280-284.

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Lee, J. H, Dolan, M.E., Istok, J., Reed R., and Field, J.A. (2004). Monitoring Bioaugmentation with Single-Well Push-Pull tests in Sediment Systems Contaminated with Trichloroethene. Fourth SETAC World Congress and 25th Annual Meeting. Portland, Oregon (November 14 – 18).

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Lee, Jae-Hyuk (2006). Anaerobic Reductive Dechlorination of TCE and TCFE in TCE-Contaminated Sediments: Enhnced Bioremediation and Bioaugmentation. Ph.D., Oregon State University.

Period Covered by the Report: October 2001 – August 2007
Date of Final Report: December 2007
EPA Agreement Number: R828772
Title: Western Region Hazardous Substance Research Center Project 1-OSU-04
Development and Evaluation of Field Sensors for Monitoring Bioaugmentation with Anaerobic Dehalogenating Cultures for In Situ Treatment of TCE
Investigator: James D. Ingle, Oregon State University
Institution: Oregon State University
Research Category: Bioremediation, groundwater
Project Period: 2001-2003

Objectives: The overall objective of this study was to refine and use redox sensors based on redox indicators as monitoring tools for assessing and optimizing redox conditions for treatment of PCE and TCE with dehalogenating cultures. Specific objectives were to 1) deploy, evaluate, and refine redox indicators for on-line monitoring of the redox conditions in two collaborative situations involving a bioaugmentation approach, 2) understand the nature of the redox conditions under which dechlorination microbial processes occur.

Rationale: Better on-line monitoring techniques for redox status are needed for 1) the initial assessment of laboratory samples or models and of subsurface conditions at a site, 2) continued assessment of the progress of remediation, and 3) control of injections of amendments (e.g., substrates, nutrients) during remediation. We have shown that redox sensors based on redox indicators exhibit promise for monitoring environmental redox levels. Research is needed to1) understand the nature of the response of these indicators, 2) improve the monitoring devices for practical use, and 3) demonstrate that these devices can be employed for on-line monitoring of the status of anaerobic dehalogenating cultures in laboratory systems.

Approach: Redox indicators immobilized on transparent films have been shown to be able to differentiate between different microbial redox levels (Fe(III)-reducing, sulfate-reducing, methanogenic). These redox indicator flow sensors were deployed in two primary situations for calibration and demonstration of their applicability: 1) continuous monitoring of redox conditions of cultures inside bioreactors or microcosm bottles as a tool for the optimizing conditions for effective dechlorination of PCE and TCE with enriched halorespiratory cultures, 2) on-line monitoring of the redox status of the material in a physical aquifer model (PAM) bioaugmented with the developed dehalogenating cultures. The design and characteristics of the redox sensor monitoring systems were improved for low oxygen permeation and portability for easy operation in the field.

Status: We have refined the portable, immobilized redox monitoring system and used it to monitor sulfate-reducing and methanogenic conditions in a PAM containing wastewater slurry and also dechlorinating cultures in bioreactors and modified microcosm bottles. The enriched dechlorinating culture (Lew Semprini lab) was loaded into our bioreactors and microcosm bottles to calibrate the response of the redox indicators to the dechlorination of PCE. The indicator data support the concept the dechlorinating process is increasingly more reducing as PCE is dechlorinated, with the most reducing step in the process being the dechlorination of vinyl chloride to ethene. Specifically, the reduction of Thionine (THI) indicates degradation of PCE and formation of TCE and cis-DCE; whereas, ~50% reduction of Cresyl Violet (CV)

correlates to the formation of vinyl chloride, and production of ethene is only observed when CV is nearly or fully reduced. We worked closely with Dr. Semprini and his students to address concerns about oxygen contamination during culture transfer steps and while monitoring with our redox indicator, flow monitoring system. Refining techniques for transfer of highly oxygen-sensitive cultures was critical for eventual column and PAM studies.

We developed a method to precipitate finely divided platinum particles into membranes with immobilized indicators through reduction of Pt^{2+} solutions. H_2 levels as low as 0.01% by volume in the headspace do reduce the indicator in the platinum embedded membranes. The indicator Phenosafranine (PSaf) is useful for monitoring dechlorinating cultures because, without Pt, PSaf is not reduced by reductants in dechlorinating cultures. Reduction of a PSaf membrane with embedded Pt indicates active fermentation and H_2 production necessary for dechlorination. The rate of reduction of the indicator changed with varying H_2 concentrations. Although the results were preliminary, this approach could be the basis of a convenient and inexpensive method to determine if H_2 concentrations in cultures are sufficient for effective dechlorination laboratory without the need to run expensive GC testing. This area of research was not further pursued.

Publications:

Journal Articles

Cantrell, K. and J. D. Ingle, Jr. (2003). The SLIM Spectrometer. *Anal. Chem.*, 75, 27-35.

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Supplemental Keywords: biotransformation; characterization; VOCs; chlorinated solvents; bioremediation; environmental chemistry

Period Covered by the Report: October 2001 – August 2007
Date of Final Report: December 2007
EPA Agreement Number: R828772
Title: Western Region Hazardous Substance Research Center Project 2-OSU-05
Aerobic Cometabolism of Chlorinated Ethenes by Microorganisms that Grow on Organic Acids and Alcohols
Investigators: P. J. Bottomley, D.J. Arp, M. Dolan, L. Semprini, Oregon State University
Institution: Oregon State University
Research Category: Cometabolism, ethenes, volatile organic compounds
Project Period: 2004-2007

Part 1: Aerobic cometabolism of chlorinated aliphatic hydrocarbon compounds with butane-grown microorganisms.

Investigators: Peter Bottomley and Dan Arp

Goal: The proposal aimed to evaluate how to maximize the chloroethene degrading potential of individual strains of hydrocarbon degrading bacteria. Specific subobjectives included identifying conditions (a) that maximize reductant flow to cometabolism, (b) that promote maximum expression of monooxygenase genes and enzyme activity, and (c) that sustain enzyme activity with minimal cell damage.

Rationale: Studies conducted under laboratory and field conditions have shown that hydrocarbon-oxidizing bacteria cometabolize a wide range of chloroethenes. Nonetheless, there is considerable variability in the properties of cometabolism shown by different types of bacteria both in terms of the range of chloroethenes degraded and in their transformation capacities. More research is needed to better understand the microbiological reasons for the range of efficiencies observed, and to use this information to improve the biotechnology of bioremediation under cometabolism conditions.

Experimental Approaches:
(a) Throughout our studies with *P. butanovora*, it became clear that despite fast initial rates of CE oxidation it was difficult to sustain maximum rates of CE degradation with propionate and butyrate as electron donors, and this was particularly true for a poor substrate like 1,2 trans DCE (Doughty et al. 2005). We continued to study the activity of BMO in *P. butanovora* in the presence of organic acids (Doughty et al. 2006; Doughty et al. 2007). Incubation of alkane-grown *P. butanovora* with butyrate or propionate led to irreversible, time-, and O_2-dependent loss of BMO activity. In contrast, BMO activity was unaffected by incubation with lactate or acetate. Chloramphenicol inhibited the synthesis of new BMO, but did not change the kinetics of propionate-dependent BMO inactivation, suggesting that the propionate effect was not simply due to it acting as a repressor of BMO transcription. BMO was protected from propionate-dependent inactivation by the presence of its natural substrate, butane.Although both the time and O_2 dependency of propionate inactivation of BMO infer that it might be a suicide substrate, no evidence was obtained for BMO-dependent propionate consumption, or ^{14}C labeling of BMO polypeptides by [2-^{14}C] propionate during inactivation. We have also examined the BMO mutant strains mentioned in project 1 of 1-OSU-02 (Halsey et al. 2006) to determine if propionate sensitivity of BMO had been changed. The effects of propionate on BMO differed among the

mutant strains. For example, in the case of the G113N mutant, in which a glycine was replaced by an asparagine residue in a region adjacent to, or contributing to the active site, BMO activity was not inactivated by propionate. In contrast, mutant strain T148C, in which a threonine in the active site region was replaced by a cysteine, showed a significant increase in propionate-dependent inactivation of BMO relative to wild type. We screened other well-studied monooxygenase enzymes for inactivation by fatty acids. *In vivo* studies showed that the diiron methane monooxygenases (sMMO) of *M. capsulatus* Bath, and *M. trichosporium* OB3b, and the toluene o-monooxygenase of *Burkholderia cepacia* G4 were not inactivated by propionate. In contrast, the toluene 4-monooxygenase (T_4MO) of the excellent TCE degrader, *Pseudomonas mendocina* KR1, was inactivated following a10 min incubation with 10 mM propionate or butyrate by ~ 60 and 94% respectively. In contrast acetate or lactate did not inactivate T_4MO activity, indicating that T_4MO was sensitive to the same range of organic acids as BMO. Furthermore, T_4MO activity was protected from propionate-dependent inactivation by toluene, the physiological substrate of T_4MO. Our data certainly showed that oxygenase containing strains with bioremediatory potential must be screened to determine if potential electron donating organic acids might have negative effects on enzyme activity prior to considering them for use as bioremediatory agents of CEs.

We discovered that BMO expression in *P. butanovora* is induced by the products of its activity i.e. alcohols, aldehydes, and epoxides, by the xenobiotic substrate, 1,2-trans DCE, but not by its natural alkane substrates (Doughty et al. 2005; Sayavedra-Soto et al. 2005). Furthermore, we showed that when butane and propane or pentane were present simultaneously, that BMO expression was repressed. This was subsequently attributed to the accumulation of propionate when cells were exposed to odd chain length alkanes and the inability of cells grown on even chain length alkanes to process propionate (Doughty et al. 2006). Although it is recognized that aerobic transformation of lesser chlorinated ethenes might occur at the aerobic/anaerobic interface of contaminant plumes undergoing reductive dechlorination, it is unclear to what extent monooxygenases like BMO can be induced under the low concentrations of O_2 found at the fringes of contaminated zones. Data were obtained to show that induction of BMO by alcohols or 1,2-trans DCE could occur at low O_2 concentrations (<1%), however, the combination of low concentrations of Cu (<1μM) and low O_2 levels (<2%) repressed induction of BMO by alcohols (Doughty et al. 2007, in review). Furthermore, a combination of a low concentration of Cu (0.5 μM), and the reducing agent Na ascorbate was an effective repressor of alcohol dependent induction of BMO under fully oxic conditions. This result was intriguingly similar to that observed in methanotrophs in which the synthesis of soluble methane monooxygenase is repressed upon exposure to low concentrations of Cu (>1μM). Again, the results indicate that care must be taken to screen strains for bioremediatory purposes that are in possession of monooxygenase genes whose induction occurs at low O_2 and is not sensitive to the presence of Cu.

