



**WESTERN REGION  
HAZARDOUS SUBSTANCE RESEARCH CENTER**

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## 1998 ANNUAL REPORT

Stanford University  
Oregon State University

December 1998

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1998 ANNUAL REPORT**

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## **WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER 1998 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 Stanford University and Oregon State University that was established in February 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, but also is supported through grants, contracts, and gifts from other federal agencies, states, municipalities, consultants, and industry.

The objectives of the Center are:

1. To promote through fundamental and applied research the development of alternative and advanced physical, chemical, and biological processes for treatment of hazardous substances in the surface and subsurface environments.
2. 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 biological approaches, represent the major focus of Center activities. The research and training functions of the Center address the major hazardous substance problems in EPA Regions 9 and 10, including chlorinated and nonchlorinated solvents, petroleum products, pesticides, and toxic inorganic compounds including heavy metals. Environmental problems from these substances, which often occur in mixtures, result largely from the production of electronic equipment, chemicals, forestry products, and food, as well as mining and military activities, all of which are important in the region pair.

The 22 faculty and 3 staff who are directing the Center's research, training, and technology transfer activities are listed in Table 1. They collectively represent an integrated research team representing five different schools (civil engineering, engineering, science, earth sciences,

medicine, and veterinary medicine), and many different disciplines (chemical engineering, chemistry, hydrogeology, hydrology, medicine, microbiology, and petroleum engineering). Perry L. McCarty is Director of the overall Center and of the research program. Kenneth J. Williamson serves as Associate Director in charge of training and technology transfer and coordinates the Center's overall activities in Oregon. Martin Reinhard, the Assistant Director, is in charge of the Center's analytical program. Sharon Parkinson is the Center's Administrative Assistant.

Table 1. KEY PERSONNEL AT THE WRHSRC

<u>Stanford University</u>	<u>Oregon State University</u>
Prof. Martin Blunt	Prof. Daniel J. Arp
Prof. Mark N. Goltz <sup>a</sup>	Prof. A. Morrie Craig
Prof. Steven M. Gorelick	Dr. Michael R. Hyman
Prof. Lynn M. Hildemann	Prof. James D. Ingle
Mr. Gary D. Hopkins	Prof. Jonathan D. Istok
Ms. Sharon Parkinson	Prof. Peter O. Nelson
Prof. Peter K. Kitanidis	Prof. John S. Selker
Prof. James O. Leckie	Prof. Lewis Semprini
Prof. Keith Loague	Prof. John C. Westall
Prof. Abdul Matin	Prof. Kenneth J. Williamson
Prof. Perry L. McCarty	Prof. Sandra L. Woods
Prof. Martin Reinhard	
Prof. Paul V. Roberts	
Prof. Alfred M. Spormann	

<sup>a</sup>Currently with the Air Force Institute of Technology, Dayton, Ohio, but still active with the WRHSRC

The Center has two major advisory groups to guide its activities. The Science Advisory Committee (SAC) has oversight for all Center research activities, and the Training and Technology Transfer Advisory Committee (TTTAC) oversees the Center's training and technology transfer activities. The members of the SAC and TTTAC during this past year are listed in Tables 2 and 3, respectively. They represent federal and state governments, industry, consulting firms, and universities. The Center budgets for the 1998 fiscal year and since the Center's inception are listed by category of support in Table 4. 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.

Table 2. SCIENCE ADVISORY COMMITTEE

<u>Member</u>	<u>Affiliation</u>	<u>Expertise</u>
Linda M. Abriola	University of Michigan	Physical Processes, Hydrology
Douglas R. Christensen	Jacobs Engineering	Engineering
David E. Ellis	Du Pont Chemicals	Chemical/Biological Processes
David Frank	Govt/EPA, Region 10	Hydrogeology
John Glaser	Govt/EPA, Cincinnati Lab	Physical/Chemical Processes
Ronald Hoepfel	Govt/U.S. Navy	Microbiology
Michael C. Kavanaugh*	Malcolm Pirnie	Physical/Chemical Processes
Richard G. Luthy	Carnegie Mellon University	Chemical/Biological Processes
Stephen Schmelling	Govt/EPA, Ada Lab	Groundwater Remediation

James M. Tiedje  
John L. Wilson†  
John Wise  
\*Chairman  
†Vice Chairman

Michigan State University  
New Mexico Technical University  
Govt/EPA, Region 9

Microbiology  
Hydrology  
Planning

Table 3. TRAINING AND TECHNOLOGY TRANSFER ADVISORY COMMITTEE

<u>Member</u>	<u>Affiliation</u>	<u>Expertise</u>
James T. Allen	Govt/ California	Treatment Technology
Michael Anderson	Govt/Oregon	Hydrology
Harry A. Ball	Govt/EPA, Region 9	Environmental Engineering
John J. Barich	Govt/EPA, Region 10	Regulations
Kenneth Bigos	Govt/EPA, Region 9	Air Pollution
Lynn Coleman	Govt/Washington	Regulations
Jerry Jones	Govt/EPA, Kerr Lab.	Groundwater Remediation
David Kennedy*	Kennedy/Jenks Consultants	Treatment Technology
Jon Kindschy	Univ. California Ext. Service	Hazardous Substances Training
Gregory Peterson	Peterson Process Engineering	Treatment Technology
Winona Victory	Govt/EPA, Region 9	Training/Tech Transfer

\*Chairman

Table 4. CENTER FUNDING

<u>Funding Sources</u>	<u>FY 1998*</u>	<u>Funds to Date</u>
EPA: Centers Program	\$1,850,000	\$10,778,015
EPA: Other	\$666,000	\$3,616,816
Other Govt: Federal†	\$559,000	\$7,869,195
Other Govt: State@	0	\$48,252
Consortium	\$462,500	\$3,579,104
Private Sector#	<u>\$102,000</u>	<u>\$2,244,452</u>
TOTAL	\$3,639,500	\$28,135,834

\*Oct. 1, 1997 - Sept. 30, 1998

†Department of Energy; Department of Navy, U.S. Air Force  
@Oregon Department of Transportation

#Aluminum Company of America, Allied Signal Corporation; BMG Engineering AG; Brown and Caldwell; CH2M HILL; Chevron; DuPont Chemicals; Electric Power Research Institute; Gas Research Institute; Hewlett-Packard Company; Metcalf&Eddy; Monsanto Company; Montgomery Watson, Inc.; Kennedy/Jenks; Kleinfelder, Inc.; McLaren/Hart; Monsanto; Orange County Water District; Schlumberger Technologies; Shell Development Corporation, Woodward-Clyde Corporation

TABLE 5. STUDENT SUPPORT

<u>Student Support</u>	<u>Number*</u>	<u>Funds to Date†</u>
B.S.	13	\$95,877
M.S.	36	\$988,424



Ph.D.	114	\$5,286,230
Post Doctoral	<u>25</u>	<u>\$1,534,718</u>
TOTAL	188	\$7,809,372

\*Total numbers in researcher-years participating on Center Projects since 1989

†Includes tuition, travel, supplies, etc.

## DIRECTOR'S REPORT

This past year marked the beginning of a new project period and the tenth year of Hazardous Substance Research Center activity. All of the projects supported through core funding from the U.S. Environmental Protection Agency and reported here are new this year with only a few exceptions. Included are nine new research projects and three new demonstration projects. These projects continue to fall under the original scope of activities of this Center on groundwater contamination. The four thrust areas included in our activities are also all covered with one new research project concerned with chemical movement, fate, and treatment, four with chlorinated solvent contamination, one with anaerobic aromatic hydrocarbon biodegradation, and three with heavy metals. These research projects are under the direction of our Science Advisory Committee. Also, in keeping with the current efforts to seek greater transfer of knowledge gained from the basic research studies, we have begun three new field demonstrations under the direction of our Training and Technology Transfer Advisory Committee. We have an additional demonstration project that is funded by a subcontract from the Air Force Institute of Technology as part of a larger project funded by the Strategic Environmental Research and Development Program (SERDP). This project is directed by Dr. Mark Goltz, a former Assistant Director of our Center.

Our Training and Technology Transfer Activities under the direction of Ken Williamson at Oregon State University have also been greatly expanded. The Technical Outreach Services for Communities (TOSC) program has had its funding doubled from \$125,000 to \$250,000 this past year. These funds come from EPA, but are not part of the core research funding for the Center. As a result of the increase, two new staff members, Mary Masters and Michael Fernandez, have been added to the program to handle the rapidly expanding number of communities that are receiving assistance from the Center to help them better deal with their hazardous waste problems. Descriptions of the problems encountered by these communities are summarized in the "Training and Technology Transfer Project Descriptions" section of this report. In addition, EPA has provided funds for us to begin a new program to aid communities in their Brownfields initiatives. These activities will start soon.

The core funding for the Center has remained constant at \$1 million since the Center's inception almost ten years ago. Because of inflation, the research and technology transfer that can be accomplished with these funds are much less than they were when we began. Such funds are inadequate to support in research all of the twenty-two principal investigators that were once active in the Center, and thus we have had to make some difficult choices. Important to our success has been the diversity of backgrounds the principal investigators have contributed in hydrogeology, environmental engineering, chemistry, and biology, disciplines that are crucial for the solution of the complex groundwater contamination problems that we are addressing. In order to keep as many of them working with the Center as possible, we have made our projects

as fellowships only for student support and their needed supplies and equipment. We cannot continue to financially support the important contributions made by the faculty and so have asked them to contribute their time for advising students without Center funds. In this manner, we have been able to maintain a core of researchers representing the various disciplines that are critical to our success.

One of the great benefits of the Center is that it has allowed us to bring together a group of engineers and scientists and their students from various disciplines to work together to help understand and to solve some of the nation's most complex and costly hazardous wastes problems. The team effort that resulted has been highly successful. Fortunately, as funds for this joint effort have decreased, many of the investigators, often working with others within the group, have successfully sought funds outside of the Center for work on hazardous waste problems. The extent of hazardous waste research being conducted by our principal investigators outside of Center funding is now much greater than within it. Thus, we expect the good work that was started here will continue at Stanford and Oregon State Universities in spite of the Center's dwindling resources.

## HIGHLIGHTS OF THE YEAR

One of the highlights this year was the publication of the "Handbook for Estimating Physicochemical Properties of Organic Compounds," by Center Assistant Director Dr. Martin Reinhard and one of his former postdoctoral scholars, Dr. Axel Drefahl. This handbook was just published by John Wiley & Sons, Inc., New York, but will have a 1999 copyright date. Coming out in early 1999 to accompany this book will be a CD-ROM "Toolkit for Estimating Physicochemical Properties of Organic Compounds." The book and CD-ROM are the result of seed funds from the first WRHSRC research project in 1989 that was designed to integrate knowledge about hazardous chemicals. Much effort has gone into their development since then. This combination provides a comprehensive survey of published property estimation methods and property-estimating capabilities that are critical for evaluating the potential for chemical movement and fate in the environment. But more broadly, they will be highly useful in new chemical synthesis and manufacturing as well as many other fields in which chemical properties are sought. The handbook gives a narrative exposition, with examples, of a comprehensive array of over 100 current property estimation methods for 15 physicochemical properties of organic compounds. The Toolkit CD is a Windows-based tool for estimating the 15 key properties according to algorithms derived from over 2,700 published literature sources. Highly important is that the software provides methods for estimating physical properties for compounds for which measured properties are not known or available in the literature. In addition, the CD-ROM contains a database of property values for thousands of organic compounds from the *Registry of Physicochemical Data*<sup>TM</sup>. One can perform a search of the database with a structural query using the SMILES notation system or by creating structures using CS ChemDraw Net, which comes with the CD.

