

**WESTERN REGION  
HAZARDOUS SUBSTANCE RESEARCH CENTER**

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**2004 ANNUAL REPORT**

Oregon State University  
Stanford University

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# WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER 2004 ANNUAL REPORT

## Administration

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## The Center at a Glance

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

1. To develop innovative technologies for the in situ treatment of volatile organic chemicals (VOCs) in groundwater, especially chlorinated solvents.
2. To increase the number, speed, and efficiency of available treatment options for both high concentration source zones and diffuse contamination plumes.
3. To disseminate the results of research to the industrial and regulatory communities, to foster exchange of information with these communities, and to promote a better understanding of the scientific capability to detect, assess, and mitigate risks associated with hazardous substance usage and disposal.

Groundwater cleanup and site remediation, with a strong emphasis on treatments that use microbes or chemical catalysts to transform VOCs into harmless substances, represent the major focus of Center activities. Research projects include biological (biotic) and physical and chemical (abiotic) treatment processes, as well as in situ characterization methods for monitoring the progress of both intrinsic and the enhanced remediation. In combination with basic laboratory and field studies, physical and mathematical models are being used to study these processes and to provide a bridge between theory and practice. The technology transfer program involves the process of taking new technologies from the laboratory to the field. Center researchers are working with other federal agencies, such as the Department of Defense (DoD)

and the Department of Energy (DoE), and private industry, in conducting field evaluations of new technologies. Technical Outreach Services for Communities (TOSC) is a technical assistance program designed to aid communities confronted with environmental contamination by hazardous waste sites. TOSC provides interested community groups with technical information and assistance that can enable early and meaningful public participation in decisions that affect health and welfare. The Center's Technical Assistance to Brownfields Communities (TAB) Program provides assistance to communities attempting to address cleanup and redevelopment of properties whose reuse has been prevented by real or perceived contamination. TAB attempts to improve involvement of all affected parties in cleanup and redevelopment process through education and training.

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

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

The Center budgets for the 2004 fiscal year and since the Center's inception are listed by category of support in Table 4. During the third year of operation, core funding totaled \$1,102,500. The distribution of the Center's \$885,000 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 third 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**

<u>Stanford University/Discipline</u>	<u>Oregon State University/Discipline</u>
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**

<u>Member</u>	<u>Affiliation</u>	<u>Expertise</u>
Dr. Richelle M. Allen-King (Vice-Chair)	Department of Geology, University at Buffalo, Buffalo, NY	Geochemistry; Hydrogeology
Dr. Harold Ball	U.S. EPA Region 9, San Francisco, CA	Environmental Engineering
Dr. Roseanne Ford	Chemical Engineering Department, University of Virginia, Charlottesville, VA	Microbial Processes; Chemical Engineering
Dr. Joe Hughes (Chair)	Department of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA	Bioremediation; Environmental Engineering
Dr. Andrea Leeson	SERDP/ESTCP Program Office, DoD, Arlington, VA	Bioremediation; Environmental Engineering
Dr. Kirk O'Reilly	ChevronTexaco Research and Technology Company, Richmond, CA	Biochemistry; Microbial Processes
Dr. Gregory D. Sayles	USEPA Office of Research and Development, Cincinnati, OH	Microbial Processes; Bioremediation
Dr. Jim Spain	Air Force Research Laboratory, Tyndall AFB, FL	Microbiology

**Table 3. Outreach Advisory Committee**

<u>Member</u>	<u>Affiliation</u>	<u>Expertise</u>
Mr. Tim Brincefield	U.S. EPA, Region 10, Seattle, WA	Superfund cleanup and brownfields
Mr. Alan Kiphut	Oregon Department of Environmental Quality, Portland, OR	Policy/law of environmental regulations
Mr. Brooks Koenig	Veritas, Vizslas, & Velos, Portland, OR	Policy/law of environmental regulations
Mr. Luis Rivera	North Coast Regional Water Quality Board, Santa Rosa, CA	Regulations
Ms. Vicki Rosen	U.S. EPA, Region 9, San Francisco, CA	Superfund community involvement
Mr. Lenny Siegel	Center for Public Environmental Oversight, Mountain View, CA	Policy/guidance for cleanup and reuse
Ms. Kathleen Veit	U.S. EPA, Region 10, Seattle, WA	Community involvement

**Table 4. Center Funding**

<u>Funding Sources</u>	<u>FY 2001</u>	<u>FY 2002</u>	<u>FY 2003</u>	<u>Funds to Date</u>
EPA: Centers Program	\$900,000	\$885,000	\$885,000	\$2,670,000
EPA: Brownfields	150,000	150,000	127,000	425,000
Oregon State University	<u>90,000</u>	<u>90,000</u>	<u>90,000</u>	<u>270,000</u>
TOTAL	\$1,140,000	\$1,125,000	\$1,102,500	\$ 3,365,000

