



**WESTERN REGION
HAZARDOUS SUBSTANCE RESEARCH CENTER**

1996 ANNUAL REPORT

Stanford University
Oregon State University

December 1996

Sponsored by the U. S. Environmental Protection Agency

**WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER
1996 ANNUAL REPORT**

TABLE OF CONTENTS

ADMINISTRATION.....	1
THE CENTER AT A GLANCE	1
DIRECTOR'S REPORT.....	4
HIGHLIGHTS OF THE YEAR	5
FUTURE DIRECTIONS	7
CENTER PROGRAM SUMMARY	9
RESEARCH PROJECT DESCRIPTIONS	14
CHEMICAL MOVEMENT, FATE, AND TREATMENT.....	14
CHLORINATED SOLVENTS.....	20
AROMATIC COMPOUNDS	26
HEAVY METALS.....	31
MINORITY ACADEMIC INSTITUTION PROJECT DESCRIPTIONS	33
TRAINING AND TECHNOLOGY TRANSFER PROJECT	
DESCRIPTIONS.....	37
DEMONSTRATION PROJECTS.....	37
TRAINING AND TECHNOLOGY TRANSFER.....	42
1996 WRHSRC PUBLICATIONS.....	45
PREVIOUS WRHSRC PUBLICATIONS	51

WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER 1996 ANNUAL REPORT

ADMINISTRATION

Perry L. McCarty
Director, WRHSRC
Department of Civil Engineering
Stanford University
Stanford, CA 94305-4020
Telephone: (415) 723-4131
FAX: (415) 725-9474
E-mail: mccarty@ce.stanford.edu

Kenneth J. Williamson
Director, Training and Technology Transfer
Department of Civil Engineering
Oregon State University
Corvallis, OR 97331
Telephone: (541) 737-6836
FAX: (541) 737-3099
E-mail: williamk@ccmail.orst.edu

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 4 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. Marilyn C. King 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	Prof. A. Morrie Craig
Prof. Steven M. Gorelick	Ms. Patricia Easley
Prof. Lynn M. Hildemann	Dr. Michael R. Hyman
Mr. Gary D. Hopkins	Prof. James D. Ingle
Mrs. Marilyn C. King	Prof. Jonathan D. Istok
Prof. Peter K. Kitanidis	Prof. Peter O. Nelson
Prof. James O. Leckie	Prof. John S. Selker
Prof. Keith Loague	Prof. Lewis Semprini
Prof. Abdul Matin	Prof. John C. Westall
Prof. Perry L. McCarty	Prof. Kenneth J. Williamson
Prof. Martin Reinhard	Prof. Sandra L. Woods
Prof. Paul V. Roberts	
Prof. Alfred Spormann	

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 1996 fiscal year and since the Center's inception are listed by category of support in Table 3. 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 4.

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
John Conomos	Govt/USGS	Hydrology
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*
Richard G. Luthy

James M. Tiedje
John L. Wilson†
John Wise

*Chairman

†Vice Chairman

ENVIRON
Carnegie Mellon University

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

Physical/Chemical Processes
Chemical/Biological
Processes
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
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	Treatment Technology
Jon Kindschy	Univ. California Ext. Service	Hazardous Substances Training
Gregory Peterson	CH2M-HILL	Treatment Technology
Winona Victory	Govt/EPA, Region 9	Training/Tech Transfer

*Chairman

Table 4. CENTER FUNDING

<u>Funding Sources</u>	<u>FY 1995*</u>	<u>Funds to Date</u>
EPA: Centers Program	\$960,000	\$8,853,015
EPA: Other	\$125,000	\$2,950,816
Other Govt: Federal†	\$180,000	\$7,310,195
Other Govt: State@	0	\$48,252
Consortium	\$136,250	\$3,102,854
Private Sector#	<u>\$105,000</u>	<u>\$2,137,452</u>
TOTAL	\$1,506,250	\$24,402,584

*Oct. 1, 1994 - Sept. 30, 1995

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

@Oregon Department of Transportation

#Aluminum Company of America, Allied Signal Corporation; 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.; MBT Environmental Engineering, Ltd.; 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.	43	\$1,269,908
Ph.D.	118	\$5,675,620
Post Doctoral	<u>29</u>	<u>\$1,834,143</u>

TOTAL 203 \$8,875,548

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

†Includes tuition, travel, supplies, etc.