Another important product resulting from Center activities this past year is the "IRP, Aerobic Cometabolic In Situ Bioremediation Technology Guidance Manual and Screening Software User's Guide," by Earth Tech, Inc., June 1998. This Manual and the accompanying screening software can be downloaded free from: <http://en.afit.af.mil/env/insitubio.htm>. One of the major activities of the WRHSRC since its beginning has been the development of biological

approaches to biodegradation of hazardous compounds in general and chlorinated solvents in particular. This activity has ranged from basic research, field pilot evaluations, and then a full scale demonstration of in situ cometabolic biodegradation of trichloroethene through toluene injection completed this past year at Edwards Air Force Base in Southern California. This demonstration was supported largely by the U.S. Air Force. The full details of the highly-successful results of this demonstration are reported in one of this year's Center publications: McCarty, P. L., M. N. Goltz, G. D. Hopkins, M. E. Dolan, J. P. Allan, B. T. Kawakami, and T. J. Carrothers, "Full-Scale Evaluation of *In Situ* Cometabolic Degradation of Trichloroethylene in Groundwater through Toluene Injection," *Environmental Science and Technology*, **32**(1), 88-100 (1998). The guidance manual represents the next step in transfer of this new technology. It was written for the U.S. Air Force by Earth Tech, the site contractor who provided field support for the full scale demonstration. The screening software was developed under the supervision of Dr. Mark Goltz, WRHSRC Manager for the demonstration, and now a faculty member at the Air Force Institute of Technology. The purpose of the manual and the screening software is to help determine if this technology is appropriate for implementation at any given site. It is based upon the full-scale demonstration at Edwards. The document concludes with a discussion surrounding regulatory acceptance of the technology and a description of case studies where the technology has been implemented in the field. The document is designed for use by project managers not necessarily experienced in treatment technology design who are exploring potential technology alternatives for groundwater treatment.

The Technical Outreach Services for Communities (TOSC) program is achieving a greatly expanded visibility in the Western Region. A home page where the activities of TOSC can be reviewed is provided at: <http://www.orst.edu/Dept/owrri/TOSC/>. Those interested in this program can keep up to date on the activities now through the web. The new Brownfields activities will be reported there as well. Also, the current activities of the WRHSRC in general can be followed at: <http://www-seep-server.stanford.edu/SEEPWeb/wrhsrc/>.

## **FUTURE DIRECTIONS**

The WRHSRC plans to continue its basic research thrusts toward cleanup of important contaminants, including petroleum hydrocarbons, halogenated aromatic compounds, chlorinated solvents, and heavy metals. They collectively pose the most extensive, difficult and expensive problems faced in restoration of contaminated sites in the United States. Some new initiatives that are just getting underway address the major problem here of nonaqueous phase liquids (NAPLs). How to contain and remediate NAPLs is perhaps the most challenging problem faced in the hazardous substance arena. No clear solutions to this difficult problem are yet evident, but because of its immensity, basic research to help better define the nature of this problem is essential to its eventual solution. The several field demonstration projects that have been conducted through Center activity represent the applications of findings from the several basic research projects that the WRHSRC has been conducting since its inception in 1989. The field demonstrations not only represent the best way to transfer the results of our basic research to show how they apply in practice, but also, the field demonstrations will undoubtedly uncover difficulties in implementation that will require basic research studies for best resolution. Thus, we anticipate that future basic research studies will result largely from our demonstration experiences.

Table 6. WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER PROGRAM SUMMARY

**RESEARCH PROGRAM SUMMARY**

<b><u>Project Description</u></b>	<b><u>Principal Investigators</u></b>	<b><u>End Date</u></b>	<b><u>Total Budget</u></b>
<b>Chemical Movement, Fate, and Treatment</b>			
Development and Characterization of Redox Sensors for Environmental Monitoring	James Ingle	1999	\$131,865
Hydrocarbon and Biology Factors Affecting Aquifer Clogging During In-Situ Bioremediation	Perry L. McCarty	1999	\$152,706
<b>Chlorinated Solvents</b>			
Cytochrome P-450: An Emerging Catalyst for the Co-metabolism of Chlorinated Aliphatic Hydrocarbons and Methyl tery-Butyl Ether?	Michael Hyman Lynda Ciuffetti	1999	\$157,968
Aerobic Cometabolism of Mixtures of Chlorinated Aliphatic Hydrocarbons by Microorganisms Grown on Butane: Kinetic, Biochemical, and Modeling Studies	Lewis Semprini Daniel J. Arp	1999	\$174,188
Effect of Chemical Structure on the Biodegradability of Halogenated Hydrocarbons	Paul V. Roberts Perry L. McCarty	1999	\$118,120
Reductive Transformation of Chlorinated Ethenes Catalyzed by Vitamin B12 – Mechanistic and Kinetic Studies	Martin Reinhard	1999	\$125,533
Mechanisms, Chemistry, and Kinetics of Anaerobic Degradation of cDCE and Vinyl Chloride	Perry L. McCarty, Alfred Spormann	1999	\$452,000
<b>Aromatic Compounds</b>			

Key Enzymes of Anaerobic Degradation of Aromatic Hydrocarbons	Alfred M. Spormann	1999	\$62,435
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**Heavy Metals**

Trace Element Adsorption in Porous Particle Packed Beds	James O. Leckie	1999	\$145,885
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Assessing Metal Speciation in the Subsurface Environment	John C. Westall	1999	\$107,574
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Simultaneous Removal of the Adsorbable and Electroactive Metals from Contaminated Soils and Groundwater	Peter O. Nelson	1999	116,591
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**TRAINING AND TECHNOLOGY TRANSFER PROGRAM SUMMARY**

**Demonstration Projects**

Field Testing of Palladium Catalyzed Hydrogenation for Chlorinated Hydrocarbon Removal: Evaluation of Catalyst Degrading Mechanism	Martin Reinhard, Paul V. Roberts	1999	\$128,585
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Demonstration of a Permeable Barrier Technology for the Bioremediation of Ground Water Contamination with Waste Mixtures	Sandra Woods, Kenneth J. Williamson	1999	\$121,577
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In Situ Measurement of TCE Degradation Using a Single Well, "Push-Pull" Test	Jack Istok, Lewis Semprini	1999	\$89,037
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Bioenhanced In-Well Vapor Stripping to Treat Trichloroethylene (TCE)	Perry L. McCarty, Mark N. Goltz, and Gary D. Hopkins	1999	\$805,000
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**Training and Technology Transfer Projects**

Technical Outreach Services for Communities (TOSC)	Kenneth J. Williamson	1999	\$485,558
Technical Assistance to Brownfields (TAB)	Kenneth J. Williamson	1999	\$135,000

## RESEARCH PROJECT DESCRIPTIONS

### CHEMICAL MOVEMENT, FATE, AND TREATMENT

#### **Development and Characterization of Redox Sensors for Environmental Monitoring. James D. Ingle, Jr., Oregon State University.**

Goal: Long-range goals: (i) to understand under what conditions redox transformations of environmental redox-active species (inorganic and organic components and contaminants) will take place and (ii) to develop and evaluate methods to determine when these conditions occur.

Rationale: A clear understanding of the redox state and speciation of chemicals is required in virtually all aspects of hazardous waste management: (i) evaluation of disposal options, (ii) risk assessments of contaminated sites, and (iii) evaluation of clean-up options for contaminated sites. Specifically, with regard to redox transformations, there are two questions to be answered: 1) what is the redox level of a system? and 2) what redox transformations are occurring?

Approach: Various components and processes are considered in characterizing redox status and transformations in groundwater. The three components are the intrinsic redox-active species or contaminants, the biogeochemical matrix, and the redox sensor. The processes are the microbial TEA processes (e.g., Fe(III)-reduction, methanogenesis) that are ultimately responsible for redox transformations of the component species. The focus has been on understanding 1) how redox sensors (e.g., Pt electrode, redox indicators) couple to components in environmental and microbial systems (e.g., groundwater, soil and wastewater slurries and microbial cultures) and 2) what information redox sensors provide about the redox status of the system in terms of the redox state of various contaminants (e.g., As, Cr) or transformations of contaminants (e.g., TCE to DCE)). In parallel experiments, novel redox sensors based on immobilized redox indicators have been developed and characterized, and the development of a field sensor for low-level H<sub>2</sub> analysis has been pursued.

Status: Progress has been made in developing thin-film configurations for immobilized redox indicators making them more amenable to field use. Techniques have been refined to covalently immobilize redox indicators to agarose thin-films and cellulose filter paper. A new spectrophotometric cell was constructed to accommodate these "redox-indicator slabs" and it was placed in the external loop filter/flow system of a special bioreactor. This arrangement allows filtered solution from a slurry or culture to interact with the indicator while the absorbance (and hence speciation) of the immobilized redox indicator is monitored with a spectrophotometer. Clogging of the cell by particles is not a problem as it had been previously when redox indicators were immobilized to beads packed in a flow cell. In earlier work, two redox indicators, thionine ( $E_{70'} = +53$  mV) and cresyl violet ( $E_{70'} = -75$  mV), were demonstrated to be effective for delineating between Fe(III)-reducing conditions and sulfate-reducing conditions in reduced soil and waste water slurries. In recent studies, phenosafranine ( $E_{70'} = -267$  mV) was reduced under methanogenic conditions in wastewater slurries and groundwater samples. These three indicators help to establish "redox windows" which can be used to identify and delineate between Fe(III)-reducing, sulfate-reducing, and methanogenic conditions. Also, these immobilized redox indicators were shown to be applicable for

determining when redox transformations of As or TCE were occurring in soil slurries and groundwater samples, respectively. In an alternative strategy, the cellulose filter-immobilized redox indicators were pressure-fit to the surface of a Pt electrode and the indicator couple appeared to poison the Pt electrode when the indicator was partially reduced.



## **Hydrocarbon and Biological Factors Affecting Aquifer Clogging During In-Situ Bioremediation: Perry L. McCarty, Stanford University**

Goals: The goals of this project are: (1) to develop a laboratory apparatus for visually observing and quantifying the physical, chemical, and biological factors affecting bacterial attachment, growth, and hydraulic conductivity change in porous media, and (2) to make preliminary observations of bacterial growth and attachment as a function of a few variables, including pore shape, Peclet number, growth substrate, and bacterial species.

Rationale: One of the major concerns in the application of in-situ bioremediation of groundwater contaminants is the reduction in hydraulic conductivity and eventual clogging of an aquifer by growth of microorganisms. There have been many empirical studies of such biofouling over the last few decades, but a good understanding of factors affecting bacterial growth and aquifer clogging is still not available. One reason is that good visualization methods for observing the processes involved were lacking until recently. The visualization approach being proposed here is expected to add significantly to our understanding of the processes of importance.