\*Oct. 1, 2003- Sept. 30, 2004

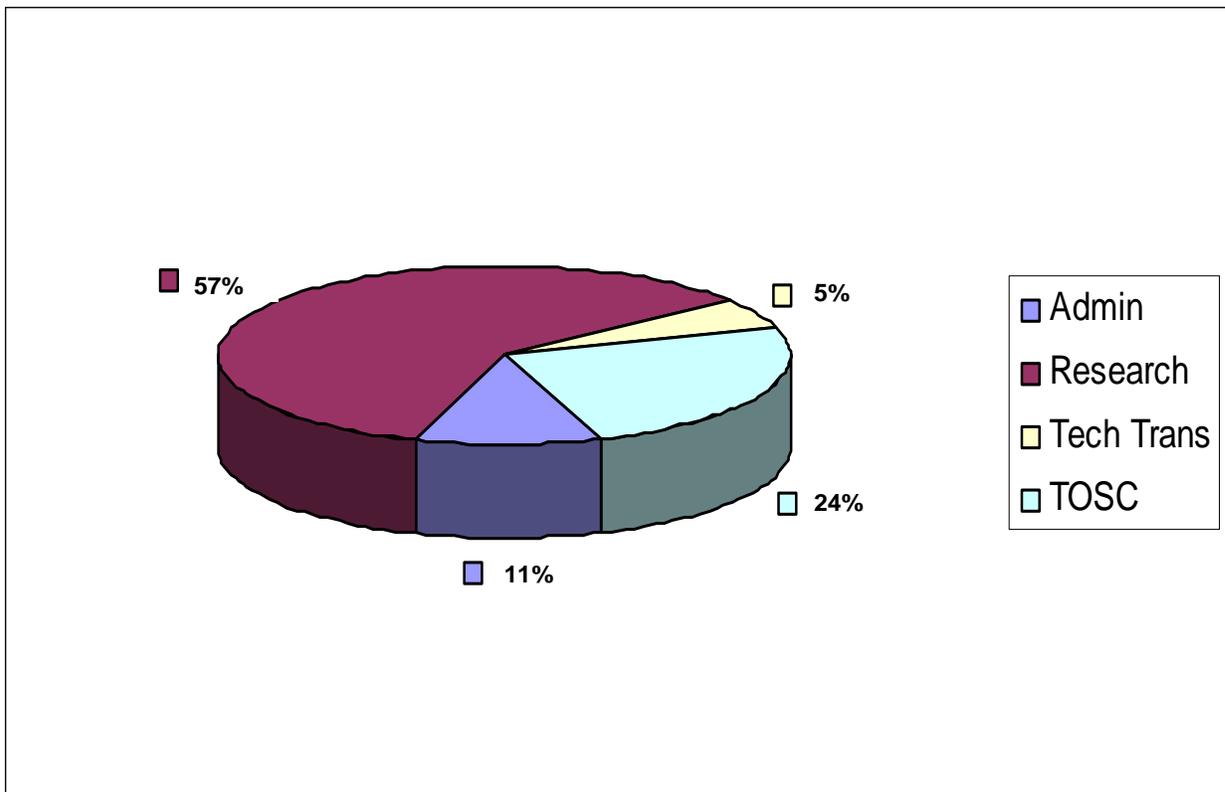
**Table 5. Student Support**

<u>Student Support</u>	<u>FY 2001</u>	<u>FY 2002</u>	<u>FY 2003</u>	<u>Funds to Date</u> <sup>†</sup>
M.S.	2	2	2	\$ 240,000
Ph.D.	9	14	10	1,330,000
Post Doctoral	<u>0</u>	<u>0</u>	<u>2</u>	<u>170,000</u>
TOTAL	11	16	14	\$ 1,740,000

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

<sup>†</sup> 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 initiated on five new research projects associated with the in situ remediation of chlorinated solvents. The projects and the researchers are summarized below.

**Table 6. RESEARCH PROJECT SUMMARY**

<b>Project</b>	<b>Title</b>	<b>PI Co-PIs</b>	<b>Year 1 Budget 2004</b>	<b>Year 2 Budget 2005</b>
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 Bioaugmentation	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	The Role of Micropore Structure in Contaminant Sorption and Desorption	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. They found in studies with *P. butanovora*, and *Nocardiodes sp. CF8*, TCE cooxidation ultimately resulted in 96% inactivation of the butane monooxygenases (BMOs). The BMO of *M. vaccae*, however, was more resistant to inactivation by TCE. They also found that the cooxidation of DCEs in *P. butanovora* can be driven by a variety of organic acids provided as exogenous electron donors; lactate supports the highest initial rates, and also sustains rates for the longest period of time.

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 cis-DCE and VC. Enrichments that are grown on ethene (Eth), VC, and potentially fluoroethene (FE), have been obtained from groundwater obtained from Ft. Lewis, WA. Genomic DNA from enrichment cultures was probed with CoM transferase primers after growing on Eth, VC or FE for 30d. CoM transferase, which is expected to be involved with the metabolism of the epoxides that were formed, was detected with growth on all three substrates. Efforts are being made to obtain pure cultures from the enrichments.

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. The research team has continued to improve portable flow monitoring systems based on immobilized redox indicators and has used them to examine redox conditions in microcosm bottles containing a dechlorinating culture (Evanite culture). A new method has been developed to determine the “reductive capacity” or “effective concentrations of reductants” in aqueous anaerobic samples taken from microcosm bottles, soil columns, and physical aquifer models (PAMs). A fiber optic probe, with immobilized redox indicator film at its tip to monitor redox status, has also been developed. The probe is used in 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 PAMs and provides for true in situ sampling.

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 Point Mugu (MU) and the Evanite (EV) cultures that have been developed and kinetically characterized in a previous WRHSRC project. 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 column. RNA-based methods are also being applied to determine energetically based TCE and VC-dehalogenating activity temporally and spatially within the column. Under the direction of Lewis Semprini and Mark Dolan, a continuous-flow

column experiment was conducted with the Evanite (EV) enrichment culture in the presence of Hanford aquifer solids. PCE dechlorination to TCE, and cis-DCE, were observed when lactate was fed as a fermentable substrate. When the lactate concentration was increased, propionate production was observed from lactate fermentation, and cis-DCE reduction to vinyl chloride (VC) and ethene occurred.

In studies being conducted at Stanford University under the direction of Alfred Spormann, a CARD-FISH protocol for the identification of *Dehalococcoides sp.* in environmental samples was developed. The protocol uses HRP-labeled 16S rRNA targeted oligonucleotide probes specific for the genus *Dehalococcoides sp.* The probe targets all 16S rRNA gene sequences of *Dehalococcoides* species currently in public databases, comprising isolated strains and environmental clones. They were able to show that HRP-labelled probes can be used to detect the *Dehalococcoides sp.* subpopulation within the Evanite enrichment culture. They were also able to demonstrate the presence of the *vcrAB* gene in total DNA extractions of the Evanite enrichment culture using the primer published by Müller et al. (2004). The results demonstrate the ability of the Evanite culture to grow on VC, consistent with results of previous Center kinetic studies.

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. In this project, new experimental protocols and methods of data analysis are being developed, and an extensive set of experiments are being performed to determine relations of transverse dispersivity with conductivity, longitudinal dispersivity, mean grain size, degree of non-uniformity, etc. New methods for the measurement of local transverse dispersion in isotropic porous media based on a helical and a cochlea-like device have been developed. Currently tracer experiments are being performed in the cochlea device and results are being compared to those obtained in a helical device.

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. The researchers have developed a novel analytical system that allows them to study simultaneously sorption and transformation of volatile organics in geological sorbents. The system consists of a soil column chromatograph that was developed in a previously funded Center project, which is directly coupled to a chromatograph for the analysis of the sorbate and transformation products. Studies with dichloropropene (DCP) obtained at different soil moisture contents confirmed that the sorption capacity decreases significantly as the moisture content increases, indicating that water displaces DCP from sorption sites. Hydrolytic transformation of DCP in the nanopores of LLNL soil was studied by measuring DCP and its hydrolysis product desorbed from soil columns. 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 essentially excluded from water.

## **Training and Technology Transfer**

The education of graduate students in the research focus area of the Center is one of our main training activities. The students who have been funded through our Center research grants are shown in Tables 5 and 7. Two M.S. students and 10 Ph.D. students received Center funds through graduate research assistantships over the past year. One student has been funded through the Center outreach program and nine through the different research projects. Through Center funding, students are being trained to do fundamental research at the Ph.D. level in a broad range of disciplines. As shown in Table 5, over half of the Center funding is devoted to the training of graduate students, with the funding going directly in tuition, stipends, travel to conferences, and supplies and materials for research. Several of the students have won awards and fellowships as a result of their Center research.