DIRECTOR'S REPORT

This past year the Center faculty and research staff were engaged in 23 separate research projects. Eleven of these projects are being supported through the Research and Re-Education for Department of Defense (R2D2) program. An additional five research projects are ongoing at minority academic institutions (MAI) through sponsorship by the WRHSRC with financial support for this purpose from the U.S. EPA. As in the past, all WRHSRC projects have a focus on groundwater cleanup and remediation of sites contaminated with chlorinated solvents, halogenated aromatic compounds such as pentachlorophenol, nonhalogenated aromatics, including petroleum derivatives such as gasoline, and heavy metals. Other projects specifically address the evaluation of factors affecting movement and fate of the above contaminants in the environment and design and management issues for site cleanup in general.

The other significant phase of WRHSRC activities is the Training and Technology Transfer (TTT) program. One of the significant outcomes of a Five Centers' Conference on Technology Transfer hosted by the WRHSRC through the efforts of Prof. Kenneth Williamson, Director of Training and Technology Transfer, was a realization that true transfer of technology to a user community does not result from oral and written presentations alone. There is a need to work closely with organizations interested in field demonstrations and further implementation of new and innovative technology developed through the Center. In addition, there is much to be learned from the act of implementation itself. Areas where implementation would benefit from additional research can thus be better identified, providing a highly beneficial feedback into the research process itself. Within this effort, there are currently six field demonstration projects underway, three of which are being supported through R2D2 funds, two at Edwards Air Force Base (in-situ cometabolic degradation of TCE and in-situ vapor stripping of TCE) are supported by U.S. Air Force with additional support for the vapor stripping project from the U.S. DOE, and finally, a project on in-situ biodegradation of petroleum hydrocarbon at Seal Beach Naval Weapons Laboratory is receiving support from the U.S. Department of Navy. Another major TTT project that has been ongoing for two years is the Technical Outreach Services for Communities (TOSC) program to aid communities impacted by hazardous waste problems. In addition, the TTT function of the Center includes sponsorship of conferences and sessions concerned with hazardous waste remediation.

One of the major findings of recent years is that chlorinated solvents are transformed in groundwater by both chemical and biological processes. The thrust group associated with the problem of chlorinated solvent contamination is multidisciplinary with active research at both Stanford and Oregon State and combined expertise in microbiology, chemistry, hydrogeology, hydrology, and engineering. The group is directing its combined efforts towards obtaining better fundamental understanding of the chemistry, microbiology, and hydrogeology involved, and in finding engineering methods for in situ treatment, including the subsurface mixing of chemicals that can help enhance natural processes for destroying the contaminants of concern or for physically removing them from the subsurface. Projects emphasize both aerobic and anaerobic biological transformations of carbon tetrachloride, tetrachloroethylene,

trichloroethylene, dichloroethylenes, and vinyl chloride, as well as reductive dehalogenations of the same compounds biotically and also abiotically by heavy metals and substituted porphyrins. A continuum of projects from basic laboratory studies through analytical simulation model development, to field demonstrations provide an integrated whole from conceptualization to implementation. Feedback between the different activities helps insure that the major technical problems in technology development and implementation are appropriately addressed.

The thrust group studying aromatic compounds and a portion of that studying chlorinated solvents have focused on anaerobic processes. A great deal of evidence has been obtained that aromatic compounds, such as the BTEX group from gasoline, and chlorinated aromatic compounds, such as pentachlorophenol, are often transformed in groundwater naturally (intrinsic bioremediation or natural attenuation) by microorganisms living under anaerobic conditions. The anaerobic conditions result when the quantity of contaminants present exceed the available oxygen supply, which is often the case. The basic research ongoing here at Stanford (with focus on BTEX compounds) and Oregon State (with focus on pentachlorophenol) is directed toward predictions of transformation potential and transformation rates, the determination of transformation products, the biochemistry and genetics of the processes involved, and the development of engineering methods to enhance the intrinsic rates of anaerobic transformations. It may be that in some cases, the most environmentally sound and least expensive alternative is the no-treatment alternative, an approach that relies on intrinsic bioremediation. However, in order to justify this, we must first learn how to make sound judgments about transformation potential at any given location. Research on these major issues is continuing. As with the chlorinated solvent studies, both laboratory and field investigations and their integration are underway.

