WESTERN REGION
HAZARDOUS SUBSTANCE RESEARCH CENTER

2005 ANNUAL REPORT

Oregon State University
Stanford University

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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 2005 fiscal year and since the Center's inception are listed by category of support in Table 4. During the fourth year of operation, core funding totaled $958,160. The distribution of the Center's $868,160 of base EPA funding is shown in Figure 1. Over 57% of the funds go directly to the research program.

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. Twelve graduate students have been supported during the fourth year of the Center, with ten of these being Ph.D. students. Over 60% of the Center core funds are being directed toward the graduate training of students through the Center's research and outreach projects.

Table 1. Key Personnel at the WRHSRC

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

Table 2. Science Advisory Committee

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

Table 3. Outreach Advisory Committee

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

Table 4. Center Funding

Funding Sources	FY 2001	FY 2002	FY 2003	FY 2004	Total Date*
EPA: Centers Program	$900,000	$885,000	$885,000	$868,160	$ 3,538,200
EPA: Brownfields	150,000	150,000	127,000	**	427,000
Oregon State University	90,000	90,000	90,000	90,000	360,000
TOTAL	$ 1,140,000	$1,125,000	$ 1,102,500	$ 958,160	$ 4,325,200

*Sept. 1, 2004- . Aug 30, 2005

**Brownfields became a separate grant in FY Sept 2004

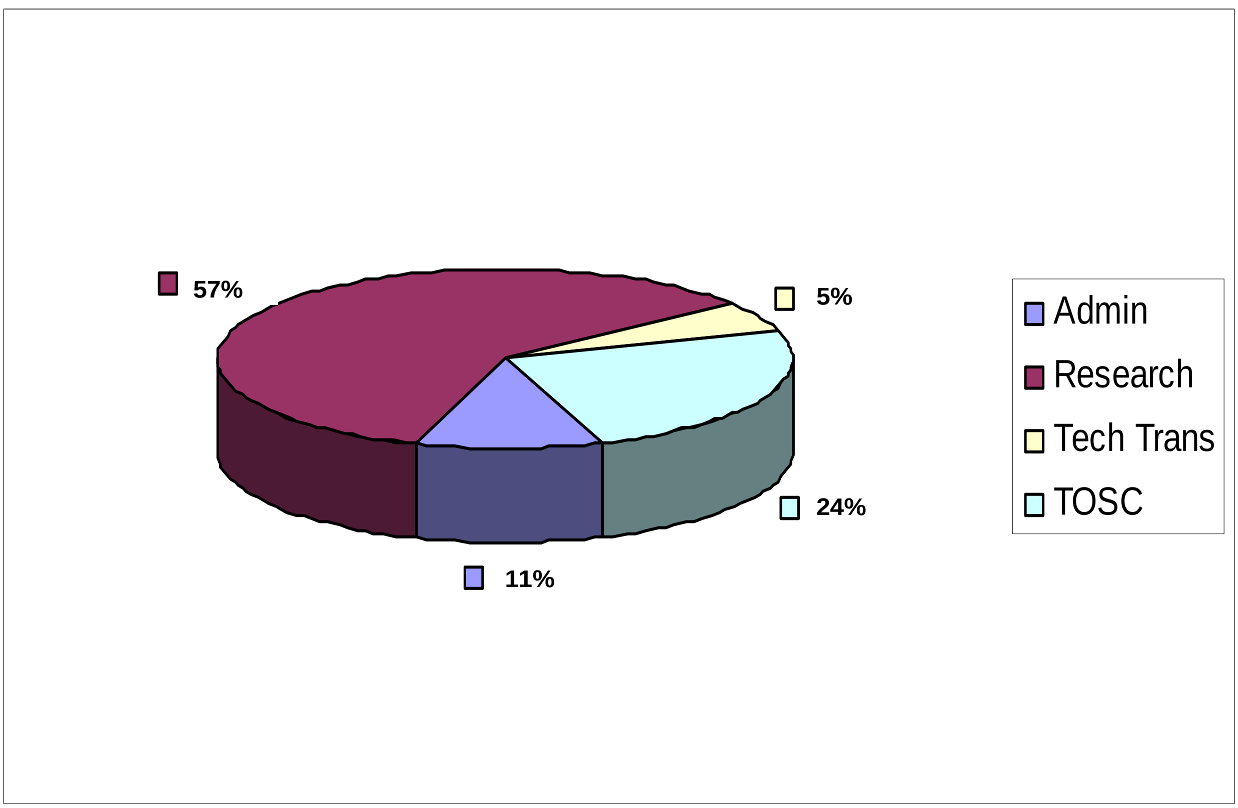
Table 5. Students Supported

Student Support	FY 2001	FY 2002	FY 2003	FY 2004	Funds to Date†
M.S.	2	2	2	2	$ 300,000
Ph.D.	9	14	10	10	$ 1,777,800
Post Doctoral	0	0	2	2	$ 270,000
TOTAL	11	16	14	14	$ 2,347,800

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

† Includes tuition, stipends travel, supplies, etc.

Figure 1.



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.

Table 6. RESEARCH PROJECT SUMMARY

Project	Title	PI Co-PIs	Year 1 Budget 2004	Year 2 Budget 2005
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
2-OSU-06	Development and Evaluation of Field Sensors for Monitoring Anaerobic Dehalogenation After Bioaugmenatation	James Ingle, PI, Oregon State University	$ 55,620	$ 59,610
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
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
2-SU-05	Sorption and Hydrolysis of Halogenated Hydrocarbons in Soil Nanopores	Martin Reinhard, PI	$ 90,000	$ 90,000

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). In a study being directed by Peter Bottomley and Daniel Arp, conditions are being identified that maximize reductant flow to cometabolism and that promote maximum expression of monooxygenase genes and enzyme activity. Mutant strains of *P. butanovora* containing single amino acid substitutions to BMO were engineered. One mutant strain, in particular, degraded a larger quantity of TCE, compared to the wild type strain, albeit at a slower rate. Currently it is being determined if mutant strains with slower turnover rates will result in sustainable TCE degradation. Preliminary *in vivo* studies *P. butanovora* indicate both propionate and butyrate irreversibly inactivated the sBMO enzyme. The possibility that organic acids act as mechanism based inactivators of the sBMO enzyme in currently being explored with a range of organic acids.

In a study being directed by Mark Dolan and Lewis Semprini, cultures are being characterized that can transform cis-DCE and VC when grown on acetate, propionate, and butyrate. In addition, cultures are being isolated and characterized that can grow directly on VC. *Mycobacterium* strain JS60 was found to grow on acetate, propionate, and butyrate, but could not grow on formate or lactate. When acetate was used an augmenting growth substrate, ethylene and VC utilization rates increased. When growing on acetate, strain JS60 cometabolized c-DCE and t-DCE, but not 1,1-DCE, with c-DCE transformed more rapidly than t-DCE. JS60 is able to cometabolize fluoroethene (FE) and directly grow 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.