Technology transfer is an important component of the WRHSRC. The goals of the training and technology transfer program are to 1) promote teamwork and information exchange among researchers using web pages and seminars; 2) provide information transfer with practitioners using web pages, electronic newsletter, video workshops, faculty presentations and publications; 3) test new technologies through pilot-scale testing, and developing online project databases; and 4) implement full-scale demonstration projects. The WRHSRC web site developed and maintained at OSU receives about 1500 visitors per month. Some of the information contained on the web site includes descriptions of research focus areas and projects; a database of WRHSRC publications and previous projects, 1989-2004; descriptions of Center outreach programs and links to the separate websites for the Western Region TOSC/TAB programs; and a News and Events page with regular postings. The website address is <http://wrhsrc.orst.edu>. The web site features Center publications and a searching capability, research briefs, demonstration projects for Technical Outreach Services for Communities, and a page that walks clients through the process of obtaining help from TOSC. Interested clients and individuals can subscribe to the new e-mail newsletter (launched in spring 2003) for WRHSRC and TOSC (started in fall 2002). Two Research Briefs, focusing on the Center Research Projects, were distributed through e-mail in 2004. The technology transfer program created three new research briefs that were distributed by e-mail, as well as posted on the WRHSRC website and the HSRC website.

The WRHSRC also houses a program to promote training activities related to lead paint contamination and disposal. The Western Regional Lead Training Center at OSU (WRLTC-OSU), originally established with U.S. EPA grant funding in 1992, is an accredited non-profit training provider of lead-based paint abatement workshops for U.S. EPA and the State of Oregon certification programs. It is the only Oregon-accredited lead-based paint (LBP) training Center and provides all of the federal LBP curricula. WRLTC-OSU is also accredited in Washington, Alaska, and Idaho, as well as all of the Pacific Northwest Indian Tribes. In 2004, nearly 200 students attended 24 workshops and received 234 certificates. Accredited workshops included Lead Inspector, Lead Risk Assessor, Combined Lead Inspector and Risk Assessor Refresher, Lead Abatement Worker, Lead Abatement Supervisor, Combined Lead Abatement Worker and Supervisor Refresher, and Lead Project Designer.

## **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 (1), Washington (2), Arizona (1), and California (9) (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 in Oregon (1), Washington (1), and Arizona (1). They have worked with the cities of Portland, OR, Spokane, WA, East Palo Alto, CA, and Richmond, CA, on various issues related to brownfields redevelopment. The TAB program as helps coordinate an annual brownfields conference in partnership with Oregon Department of Environmental Quality and Oregon Economic and Community Development Department.

## **Center Annual Research Meeting**

In November 2004, Center researchers, graduate students, and Science Advisory Committee members met for the third annual meeting of the WRHSRC while attending the NERL/HSR Meeting on Superfund Research in Las Vegas, Nevada. A student poster session on the Center's research projects was held on the evening of November 4, and during the meeting the SAC reviewed the progress of the Center's research programs over the past year.

## **Research Project Reports**

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

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

### Part I: Aerobic cometabolism of chlorinated aliphatic hydrocarbon compounds with butane-grown microorganisms

P. J. Bottomley, PI; D.J. Arp, Co-PI

**Goal:** The project aims to evaluate how to maximize the chloroethene degrading potential of individual strains and mixed communities of hydrocarbon-degrading bacteria. Specific subobjectives 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) While co-oxidation of TCE by *P. butanovora*, and *Nocardioides* sp. CF8 ultimately results in 96% inactivation of the butane monooxygenases, the BMO of *M. vaccae* is more resistant to inactivation. Although the rates of TCE transformation by *P. butanovora* increase with increasing TCE concentrations (up to 165 $\mu$ M), cell viability is concomitantly reduced to 17% of the control. In the case of *M. vaccae* and *Nocardioides* CF8, rates of TCE transformation do not increase in response to TCE concentrations > 22  $\mu$ M, and viability is unaffected. These findings indicate that situations might be identified where the use of strains (such as *M. vaccae*) possessing slower rates of CAH degradation without cell death, might be more appropriate bioremediatory agents than strains that show high transitory rates of TCE degradation that are accompanied by substantial loss of cell viability.

(b) Although cooxidation of DCEs in *P. butanovora* can be driven by a variety of organic acids provided as exogenous electron donors, lactate supports the highest initial rates, and also sustains rates for the longest period of time. Using lactate as electron donor, butane-grown *P. butanovora* co-oxidize cis-DCE, 1,2, trans-DCE, and 1,1-DCE at distinctly different rates, with 1,1-DCE being the best and 1,2-trans DCE the worst substrates. After exposure of *P. butanovora* to DCEs, BMO activity was reduced in a time-dependent manner that varied with the specific DCE. BMO activity decreased by 50% after 15 min exposure to cis-DCE, after 6 min exposure to trans-DCE, and after only 30 sec exposure to 1,1-DCE. However, because the velocity of 1,1-DCE oxidation was ~10x faster than that of 1,2-trans DCE, the cells actually consumed about equal amounts of the two DCEs to achieve similar inactivation of sBMO. We conclude that the products of oxidation of both 1,1 and 1,2 trans-DCEs were equally toxic to sBMO activity despite being generated at different rates. Although the same amounts of 1,1, DCE and 1, 2-trans DCE were consumed by *P. butanovora* in 30sec and 6 min, respectively, the declines in lactate-dependent O<sub>2</sub> consumption was delayed to follow the same kinetics of decline as the 1,2-trans DCE treatment. This observation indicates that, in contrast to effects on sBMO activity, the products of 1,2-trans DCE oxidation are more toxic to general respiratory activity than are those of 1,1-DCE.

Recent studies have been 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. Propane is a slower inducer of sBMO than butane, and also negatively interferes with the induction of sBMO by butane. The products of propane metabolism, propanol, propanaldehyde and propionate are potent repressors of BMO induction. We hypothesize that the pathway for metabolizing propionate is repressed during growth on even chain length alkanes, organic acids and alcohols, and that addition of odd chain length hydrocarbons, alcohols and organic acids cause the accumulation of propionate and prevent expression of sBMO. This work is being further investigated.

Not only are the organic acids, butyrate and propionate, less efficient electron donors than lactate, they have been shown to inactivate sBMO when turning over poorer substrates like 1,2-trans DCE. Although the aliphatic organic acids provide reductant to sBMO, they seem to compete with substrates for the active site of the enzyme. The mechanism of BMO inactivation by aliphatic organic acids is currently being studied.

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

M. Dolan and L. Semprini, Co-PIs

**Goal:** A primary goal of the project 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* strains capable of growth on VC have been obtained from the researchers that isolated the cultures (Coleman et al.2000a) , as well as the culture JS666 (Coleman et al. 2000b), the only know organism capable of growth on cis-DCE. Studies will be 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 will 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. A proposal was submitted to the ESTCP program that would incorporate the findings from this research for use in field tests to evaluate the potential for direct VC or cis-DCE metabolism in contaminated aquifers.