A third associated thrust area is the modeling of transport and fate processes and groundwater-management strategies. These studies are conducted partly independent of the other thrust groups, but increasingly are becoming integrated into them as the need arises. In order to remove polluted groundwater and associated contaminants, improved mathematical models are desirable that consider the great uncertainties in the hydrogeological structure of a contaminated site, and the relative chemical and physical properties of both the contaminant and the aquifer materials that impact on contaminant distribution and movement. An additional highly complex need is for methods to introduce nutrients or other chemicals required to enhance biological or chemical transformation, and to mix these chemicals with groundwater contaminants as noted above. The absence of good methods for this is one of the major barriers to in-situ treatment. The activities here are being integrated quite closely into the chlorinated solvent field activities.

A fourth perhaps small, but nevertheless important thrust area of the WRHSRC is concerned with heavy metals. Chromium and lead are among the most frequently found hazardous substances found at contaminated sites. The WRHSRC has been conducting research both on improved procedures for removing heavy metals from groundwater, and also on surface treatment of heavy-metal-contaminated groundwater.

HIGHLIGHTS OF THE YEAR

A major highlight of this year is the startup and near completion of the full-scale evaluation of in situ biodegradation of trichloroethene (TCE) through toluene, hydrogen peroxide, and oxygen addition at a Superfund site at Edward's Air Force Base in southern California. This

represents the culmination of basic laboratory and field experimental work conducted during the first eight years of WRHSRC activity. Twenty-three faculty and staff members, not only from Stanford and Oregon State, but also from Michigan State University, the University of Western Florida, and the University of Minnesota, together with 30 graduate students participated in this research. To date there have been 40 WRHSRC sponsored journal publications, 14 conference reports, 6 book chapters, 12 doctoral dissertations, and 3 patents issued that are related to this technology. The full scale demonstration required support and administrative approvals from a variety of agencies, including the U.S. EPA Region IX, California EPA, the Lahonton Regional Water Quality Control Board, and the U.S. Air Force. The site demonstration itself is being financially supported by the Armstrong Laboratory Environics Directorate, Tyndall Air Force Base, the Headquarters U.S. Air Force Environmental Quality Division, and Woodward-Clyde Consultants. Technical support for construction and operation of the demonstration site was provided by EarthTech, and support for all phases was provided by the Edwards Air Force Base.

In situ biodegradation of TCE was achieved at Edwards through injection of toluene (7 to 14 mg/l) as a primary substrate, oxygen, and hydrogen peroxide into two groundwater circulation wells located 10 m apart. This novel system was conceived by Stanford graduate students in a seminar class in 1993. The wells penetrated two contaminated aquifers, 8 and 5 meters deep, with initial TCE concentration between 500 and 1,400 $\mu\text{g/L}$. Pumpage at 25 to 38 liters/min through each well resulted in groundwater circulation between the aquifers through two bioremediation zones. At steady-state, 75 to 87 percent TCE removal with each pass through a treatment zone was achieved, providing a reduction from over 1,000 $\mu\text{g/L}$ in water entering the 22 meters square monitored zone to less than 50 $\mu\text{g/L}$ in water leaving the zone. Toluene concentration of less than 2 $\mu\text{g/L}$ average was maintained at the zone boundaries, much below the 20 $\mu\text{g/L}$ limit that was agreed upon by regulatory agencies and Stanford University for this study. The in situ system in effect treated an estimated 60 to 80 meter width of the contaminated plume based upon regional flow, reducing the TCE concentration by about 97 to 98 percent as the plume passed through the system. The treatment system performed as anticipated based upon extensive prior laboratory and field investigations. The evaluation is still ongoing through various modifications to optimize system performance, with a completion date scheduled for the end of March 1997. There will be several reports resulting from this evaluation, including one that will address the engineering issues involved in application elsewhere.

