



**WESTERN REGION  
HAZARDOUS SUBSTANCE RESEARCH CENTER**

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**2006 ANNUAL REPORT**

Oregon State University  
Stanford University

April 2007

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# WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER 2006 ANNUAL REPORT

## Administration

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## The Center at a Glance

The Western Region Hazardous Substance Research Center (WRHSRC) is a cooperative activity between Oregon State University and Stanford University that was established in October 2001. The Center is 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 receives its base financial support from the U.S. Environmental Protection Agency.

The objectives of the Center are

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 use microbes or chemical catalysts to transform VOCs into harmless substances, represent the major focus of Center activities. Research projects include 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 are being used to study these processes and to provide a bridge between theory and practice. The technology transfer program involves the process of taking new technologies from the laboratory to the field. Center researchers are working with other federal agencies, such as the Department of Defense (DoD) and the Department of Energy (DoE), and private industry, in

conducting field evaluations of new technologies. Technical Outreach Services for Communities (TOSC) is a technical assistance program designed to aid communities confronted with environmental contamination by hazardous waste sites. TOSC provides interested community groups with technical information and assistance that can enable early and meaningful public participation in decisions that affect health and welfare. The Center's Technical Assistance to Brownfields Communities (TAB) Program provides assistance to communities attempting to address cleanup and redevelopment of properties whose reuse has been prevented by real or perceived contamination. TAB attempts 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 are currently involved in the Center. They collectively represent 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 is director of the Center and of the research program. Kenneth J. Williamson serves as associate director in charge of training, technology transfer and community outreach. Martin Reinhard, the assistant director, is in charge of the Center's quality control program. Garrett Jones is the Center's administrative assistant.

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

The Center budgets for the 2006 fiscal year and since the Center's inception are listed by category of support in Table 4. During the fifth year of operation, core funding totaled \$225,000 which was directed to the TOSC program. No additional funds were received for the Center's research activities. The research however continued on funds received in the 2005 fiscal year.

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. Thirteen graduate students were supported during the fifth year of the Center, with nine of these being Ph.D. students.

**Table 1. Key Personnel at the WRHSRC**Stanford University/Discipline

Craig C. Criddle, Environmental Engineering  
 Peter K. Kitanidis, Hydrogeology  
 Martin Reinhard, Environmental Chemistry  
 Alfred Spormann, Microbiology/Biochemistry

Oregon State University/Discipline

Daniel J. Arp, Biochemistry  
 Peter Bottomley, Microbiology  
 Linda Ciufetti, Microbiology  
 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**

<u>Member</u>	<u>Affiliation</u>	<u>Expertise</u>
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**

<u>Member</u>	<u>Affiliation</u>	<u>Expertise</u>
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**

<u>Funding Sources</u>	<u>FY 2001</u>	<u>FY 2002</u>	<u>FY 2003</u>	<u>FY 2004</u>	<u>FY 2005*</u>	<u>Total</u>
EPA: Centers Program	\$900,000	\$885,000	\$885,000	\$868,160	\$225,000	\$3,763,200
EPA: Brownfields	150,000	150,000	127,000	**		\$ 427,000
Oregon State University	<u>90,000</u>	<u>90,000</u>	<u>90,000</u>	<u>90,000</u>	<u>\$ 45,000</u>	<u>\$ 405,000</u>
TOTAL	<u>\$ 1,140,000</u>	<u>\$1,125,000</u>	<u>\$ 1,102,500</u>	<u>\$ 958,160</u>	<u>\$170,000</u>	<u>\$4,595,200</u>

\*Sept. 1, 2005- Aug 30, 2006

\*\*Brownfields became a separate grant in FY Sept 2004

**Table 5. Students Supported**

<u>Student Support</u>	<u>FY 2001</u>	<u>FY 2002</u>	<u>FY 2003</u>	<u>FY 2004</u>	<u>FY 2005</u>	<u>Funds to Date†</u>
M.S.	2	2	2	2	2	\$ 375,000
Ph.D.	9	14	10	10	9	\$ 2,150,000
Post Doctoral	<u>0</u>	<u>0</u>	<u>2</u>	<u>2</u>	<u>2</u>	<u>\$ 337,500</u>
TOTAL	11	16	14	14	13	<u>\$ 2,862,500</u>

\*Total numbers in researcher-years participating on Center Projects since 2001

† Includes tuition, stipends travel, supplies, etc.

## Director's Report

### Project Highlights of the Year

The major focus of research activities for the OSU-Stanford WRHSRC, and indeed its major mission, has been the conduct of basic research related to the in situ treatment of VOC subsurface contamination. During the past year research was continued on five center projects associated with the in situ remediation of chlorinated solvents. The projects and the researchers are summarized below. A total of 210 K in new funds were added to these projects from unallocated funds received in 2005.

**Table 6. RESEARCH PROJECT SUMMARY**

Project	Title	PI Co-PIs	Year 1 Budget 2004	Year 2 Budget 2005	Year Budget 2006
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	\$115,520	\$119,020	\$60,000
2-OSU-06	Development and Evaluation of Field Sensors for Monitoring Anaerobic Dehalogenation After Bioaugmentation	James Ingle, PI, Oregon State University	\$ 55,620	\$ 59,610	\$30,000
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	\$142,060	\$168,790	\$90,000
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	\$ 70,000	\$ 70,000	\$30,000
2-SU-05	Sorption and Hydrolysis of Halogenated Hydrocarbons in Soil Nanopores	Martin Reinhard, PI	\$ 90,000	\$ 90,000	\$0

Research projects include both aerobic and treatment processes (physical and reactive transport processes), as well as in situ characterization methods for monitoring the progress of both intrinsic and the enhanced remediation. Three project PIs are at OSU and three are at Stanford University. Project 2-OSU-07 represents a joint project between Stanford University and Oregon State University on the anaerobic transformation of chlorinated solvents.

### **Project Highlights of the Year Summary**

**Project 2-OSU-05**, which is being conducted at Oregon State University, is a collaborative project among microbiologists and engineers headed by Peter Bottomley and Mark Dolan. The goal of the project is to study the aerobic cometabolism of chlorinated ethenes by microorganisms that are grown hydrocarbons as well as organics acids, and also to study the direct aerobic metabolism of vinyl chloride (VC) and cis-dichloroethene (cis-DCE).

Peter Bottomley's researchers have genetically engineered strains of *Pseudomonas butanovora* by replacing specific amino acids associated with the BMO hydroxylase alpha subunit. They have examined the chloroethene degrading properties of these mutants and examined the impact of cometabolism of different chloroethenes on monooxygenase activity, and assessed the effect of cometabolism on cell viability. The mutants were found to have lower specific activities of BMO than the wild type. Upon examination of chloroethene oxidation by these mutants they observed that

the mutants that produce 2-butanol were able to degrade 1,1-DCE, 1,2, cis DCE and TCE and not suffer the cell death observed with the wild type strain. They hypothesize that strains possessing slower rates of CAH degradation without cell death might be more appropriate bioremediatory agents than strains that show high transitory rates of TCE degradation that are accompanied by substantial loss of cell viability. Mutant strains of *P. butanovora* that do not show cytotoxic effects of 1,1,DCE and TCE metabolism metabolize propane and butane primarily to 2-propanol and 2-butanol, respectively. They are currently conducting studies to examine how *P. butanovora* metabolizes 2-alcohols.

Dr. Dolan's researchers are studying three microorganism strains EE13A, JS60, and JS614, that cometabolize or directly metabolize vinyl chloride as a carbon and energy source. They are also evaluating fluoroethene (FE) as a reactive surrogate of VC. EE13A, isolated from Ft. Lewis groundwater, utilizes Eth as a growth substrate and will cometabolize VC and FE. Strain EE1 JS60 utilizes Eth and VC as growth substrates and cometabolically degrades FE, and JS614 utilizes ethene, VC and FE as growth and energy substrates. Despite the fact that the three VC-degrading isolates responded differently to Eth, VC and FE as growth substrates, there were no significant differences between the  $K_s$  or  $k_{max}$  values of each of the isolates for FE and VC, and there was little difference between the 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. Their results have shown that FE is an effective surrogate for studying VC utilization and transformation, and might be used as an in situ reactive tracer at contaminated sites.