**Status:** T-RFLP analyses of the enrichment cultures produced from the same field groundwater sample showed that each substrate yielded distinct microbial communities. Although there was much diversity and many common TFLs between the enrichments, the dominant fragments produced from each enrichment substrate were different. Similarities in dominant fragments were observed in enrichments of different groundwater samples with the same substrate. Microcosms enriched with toluene or ethene produced turbid cultures upon repeated feeding. The groundwater microcosms enriched with cis-DCE did not grow dense cultures, but slow transformation of the cis-DCE was observed over time. Back transfers of the cis-DCE enriched

cultures into MSM media have not successfully retained cis-DCE transformation ability. To ensure that ammonia oxidizers were not being enriched in the microcosms, the ammonium in the MSM was replaced with nitrate for further attempts.

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

Attempts to isolate organisms out of the ethene, VC, and FE amended cultures began with streak plating on tryptic soy agar. Isolates were obtained from the ethene enriched microcosms, were back transferred into MSM and retained their ability to utilize ethene. They are now being tested in MSM for the ability to grow on VC or FE, a fluorinated VC surrogate. 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.

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## **Highlights**

David Doughty received the Janet Ford studentship award for outstanding graduate research in the Department of Microbiology.

Anne Taylor received an NSF IGERT Fellowship from the OSU Subsurface Biosphere Grant..

## **Field projects**

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

## **Students Working on the Project**

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

David M. Doughty, Ph.D. candidate, Microbiology Graduate Program, Oregon State University.

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

Cecilia Razzetti, visiting Ph.D. scholar, Ph.D. candidate, University of Bologna, Civil Construction and Environmental Engineering.

## **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, PI

**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 two collaborative situations involving a bioaugmentation approach in soil columns and physical aquifer models (PAMs).

**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 be able to differentiate between different microbial redox levels and to predict whether conditions are appropriate for reductive dechlorination to occur. These redox indicators, which are incorporated into flow sensors and fiber optic probes, will be deployed in collaborative experiments for calibration and demonstration of their applicability. These experiments will involve continuous monitoring of the redox conditions of cultures inside columns and PAMs packed with soil and enriched with halo-respiratory cultures as a tool for spatial monitoring of dechlorination and to improve conditions necessary for effective dechlorination of PCE and TCE. 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 continued to improve portable flow monitoring systems based on immobilized redox indicators and used them to examine redox conditions in microcosm bottles containing a dechlorinating culture (Evanite culture). Oxygen contamination has been reduced to the point that complete dechlorination is now achieved in microcosm bottles with continual redox monitoring in a time period about 50% longer than without monitoring. Reduction of the indicator thionine indicates conditions are appropriate for dechlorination and ethene production is typically observed with the indicator cresyl violet is approximately half reduced.

We developed a new method to determine the “reductive capacity” or “effective concentrations of reductants” in aqueous anaerobic samples taken from microcosm bottles, soil columns, and physical aquifer models (PAMs). 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. 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  $\mu\text{M}$  for active microcosms. We anticipate the eventual automation of this measurement to allow for continuous monitoring.

We have begun to employ a fiber optic probe with immobilized redox indicator film at its tip to monitor redox status. The probe is used in 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 PAMs and provides for true in situ sampling. Redox indicators placed on the fiber optic probe respond comparably to those installed in flow cells and connected by flow loops.

To evaluate sensors and probes, we have constructed a PVC-based column with suitable  $\frac{1}{4}$ -28 ports for installation of redox flow monitoring setups and  $\frac{1}{2}$ -20 ports to install the fiber optic probe. The column was recently 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 reduced at all ports indicating suitable conditions for dehalogenation. Also we have successfully used the redox flow sensor for monitoring conditions in PAMs and columns in labs in environmental engineering.

We have begun to probe the applications of other redox indicators, particularly quinones, 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 promising for determining “reductive capacity”. Also we have begun evaluating long-path 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.

### **Awards**

Tartar Summer Research Fellowship, Peter Ruiz-Haas (2003).

### **Students Working on the Project**

Peter Ruiz-Haas, Ph.D. student, Department of Chemistry, Oregon State University, the primary student working on the project.

Defne Cakin, Ph.D. student, Department of Chemistry, Oregon State University, working on developing new monitoring techniques for the project.

Anthony Scott, Ph.D. student, Department of Chemistry, Oregon State University, working on automating procedures for the project.

### **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, PIs; Alfred Spormann, Stanford University, Co-PI

**Goals:** This project is evaluating the transformation of chlorinated ethenes in sequencing batch reactors and continuous-flow column studies with the Point Mugu (MU) and the Evanite (EV) cultures that have been developed and kinetically characterized in our earlier WRHSRC project (Yu and Semprini, 2004; Yu et al. in press). 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 MU culture 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 dehalogenation of PCE and TCE to ethene. Bioaugmentation of microbial consortium that contain phylogenetic relatives of *Dehalococcoides 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**

Lewis 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; and 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 anaerobic 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 were conducted with the Evanite (EV) enrichment culture in the presence of Hanford aquifer solids to evaluate the sequential reductive dechlorination of chlorinated ethenes. Three glass columns (30 cm length x 4.5 cm i.d.) with Teflon endcaps were connected in series. Each column was packed with about 1 kg Hanford soil, resulting in a porosity of 0.35 with 190 ml pore volume, and sequentially connected under anaerobic conditions. After packing the columns, synthetic Hanford groundwater was pumped into the bottom of the first column using a gradient HPLC pump. Tracer tests were performed by pumping 0.6 mM Br and 0.07 mM PCE through the column at flow rate of 0.1 mL/min and measuring breakthrough of bromide and PCE. PCE retardation factors of 6.8 - 7.4 were calculated from bromide and PCE tracer breakthrough curves by using CXTFIT two-site-non-equilibrium model. After four weeks of PCE and bromide addition the columns were amended

with nutrients, PCE 0.07 mM, lactate 0.34 mM, and 0.2 mM sulfate in synthetic Hanford groundwater. Little microbial activity was observed during this period with indigenous microbes from the Hanford soil, based on the absence of sulfate reduction and lactate removal. After six weeks, the columns were inoculated with the EV enrichment culture by direct injection of the culture into the bottom, inlet end, of each column. The injected lactate concentrations were increased from 0.34 mM to 0.67 mM, and to 1.34 mM, after six, eight, and sixteen weeks, respectively.

PCE dechlorination to TCE, and cis-DCE was observed when lactate concentrations increased from 0.35 mM to 0.67 mM on week eight. Rapid increases in cis-DCE concentration in the effluent compared to influent PCE concentration suggested enhanced PCE desorption and consequent reduction to cis-DCE. All the PCE was transformed to cis-DCE in the first column. When the lactate concentration was increased to 1.34 mM, propionate production was observed from lactate fermentation, and cis-DCE reduction to vinyl chloride (VC) occurred. VC transformation to ethene was observed in column three once the cis-DCE concentrations were reduced to low levels, and enhanced cis-DCE desorption was also indicated. The results are consistent with previous laboratory and modeling studies with the EV culture (Yu et al., in press) that showed cis-DCE strongly inhibits VC transformation. The reductive capacity increased over time as the columns became more biologically active and production of VC and ethene was observed. These results suggest that the EV culture is able to transform PCE to ethene under controlled reducing conditions in a continuous-flow system. Methane production has not been detected in the columns.