Part 2: Aerobic metabolism and cometabolism of vinyl chloride and fluoroethene on microorganisms that grow on ethene

Investigators: Lewis Semprini, Peter Bottomley and Mark Dolan

Considerable attention has been given to bacteria that degrade VC aerobically because it is often a persistent product of reductive dechlorination and can move out of the anoxic contaminated plume into the adjacent oxic zone. It is difficult to estimate rates of aerobic VC transformation in situ because the mineralization of VC yields CO_2 and Cl^-; neither of which can be tied solely to VC transformation. Fluoroethene (FE) is a stable molecule in aqueous solution and its aerobic degradation yields fluoride (F^-), which is a unique signature in most aquifers.Work with cytochrome P450-dependent monooxygenases suggests that FE is aerobically degraded in a manner similar to VC; an epoxide is formed from the initial oxidation. FE-epoxide is unstable and is expected to yield spontaneous degradation products analogous to VC-epoxide. Laboratory experiments were carried out with various alkene monooxygenase-containing bacteria that either cometabolically or catabolically metabolize VC, to evaluate if (i) rates of FE transformation are similar to those of VC transformation, (ii) VC and FE have similar affinities for the monooxygenase that mediates the initial transformation, (iii) a competitive inhibition kinetic model accurately simulates concurrent FE and VC degradation, and (iv) the rate of F^- accumulation can be correlated with that of VC utilization. In addition the potential for bacteria to use FE as a carbon and energy source was evaluated (Taylor et al. 2007, in press). Despite the fact that the three VC-degrading isolates responded differently to Eth, VC and FE as growth substrates, there were no differences between the $K_{s/c}$ or k_{max} values for FE and VC of any individual isolate, and there was little difference between the three isolates in their rates of transformation or affinity for the halogenated substrates. Additionally, rates of maximum VC and FE transformation or utilization were similar for all three isolates, indicating that the presence of the smaller F atom did not affect the alkene monooxygenase's ability to accept FE as a substrate. In a separate experiment we also determined if it was possible to monitor VC transformation by modeling the rates of F^- accumulation. During cotransformation, the initial rates of halide release matched that of substrate transformed for each VC-degrading isolate, and in separate experiments where the degradation of individual substrates was followed, halide release ceased as soon as substrate transformation was complete. This demonstrated that there was no further halide release from halogenated products that might have been formed during cometabolism. For VC, mass balance of the substrate transformed and halide released were nearly stoichiometric regardless of whether it was a growth or nongrowth substrate and averaged 0.98 (±0.11) on a mole fraction basis. There was a trend for stoichiometric release of F^- during transformation of FE by JS614 (1.0 ±0.16 mole percent), while F^- released during cometabolic transformation of FE by EE13a and JS60 averaged 0.84 (±0.04) mole percent. Competitive inhibition between substrates, halide release rates equivalent to substrate transformation rates, a known mole fraction of halide released, and previously determined X, k_{max} and $K_{s/c}$ values were incorporated into the model. This model accurately estimated the accumulation of halide in the batch reactors using heuristically fit K_i values. For the three aerobic VC-degrading isolates studied, both the rates of FE transformation and F^- accumulation could be correlated with the rate of aerobic degradation of VC. FE therefore has the potential to be used as a surrogate reactive tracer for estimating rates of VC degradation in situ. In a VC contaminated aquifer, for example, in single-well push-pull tests, FE addition and subsequent cotransformation could be utilized as an indicator of in situ VC transformation rates.

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Halsey, K. H., D. M. Doughty, L. A. Sayavedra-Soto, P. J. Bottomley and D.J. Arp. (2006). Investigating the Basis of Substrate Specificity of Butane Monooxygenase and Chlorinated Ethene Toxicity in *Pseudomonas butanovora. Molecular Basis for Microbial One-carbon Metabolism, Gordon Research Conference at Magdalen College,* Oxford, UK.

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Supplemental Keywords: biotransformation; characterization; VOCs; chlorinated solvents; bioremediation; in situ

Period Covered by the Report: October 2001 – August 2007
Date of Final Report: December 2007
EPA Agreement Number: R828772
Title: Western Region Hazardous Substance Research Center Project 2-OSU-06
Development and Evaluation of Field Sensors for Monitoring Anaerobic Dehalogenation after Bioaugmentation for In-Situ Treatment of PCE and TCE
Investigator: James D. Ingle, Jr.; Oregon State University
Institution: Oregon State University
Research Category: Dehalogenation, bioaugmentation, PCE, groundwater
Project Period: 2004-2007

Goals: The purpose of this study was to develop, refine, and use sensors and field instruments, based on redox indicators and other reagents as on-site, on-line, or in-situ monitoring tools for assessing and optimizing redox and related conditions for treatment of PCE and TCE with dehalogenating and other cultures. These sensors and field instruments were calibrated for evaluating redox conditions and the effectiveness of dechlorination in collaborative situations involving a bioaugmentation approach in packed sediment columns.

Rationale: Better field and portable monitoring techniques for redox status and related conditions for bioremediation are needed 1) for the evaluation of laboratory samples, models such as columns and PAMs, and subsurface conditions at a site, 2) for continued assessment of the progress of remediation, and 3) for examination of the effects of bioaugmentation in field and laboratory experiments. We have demonstrated that redox sensors based on redox indicators exhibit promise for monitoring environmental redox levels. Research is needed to 1) identify and compare the response of these indicators during bioaugmentation, 2) improve the monitoring devices and methodology (flow cells, fiber optic probes, sampling) for practical use, 3) demonstrate that these devices and methodology can be employed for on-line or in situ monitoring of the status of anaerobic dehalogenating cultures in laboratory systems, and 4) develop new sensing species, methods, instrumental components and sensor designs for on-line monitoring of the status of dechlorinating and other anaerobic systems in columns and PAMs packed with soil, microcosm bottles, and sub-surface systems in the field.

Approach: Redox indicators immobilized on transparent, polymer films have been shown to be able to differentiate between different microbial redox levels and to predict whether conditions are appropriate for reductive dechlorination to occur. These redox indicators, which are incorporated into specially constructed flow sensors and fiber optic probes, were deployed in collaborative experiments for calibration and demonstration of their applicability. These experiments involved continuous monitoring of the redox conditions of cultures inside columns and PAMs packed with soil and enriched with halorespiratory cultures as a tool for spatial monitoring of dechlorination and to improve conditions necessary for effective dechlorination of PCE and TCE. In addition, we sought to investigate alternative sampling/reagent/detection systems, quantitative measurement of concentrations of reductants, O_2, and fiber optic sensors.

Status: We improved portable, flow-based monitoring systems based on measuring the absorbance of immobilized redox indicators. The design and characteristics of the redox sensor monitoring systems were significantly modified to minimize oxygen permeation (contamination) and provide portability for easy operation in the lab and field. The flow sensors were used to successfully examine redox conditions in microcosm bottles containing a dechlorinating culture

(Evanite culture) and in packed columns augmented with the culture. Consistently, we demonstrated that the rapid reduction of the indicator immobilized thionine (THI) indicated that conditions were appropriate for dechlorination and occurred with hours of contact with an active dechlorinating culture. The redox indicator cresyl violet (CV) was slowly but consistently reduced (about 25 to 75% reduced) as the culture became more reducing, typically during the dechlorination of cis-DCE and VC. It appears likely that reduced species other than S(-II) or Fe(II) contributed to the reduction of the indicators and preliminary results suggested some of these reductants may be products of cellular energy generation, mediators, or co-factors.

We constructed a fiber optic redox probe with immobilized redox indicator film at its tip and used it to monitor redox status by measuring the indicator absorbance providing in situ information about the redox conditions in the center of the columns in two laboratories. The fiber optic redox probe and flow redox sensor were installed in the same column and shown to respond comparably to the same redox conditions.

A new method was developed for determining reductive capacity (RC) with redox indicators. Based on a relatively uncomplicated concept, namely through determination of the total number of moles of indicator that react with an anaerobic sample, the concentration of reductants in an anaerobic sample is determined. This novel technique provides a new tool to evaluate redox status of anoxic and anaerobic samples in laboratory and field studies. RC provides information that is different from the "redox level" sensed by immobilized indicators. An immobilized indicator may be totally reduced, but the RC for the same indicator could still be increasing if microbial activity increases and concentrations of reductants increase in the sample.

In microcosm bottles inoculated with an active EV culture, RC (THI) ranged from about 100-400 μM and increased as the culture dechlorinated PCE to ETH. A large relative drop in RC likely suggests that the concentration of a critical species such as an electron donor has dropped and significantly impaired microbial activity or that the culture may have been compromised by contamination with O_2. Data suggest that measurements of RC likely probe reductants that are associated with outer cell membranes or within cells. RC values are much greater than S(-II) or Fe(II) concentrations and the measured RC drops considerably when solutions obtained from a microcosm bottle are filtered. Reductive capacities were also measured in packed columns where the overall RC(THI) observed was lower (100-200 μM), which suggests that some of the RC measured in free cultures was not present due to cells or particles becoming trapped or attached in the column. Upon filtration of column samples, RC(THI) decreased by ~50%, which suggests that a larger fraction of the RC was due to reductants in solution compared to cultures in microcosm bottles.

We have a simple system to determine very low oxygen levels in laboratory samples and groundwater in the field. It is based on measuring the increase in absorbance of the redox indicator indigo carmine at 610 nm. First, 2 mL of the indigo carmine that is pre-reduced with H_2 and a Pt catalyst is added to the a spectrometer sample cell and then 0.5 mL of the sample is added with a manual syringe or an automated miniature syringe pump. The method provides detection of O_2 at levels below 1 ppm which cannot be accurately measured with a DO probe and the detection limit is 0.04 ppm. All components in the sample transfer system and sample cell were optimized to minimize O_2 contamination. Several novel innovations were incorporated. Anaerobic cultures were grown in septum bottles and were tested for DO. Microcosms dominated by Fe(III)-reducing, sulfate reducing or methanogenic conditions were prepared by

seeding appropriate media with fresh wastewater sludge. As expected, none of the sulfate reducing and methanogenic cultures had detectable DO concentrations (above 0.04 mg/L). Interference of Fe(III) was observed in Fe(III)-reducing cultures possibly due to excess amount of iron hydroxide colloids.

We have constructed a versatile, integrated, microfluidic analysis platform fabricated using standard photolithography techniques and polydimethylsiloxane (PDMS) replica molding. This platform is designed for laboratory and field analysis of species (e.g., redox-active species such as S(-II) or Fe(II)) based on mixing a sample with a reagent and measuring the absorbance of the colored product formed. Various fluidic components have been developed individually and then integrated on one platform. These components include lateral percolation filters of different post sizes, micromixers that utilize lamination and geometric focusing to reduce diffusion distances, and a 1-cm long micro-flow cell with integrated fiber optics for spectroscopic detection. The small size of the platform (centimeters) and fluid channels of dimensions 50 to 200 μm make quite portable and suitable for long term monitoring because flow rate and sample and reagent consumption are very small (i.e., microliters).