**Project 2-OSU-06** is being conducted at Oregon State University under the direction of James Ingle. The goal of this study is 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 cultures. 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. . 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. They have also developed 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. The method provides detection of  $O_2$  at levels below 1 ppm that cannot be accurately measured with a DO probe and the detection limit is 0.04 ppm.

**Project 2-OSU-07**, a joint project of Oregon State University and Stanford University, is evaluating the transformation of chlorinated ethenes in continuous-flow column studies with the Victoria Strain (VS) and the Evanite Strain (EV) cultures that have been developed and kinetically characterized in previous WRHSRC projects. Molecular methods are being applied such as FISH and Real-Time PCR to determine the spatial distribution of the cultures and quantify the dehalogenating biomass within the column. RNA-based methods are also being applied to determine energetically based TCE and VC-dehalogenating activity temporally and spatially within the columns.

The results of column studies showed that the transformation proceeded more rapidly from PCE or TCE to ethene when the aquifer material had been chemically pre-reduced using NaS. Pre-reduction

of ferric iron to ferrous iron likely resulted in less competition for hydrogen by iron reducing microorganisms for required as an electron donor to drive c-DCE transformation to VC and ethene.

Based on the column experiments and the microbial community analysis, a working hypothesis of the flow column ecosystem as a whole and the interspecies interactions was developed which is likely essential to microbial reductive dehalogenation. In the model, the primary electron donor lactate is fermented to propionate, acetate, CO<sub>2</sub>, and hydrogen by a diverse population of fermenting bacteria, including *Clostridia*. Homoacetogenic bacteria like *Acetobacterium* relatives use CO<sub>2</sub> and hydrogen to produce acetate. Acetate and hydrogen can serve as electron donor for the groups of dehalogenating microorganisms, which compete with the homoacetogenic bacteria for hydrogen. While reductively dechlorinating microorganisms like *Desulfuromonas* species require acetate as electron donor, *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.

**Project 2-SU-04**, a project at Stanford University under the direction of Peter Kitanidis and Craig Criddle, is investigating novel methods for the measurement of transverse dispersion in homogeneous isotropic unconsolidated porous media. 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. The researchers developed new methods for the measurement of local transverse dispersion in isotropic porous media based on a helical and a cochlea-like device. The principle was to induce shear flow inside the device that creates strong longitudinal dispersion in the observed breakthrough curve; transverse mixing tends to negate the effects of shear flow and thus reduce the observed column-scale longitudinal dispersion. Then, from the spreading of the observed breakthrough curve, they estimated the unknown, the pore-scale transverse dispersion.

The project is now completed and a PhD dissertation has been submitted that will serve as final report. The numerical simulation and parameter estimation methods used to analyze the experimental data are presented along with the relative advantages of each device, instrument, and methodology that they 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 estimated ratio of transverse dispersivity to longitudinal dispersivity agrees with the higher ratios reported in the literature.

**Project 2-SU-05** is located at Stanford University under the direction of Martin Reinhard and is evaluating the role of microstructure on contaminant sorption and desorption, as well as abiotic transformations. The overall goal of this project is to develop a better understanding of the impact of soil nanopores on the fate and transport of halogenated hydrocarbon contaminants. Specific project goals are 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. The researchers found that 2,2-dichloropropene (2,2-DCP) which reacts with water to 2-chloropropane when sorbed in hydrophobic micropores, reacts more slowly 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.

In studies of TCE sorption 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 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.

## **Training and Technology Transfer**

The goals of the technology transfer program are to promote teamwork and information exchange among researchers through listservs, Web pages, and seminars; promote information transfer with practitioners using Web pages, electronic newsletter, workshops, faculty presentations and publications; test new technologies through laboratory and pilot-scale testing, demonstrations, and an online project database; and implement full-scale demonstration projects.

In 2006, 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. Research Brief #10 was added this year. It describes work by Alfred Spormann and Sebastian Behrens (Stanford University) to apply polymerase chain reaction (PCR) and fluorescence in situ hybridization (FISH) for monitoring bioremediation in situ ([http://wrhsrc.oregonstate.edu/briefs/brief\\_10.htm](http://wrhsrc.oregonstate.edu/briefs/brief_10.htm)). During 2006, the web site received an average of 1400 visits per month.

## **TOSC and TAB Programs**

The two outreach programs of importance are Technical Outreach Services for Communities (TOSC) and Technical Assistance to Brownfields (TAB). These programs are directed by Ken Williamson and Denise Lach at Oregon State University.

TOSC provides interested community groups with technical information and assistance that can enable early and meaningful public participation in decisions that affect health and welfare. The TOSC program provides a viable alternative strategy for communities that do not qualify for a Technical Assistance Grant (TAG) from the US Environmental Protection Agency. The TOSC team is comprised of 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. Currently the TOCS program is actively working with communities in Region 10 (4) and Region 9 (10).

The TAB program provides assistance to communities attempting to address cleanup and redevelopment of properties whose reuse has been prevented by real or perceived contamination. TAB attempts to improve involvement of all affected parties in cleanup and redevelopment process through education and training. The TAB program is currently working with three communities in Region 10.

## Research Project Reports

Summary reports are presented below for each of the Center's projects and outreach and technology transfer activities.

### **2-OSU-05 Aerobic Cometabolism of Chlorinated Ethenes by Microorganisms that Grow on Organic Acids and Alcohols**

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

Investigators: P. J. Bottomley and D.J. Arp.

**Goal:** The proposal aims to evaluate how to maximize the chloroethene degrading potential of individual strains and mixed communities of hydrocarbon-degrading bacteria. Specific sub-objectives include 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 cytotoxic damage to the cells.

**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) We have genetically engineered strains of *Pseudomonas butanovora* by replacing specific amino acids associated with the BMO hydroxylase alpha subunit. We have examined the chloroethene degrading properties of these mutants and examined the impact of cometabolism of different chloroethenes on monooxygenase activity, and assessed the effect of cometabolism on cell viability.

(b) We have continued to study the inactivation of BMO in *P. butanovora* by organic acids. We have carried out a series of studies to determine if organic acids such as propionate and butyrate are suicide substrate inactivators of BMO, and to generally characterize the nature of the inhibition. Additional studies have been conducted with other alkane degrading bacterial species with the potential to cooxidize chloroethenes to determine how widespread organic acid inactivation really is. We have also examined the mutant strains mentioned in (a) to determine if propionate sensitivity of BMO can be reduced.

(c) Because we showed that the mutant strains that metabolize butane to 2-butanol are not inactivated during cooxidation of chloroethenes, we have examined metabolism of 2-butanol and acetone to gain insight into how these compounds are metabolized in *P. butanovora*, and to elucidate their propensity to serve as electron donors of cooxidation.

**Status:**

(a) The mutants characterized showed lower specific activities of BMO than the wild type. Interestingly, two of the mutants showed an increased proportion of 2-butanol relative to 1-butanol as the primary product of BMO activity. These phenotypes were similar to what has been observed before with *M. vaccae*. Upon examination of chloroethene oxidation by these mutants we observed that the mutants that produce 2-butanol were able to degrade 1,1-DCE, 1,2, cis DCE and TCE and not suffer the cell death observed with the wild type strain. Furthermore, the fact that much less chloride was released in the mutants than from the wild type indicated that the chloroethenes were being oxidized by a different mechanism to wild type, and which might be accounting for the lack of cytotoxic effects. Strains possessing slower rates of CAH degradation without cell death might be more appropriate bioremediatory agents than strains that show high transitory rates of TCE degradation that are accompanied by substantial loss of cell viability.