Approach: An apparatus for visualizing the growth of bacteria on surfaces that simulate a groundwater aquifer and for measurement of fluid velocities as well has been constructed. The apparatus consists of simulated aquifer pore structure with defined channel widths etched on a silicon wafer. Growth is visualized by a light microscope with moving stage, with images stored in a computer. The chip is termed a Silicon Pore Imaging Element (SPIE). The SPIE is seeded initially with a bacterial culture, and liquid containing nutrients and substrate is pumped through the SPIE with a syringe pump. The resulting growth, its distribution and pressure losses across the SPIE are recorded over time. From the understanding that is obtained, cultures with different adhesion properties will be evaluated under a variety of growth substrates at different concentrations and with different fluid velocities and aquifer system geometries. The effect of disinfectants on the biofilms that develop will also be evaluated.

Status: The visual system works well. Two case studies have now been conducted using a defined pore structure with a checkerboard layout, and with randomly varying pore widths. The first study with mixed culture fed acetate developed fungal growth due to a low pH. The second study seeded and fed in a similar way, but with neutral pH produced attached bacterial colonies. The fungal culture growth resulted dominantly in an upgradient direction. This suggests chemotaxis is important. The growth was patchy, leaving some portions of the SPIE open for liquid flow while others were filled with biological growth. The effect of disinfectant on growth has also now been observed. Interpretation of results is underway.

## CHLORINATED SOLVENTS

### **Cytochrome P-450: An Emerging Catalyst for the Cometabolism of Chlorinated Aliphatic Hydrocarbons and Methyl *tert*-butyl Ether?**

Principal Investigators: Michael Hyman and Lynda Ciuffetti

Goals: This project has three main goals. The first is to examine the ability of the filamentous fungus, *Graphium*, to degrade the chlorinated solvent, chloroform. Second, the project aims to provide a quantitative description of the cometabolism of the gasoline oxygenate, methyl *tert*-butyl ether (MTBE) by *Graphium*. Third, the project aims to examine whether cytochrome P-450 type enzymes are consistently associated with the ability of microorganisms to degrade MTBE.

Rationale: The ability of diverse microorganisms to degrade non-growth supporting substrates through cometabolism represents one of the most promising avenues for the bioremediation of many persistent environmental pollutants. The aim of this project is to examine the role of cytochrome P-450 enzymes as useful catalysts for this purpose.

Approach: Our experimental approach typically involves short-term (<12 h) laboratory-based experiments using axenic cultures of individual organisms grown on a variety of hydrocarbon substrates. From these types of studies we are able to identify both the initial products and initial rates of cometabolic transformations. Furthermore, the use of short-term studies using resting cells typically allows us to minimize the effects of new enzyme synthesis or pathway induction on the part of the test organism in response to new substrate, cosubstrate and inhibitors.

Status: Our studies of chloroform by *Graphium* have been completed. *Graphium* was found to reductively dehalogenate CT to CF in the absence of oxygen through the activity of a propane-inducible cytochrome P-450 activity. The CF generated under anaerobic conditions is rapidly oxidized by the same enzyme when oxygen is reintroduced into the reaction. This suggests that it is possible to achieve full mineralization of CT and potentially other halogenated hydrocarbons using a single microorganism. We have also demonstrated that the aerobic bacterium *Nitrosomonas europaea* can catalyze the same sequence of reactions. In this case the reductive transformation of CF is then catalyzed by the constitutive and highly non-specific ammonia microorganism. The oxidation of CF is then catalyzed by the constitutive and highly non-specific ammonia monooxygenase enzyme

Our evidence indicates that MTBE is oxidized by both prokaryotes and eukaryotes by the same pathway which involves *tert*-butyl formate and *tert*-butyl alcohol as the initial products. Using bacterial MTBE-degraders we have also established that there are potentially four oxygenase-catalyzed reactions involved in the oxidation of MTBE to the level of acetaldehyde.

We examined all of the major classes of TCE-degrading microorganisms and have demonstrated that MTBE is only oxidized by microorganisms capable of growing on propane as a sole source of carbon and energy. MTBE-degrading activity is also consistently associated with the ability of microorganisms to grow on branched hydrocarbons that are structural analogs

of MTBE. Our studies using selective mechanism-based inactivators of heme- and non-heme-containing oxygenases also suggest that cytochrome P-450 enzymes are not the only oxygenases capable of MTBE oxidation. Toluene was found to be a potent inhibitor of bacterial MTBE cometabolism.

## **Aerobic Cometabolism of Mixtures of Chlorinated Aliphatic Hydrocarbons by Microorganisms Grown on Butane; Kinetic, Biochemical, and Modeling Studies: Lewis Semprini and Daniel J. Arp, Oregon State University**

Goal: The objectives of this study are: 1) to examine the ability of pure cultures (CF8, *Mycobacterium vaccae* and *Pseudomonas butanovora*) and butane enrichment culture to transform mixtures of chlorinated aliphatic hydrocarbons (CAHs), with a focus on 1,1,1-trichloroethane (1,1,1-TCA), 1,1-dichloroethylene (1,1-DCE), and 1,1-dichloroethane (1,1-DCA), 2) to perform pilot scale bioreactor biotransformation studies of CAH mixtures, 3) to determine products formed from the transformation of 1,1,1-TCA, 1,1-DCE, 1,1-DCA, and 4) to determine if propane-grown cultures of the butane-enrichment, CF8, *M. vaccae*, and *P. butanovora* have identical transformation patterns.

Rationale: In previously funded Center research microorganisms grown on butane and propane were found to have good potential for the aerobic treatment of chlorinated methanes, chlorinated ethanes, and some chlorinated ethenes (1,1-DCE). Effective transformation was observed for chloroform (CF), 1,1,1-TCA, and 1,1-DCE that had previously been shown to be fairly difficult contaminants to degrade via aerobic cometabolism. These studies were performed with an enrichment culture obtained from the Hanford DOE site, and pure cultures CF8 (obtained from the Hanford enrichment), *M. vaccae* and *P. butanovora*. The current study extends that work to investigate the cometabolism of CAH mixtures using the enrichment and the pure cultures.

Approach: Detailed batch kinetic studies will be performed with the butane enrichment on 1,1,1-TCA, 1,1-DCE, and 1,1-dichloroethane (1,1-DCA) transformation, since these contaminants are often found together in the subsurface due to the abiotic and biotic transformations of 1,1,1-TCA. The maximum degradation/ transformation rates ( $k$ ), half-saturation coefficient ( $K_s$ ), inhibition types (competitive, non-competitive, and mixed inhibition), and inhibition coefficients ( $K_I$  and  $K_I'$ ) for 1,1,1-TCA, 1,1-DCE, 1,1-DCA, and butane will be determined. Kinetic and modeling studies will be performed with complex mixtures of these contaminants. Biochemical studies will be performed with the pure cultures to learn more about the range of substrates that induce CAH transformation and products formed during cometabolism. The diversity of oxygenase enzymes expressed in the cultures will be investigated through an analysis of the reversible and irreversible effects of selective monooxygenase inhibitors.

Status: In batch kinetic studies with the butane-grown enrichment the maximum degradation/transformation rates ( $k$ ), half-saturation coefficient ( $K_s$ ), inhibition types (competitive, non-competitive, and mixed inhibition), and inhibition coefficients ( $K_I$  and  $K_I'$ ) of 1,1,1-TCA, 1,1-DCE, 1,1-DCA, and butane have been determined. A direct linear plot method was used to identify the types of inhibition. 1,1,1-TCA competitively inhibited the transformation of 1,1-DCA, and vice versa. However, butane (growth substrate) showed different inhibition types, that is, non-competitive inhibition on 1,1,1-TCA and 1,1-DCA and mixed inhibition on 1,1-DCE transformation. Inhibition model parameters have been determined using non-linear least square regression methods. *P. butanovora* was grown on a variety of alkane substrates. Chloroform was degraded at similar rates whether grown on propane, butane, or pentane. In *P. butanovora*, *M. vaccae*, and the Hanford environmental isolate CF8, the  $K_s$  for butane was examined. The results indicate that the  $K_s$  values are well below 5  $\mu\text{M}$ . The substrate range of all three bacteria was further investigated. None of the three bacteria oxidized methane when grown on butane and none could grow on methane. Ammonia was oxidized to hydroxylamine or nitrite by all three bacteria. Ethylene was oxidized to ethylene oxide by all three bacteria, though *P. butanovora* could not sustain the rate as the butane monooxygenase was inactivated by ethylene.

## **Effect of Chemical Structure on the Biodegradability of Halogenated Hydrocarbons: Paul V. Roberts and Perry L. McCarty, Stanford University**

Goal: Halogenated hydrocarbons have been found to be transformed by a variety of microorganisms. Much experimental data has been generated for biodegradation rates of halogenated hydrocarbons. However, there are still gaps in both the data and our understanding of why certain halogenated compounds are more readily transformed than others. The goal of this research is to improve our ability to estimate biodegradation potential for halogenated hydrocarbons and to improve conceptual understanding of the factors that affect biodegradability.

Rationale: Transformation of a compound by biological processes is a function of the properties of the compound, such as the presence and accessibility of readily transformed chemical bonds (biophore), among other factors. By understanding the relative contributions of such properties, the biodegradation potential of a given compound can be estimated based on knowledge of that compound's properties. Biological transformation of halogenated compounds is also governed at the molecular level by the interaction between key enzymes and the compounds (substrate). Thus, biological transformation of halogenated compounds is both a function of the internal reactivity of a molecule and how this compound interacts with the transforming enzyme. Estimation of biodegradability for a given compound must account for both.

Approach: Computational tools are being used to leverage the experimental data already obtained on halogenated compounds. First, statistical methods are being used to develop correlative relationships between various independently measurable properties of a compound and their observed biotransformation rate. These are known as quantitative structure-activity relationships (QSARs). Molecular modeling tools are also being used to develop better fundamental understanding of the importance of enzyme-substrate interactions in determining bioactivity.

Status: Structure activity relationships have been developed for biotransformation of halogenated hydrocarbons by soluble methane monooxygenase. Various electronic descriptors were employed along with comparative molecule field analysis (CoMFA) which measures the steric and electrostatic fields surrounding a molecule. Molecular docking simulations of the interactions between halogenated compounds and a three dimensional model of soluble methane monooxygenase obtained from the Brookhaven Protein Data Bank were also performed. These simulations are being used to develop understanding of how steric interactions between substrate and enzyme may affect the ability of methane monooxygenase to transform a given compound. Similar studies have begun with haloalkane dehalogenase, another enzyme known to transform halogenated compounds. Experimental data with chloro- and bromo- substituted analogues is available and used for developing structure-activity relationships. Docking simulations are also being performed with haloalkane dehalogenase. Docking results with dichloroethane positioned the substrate roughly similar to results obtained from crystallographic analysis, demonstrating the usefulness of docking programs to help simulate actual enzyme substrate interactions

## **Reductive Transformation of Chlorinated Ethenes Catalyzed by Vitamin B12 and in Biological Systems: Martin Reinhard, Stanford University.**

Goal: This project aims to get a better understanding of the role of vitamin B12 in biological dehalogenation reactions.