We have successfully developed and evaluated the majority of the fluidic components necessary to in the future make an integrated microfluidic system that can be lowered into wells for chemical analysis. A microfabricated filter has been developed and characterized with the intent of allowing raw sample reagent to be pumped into the microfluidic device without pre-treatment. The filter was also used with a real environmental sample, Willamette River water, to demonstrate the application of pretreatment in environmental monitoring. A microfluidic reactor capable of mixing a sample and a reagent (or reagent mixture) by diffusion has been fabricated and the mixing efficiency has been determined in terms of the length needed for a given continuous flow rate. The fluid channels have dimensions 50 to 200 mm. An optical flow cell for performing UV/Visible spectrometry was developed, coupled to optical fibers implanted into the microfluidic chip, and evaluated a with miniaturized Ocean Optics light source and CCD array detector.

A method for packaging about 1 mL of reagent into a heat-sealed, collapsible reagent bag has been developed. A novel micropump (peristaltic) prototype that also provides valve functionality has been constructed and characterized and provides a relatively constant flow rate in the range of 1 to 80 μL/min. The pump requires low power to operate. Miniature light sources, detectors, and specific electronics components (microprocessors, pump driver circuitry, power management, miniature power supplies, etc.) have been purchased and evaluated

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Ruiz-Haas, P. (2006). Monitoring Redox Conditions with Redox Indicators during Microbial Reductive Dechlorination in Microcosms and Bioaugmented Columns, Ph.D., Oregon State University.

Supplemental Keywords: biotransformation; characterization; VOCs; chlorinated solvents; bioremediation; environmental chemistry

Period Covered by the Report: October 2001 – August 2007
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EPA Agreement Number: R828772
Title: Western Region Hazardous Substance Research Center Project 2-OSU-07. Continuous Flow Column Studies of Reductive Dehalogenation with Two Different Enriched Cultures: Kinetics, Inhibition, and Monitoring of Microbial Activity
Investigators: Lewis Semprini, Mark E. Dolan, and Alfred Spormann
Institution: Oregon State University and Stanford University
Research Category: Groundwater, TCE, PCE, Vinyl Chloride, DNAPL, Bioremediation
Project Period: January 2003 – August 2007

Objectives: This joint project between Oregon State University and Stanford University evaluated the transformation of chlorinated ethenes in continuous-flow column studies with the Evanite (EV), Point Mugu (PM) and Victoria Strain (VS) cultures that have been developed and kinetically characterized in previous WRHSRC studies. The overall objectives of the project were to 1) evaluate if the predicted performance of the enrichment cultures was achieved and to test methods that may distinguish the VS from the EV culture; 2) apply molecular methods, such as FISH and Real-Time PCR, to determine the spatial distribution of the cultures and quantify the dehalogenating biomass within the column; 3) apply RNA-based methods to determine energetically based TCE and VC-dehalogenating activity temporally and spatially within the columns; 4) apply molecular based activity tests, such as transformation of fluorinated analogs, to determine dehalogenating activity that develops within the column; and 5) compare the results from modeling, molecular, and activity based results.

Summary of Findings:

Evanite Strain (EV) Culture

Continuous-flow column experiments were conducted to evaluate the reductive dechlorination of tetrachloroethene (PCE) in Hanford aquifer material after bioaugmentation with the Evanite (EV) culture. Three sets of column studies were performed with the Evanite culture. In Study-1 the culture was added to Hanford aquifer material where the iron content of the solids had not been pre-reduced. In Study-2 the effects of pre-reduction of the aquifer material with Na_2S solution was evaluated and a series of transient tests were performed to determined how the columns responded to gradual increases in PCE and TCE concentrations. In Study-3 the aquifer material was changed to silica sand that had low iron content.

Study-1. In Study-1, prior to culture addition, PCE (0.07 mM) and bromide were added to a column amended with synthetic Hanford groundwater that contained 0.2 mM sulfate and 0.34 mM lactate. Little microbial activity was observed during this period with indigenous microbes from the Hanford aquifer material based on the absence of sulfate reduction and lactate removal. After six weeks, the column was inoculated with the EV enrichment culture. The injected lactate concentrations were increased from 0.34 mM to 0.67 mM, and to 1.34 mM, after six, eight, and sixteen weeks, respectively. PCE dechlorination to TCE and cis-DCE was observed when lactate concentrations increased from 0.35 mM to 0.67 mM on week eight. Rapid increases in cis-DCE concentration in the effluent to levels higher than the influent PCE concentration indicated enhanced PCE desorption and subsequent reduction to cis-DCE. When the lactate concentration was increased to 1.34 mM, propionate production was observed from lactate fermentation, and cis-DCE reduction to vinyl chloride (VC) and ethene occurred. The results indicated that

competing electron acceptors, such as ferric iron, may have been responsible for the stall in cis-DCE transformation, but with prolonged treatment transformation to ethene occurred. At the end of 170 days, the column was destructively tested in an anaerobic glove box. Microcosms were constructed with spatial samples from the column and rates of PCE and VC transformation were determined. Spatial samples of the aquifer material were shipped to Stanford for molecular-based analysis.

Microcosm results indicated that most of the PCE and TCE transformation occurred near the column inlet. Microcosms supplied with PCE had the highest TCE and c-DCE formation rates in the sample taken closest to the column inlet and exhibited an essentially exponential decrease of an order of magnitude along the column length. VC transformation rates obtained from microcosms fed VC showed essentially uniform ETH production rates along the column length. The uniform spatial distribution of VC transformation activity compared to PCE transformation activity may have been due in part to the time chosen for column solids analysis. At 170 days, VC transformation to ETH was just being completely developed in the column and VC was present throughout the column, whereas rapid PCE transformation most likely depleted PCE concentrations within the first few centimeters from the inlet. Results of the molecular-based analysis of parallel column samples are provided below.

Study-2. In column Study-2, the effects of the pre-reduction of iron in the aquifer material was evaluated along with a series of transient tests where PCE (0.09-0.27 mM) and TCE (0.38-1.52 mM) concentrations were gradually increased. The aquifer material was chemically pre-reduced with a 5 mM Na_2S solution to eliminate easily excessable Fe(III) in the aquifer material. With pre-reduced aquifer material, PCE was rapidly dechlorinated to cis-DCE, VC and ETH, with essentially no stall at the cis-DCE stage of transformation. Redox capacity measurements showed highly reducing conditions were more rapidly achieved in the column and sulfate reduction was immediately observed after lactate addition was initiated. Also, complete reduction to ETH was observed at lower lactate injection concentrations. Immediately upon switching to TCE addition (0.38 mM), 94% transformation to ETH was observed. TCE was essentially completely transformed to ETH at a concentration of 1.52 mM at a lactate concentration of 1.42 mM. Electron mass balances showed that in Study-1, where aquifer solids were not pre-reduced only 4.4 % of the electron flow was associated with dechlorination reactions, while in the pre-reduced column about 22% of the electron flow was associated with dechlorination reactions. The results indicate that when aquifer solids were not pre-reduced, the dehalogenating microorganisms were likely being outcompeted by iron reducing microorganisms for the available hydrogen required as an electron donor to drive c-DCE transformation to VC and ethene.

Study- 3. In column study 3, the EV culture was added to quartz sand (99.5% SiO_2) with low iron content (40 mg/kg). PCE (0.09 mM) and bromide were added to the columns amended with synthetic Hanford groundwater that contained 0.2 mM sulfate. No retardation of PCE transport through the column was observed. After 13 days, the columns were inoculated with the EV enrichment culture and 0.67 mM lactate. Immediately after EV culture addition, PCE dechlorination to TCE, and cis-DCE was observed. Effluent c-DCE concentrations did not exceed the influent PCE concentrations indicating no PCE was sorbed to the sand. Sulfate reduction occurred after increasing the lactate concentration to 1.0 mM and sulfide concentration increased to about 160 uM in the column. Complete PCE transformation to 89% ETH and 11% VC was achieved after 100 days of column operation. High sulfide concentration in the aqueous

phase may have inhibited VC transformation to ETH. After 267 days, lactate was replaced with 2.14 mM formate as an electron donor. No major improvement was observed in the extent of PCE dechlorination to VC and ETH, but a higher percentage of total electrons were used for dehalogenation reactions than with lactate as the electron donor.

Mass and electron balance calculations were performed for the CAHs, ETH, and organic acids exiting the different columns. Approximately 60% to 70% of the electron donor addition could be accounted with 4.4% being used in dehalogenation reactions in the column study with high iron-content aquifer material. Approximately 95% of the electron donor addition could be accounted for with 7.8% associated with dehalogenation reactions in the lactate-fed low iron-content aquifer material. With formate addition in the same column, 65% of the electron donor could be accounted for but 15.6% of the donor was associated with dehalogenation reactions. The results indicate where the aquifer solids with high iron were used, hydrogen produced from lactate fermentation was partly consumed by ferric iron reducing bacteria. Formate was a more efficient electron donor for dechlorination than lactate, possibly due to supporting less iron reduction or less growth of iron reducing populations.

Victoria Strain (VS) Culture

Study-4. In column Study-4 the reductive dechlorination of TCE (0.17-0.37 mM) with Hanford aquifer material bioaugmented with the Victoria (VS) culture, was evaluated. The VS culture can dehalogenate TCE to ethene and obtains energy from all steps of the transformation process. The aquifer solids were chemically pre-reduced with a 5 mM Na_2S solution. Shortly after VS culture addition, cis-DCE concentrations in the column effluent exceeded the influent TCE concentration indicating enhanced TCE desorption and transformation. The lactate concentration was increased from 0.67 to 1.24 mM to compensate for hydrogen consumption and sulfate reduction and increases in the influent TCE concentration. About 98% of TCE was transformed to ETH at concentrations of 0.17 and 0.37 mM within a hydraulic residence time of 3.6 days. After 152 days of operation the column was destructively sampled and spatial samples were sent to Stanford for molecular analysis.