CAH recoveries were 98.8%, 90.4% , and 113.5% of the PCE injected in columns #1, #2 and #3, respectively. Lactate mass recoveries were 43%, 59%, and 45.7%, and sulfate recoveries were 36.1%, 31.1%, and 26.1%. Mass balances on lactate reduction as an electron donor were calculated using chemical reactions for CAH and sulfate reduction, and the production of propionate. Results show that only 31.1% of reduced lactate could be accounted for by the reduction of CAHs, sulfate, and production of propionate. We have not observed acetate production, and are currently determining if we have had an analytical problem with our acetate measurement.

**Future Plans:** Aquifer solids from the three columns will be sampled for molecular analysis as soon as VC is completely transformed to ethene in the third column. Of particular interest will be the first column where PCE is added and is currently being transformed to TCE, cis-DCE and VC. We have also fabricated three stainless steel columns that will permit simultaneous experiments with the EV and MU cultures. In the third columns we may study a mixture of the EV and MU cultures, or the *Dehalococcoides* sp. strain VS that has been studied in detail at Stanford University in Dr. Spormann's laboratory.

## **Part II: Development and application of molecular methods for the detection and quantification of microbial populations capable of reductive dehalogenation**

Alfred M. Sporman, PI, and Sebastian Behrens, postdoctoral researcher and Co-PI, Stanford University

A. Monitoring and quantifying dehalogenating microorganisms.

We developed and evaluated the specificity of a new primer set targeting the 16S rRNA gene of *Dehalococcoides* sp. These primers will be used in real-time quantitative PCR studies to quantify 16S rRNA gene copy numbers in total DNA from different sections of the column and in the aqueous phase.

B. Determining the spatial distribution of *Dehalococcoides* sp. on the bioreactor columns.

Fluorescence in situ hybridization (FISH) with rRNA-targeted probes is, among other things, a staining technique that allows phylogenetic identification of bacteria in mixed assemblages without prior cultivation by means of epifluorescence and confocal laser scanning microscopy (1). In theory, each ribosome within a bacterial cell, containing one copy each of 5S, 16S and 23S rRNA, is stained by one probe molecule during the hybridization procedure. For FISH with mono-labeled fluorescent oligonucleotide probes this is often the critical point because the majority of environmental bacteria are small, slowly growing or starving, and contain only low amounts of ribosomes. Therefore signal intensities are frequently below detection limits or lost in high levels of background fluorescence.

These limitations can be overcome by the use of horseradish peroxidase (HRP) labeled oligonucleotide probes and tyramide signal amplification (TSA), also known as catalyzed reporter deposition (CARD) (4). CARD is based on the deposition of a large number of fluorochrome-labeled tyramine molecules by peroxidase activity. In this way numerous fluorescent molecules can be introduced at the hybridization site in situ. This results in greatly enhanced FISH sensitivity compared to probes with a single fluorochrome.

We developed a CARD-FISH protocol for the identification of *Dehalococcoides* sp. in environmental samples. The protocol uses HRP-labeled 16S rRNA targeted oligonucleotide probes specific for the genus *Dehalococcoides* sp. (Fig. 1). The probe targets all 16S rRNA gene sequences of *Dehalococcoides* species currently in public databases, comprising isolated strains and environmental clones. All 16S rRNA gene sequences are closely related and form a unique cluster of *Dehalococcoides* species.



Fig. 1: Photomicrograph of *Dehalococcoides* sp. strain VS stained with DAPI and hybridized with HRP-labelled oligonucleotide probes. A DAPI image. B phase contrast image. C *Dehalococcoides* sp. genus-specific probe image. Scale bar 10  $\mu$ m.

We could show that our HRP-labeled probes can be used to detect the *Dehalococcoides* sp. subpopulation within the Evanite enrichment culture (Fig. 2). By using the CARD-FISH protocol and the evaluated *Dehalococcoides*-genus specific probes we will map the spatial distribution of these organisms throughout the length of the column.

### C. Determining the spatial distribution of *vcrA*

Müller et al. (2004) showed a strong correlation of the presence of a *vcrA* homolog with reductive VC dehalogenation (2). They used the *vcrAB* sequence as molecular probe to test for in situ VC reduction potential in environmental samples. We were able to demonstrate the presence of the *vcrAB* gene in total DNA extractions of the Evanite enrichment culture using the primer published by Müller et al. (2004)

We developed two new primer sets that target the *vcrA* gene of *Dehalococcoides* sp. strain VS for application in real-time quantitative PCR. The specificity of the primers was tested by standard PCR. The primer will be used in real-time quantitative RT-PCR assays to quantify *vcrA* copy numbers in total RNA from column matrix and pore water samples. This is a 'straightforward' approach since we showed the presence of *vcrA* in the Evanite enrichment culture that has been used to inoculate the flow columns.

In parallel we are working on developing a protocol for the detection of *vcrA* mRNA gene transcripts by fluorescence in situ hybridisation (3). By combining the sensitivity of the CARD-FISH approach with the use of polyribonucleotide transcript probes of *vcrA* our aim is to visualize microorganisms that are actively transcribing *vcrA* in the column material.

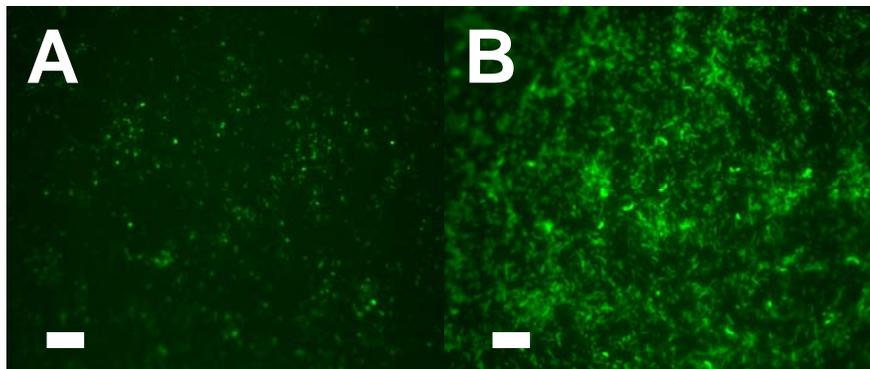


Fig. 2: Photomicrograph of the Evanite enrichment culture hybridized with HRP-labelled oligonucleotide probes. A *Dehalococcoides* sp. genus-specific probe image. B general Eubacteria probe image. Scale bar 10  $\mu$ m.

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### **Students Working on the Project**

Sebastian Behrens, post-doctoral student, Stanford University.