Rationale: Transition-metal coenzymes such as vitamin B12 have been shown to mediate the dehalogenation of chlorinated hydrocarbons in microbial systems. The mechanism of abiotic dehalogenation by vitamin B12 was studied in aqueous solutions in the presence of bulk reductants (Ti(III) or thio-reductants). These studies indicate the formation of relatively stable Co-C bonds and the formation of radical intermediates. The significance of these findings for biological remediation is not yet understood. Biotic systems differ from abiotic systems in several respects, including (1) no (chloro-)acetylene formation, (2) generally no trans-DCE or 1,1-DCE formation, (3) dehalogenation stops at cis-DCE in most pure cultures, and (4) no catalyst deactivation. Detailed mechanistic understanding of these observations can help optimize biological detoxification processes and promote the design of novel remediation approaches.

Approach: The kinetic and product distribution data from microbial, enzymatic, and abiotic dehalogenation experiments will be compared using chlorinated ethylenes as the substrates. This approach will determine what factors affect the stability of various Co-(Cl-) ethylene complexes and will identify such complexes in enzymatic and biological systems. Commercially available cobalamin-containing enzymes and enrichment cultures from a dehalogenating site will be used. The presence and stability of Co-(Cl-)ethylene complexes will be investigated using UV-spectroscopy,

Status: The methodology for quantifying products and intermediates has been tested, and enrichment cultures have been developed using sediment from a dehalogenating site. The pertinent literature has been reviewed and summarized in the form of a book chapter that is now in press.

**Mechanisms, Chemistry, and Kinetics of Anaerobic Degradation of cDCE and Vinyl Chloride: Perry L. McCarty and Alfred Spormann, Stanford University (Supported by DuPont Chemicals and U.S. Department of Energy)**

Goal: The objectives of this study are to describe the bacterium or groups of bacteria that are responsible for conversion of tetrachloroethene (PCE) to ethene in aquifer samples taken from a contaminated site in Victoria, Texas, and to examine the factors affecting the rate and extent of transformation.

Rationale: Several species of bacteria have been isolated and identified by others that have the ability to reductively dehalogenate chlorinated aliphatic hydrocarbons (CAHs). However, little is known about individual or groups of organisms responsible for the complete dehalogenation process from tetrachloroethylene (PCE) to ethene. Whether or not the complete dehalogenation or all of the individual dehalogenation steps are the result of cometabolism or of energy metabolism is also not known. In addition, the various factors that affect the rates of dehalogenation have not been adequately evaluated. Such factors may include the electron donor used, the presence or absence of alternative electron acceptors, pH, and temperature. In order to understand and apply the process better, definitive studies concerning the nature of the organisms involved and factors affecting transformation rates are needed.

Approach: Anaerobic aquifer material for this study was obtained by DuPont Chemical Company from a contaminated site in Victoria, Texas. An enrichment culture fed PCE and benzoate was developed in a chemostat. This culture completely converts 1 mM PCE to ethene, while being fed about 1.5 mM benzoate. Subcultures are taken from the chemostat to analyze the effects of different variables on kinetics of the reactions. Special emphasis is being placed on the conversion of cis-1,2-dichloroethylene (cDCE) to ethene as this tends to be the rate limiting reaction in the overall conversion of PCE to ethene. An enrichment culture has also been developed on vinyl chloride for biochemical and enzymatic studies.

Status: The original project was funded only by DuPont, but additional support has now been received from the U.S. Department of Energy. This overall study has demonstrated that there are two separate groups involved in the overall dehalogenation of PCE to ethene in our culture, the first group converts PCE to cDCE, and the second converts cDCE to vinyl chloride and ethene. The rates of transformation of PCE and TCE to cDCE were higher than for the conversion of cDCE to vinyl chloride and vinyl chloride to ethene. Organisms appear to obtain energy from the dehalogenation reaction while using the chlorinated organics as electron acceptors. Kinetic studies of the reactions involved have been completed. These studies indicate the threshold level of hydrogen for dehalogenation, and the effects of CAH concentration on the reaction rates. Also, enzymatic studies are being conducted on VC dehalogenation in order to better understand the biochemistry of the reactions involved.



## AROMATIC COMPOUNDS

### **Key Enzymes of Anaerobic Degradation of Aromatic Hydrocarbons: Alfred M. Spormann, Stanford University.**

Goal: To isolate key enzymes of anaerobic toluene and ethylbenzene degradation (benzylsuccinate synthase and ethylbenzene dehydrogenase), to clone the genes involved, and to develop gene probes.

Rationale: Anaerobic microorganisms capable of complete mineralization of aromatic hydrocarbons (specifically of BTEX compounds) are important players for in situ remediation of anoxic, fuel hydrocarbon contaminated aquifers. Research in this laboratory, that was initially funded by the WRHSRC and more recently by two grants from the NSF Metabolic Biochemistry program, led to the identification of the initial reactions of the pathways of anaerobic toluene, *m*-xylene, and ethylbenzene mineralization. The key enzyme of toluene mineralization is benzylsuccinate synthase, and ethylbenzene oxidation is initiated by ethylbenzene dehydrogenase. Understanding these key enzymes will allow to develop metabolic and molecular probes to monitor and to predict intrinsic degradation of these compounds in the field and laboratory studies.

Approach: Pure cultures of denitrifyins *Azoarcus* sp. Strain T and *Azoarcus* sp. Strain EBI were used in in vitro studies to investigate the initial enzymatic reactions involved in anaerobic toluene and ethylbenzene mineralization, respectively. Cells of these strains served as the source to isolate the key enzymes. Reverse genetics is used to determine the nucleotide sequence of the genes involved.

#### Status:

1) Key enzyme in anaerobic toluene mineralization, Benzylsuccinate synthase was isolated to more than 70% purity from toluene grown *Azoarcus* sp. Strain T. The native enzyme has a molecular weight of ca 260 kDa., consistent with  $\alpha_2\beta_2\gamma_2$  composition as was observed in studies by others. Based on this finding and on sequence of benzylsuccinate synthase from *Thauera* sp., the complete benzylsuccinate synthase operon of *Azoarcus* sp. Strain T was cloned and the nucleotide sequence determined. Comparison of this sequence with that of a previously determined sequence of benzylsuccinate synthase from strain T1 and *Thauera aromatica* revealed regions of homology. Design of molecular probes for this key enzyme derived from these sequences and testing of these probes in progress.

2) Key enzyme in anaerobic ethylbenzene mineralization. Ethylbenzene dehydrogenase activity was characterized in cell-free extracts, and an assay was developed that will be used to purify the membrane-bound enzyme activity. Isolation of the enzyme is in progress.

This project was funded for one year. Because a NSF grant was obtained that would overlap with this project, funds for the second year of this project were not required.

## HEAVY METALS

### **Trace Element Adsorption in Porous Particle Packed Beds: James O. Leckie, Stanford University**

Goal: The objective of this project is to develop the empirical data base and mathematical model requisite to trace element treatment technology based on trace element adsorption onto packed beds of high adsorption capacity, porous alumina particles, to bring waste streams into compliance with increasingly stringent NPDES standards. Research activities are to be directed toward establishing optimal design and operational characteristics for the proposed technology.

Rationale: Chemical precipitation has been the most widely used technique of trace element removal from wastewater for several decades. Though chemical precipitation has proved to be effective over a narrow range of operating conditions, it is beset with a number of problems, including the inability of achieving increasingly stringent NPDES in-stream permit standards. Adsorption promises to be a more efficient method of trace element removal, especially over a wider pH range and to concentrations lower than those achievable by precipitation. Additional advantages include the ability to remove cations and anions simultaneously and the ability to regenerate the adsorbent by adjusting contacting solution conditions (e.g., pH, presence of ligands, etc.). Despite inherent advantages over precipitation, the potential of developing packed bed adsorption into a feasible treatment technology has not yet been thoroughly investigated.

Approach: The proposed technology will use packed beds of adsorbent particles for treating waste solutions as such a technology can be modularized and requires a small footprint and low maintenance. The project will use commercially available alumina particles that exhibit strong adsorption characteristics along with fast uptake rates for the target contaminants. Our studies have shown that for fast adsorption, the adsorbent should have significant pore volume in the macro- and mesoporous regions. Cadmium and selenite are being used as model contaminants for the study. The study will generate a high quality empirical database from extensive batch and column studies of adsorption. A mathematical model will be developed for simulating adsorption processes in continuous-flow column reactors. The model, once developed and verified, can be used to predict column adsorber performance and is intended to serve as a tool in designing and operating the proposed treatment scheme.

Status: Experimental batch studies of equilibrium sorption and rate of uptake of cadmium and selenite by the porous alumina solids (DD660 and DD431) over a wide range of chemical conditions have been completed. We have initiated column sorption experiments to study cadmium and selenite sorption under the influence of hydrodynamic conditions (which is the target configuration for the proposed technology). Equilibrium sorption results have been modeled using the Triple-Layer Model (TLM) and satisfactory fits to the data have been obtained in most cases. However, modeling of equilibrium sorption of EDTA and its effect on cadmium sorption characteristics (with the TLM) has yielded unsatisfactory fits to data. We are now utilizing a charge-distribution approach in conjunction with a Four-Layer model to describe this sorption behavior. The modeling of the rate of uptake data of cadmium in the absence and presence of ligands is currently being done using the KINEQL approach. The

completion of both these modeling tasks will allow us to build and calibrate the mathematical model that will be utilized in interpreting the column sorption data.

## **Assessing Metal Speciation in the Subsurface Environment: John C. Westall, Oregon State University.**

Goals: The long-range goals of this research are (i) to advance the state of knowledge about the binding of metal (and metalloid) ions to heterogeneous environmental sorbents; and (ii) to advance the ability to model these interactions in a way that is useful for field applications.

Rationale: Speciation models are needed in virtually all aspects of management of metal contamination of the subsurface environment, including risk assessment, site remediation, and waste disposal. One of the greatest obstacles to the practical application of metal speciation models in field systems is the heterogeneity of environmental sorbents, such as humic substances or the surfaces of rocks and minerals. In this project, metal speciation models are to be developed that are much better suited to real, complex, heterogeneous materials from the field. Better knowledge of metal speciation will lead to cheaper and better decisions about disposal options, risk assessment, and clean-up procedures.

Approach: In this project, two fundamental barriers to successful description of the association of metal ions with heterogeneous environmental sorbents are addressed: (i) the paucity of multidimensional datasets (i.e., datasets with variations in many solution chemistry properties such as pH, salt concentration, total metal concentration, etc.) for metal ion adsorption by these materials, and (ii) the inadequacy of detailed mechanistic models in dealing with these multidimensional datasets. First, multidimensional data will be gathered for the behavior of metals with heterogeneous environmental complexing agents, with initial focus on inorganic priority pollutants common to groundwater pollution problems. Then the "discrete log K spectrum" or "affinity spectrum" approach to modeling the interactions in these systems will be developed and applied. Finally, as a point of reference, the results will be compared to the traditional "surface complexation - electrostatic" approach.