Point Mugu (PM) Culture

Study-5. Column Study-5 was conducted to evaluate the anaerobic transformation of trichloroethene (TCE) and trichlorofluoroethene (TCFE) in a continuous flow column packed with aquifer material and bioaugmented with the Point Mugu (PM) mixed culture that transforms TCE to ethene. The effect of adding different concentrations of lactate as an electron donor and changing electron donor addition to formate was also evaluated. TCE (0.17 mM) was initially fed along with lactate as an electron donor. Upon addition of the PM culture, sulfate reduction and lactate fermentation occurred and, after 130 days of flow through the column, TCE was converted to VC (50%) and ethene (50%) within a hydraulic residence time of 1.5 days. Upon adding TCFE (0.038 mM), ethene and VC concentrations decreased and 1,2-*cis*-dichloroethene (*c*-DCE) was produced. This coincided with TCFE being transformed to dichlorofluoroethene (DCFE), chlorofluoroethene (CFE) and fluoroethene (FE). With prolonged addition, TCE and TCFE were transformed to VC (80%) and ethene (20%) and CFE (80%) and FE (20%), respectively. Significant inhibition of TCE transformation resulted from TCFE addition, likely due to competitive inhibition kinetics among the chlorinated and chloro-fluorinated compounds. However, consistent with previous studies, DCFE, CFE and FE production correlated with *c*-DCE, VC and ethene production, respectively, showing that fluorinated analogs successfully tracked the transient transformation conditions. In this column experiment, the effect of electron

donors as lactate and formate concentrations were tested during the course of the experiment to address limitations on TCE dechlorination. About 96% of the TCE was transformed to ETH at a lactate concentration of 1.0 mM, but at a reduced lactate concentration of 0.67 mM, partial transformation of TCE to cis-DCE and VC was observed. Addition of formate lessened iron reduction and resulted in more effective electron flow into dehalogenation reactions.

Molecular Studies: Spatial Distribution of the genus *Dehalococcoides* and species subpopulations

Study-1. The microbial community composition in column study 1 with the EV culture was analyzed with special focus on the genus *Dehalococcoides*. Aquifer solids from the column were sampled for molecular analysis after 170 days of column operation. The column was split in six 5 cm sections.. DNA extracted from each section served as template in real-time PCR assays to quantify *Dehalococcoides* organisms along the column profile. The relative abundance of *Dehalococcoides* species as a percentage of total Eubacteria increased from 0.5% in the first 5 cm to about 4% towards the column outflow.

The population composition of the genus *Dehalococcoides* was analyzed with the help of functional gene primers specific to certain *Dehalococcoides* strains, e.g. strain VS, and strain BVA-1. Targeted were key genes of reductive dehalogenation that have been genetically and biochemically characterized to catalyze the complete dechlorination of trichloroethene (TCE) and/or vinyl chloride (VC) to ethene. Primer sets were designed to target the vinyl chloride reductase of *Dehalococcoides* sp. strain VS (*vcrA*_VS), the vinyl chloride reductase of *Dehalococcoides* sp. strain BVA-1 (*vcrA*_BVA-1), and the trichloroethene reductase of *Dehalococcoides ethenogenes* strain 195, *Dehalococcoides* sp. strain FL2, and Bacterium PM-VC1, RC-VC2, and YK-TCE1 (*tceA*_195+).
The three *Dehalococcoides* subpopulations differ dramatically in abundance along the column profile. Whereas strain BVA-1 makes up to two third of all *Dehalococcoides* cells in the last 10 cm closest to the column outflow, *tceA* containing relatives of *Dehalococcoides ethenogenes* strain 195 decrease in abundance from 6% to 0.2% and 1% towards the column end. The *vcrA* gene of *Dehalococcoides* strain VS showed a more equal distribution over the column profile decreasing from 20% to about 10% with a low of 4% around 25 cm from the column inflow.

Monitoring of gene expression associated with reductive dehalogenation

Study-1. The primers for the TCE and VC reductive dehalogenases were further used in real-time reverse transcription (RT-) PCR experiments to study gene expression along the vertical horizon of the column. RNA was extracted from each 5 cm section. The RNA was reverse transcribed into cDNA which served as template in real-time PCR assays to estimate the relative expression of the described reductive dehalogenases. The expression data for each gene was normalized to the gene abundance determined in the DNA quantification experiments as described in the previous paragraph. Despite their low gene abundance the trichloroethene reductase (*tceA*) showed the highest expression of all three dehalogenases under investigation. The relative expression of *tceA* from *Dehalococcoides ethenogenes* strain 195 and others peaked 10 to 15 cm from the column inflow. The highest PCE reduction rates were measured in sediments obtained within 5 cm of the column inflow, so it is consistent to observe the highest *tceA* expression further into the column where most of the PCE has already been reduced. Vinyl chloride reduction as measured by *vcrA* expression of strain VS and BVA-1 showed a more uniform activity pattern over the column profile with slightly elevated expression of the *vcrA* of strain BVA-1 towards the reactor outflow.

Our results show that real-time (RT-) PCR can be used to quantify abundance and activity of genes involved in reductive dehalogenation of tetrachloroethene under bioremediation conditions. The protocol and oligonucleotide primers developed in this study assemble a powerful tool for the in situ monitoring and evaluation of laboratory scale bioreactors and field sites undergoing bioremediation.

Microbial diversity of PCE/TCE dechlorinating continuous flow columns

Study-2 and Study-4. The microbial community composition of two different dechlorinating column studies was analyzed by constructing 16S rRNA gene clone libraries. As described above, the columns were bioaugmented with either the TCE reducing *Dehalococcoides* sp. strain VS or the PCE reducing Evanite enrichment culture that contains at least two other *Dehalococcoides* strains in addition to strain VS. Both flow systems were operated with lactate as electron donor. Details of the operation of the columns are described in the previous section, column study 2 and column study 4. The clone libraries were constructed from 3 different horizontal sections of each of the two flow columns. Samples were taken from a location close to the column inflow port (0-2 cm), a middle section (8-10 cm) and a section near the column outflow (25-30 cm). Column solids from each section were used for DNA extraction. DNA from each column section served as template in PCR reactions with general bacterial 16S rRNA gene primers. The resulting PCR products were cloned and sequenced. No PCR products were produced using general archaeal primers indicating that *Archaea* were not detectable by these methods.

For the PCE-column (EV culture) a total of 200 full length 16S rRNA gene sequences comprising 29 operational taxonomic units (OTUs) were obtained, where an OTU represents a collection of sequences not more than 3% different from each other. Based on the rarefaction curve for this clone library, the probability of observing a novel OTU through additional sequencing was about 14.5%. From the column operated with TCE (VS culture) a total of 281 full length 16S rRNA gene sequences were obtained. The 16S rRNA gene sequences from the TCE-column comprise 32 OTUs with an 11.4% probability of an additional clone sequence falling into a not yet targeted OTU. Based on a chao1-estimate the total microbial community richness is 41-58 OTUs for the PCE column and 42-50 OTUs for the TCE column. For the PCE reducing column 106 clone sequences were obtained for the 2 cm section and 47 clone sequences were obtained from each of the two other column locations. In the TCE dechlorinating flow column 92 clones were retrieved from the inflow section, 94 from the middle and 95 from the outflow segment.

Both column libraries revealed a similar overall abundance of *Acetobacterium* and *Clostridium novyi* relatives, although their distribution among the different sections of each column varied. Both OTUs have their highest sequence representation in the inflow sections of the columns. OTUs found in the PCE and TCE dechlorinating column libraries with different abundance were *Sedimentibacter*, uncultured *Thermomicrobia*, *Desulfitobacterium*, *Dehalococcoides* and *Clostridium sphenoides* relatives. Small subunit rRNA sequences of *Azospira-Dechlorosoma* relatives were only found in the PCE dechlorinating column library, whereas relatives of *Geobacter gribiciae*, uncultured *Bacteroidetes*, and *Desulfovibrio alcoholovorans* could only be found in the TCE dechlorinating soil column. The middle and outflow section of the PCE dechlorinating column were dominated by uncultured *Thermomicrobia* and *Dehalococcoides* relatives. Sequences of both OTUs account for more than 50% of all clones obtained from these

two sections. The middle and outflow section of the TCE dechlorinating column is dominated by *Sedimentibacter* and *Geobacter gribiciae* relatives, making up two thirds of all clone sequences retrieved from these two column segments.

Based on the column experiments and the microbial community analysis, we developed a working hypothesis of the flow column ecosystem as a whole and the interspecies interactions we think are essential to microbial reductive dehalogenation. In our model, the primary electron donor lactate is fermented to propionate, acetate, CO_2, and hydrogen by a diverse population of fermenting bacteria, including *Clostridia*. Homoacetogenic bacteria, like *Acetobacterium* relatives, use CO_2 and hydrogen to produce acetate. Acetate and hydrogen can serve as electron donors for the groups of dehalogenating microorganism, which compete with the homoacetogenic bacteria for hydrogen. While reductively dechlorinating, microorganisms like *Desulfuromonas* species require acetate as electron donor whereas *Dehalococcoides* spp. are solely dependent on the use of hydrogen as electron donor for halorespiration. In addition to hydrogen supply by the community, survival and sustained dechlorination activity of *Dehalococcoides* spp. is also dependent on the supply of several limiting cofactors.

B. Unusual codon usage in vinyl chloride reductase genes of *Dehalococcoides* species
The enzymes responsible for catabolic reduction of vinyl chloride, vinyl chloride reductases (VC-RDase), are the key enzymes for complete microbial reductive dehalogenation of chloroethenes, including the groundwater pollutants tetrachloroethene and trichloroethene. Analysis of codon usage of VC-RDase genes showed that these genes are highly unusual, characterized by a low fraction of G+C at the third position (GC3). The third position of codons in VC-RDase genes is biased toward the nucleotide T, even though available *Dehalococcoides* genome sequences lack tRNAs that match to codons with T at the third position. The codon usage of VC-RDase genes is clearly distinct from genes shown to be highly expressed in recent proteomic analysis for *Dehalococcoides* strain 195. The comparatively high level of abnormality in codon usage of VC-RDase genes suggests a recent evolutionary history that is different from most *Dehalococcoides* genes, including those encoding other reductive dehalogenases. One explanation is that VC-RDase genes may have been recently acquired from a heretofore unknown microorganism.

Publications:

Journal Articles

Azizian, M., S. Behrens, A. Sabalowsky, M. Dolan, A. Spormann, L. Semprini. Continuous-Flow Column Study of Reductive Dehalogenation of PCE upon Bioaugmenation with the Evanite Enrichment Culture. (in review 2007).

McMurdie, P. J., S. F. Behrens, S. Holmes, and A. M. Spormann (2007). Unusual Codon Bias in Vinyl Chloride Reductase Genes of Dehalococcoides Species. *Appl. Environ. Microbiol.* 73:2744-2747.

Conference Abstracts and Presentations

Azizian, Mohammad F., Mark E. Dolan, Peter Ruiz-Haas, James D. Ingle, and Lewis Semprini. (2007). Effect of pre-reduction of aquifer material on PCE reductive dechlorination in a continuous-flow column study. *American Chemical Society, Division of Environmental Chemistry,* Vol. 47 No.1, 560-565.