Seungho Yu, post-doctoral student, Department of Civil, Construction, and Environmental Engineering, Oregon State University.

Andrew Sabolwsky, Ph.D. graduate 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, Stanford University, PI; Craig Criddle, Stanford University, Co-PI

**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:** We have been conducting experiments with a newly constructed cochlear device to evaluate the transverse dispersion for glass beads at the size of coarse sand. The cochlear device is easier to pack (i.e., fill with the tube with the granular material) than the helical one and the analysis of the data is easier. Nevertheless, it is always a challenge to pack a device in a uniform fashion and to prevent the entrapment of gas in the porous medium. We are comparing the results with those obtained in a helix.

### **Student Working on the Project**

Ioannis Benekos, Ph.D. candidate, Department of Civil and Environmental Engineering, Stanford University.

### **2-SU-05 The Role of Micropore Structure in Contaminant Sorption and Desorption**

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 and equilibrated for weeks to months. 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 can be calibrated using sorbents with known porosity (silica gel) and sorbates with known hydrolysis rates—trichloroethylene (TCE), benzyl chloride (BzC) and 2,2-dichloropropene (DCP).

**Status:** Initial studies focused on the non-reactive (TCE) and the two reactive model substrates (BzC and DCP) as the sorbates, and the clay and silt fraction (< 50 μm) of soil from a site at the Lawrence Livermore National Laboratory (LLNL) as the sorbent. DCP sorption data obtained at different soil moisture contents confirmed that the sorption capacity decreases significantly as the moisture content increases, indicating that water displaces DCP from sorption sites as the moisture content increases. However, water did not completely eliminate the sorption capacity for DCP, and a small but significant amount of 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.

The effect of competitive cosorbates on desorption of nanopore-sorbed contaminant was studied by adding water, methanol, or methane to the dry helium stream during TCE desorption. TCE desorption fluxes increased significantly with water (1.6 times) and methanol (8.2 times), whereas methane showed no effect, indicating that small relatively polar molecules can dislodge TCE from the sorption sites. More data is needed to conclusively interpret these results but a preliminary analysis suggests that sorption in hydrophobic nanopores could play a role. Hydrophobic nanopores are pores of the size of a TCE molecule in which water sorption is energetically and entropically unfavored. The fact that the impact of water and methanol on desorption flux is evident within a relatively short time (~42 min) suggests that the diffusion from such nanopores into larger pores is a relatively rapid process.

Hydrolytic Transformation of DCP in the nanopores of LLNL soil was studied by measuring DCP and its hydrolysis product desorbed from soil columns. In bulk solution, DCP hydrolyzes mainly via dehydrohalogenation:



At 25°C, the rate in bulk water is  $3.18 \times 10^{-4} \text{ min}^{-1}$  with an activation energy ( $E_a$ ) of  $111.1 \pm 2.0$  kJ/mol. The compound has been chosen because the rate is pH independent and the half-life ( $t_{1/2}$ ) of 45.9 hrs at 25 °C is within the range of interest of these experiments. The rate increases with temperature and  $t_{1/2}$  at 50 °C is 1.06 hrs. With a 10-hour reaction time at 50°C (equivalent to 9.4 times  $t_{1/2}$ ), DCP is expected to transform nearly completely (to > 99.99%). In contrast to this prediction, desorption profiles indicate that the conversion of DCP to 2-chloropropene is

significantly slowed. In the fully wet (5.10% water) and semi-wet (1.94% water) soil columns, only 15.5% and 21.3% of the total DCP is converted, respectively. The fact that the DCP desorption profiles show unhydrolyzed DCP being released from slow desorbing sites is consistent with the hypothesis of DCP sorption in water-free nanopores.

Hydrolysis of DCP sorbed on a silica gel (nanoporous Davisil 60 Å) with known pore characteristics was studied under fully wet condition, which was achieved by draining the water for 2 hours from a column that has been previously saturated with water for two days. Water content in the silica gel column is relatively high (0.48 g/g dry silica), and small water droplets and meniscus were visible in the column. After 10 hrs' reaction time at 50°C, 10.5% DCP was recovered from this water-rich system. This observation also confirms that DCP hydrolysis in the columns was not limited by the availability of liquid phase water. Hydrolysis of DCP in LLNL soil (previously equilibrated with water vapor for more than 3 months) at room temperature (for 2 months, which is equivalent to 31.2 times  $t_{1/2}$ ) and at elevated temperature (50°C for 10 hrs, which is equivalent to 9.4 times  $t_{1/2}$ ) was also compared. The mass balances of DCP recovered from the two columns are comparable (81.0% and 82.6%). A greater fraction of DCP was recovered as unhydrolyzed from the column set at the room temperature (30.2%) than from the one that had been heated to 50°C (6.1%), although DCP under the former condition should have undergone greater extent of hydrolytic transformation. The greater extent of DCP hydrolysis observed in the heated columns results from the experimental procedure of heating the column in 50°C immediately after DCP sorption. There was not sufficient time for DCP molecules to diffuse into nanopores when the heating started, which accelerated the reaction rate of DCP by ~43 times. Although the diffusivity of DCP molecules is also higher at 50°C, more ended up undergoing dehydrochlorination before diffusing into the nanopores, where they could be preserved.

The sorption and hydrolytic transformation of BzC on LLNL soil was also studied. BzC desorption fluxes at 50°C and 90°C were higher than expected based on hydrolysis data, which is consistent with BzC sorption in nanopores.

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 essentially 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 not in contact with water molecules and are prevented from hydrolysis.

**Future work:** We plan to substantiate and quantify these findings by conducting further column experiments using a wider range of materials and conditions.

### **Students Working on the Project**

Hefa Chang, Ph.D. candidate, Department of Civil and Environmental Engineering, Stanford University.

# Outreach Project Reports

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

Kenneth J. Williamson, Director; Denise Lach, Co-Director, Oregon State University

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

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## Technical Outreach Services for Communities (TOSC)

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

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

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

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## TOSC Active Communities

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### ***Region 10***

#### **OREGON**

**Community Group:** Portland Harbor Community Advisory Group

**Site:** Portland Harbor

**Location:** Portland, OR

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

**Date TOSC learned about the community:** May 2003

**Contaminants:** Metals, PAHs, pesticides, VOCs

**Description:** The Portland Harbor Community Advisory Group asked TOSC to review the April 17, 2003, field sampling plan which is intended to support site characterization and risk assessment activities. The CAG has also asked TOSC to consider providing additional technical support in the future.

**Notes on TOSC Activities this Quarter:** TOSC is waiting to hear from DEQ about updating the 2000 Environmental Finance Committee report to recommend funding mechanisms for the cleanup.

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#### **WASHINGTON**

**Community Group:** Skykomish Environmental Coalition

**Site:** Burlington Northern Sante Fe yard

**Location:** Skykomish, WA

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

**Date Letter of Agreement signed:** 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 on 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:** TOSC recently reviewed BNSF's proposed revisions to the feasibility study and met with the community to discuss the report and future site activities.