(b) We have shown that not only are the organic acids, butyrate and propionate, less efficient electron donors than either acetate or lactate, they have been shown to inactivate BMO when turning over poorer substrates like 1,2-trans DCE. Although the aliphatic organic acids provide reductant to BMO, they seem to compete with substrates for the active site of the enzyme. The mechanism of BMO inactivation by aliphatic organic acids is currently being studied. We have determined that propionate-dependent inactivation is time dependent, requires O<sub>2</sub> and can be protected by butane. In contrast to acetylene, which is a suicide substrate that binds to the monooxygenase, we obtained no evidence that propionate binds covalently to BMO polypeptides. Preincubation of propionate with cells substantially reduced the amount of acetylene labeled enzyme adding further proof of propionate-dependent irreversible inactivation. Propionate inactivation cannot be prevented by adding more lactate or acetate.

Mutant strains of BMO modified in their catalytic activity showed less sensitivity to propionate, and could not be irreversibly inactivated implying that propionate needs to bind specifically into the active site of BMO to have an effect, and that specific amino acid residues are required for the inactivating interaction to take effect. A model has been developed that suggests that the carboxyl group of propionate binds to the diiron site, and that the tail of propionate sticks into the hydrophobic pocket of BMO. Several other monooxygenase containing strains were evaluated for propionate inactivation. Only one strain *Pseudomonas mendocina* KR1 showed a strong negative effect of propionate, but this has been a favorite strain for cooxidation of TCE, and possesses a multicomponent toluene 4-monooxygenase.

(c) It is well recognized that some bacteria that possess monooxygenases and that cooxidize chloroethenes do not suffer inactivation or cytotoxic effects as strongly as occur in other strains. Mutant strains of *P. butanovora* that do not show cytotoxic effects of 1,1,DCE and TCE metabolism metabolize propane and butane primarily to 2-propanol and 2-butanol, respectively. We have been conducting studies to examine how *P. butanovora* metabolizes 2-alcohols. Preliminary results have shown that *P. butanovora* can effectively metabolize 2-alcohols to ketones, and that a carboxylase (CO<sub>2</sub> fixing) enzyme is required to process the ketones generated by the secondary alcohol oxidation. Interestingly, we have shown that growth on 2-propanol or acetone is dependent upon yeast extract, whereas growth on 1-propanol or 2-butanol is not. Further studies are underway to determine the effectiveness of 2-alcohol and ketone metabolism at supporting CE oxidation.

## **Part II.** Isolation and investigation of cultures capable of direct metabolism of VC and cis DCE.

Investigators: M. Dolan and L. Semprini, Oregon State University

**Goal:** A primary goal of this work is to isolate and characterize pure cultures that directly metabolize cis-DCE and VC and to develop surrogates reactive tracers to study their transformation in-situ.

**Rationale:** The recent identification of microorganisms capable of aerobic metabolic growth on cis-DCE and VC illustrate the potential for these organisms in the aerobic remediation of distal areas of chlorinated ethene contaminated plumes.

**Experimental Approaches:** *Mycobacterium* and *Nocardioides* strains capable of growth on VC have been obtained from the researchers that isolated the cultures (Coleman et al.2000a), as well as the culture JS666 (Coleman et al. 2000b), the only know organism capable of growth on cis-DCE. Studies were conducted on these strains to determine their substrate range and possible ability to grow on natural fermentation products such as organic acids or alcohols and whether they retain their ability to utilize VC or cis-DCE. Additionally, the VC utilizers were be screened for their ability to grow on the VC surrogate compound, FE, to determine if FE could be a useful surrogate to investigate the potential for VC metabolism at VC-contaminated sites. *Mycobacterium* strain JS60 is able to cometabolize FE and direct growth on ethene and VC. Currently no bacteria have been described in the literature that are capable of utilizing FE as a sole carbon and energy source, but preliminary work with *Nocardioides* strain JS614 shows growth on FE along with ethene and VC.

**Status:** T-RFLP analyses of the enrichment cultures produced from the same field groundwater sample showed that each substrate yielded distinct microbial communities. Although there was much diversity and many common TFLs between the enrichments, the dominant fragments produced from each enrichment substrate were different. Similarities in dominant fragments were observed in enrichments of different groundwater samples with the same substrate.

Groundwater microcosms enriched with ethene were combined and subdivided for incubated with ethene, VC, or FE as the sole carbon source to test for evidence of direct VC metabolism and the ability to transform or grow on FE. In some of the tests the enrichment culture was diluted so that VC or FE disappearance would likely indicate growth on these compounds. Compound disappearance and concomitant increases in optical density were observed with all three compounds over repeated feedings with similar rates of utilization for VC and FE and ethene rates about 5 times greater. After about 30 days of exposure to the respective substrates, T-RFLP analyses showed the VC and FE communities had shifted relative to the ethene-fed cultures. Genomic DNA from the ethene enrichment culture was probed with CoM transferase primers after growing on Eth, VC or FE for 30d. CoM transferase, which is expected to be involved with the metabolism of the epoxides (Coleman et al., 2003) that were formed, was detected with growth on all three substrates.

Attempts to isolate organisms out of the ethene, VC, and FE amended cultures began with streak plating on tryptic soy agar. Representative colonies were back transferred into MSM with either ethene, FE or VC as growth substrates. Resumption of utilization and growth on these substrates was slow, and in all but one case the cultures that retained their ability to utilize ethene, FE or VC were mixed. One isolate *Mycobacterium* strain EE13A, which grows on ethene and cometabolizes VC and FE, was obtained. The partial 16S rDNA of strain EE13a (900 bp) shared 99% sequence identity with *Mycobacterium* sp. O228YA isolated from a contaminated site in Japan.

Further studies are being conducted with three phenotypes of Eth utilizing bacteria which will be used in assessing the potential for FE to serve as a surrogate of VC in the subsurface. EE13A was isolated from Ft. Lewis groundwater, utilizes Eth as a growth substrate and will cometabolize VC and FE. JS60 utilizes Eth and VC as growth substrates and cometabolically degrades FE, and JS614 utilizes ethene, VC and FE as growth and energy substrates. Despite the fact that the three VC-degrading isolates responded differently to Eth, VC and FE as growth substrates, there were no significant differences between the  $K_s$  or  $k_{max}$  values of each of the isolates for FE and VC, and there was little difference between the 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. During co-transformation, the initial rates of halide release matched that of substrate transformed for each VC-degrading isolate, and degradation of individual substrates demonstrated that halide release ceased as soon as substrate transformation was complete and showed that there was no further halide release from halogenated degradation products that might result during cometabolism. For VC, mass balance of the substrate transformed and halide released were nearly stoichiometric regardless of whether transformation was direct or cometabolic and averaged 98.9 ( $\pm 11.1$ ) %. Stoichiometric release of  $F^-$  during direct metabolism of FE by JS614 was also achieved (99.5  $\pm$  15.7) %, but during cometabolic transformation of FE by EE13a and JS60 mass balance averaged 83.5 ( $\pm 4.0$ ) %. Competitive inhibition best described the interaction of VC and FE when these substrates were simultaneously transformed for all three isolates. Transformation of substrates and accumulation of halide were modeled and fit closely to experimental results. For the three aerobic VC-degrading isolates studied, both the rates of FE transformation and  $F^-$  accumulation (two unique signals and two different analytical methods) could be correlated with the rate of aerobic degradation of VC. FE therefore can be used as a surrogate reactive tracer for estimating rates of VC degradation in situ.

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### **Publications:**

#### **(a) Journal articles**

- Halsey, K.H., L.A. Sayavedra-Soto, P.J. Bottomley, and D.J. Arp. 2005. TCE degradation by butane-oxidizing bacteria causes a spectrum of toxic effects. *Appl. Microbiol. & Biotechnol.* 68: 794-801.