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 fiber optic probe to monitor redox status has been developed with immobilized redox indicator film at its tip. The probe is used is conjunction with a light source and CCD spectrometer to monitor the absorbance of the indicator. This probe can be easily positioned in soil columns and provides for true in-situ monitoring. Redox indicators placed on the fiber optic probe respond comparably to those installed in flow cells and connected by flow loops and allow for true in-situ monitoring. A new method has been developed to determine the "reductive capacity" (RC) or "effective concentrations of reductants" in aqueous anaerobic samples taken from microcosm bottles, soil columns, and reactors. This technique has been successful at identifying significant changes in the redox conditions in columns and microcosms (e.g., a 50% drop in RC when the column is disturbed). The column was packed with sediment from the Hanford site and inoculated with the Evanite culture. As observed during previous microcosm experiments with the Evanite culture, the indicator cresyl violet is about ½ or fully reduced at all ports indicating suitable conditions for dehalogenation. The researchers have successfully used the redox flow sensor and the fiber optic probe to monitoring redox conditions in the columns studies of project 2-OSU-7, described below. The researchers anticipate the eventual automation of this measurement to allow for continuous monitoring.

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, such as FISH and Real-Time PCR, are being used to determine the spatial distribution of the cultures and quantify the dehalogenating biomass within the columns. RNA-based methods are also being applied to determine energetically based TCE and VC-dehalogenating activity temporally and spatially within the column. A second series of column tests were initiated this year. The columns were sized so that samples could be obtained for concentration measurements are three spatial locations along the column as well as the column exit, and so solid coupons could be collected at three different spatial locations to enumerate microbial activity. A series of transient tests have been performed with gradual increases in PCE concentration from 10 mg/L to 50 mg/L. Compete transformation of VC to ethene was achieved with a hydraulic residence time of 4.5 days, and PCE is transformed to VC and ethene within a hydraulic residence to o 1.5 days.

Relative abundance of selected genes catalyzing the stepwise dechlorination of PCE to ethene in the first column study were determined . The tracked genes are specific to subpopulations of the genus *Dehalococcoides* indicating functional and spatial differences in the localization of different *Dehalococcoides* strains along the columns vertical profile. The primer for the TCE and VC reductive dehalogenases were further used in real-time reverse transcription (RT-) PCR experiments to study gene expression along the vertical horizon of the first reactor. The results showed that RT-PCR could be used to quantify the abundance and activity of genes involved in the reductive dehalogenation of PCE under bioremedination conditions. The protocol and oligonucleotide primer developed in this study are powerful tools for monitoring of intrinsic transformations and the evaluation of laboratory scale bioreactors and field sites undergoing bioremediation.

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. New methods were developed for the measurement of local transverse dispersion in isotropic porous media based on helical and cochlea-like devices. Experiments were performed similar to the tracer test through a laboratory column packed with a porous medium and to measure the breakthrough curve; however, the objective was not to determine the column-scale longitudinal dispersion but the transverse dispersion. The experiments showed relative advantages of each device, instrument, and methodology that were used to estimate transverse dispersivity. 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 were estimated agrees with the higher ratios reported in the literature.

Project 2-SU-05 is a project at Stanford University under the direction of Martin Reinhard that 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. Recent work focused on sorption of TCE in zeolites with a range of hydrophobic surface properties. 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 the micropores. Their 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. As a result, the halogenated hydrocarbon molecules in hydrophobic nanopores are less exposed to water molecules and are prevented from hydrolysis.

Training and Technology Transfer

Training and technology transfer activities included providing information on the Center's activities through our Website, the development of Research Briefs on the results of Center research projects, development of online tutorials, and presentations at conferences and workshops. Our Website usage has continued to increase, with over 3000 visits during the month of September 2005. Four Research Briefs, representing short summaries of Center projects, were developed to communicate our research results to practitioners and others interested in emerging cleanup technologies. The Research Briefs were advertised through our Center's electronic newsletter, Tech Direct, USEPA Region 10 Science Forum, and USEPA Region 9 Hazardous Substance Technical Liaison Newsletter, as well as the NIEHS research briefs distribution list. A tutorial was developed for simulating groundwater remediation using Interactive Groundwater Model 3.5.6, with a focus on modeling pump-and-treat. WRHSRC researchers continue to be active in conferences and workshops. For example, eight presentations were given by WRHSRC researchers at the Joint International Symposium for Subsurface Microbiology (ISSM 2005) and Environmental Biogeochemistry (ISEM XVII) in Jackson Hole, Wyoming, last August. The WRHSRC continues to training of graduate students. Over the past year two MS theses and three PhD theses were completed on research funded by the WRHSRC.

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 Oregon (3), Washington (2), and

California (8) (the number following the state designates the number of communities in each state).

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 two communities in Oregon and one in Idaho. TAB participated in the 2005 Oregon Brownfields Conference by organizing two sessions (Brownfields Basics and Meaningful Community Involvement) and making a short movie about brownfields redevelopment in three communities in Oregon.

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. Peter Bottomley, PI; Daniel Arp, Mark Dolan, Lewis Semprini, Co-PIs, Oregon State University

Part I: Aerobic cometabolism of chlorinated aliphatic hydrocarbon compounds with butane-grown microorganisms. Investigators: P. J. Bottomley and D.J. Arp.

Goal: This research 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 that maximize reductant flow to cometabolism, and that promote maximum expression of monooxygenase genes and enzyme activity.

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 examined the chloroethenes degrading properties of several individual strains of butane-oxidizing bacteria (*Pseudomonas butanovora*, *Nocardioides* CF8, and *Mycobacterium vaccae* JOB5) that are genotypically distinct from each other, and that are known to possess distinctly different butane monooxygenases (BMO). We have examined the impact of cometabolism of different chloroethenes on monooxygenase activity, and assessed the effect of cometabolism on cell viability.

(b) We have conducted an examination of the cometabolism of the lesser-chlorinated dichloroethenes (DCEs) by *P. butanovora*, because they are often persistent products of reductive dechlorination at field sites. In this study we have focused upon the abilities of different electron donors to drive DCE cooxidation by butane and propane-grown cells, and to study why different electron donors show different efficacies in sustaining cooxidation. In addition, we have examined the ability of DCEs to induce the alkane monooxygenase of *P. butanovora*.

Status:
a) Mutant strains of *P. butanovora* containing single amino acid substitutions to BMO were engineered. Studies of TCE degradation using these mutants have revealed differences in rates of TCE turnover between mutant strains. One mutant strain, in particular, degraded a larger quantity of TCE, compared to the wild type strain, albeit at a slower rate. We are interested in determining if mutant strains with slower turnover rates will result in sustainable TCE degradation.

b) Recent studies have focused upon a comparison of the efficacy of different electron donors for cooxidation of DCEs by butane and propane-grown *P. butanovora*. Although propionate is an effective electron donor that supports DCE oxidation in propane-grown cells, it does not support cooxidation in butane-grown cells. Further studies have shown propionate catabolism was inactive following growth on either butane or ethane. In contrast, propionate consumption was induced (about 80 nmoles propionate consumed$\times$min$^{-1}\times$mg protein^{-1}) following growth on the odd chain-length alkanes, propane and pentane. Using degenerate primers genes have been identified on a 30 Kb fragment of DNA that show close homology to propionyl-CoA carboxylase subunits, and to methylmalonyl-CoA mutase subunits. If these genes produce active protein products then it seems reasonable to infer that propionate utilization in *P. butanovora* proceeds via methylmalonyl-CoA and succinyl-CoA into the TCA cycle.