Another major field evaluation that is part of continuing studies is that of natural attenuation of gasoline contamination at the Naval Weapons Laboratory, Seal Beach, California, a study being directed by Prof. Martin Reinhard, Stanford University. The interest here is in the fate of aromatic hydrocarbons resulting from the soluble components of gasoline that contaminate groundwaters. These contaminants are generally termed the BTEX compounds (benzene, toluene, ethylbenzene, and xylenes). Prof. Reinhard and coworkers reported upon a noted disappearance of xylenes in a landfill leachate plume in 1984 (*Environmental Science & Technology*, 18: 953-961) and concluded, "The selective removal of the xylenes was hypothesized to be due to biotransformation." This greatly disputed hypothesis was convincingly confirmed by the late Stanford Professor Dunja Grbic-Galic and Dr. T. Vogel in 1986 (*Applied and Environmental Microbiology*, 5:200-202). They noted oxygen was incorporated into the aromatic ring under anaerobic conditions from water, rather than from molecular oxygen, the then believed only route by which this group of compounds could be

transformed. Now, many microorganisms have been isolated that are capable of anaerobic decomposition of BTEX compounds. The scientific underpinning these studies provided together with frequently observed natural shrinkage of hydrocarbon plumes with time once the contamination source has been removed, has now led to a major change in the thinking by regulatory agencies. In many instances, remediation of BTEX plumes is no longer required as natural attenuation itself is believed to be sufficiently protective. This change in practice resulting from this good science is now saving billions of dollars.

Nevertheless, the story is not felt by WRHSRC researchers to be complete. Students advised by Prof. Reinhard and Prof. Alfred Spormann are addressing several important questions of how anaerobic degradation of BTEX compounds occurs, what are the biochemical pathways for these novel and highly unexpected reactions, what intermediate compounds may be formed, whether they pose an environmental or health risk, and how can the rate and extent of natural attenuation of BTEX compounds be evaluated at a given site? Basic laboratory studies together with the Seal Beach demonstration are directed towards finding answers to these important questions. At Seal Beach it was demonstrated that under denitrifying and sulfate-reducing conditions, toluene, m-xylene, and ethylbenzene were rapidly degraded (within days) while o-xylene was degraded at a slower rate. Benzene transformation under sulfate-reducing conditions was much slower, but significant. The specific compounds degraded and their fate were functions of environmental factors, and differed under denitrifying, sulfate reducing, and methanogenic conditions.

Prof. Spormann and his students Harry A. Ball, Harry R. Beller, and Hope A. Johnson have now provided evidence for the major pathways of anaerobic degradation of ethylbenzene and toluene. The initial step in ethylbenzene degradation was found to be the formation of 1-phenylethanol, an important step that activates this aromatic hydrocarbon. The oxygen atom in 1-phenylethanol was demonstrated to come from water rather than from molecular oxygen, an important finding that confirms the anaerobic pathway. The remaining steps in the pathway to complete destruction have now also been found. The pathway for toluene degradation under denitrifying conditions has now also been elaborated. This includes the addition of toluene to fumarate as the first step to form benzylsuccinate. Benzylsuccinate is then degraded under anaerobic conditions. A similar pathway occurs presumably for xylenes as well. Intermediates from toluene and xylene degradation, such as benzylsuccinate and the corresponding methylbenzylsuccinates, have all been found at the Seal Beach site, suggesting that monitoring for such intermediates may serve as a tool for evaluating biodegradation potential and rates. Potential health effects of the intermediates found now needs evaluation.

Another major highlight of the year is the results of seed funding for a project by Dr. Michael Hyman and his colleague, Prof. Lynda Ciuffetti from the Department of Botany and Plant Pathology, at Oregon State University to study the potential for cometabolism of contaminants by an interesting filamentous fungus *Graphium* sp. This organism can grow through oxidation of n-alkanes, which is initiated by cytochrome P-450. In addition to its ability to cometabolize chloroform, 1,2-dichloroethane, and 1,1,2,2-tetrachloroethane, it also was found to degrade methyl tert-butyl ether (MTBE). MTBE is a gasoline additive that is resistant to biodegradation and has become a major groundwater contaminant of growing concern. However, MTBE biodegradation by *Graphium* sp. is not complete, and tertiary butyl alcohol is formed as a major intermediate. This novel reaction is being explored further to determine its potential for in situ biodegradation.