Status: The initial data set for adsorption of selenite and selenate onto a well characterized kaolinitic soil clay from the Cecil-Pacolet (CP) soil series in North Carolina has been obtained. The clay fraction of the CP soil is dominated by kaolinite and crystalline Fe and Al oxides. Adsorption data are available for a wide range of pH and total Se concentration. The affinity spectrum approach is successful at representing the data over a broad range of solution composition, but the affinity distribution is not unique. The Atraditional@ triple layer model fails to represent the data as a function of total Se concentration and is not internally consistent with the composition of the CP clay; however, it may provide some insight in to the relative importance of the component mineral phases. Future work includes further development of the affinity spectrum approach (determination of the optimum means of discretizing the affinity spectrum, relating the affinity spectrum properties to systematically varying properties of the sorbent, and coupling of the affinity spectrum models to transport models) and testing of additional data sets.

## **Simultaneous Removal of the Adsorbable and Electroactive Metals from Contaminated Soils and Groundwater: Peter O. Nelson, Oregon State University**

Goal: The goal of this research is to examine the applicability of a permeable barrier technology for the simultaneous removal of adsorbable and electroactive metals from soils and contaminated groundwaters.

Rationale: In-situ permeable reactive barrier technologies have been proposed as a lower cost alternative to traditional methods of groundwater remediation such as pump-and-treat. Many hazardous waste sites have more than a single contaminant. Groundwater contamination at wood preservative facilities often contains metals mixtures. Among common metals formulations used as wood preservatives are copper, chromium, and arsenic (CCA), and ammoniacal copper zinc arsenate (ACZA).

Approach: Metals can be removed from contaminated groundwater by adsorption and/or reduction onto iron-oxide minerals (e.g. hematite and biotite). Iron-oxide-coated sand (IOCS) has been successfully used in our lab in both batch and column reactor experiments to remove lead from aqueous solution. Magnetite-coated sand (MCS) is a mixed oxidation state (ferrous-ferric) iron oxide, providing both adsorption and reduction capacities for simultaneous removal of Pb(II) and Cr(VI) from aqueous solution. In a mixed metals contaminated ground water (e.g. Cu(II), Cr(VI), and As(V)), MCS can be used to for simultaneous removal of Cu(II) by adsorption, Cr(VI) by reduction to Cr(III), and As(V) by adsorption without reduction to As(III). One possible advantage of MCS is that it adsorbs As(V) and prevents it from reducing to the more toxic and mobile As(III) species, compared to metallic iron ( $\text{Fe}^0$ ), for which reduction of both Cr(VI) and As(V) is thermodynamically favored.

Status: The effects of competitive adsorption of strongly a adsorbed anion, arsenate, and cation, copper, on adsorption and reduction of a weakly (outer-sphere) adsorbed anion, chromate, on IOCS and MCS has been completed. The effect of competitive adsorption among CCA metals has been completed. The environmental effects on adsorption of CCA onto MCS and IOCS including pH, ionic strength, and metals concentration has been completed. Surface complexation modeling has successfully predicted arsenate, copper, and chromium adsorption on IOCS and MCS in single-solute systems. Equilibrium adsorption constants and surface site density for each metal have been determined. The effects of the enhancement and competitive effects among CCA metals and the use of the triple-layer model to predict adsorption for multi-solute systems are in progress. Determination of precipitates such as  $\text{Cr}(\text{OH})_3$ ,  $(\text{Cr}_x\text{Fe}_{1-x})(\text{OH})_3(\text{s})$ , and  $\text{Cu}(\text{OH})_2$  that occur on the surface, and regeneration processes for IOCS and MCS, will be investigated

## TRAINING & TECHNOLOGY TRANSFER PROJECT DESCRIPTIONS

### DEMONSTRATION PROJECTS

#### **Field Testing Of Palladium Catalyzed Hydrogenation For Chlorinated Hydrocarbon Removal: Martin Reinhard and Paul Roberts, Stanford University**

Goals: This project aims (1) to evaluate the effectiveness of hydrogen/palladium treatment for the removal of halogenated hydrocarbons, by determining the catalyst lifetime in a packed bed reactor; (2) to identify competitors/inhibitors in the process and minimize their effects; and (3) to scale-up, optimize and implement the process at the field-scale.

Rationale: Batch studies with supported palladium catalysts have demonstrated the potential of the palladium/hydrogen process for treating groundwaters or effluent streams that are contaminated with halogenated compounds. These studies yielded virtually complete reductive dehalogenation of chlorinated ethylenes to ethane at room temperature in short contact times, with reaction rates that are orders of magnitude higher than zero-valent iron. Other batch studies have shown the ability of palladium to catalyze the reaction of a range of compounds: tetrachloroethylene, trichloroethylene, the DCE isomers, carbon tetrachloride, 1,2 dibromo-3-chloropropane, Freon 113, and chloroform. An initial feasibility model indicated that the process is economically competitive with GAC adsorption for catalyst lifetimes of at least two months. However, research is needed to determine the catalyst lifetime and to understand the factors that affect it under field conditions.

Approach: For this project, two bench-scale continuous-flow packed bed column reactors were constructed. The columns are used to remove trichloroethylene (TCE) in waters of various quality: deionized (DI) water, DI water which was artificially contaminated with known quantities of substances, or groundwater obtained from Lawrence Livermore National Laboratories (LLNL). From these tests, it may be possible to deduce some substances which can harm the catalyst. In addition, through comparisons of spectroscopic analyses of fresh and spent catalysts, more information on the nature of the deactivation can be obtained. In conjunction with this laboratory work, a field-scale test is being conducted by LLNL at their Livermore site.

Status: In column studies, no effects were seen when the catalyst was exposed to DI water. Nitrate and oxygen competed with TCE for hydrogen or catalyst sites, thereby lowering the TCE removal; however, no permanent effects to the catalyst were observed. Exposure to carbonate, carbon dioxide, and LLNL groundwater have all been found to deactivate the catalyst. The field-scale test at LLNL is currently underway; a packed bed column has been in operation for over six months, without any apparent deactivation. (Note that the bench-scale columns are designed to run at incomplete TCE removal, so that any deactivation can be easily observed. The LLNL column is designed with excess palladium for complete removal of TCE, even if some deactivation occurs.) Current efforts focus on spectroscopic analysis of used catalyst to investigate changes in the catalyst due to exposure to various waters. Through the use of x-ray photoelectron spectroscopy (XPS), the palladium appears to be mobile; its concentration on the support can vary with location and exposure conditions. In addition, carbon and nitrogen have been found on the catalyst surface after exposure to column

conditions, presumably due to the accumulation of organics. Finally, chlorine has been found on all active catalysts, but is nondetectable on deactivated catalysts.

## **Demonstration of a Permeable Barrier Technology for the Bioremediation of Ground Water Contaminated with Waste Mixtures: Sandra Woods, Kenneth J. Williamson.**

Goal: The intent of this project is to demonstrate a passive, in-situ, treatment strategy for the bioremediation of ground water contaminated with waste mixtures. The project, incorporating laboratory and field components, focuses on the design, implementation and optimization of a permeable barrier reactor capable of pentachlorophenol (PCP) and naphthalene degradation.

Rationale: In-situ bioremediation schemes often fail because a suitable substrate, the contaminant and viable microorganisms lack adequate mixing in the subsurface. In an effort to improve contaminant removal, minimize cost and maximize process control, a down-hole permeable reactor was developed. In-situ groundwater treatment is achieved using a large diameter well and a permeable biological reactor installed within a screened interval of the contaminated aquifer. The reactor assembly is equipped with sensors, nutrient delivery, and mixing systems for the support of a subsurface biological population. Reactor environmental conditions are controlled from the surface and allow the operation of three unique (e.g. anaerobic, aerobic) biological treatment zones. Biodegradation of the aqueous phase organic compounds occurs over the length of the reactor.

Approach: Biological degradation of PCP generally occurs under the reduced conditions favored by anaerobic systems. PCP degradation by anaerobic reductive dechlorination is rapid and often results in complete mineralization. However, overall rates of PCP mineralization can be increased if the dechlorinated intermediates produced under anaerobic conditions are exposed to an oxidizing aerobic environment. Complimentary to increased rates of PCP removal, naphthalene degradation is also favored by aerobic conditions. An active wood treating facility with PCP and naphthalene ground water contamination was chosen to demonstrate the effectiveness of an in-situ permeable barrier treatment system operating under sequential anaerobic/aerobic treatment zones.

Status: Commencing with the initial installation in August 1997, PCP removal has been evaluated as a function of environmental conditions imposed on the permeable reactor. During the first week of December 1997, the reactor was removed, inoculated with 50/50 (vol.) mixture of anaerobic digester and activated sludge and returned to the well in the absence of electron donor. Approximately 5 weeks of data collection showed little change in chlorophenol or naphthalene concentrations. Continuous flow cell data also indicated no major changes in the overall environmental conditions within the reactor. In early January 1998, injection of the electron donor, imitation vanilla flavoring began. Initially, oxygen was supplied to induce aerobic conditions suitable for naphthalene removal and to increase degradation rates of anaerobic degradation products of PCP. The response to oxygen addition was immediate and evidenced by the rapid reactor wide increase in oxidation-reduction (EH) potential. Aerobic conditions prevailed in the unit for one month, however no change was observed in the in-situ PCP or naphthalene concentrations. The oxidant was removed and the system EH rapidly fell and eventually stabilized near -270 mv. Concurrent with the decline in the system EH, production of dechlorinated intermediates was observed. With time, complete degradation of PCP under anaerobic conditions was observed in the permeable barrier reactor. Laboratory efforts are underway to better understand the interactions of PCP with the co-contaminants present in the site ground water. Subsequent experiments will focus on naphthalene removal.



Batch bottle studies will hopefully provide the information needed to make a reality the overall project goal of remediation of waste mixtures.

**In-Situ Measurement of TCE Degradation Using a Single-Well “Push-Pull” Test:  
Jonathan D. Istok, Lew Semprini, Oregon State University**

Goal: The overall goal of this project is to further develop the single-well, push-pull test method for use in quantifying rates of anaerobic microbial transformations of chlorinated aliphatic hydrocarbons (CAHs) such as trichloroethene (TCE) in groundwater aquifers.

Rationale: It is becoming increasingly apparent that in-situ testing methods will be required to fully understand microbial processes occurring in the deep subsurface, especially in contaminated environments, which typically display steep geochemical gradients. The recently developed, single-well, “push-pull” test method is a potentially powerful method for obtaining quantitative information about microbial metabolic activities in groundwater aquifers. A push-pull test consists of the controlled injection of a prepared test solution into an aquifer followed by the recovery of the test solution/groundwater mixture from the same location.

Approach: Microcosm experiments constructed with groundwater and sediment from a TCE-contaminated field site are used to select combinations and concentrations of cometabolic substrates to use in field push-pull tests. Field push-pull tests are then conducted in wells located in both pristine and TCE-contaminated portions of the field site to obtain in situ rates of introduced substrate transformation and TCE degradation. In situ rates are compared with rates observed in microcosm experiments and with various geochemical indicators including contaminant concentrations and concentrations of potential electron donors, electron acceptors, and metabolic products.