Azizian, M., S. Behrens, A. Sabalowsky, M. Dolan, A. Spormann, L. Semprini (2006). Continuous-Flow Column Studies of Reductive Dehalogenation of CAHs with Evanite Enriched Culture: Kinetics, Inhibition, and Monitoring of Microbial Activity (abstract and poster), *Subsurface Biosphere Initiative (SBI) workshop/IGERT Retreat,* Newport, Oregon (June 18-21).

Behrens, S., J. McMurdie, G. Meshulam, A. Spormann (2005). Evaluation of a CARD-FISH Protocol for the Quantification of *Dehalococcoides* sp. in Soil. *2005 ASM General Meeting* (June 5-9).

Behrens, S., Azizian, M., McMurdie, J., Sabalowsky, A. Dolan, M., Semprini, L., Spormann, A. M. (2006). Monitoring Gene Abundance and Expression of Reductive Dehalogenases Involved in Complete Dechlorination of PCE Under Continuous Flow Conditions. Poster presentation at the *11th International Symposium on Microbial Ecology,* Vienna, Austria (August).

Sabalowsky, A.R. and L. Semprini (2005). Alkynes as Reversible Inhibitors for Probing Mechanisms of Reductive Dehalogenation of Chloroethenes. *Joint International Symposia for Subsurface Microbiology (ISSM 2005) and Environmental Biogeochemistry (ISEB XVII)*, Jackson Hole, Wyoming (August 14-19).

Semprini, L., M. Azizian, A. Sabalowsky, M. Dolan, P. Ruiz-Hass, J. Ingle, S. Behrens, A. Spormann (2005). A Continuous Flow Column Study of Anaerobic PCE Transformation with the Evanite Culture and Hanford Aquifer Solids. *Joint International Symposia for Subsurface Microbiology (ISSM 2005) and Environmental Biogeochemistry (ISEB XVII)*, Wyoming (August 14-19).

Semprini, L. Mark E. Dolan and M. F. Azizian (2007). Anaerobic transformation of trichloroethene and trichlorofluoroethene in a continuous flow column study. *American Chemical Society, Division of Environmental Chemistry, ACS national meeting,* Boston, MA (August 19-23).

Semprini, L. and M. Azizian. Continuous-Flow Column Studies to Evaluate the Effects of Different PCE and TCE Concentrations and Lactate Addition on Reductive Dehalogenation (2007). (abstract and poster), *Partners in Environmental Technology Technical Symposium and Workshop,* Washington D.C. (December 4-6).

Supplemental Keywords: biotransformation; groundwater; NAPL; VOCs; bioremediation; chlorinated solvent; remediation technologies; in-situ; characterization

Period Covered by the Report: October 2001 – August 2007
Date of Final Report: December 2007
EPA Agreement Number: R828772
Title: Western Region Hazardous Substance Research Center Project 2-SU-04
Novel Methods for Laboratory Measurement of Transverse Dispersion in Porous Media
Investigators: Peter K. Kitanidis and Craig Criddle, Stanford University
Institution: Oregon State University
Research Category: Groundwater, transport
Project Period: 2004-2007

Goal: (1) Develop, refine, and critically evaluate novel methods for the laboratory measurement of transverse dispersion in homogeneous isotropic unconsolidated porous media; (2) develop experimental protocols and methods of data analysis; (3) independently verify the accuracy of the new methods; (4) perform extensive experiments to determine relations of transverse dispersivity with conductivity, longitudinal dispersivity, mean grain size, degree of non-uniformity, etc.

Rationale: Transverse dispersion in porous media measures the rate of spreading of a solute in the direction perpendicular to flow. Pore-scale transverse dispersion is widely accepted as playing a dominant role in determining the actual rate of dilution of solutes and mixing of reactants in porous media. For example, consider a long plume of contaminants emanating from a constant source. The rate of intrinsic remediation is determined by the rate of transverse mixing of contaminants in the plume with reactants from the surrounding groundwater. The rate may be primarily determined by the value of the transverse dispersion coefficient. Better understanding of transverse dispersion would ultimately improve our understanding of diffusion-limited processes, such as intrinsic remediation. Despite its importance, transverse dispersion remains insufficiently understood.

Approach: Part of the difficulty has been the lack of accurate and efficient methods for laboratory measurements. In most existing methods for the determination of transverse dispersion, the measured quantity is proportional to the dispersion coefficient, and thus small and swamped by experimental error. However, we developed new methods for the measurement of local transverse dispersion in isotropic porous media based on a helical and a cochlea-like device. The idea was to perform an experiment similar to the tracer test through a laboratory column packed with a porous medium and to measure the breakthrough curve; however, the objective was not to determine the column-scale longitudinal dispersion but the transverse dispersion. The principle was to induce shear flow inside the device that creates strong longitudinal dispersion in the observed breakthrough curve; transverse mixing tended to negate the effects of shear flow and thus reduced the observed column-scale longitudinal dispersion. Then, from the spreading of the observed breakthrough curve, we could estimate the unknown, the pore-scale transverse dispersion. The measured quantity varies inversely with transverse dispersion coefficient.

Summary of Findings: The project was completed and a PhD dissertation has been submitted that has served as final report. Highlights of the dissertation: We discuss instrumentation and tracers that we used to obtain experimental concentration breakthrough curves. We describe the numerical simulation and parameter estimation methods used to analyze the experimental data.

We discuss the results and describe the relative advantages of each device, instrument, and methodology that we have used to estimate transverse dispersivity. Perhaps the most noteworthy conclusions of this research are that the results from the two devices, helix and cochlea, are in agreement and that the ratio of transverse dispersivity to longitudinal dispersivity that we estimate agrees with the higher ratios reported in the literature.

Publications:

Journal Articles

Benekos, I. D., O. A. Cirpka, and P. K. Kitanidis (2006). Experimental determination of transverse dispersivity in a helix and a cochlea. *Water Resources Research*, *42*, W07406, 10.1029/2005WR004712.

Conference Proceedings and Presentations

Benekos, I., P.K. Kitanidis, M.A. Rahman, and O.A. Cirpka (2001). Experimental and Mathematical Studies of Pore-Scale Transverse Dispersion in a Helical Soil Column. *AGU Fall Meeting,* December 10-14, San Francisco, CA.

Benekos, I., and P.K. Kitanidis (2004). Experimental Determination of Transverse Dispersivity in a Cochlear Device. *Western Pacific Geophysics Meeting,* August 15-21, Honolulu, HI.

Benekos, I., and P.K. Kitanidis (2005). An Optimization Approach Using Tracer Concentration Breakthrough Curves for Determining the Transverse Dispersivity in a Cochlear Device. *EPA-HSRC Workshop on Risk Assessment and Monitoring Research,* November 4-5, Las Vegas, NV.

Benekos, I., and P. Kitanidis (2005). On the Determination of Transverse Dispersivity: Experiments and Simulations in a Helix and a Cochlea. *AGU Fall Annual Meeting*.

Theses

Benekos, Ioannis D. (2005). On the Determination of Transverse Dispersivity: Experiments and simulations in a helix and a cochlea. Ph.D, Stanford University.

Supplemental Keywords: **characterization; groundwater; chemical wastes**

Period Covered by the Report: October 2001 – August 2007
Date of Final Report: December 2007
EPA Agreement Number: R828772
Title: Western Region Hazardous Substance Research Center Project 2-SU-05
Sorption and Hydrolysis of Halogenated Hydrocarbons in Soil Nanopores
Investigator: Martin Reinhard
Institution: Stanford University
Research Category: Groundwater, transport
Project Period: January 2003- August 2007

Objectives: The overall goal of this project was to develop a better understanding of the impact of soil nanopores on the fate and transport of halogenated hydrocarbon contaminants. Specific project objectives were to: (1) study the kinetics of slow sorption and desorption of halogenated hydrocarbons in aquifer sediment, and (2) determine effect of sorption on contaminant reactivity. Results allow us to better predict natural attenuation of hydrocarbon compounds in aquifers and assess the risks associated with groundwater aquifers contaminated by halogenated hydrocarbons.

Rationale: Geological solids contain nanopores because of material imperfections or weathering, cracking, or turbostratic stacking. Previous work has demonstrated that sorption of hydrophobic organic compounds in nanopores can be a significant sequestering process. Sorption in nanopores is reversible but rates are very slow (weeks to months) and difficult to quantify, especially in the field. Our understanding of geosorbent nanoporosity and how it affects the sorption and chemical transformations of organic contaminant is very limited. The fundamental hypothesis is that water is unable to compete for sorption sites in hydrophobic nanopores and unable to displace sorbed hydrophobic contaminants. We hypothesize that inside such nanopores, halogenated hydrocarbon compounds are prevented from reacting with water and that this phenomena leads to long residence times of reactive contaminants in soils and aquifers.

Summary of Findings: A novel analytical system was developed that allowed us to simultaneously study sorption and transformation of volatile organics in geological sorbents. The system consists of the previously developed soil column chromatograph (Project 1-SU-03), which is directly coupled to a chromatograph for the analysis of the sorbate and transformation products. The procedure involves first loading contaminant onto the soil column by passing a stream of contaminant vapor through the column until breakthrough using helium (1.00 mL/min) as the carrier gas. The column is then disconnected, sealed, equilibrated, and incubated for weeks to months at predetermined temperatures. Following equilibration, the columns are purged with a helium stream (1.00 mL/min) that is fed directly to the on-line gas chromatograph (GC), which quantifies the concentrations of the sorbate and the transformation products. Desorption and transformation concentration-time profiles are obtained as a function of temperature, humidity, and competitive cosorbates or cosolvents. The procedure has been calibrated using sorbents with known porosity (silica gel), zeolites with surface properties ranging from polar to hydrophobic, and sorbates with known hydrolysis rates—trichloroethylene (TCE) which is practically unreactive, and 2,2-dichloropropene (2,2-DCP) which reacts with water to 2-chloropropane.

Initial studies focused on the non-reactive (TCE) and the one reactive model substrates (2,2-DCP) as the sorbates, (synthetic) silica, zeolites, and the clay and silt fraction (< 50 μm) of soil

from a site at the Lawrence Livermore National Laboratory (LLNL), as the sorbent. 2,2-DCP sorption data obtained at different soil moisture contents confirmed that the sorption capacity decreases significantly as the moisture content increases. Data indicate that water displaces 2,2-DCP from sorption sites in micropores as the moisture content increases. However, water did not completely eliminate the sorption capacity for 2,2-DCP, and a small but significant amount of 2,2-DCP (~0.1 mg/g dry soil) could still be sorbed when the soil was wet. Most of this fraction was desorbing very slowly, which is consistent with sorption in hydrophobic nanopores. More recent sorption data obtained using zeolites and TCE shows that hydrophobic compounds displace water from hydrophobic micropores.