- Doughty, D.M., L.A. Sayavedra-Soto, D.J. Arp, and P.J. Bottomley. 2005. Dichloroethenes as substrates and inducers of butane monooxygenase in *Pseudomonas butanovora*. *Appl. Environ. Microbiol.* 71:6054-6059.
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- Halsey, K.H., L.A., Sayavedra-Soto, Bottomley, P.J., and D.J. Arp. 2006. Site-directed amino acid substitutions in the hydroxylase  $\alpha$  subunit of butane monooxygenase from *Pseudomonas butanovora*: implications for substrates knocking at the gate. *J. Bacteriol.* 188: 4962-4969.
- Taylor, A.E., M. E. Dolan, P.J. Bottomley, and L. Semprini . Utilization of Fluoroethene as a Surrogate for Aerobic Vinyl Chloride Degradation. Submitted to *Environ. Science and Tech.*

### **(b) Abstracts and Posters**

1. K.H. Halsey, L.A. Sayavedra-Soto, P.J. Bottomley, and D.J. Arp. 2004. Kinetics of TCE transformation and comparisons of TCE transformation-dependent toxicities in butane-oxidizing bacteria. *Annu. Mtg. Amer. Soc. Microbiol. New Orleans.*
2. D.M. Doughty, L.A. Sayavdero-Soto, P.J. Bottomley, and D.J. Arp. 2004. Metabolism of dichloroethenes by *Pseudomonas butanovora*. *Annu Mtg. Amer. Soc. Microbiol. New Orleans*
3. C. Razzetti, M.E. Dolan, and L. Semprini. 2004. Cometabolic degradation of 1,1,1-trichloroethane and 1,1-dichloroethane by a *Rhodococcus* species. SETAC Annual Meeting, Portland, OR.
- 4) L. Semprini, M.E. Dolan. M. Mathias, G.D. Hopkins, and P.L. McCarty. Laboratory and Field Studies of Bioaugmentation of Butane-Utilizing Microorganisms for the In-situ Cometabolic Treatment of Treatment of 1,1-Dichloroethene, 1,1-Dichloroethane, and 1,1,1-Trichloroethane. *Proceedings of the 3<sup>rd</sup> European Conference on Bioremediation, Crete, July 3-5, 2005.*
5. A.E. Taylor, L. Semprini, P.J. Bottomley, M. Dolan. 2005. Evaluation of fluoroethene as an analogue for aerobic vinyl chloride degradation. *The Joint International Symposia for Subsurface Microbiology and Environmental Biogeochemistry. Jackson Hole, Wyoming.*
6. C. Razzetti, M.E. Dolan, and L. Semprini, Cometabolic degradation of 1,1,1-trichloroethane and 1,1-dichloroethane by a *Rhodococcus* species: kinetic studies and model simulations, 10th EuCheMS Conference on Chemistry and the Environment, Rimini, September 2005
7. C. Razzetti, M.E. Dolan, and L. Semprini, Cometabolic degradation of 1,1,1-trichloroethane and 1,1-dichloroethane by a *Rhodococcus* species in a soil column reactor, 10th EuCheMS Conference on Chemistry and the Environment, Rimini, September 2005.

### **3a. Graduate students supported on this project**

Kimberley Halsey, Ph.D. candidate, Molecular and Cellular Biology Program.

David M. Doughty, Ph.D. candidate, Microbiology Graduate Program.

Anne Taylor, Ph.D. candidate, Civil Construction and Environmental Engineering.

Cecilia Razzetti, Visiting Ph.D. Scholar, Ph.D. candidate, University of Bologna, Civil Construction and Environmental Engineering.

Christina Blatchford . M.S. candidate, Civil Construction and Environmental Engineering.

Kristin Skinner, Ph.d. Candidate, Botany and Plant Pathology

### **3b. Theses completed**

Doughty, D.M. 2004. Department of Microbiology, MS thesis, Metabolism of dichloroethenes by the butane-oxidizing bacterium, *Pseudomonas butanovora*.

Sattayatewa, C. 2004. Department of Civil Construction and Environmental Engineering, MS project, Growth Characteristics and Chlorinated Hydrocarbon Transformation Ability of a *Rhodococcus* sp. Isolate.

Li, J. 2004. Department of Civil Construction and Environmental Engineering, MS thesis, Molecular Analysis of Bacterial Community Dynamics During Bioaugmentation Studies in a Soil Column and at a Field Test Site.

Maremanda, B. 2004. Department of Civil Construction and Environmental Engineering, MS thesis, Aerobic cometabolism of 1, 1, 1-Trichloroethane and 1, 1-Dichloroethene by a bioaugmented butane-utilizing culture in a continuous flow column.

Blatchford, C. 2005. Department of Civil Construction and Environmental Engineering, MS thesis, Aerobic Degradation of Chlorinated Ethenes by *Mycobacterium* Strain JS60 in the Presence of Organic Acids.

Razzetti, C. 2005. Cometabolic degradation of 1,1,1-trichloroethane and 1,1-dichloroethane by a butane grown *Rhodococcus* species: kinetic studies, reactor operation and modeling. Ph.D. Thesis, Department of Chemical Engineering, University of Bologna

#### **4. Field projects**

Development of Effective Aerobic Cometabolic Systems for the In-situ Transformation of Problematic Chlorinated Solvent Mixtures, DoD SERDP Program.

#### **2-OSU-06 Development and Evaluation of Field Sensors for Monitoring**

##### **Anaerobic Dehalogenation after Bioaugmentation for In-Situ Treatment of PCE and TCE**

James D. Ingle, Jr.; Oregon State University

**Goals:** The purpose of this study is 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 will be 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 1) to identify and compare the response of these indicators during bioaugmentation, 2) to improve the monitoring devices and methodology (flow cells, fiber optic probes, sampling) for practical use, 3) to 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) to 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 subsurface 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, will be deployed in collaborative experiments for calibration and demonstration of their applicability. These experiments will involve continuous monitoring of the redox conditions of cultures inside columns and PAMs packed with soil and enriched with halo-respiratory cultures as a tool for spatial monitoring of dechlorination and to improve conditions necessary for effective dechlorination of PCE and TCE. In addition, we seek to investigate alternative sampling/reagent/detection systems, quantitative measurement of concentrations of reductants, O<sub>2</sub>, and fiber optic sensors.

**Status:** We have 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. We have used the flow sensors to successfully examine redox conditions in microcosm bottles containing a dechlorinating culture (Evanite culture) and in packed columns augmented with the culture. Consistently, we have demonstrated that the rapid reduction of the indicator immobilized thionine (THI) indicates that conditions are appropriate for dechlorination and occurred when hours of contact with an active dechlorinating culture. The redox indicator cresyl violet (CV) is slowly but consistently reduced (about 25 to 75% reduced) as the culture becomes more reducing, typically during the dechlorination of cis-DCE and VC. It appears likely that reduced species other than S(-II) or Fe(II) are contributing to the reduction of the indicators and preliminary results suggest some of these reductants may be products of cellular energy generation, mediators, or co-factors.

We have 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  $\mu$ M 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<sub>2</sub>. 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  $\mu\text{M}$ ), which suggests that some of the RC measured in free cultures is not present due to cells or particles becoming trapped or attached in the column. Upon filtration of column samples, RC(THI) decreased by  $\sim 50\%$ , which suggests that a larger fraction of the RC is 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  $\text{H}_2$  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  $\text{O}_2$  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  $\text{O}_2$  contamination. Several novel innovations were incorporated. The new method was applied to Fe-reducing, sulfate-reducing, and methanogenic cultures.

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  $\mu\text{m}$  make quite portable and suitable for long term monitoring because flow rate and sample and reagent consumption are very small (i.e., microliters). Future research is focused on applications and on valves, pumps, reagent reservoirs, and control/data acquisition electronics with an emphasis on low power consumption and portability.