Although the aliphatic organic acids, propionate and butyrate, initially enhanced the rate of ethene and chloroethene cooxidation by sBMO, these initial rates of ethene cooxidation could not be sustained for greater than 10 min. Preliminary *in vivo* studies indicate both propionate and butyrate irreversibly inactivated the sBMO enzyme. The rate of ethene cooxidation by BMO was reduced to one half of the initial rate following 15 min. of exposure to either 10mM butyrate or propionate. Furthermore, the physiological substrate, butane, was able to protect the sBMO enzyme from the effect of either propionate or butyrate. The possibility that organic acids act as mechanism based inactivators of the sBMO enzyme in currently being explored. This project will characterize the effects of a range of organic acids on sBMO and to extend our observations of organic acid-dependent inactivation to other monooxygenase enzymes. The long range plans for this project will be to explore the mechanism of inactivation. Both a bacterial aromatic oxygenase and a peroxidase were irreversibly inactivated during the catalytic oxidation of either phenylpropionaldehyde and phenylbutyraldehyde (Raner et al., 2000) or by aliphatic fatty acids, in general (Huang et al., 2004). If a similar phenomenon occurs in sBMO, it would provide a mechanism to explain why the aliphatic fatty acids, in particular, did not sustain BMO activity.

Because the potential of *P. butanovora* to degrade chlorinated ethenes is intimately linked to the expression of the sBMO enzyme we have studied the regulation of sBMO. Preliminary experiments showed that cells of *P. butanovora* grown on alkanes C2 through C5 achieved similar maximum levels of sBMO activity (~160 nmoles ethene oxide x min^{-1} x mg protein^{-1}), the up-regulation of sBMO activity in lactate-grown, sBMO repressed cells was consistently delayed in propane-exposed cells relative to butane. Furthermore, when lactate-grown cells were exposed to butane and propane simultaneously, the presence of propane reduced the ability of butane to induce sBMO activity, indicating that the lag in sBMO induction during exposure to propane is due to repression by propane rather than to its inability to induce sBMO. The repressive behavior of propane was extended to other odd chain alkanes when it was shown that butane induction of sBMO activity could be aborted by additions of either propane, or pentane to cultures already actively inducing sBMO activity. In contrast, the increase in sBMO activity was unaffected by additions of ethane or more butane. These data indicate that propane and pentane were capable of suppressing sBMO activity in *P. butanovora*, despite their ability to promote sBMO activity when provided as sole growth substrates. As mentioned above, the pathway of propane and pentane consumption is blocked at propionate during growth on either ethane or butane. We believe sBMO expression, in *P. butanovora*, is repressed in situations where propionic acid accumulates. This need to have the propionate catabolic pathway induced led to the striking disparity in the ability of even chain-length vs. odd chain-length alkanes and alcohols to induce BMO expression.

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

Investigators: M. Dolan and L. Semprini

Goal: A primary goal of the proposed work is to isolate and characterize pure cultures that can transform cis-DCE and VC when grown on acetate, propionate, and butyrate. Initially, the proposal was to attempt isolation of members of an enrichment culture, BA-1, known to co-oxidize cis-DCE when grown on organic acids. However, upon recommendation of the SAC, our initial focus has changed to isolation and metabolic evaluation of cultures able to directly metabolize cis-DCE and VC.

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. Unlike the VC-utilizing organisms, to date, no organisms capable of direct metabolism of cis-DCE have been isolated from aquifer solids or groundwater samples. Two recent field studies on co-metabolic transformation of chlorinated ethenes performed in contaminated zones at Ft. Lewis, WA, and McClellan AFB, CA, showed effective TCE and cis-DCE transformation upon stimulation of the microbial population with toluene or propane. However, after terminating substrate addition, TCE concentrations were observed to rebound to near pre-treatment levels while cis-DCE concentrations remained very low over extended periods. Therefore, organisms may exist at the sites capable of direct metabolism of cis-DCE which may have been directly or indirectly stimulated by the addition of toluene or propane.

Experimental Approaches: Groundwater samples were obtained at Ft. Lewis, WA, from stimulated and control monitoring wells used in a push-pull experiment to investigate cometabolic TCE and cis-DCE transformation upon stimulation with toluene. Microbial community composition as measured by terminal restriction fragment length polymorphism (T-RFLP) analysis showed considerable community shift as a result of toluene stimulation. Groundwater samples from non-perturbed wells and from toluene-stimulated wells were amended with mineral salts medium (MSM) and a single (carbonaceous) substrate of cis-DCE, ethene, or toluene and monitored for substrate depletion and increased turbidity as an indication of microbial growth. After repeated cycles of enrichment, the cultures were again analyzed for microbial community composition and efforts were begun to isolate cultures from these enrichments. Also, sub-samples of the ethene-enriched systems were used to test for the ability of the enrichments to grow on either VC or fluoroethene (FE), a fluorine-substituted analog of VC.

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 known 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: 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, EE13A, which grows on ethene and cometabolizes VC and FE, was obtained. The mixed cultures were streaked on MSM plates and incubated in jars with ethene, VC or FE in the headspace. Colonies that grew under these conditions were streaked to tryptic soy agar plates to check for purity, and representative colonies inoculated into MSM with either ethene, FE or VC as growth substrates. Isolated cultures will be checked for purity and phylogeny will be established based on their 16S rDNA sequence and compared to known cultures of VC utilizing organisms. Further study 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 will be conducted.

Further studies are being conducted with three phenotypes of Eth utilizing bacteria that 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. Preliminary kinetic studies with JS60, JS614 and EE13A show that FE and VC are transformed at approximately the same rate regardless of whether degradation is achieved by direct or co-metabolism. Additionally, significant halogen release was observed for both direct and cometabolic degradation of FE and VC. Degradation intermediates during cometabolism are being determined to allow for a mass balance of halide-associated compounds, and to assist in the development of a hypothetical pathway for degradation during cometabolism.

Mycobacterium strain JS60 was also found to grow on acetate, propionate, and butyrate, but could not grow on formate or lactate. Acetate was chosen for further study because strain JS60 consumed acetate the most rapidly of all the organic acids tested, and acetate is a common product of fermentation reactions in the subsurface. Comparatively, strain JS60's rate of growth on VC is much slower than that of ethylene. With acetate as an augmenting growth substrate, ethylene and VC utilization rates increased by 30% and 48%, respectively. When strain JS60 was exposed to the isomers of DCE (trans-1,2-dichloroethylene (t-DCE), cis-1,2-dichloroethylene (c-DCE), and 1,1-dichloroethylene (1,1-DCE)) the cells were unable to grow on these compounds. However, when growing on acetate, strain JS60 cometabolized c-DCE and t-DCE, but not 1,1-DCE, with c-DCE transformed more rapidly than t-DCE.

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(a) Journal articles

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2. Halsey, K.H., P.J. Bottomley and D.J. Arp. 2005. TCE degradation by butane-oxidizing bacteria causes a spectrum of toxic effects. Applied Microbiology and Biotechnology 68:794-801
3. Doughty, D.M., L.A. Sayavedro-Soto, D.J. Arp, and P.J. Bottomley. 2005. Effects of dichloroethene isomers on the induction and activity of butane monooxygenase in the alkane-oxidizing bacterium, *'Pseudomonas butanovora'*. Applied and Environmental Microbiology 71(10): 6054-6059.
4. Doughty, D.M., L.A. Sayavedro-Soto, D.J. Arp, and P.J. Bottomley. 2004. In review. Product repression of alkane monooxygenase expression in *Pseudomonas butanovora*. J. Bacteriol.
5. Kim, Y. and L. Semprini. Cometabolic Transformation of cis-1,2-dichloroethylene and cis-1,2-dichloroethylene epoxide by a butane-grown mixed culture. 2005. *Water Science & Technology* Vol 52 (8): 125-131.
6. Razzetti,C. L. Semprini, M. Dolan, Cometabolic Degradation Of 1,1,1-Trichloroethane And 1,1-Dichloroethane By A Butane Grown Rhodococcus Sp.: Kinetic, Toxicity and Inhibition Studies, accepted by Environmental Science and Pollution Research.