Method development and system evaluation using a model silica gel and real sediment from a previously studied aquifer has been completed and reported (submitted). It was confirmed that hydrophobic micropores play a significant role in controlling the long-term release of hydrophobic organic contaminants. This is a significant factor affecting the times it takes to remediate sites. We developed a technique for quantifying the total and the hydrophobic micropore volumes based on the mass of TCE sorbed in the slow-releasing pores under dry and wet conditions. The micropore environment in which organic molecules were sorbed in the presence of water was probed by studying the transformation of a water-reactive compound (2,2-DCP). For sediment from an alluvial aquifer, the total micropore volume was estimated to be between 1.56 and 3.75 μL/g, while its hydrophobic micropore volume was only 0.022 μL/g. In a microporous silica gel, a hydrophobic micropore volume of 0.038 μL/g was measured.

Dehydrohalogenation rate of 2,2-DCP sorbed in hydrophobic micropores was slower than that reported in bulk water, which is indicative of an environment of low water activity. The results suggest that hydrolyzable organic contaminants sorbed in hydrophobic micropores may be preserved for many times longer than their half-lives in water, consistent with the reported persistence of reactive contaminants in natural soils. Although the hydrophobic micropores represent a small fraction of the total micropore volume, the significant amounts of hydrophobic contaminants stored in them may pose long-term risk to groundwater quality.

More recent work focused on sorption of TCE in zeolites with a range of hydrophobic surface properties. We have elucidated the mechanism of hydrophobic organic compound sorption in mineral micropores by studying the water sorption and thermal dehydration behaviors of three dealuminated Y zeolites, and sorption of TCE in partially dehydrated zeolites and wet zeolites (equilibrated with saturated water vapor). Zeolites of higher Si/Al ratios exhibited lower affinity for water sorption and lost water more easily during dehydration. It was also observed that the high silica zeolites, both partially dehydrated and wet, could sorb more TCE than the low Si/Al zeolite under the same conditions. Experimental results suggest that the density of hydrophilic centers (surface cations and hydrogen bonding sites) on the pore wall surface of micropores plays a key role in water sorption and determines their hydrophobicity. The enhanced dispersion interactions of TCE molecules are only strong enough to displace the loosely bound water molecules from the hydrophobic micropores, while water molecules coordinated to surface cations and the hydrogen bonded water molecules are unaffected. The results indicate that sorption of hydrophobic organic molecules in hydrophobic micropores occurs through displacing the weakly sorbed water molecules in them and organic molecules co-exist with the strongly sorbed water molecules in them.

In summary, our experimental data showed that reactive, i.e., hydrolysable contaminants sorbed in slow desorbing sites of geological solids reacted significantly slower than in bulk solution, suggesting that the contaminants reside in an environment that is to some extent excluded from water. Conversely, steric and energetic factors hindered exchange between the sorption sites and bulk solution, thus preventing hydrolysis. As a result, the halogenated hydrocarbon molecules in hydrophobic nanopores were less exposed to water molecules and were prevented from hydrolysis.

Publications:

Journal Articles

Cheng, H. and M. Reinhard (2006). Quantifying the Volume of Hydrophobic Micropores from Trichloroethylene Desorption. *Environmental Science and Technology,* 40 (11), 3595-3602.

Cheng, H. and M. Reinhard (2006). Sorption of Trichloroethylene in Hydrophobic Micropores of Dealuminated Y Zeolites and Natural Minerals, *Environmental Science and Technology,* 40 (24), 7694-7701.

Cheng, H. and M. Reinhard (2007). Sorption and Inhibited Dehydrohalogenation of 2,2-Dichloropropane in Micropores of Dealuminated Y Zeolites. *Environmental Science and Technology,* 41 (6), 1934-1941.

Cunningham, J.A., J.J. Deitsch, J.A. Smith and M. Reinhard (2005). Quantification of Contaminant Sorption-Desorption Time-Scales from Batch Experiments. *Environmental Toxicology and Chemistry*, 24 (9), 2160-2166.

Abstracts and Posters

Cheng, H. and M. Reinhard (2005). Inhibition of 2,2-dichloropropane dehydrohalogenation by micropore sorption. *The 230th ACS National Meeting,* Washington, DC (Aug 28-Sept 1).

Theses

Cheng, H. (2006). Sorption and Hydrolysis of Chlorinated Aliphatic Hydrocarbons in Hydrophobic Micropores, Ph.D., Stanford University.

Supplemental Keywords: groundwater; soil; characterization; chlorinated solvent; VOCs; environmental chemistry

Training and Technology Transfer

WRHSRC training focuses on educating graduate students. As shown in the table below, a total of 19 students have been funded through the Center: three at the master's level and 13 at the Ph.D. level. Through Center funding, students are trained to do fundamental research and outreach activities in a broad range of disciplines

Table 7. Graduate Students Funded through the WRHSRC

Student	Field	Degree/ Institution/Graduation	Project
Sebastian Behrens	Environmental Engineering	Post-Doctoral/Stanford	2-OSU-07
Ioannis Benekos	Environmental Engineering	Ph.D./Stanford/2005	2-SU-04
Christina Blatchford	Environmental Engineering	M.S./Oregon State University/2005	1-OSU-02
Defne Cakin	Chemistry	Ph.D./ Oregon State University /2008	2-OSU-06
Kevin Cantrell	Chemistry	Ph.D./Oregon State University /2002*	1-OSU-04
Hefa Cheng	Environmental Engineering	Ph.D./Stanford/2006	2-SU-05
David Doughty	Microbiology	M.S./2004 Ph.D./2008/ Oregon State University	2-OSU-05
Michael Norman Fienen	Environmental Engineering	Ph.D./ Stanford University /2006	1-SU-01
Kimberly Halsey	Molecular and Cellular Biology	Ph.D./ Oregon State University /2006	2-OSU-05
Corey Koch	Chemistry	Ph.D./ Oregon State University/2008	2-OSU-06
Jae-Hyuk Lee	Environmental Engineering	Ph.D./ Oregon State University /2006	1-OSU-03
Jun Li	Environmental Engineering	M.S./Oregon State University/2004	
Jian Luo	Environmental Engineering	Ph.D./ Stanford University /2006	1-SU-01
Bhargavi Maremanda	Environmental Engineering	M.S./Oregon State University /2004	1-OSU-02
Naoko Munakata	Environmental Engineering	Ph.D./ Stanford University /2005	1-SU-02
George Pon	Environmental Engineering	Ph.D./Oregon State University/2004	

Sanchai Prayoonpokarach	Chemistry	Ph.D./ Oregon State University /2003	1-OSU-04
Cecillia Razzetti	Environmental Engineering	Ph.D./ University of Bologna, Italy/2005	2-OSU-05
Peter Ruiz-Haas	Chemistry	Ph.D./ Oregon State University /2006	2-OSU-06
Andy Sabalowosky	Environmental Engineering	Ph.D./ Oregon State University /2008	2-OSU-07
C. Sattayatewa	Environmental Engineering	M.S. Project/Oregon State University/2004	
Kristin Skinner	Molecular and Cellular Biology	Ph.D./ Oregon State University /2007	1-OSU-02
Watanee Sriwatanapongse	Environmental Engineering	Ph.D./ Stanford University /2005	1-SU-02
Anne Taylor	Environmental Engineering	Ph.D./ Oregon State University /2008	1-OSU-05
Seungho Yu	Environmental Engineering	Ph.D./ Oregon State University /2004	2-OSU-07

* This student received funding from the previous Center (Stanford) as well as the OSU WRHSRC.

Technology Transfer

The goals of the technology transfer program were the following:

- ◊ To promote teamwork and information exchange among researchers.
  - Tools: listservs, web pages, seminars
- ◊ To promote information transfer with practitioners.
  - Tools: web pages, electronic newsletter, workshops, faculty presentations and publications
- ◊ To test new technologies.
  - Tools: laboratory and pilot-scale testing, demonstrations, online project database
- ◊ To implement full-scale demonstration projects.

Rational: In order for research advances to be effective, information must be effectively transferred among researchers and between researchers and practitioners.

Status: Tech transfer activities included maintenance of the WRHSRC website, writing and distribution of Research Briefs and WRHSRC News by e-mail, workshop presentations, and continuation of several technology demonstration projects.

Web Site

The web site http://wrhsrc.oregonstate.edu/ provided an overview of the WRHSRC and links to publications and project information. The website included:

- ◊ A description of the HSRC program and WRHSRC goals and management.
- ◊ Links and contact information for center research and outreach staff.
- ◊ Descriptions of research focus areas and projects.

- ◊ A database of WRHSRC publications and previous projects. This database has been made available in a searchable format (http://wrhsrc.oregonstate.edu/publications/index.htm)
- ◊ Descriptions of center outreach programs and links to the separate websites for the Western Region TOSC/TAB programs.
- ◊ A News and Events page with regular postings.
- ◊ An opportunity to sign up to receive electronic newsletters from the WRHSRC and the TOSC/TAB programs.
- ◊ Research Briefs – short summaries of Center projects that emphasize research applications and demonstration projects. Research Briefs are advertised through the Center electronic newsletter and announced on other electronic mailing lists read by the groundwater remediation community such as: Tech Direct, USEPA Region 10 Science Forum (intranet site), USEPA Region 9 Hazardous Substance Technical Liaison Newsletter. Over the life of the Center, ten Research Briefs #10 were written. These Research Briefs at located at the following Web Address: (http://wrhsrc.oregonstate.edu/briefs/index.htm)

The web site received an average of 1400 visits per month. Common search terms that lead visitors to the site include: reductive dechlorination, cometabolism, anaerobic processes, palladium catalysts, and individual researchers' names. The Research Briefs are among the most commonly viewed pages. Most web site visitors are from US domains with visitors from network (.net), commercial (.com), and educational (.edu) domains dominating. Site visitors also come from many international locations–in a typical month the website will be viewed by visitors in more than 30 different countries.

Field Scale Demonstration Projects

Several field scale demonstration projects were conducted that were based on basic and applied research preformed by the WRHSRC. These projects were funded by the Environmental Security Technology Certification Program (ESTCP) and the Strategic Environmental Research and Development Program (SERDP) of the Department of Defense. Several of the final project reports and protocol documents that resulted from these field demonstration projects are provided below, as well as Journal Articles. Two of the projects, and ESTCP and SERDP were associated with demonstrations and the development of protocols to assess aerobic cometabolism. Another project ESTCP was centered around using radon-222 as in-situ tracer for monitoring the remediation of NAPL contamination. A third project ESTCP was evaluating Pd-catalyst for the in-situ treatment of TCE using horizontial wells. Support from Chevron-Texaco and Textron Corporation supported the field push-pull tests using trichlorofluoroethene as a reactive tracer for evaluating TCE remediation.