Oregon State University researchers are also exploring the potential for other novel microorganisms for cometabolism of troublesome chlorinated solvents. Profs. Lewis Semprini and Daniel J. Arp are exploring bacteria that use n-alkanes such as butane and propane for energy and growth, perhaps with some similarities to the fungus described above. They also found that chloroform was cometabolized through n-alkane oxidation, this time using butane. Also of interest is that both 1,1,1-trichloroethane (TCA) and its anaerobic degradation product, 1,1-dichloroethane (1,1-DCA) are readily degraded by these microorganisms. Neither TCA nor 1,1-DCA are cometabolized well by most organisms studied to date, and thus use of butane when TCA and/or 1,1-DCA are present is of significant interest. Field studies using bioaugmentation with both *Graphium* sp. and the butane-oxidizing bacteria represent the next step in evaluation of the potential of these organisms.

Many other research projects and field demonstrations are being conducted through the WRHSRC. Space does not allow featuring them all, but they are described briefly in the back of this report. We believe the Center research is moving well between the laboratory and the field, and there is much to be gained by interactions between the two. Such efforts will be continued.

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 are now ongoing 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. We also anticipate that with success in the field, we will be called upon with greater frequency by the engineering community to assist them in implementing our findings on a broader scale. This we must be prepared to do and will put some of our future efforts in this direction.

Table 6. WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER PROGRAM SUMMARY

RESEARCH PROGRAM SUMMARY

<u>Project Description</u>	<u>Principal Investigators</u>	<u>End Date</u>	<u>Total Budget</u>
Chemical Movement, Fate, and Treatment			
Three-phase Flow in Fractured Media	Martin Blunt and Paul Roberts	1997	\$302,449
Physics of Dissolution of Nonaqueous Phase Liquids: Pore Networks and Field Simulations	Steven M. Gorelick	1997	\$159,610
Modeling VOC Emissions from Hazardous Waste Sites	Lynn M. Hildemann	1997	\$166,507
Upscaling Pore-Scale Hydrodynamics and the Transport of Reactive Solutes	Peter K. Kitanidis	1997	\$159,709
Effects of Redox Zones on the Fate and Transport of Contaminants in the Saturated Subsurface; Characterization and Simulation	Keith Loague	1997	\$121,422
The "Bubble Wall": A Passive In Situ System for Treatment and/or Containment of Contaminated Groundwater	J.D. Istok, V.A. Fry, J.S. Selker, and S.M. Gorelick	1997	\$297,685
Redox Transformations of Organic and Inorganic Contaminants in the Subsurface Environment	John C. Westall and James D. Ingle	1997	\$117,804
Hydrocarbon and Biological Factors Affecting Aquifer Clogging During In-Situ Bioremediation	Perry L. McCarty	1997	\$190,883
Chlorinated Solvents			

Reductive Transformation of Chlorinated Hydrocarbons by Reduced Ethenes Catalyzed by Vitamin B12--Mechanistic and Kinetic Studies	Martin Reinhard	1997	\$178,567
---	-----------------	------	-----------

<u>Project Description</u>	<u>Principal Investigators</u>	<u>End Date</u>	<u>Total Budget</u>
Inhibition, Inactivation and Recovery: A Universal Model for Aerobic Cometabolic Degradation of Aliphatic Compounds	Daniel Arp, Michael Hyman, and Ken Williamson	1997	\$168,097
An Investigation of the Chlorinated Hydrocarbon Substrate Range of the Filamentous Fungus, <i>Graphium</i> sp	Michael Hyman	1997	\$24,045
<i>Pseudomonas</i> Starvation Promoter-Driven In Situ Bioremediation	Abdul Matin	1997	\$114,721
Aerobic Cometabolism of Chloroform, 1,1,1-trichloroethane, 1,1-dichloroethylene, and Other Chlorinated Aliphatic Hydrocarbons by Microbes Grown on Butane and Propane	Lewis Semprini and Daniel J. Arp	1997	\$234,502
Mechanisms, Chemistry, and Kinetics of Anaerobic Biodegradation of cDCE and Vinyl Chloride	Perry L. McCarty and Alfred Spormann	1996	\$225,000
Biochemical Mechanisms of PCE Dehalogenation by Strain MS-1, and its Potential for In-Situ Bioaugmentation	Pramod K. Sharma and Perry L. McCarty	1997	\$337,323
Aromatic Compounds			
The Effect of Environmental Conditions on Reductive Dechlorination Rates	Sandra Woods	1996	\$ 154,818