(b) Abstracts

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 3rd 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.

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 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 systems in columns and PAMs packed with soil, microcosm bottles, and sub-surface systems in the field.

Approach: Redox indicators immobilized on transparent films have been shown to 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 halorespiratory cultures as a tool for spatial monitoring of dechlorination and to improve conditions necessary for effective dechlorination of PCE and TCE. The design and characteristics of the redox sensor monitoring systems will be improved for low oxygen permeation and portability for easy operation in the lab and field. In addition, we seek to investigate alternative sampling/reagent/detection systems, quantitative measurement of concentrations of reductants, and fiber optic sensors. Other probe species such as quinones may provide unique information about dechlorinating activity.

Status: We have improved portable, flow-based monitoring systems based on immobilized redox indicators and used them to successfully examine redox conditions in microcosm and bottles containing a dechlorinating culture (Evanite culture). We developed a new method to determine the "reductive capacity" (RC) or "effective concentrations of reductants" in aqueous anaerobic samples taken from microcosm bottles and aquifer columns. A small volume of deaerated redox indicator solution in a septum-sealed, spectrometer cuvette is mixed and reacted with an aqueous sample obtained with a gas-tight syringe from a packed column or microcosm bottle. Reductants in the sample reduce the indicator if the formal potential of the reductant couple is equal or below that of the indicator. From the decrease in absorbance of the indicator, the "reductive capacity" can be calculated which is typically 200 to 600 μM for active microcosms and 50-300 μM in bioaugmented packed columns under active reductive dehalogenation. This technique has been successful at identifying significant changes in the redox conditions in columns and microcosms (e.g., a 50% drop in RC when the column is disturbed). We anticipate the eventual automation of this measurement to allow for continuous monitoring.

We have employed a fiber optic probe with immobilized redox indicator film at its tip to monitor redox status. The probe is used is conjunction with a light source and CCD spectrometer to monitor the absorbance of the indicator. This probe can be easily positioned in soil columns and provides for true in-situ monitoring. Redox indicators placed on the fiber optic probe respond comparably to those installed in flow cells and connected by flow loops and allow for true in-situ monitoring.

To evaluate sensors and probes, we have constructed a PVC-based column with suitable ¼-28 ports for installation of redox flow monitoring setups and ½-20 ports to install the fiber optic probe. The column was packed with sediment from the Hanford DOE site and inoculated with the Evanite culture. As observed during previous microcosm experiments with the Evanite culture, the indicator cresyl violet is about ½ or fully reduced at all ports indicating suitable conditions for dehalogenation. Also, we have successfully used the redox flow sensor and the fiber optic probe for monitoring conditions in and columns in labs in environmental engineering.

We have begun to probe the applications of other redox indicators, particularly quinones, and vitamin B_{12} to the array of indicators available to study environmental redox processes. Quinones are of particular interest as they participate in numerous cellular reactions and appear to influence "reductive capacity" measurements.

Also, we have evaluated long-path flow cells and automated syringes for improved on-line measurements of redox-active species (e.g., S(-II), Fe(+II)) and for push-pull sensing methods. We have begun research on construction of a lab-on-a-chip device that can be employed for on-site or in-situ measurements of environmentally relevant redox species.

Journal Publications:
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..2005. Monitoring of Redox State in a Dechlorinating Culture with Redox Indicators., *Journal of Environmental Monitoring*, in preparation.

Ruiz-Haas, P. and Ingle, J.D, Jr.. 2005. 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*, invited paper in preparation.

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. Ruiz-Haas, P. and Ingle, J.D., 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 Thesis, Department of Chemistry, Oregon State University.

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 Ph.D Thesis, Department of Chemistry, Oregon State University.

Current students on project:
Peter Ruiz-Haas is the primary Ph.D. graduate student on the project.

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

Goals: This project is evaluating the transformation of chlorinated ethenes in continuous-flow column studies with the Evanite (EV) culture and the Victoria Strain (VS) culture that have been developed and kinetically characterized by Yu and Semprini, 2004; Yu et al. 2005; and Cupples et al 2004a; 2004b. The overall goals of the project are to 1) determine if kinetic parameters that were derived under batch conditions can be used to model the sequential transformation of chlorinated ethenes spatially in the columns; 2) evaluate if the predicted performance of the two enrichment cultures is achieved and to test methods that may distinguish the VS from the EV culture; 3) apply molecular methods such as FISH and Real-Time PCR to determine the spatial distribution of the cultures and quantify the dehalogenating biomass within the column; 4) apply RNA-based methods to determine energetically based TCE and VC-dehalogenating activity temporally and spatially within the column; 5) apply molecular based activity tests, such as transformation of fluorinated analogs, to determine dehalogenating activity that develops within the column; 6) study toxicity and inhibition that may result from the presence of co-contaminants, such as chloroform or acetylene; and 7) compare the results from modeling, molecular, and activity based results.

Rationale: Biologically driven reductive dehalogenation is becoming a commonly used process for remediating groundwater contaminated with chlorinated ethenes and mixtures of other chlorinated aliphatic hydrocarbons. Several studies have now demonstrated that engineered systems of enhanced reductive dehalogenation can result in complete dehaogenation of PCE and TCE to ethene. Bioaugmentation of microbial consortium that contain phylogenetic relatives of *Dehaloccoides etheneogenes* has promoted the complete dehalogenation of PCE or TCE to ethene. Remediation of source zones containing high concentrations of PCE and TCE via reductive halogenation is also being considered. Few studies have been performed that have evaluated changes in community structure and function under flow conditions where spatial and temporal changes in transformation and community structure can result. Column studies to date have not been performed with cultures with well defined kinetic parameters or have employed RNA-based methods to characterize the microbial activity. This study will therefore compare results of modeling, molecular, and activity based measurements in a series of continuous flow column studies.

Part 1. Continuous Flow Column Studies (Semprini (PI) and Dolan (Co-PI) (Oregon State University)

Experimental Approach: Studies are being conducted in continuous flow columns that are packed with aquifer solids from Hanford, Washington. The size of the columns allow packing and unpacking of the columns within an anaerobic glove-box. Initial studies were conducted with glass columns connected in series. These initial tests helped determine the column size needed to observe all the steps of the transformation within one column. We have now fabricated three columns from stainless steel, with sampling ports along the columns to permit spatial sampling. Three continuous flow column experiments can now be performed simultaneously. The experimental approach for these column studies was to study the transport of the CAHs prior to biostimulation; add the cultures and biostimulate through electron donor addition; continue electron donor and CAH addition until desired spatial transformations were observed. During the course of the experiments the aqueous concentrations of the CAHs, the electron donor, fermentation products, sulfate, iron, methane, and hydrogen are being monitored. In addition, the redox status of the columns are being monitored through Dr. Ingle's Center Project. After the desired spatial distribution of CAH transformation is achieved, the column's aquifer material will then be sampled in an anerobic glove box and molecular analyses will be performed at Stanford University, under the direction of Dr. Spormann, using FISH, Real Time PCR, and RNA-based methods.