#### ***Journal Publications:***

- K. Cantrell and J. D. Ingle, Jr. (2003) "The SLIM spectrometer", *Anal. Chem.*, 75, 27-35.
- B. D. Jones and J.D. Ingle, Jr. (2005) "Evaluation of redox indicators for determining sulfate-reducing and dechlorinating conditions", *Water Research*, 39, 4343-435.
- Ruiz-Haas, P. and Ingle, J.D, Jr. (2006) "Applications of Sensing Devices based on Redox Indicators for Non-invasive and In-situ Monitoring of Redox Conditions during Reductive Dehalogenation of PCE in Packed Columns.", *Geomicrobiology Journal*, in press .
- Ruiz-Haas, P. and Ingle, J.D, Jr. (2005) "Monitoring of Redox State in a Dechlorinating Culture with Redox Indicators.", *Journal of Environmental Monitoring*, in preparation

#### ***Conference Abstracts:***

- Defne Cakin and J.D. Ingle, Jr. (2004). "Design and Characterization of a Liquid Core Waveguide based Analytical Device for the Analysis of Anaerobic Systems." EPA/ORD/HSRC Superfund Research on Risk Characterization and Monitoring. Las Vegas, NV
- Peter Ruiz-Haas and J.D. Ingle, Jr. (2004). "Evaluation of Redox Conditions with Redox-Indicator Based Sensors in Soil and Microcosms Bioaugmented with Reductive Dehalogenating Bacteria." EPA/ORD/HSRC Superfund Research on Risk Characterization and Monitoring. Las Vegas, NV
- Peter Ruiz-Haas and J.D. Ingle, Jr. (2005). "Monitoring of Redox Conditions with Redox-Indicator Based Sensors in Soil Columns and Microcosms Bioaugmented with Reductive Dehalogenating Bacteria." Joint International Symposia for Subsurface Microbiology and Environmental

Biogeochemistry, Jackson, WY; and American Chemical Society (ACS) Fall National Meeting, Washington, DC.

***Theses:***

Kevin Cantrell (2002), The Development and Characterization of Miniature Spectrometers for Measuring the Redox Status of Environmental Samples, Ph.D.

Sanchai Prayoonpokarach (2003), Development and Evaluation of Sampling Techniques, Instrumentation, and Pyridine Derivative Reagents for Fluorometric Determination of Chloroform and TCE in Water with a Portable Fluorometer

Peter Ruiz-Haas (2006), Monitoring Redox Conditions with Redox Indicators during Microbial Reductive Dechlorination in Microcosms and Bioaugmented Columns

***Students:***

Defne Cakin is a Ph.D. graduate student and is working on developing new monitoring techniques for the project, with a particular emphasis on low level detection of reductants and oxidants with specially developed long-path cells.

Corey Koch is a Ph.D. graduate student and is working on automation and development of microfluidic devices and procedures for field and in-situ determination of environmental information

**2-OSU-07 Continuous Flow Column Studies of Reductive Dehalogenation with Two Different Enriched Cultures: Kinetics, Inhibition, and Monitoring of Microbial Activity**

Lewis Semprini and Mark Dolan, Oregon State University

Alfred Spormann, Stanford University

***Goal:*** In this joint project between Oregon State University and Stanford University, the transformation of chlorinated ethenes in continuous-flow columns is being evaluated with the Victoria Strain (VS) and the Evanite Strain (EV) cultures that have been developed and kinetically characterized in previous WRHSRC projects. Molecular methods are being applied such as FISH and Real-Time PCR to determine the spatial distribution of the cultures and quantify the dehalogenating biomass within the column. RNA-based methods are also being applied to determine energetically based TCE and VC-dehalogenating activity temporally and spatially within the columns.

***Status:*** 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. The results of that work are presented in last year's report. In the second column study performed this year the effects of the pre-reduction of aquifer material was evaluated and a series of transient tests have been performed under flow conditions to determine how the columns responded to gradual increases in PCE concentrations from 0.09-0.27 mM and TCE 0.38-1.52 mM. The aquifer material was chemically pre-reduced *in situ* with a 5 mM Na<sub>2</sub>S solution to reduce excessive Fe(III). With the 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 pre-reduced column. Complete reduction to ETH was observed at lower lactate injection concentrations. The results indicate that in the column study where the 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.

The third column (stainless steel) experiments were conducted to evaluate the reductive dechlorination of TCE 0.17-0.37 mM in Hanford aquifer material after bioaugmentation with the Victoria (VS) culture. The column was chemically pre-reduced with a 5 mM Na<sub>2</sub>S solution. Shortly after VS culture addition, cis-DCE concentrations in the column effluent exceeded the influent TCE concentration indicating enhanced TCE desorption and transformation.. Lactate concentration was increased from 0.67 to 1.24 mM to compensate for hydrogen consumption and sulfate reduction. About 98% of TCE was transformed to ETH at concentrations of 0.17 and 0.37 mM with 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. for molecular analysis

**Microbial diversity of PCE/TCE dechlorinating continuous flow column bioreactors .** The microbial community composition of two different dechlorinating columns 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. 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 *Bacteria* primer. Obtained PCR products were cloned and sequenced. No PCR products were obtained with general archaeal primer indicating that *Archaea* were not detectable by these standard methods.

For the PCE-column 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 as electron a total of 281 full length 16S rRNA gene sequences was 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 are 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<sub>2</sub>, and hydrogen by a diverse population of fermenting bacteria, including *Clostridia*. Homoacetogenic bacteria like *Acetobacterium* relatives use CO<sub>2</sub> and hydrogen to produce acetate. Acetate and hydrogen can serve as electron donor 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, *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 ground water 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.

#### ***Published articles:***

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 Presentations and Abstracts:***

- Behrens, S., J. McMurdie, G. Meshulam, A. Spormann. Evaluation of a CARD-FISH Protocol for the Quantification of *Dehalococcoides* sp. in Soil 2005 ASM General Meeting (6/5/2005 through 6/9/2005).
- 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, 2005.
- 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, 2005.
- Behrens, S., Azizian, M., McMurdie, J., Sabalowsky, A. Dolan, M., Semprini, L., Spormann, A. M. "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, Aug 2006  
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.

***Students Working on the Project:***

Sebastian Behrens (Post doctoral student, Stanford University)

Andrew Sabolowsky (Ph.D. student, Department of Civil, Construction, and Environmental Engineering, Oregon State University)

**2-SU-04 Novel Methods for Laboratory Measurement of Transverse Dispersion in Porous Media**

Peter K. Kitanidis and Craig Criddle, Stanford University

**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 have 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 is 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 is not to determine the column-scale longitudinal dispersion but the transverse dispersion. The principle is to induce shear flow inside the device that creates strong longitudinal dispersion in the observed breakthrough curve; transverse mixing tends to negate the effects of shear flow and thus reduce the observed column-scale longitudinal dispersion. Then, from the spreading of the observed breakthrough curve, we can estimate the unknown, the pore-scale transverse dispersion. The measured quantity varies inversely with transverse dispersion coefficient.

**Status:** The project is now completed and a PhD dissertation has been submitted that will serve 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.

***PhD Dissertations Completed in 2005:***

Benekos, I. D., On the determination of transverse dispersivity: Experiments and simulations in a helix and a cochlea, August 2005, Stanford University.

Luo, J., December 2005, Hydraulic control and reactive transport simulations for in situ bioremediation of uranium-contaminated groundwater, Stanford University.

***Archival Journal Articles:***

Luo, J., and P.K. Kitanidis, Fluid residence times within a recirculation zone created by an extraction-injection well pair, *Journal of Hydrology*, 295, 149-162, doi: 10.1016/j.jhydrol.2004.03.006, 2004.

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

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, doi:10.1029/2005WR004712.

***Presentations at Meetings and Symposia:***

Luo, J., Weber, F.-A., Cirpka, O.A., Wu, W-M., Carley, J., Nyman, J., Jardine, P., Criddle, C.S., Kitanidis, P.K. Reactive transport simulation of a field-scale U(VI) bioremediation experiment. Annul DOE-NABIR FRC Conference, 2005, Oak Ridge, TN.

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

**2-SU-05 Sorption and Hydrolysis of Halogenated Hydrocarbons in Soil Nanopores**

Martin Reinhard, Stanford University, PI

***Goal:*** The overall goal of this project is to develop a better understanding of the impact of soil nanopores on the fate and transport of halogenated hydrocarbon contaminants. Specific project goals are 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 will 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.