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**Community Group:** Spokane Indian Tribe

**Site:** Midnite Mine

**Location:** Spokane, WA

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

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

**Contaminants:** uranium, heavy metals

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

**Notes on TOSC Activities this Quarter:** TOSC participated in a workshop on risk assessment in Wellpinit, WA on August 24, 2004. The workshop was organized by TOSNAC and included presentations on various aspects of risk assessment by TOSNAC, TOSC, and EPA Region 10 staff.

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## ***Region 9***

### **ARIZONA**

**Community Group:** Downtown Southwest Neighborhood Association

**Location:** South Phoenix, AZ

**TOSC Contact:** Stephanie Sanford, stephanie.sanford@oregonstate.edu, 541-737-5861

**Date Letter of Agreement signed:** 11/20/02

**Contaminants:** air pollutants from traffic, industry, and waste treatment

**Description:** The community is concerned about the health of its residents particularly as it is affected by air quality and other environmental impacts.

**Items in Letter of Agreement:** Seek to establish air monitoring and appropriate data collection and analysis within the boundaries of the neighborhood; produce summary educational

materials for community members from the technical report, "Environmental Review for Neighborhoods for Justice"; assist the community in tracking the status of air quality permits for entities located in or near the neighborhood.

**Notes on TOSC Activities this Quarter:** TOSC is establishing a relationship with a new community contact after the death of its community leader, Ethel Lane.

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## CALIFORNIA

**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 has prepared a report to explain the process by which the University of California at Irvine evaluated the incidence of cancer in Valley Center. The purpose of the report is to help the community understand that study. TOSC's resources in this effort include an epidemiologist from Oregon Health and Sciences University and the Department of Statistics at OSU. TOSC expects to provide its final report to the community by December 2004.

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

**Site:** Air Force Plant 42

**Location:** Palmdale, CA

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

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

**Contaminants:** TCE in groundwater

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

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

**Notes on TOSC Activities this Quarter:** TOSC expects to review a draft feasibility study this fall.

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**Community Group:** Tustin RAB

**Site:** Marine Corps Air Facility

**Location:** Orange County, CA

**TOSC Contact:** Ken Williamson, ken.williamson@oregonstate.edu, 541-737-6836

**Date Letter of Agreement signed:** 8/21/97

**Contaminants:** TCE and other VOC's in groundwater

**Description:** TOSC is providing assistance to an established Restoration Advisory Board regarding remediation activities at a Marine Corps Air Station. RI/FS completed.

**Items in Letter of Agreement:** Regular attendance at Restoration Advisory Board meetings; review and comment on RI/FS and draft and final ROD documents at OU-3; review and comment on draft RI/FS at OU-1; ongoing educational programs for RAB members related to remediation plans and activities; TOSC presentation on viability of bioremediation for the site.

**Notes on TOSC Activities this Quarter:** None

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

**Site:** Abex-Remco Hydraulics

**Location:** Willits, CA

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

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

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

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

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

**Notes on TOSC Activities this Quarter:** TOSC gave a presentation on risk assessment at the September 21, 2004, community meeting. TOSC is helping the community to create a web site. The community has also asked TOSC to review the final Public Health Assessment report which is being prepared by the CA Department of Health Services.

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

**Location:** Oakland, CA

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

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

**Contaminants:** Lead and vinyl chloride

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

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

**Notes on TOSC Activities this Quarter:** TOSC sent results of its review of a memo from DTSC to the community summarizing the status of issues related to the community's environmental justice complaint. TOSC also made recommendations to EPA regarding locations for soil gas sampling in the vicinity of the play structure at South Prescott Neighborhood Park. The community requested that EPA collect soil vapor and ambient air samples to determine if park visitors were being exposed to vinyl chloride at the park.

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

**Site:** Fort Ord

**Location:** Marina, CA

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

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

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

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

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

**Notes on TOSC Activities this Quarter:** TOSC has been working with the community to find funding for activities not covered by its TAG. TOSC will make a presentation on risk assessment at the October 11, 2004, community group meeting.

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**Community Group:** South Bay Cares

**Site:** Palos Verdes Landfill

**Location:** Palos Verdes, CA

**TOSC Contact:** Michael Fernandez, michael.fernandez@oregonstate.edu, 541-737-4023  
**Date Letter of Agreement signed:** 10/3/03

**Contaminants:** Landfill leachate, mixed contaminants

**Description:** Community is concerned about health effects of developing an old landfill as a golf course. The Los Angeles County Sanitation Districts are preparing a draft environmental impact report on this proposed development.

**Notes on TOSC Activities this Quarter:** None

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**Community Group:** Perchlorate Citizens Advisory Board

**Location:** San Martin , CA

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

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

**Contaminants:** perchlorate

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

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

**Notes on TOSC Activities this Quarter:** TOSC is evaluating its relationship with this community. The community is receiving much attention and assistance from local agencies, diminishing TOSC's role. Talks are underway to adjust TOSC assistance.

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**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 summarized the Site Inspection Report and

presented the summary to the community. TOSC then attended the BRAC meeting later that day where the report was presented by the consultant that produced it.

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**TOSC**  
**Inactive Communities**

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***Arizona***

Don't Waste Arizona (Phoenix)

***California***

Elem Tribe (Clearlake)

## Technical Assistance to Brownfields Communities (TAB)

**Goal:** 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. The TAB program makes use of the same faculty, consultant, and research assistant staff as those involved in the TOSC program. TAB attempts to improve involvement of all affected parties in cleanup and redevelopment process through education and training. TAB also attempts to accelerate the redevelopment process through the application of HSRC and other research and through improved community involvement.

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### TAB Active Communities

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#### *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:** TAB participated in the September workgroup meeting. At that meeting TOSC facilitated a lengthy discussion about redevelopment of two brownfields sites in St. Helens, OR.

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#### WASHINGTON

**Organization:** City of Spokane, Mayor's Office

**City:** Spokane, WA

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

**Date Letter of Agreement signed:** 5/12/03

**Description:** The City of Spokane received an EPA assessment grant in May 2002 to address two economically depressed sections of the city. TAB has been assisting in various aspects of the city's brownfields activities.

**Items in Letter of Agreement:** To design and help implement a systematic process of prioritizing and selecting brownfields properties for redevelopment. The process includes the use of surveys and workshops designed and facilitated by TAB. Letter signed in May 2003.

**Notes on TAB Activities This Quarter:** TAB continues to assist Spokane with aspects of their assessment grant. Specifically, TAB is trying to assist the city with encouraging property owners to take part in the brownfields program.

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## *Region 9*

### ARIZONA

**Organization:** Salt River Pima-Maricopa Indian Community (SRPMIC)

**Location:** Scottsdale, AZ

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

**Date TOSC learned about the community:** 4/27/04

**Description:** EPA has selected the Salt River Pima-Maricopa Indian Community for a brownfields assessment grant. The Indian Community has identified a 200-acre abandoned landfill site as its target area. Grant funds will be used for assessment planning, site characterization and analysis, etc.