Status: Anaerobic continuous-flow column experiments initiated last year with the Evanite (EV) enrichment culture in the presence of Hanford aquifer solids were completed. Three columns were connected in series for these tests. At the highest injected lactate concentration of 1.34 mM, PCE was completely transformed to ethene with a hydraulic residence time of 4.6 days. Within a hydraulic residence time of 1.5 days, PCE was transformed to VC and ethene. Electron mass balances showed that about 70% of the lactate added could be accounted for, with about 2% going to CAH reduction, 5% iron reduction, 16% sulfate reduction, and 38% to the production of acetate and propionate. The 2% of the electron donor associated with dehalogenation reactions is consistent with microbial enumerations described below that shown *Dehalococcoides sp.* gene copy numbers represented 0.5 to 4.0% of the Eubacterial 16S rRNA genes present.

Redox capacity measurements (see report of Ingle), showed more reducing conditions were associated with the stage of the experiment when VC was being reduced to ethene. During the stage of the test when PCE transformation was stalled at cis-DCE, reducing conditions in the column were associated with iron reduction.

At the end of the tests the columns were destructively sampled, and solid samples were obtained spatially from columns. The solids were used in microcosm construction to evaluate for rate of PCE and VC transformation, as well as rates of lactate fermentation. Spatial samples for molecular analysis were sent to Stanford for molecular methods analysis described below.

Microcosm tests showed that PCE was most rapid transformed by microbes attached to aquifer solids near the entrance to the column 1 and rates rapid decreased with distance from the column entrance. The results are consistent concentration observations that showed PCE was rapidly transformed to VC and ethene in this column. Rates of VC transformation in the microcosms were similar spatially in column 1, with slight higher rates near the column entrance. The results were consistent with the column concentration measurements that indicated VC was being transformed to ethene in all three columns.

A second series of column tests were initiated this year in columns fabricated with stainless steel. The columns were sized so that samples could be obtained for concentration measurements on three spatial locations along the column as well as the column exit. The columns were also fabricated so that solid coupons could be collected during the course of the study at three different spatial locations to enumerate microbial activity. The columns were again packed with Hanford aquifer solids and bioagumented with the Evanite culture.

Prior to bioaugmenting the culture that aquifer solids were reduced by injecting groundwater that contained sulfide. Upon bioaugmentation of the Evanite culture, the transformation of PCE to VC and ethene proceeded rapidly, and no stall with cis-DCE formation was observed. Then injected lactate concentration was also greatly reduced compared to the amount required in the previous tests to achieve VC transformation to ethene. A series of transient tests have been performed with gradual increases in PCE concentration from 10 mg/L to 50 mg/L. Compete transformation of VC to ethene is achieved with a hydraulic residence time of 4.5 days, and PCE is transformed to VC and ethene within a hydraulic residence to o 1.5 days. In order to effectively transform PCE to ethene, increased lactate addition was needed when PCE concentrations were raised.

Part II: Real-Time (RT-)PCR for Monitoring of Reductive Dehalogenation of Chlorothenes

Alfred Spormann (PI) and Sebastian Behrens (postdoctoral researcher) (Stanford University)

<u>A. Spatial Distribution of the genus *Dehalococcoides* and species subpopulations in a continuous flow column bioreactor</u>

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

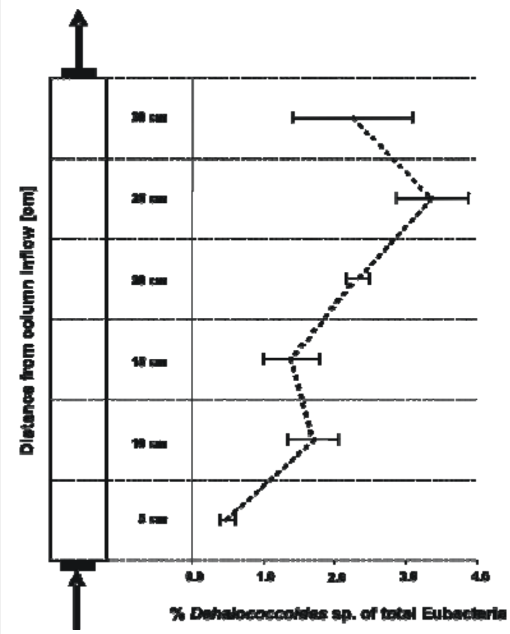

Figure 1. Relative abundance and spatial distribution of *Dehalococcoides* sp. over the vertical profile of column 1.

We further analyzed the population composition of the genus *Dehalococcoides* with the help of functional gene primer specific to certain *Dehalococcoides* strains, e.g. strain VS, and strain BVA-1. This is possible because advanced sequencing efforts revealed that reductive dehalogenases despite their functional homogeneity contain diverse nucleotide sequences regions that allow for highly specific gene probing. We chose to target key genes of reductive dehalogenation that have been genetically and biochemically characterized to catalyze the complete dechlorination of trichloroethene (TCE) and/or vinyl chloride (VC) to ethene. Primer sets were designed to target the vinyl chloride reductase of *Dehalococcoides* sp. strain VS (*vcrA*_VS) (4), the vinyl chloride reductase of *Dehalococcoides* sp. strain BVA-1 (*vcrA*_BVA-1) (1), and the trichloroethene reductase of *Dehalococcoides ethenogenes* strain 195, *Dehalococcoides* sp. strain FL2, and Bacterium PM-VC1, RC-VC2, and YK-TCE1 (*tceA*_195+) (2). The distribution of the selected functional genes over the vertical profile of column 1 is shown in figure 2.

The reductive dehalogenase gene abundances are displayed as percentage of the 16S rRNA gene pool of all *Dehalococcoides* species present in the respective section. These gene comparisons are possible because *Dehalococcoides* cells have only one ribosomal RNA operon per genome. The three *Dehalococcoides* subpopulations differ dramatically in abundance along the column profile. Whereas strain BVA-1 makes up to two third of all *Dehalococcoides* cells in the last 10 cm closest to the column outflow, *tceA* containing relatives of *Dehalococcoides ethenogenes* strain 195 decrease in abundance from 6% to 0.2% and 1% towards the column end. The *vcrA* gene of *Dehalococcoides* strain VS showed a more equal distribution over the column profile decreasing from 20% to about 10% with an numerical low of only 4% around 25 cm from the column inflow (figure 2).

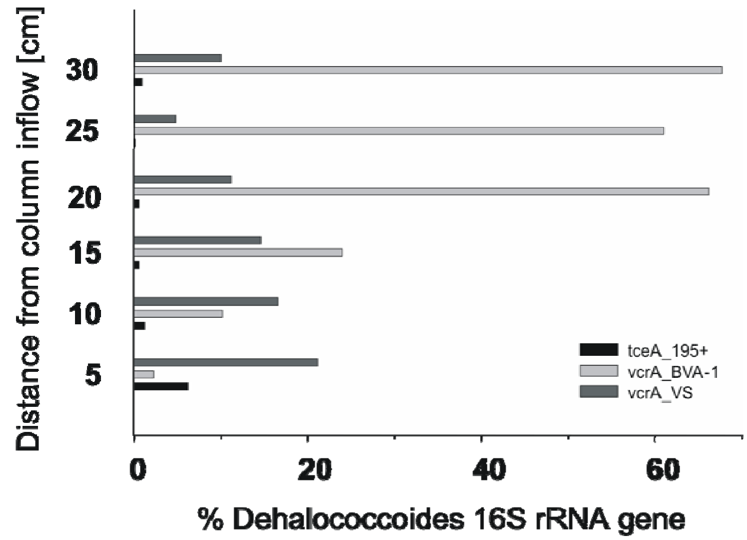

Figure 2. Relative abundance of selected genes catalyzing the stepwise dechlorination of trichloroethene to ethene on bioreactor 1. The tracked genes are specific to subpopulations of the genus *Dehalococcoides* indicating functional and spatial differences in the localization of different *Dehalococcoides* strains along the columns vertical profile.