**Approach:** A novel analytical system has been developed that allows us to study simultaneously sorption and transformation of volatile organics in geological sorbents. The system consists of the previously (Project 1-SU-03) developed soil column chromatograph, 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.

**Status:** 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  $\mu\text{m}$ ) 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 a 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 have 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  $\mu\text{L/g}$ , while its hydrophobic micropore volume was only 0.022  $\mu\text{L/g}$ . In a microporous silica gel, a hydrophobic micropore volume of 0.038  $\mu\text{L/g}$  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 show that reactive, i.e., hydrolysable contaminants sorbed in slow desorbing sites of geological solids react 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 hinder exchange between the sorption sites and bulk solution thus preventing hydrolysis. As a result, the halogenated hydrocarbon molecules in hydrophobic nanopores are less exposed to water molecules and are prevented from hydrolysis.

**Future work:** With method development completed and reported, results of sorption in hydrophobic micropores from model solids and sediments evaluated and reported, we are now moving towards the study of redox reactions in model systems

***Student working on the project:***

Hefa Cheng

Degree: Ph.D. in Environmental Engineering & Science (2006)

***Publications:***

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

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

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

Cheng, H. and M. Reinhard, "Sorption and Inhibited Dehydrohalogenation of 2,2-Dichloropropane in Micropores of Dealuminated Y Zeolites", Environmental Science & Technology, *accepted December 2006*.

Cheng, H., September 2006, “Sorption and Hydrolysis of Chlorinated Aliphatic Hydrocarbons in Hydrophobic Micropores”, Ph.D. thesis, Department of Civil and Environmental Engineering, Stanford University.

## Outreach Project Reports

### **Technical Outreach Services for Communities (TOSC) and Technical Assistance to Brownfields Communities (TAB) Programs: Kenneth J. Williamson, Director, Oregon State University**

The TOSC and TAB programs involve a staff of faculty, consultants, and graduate research assistants including:

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## Technical Outreach Services for Communities (TOSC)

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

**Rationale:** TOSC provides interested community groups with technical information and assistance that can enable early and meaningful public participation in decisions that affect health and welfare. The TOSC program provides a viable alternative strategy for communities that do not qualify for a Technical Assistance Grant (TAG) from the US Environmental Protection Agency.

**Approach:** The Western Region's outreach program is one of five nationally instituted community outreach programs. Centered at Oregon State University, the TOSC team is comprised of 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 provides communities with technical assistance related to understanding the effects of hazardous waste sites. Where appropriate, WR TOSC partners with staff of the Technical Outreach Services for Native American Communities (TOSNAC).

### WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER

#### Technical Outreach Services for Communities (TOSC) & Technical Assistance to Brownfields Communities (TAB)

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#### TOSC Active Communities

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#### *Region 10*

#### OREGON

**Community Group:** Concerned Citizens for Clean Air

**Site:** Georgia-Pacific Kraft Mill

**Location:** Toledo, OR

**TOSC Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023

**Date TOSC learned about the community:** 9/15/05

**Contaminants:** pulp mill emissions

**Description:** The community is concerned about the health effects of the pulp mill emissions. Community members experience headaches, burning eyes, nausea, and dizziness. They are also concerned about the incidence of cancer.

**Notes on TOSC Activities This Quarter:** TOSC continued to meet with the community group to promote emissions reductions at the Toledo Pulp Mill.

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**Community Group:** Railroad Pollution Coalition

**Site:** Union Pacific Rail Yard

**Location:** Eugene, OR

**TOSC Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023

**Date of Letter Agreement:** December 2004

**Contaminants:** Petroleum, PAHs, chlorinated solvents

**Description:** The community is concerned about cleanup of a Union Pacific Rail Yard in Eugene, OR.

**Items in Letter Agreement:** Review background site investigation documents and revised risk assessment report when available; participate in community group meetings as appropriate.

**Notes on TOSC Activities This Quarter:** No activity this quarter.

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**Community Group:** OSP Community Group

**Site:** Oregon State Penitentiary

**Location:** Salem, OR

**TOSC Contact:** Michael Fernandez, michael.fernandez@orst.edu, 541-737-4023

**Date Letter of Agreement signed:** 8/31/98

**Contaminants:** PCE and TCE

**Description:** TOSC provided assistance to the Oregon State Penitentiary (OSP) community group regarding an imminent 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 through ingestion of local produce, soil contact, and incidental ingestion of soil.

**Items in Letter of Agreement:** Evaluate and comment on IRAM and air stripping towers; provide information on the long and short-term health effects of P/TCE exposure; review and comment on the Human Health Risk Assessment; evaluate air quality concerns in local residential basements; evaluate possible exposures through ingestion of local produce, soil contact, and incidental ingestion of soil.

**Notes on TOSC Activities This Quarter:** No activity this quarter.

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## WASHINGTON

**Community Group:** Skykomish Environmental Coalition

**Site:** Burlington Northern Sante Fe yard

**Location:** Skykomish, WA

**TOSC Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023

**Date Letter of Agreement:** 5/1/04

**Contaminants:** Petroleum (diesel, Bunker C)

**Description:** Community contacted TOSC for assistance in reviewing an RI/FS for this closed railroad facility. There is free product in groundwater as well as lead and arsenic contamination in surface soil.

**Items in Letter of Agreement:** Review investigation and cleanup documents. Participate in community group and formal public meetings.

**Notes on TOSC Activities This Quarter:** No activity this quarter.

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**Community Group:** Spokane Indian Tribe

**Site:** Midnite Mine

**Location:** Spokane, WA

**TOSC Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023

**Date TOSC learned about the community:** 1/1/04

**Contaminants:** uranium, heavy metals

**Description:** TOSC and TOSNAC are providing assistance to the community related to risk assessment and feasibility studies for the mine cleanup.

**Notes on TOSC Activities This Quarter:** No activity this quarter.

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## ***Region 9***

### **CALIFORNIA**

**Community Group:** Perchlorate Citizens Advisory Group (PCAG)

**Location:** San Martin, CA

**TOSC Contact:** Jerry Orlando, Jerry.Orlando@oregonstate.edu, 541-737-5736

**Letter of Agreement:** 6/9/03

**Contaminants:** perchlorate

**Description:** A large number of wells in the San Martin area are contaminated by perchlorate from a now-closed flare manufacturing facility.

**Items in Letter of Agreement:** Review and summarize technical documents and communicate such information. Attend and present information at PCAG meetings as mutually agreed upon.

**Notes on TOSC Activities This Quarter:** TOSC reviewed a feasibility study for cleanup of the Llagas Subbasin prepared by the consultant for the RP and presented it to the community. TOSC also facilitated an open discussion of the Study at the August PCAG meeting.

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**Community Group:** Valley Center

**Location:** Valley Center, CA

**TOSC Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023

**Date Letter of Agreement signed:** 3/14/04

**Contaminants:** pesticides and MTBE

**Description:** The community is concerned about their health and the number of cancer cases among neighborhood children.

**Items in Letter of Agreement:** Help the community understand the epidemiological report concerning childhood cancer and explain the methodology used for data collection and analysis as well as the inclusion/exclusion criteria; explain the results from the MTBE water testing at local schools and the collection and analysis procedures; support the community's request for city and state to test the city water and well water in schools for MTBE and pesticides; help the community convince the City and State to test the soil around the school grounds for pesticides and MTBE; and evaluate the regulatory status of Dursban (chlorpyrifos) and report to the community.

**Notes on TOSC Activities This Quarter:** No activity this quarter.

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**Community Group:** Air Force Plant 42 ERAB

**Site:** Air Force Plant 42

**Location:** Palmdale, CA

**TOSC Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023

**Date Letter of Agreement signed:** 3/1/02

**Contaminants:** TCE in groundwater

**Description:** TOSC is reviewing documents related to cleanup of groundwater contamination.

**Items in Letter of Agreement:** Review remedial investigation and feasibility study and participate in RAB meetings.

**Notes on TOSC Activities This Quarter:** TOSC participated in the September 13 ERAB meeting where the main topics of discussion involved the results of a pilot SVE study on the suspected TCE source area at Site 29, the hanger where TEC was released.