**Items in Letter of Agreement:** Draft LOA presented to community.

**Notes on TAB Activities this Quarter:** TAB is assisting the SRPMIC Cultural and Environmental Services Division with outreach efforts. TAB has produced a brochure about the brownfields project that will be distributed along with an invitation to a community open house tentatively scheduled for November 18. TAB is actively involved in the planning of the open house and is coordinating the production of materials and content.

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## TAB Potential Communities

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### *California*

**Organization:** Lula Washington Dance Company

**Location:** Los Angeles, CA

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

**Description:** TAB recently checked in with this community, which received a cleanup grant in 2003. Activity on this project was delayed by the phase 2 assessment. However, the phase 2 is now complete and the community anticipates needing TAB assistance in issuing an RFP for a consultant and assistance with planning and executing a community open house.

**Organization:** San Benito County

**Location:** Hollister, CA

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

**Description:** 2004 recipient of an EPA assessment grant to investigate the New Idria mercury mine. TAB was contacted for community outreach assistance. TAB has provided this community with guidance documents on the Triad Approach and writing RFPs that encourage innovative technology. TAB is planning a site visit for November where future TAB assistance will be discussed.

**TAB**  
**Communities/Entities Seeking Information, Resources or Support**

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City of Glendale, Glendale AZ  
Business Environmental Resource Center, Sacramento CA  
City of Goleta, Goleta CA  
ACME Scenic and Display, Portland OR  
Tacoma-Pierce County Health Department, Tacoma WA

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**TAB**  
**Inactive Communities**

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***California***

Padres Unidos de Maywood (PUMA)  
Richmond

***Idaho***

Capital City Development Corporation (Boise)

***Oregon***

Clackamas County  
Corbett, Terwilliger, Lair Hill Neighborhood (Portland)  
Gresham  
Portland Development Commission  
Portland Showcase (Portland)  
Eliot Neighborhood

**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)**

<u>Student</u>	<u>Field</u>	<u>Degree/ Institution/Graduation</u>	<u>Project</u>
Sebastian Behrens	Environmental Engineering	Post-Doctoral/Stanford	2-OSU-07
Ioannis Benekos	Environmental Engineering	Ph.D./Stanford/2005	2-SU-04
Tina Blatchford	Environmental Engineering	M.S./Oregon State University/2005	1-OSU-02
Defne Cakin	Chemistry	Ph.D./ Oregon State University /2006	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 /2005	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 /2005	2-OSU-06
Andy Sabalowosky	Environmental Engineering	Ph.D./ Oregon State University /2006	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 is an important component of the WRHSRC. The goals are as follows:

- Promote teamwork and information exchange among researchers
  - Tools: listservs, webpages, seminars
- Promote information transfer with practitioners
  - Tools: webpages, electronic newsletter, video workshops, faculty presentations and publications
- Test new technologies
  - Tools: laboratory and pilot-scale testing, demonstrations, online project database
- 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 2004, tech transfer activities included further development of the WRHSRC website, creation of three new Research Briefs and WRHSRC News distributed by e-mail, and continuation of several technology demonstration projects.

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 increased to about 1500 visitors per month. 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.

The website features Center publications and a searching capability, research briefs, demonstration projects for Technical Outreach Services for Communities, and a page that walks clients through the process of obtaining help from TOSC. Interested clients and individuals can subscribe to the new e-mail newsletter (launched in spring 2003) for WRHSRC and TOSC (started in fall 2002). The following new e-mail Research Briefs, which can be found at <http://wrhsrc.oregonstate.edu/briefs/index.htm>, were distributed during 2004:

**Brief # 4:** Defining the kinetics and inhibition of anaerobic reductive dechlorination of PCE and TCE. (Profile of research by Dr. Semprini of Oregon State University.)

**Brief #5:** Developing "Push-pull" tests for monitoring bioaugmentation with reductive dechlorinating cultures. (Profile of research by Drs. Istok, Field, and Dolan of Oregon State University.)

**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.)

# Western Regional Lead Training Center, OSU

## Hazardous Waste Training

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

**Approach:** 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 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 for US Departments of Housing and Urban Development (HUD) and Energy (DOE).

**Status:** In 2004, WRLTC-OSU offered 24 certification courses that were attended by 194 students who received 234 certifications. These courses were held in Oregon City, OR; Salem, OR; Pocatello, ID; Vancouver, WA; and Bonneville Dam & Locks, OR. Under the Oregon DHS Lead Program/EPA Community Outreach Training Grant, 218 students attended 10 workshops offered by WRLTC-OSU. These workshops were held in Portland-Metro (3), North Bend, Astoria, Roseburg, Klamath Falls, Salem, Lincoln City, Bend, and Yamhill. This Outreach Training Grant has been extended an additional year through September 2005 for WRLTC-OSU to provide additional LBP abatement training and outreach activities to the State of Oregon.

## 2004 WRHSRC Publications

Publications for 2004 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 2004 a total of 13 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.

In 2003-2004 there have been four master's theses/projects and four Ph.D. dissertations submitted.

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

Cupples, A. M., Spormann, A. M., and McCarty, P. L., "Comparative Evaluation of Chloroethene Dechlorination to Ethene by *Dehalococcoides*-like Microorganisms," *Environmental Science & Technology*, **38**, 4768-4774 (2004).

Lee, I., Bae, J., Yang, Y., McCarty, P. L., "Simulated and Experimental Evaluation of Factors Affecting the Rate and Extent of Reductive Dehalogenation of Chloroethenes with Glucose," *Journal of Contaminant Hydrology*, **74**, 313-331 (2004).

Luo, J., and Kitanidis, P. K., "Fluid Residence Times Within a Recirculation Zone Created by and Extraction - Injection Well Pair," *Journal of Hydrology*, **295**, 149-162 (2004).

Cunningham, J.A., J. J. Deitsch, J.A. Smith, and M. Reinhard, "Quantification of contaminant sorption-desorption time-scales from batch experiments," submitted to *Environmental Chemistry and Toxicology*.

Doughty, D.M., L., Sayavedro-Soto, D.J. Arp, and P.J. Bottomley. Metabolism of dichloroethylenes by butane-grown *Pseudomonas butanovora*. *Applied and Environmental Microbiology*. In review (2004).

Doughty, D.M., L. Sayavedro-Soto, D.J. Arp, and P.J. Bottomley. Link between alkane chain length and propionate metabolism on the regulation of butane monooxygenase genes in *Pseudomonas butanovora*. *Microbiology*. In review (2004).

Halsey, K.H., P.J. Bottomley and D.J. Arp. TCE degradation by butane-oxidizing bacteria causes a spectrum of toxic effects. *Applied Microbiology and Biotechnology*. In review (2004).

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