<u>B. Monitoring of gene expression associated with reductive dehalogenation under continuous flow conditions</u>

The primer for the TCE and VC reductive dehalogenases were further used in real-time reverse transcription (RT-) PCR experiments to study gene expression along the vertical horizon of the first reactor. Therefore RNA was extracted from each 5 cm section. The RNA was reverse transcribed into cDNA which served as template in real-time PCR assays to estimate the relative expression of the described reductive dehalogenases. Figure 3 shows the expression profile of the three reductive dehalogenases followed in this study. The expression data for each gene was normalized to the gene abundance determined in the DNA quantification experiments as described in the previous paragraph. Despite their low gene abundance the trichloroethene reductase (tceA) showed the highest expression of all three dehalogenases under investigation. The relative expression of the tceA from *Dehalococcoides ethenogenes* strain 195 and others peaked 10 to 15 cm from the column inflow. With the highest PCE reduction rates measured within the 5 cm from the column inflow, TCE reduction starts further up the column, where most of the PCE has already been reduced. Vinyl chloride reduction as measured by the vcrA expression of strain VS and BVA-1 showed a more uniform activity pattern over the column profile with slightly elevated expression of the vcrA of strain BVA-1 towards the reactor outflow (Figure 3).

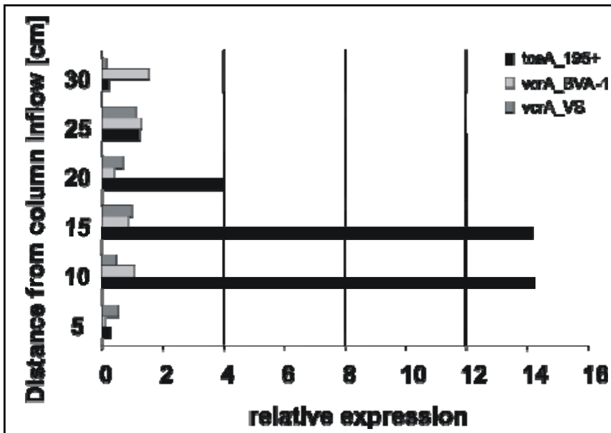

Figure 3. Relative expression of reductive dehalogenases on column 1. The expression data was normalized to the gene abundance based on genomic DNA extractions from each section.

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

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Publications:

Pon, G. and L. Semprini. 2004. Anaerobic Reductive Dechlorination of 1-chloro-1-fluoroethene to Track the Transformation of Vinyl Chloride," *Environ. Sci. Technol* Vol.38 6803-6808.

Yu, S. M.E. Dolan, and L. Semprini. 2005. "Kinetics and Inhibition of Reductive Dechlorination of Chlorinated Ethylenes by Two Different Mixed Cultures," *Environ. Sci. Technol.Vol.* 39 195-205.

Conference Presentations:

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).

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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.

Students Working on the Project:
Sebastian Behrens, post-doctorial 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 near completion 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. August 2005. On the determination of transverse dispersivity: Experiments and simulations in a helix and a cochlea, Stanford University.

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

Journal Articles:

Luo, J., and P.K. Kitanidis. 2004. 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. 03.006.
Luo, J., Cirpka, O.A., Kitanidis. 2005. P.K. Temporal-moment matching for truncated breakthrough curves for step or step-pulse injection. *Adv. Water Resour.* (in press).

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

Method development and system evaluation using a model silica gel and 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 μL/g, while its hydrophobic micropore volume was only 0.022 μL/g. In a microporous silica gel, a hydrophobic micropore volume of 0.038 μL/g was measured. Dehydrohalogenation rate of 2,2-DCP sorbed in hydrophobic micropores was slower than that reported in bulk water, which is indicative of an environment of low water activity. The results suggest that hydrolyzable organic contaminants sorbed in hydrophobic micropores may be preserved for many times longer than their half-lives in water, consistent with the reported persistence of reactive contaminants in natural soils. Although the hydrophobic micropores represent a small fraction of the total micropore volume, the significant amounts of hydrophobic contaminants stored in them may pose long-term risk to groundwater quality.

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

In summary, our experimental data 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, PhD student in Environmental Engineering & Science

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

Cheng, H., and M. Reinhard. Quantifying the Volume of Hydrophobic Micropores from Trichloroethylene Desorption. Environmental Science and Technology, submitted October 2005.

Cheng, H., and M. Reinhard. Displacement of Water by Trichloroethylene in Hydrophobic Micropores of Dealuminated Y Zeolites, in preparation, 2005.

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 WRHSRC 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).

TOSC
Active Communities

Region 10

OREGON

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: TOSC plans to review the risk assessment from UPPR when it is available.

Community Group: Dallas
Site: Boise Cascade
Location: Dallas, OR
TOSC Contact: Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023
Date TOSC learned about the community: 02/01/01

Contaminants: herbicides and pesticides

Description: Members of the Dallas community are concerned about aerial pesticide applications in neighboring Boise Cascade tree plots and their impact on human and animal health.

Notes on TOSC Activities This Quarter: TOSC met with the community contact to review her documentation and discuss a possible health survey. TOSC also met with the Pesticide Analytical and Response Center (PARC) Board to discuss TOSC activities and how the PARC member agencies may support them.

Community Group: Oregon State Penitentiary
Location: Salem
TOSC Contact: Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023
Date Letter of Agreement: 08/31/98

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 helped evaluate air quality concerns in local residential basements and possible exposures though ingestion of local produce, soil contact, and incidental ingestion of soil.

Notes on TOSC Activities This Quarter: TOSC reviewed and commented on the work plan for the facility's remedial design.

Community Group: Concerned Citizens for Clean Air
Site: Georgia Pacific Kraft Mill
Location: Toledo
TOSC Contact: Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023
Date TOSC learned about the community: 09/15/05

Contaminants: The community is concerned about emissions from the pulp mill and human health effects. Community members experience headaches, burning eyes, nausea, and dizziness. The community wants technical assistance to provide feedback to the ODEQ at this time when the air and water permits are up for renewal.

Description: Notes on TOSC Activities This Quarter: TOSC has met with the community and attended several public meetings regarding the renewal of air and water permits. The community is reviewing the draft Letter of Agreement for TOSC assistance.

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.

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: TOSC made presentations on the CERCLA process and air considerations in the site cleanup at the 11/17/05 workshop on the reservation. TOSC is also providing comments on EPA's proposed plan for cleaning up the mine site.

Region 9

CALIFORNIA

Community Group: Alameda Point Collaborative
Location: Alameda, CA
TOSC Contact: Jerry Orlando, Jerry.Orlando@oregonstate.edu, 541-737-5736
Letter of Agreement: 3/1/02

Contaminants: Previously PAHs, now unspecified

Description: Former naval base housing now leased to the APC as transitional housing for groups in need (homeless, etc). Originally contacted TOSC about a cleanup of PAH contaminated soil that occurred in the Spring of 2003. At the request of the Navy cleanup manager, TOSC attended a community open house to answer residents concerns about health risks and exposures that may have occurred.