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**Community Group:** Willits Citizens for Environmental Justice

**Site:** Abex-Remco Hydraulics

**Location:** Willits, CA

**TOSC Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023

**Date Letter of Agreement signed:** 4/1/00

**Contaminants:** Hexavalent Chromium in soils and groundwater; TCE and other VOCs in groundwater

**Description:** TOSC has assisted this community during the investigation and remediation of the

Abex-Remco facility. A TOSC member has served on the Site Team, which includes representatives from the community, the Regional Water Quality Control Board, and the California Department of Health Services. TOSC is providing assistance related to health impacts and cleanup of chromium and VOC contamination.

**Items in Letter of Agreement:** Review and comment on remedial investigation reports, sampling plans, health risk assessments; conduct public environmental education workshops.

**Notes on TOSC Activities This Quarter:** TOSC reviewed the Baseline Risk Assessment and submitted comments to the Willits Environmental Remediation Trust.

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**Community Group:** Chester Street Block Club Association

**Location:** Oakland, CA

**TOSC Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023

**Date Letter of Agreement signed:** 9/1/01

**Contaminants:** Lead and vinyl chloride

**Description:** TOSC currently is participating in a series of mediation sessions with community and PRP representatives. The mediation is related to the cleanup of contaminated properties and subsequent development as a neighborhood park. The neighborhood association has filed a Title VI environmental justice complaint against the State of California and the mediation sessions are an attempt to resolve the community's concerns.

**Items in Letter of Agreement:** Agreed to provide technical support for community during the alternative dispute resolution process; this support includes reviewing investigation and cleanup documents for South Prescott Neighborhood Park and participating in mediation meetings.

**Notes on TOSC Activities This Quarter:** No activity this quarter.

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**Community Group:** Fort Ord Environmental Justice Network

**Site:** Fort Ord

**Location:** Marina, CA

**TOSC Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023

**Date Letter of Agreement signed:** 4/1/00

**Contaminants:** Ordnance and explosives, landfill gases, carbon tetrachloride, TCE

**Description:** TOSC is assisting the community in participating in the base cleanup and redevelopment process. TOSC will also assist the community by providing document review and information on health effects.

**Items in Letter of Agreement:** Review and comment on technical documents; assistance in preparing for community meetings with the Army and regulatory agencies; and attending community group meeting and relevant public meetings when possible.

**Notes on TOSC Activities This Quarter:** TOSC continued to provide assistance in preparing the FOEJN quarterly newsletter.

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**Community Group:** Elem Tribe  
**Site:** Sulphur Bank Mercury Mine  
**Location:** Clearlake, CA  
**TOSC Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023  
**Date TOSC learned about the community:** 6/1/2001 (LOA with TOSNAC in 1/99)

**Contaminants:** Mercury and other heavy metals

**Description:** TOSC is providing assistance to the Elem regarding contamination on their reservation and in Clear Lake.

**Items in Letter of Agreement:** Reviewing remedial investigation and feasibility study for the Sulphur Bank Mercury Mine.

**Notes on TOSC Activities This Quarter:** TOSC submitted materials to TOSNAC for a training workshop that will be presented this month.

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**Community Group:** Quemetco  
**Site:** Quemetco  
**Location:** City of Commerce, CA  
**TOSC Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023  
**Draft Letter of Agreement:** 11/8/05

**Contaminants:** lead, sulfuric acid, antimony, arsenic, barium, cadmium, chromium and zinc

**Description:** The community is concerned about hazardous waste releases and air emissions from this battery recycling facility.

**Items in Letter of Agreement:** Review and comment on a risk assessment prepared by the local air quality management district; review and comment on hazardous waste permitting and cleanup documents as needed; participate in community group meeting and make presentations to the group as appropriate.

**Notes on TOSC Activities This Quarter:** No activity this quarter. TOSC will assist the group in holding a public meeting to update local residents on facility activities, the permit appeals process, and possible future community actions.

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**Community Group:** CIAAO  
**Site:** Univar  
**Location:** City of Industry, CA  
**TOSC Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023  
**Draft Letter of Agreement:** 1/12/06  
**Contaminants:** VOCs

**Description:** The community is concerned about the health effects of the contaminants, the best methodology for the cleanup, and the safety issues involved in tearing down contaminated buildings

in the area.

**Items in Letter of Agreement:** Give presentations to the community describing the investigation and cleanup work at the facility and provide information on potential health effects from exposure to site contaminants.

**Notes on TOSC Activities This Quarter:** TOSC provided comments to the community on an air sampling report submitted to the Department of Toxic Substances Control.

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**Community Group:** Wyle Labs CAG

**Site:** Wyle Labs

**Location:** Norco, CA

**TOSC Contact:** Jerry Orlando, jerry.orlando@orst.edu, 541-737-5736

**Date Letter of Agreement Signed:** 03/21/05

**Contaminants:** VOCs, metals

**Description:** Wyle Labs tests products for the military, aerospace and consumer industries on its 429 acre facility in Norco California. In the past two years the area has attracted more development with homeowners expressing concern over suspected contamination from Wyle. Some residents believe adverse health effects can be traced to contamination caused by Wyle's operations.

**Items in Letter of Agreement:** Responding to requests from the CAG for technical information and TOSC activities that will assist the CAG in meeting their goals and mission. Examples of such activities are information retrieval, technical document review and technical training sessions. Attendance at CAG meetings or community meetings where appropriate to assist in the dissemination of information or to perform technical training.

**Notes on TOSC Activities This Quarter:** TOSC reviewed a radiation scan done on the Wyle property and answered community questions about detecting radiation, specifically depleted uranium in soil.

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**Community Group:** Collective Roots

**Location:** East Palo Alto, California

**TOSC Contact:** Jerry Orlando, jerry.orlando@oregonstate.edu, 541-737-5736

**Date TOSC learned about the community:** July 2006

**Contaminants:** hydroxylamine, monoethanolamine, toluene and acetonitrile.

**Description:** An accidental release of a 4000 gallon mixture of waste chemicals occurred at the Romic recycling site in the form of a fine mist that spread across 2 acres of the community.

**Notes on TOSC Activities This Quarter:** TOSC worked briefly with this community in August, reviewing data and answering questions about the explosion's possible effect on the garden.

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**ARIZONA**

**Community Group:** Dewey-Humboldt Town Council/ Dewey Humboldt Community Organization

**Location:** Dewey-Humboldt, AZ

**TOSC Contact:** Jerry Orlando, jerry.orlando@oregonstate.edu, 541-737-5736

**Date Letter of Agreement signed:** 07/06/06

**Contaminants:** Lead, Arsenic

**Description:** The Arizona DEQ alerted TOSC about this community. The community has several environmental issues pending including a voluntary cleanup in process, an old mine site and an EPA NPL listing investigation. After speaking with the EPA project manager for the area, TOSC decided to see if there is a need for their services.

**Notes on TOSC Activities This Quarter:** TOSC reviewed a Dust Control Plan prepared by an industry in the area and offered comments and a comparison to the OSHA Dust Control Handbook.

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**TOSC  
Potential Communities**

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***Region 10***

**CALIFORNIA**

**Community Group:** Citizens for Real Economic Growth

**Location:** Eureka Rail Yard Site

**TOSC Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023

**Date TOSC learned about the community:** July 2006

**Contaminants:** TOSC has not received information about site-specific conditions but it is anticipated that the site will have petroleum fuels (diesel and/or Bunker C), PAHs, metals, and solvents in soil and groundwater.

**Description:** CREG is concerned about potential cleanup redevelopment activities at the site. TOSC expects to review a draft EIR and other site investigation and cleanup documents.

**Notes on TOSC Activities This Quarter:** TOSC contacted the North Coast Regional Water Quality Control Board to gather information about the site and prepare for EIR review.