Status: Laboratory and microcosm experiments have been completed to identify appropriate cometabolic substrates for use in field push-pull test measurements of reductive transformations of TCE. Laboratory microcosm experiments and field push-pull tests have been conducted to study the transport and transformation behavior of a fluorinated analog for TCE, trichlorofluoroethene (TCFE). Laboratory microcosm and field push-pull tests have been used to quantify spatial variability in glucoamidase activity and lipid-bound phosphate as indicators of microbial biomass and to develop correlations between these parameters and measured rates of TCE and TCFE transformations.

**Bioenhanced In-Well Vapor Stripping to Treat Trichloroethylene (TCE): Mark N. Goltz, Department of Engineering and Environmental Management, Air Force Institute of Technology; Perry L. McCarty and Steve M. Gorelick, Stanford University**

Goal: The purpose of this study is to evaluate the potential for removal of chlorinated organic solvents at their source in an aquifer by combining two processes, in-well vapor stripping and in situ aerobic cometabolic biodegradation. The combined system is termed BioEnHanced In Well Vapor Stripping (BEHIVS). The system will be evaluated at full scale in the field at a trichloroethylene (TCE) contaminant groundwater site at Edwards Air Force Base.

Rationale: Removal of chlorinated solvent contaminants at their subsurface source is one of the most challenging problems for remediation of these prevalent contaminants. Here, the solvents are generally present as dense non-aqueous phase liquids (DNAPLs). The potential for application of innovative processes is thus of great interest. The BEHIVS system is believed to be applicable to this problem, at least at some sites. The efficacy of each of the two innovative remediation technologies that comprise BEHIVS were successfully demonstrated during field trials for TCE removal at Edwards AFB. Both technologies make use of groundwater recirculation wells, which may be defined as wells with injection and extraction screens that create groundwater circulation cells in the surrounding aquifer. The in-well vapor stripper established a vertical circulation cell in the aquifer, whereas the aerobic cometabolism system employed a pair of vertical recirculation wells, one operated in an upflow mode, the other in a downflow mode, to establish two horizontal cells. By combining the two in situ treatment systems, the advantages of each can be captured in a way that enhances the performance of the other.

Approach: Using the BEHIVS concept, an in-well vapor stripper, operating in an upflow mode, will be augmented by a biotreatment well that promotes aerobic cometabolic bioremediation, operating in a downflow mode. The well pair will be used to establish horizontal circulation cells to remediate the source of TCE in a single contaminated aquifer at Edwards AFB. Model simulations are being used to illustrate how this configuration may be applied to remediate a chlorinated solvent source area under typically encountered conditions of anisotropy. The model simulations are also being used for system design.

Status: A numerical model has been developed that simulates the important processes relevant to performance of a BEHIVS system. The model simulates recirculating groundwater flow through the two wells; transport of dissolved TCE, toluene, and oxygen; cometabolism; microbial growth; TCE dissolution kinetics; gas-phase TCE transport; and in-well volatilization of dissolved TCE. Model simulations show that BEHIVS can achieve a several order-of-magnitude reduction of TCE concentrations. In addition, the results of laboratory microcosm studies show indigenous microorganisms are capable of supporting aerobic cometabolism of TCE when they are supplied with toluene as a carbon source. The design of the BEHIVS system for demonstration at Edwards AFB is underway. Construction is expected to be complete in Spring 1999, and then operation will commence.

## **TRAINING AND TECHNOLOGY TRANSFER**

### **Hazardous Waste Training: Peter O. Nelson, Ann Kimlering, and Kenneth Williamson, Oregon State University.**

Goal: To promote training activities related to lead paint contamination and disposal.

Rationale: The Center can effectively promote training activities desired by other Federal agencies within EPA Regions 9 and 10.

Approach: The Lead Training Center is a consortium with the University of California Extension Program in Hazardous Materials Management and is funded by the EPA.

Status: The Center conducted 12 workshops within Oregon, Washington, Alaska and Idaho in 1998. Funding for the Center has been extended through 1999. The Center has been EPA certified for lead training and is the only certified training program for lead abatement in the Pacific Northwest.

### **Continuing Education: Kenneth J. Williamson, Oregon State University.**

Goal: To actively promote conferences to address technical aspects of hazardous substance research and education.

Rationale: Conferences are a highly effective way to achieve technology transfer.

Status: The Center sponsored or organized sessions for the following conferences:

The 1997 Idaho Regional Conference on Hazardous Materials, Pocatello, ID, April, 1998.

Remediation of Chlorinated and Recalcitrant Compounds, Battelle, San Diego, CA, May 18-21, 1998. WRHSRC participated in organizing the conference, and WRHSRC researchers presented 11 papers including one of the keynote addresses.

1998 Joint Conference on the Environment, Albuquerque, NM, March 31-April 1, 1998. WRHSRC cosponsored the conference and its researchers presented 6 papers.

## **TECHNICAL OUTREACH SERVICES FOR COMMUNITIES (TOSC) PROGRAM**

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

The TOSC program is conducted with a staff of faculty, consultants, and research assistants including:

- Kenneth J. Williamson, Director
- Anna Harding, Associate Professor, Department of Public Health, Oregon State University
- Christopher Blakeman, Graduate Student, Department of Public Health, Oregon State University
- Alexandra Degher, Graduate Student, Department of Civil, Construction, and Environmental Engineering, Oregon State University
- Mary Masters, Technical Outreach Specialist
- Janet Gillaspie, Consultant

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

The TOSC program provides assistance to communities with an emphasis upon the following areas:

- remediation technologies
- health effects of exposure to hazardous substances
- risk assessment
- hazardous waste regulations and legislation

Approach: The Western Region's TOSC program is one of five nationally instituted community outreach pilot programs. Centered at Oregon State University, the TOSC team is comprised of university faculty and staff, as well as contracted environmental professionals, who specialize in civil and environmental engineering, risk communication, public health, information transfer, and community relations. Since its inception in 1994, the TOSC team has provided communities in northern and southern California and central Washington with technical assistance in efforts to help community residents gain a greater understanding of the effects of hazardous waste sites in their communities. Such information is provided through workshops, community meetings, and various other educational resources. TOSC also aided these communities in breaking the barriers of communication with local, state, and federal agencies.

Insert here

**ACTIVITIES:**

**South Phoenix, Arizona:** Assistance is being provided to Concerned Residents of S. Phoenix concerning impacts upon the community from a fire at the Quality Printed Circuits, Inc. (QPC) site. Work will include:

- Review EPA sampling plan and sampling results
- Develop cluster analysis of community mortalities
- Review mortality report by Arizona Dept. of Health Services
- Tabulate and analyze questionnaires from existing health study
- Obtain data about chemical inventories before fire at QPC facility

Progress to Date: Provided review and comment on DRAFT Final Report from EPA. TOSC's statistical analysis of existing 1993 health study indicates greater health symptom prevalence with increased proximity to the fire. 1996 EPA sample results for 35 households were analyzed by TOSC personnel and showed that all chemicals were below Health Based Comparison Levels.

TOSC plans to map mortality data from 1988-98 (name, address, and year of death for residents within the census tract closest to the smoke plume) for each year to determine if there are cluster effects within the community.

**Tempe, Arizona:** Assistance is being provided to the Kiwanis Park Neighborhood Association regarding the operational air permitting process for the nearby ME West Castings foundry. TOSC work will include:

- Review of air emissions sampling plan and report

Progress to Date: Reviewed several documents regarding the facility, and have attended meetings of local agency, facility, and community representatives. Assisted in reaching agreement on majority of community concerns. Facility to undergo further emissions sampling prior to permitting.

**Union Hills Subdivision, Phoenix, Arizona:** Assisting neighborhood citizens group to investigate symptoms related to possible chemical exposures. These exposures are likely a causative factor of some resident morbidity, however the source(s), are unknown following Arizona Dept. of Environmental Quality and U.S. EPA investigations. Work to include:

- Provide information regarding chemical sensitivities, and names of experts in this field to community leaders
- Review agency investigation documents

Progress to Date: Information from chemical sensitivity literature search and review, names of nationally recognized researchers and physicians sent to community. Document review completed. Conducted a health survey of community volunteers on November 11, 1998 to document reported health symptoms and examine any patterns/similarities of symptoms. A final report will be written and a summary will be sent to the state environmental and health agencies, as well as to Region 9 EPA and ATSDR.

**Sitka, Alaska:** Continue work with Citizen's Advisory Committee, Sitka, Alaska (Bill Janes, DEC), related to hazardous material releases from Alaska Pulp Corporation pulp mill. Work involves:

- Review sampling plan and results from Foster Wheeler, consultants for Alaska Pulp Corp.
- Educate community related to health problems associated with low level dioxin exposure and recommended residual concentrations
- Review health analyses by Alaskan Department of Health
- Review of remediation plan to be developed

Progress to Date: Reviewed sampling plan for land and water sampling sites. Conducted educational program related to hazards associated with low level dioxin exposure. Attended three CAC meetings. Provided review and comment on sampling results, and review of Alaska Dept. of Health, Health Analysis. Awaiting local planning decisions, closure imminent.

**Alameda Naval Air Station, San Francisco, California:** Providing assistance to the Restoration Advisory Board (RAB) regarding issues related to the cleanup of Operable Unit-1 (OU-1) at this former Naval Air Station. Services to include:

- Review and comment on the Remedial Investigation, Human Health Risk Assessment, and the Ecological Risk Assessment for OU-1

Progress to Date: Document review and comment completed. TOSC presented its findings at a November 3<sup>rd</sup> RAB meeting. Awaiting possible revisions to documents, may conduct follow-up review and/or additional work if RAB seeks continued TOSC involvement.

**Bay Area Drum, San Francisco, California:** Providing assistance to the Southeast Alliance for Environmental Justice (SAEJ) regarding remediation of the Bay Area Drum hazardous materials site. Work to include:

- Review and evaluation of soil and groundwater sampling results
- Conduct a community educational workshop focused on potential health concerns associated with contaminants found in local residential soils
- Review and comment on Remedial Investigation and Feasibility Study documents

Progress to Date: Completed document review and have submitted comments. Additional comments provided to the California Dept. of Toxic Substance Control regarding the proposed neighborhood soil removal plan.

**Bayview-Hunters Point, California:** In process of initiating assistance to the Southeast Alliance for Environmental Justice (SAEJ) community advocacy group. Issues of concern relate to planned Brownfields redevelopment of Hunters Point Naval Base near San Francisco. TOSC may provide:

- Review of Phase I site assessment data being used to direct redevelopment plans
- Other potential assistance being negotiated at this time

**Casmalia, California:** Continue work with Citizen's Advisory Committee (CAC) for the PRP's associated with the Casmalia Resources Hazardous Waste facility. Work involves:

- Assistance with development of active involvement in the CAC
- Review of consent decree issued by Region 9, EPA, for the Casmalia facility

Progress to Date: Reviewed background material. Assisted the community in review of consent decree. Provided information to the community on the necessary steps to become incorporated as a non-profit organization. Have renewed outreach efforts, but progress slowed by lack of local organization, working with EPA CIC staff to arrange possible solution(s). Have offered assistance to community group regarding their application for a Technical Support Grant, awaiting their response.