Items in Letter of Agreement: Review of the EDC-5 Draft Site Inspection Report issued June 30, 2004 and future documents as needed. Present information and training in technical matters related to environmental conditions in the community. Attend BRAC meetings and provide support as needed.

Notes on TOSC Activities This Quarter: TOSC attended the September RAB meeting where the ATSDR gave a presentation on the Public Health Assessment of the base that they released in 2004. TOSC met with a representative of the APC to discuss the future plans for the base and how that affects the residents of the APC.

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: TOSC discussed "next steps" with the community, following their review of TOSC's cancer evaluation report. TOSC also solicited comments from U.C. Irvine regarding the TOSC summary report and will incorporate the comments into the final report as appropriate.

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 quarterly ERAB meeting and will review groundwater monitoring data and proposed additional work for Site 29.

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 continues to seek grant opportunities to support a workshop on medical monitoring for Willits residents who were exposed to hexavalent chromium and VOCs from the Abex-Remco facility.

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: TOSC has agreed to assist the community by reviewing soil vapor, ambient air quality, and groundwater monitoring data when they become available.

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 continues to search for funding opportunities for environmental health support and in finding local partners to work with FOEJN on environmental health issues.

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

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 and TOSNAC are exploring opportunities for ongoing community education and outreach.

Community Group: Perchlorate Citizens Advisory Group
Location: San Martin, CA
TOSC Contact: Jerry Orlando, jerry.orlando@oregonstate.edu, 541-737-5736
Date Letter of Agreement: 06/09/03

Contaminants: perchlorate

Description: A large number of domestic water supply wells in the San Martin area are contaminated with perchlorate from a now closed flare manufacturing facility.

Notes on TOSC Activities This Quarter: TOSC made a presentation at the November 4 PCAG meeting summarizing two proposals by the Santa Clara Valley Water District dealing with forensic studies of perchlorate. The community has asked for assistance summarizing additional documents.

TOSC
Potential Communities

Region 9

ARIZONA

Community Group: Dewey-Humboldt Town Council
Location: Dewey-Humboldt, AZ
TOSC Contact: Jerry Orlando, jerry.orlando@oregonstate.edu, 541-737-5736
Date TOSC learned about the community: August 2005

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 plans to visit the community in mid-December to assess the situation and determine how TOSC can help. TOSC will be meeting with both the town council and members of the community organization. In preparation for the visit, TOSC has reviewed a document submitted as part of the voluntary cleanup by one of the entities whose property is under investigation by the EPA.

Region 10

OREGON

Community Group: Mattel Community Advisory Group
Location: Beaverton, OR
TOSC Contact: Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023
Date TOSC learned about the community: 3/13/2003

Contaminants: TCE

Description: TOSC has been asked to consider providing technical services to the DHS CAG regarding former employees of the Mattel View Master plant who were exposed to well water contaminated with high levels of TCE.

Notes on TOSC Activities This Quarter: TOSC continues to attend CAG meetings and expects to provide peer review of health study protocols as they are developed.

TOSC
Inactive Communities

ARIZONA
Downtown Southwest Neighborhood Association

OREGON
Portland Harbor Community Advisory Group

CALIFORNIA
Tustin RAB
South Bay Cares
Protect Our Neighborhood Committee
Wyle Labs CAG

TAB
ACTIVE COMMUNITIES

Region 10

OREGON

Organization: Oregon DEQ Brownfields Program
TAB Contact: Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023

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

Notes on TAB Activities This Quarter: 1) TAB participated in the 2005 Oregon Brownfields Conference by organizing two sessions (Brownfields Basics and Meaningful Community Involvement) and making a short movie about brownfields redevelopment in three communities in Oregon.
2) TAB is working with the DEQ Brownfields Program to take the St. Helens RSVP project (see below) to another small community in Oregon. In November TAB met with the DEQ and four RSVP directors from southern Oregon to discuss the brownfields inventory program and gauge their interest in participating. It is anticipated that one or more volunteer groups will be organized in the spring of 2006.

Organization: Retired and Senior Volunteer Program
TAB primary contact: Jerry Orlando, jerry.orlando@oregonstate.edu, 541-737-5736

Description: A joint project of the Oregon DEQ and RSVP. Volunteers developed a brownfields inventory then selected two publicly held sites for targeted redevelopment.

Notes on TAB Activities This Quarter: TAB is preparing to assist this group in expanding their brownfields inventory and promotional efforts to include privately held sites they believe are good candidates for redevelopment. Weekly meetings resumed in September.

Organization: Housing Development Center (HDC)
Location: Bertha Station, Portland
TAB primary contact: Jerry Orlando, Jerry.Orlando@oregonstate.edu, 541-737-5736

Description: HDC is working with the Community Partners for Affordable Housing, a 2005 petroleum cleanup grant recipient, and wants general technical assistance with a brownfield project in SW Portland. Plans are to build affordable senior housing. Assistance anticipated- technology options, RFP writing, consultant selection, some community outreach.

Notes on TAB Activities This Quarter: TAB has been gathering information for the preparation of an RFP/ Work Order that will be used to procure a contractor to oversee the cleanup of the site. TAB participated in the kick-off meeting with the EPA, HDC and CPAH and has assisted with the RFP.

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 met with representatives of the IDEQ and an RSVP director on a visit to Boise in September. Future plans include presenting the project idea to a meeting of Idaho RSVP directors, training the volunteers and helping the IDEQ manage the project activities. The first training of RSVP volunteers will be held in Boise in late winter. TAB is adapting the Oregon training materials for Idaho.

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 (2004)

Student	Field	Degree/ Institution/Graduation	Project
Sebastian Behrens	Environmental Engineering	Post-Doctoral/Stanford	2-OSU-07
Ioannis Benekos	Environmental Engineering	Ph.D./Stanford/2005	2-SU-04
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
Anthony Scott	Chemistry	Ph.D./ Oregon State University	2-OSU-06
Kristin Skinner	Molecular and Cellular Biology	Ph.D./ Oregon State University /2006	1-OSU-02
Anne Taylor	Environmental Engineering	Ph.D./ Oregon State University	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 2005, tech transfer activities included maintenance of the WRHSRC website, writing and distribution of Research Briefs and WRHSRC News by e-mail, development of a groundwater remediation online tutorial and workshop presentations.

Website

The website http://wrhsrc.oregonstate.edu/ provides an overview of the WRHSRC and links to publications and project information. Since its launch in January 2001, usage has steadily increased. During the past three years, the number of visits has more than tripled, from about 800 visits per month in September 2002 to 3000 visits per month in September 2005. Web usage statistics demonstrate that site visitors are a diverse group. In a typical month, about 70% of visitors' affiliation can be determined. Of these, about 30% are from U.S. educational institutions (.edu), about 20-25% are from U.S. commercial domains (.com), 1-5% are from the U.S. government (.gov and .mil), and 15% are from other countries (for example Canada, the Netherlands, Poland, Germany, India, and Great Britain).

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, 1989-2004. 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.
- ◊ Research Briefs – short web articles that summarize Center research projects.
- ◊ An opportunity to sign up to receive electronic newsletters from the WRHSRC and the TOSC/TAB programs.