Publications:

Reports

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Journal Articles

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Western Regional Lead Training Center, OSU
Hazardous Waste Training

Peter O. Nelson, Ann Kimerling, and Kenneth Williamson, Oregon State University

The Western Regional Lead Training Center at Oregon State University (WRLTC-OSU), originally established with U.S. EPA grant funding in 1993, is an accredited non-profit training provider of lead-based paint (LBP) abatement training and inspection courses. All WRLTC-OSU certification courses are accredited by USEPA, the State of Oregon DHS Lead Program, and the State of Washington CTED Lead Program. Additional WRLTC-OSU lead abatement training courses are provided with US Department of Housing and Urban Development (HUD) and US Department of Energy (DOE) curriculum.

Status: WRLTC-OSU offered 20 to 30 certification courses each year which were attended by 200 to 300 students. These courses were conducted in Oregon,Washington, and Alaska. Under the Oregon DHS Lead Program/EPA Community Outreach Training Grant, students attended Lead-Safe Work Practices. The State of Oregon DHS Lead Program funding these activites through September 2007 for additional LBP abatement training and outreach activities throughout Oregon.

Technical Outreach Services for Communities (TOSC) and Technical Assistance to Brownfields Communities (TAB) PROGRAMS, October 2001-August 2007

Kenneth J. Williamson, Director, Oregon State University

The TOSC and TAB programs involved a staff of faculty, consultants, and graduate research assistants. In the last year of the programs, the staff included:

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Tarek Kassim, Instructor; Civil, Construction, and Environmental Engineering

Technical Outreach Services for Communities (TOSC)

Goal: The Technical Outreach Services for Communities (TOSC) Program was a technical assistance project designed to aid communities confronted with environmental contamination by hazardous waste sites.

Rationale: TOSC provided interested community groups with technical information and assistance to encourage early and meaningful public participation in decisions that affect their health and welfare. The TOSC program provided a viable alternative source of assistance for communities that did not qualify for a Technical Assistance Grant (TAG) from the US Environmental Protection Agency.

Approach: The Western Region's outreach program was one of five nationally instituted community outreach programs. Centered at Oregon State University, the TOSC team involved university faculty and students, as well as contracted environmental professionals with specialization in environmental engineering, risk communication, public health, information transfer, environmental justice, and community relations. The TOSC team provided communities with technical assistance related to understanding the effects of hazardous waste sites. Where appropriate, WR TOSC partnered with staff of the Technical Outreach Services for Native American Communities (TOSNAC) to provide assistance to tribal communities.

TOSC Communities
October 2001-August 2007

Region 10

OREGON

Community : Blair Community, Eugene
Contaminants of Concern: Pesticides and other hazardous substances
Overview of TOSC Assistance: Provided assistance to the Blair Community related to health concerns from unknown sources. Services included meeting with community to discuss health symptoms and concerns, reviewing regulatory agency records to determine if potential contaminant sources existed in the neighborhood, and preparing a report based on the agency file review with recommendations for further action.

Community: Concerned Citizens for Clean Air
Site: Georgia-Pacific Kraft Mill, Toledo
Contaminants of Concern: pulp mill emissions
Overview of TOSC Assistance: Provided comments on the facility's draft Title V permit; worked with the community to negotiate emissions reduction actions with Georgia-Pacific.

Community: Dallas, Oregon
Contaminants of Concern: aerial pesticide applications
Overview of TOSC Assistance: Met with the community contact to review her documentation and discuss a possible health survey. TOSC also met with the Pesticide Analytical and Response Center (PARC) Board to discuss TOSC activities and how the PARC member agencies may support them.

Community: OSP Community Group
Site: Oregon State Penitentiary, Salem
Contaminants: PCE and TCE
Overview of TOSC Assistance: TOSC provided assistance to the Oregon State Penitentiary (OSP) community group regarding investigation and cleanup of a chlorinated solvent release, an interim removal action measure (IRAM), and health concerns related to PCE and TCE groundwater contamination and cleanup. TOSC also helped evaluate air quality concerns in local residential basements and possible exposures though ingestion of local produce, soil contact, and incidental ingestion of soil.

Community: Portland Harbor Community Advisory Group
Contaminants of Concern: Metals, PAHs, pesticides, VOCs
Overview of TOSC Assistance: Reviewed the April 17, 2003 field sampling plan which is intended to support site characterization and risk assessment activities. The CAG has also asked TOSC to consider providing additional technical support in the future.

Community: Railroad Pollution Coalition
Site: Union Pacific Rail Yard, Eugene, Oregon
Contaminants of Concern: Petroleum, PAHs, chlorinated solvents
Overview of TOSC Assistance: Reviewed background site investigation documents and revised risk assessment reports; provided comments on various site cleanup and public health-related documents; participated in community group meetings as appropriate.

WASHINGTON

Community: Klickitat County
Site: Champion International Corporation mill site, Goldendale
Contaminants of Concern: Pentachlorophenol, petroleum products, metals
Overview of TOSC Assistance: TOSC reviewed and provided comments on documents related to the investigation and cleanup of an abandoned wood treatment facility; TOSC also provided comments on a health consultation prepared by WA Department of Health.

Community: Quincy Concern
Site: CENEX Facility, Quincy
Contaminants of Concern: Dichloropropopane
Overview of TOSC Assistance: TOSC reviewed and provided comments on a health consultation prepared by the WA Dept of Health. Reviewed site investigation reports on the CENEX facility; attended meetings with community members as necessary.

Community: Skykomish Environmental Coalition
Site: Burlington Northern Sante Fe yard, Skykomish
Contaminants of Concern: Petroleum (diesel, Bunker C)
Overview of TOSC Assistance: Reviewed investigation and cleanup documents. Participated in community group and formal public meetings.

Community: Spokane Indian Tribe
Site: Midnite Mine, Spokane
Contaminants of Concern: uranium, heavy metals
Overview of TOSC Assistance: TOSC and TOSNAC provided assistance to the community related to risk assessment and feasibility studies for the mine cleanup.

Region 9

CALIFORNIA

Community: Air Force Plant 42 ERAB
Site: Air Force Plant 42, Palmdale
Contaminants of Concern: TCE in groundwater
Overview of TOSC Assistance: Reviewed and commented on remedial investigation and feasibility study and other site investigation and cleanup documents; participated in RAB meetings.

Community: Alameda Point Collaborative
Site: Alameda Naval Air Station, Alameda
Contaminants of Concern: PAHs, lead, pesticides
Overview of TOSC Assistance: Reviewed the EDC-5 Draft Site Inspection Report; presented information and training in technical matters related to environmental conditions at the former air station.

Community: Alameda Restoration Advisory Board (RAB)
Site: Alameda Naval Air Station, San Francisco
Contaminants of Concern: VOCs, PAHs, Pesticides, PCBs, and Metals identified in soils and groundwater
Overview of TOSC Assistance: Provided assistance to the RAB regarding issues related to the cleanup of Operable Unit 1 (OU-1) at this former Naval Air Station. Completed OU-1 document review and comments.

Community: CIAAO
Site: Univar, City of Industry
Contaminants of Concern: VOCs
Overview of TOSC Assistance: Provided comments to the community and to the Department of Toxic Substances Control on air sampling, soil vapor sampling, and groundwater monitoring reports submitted to the DTSC by Univar, Inc. consultants.

Community: Chester Street Block Club Association, Oakland
Contaminants of Concern: Lead, pesticides, and vinyl chloride
Overview of TOSC Assistance: Provided technical support for community during the alternative dispute resolution process, including reviewing investigation and cleanup documents for South Prescott Neighborhood Park and participating in mediation meetings.

Community: Collective Roots, East Palo Alto
Contaminants of Concern: hydroxylamine, monoethanolamine, toluene and acetonitrile.
Overview of TOSC Assistance: TOSC reviewed data and answered questions about the explosion's possible effect on the garden.

Community: Community Advisory Group (CAG)
Site: Aerojet, Rancho Cordova
Contaminants of Concern: Trichloroethylene, perchlorate
Overview of TOSC Assistance: TOSC reviewed the site assessment report for candidate carve-out lands at the Aeroject Superfund Site and provided comments to the Aeroject Community Advisory Group. Attended monthly community meetings as necessary.

Community: Del Amo Action Committee
Site: Montrose Chemical DDT plant, Montrose
Contaminants of Concern: DDT
Overview of TOSC Assistance: TOSC reviewed a soil removal action along Kenwood Avenue, in the neighborhood next to the Montrose Chemical DDT plant. The removal action followed the discovery of high concentrations of DDT in a drainage channel originating near the Montrose facility. Researchers from the Western Region Hazardous Substance Research Center, worked with TOSC to conduct the review. TOSC and HSRC personnel attended a community meeting to provide comments on the report.

Community: Elem Tribe
Site: Sulphur Bank Mercury Mine, Clearlake
Contaminants of Concern: Mercury and other heavy metals
Overview of TOSC Assistance: Reviewed and commented on remedial investigation and feasibility study documents for the Sulphur Bank Mercury Mine; worked with TOSNAC to develop and conduct training workshops related to the mine cleanup.

Community: Fort Ord Environmental Justice Network
Site: Fort Ord, Marina
Contaminants of Concern: Ordnance and explosives, landfill gases, carbon tetrachloride, TCE
Overview of TOSC Assistance: Reviewed and commented on various site investigation and cleanup documents; assisted FOEJN in preparing for community meetings with the Army and regulatory agencies; attended community group meetings and relevant public meetings.

Community: Perchlorate Citizens Advisory Group (PCAG), San Martin
Contaminants of Concern: perchlorate
Overview of TOSC Assistance: Reviewed and summarized technical documents and communicated results. Attended and presented information at PCAG meetings.

Community: Protect Our Neighborhood Committee
Site: Waste Disposal Inc., Santa Fe Springs
Contaminants of Concern: Metals, VOCs, PAHs, PCBs, and Pesticides in Soils, Methane, Benzene, Vinyl Chloride, TCE and Other VOCs in Soil Gas, VOCs in Groundwater
Overview of TOSC Assistance: Reviewed the Record of Decision; determined health related concerns of residents living adjacent to the site; provided an updated list of contaminants affecting groundwater, soil, and air both at the site and in the adjacent community; reviewed and commented on investigation and feasibility studies.

Community: Quemetco
Site: Quemetco, City of Commerce
Contaminants of Concern: lead, sulfuric acid, antimony, arsenic, barium, cadmium, chromium and zinc
Overview of TOSC Assistance: Reviewed and commented on a risk assessment prepared by the local air quality management district; reviewed and commented on hazardous waste permitting and cleanup documents as needed. Provided information on potential combustion products from burning of plastics and potential health effects from exposure to those byproducts as well as on the feasibility of in-stack monitoring for VOC emissions from the Quemetco furnace.