**North Island Naval Station, San Diego, California:** Continue work the North Island Naval Station Restoration Advisory Board (RAB). Work involves:

- Review of contractor reports and documents for RAB
- Attend RAB meeting to comment on proposed remediation plans

Progress to Date: Attended several RAB meetings. Reviewed background information. Provided technical assistance related to incineration option, risk of worker exposure to TCE, and management of migration of contaminants to Bay. Provided review and assessment of proposed remediation plans, awaiting similar plans and response for other areas of site. RAB recently opted against applying for TAPP funding in order to retain TOSC assistance.

**Santa Fe Springs, California:** Providing assistance to the Protect Our Neighborhood Committee (PONC) in their involvement with the Waste Disposal, Inc. Superfund Site, a former landfill. Services to include:

- Review the Record of Decision
- Review past and current data associated with groundwater, soil, and air sampling
- Attempt to ascertain health related concerns of residents living adjacent to the site
- Provide an updated list of contaminants affecting groundwater, soil, and air both at the site and in the adjacent community

Progress to Date: Attended several meetings, completed document review and comment. Facilitating community effort to have the state Dept. of Health Services (DHS) conduct a survey of health symptoms and concerns of local residents, TOSC is committed to conduct its own health survey of the community if DHS is not forthcoming by mid February 1999.

**Tustin, California:** Providing assistance to established RAB dealing with remediation activities at a Marine Corps Air Station. RI/FS completed. TOSC assistance involves:

- Review and comment on RI/FS and Draft ROD documents
- Ongoing educational programs for RAB members related to remediation plans and activities

Progress to Date: Document review ongoing, some comment provided. Awaiting revised documentation from the Army Corps of Engineers regarding site-related flood. TOSC conducted presentation at a January '98 RAB meeting, and have attended additional regular RAB meetings.

**Vallejo, CA** - Providing assistance to an established RAB in dealing with remediation activities at the Mare Island Naval Shipyard where the RI/FS is being completed. Work will include:



- Review and evaluation of the Human Health Risk Assessment and the Ecological Risk Assessment

Progress to Date: Completed review and comment on Risk Assessments. Attended RAB meeting on July 30, 1998. May be asked to conduct additional review and comment, awaiting RAB decision.

**Makua Military Reservation, Oahu Island, Hawaii:** In the process of negotiating assistance to the Malama Makua community group regarding cleanup and possible closure activities at the Makua Military Reservation, a live ordinance testing and training facility. Services to include:

- Review and comment on documents related to the potential closure of a former Open-Burn Open-Detonation (OBOD) site on this base
- Review and comment on Army's investigation of area groundwater and near-shore water contamination

Progress to Date: Have provided contact information for Unexploded Ordinance expertise. TOSC personnel attended meeting of all stakeholders, and toured site in December, 1998.

**Eastern Michaud Flats NPL Site, Pocatello, Idaho:** Assistance is being provided to a local non-profit community group, the Portnuef Environmental Council (PEC), concerning the recently closed Eastern Michaud Flats Superfund site. Remediation involves contaminant assessment and mitigation at two operating commercial phosphorus production facilities. Work to include:

- Review of RI/FS documents and proposed remediation actions

Progress to Date: Document review completed. Assessment of EPA airborne contaminant sampling and analysis complete, assessment report drafted, final version forthcoming. Graduate thesis project investigating local radiation exposure risks from off-site surface soils ongoing. May provided additional services related to completion of cleanup activities articulated in ROD and RCRA settlement.

**Bunker Hill NPL Site, Kellogg, Idaho:** In process of negotiating assistance to the Silver Valley People's Action Coalition/CRC Co. regarding the adjacent Bunker Hill Superfund site. Services likely to include:

- Review and comment on various EPA documents (to be determined by the community);
- Review and comment on Dr. Joel Hirschhorn's report, and the 1990 House Dust Pilot Study;
- Research and provide information on current metals remediation technologies, expectations for post remediation residual contamination, and possible costs involved.

Progress to Date: Evaluation of community needs assessment and draft of outreach service agreement. Site visit planned for near future.

**Oregon State Penitentiary, Salem, Oregon:** Providing assistance to the Oregon State Penitentiary (OSP) community group regarding an imminent interim removal action measure (IRAM) and health concerns related to a PCE and TCE groundwater contamination and cleanup. TOSC services to include:

- Evaluate and comment on IRAM and air stripping towers with regard to their safety and effectiveness
- Provide information on the long and short-term health effects of P/TCE exposure
- Review and comment on the Human Health Risk Assessment
- Provide information on potential effect(s) which the contaminated gw plume might have on the local water district drinking water supply
- Evaluate air quality concerns in local residential basements
- Evaluate possible exposures though ingestion of local produce, soil contact, and incidental ingestion of soil

Progress to date: Review of RI report and modeling of stripping tower air discharge completed. Attended several community meetings. Presented detail on alternative bioremediation protocol, environmental partitioning of P/TCE, and fabrication of activated charcoal filters for air strippers.

**Brewster, WA** – Providing assistance to community members who have been exposed to pesticides due to improper application and storage practices in nearby orchards. Work will include:

- Follow up with state Dept. of Agriculture regarding their investigation into local exposures and review their findings
- Provide information on reporting pesticide exposures and incidents of improper application

Progress to date: Attended community meeting on August 20, 1998. Contacted WA Department of Agriculture to discuss pesticide application practices of local orchard owners, and most effective means of reporting pesticide exposures. Awaiting their report. Agreement from Region 10 ATSDR representative to assist as needed following report review.

**Rayonier Mill Site, Port Angeles, Washington:** Working cooperatively with Haskell Indian Nations University to provide services to the Lower Elwha Klallam Tribal community regarding the ongoing remedial investigation and proposed cleanup of the former Rayonier Paper Mill, a possible USEPA Superfund site. Services may include:

- Conduct a needs assessment of the tribal community
- Review various technical reports associated with the investigation and remediation plans

Progress to Date: Provided review and comment on the Expanded Site Investigation report. Awaiting finalization of working agreement with tribe.

## **TECHNICAL ASSISTANCE TO BROWNFIELDS (TAB) PROGRAM**

**TAB Staff: Kenneth Williamson, Director; Michael Fernandez, Oregon State University.**

The TAB program just started in the fall, 1998. Various activities are being initiated to work with Brownfields communities in EPA Regions 9 and 10. A major focus is upon developing outreach tools that can be used by all five HSRCs.

**Oregon Brownfields Workshops** - Working with Oregon DEQ and the EPA Region 10 Brownfields Coordinator in developing a series of one day workshops to be held in communities around Oregon. Workshops are targeted to area municipal and development leaders, and will provide Brownfields basics, a handbook of tools and information resources, as well as consideration of some local/regional Brownfield issues. First workshop was held in September in Klamath Falls, OR and was attended by 20 local and state economic development personnel.

Goal: To promote training activities related to lead paint contamination and disposal.

Rationale: The Center can effectively promote training activities desired by other Federal agencies within EPA Regions 9 and 10.

Approach: The Lead Training Center is a consortium with the University of California Extension Program in Hazardous Materials Management and is funded by the EPA.

## 1998 WRHSRC PUBLICATIONS

Documents either published or submitted for publication during this year of WRHSRC activity are as follows:

### A. Refereed Journal Articles

- Abrams, R.H., K. Loague, and D.B. Kent, "Development and Testing of a Compartmentalized Reaction Network Model for Redox Zones in Contaminated Aquifers," *Water Resources Research*, **34** (6), 1531-1541 (1998).
- Beller, H.R., and A. M. Spormann, "Analysis of the Novel Benzylsuccinate Synthase Reaction for Anaerobic Toluene Activation Based on Structural Studies of the Product." *Journal of Bacteriology* **180**(20), 5454-5457 (1998).
- Cao, J., and P. K. Kitanidis, "Pore-scale Dilution of Conservative Solutes: An Example," *Water Resour. Res.*, **34**(8), 1941-1949, (1998).
- Donaldson, J.H., J.D. Istok, and K.T. O'Reilly, "Dissolved Gas Transport in the Presence of a Trapped Gas Phase: Development and Laboratory Testing of a Two-Dimensional Kinetic Model", *Ground Water*, **36**(1), 133-142 (1998).
- Donaldson, J.H., J.D. Istok, M.D. Humphrey, K.T. O'Reilly, C.A. Hawelka, and D.H. Mohr, "Development and Laboratory Testing of a Kinetic Model for Dissolved Oxygen Partitioning and Transport in Porous Media in the Presence of Trapped Gas,". *Ground Water*, **40**(1), 113-119 (1997).
- Fraley, C.D., J.H. Kim, M.P. McCann, and A. Matin. "The Escherichia Coli Starvation Gene, *cstC*, is Involved in Amino Acid Catabolism." *Journal of Bacteriology*: **180**, 4287 (1998).
- Fry, V. A., J. S. Selker, and S.M. Gorelick, "Experimental Investigations for Trapping Oxygen Gas in Saturated Porous Media for in situ Bioremediation," *Water Resources Research*. **33**(12), 2687-2696 (1997).
- Haggerty, R. and S.M. Gorelick, "Modeling Mass Transfer Processes in Soil Columns with Pore-Scale Heterogeneity," *Soil Sci. Soc. Am. J.*, **62**(1), 62-74 (1998).
- Haggerty, R., M.H. Schroth, and J.D. Istok, "Simplified Method of "Push-Pull" Test Data Analysis for Determining In Situ Reaction Rate Coefficients," *Ground Water*, **36**(2), 314-324 (1998).
- Istok, J.D., M.D. Humphrey, M.H. Schroth, M.R. Hyman, and K.T. O'Reilly, "Single-Well, "Push- Pull" Test for In Situ Determination of Microbial Metabolic Activities", *Ground Water*, **35**(4), 619-631 (1997).
- Keller, A. A., "High Resolution, Non-Destructive Measurement and Characterization of Fracture Apertures," *Int. J. Rock Mech. Min. Sci.*, **35**(8), 1037-1050 (1998).
- Kitanidis, P. K., "How Observations and Structure Affect the Geostatistical Solution to the Steady-State Inverse Problem", *Ground Water*, **36**(5), 754-763 (1998).