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**TOSC  
Inactive Communities**

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**ARIZONA**

Downtown Southwest Neighborhood Association

**OREGON**

Dallas

Portland Harbor Community Advisory Group

## CALIFORNIA

Tustin RAB  
South Bay Cares  
Protect Our Neighborhood Committee  
Alameda Point Collaborative

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## TAB ACTIVE COMMUNITIES

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### *Region 10*

## OREGON

**Organization:** Oregon DEQ Brownfields Program

**TAB Contact:** Jerry Orlando, jerry.orlando@oregonstate.edu, 541-737-5736

**Description:** TAB acts as a resource to the ODEQ brownfields program.

**Notes on TAB Activities This Quarter:** TAB attends the quarterly networking meetings. TAB is also working with the DEQ on their volunteer-based brownfields inventory project, trying to locate a student group or RSVP volunteer group to participate in another small city in Oregon.

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**Organization:** Oregon Brownfields Conference

**TAB Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023

**Description:** TAB acts as a resource to the Oregon Brownfields Conference.

**Notes on TAB Activities This Quarter:** TAB is on the planning committee for the latest conference to be held in March of 2007.

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## IDAHO

**Organization:** Idaho Department of Environmental Quality

**Location:** Six communities throughout Idaho

**TAB primary contact:** Jerry Orlando, Jerry.Orlando@oregonstate.edu, 541-737-5736

**Description:** The Idaho DEQ is planning on taking the Oregon DEQ St. Helens RSVP project (using retired community volunteers to develop a brownfields inventory and promote brownfields redevelopment in their communities) to six cities in Idaho.

**Notes on TAB Activities This Quarter:** TAB participated in the training of 4 volunteers in Sandpoint, Idaho. The volunteers are going to build a brownfields inventory for the small adjacent cities of Sandpoint, Kootenai, Dover and Ponderay.

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**TAB**  
**Potential Communities**

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**OREGON**

**Organization:** Metro Regional Government (Portland, Oregon)

**TAB Contact:** Jerry Orlando, jerry.orlando@oregonstate.edu, 541-737-5736

**Description:** Metro, the regional government with jurisdiction over the Portland metro area, received a 2006 EPA Brownfields Assessment Grant.

**Notes on TAB Activities This Quarter:** The brownfields contact at Metro has moved to another position but has told TAB that the new person will be in contact when the position is filled.

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**TAB**  
**Inactive Communities**

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Retired and Senior Volunteer Program (St. Helens, Oregon)

Lula Washington Dance Theatre (Los Angeles, CA)

## Training and Technology Transfer

WRHSRC training focuses on educating graduate students. As shown in Table 7 below, a total of 16 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**

<u>Student</u>	<u>Field</u>	<u>Degree/ Institution/Graduation</u>	<u>Project</u>
Sebastian Behrens	Environmental Engineering	Post-Doctoral/Stanford	2-OSU-07
Ioannis Benekos	Environmental Engineering	Ph.D./Stanford/2005	2-SU-04
Tina Blatchford	Environmental Engineering	M.S./Oregon State University/2005	1-OSU-02
Defne Cakin	Chemistry	Ph.D./ Oregon State University /2007	2-OSU-06
Hefa Cheng	Environmental Engineering	Ph.D./Stanford/2006	2-SU-05
David Doughty	Microbiology	M.S./Oregon State University /2004	2-OSU-05
Kim Halsey	Molecular and Cellular Biology	Ph.D./ Oregon State University /2006	2-OSU-05
Jae-Hyuk Lee	Environmental Engineering	Ph.D./ Oregon State University /2006	1-OSU-03
Bhargavi Maremanda	Environmental Engineering	M.S./Oregon State University /2004	1-OSU-02
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 /2007	2-OSU-07
Corey Koch	Chemistry	Ph.D./ Oregon State University	2-OSU-06
Kristin Skinner	Molecular and Cellular Biology	Ph.D./ Oregon State University /2007	1-OSU-02
Anne Taylor	Environmental Engineering	Ph.D./ Oregon State University /2007/	1-OSU-05
Seungho Yu	Environmental Engineering	Ph.D./ Oregon State University /2004	2-OSU-07

## Technology Transfer

The goals of the technology transfer program are 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:** In 2006, 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/> provides an overview of the WRHSRC and links to publications and project information. The website includes:

- ◇ 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. Research Brief #10 was added this year. It describes work by Alfred Spormann and Sebastian Behrens (Stanford University) to apply polymerase chain reaction (PCR) and fluorescence in situ hybridization (FISH) for monitoring bioremediation in situ ([http://wrhsrc.oregonstate.edu/briefs/brief\\_10.htm](http://wrhsrc.oregonstate.edu/briefs/brief_10.htm)).

During 2006, 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.

# 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:** In 2006, WRLTC-OSU offered 24 certification courses which were attended by 212 students. These courses were conducted in Oregon City, OR (20), Yakima, WA (2), Anchorage, AK (2).

Under the Oregon DHS Lead Program/EPA Community Outreach Training Grant, 36 students attended 3 Lead-Safe Work Practices. These workshops were held in Oregon City (1), Medford (2). The State of Oregon DHS Lead Program has extended grant funding through September 2007 for additional LBP abatement training and outreach activities throughout Oregon.

## 2006 WRHSRC Publications

Publications for 2006 listed below have resulted from work funded by the WRHSRC. We continue to maintain the database for publications from both the original and current Center.

During 2006 a total of 10 journal articles have appeared, accepted, or have been submitted for publication. All or part of the research was supported through Center funds. Center researchers have also published in-bound conference proceedings, and have been active in conference participation. Also during 2006, three Ph.D. theses were submitted.

### Journal Articles (also includes those in press and submitted)

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, doi:10.1029/2005WR004712.

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, "Sorption and Inhibited Dehydrohalogenation of 2,2-Dichloropropane in Micropores of Dealuminated Y Zeolites", *Environmental Science & Technology*, accepted December 2006.

- Doughty, D.M., L.A., Sayavedra-Soto, D.J. Arp and P.J. Bottomley (2006) Product repression of butane monooxygenase expression in '*Pseudomonas butanovora*'. *J. Bacteriol.* 188: 2586-2592.
- Halsey, K.H., L.A., Sayavedra-Soto, Bottomley, P.J., and D.J. Arp (2006) Site-directed amino acid substitutions in the hydroxylase  $\alpha$  subunit of butane monooxygenase from *Pseudomonas butanovora*: implications for substrates knocking at the gate. *J. Bacteriol.* 188: 4962-4969.
- Luo, J., Cirpka, O.A., Kitanidis, P.K. (2006) Temporal-moment matching for truncated breakthrough curves for step or step-pulse injection. *Adv. Water Resources.* 29(9), 1306-1313.
- 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.
- Ruiz-Haas, P. and Ingle, J.D, Jr. (2006) "Applications of Sensing Devices based on Redox Indicators for Non-invasive and In-situ Monitoring of Redox Conditions during Reductive Dehalogenation of PCE in Packed Columns.", *Geomicrobiology Journal*, in press .
- Taylor, A.E., M. E. Dolan, P.J. Bottomley, and L. Semprini . Utilization of Fluoroethene as a Surrogate for Aerobic Vinyl Chloride Degradation. Submitted to *Environ. Science & Technology*.

### **Conference 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.
- Behrens, S., Azizian, M., McMurdie, J., Sabalowsky, A. Dolan, M., Semprini, L., Spormann, A. M. "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, Aug 2006.

### **Thesis**

- Cheng, H., September 2006, "Sorption and Hydrolysis of Chlorinated Aliphatic Hydrocarbons in Hydrophobic Micropores", Ph.D. thesis, Department of Civil and Environmental Engineering, Stanford University.
- Lee, Jae-Hyuk (2006). Anaerobic reductive dechlorination of TCE and TCFE in TCE contaminated sediments : enhanced bioremediation and bioaugmentation. Ph.D. Oregon State University.
- Ruiz-Haas, Peter (2006), Monitoring Redox Conditions with Redox Indicators during Microbial Reductive Dechlorination in Microcosms and Bioaugmented Columns, Ph.D. Oregon State University.