In-Situ Anaerobic Biological Treatment of Aromatics in Groundwater	Martin Reinhard, Gary D. Hopkins, and Alfred M. Spormann	1996	\$456,250
The Effect of Apparent EH, Compound Structure, and Electron Donor on Anaerobic Biotransformation of Trinitrotoluene and its Metabolites	Sandra Woods, A. Morrie Craig, Kenneth J. Williamson	1997	\$126,434
Pathways of Anaerobic Toluene Metabolism by a Sulfate-Reducing Bacterium, Strain PRTOL1	Alfred M. Spormann	1997	\$189,616

<u>Project Description</u>	<u>Principal Investigators</u>	<u>End Date</u>	<u>Total Budget</u>
Anaerobic Ethylbenzene Oxidation in Denitrifying Strain EB1	Alfred M. Spormann	1997	\$151,585
Development of a Vitamin B12-Amended Bioremediation Process for the Reductive Dechlorination of Chlorobiphenyls at all Chlorine Positions	Sandra Woods	1997	\$144,357

Heavy Metals

Incorporation of Nickel in a Ceramic Matrix - A method for Treatment and Disposal of Heavy Metal Containing Wastes	George Redden and James Leckie	1996	\$299,450
Development, Characterization, and Performance Evaluation of Ferrous-Ferric Oxide Adsorbents for Metal Removal from Contaminated Groundwater	Peter O. Nelson	1997	\$111,668
Trace Element Adsorption in Porous Particle Packed Beds	James O. Leckie	1997	\$169,871

Minority Academic Institution Projects

Biotransformation of Lead and Chromate by Bacteria	Robert C. Blake Xavier University	1996	\$115,000
Magnetic Resonance Studies of Heavy Metals in Clays, Zeolites and Ceramics	Cynthia J. Hartzell and Michael P. Eastman Northern Arizona University	1996	\$ 79,658
Probing the Redox Properties of Environmental Systems: Natural Phenolic Materials	Robin Helburn Northern Arizona University	1996	\$ 70,357
Reductive Dehalogenation at Carbon and Derivatized Carbon Electrodes	Merritt Helvenston New Mexico Highlands University	1996	\$149,394
Detection of Microorganisms Capable of Anaerobic Degradation of Hazardous Substances in Natural Environments	Sara E. Silverstone California St. Univ., Bakersfield/State U. of New York	1996	\$111,405

TRAINING AND TECHNOLOGY TRANSFER PROGRAM SUMMARY

<u>Project Description</u>	<u>Principal Investigators</u>	<u>End Date</u>	<u>Total Budget</u>
Demonstration Projects			
Field Test of In-Situ Vapor Stripping for Removal of VOCs from Groundwater	Steven M. Gorelick	1997	\$641,709
Full-Scale Evaluation of an Apparatus for Down-well Oxygen Transfer to Implement <i>In situ</i> Bioremediation at Edwards AFB	Perry L. McCarty and Mark N. Goltz	1996	\$142,334
Field Testing of Palladium-Catalyzed Hydrodehalogenation for Chlorinated Hydrocarbon Removal from Groundwater	Martin Reinhard and Paul V. Roberts	1996	\$321,149
Full-Scale Evaluation of <i>In Situ</i> Bioremediation of Chlorinated Solvent Groundwater Contamination	Perry L. McCarty, Mark N. Goltz, and Gary D. Hopkins	1996	\$611,061
Demonstration of an Interceptor Trench Technology for the Bioremediation of a Pentachlorophenol-Contaminated Ground Water	Sandra Woods	1997	\$284,800
Minority Academic Institution Training and Technology Projects			
Faculty Enhancement on Hazardous Contaminant Research with Specific Emphasis on In-Situ Anaerobic Biodegradation of BTEX Hydrocarbon Contaminant	Aregai Tecele Northern Arizona U.	1996	\$86,315
Bioremediation Technology Transfer Conferences at Albuquerque Technical-Vocational Institute	R. Barry King Albuquerque Technical-Vocational Institute	1996	\$34,375