- Lotrario, J.B. and S.L. Woods, "Comparison of the Correlation of MEP and the Free Energies of Reaction with Nitro Group Reduction of Nitrotoluene Congeners," *Bioremediation*, **1**(2), 115-122 (1997).
- McCarty, P. L., M. N. Goltz, G. D. Hopkins, M. E. Dolan, J. P. Allan, B. T. Kawakami, and Carrothers, T. J., "Full-Scale Evaluation of *In Situ* Cometabolic Degradation of Trichloroethylene in Groundwater through Toluene Injection," *Environmental Science and Technology*, **32**(1), 88-100 (1998).
- Pinto, M., H. Gvirtzman, and S.M. Gorelick, "Aquifer Remediation By In-Well Vapor Stripping: 2. Modelling Results," *J. of Contaminant Hydrology*, **29**(1), 41-58 (1998).
- Schroth, M.H., J.D. Istok, G.T. Conner, M.R. Hyman, R. Haggerty and K.T. O'Reilly, "Spatial Variability in Aerobic Respiration and Denitrification Rates in a Petroleum Contaminated Aquifer", *Ground Water*, **36**(6), 924-937 (1998).
- Semadeni, M., P.-C. Chiu, and M. Reinhard. "Reductive Transformation of Trichloroethene Catalyzed by Cobalamin: Reactivities of the Intermediates, Acetylene, Chloroacetylene, and the DCE Isomers," *Environmental Science and Technology*, **32**(9), 1207-1213 (1998).
- Smith, L. H., P. L., McCarty, and P. K. Kitanidis, "Spreadsheet Method for Evaluation of Biochemical Reaction Rate Coefficients and Their Uncertainties by Weighted Nonlinear Least-Squares Analysis of the Integrated Monod Equation," *Applied and Environmental Microbiology*, **64**(6), 2044-2050 (1998).
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- Stuart, S.L. and S.L. Woods, "Kinetic Evidence for Pentachlorophenol-dependent Growth of a Dehalogenating Population in a Pentachlorophenol- and Acetate-fed, Methanogenic Culture," *Biotechnology and Bioengineering*, **57**(94), 420-429 (1998).
- Workman, D.J., S.L. Woods, Yuri A. Gorby, J.K. Fredrickson, and M.J. Truex, "Microbial Reduction of Vitamin B12 by *Shewanella* alga Strain BrY with Subsequent Transformation of Carbon Tetrachloride," *Environmental Science and Technology*, **31**(8), 2292-2297 (1997).
- Yang, Y. and P. L. McCarty, "Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Mixed Culture," *Environmental Science and Technology*, **32**(24), 3591-3597 (1998).

## **B. Articles Submitted or in Press**

- Cao, J., and P. K. Kitanidis, "Adaptive-Grid Simulation of GroundWater Flow in Heterogeneous Aquifers." *Adv. in Water Resour.*, accepted for publication (1998).
- Cao, J., and P. K. Kitanidis, "Adaptive Finite-Element Simulation of Stokes Flow in Porous Media," *Adv. in Water Resour.*, **22**(1), submitted 1999.
- Dupin, J. J. and P.L. McCarty, "Mesoscale and Microscale Observations of Biological Growth and Clogging in a Silicon Pore Imaging Element" (SPIE), submitted 1998.

- Goltz, M.N., G.C. Mandalas, G.D. Hopkins, and P.L. McCarty, "Technology Transfer of an Innovative Remediation Technology from the Laboratory to the Field: A Case Study of In Situ Aerobic Cometabolic Bioremediation," *Environmental Engineering and Policy*, in press.
- Haston, Z. C. and P. L. McCarty, "Chlorinated Ethene Half-Velocity Coefficients ( $K_s$ ) for Reductive Dehalogenation," *Environmental Science and Technology*, in press.
- Keller, A. A., M. J. Blunt and P. V. Roberts, "Behavior of Dense Non-Aqueous Phase Liquids in fractured porous media under two-phase flow conditions", *Transport in Porous Media*, in press.
- Keller, A. A., P. V. Roberts and M. J. Blunt, "Effect of Fracture Aperture Variations on Dispersion of Contaminants, *Water Resources Research*," in press.
- Khaodhiar, S., M. F. Azizian, and P. O. Nelson, "Copper, Chromium, and Arsenic Adsorption and Equilibrium Modeling in an Iron-Oxide-Coated Sand, Synthetic Groundwater System," submitted 1998.
- Stuart, S. L., S L. Woods, T. L. Lemmon, J. D. Ingle, Jr, "The Effect of Redox Potential Changes on Reductive Dechlorination of Pentachlorophenol and Degradation of Acetate by a Mixed, Methanogenic Culture", *Biotechnology and Bioengineering*, in press.

### **C. Chapters in Books or Bound Proceedings**

- Azizian, M.F., and P.O. Nelson, "Lead Adsorption, Chemically-Enhanced Desorption, and Equilibrium Modeling in an Iron-Oxide-Coated Sand, Synthetic Groundwater System", in *Adsorption of Metals by Geomedia*, E.A. Jenne, Ed., American Chemical Society Symposium, Series, chapter 6, 165-180.(1998),
- Cole, J.D., S.L. Woods, P.J. Kaslik, K.J. Williamson, and D.B. Roberts, "Bioremediation In Situ and On-Site Bioremediation: Volume 4, Fourth International In Situ and On-Site Bioremediation Symposium, New Orleans, Louisiana, April 28-May 1, 1997, Symposium Chairs Bruce C. Alleman and Andrea Leeson, Battelle Press, Columbus, Richland, pg. 239, 1997.
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- Cole, J., S. Woods, K. Williamson, and D. Roberts, "Anaerobic Treatment of a Pentachlorophenol-Contaminated Aquifer using a Borehole Reactor," 1998 Joint Conference on the Environment, WERC-WRHSRC-NMHWMS, Crowne Plaza Pyramid, Albuquerque, New Mexico, pgs. 57-62 (1998).

- Degher, A.B., K.G. Wang, K.J. Williamson, S.L. Woods, and R. Strauss, "Bioremediation of Soils Contaminated with 2,4-D, 2,4,5-T, Dichlorprop, and Silvex," In Situ and On-Site Bioremediation: Volume 2, Fourth International In Situ and On-Site Bioremediation Symposium, New Orleans, Louisiana, April 28-May 1, 1997, Symposium Chairs Bruce C. Alleman and Andrea Leeson, Battelle Press, Columbus, Richland, 279 (1997).
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- Havighorst, M.B., S.L. Woods, and K.J. Williamson, "Bioremediation of Pentachlorophenol-Contaminated Soil in Laboratory and Full-Scale Processes," In Situ and On-Site Bioremediation: Volume 2, Fourth International In Situ and On-Site Bioremediation Symposium, New Orleans, Louisiana, April 28-May 1, 1997, Symposium Chairs Bruce C. Alleman and Andrea Leeson, Battelle Press, Columbus, Richland, pg. 455, 1997.
- Hyman, M.R., T. Musaffika-Jeknic, P. Kwon, K. Williamson and K. O'Reilly "Cometabolism of MTBE by Alkane-Utilizing Microorganisms; In: Proceedings Joint Conference on the Environment" WERC, New Mexico State University 197-201 (1998).
- Kawakami, B.T., J.A. Christ, M.N. Goltz, and P.L. McCarty, "Hydraulic Design of an In Situ Injection/Extraction Bioremediation System, Designing and Applying Treatment Technologies--Remediation of Chlorinated and Recalcitrant Compounds, Wickramanayake, G.B. and R.E. Hinchee, Eds., pp. 33-38, Battelle Press, Columbus, OH, 1998.
- Keller, A. A. "Steam Injection to Displace DNAPLs From Fractured Media", in Int. Assoc. of Hydrological Sciences, Publication no. 250, Oxfordshire, UK.1998.
- Khaodhiar, S., M.F. Azizian, and P.O. Nelson, "Equilibrium Modeling of Arsenic, Chromium, and Copper Adsorption on an Iron-Oxide-Coated Sand," Extended Abstracts, Vol. 38, No. 1, March 1998, American Chemical Society, Environmental, Chemistry Division, 215th ACS National Meeting, Dallas, Texas.
- Lotrario, J.B, S.L. Woods, A.M. Craig, and K.J. Williamson, "The Anaerobic Transformation of TNT and other Nitroaromatic Compounds," In Situ and On-Site Bioremediation: Volume 2, Fourth International In Situ and On-Site Bioremediation Symposium, New Orleans, Louisiana, April 28-May 1, 1997, Symposium Chairs Bruce C. Alleman and Andrea Leeson, Battelle Press, Columbus, Richland, pgs. 41-46, 1997.
- Mandalas, G.C., J.A. Christ, G.D. Hopkins, P.L. McCarty, and M.N. Goltz, "Remediation Technology Transfer from Full-scale Demonstration to Implementation: A Case Study of Trichloroethylene Bioremediation, Risk, Resource, and Regulatory Issues--Remediation of Chlorinated and Recalcitrant Compounds, Wickramanayake, G.B. and R.E. Hinchee, Eds., pp. 235-240, Battelle Press, Columbus, OH, 1998.
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Reinhard, M., M. Semadeni, P.K. Sharma, and W.R. Haag, "Dehalogenation of Haloaliphatic Hydrocarbon Compounds in the Aquatic and Terrestrial Environment," Chapter 7 in American Society of Agronomy Monograph "Bioremediation of Contaminated Soils." D. Adriano, J.-M. Bollag, EDS., in press, 1998.

Stuart, S.L. and S.L. Woods, "Apparent Pentachlorophenol-Dependent Growth of a Subpopulation in a Mixed, Methanogenic Culture," In Situ and On-Site Bioremediation: Volume 2, Fourth International In Situ and On-Site Bioremediation Symposium, New Orleans, Louisiana, April 28-May 1, 1997, Symposium Chairs Bruce C. Alleman and Andrea Leeson, Battelle Press, Columbus, Richland, pgs. 475-480, 1997

Trobaugh, D.J., S.L. Woods, K.J. Carter, and D. Workman, "Development of a Vitamin B12-Amended PCB Bioremediation Strategy," In Situ and On-Site Bioremediation: Volume 2, Fourth International In Situ and On-Site Bioremediation Symposium, New Orleans, Louisiana, April 28-May 1, 1997, Symposium Chairs Bruce C. Alleman and Andrea Leeson, Battelle Press, Columbus, Richland, pgs. 413-418, 1997.

#### **D. Book**

Reinhard, M. and Drefahl, A., *Handbook for Estimating Physicochemical Properties of Organic Compounds*, John Wiley & Sons, Inc., New York (1999).

#### **E. CD ROM**

Reinhard, M. and Drefahl, A. *Toolkit for Estimating Physicochemical Properties of Organic Compounds*, CD-ROM, John Wiley & Sons, Inc., New York (1999).

Heider J, Spormann AM, Beller HB, Widdel F 1998: Anaerobic bacterial metabolism of hydrocarbons. FEMS Microbiol. Review, in press.

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Khaodhiar, S., "Removal of Chromium, Copper, and Arsenic from Contaminated Groundwater Using Iron-Oxide Composite Adsorbents", Ph.D. Thesis, 125 p., Oct. 1997.

Osathaphan, K., "Adsorption of Arsenate on Iron-Oxide-Coated Sand and Magnetite-Coated Sand", MS Report, 88 p., Dec. 1997.



## PREVIOUS WRHSRC PUBLICATIONS

### A. Refereed Journal Articles

- Adriaens, P., "Evidence for Chlorine Migration During Oxidation of 2-Chlorobiphenyl by a Type II Methanotroph," *Applied and Environmental Microbiology*, **60**(5), 1658-1662 (1994).
- Adriaens, P. and D. Grbić -Galić, "Cometabolic Transformation of Mono- and Dichlorobiphenyls and Chlorohydroxybiphenyls by Methanotrophic Groundwater Isolates," *Environmental Science and Technology*, **28**(7), 1325-1330 (1994).
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