Research Briefs

The Center's Research Briefs are one way that the Center hopes to communicate with cleanup practitioners and others interested in emerging cleanup technologies. They are short summaries of Center projects that emphasize the research applications and demonstration projects. Four

Research Briefs were produced during 2005 and can be found at http://wrhsrc.oregonstate.edu/briefs/index.htm. They included:

- Brief #6 -- Strategies for cost-effective chemical delivery and mixing for bioremediation. (Profile of research by Dr. Kitanidis and his research team at Stanford University.)
- Brief #7 -- Soil and mineral nanopores and their role in contaminant fate and transport. (Profile of research by Dr. Reinhard and his research team at Stanford University.)
- Brief #8 -- Bioremediation by aerobic cometabolism with butane-grown microorganisms. (Profile of research by Drs. Arp and Bottomley and their research team at Oregon State University.
- Brief #9 -- A novel approach for determining transverse dispersion, a process that facilitates dilution and mixing of contaminants in groundwater. (Profile of research by Dr. Kitanidis and his research team at Stanford University.)

Research Briefs are advertised through the Center electronic newsletter and announced on other electronic mailing lists read by the groundwater remediation community. In 2005, briefs were advertised on mailing lists such as: Tech Direct, USEPA Region 10 Science Forum (intranet site), USEPA Region 9 Hazardous Substance Technical Liaison Newsletter. We have also coordinated with the other HSRC's to run special announcements about HSRC Research Briefs on the NIEHS research brief distribution list.

Online Tutorial

In 2005, we also launched a new website with lessons and tutorials for simulating groundwater remediation with a free, windows-based model called the Interactive Groundwater Model 3.5.6 (IGW) (http://ccee.oregonstate.edu/enve/igw/). The website contains explanations of fundamental groundwater cleanup principles and step-by-step instructions for running several simulations, including a pump-and-treat example where the model is used to compare various pumping scenarios. IGW's accessibility and easy operation make it a useful tool for visualizing the physical processes involved in groundwater cleanup. It was developed by Michigan State University.

Presentations and Workshops

In August, many WRHSRC researchers presented at the Joint International Symposia for Subsurface Microbiology (ISSM 2005) and Environmental Biogeochemistry (ISEB XVII) in Jackson Hole, Wyoming. Their presentations included the following:

- Solvent Mixtures by Bioaugmented Butane-Utilizing Microorganisms -- M. E. Dolan, L. Semprini, Oregon State University; P. L. McCarty, G. Hopkins, Stanford University.
- Factors Controlling In Situ Uranium and Technetium Bio-Reduction and Reoxidation -- J. D. Istok, Oregon State University; L. Krumholz, University of Oklahoma; A. Peacock, University of Tennessee; J. McKinley, Pacific Northwest National Laboratories.
- Haldane Inhibitions of Reductive Dechlorination at High CAH Concentrations -- S. Yu, L. Semprini, Oregon State University.
- Alkynes as Reversible Inhibitors for Probing Mechanisms of Reductive Dehalogenation of Chloroethenes -- A. R. Sabalowsky, L. Semprini, Oregon State University.
- A Continuous Flow Column Study of Anaerobic PCE Transformation with the Evanite Culture and Hanford Aquifer Solids -- L. Semprini, A. Mohammad, A. Sabalowsky, M.

Dolan, P. Ruiz-Hass, J. Ingle, Oregon State University; S. Behrens, A. Spormann, Stanford University.

- Much Ado About Upscaling: Microbiology to Engineering Science and Back Again -- T. Ginn, K. Scow, University of California at Davis; B. Wood, Oregon State University.
- Evaluation of Fluoroethene as an Analogue for Aerobic Vinyl Chloride Degradation -- A. E. Taylor, L. Semprini, P. J. Bottomley, M. Dolan, Oregon State University.
- Evaluation of In Situ Aerobic Cometabolism of Chlorinated Ethenes by Toluene-Utilizing Microorganisms Using Push-Pull Tests -- M. F. Azizian, J. Istok, L. Semprini, Oregon State University.

For abstracts and the complete symposium program visit http://www.issm-iseb.org/ .

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 2005, WRLTC-OSU offered 29 certification courses which were attended by 266 students. These courses were conducted in Oregon City, OR (16), Spokane, WA (2), Medford, OR (3), and Salem (3).

Under the Oregon DHS Lead Program/EPA Community Outreach Training Grant, 208 students attended 12 Lead-Safe Work Practices and EBLL workshops. These workshops were held in Baker City, Oregon City (3), Bend, Medford (2), Tigard, Astoria, and Salem (3). The State of Oregon DHS Lead Program has extended grant funding through September 2006 for additional LBP abatement training and outreach activities throughout Oregon.

2005 WRHSRC Publications

Publications for 2005 listed below have resulted from work funded by the new WRHSRC. We continue to maintain the database for publications from both the original and current Center.

During 2005 a total of 12 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.

During 2005 there have been one master's theses/projects and three Ph.D. dissertations submitted.

Journal Articles (also includes those in press and submitted)

Cheng, H., and M. Reinhard, Quantifying the Volume of Hydrophobic Micropores from Trichloroethylene Desorption, Environmental Science and Technology, submitted October 2005.

Cheng, H., and M. Reinhard, Displacement of Water by Trichloroethylene in Hydrophobic Micropores of Dealuminated Y Zeolites, in preparation, 2005.

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

Doughty D.M., Sayavedra-Soto L.A., Arp D.J., and Bottomley P.J. 2005. Effects of dichloroethene isomers on the induction and activity of butane monooxygenase in the alkane-oxidizing bacterium, '*Pseudomonas butanovora*', *Applied and Environmental Microbiology,* 71(10): 6054-6059.

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

Halsey K.H., Sayavedra-Soto L.A., Bottomley P.J., Arp D.J. 2005. Trichloroethylene degradation by butane-oxidizing bacteria causes a spectrum of toxic effects. *Applied Microbiology and Biotechnology,* 68(6): 794-801.

Jones, B.D., and J.D. Ingle, Jr. 2005. Evaluation of redox indicators for determining sulfate-reducing and dechlorinating conditions, *Water Research,* 39, 4343-435.

Kim, Y. and L. Semprini. 2005. Cometabolic Transformation of cis-1,2-dichloroethylene and cis-1,2-dichloroethylene epoxide by a butane-grown mixed culture, *Water Science & Technology,* 52 (8), 125-131.

Luo, J., Cirpka, O.A., Kitanidis, P.K. 2005. Temporal-moment matching for truncated breakthrough curves for step or step-pulse injection. *Adv. Water Resour.* (in press).

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

Niemet, M.R., and L. Semprini. 2005. Column Studies of Anaerobic Carbon Tetrachloride Biotransformation with Hanford Aquifer Material, *Ground Water Monitoring Remediation*, 25(3), 82-92.

Pon, G. and L. Semprini. 2004.Anaerobic Reductive Dechlorination of 1-chloro-1-fluoroethene to Track the Transformation of Vinyl Chloride, *Environ. Sci. Technol* Vol.38 6803-6808.

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.

Ruiz-Haas, P. and Ingle, J.D, Jr. 2005. 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*, invited paper in preparation.

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

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

Theses and Dissertations

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

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

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

Conference 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).

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.

Cakin, Defne 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.

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.

Razzetti, C., 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

Razzetti, C., 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

Ruiz-Haas, Peter 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

Ruiz-Haas, Peter and J.D. Ingle, Jr. 200. Ruiz-Haas, P. and Ingle, J.D., 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.

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.

Semprini, L., 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 3rd European Conference on Bioremediation, Crete, July 3-5, 2005.

Taylor, A.E., 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.