Community: Restoration Advisory Board (RAB)
Site: Naval Air Station North Island, Coronado
Contaminants of Concern: Primarily TCE and Metals
Overview of TOSC Assistance: Attended several RAB meetings. Reviewed background information. Provided technical assistance related to incineration option, risk of worker exposure to TCE, and management of migration of contaminants to San Diego Bay. Reviewed and commented on Site 9 Draft Feasibility Study.

Community: South Bay Cares, Palos Verdes
Contaminants of Concern: Landfill byproducts.
Overview of TOSC Assistance: Reviewed and commented on an environmental impact report for a proposed golf course project on the closed Palos Verdes Landfill.

Community: Southeast Alliance for Environmental Justice (SAEJ)
Site: Bay Area Drum, San Francisco
Contaminants of Concern: PCE, PCBs, Pesticides, Arsenic, and Lead in soils and groundwater.
Overview of TOSC Assistance: Completed historical document review and provided comments to the California Dept. of Toxic Substances. Commented on groundwater monitoring results provided to SAEJ.

Community: Tustin RAB
Site: Marine Corps Air Facility, Tustin
Contaminants of Concern: TCE and other VOCs in groundwater
Overview of TOSC Assistance: Provided assistance to established RAB dealing with remediation activities at a Marine Corps Air Station. Reviewed and commented on RI/FS documents. Attended RAB meetings; made presentation on viability of bioremediation for groundwater.

Community: Valley Center, Valley Center
Contaminants of Concern: pesticides and MTBE
Overview of TOSC Assistance: Helped the community understand an epidemiological report concerning childhood cancer; prepare a report explaining the methodology used for data collection and analysis as well as case inclusion/exclusion criteria; explained the results from the MTBE water testing at local schools and the collection and analysis procedures; evaluated the regulatory status of Dursban (chlorpyrifos).

Community: West College Neighborhood Association, Santa Rosa
Contaminants of Concern: PCE and its breakdown products
Overview of TOSC Assistance: Provided information related to water treatment options; reviewed and commented on reports and other materials related to soil gas sampling, leaks from sewer systems, and groundwater sampling; provided information on health effects of exposure to PCE and medical outreach protocol; provided information on agencies' roles in cleaning up hazardous substances in the environment; provided information on cleanup technologies for PCE in soil and groundwater.

Community: Willits Citizens for Environmental Justice
Site: Abex-Remco Hydraulics, Willits
Contaminants of Concern: Hexavalent Chromium in soils and groundwater; TCE and other VOCs in groundwater
Overview of TOSC Assistance: Reviewed and commented on remedial investigation reports, sampling plans, and health risk assessments; conducted public environmental education workshops; served on the Site Team, which included representatives from the community, the Regional Water Quality Control Board, and the California Department of Health Services.

Community: Wyle Labs CAG
Site: Wyle Labs, Norco
Contaminants of Concern: VOCs, metals
Overview of TOSC Assistance: Responded to CAG member requests for technical information and assisted with information retrieval, technical document review and technical training sessions. Reviewed a radiation scan done on the Wyle property and answered community questions about detecting radiation, specifically depleted uranium in soil.

ARIZONA

Community: Barrios Unidos, Phoenix
Contaminants of Concern: Various air pollutants including VOCs, NO_x, and particulate matter
Overview of TOSC Assistance: Reviewed and summarized consultant's environmental report.

Community: Dewey-Humboldt Town Council/ Dewey Humboldt Community Organization, Dewey-Humboldt
Contaminants of Concern: Lead, Arsenic
Overview of TOSC Assistance: Reviewed a Dust Control Plan prepared by an industry in the area and offered comments and a comparison to the OSHA Dust Control Handbook.

Community: Don't Waste Arizona, South Phoenix
Contaminants of Concern: Anions, Metals, PAH's, and Dioxins in Soils, House Dusts, and Ventilation/Air Conditioning Systems.
Overview of TOSC Assistance: Provided review and comment on sampling plans and DRAFT Final Report from EPA. TOSC's statistical analysis of existing 1993 health study and

mortality mapping project indicated greater health symptom prevalence with increased proximity to the fire. EPA's 1996 sample results for 35 households were analyzed by TOSC personnel and showed that all chemicals were below health-based comparison levels.

Community: Downtown Southwest Neighborhood Association, South Phoenix
Contaminants of Concern: Various air pollutants including VOCs, NO_x, and particulate matter
Overview of TOSC Assistance: Assisted the community in obtaining a local air monitoring station; helped the community track permits for local facilities' air emissions; reviewed and summarized consultant's environmental report.

Community: Grant Park, Phoenix
Contaminants of Concern: Various air pollutants including VOCs, NO_x, and particulate matter
Overview of TOSC Assistance: Reviewed and summarized consultant's environmental report.

Community: Kiwanis Park Neighborhood Association (KPNA), Tempe
Contaminants of Concern: Benzene, Hexavalent Chromium, and Other Metals.
Overview of TOSC Assistance: Reviewed facility emissions data and risk assessment reports from 1994 and earlier years, reviewed multiple parts of the proposed air permit, attended meetings of local agency, facility, and community representatives to negotiate air permit provisions, assisted in reaching agreement on majority of community concerns, and, at the request of KPNA, TOSC has developed a draft air sampling plan to monitor emissions in the community.

Community: Union Hills
Site: Union Hills Subdivision, Phoenix
Contaminants of Concern: Unknown but suspected to include Pesticides, Chlorinated Solvents, and other VOCs
Overview of TOSC Assistance: Provided information from chemical sensitivity literature search and review, names of nationally recognized researchers and physicians, and information on cancer clusters sent to community. Conducted a health survey of community volunteers on November 11, 1998 to document reported health symptoms and examine any patterns/similarities of symptoms.

HAWAII

Community: Malama Makua
Site: Makua Military Reservation, Oahu Island
Contaminants of Concern: Lead, Arsenic, 2,4-DNT, and 2,6-DNT in Soils.
Overview of TOSC Assistance: Provided contact information for Unexploded Ordinance (UXO) expertise. Attended a meeting of all stakeholders, and toured the site in December 1998. Completed RI review and submitted comments in February 1999. Submitted comments on beach ponds sampling plan April 1999. Submitted comments on beach pond sampling results summer 1999.

TAB Communities
October 2001-August 2007

Region 10

OREGON

Community: City of Astoria
Site: Former gasification plant
Overview of TAB Assistance: Visited the community and provided the grantee with information on how to write an RFP for environmental services.

Community: Clackamas County
Overview of TAB Assistance: Assisted the County in its community outreach and education program.

Community: Eliot Neighborhood
Overview of TAB Assistance: Prepared a summary of the cleanup activities undertaken at the site after demolition and presented the summary at a community meeting.

Community: Oregon Brownfields Conference
Overview of TAB Assistance: Acted as an organizing partner and as a resource to the Oregon Brownfields Conference.

Community: Oregon DEQ Brownfields Program
Overview of TAB Assistance: Acted as a resource to the ODEQ brownfields program. Attended quarterly networking meetings.

Community: Portland Development Commission
Site: South Waterfront/ North Macadam
Overview of TAB Assistance: Attended neighborhood meetings; reviewed and commented on an RFP prepared by PDC for a previous EPA assessment grant project in Portland. TAB also brought together PDC staff and a consultant whose unique services were beneficial to the project.

Community: Portland Showcase, Portland
Overview of TAB Assistance: Invited the Showcase to participate in the Oregon Brownfields Conference planning process as a means of facilitating achievement of their community outreach goals. Prepared "master" site cleanup fact sheet; evaluated current state/federal interagency workgroup for opportunities to expand and build improved partnerships; reviewed existing Showcase Program web links and suggested improvements and additions; recommended 10-12 essential brownfields library documents; evaluated the existing Regulatory Innovation Action Plan and suggested how to build upon DEQ reforms; evaluated implications of the Endangered Species Act.

Community: Retired and Senior Volunteer Program, St. Helens

Overview of TAB Assistance: Attended weekly meetings and provided support at several presentations before various civic groups, iincluding the League of Women Voters and Kiwanis Club; helped plan and hold an open house to showcase a major site to developers; helped this group expand their brownfields inventory and promotional efforts.

WASHINGTON

Community: City of Spokane
Overview of TAB Assistance: Helped produce surveys and conduct a workshop to assist in the selection and prioritization of brownfield sites within the areas specified in the grant.

IDAHO

Community: Idaho Department of Environmental Quality
Overview of TAB Assistance: Participated in the training of 4 volunteers in Sandpoint, Idaho to build a brownfields inventory for the small adjacent cities of Sandpoint, Kootenai, Dover and Ponderay.

Region 9

ARIZONA

Community: Organization: Arizona Department of Environmental Quality
Overview of TAB Assistance: Met with the grant writing team and the city officials to discuss strategy for writing a grant and reviewed a draft they had prepared.

CALIFORNIA

Community: Brisbane Baylands Community Advisory Group
Overview of TAB Assistance: Reviewed leachate management plan submitted by the landowner and provided comments to the CAG.

Community: Lula Washington Dance Theatre (Los Angeles, CA)
Overview of TAB Assistance: Worked with the grantee, the EPA and the environmental consultant performing the work in designing and implementing a community outreach program. Created a flyer, made presentations before neighborhood groups and assisted with an Open House for the community.

Community: Richmond Redevelopment Agency
Overview of TAB Assistance: Helped gauge interest in a specific brownfields project through community contact, assisted in conducting community meetings, and helped write a community relations plan.

Community: Richmond Southeast Shoreline Area (RSSA) CAG, Richmond
Overview of TAB Assistance: Met with this CAG several times to discuss their concerns and TAB's assistance; assisted the CAG with questions about environmental policy, remediation and other issues surrounding the site under their purview.

Community: Susanville Indian Rancheria
Overview of TAB Assistance: Worked with TOSNAC to provide information and assistance to the tribe in its efforts to redevelop a brownfield site on the rancheria.

NEVADA

Community: Nevada Division of Environmental Protection (NDEP)
Overview of TAB Assistance: Met with NDEP to discuss helping plan and present a rural brownfields conference; participated in and facilitated a session of the first rural brownfields conference.

Conference Sponsorship
October 2001-August 2007

Oregon Rural Brownfields Conference, Bend, Oregon; October 2001
Oregon Rural Brownfields Conference, Bend, Oregon; September 2002
Oregon Brownfields Conference: *Putting Brownfields Back to Work,* Portland, Oregon; August 2005
Oregon Brownfields Conference, Salem, Oregon; March 2007

Supplemental Keywords: groundwater; soil; risk management; risk assessment; brownfield sites, region 9, region 10,

WRHSRC Publications 2001-2007

A. Journal Articles

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