<u>Project Description</u>	<u>Principal Investigators</u>	<u>End Date</u>	<u>Total Budget</u>
Training and Technology Transfer Projects			
Hazardous Waste Training	Kenneth J. Williamson Peter O. Nelson	1996	\$120,000
Continuing Education	Kenneth J. Williamson	1996	\$120,000
Conference Sponsorship	Kenneth J. Williamson	1996	\$120,000
Technical Outreach Services for Communities	Kenneth J. Williamson	1997	\$562,500

RESEARCH PROJECT DESCRIPTIONS

CHEMICAL MOVEMENT, FATE, AND TREATMENT

Three-phase Flow in Fractured Media: Martin Blunt and Paul Roberts, Stanford University (Supported by R2D2 Program)

Goal: To obtain a validated understanding of three-phase flow behavior of immiscible organic liquids (NAPLs) in fractured media, with particular application to steam injection. The project is oriented towards the study of steam injection as a remediation alternative both because of its relevance in treating fractured bedrock and low permeability lenses as well as the three-phase nature of steam injection displacements.

Rationale: Many DNAPL spill sites are underlain by fractured aquitards or bedrock. Treatment of such sites with a conventional pump and treat approach is likely to result in a long and costly remediation. Steam injection is an alternative clean-up option for such sites. However, our understanding of three-phase flow behavior is very incomplete. With this research, we plan to develop a set of relationships between phase saturation and relative permeability and capillary pressure of each phase in fractured media, as well as mass transfer relationships between fracture and matrix, based on our physical understanding of three-phase flow and on the results of visualization experiments both in micromodels and at the core scale using CT scanning.

Approach: Given our previous understanding of the physics of two and three phase flow, which has been captured in a three-dimensional, three phase network model, we have postulated the shape of the relative permeability and capillary pressure curves. The experimental research being carried out in this work seeks to validate the displacement mechanisms predicted for three phase flow, specifically double drainage, double imbibition and flow in thin layers, and the effect of the spreading coefficient on the importance of these mechanisms, by observing them in micromodels which have been etched with a realistic replica of a fractured sandstone matrix. In addition, at the core scale we are seeking to validate the shape of the relative permeability and capillary pressure curves, using CT scanning to determine the phase saturation before, during and after a sequence of displacements, including imbibition, drainage and steam injection.

Status: The shape of the relative permeability and capillary pressure relationships has been predicted by the network model. The micromodel experiments have shown that double drainage exists and is important even for non-spreading NAPLs (such as decane and higher hydrocarbons as well as many chlorinated solvents), and that flow in thin layers is an important mechanism for movement of small amounts of NAPLs in porous and fractured media, even at low NAPL saturations. The CT scanning experiments have been performed with three fractured cores. Analysis and numerical modeling of the experiments continues and should be completed early in 1997, as planned.

Physics of Dissolution of Nonaqueous Phase Liquids: Pore Networks and Field Simulations: Steven M. Gorelick, Stanford University (Supported by R2D2 Program)

Goal: The objectives of this study are: (1) to contribute to the understanding of interphase mass transfer by using pore network models to investigate NAPL dissolution; (2) to analyze how the rate of dissolution varies with multiphase properties ranging from pore-scale interfacial area to measurable entities such as fluid saturation and concentration; (3) to develop a physically-based relation between dissolution rate and measurable entities that reflects dynamic processes, such as varying interfacial area, fluid saturation, and the mass fraction of soluble species; (4) to enhance a 3-dimensional multiphase compositional simulator to account for nonequilibrium NAPL dissolution; (5) to explore the range of applicability of the local equilibrium assumption at the field scale and determine the impact of kinetically-mediated NAPL dissolution; and (6) to understand the role of intrinsic bioremediation at a NAPL-contaminated site and to investigate its effects in the context of nonequilibrium NAPL dissolution.

Rationale: During remediation of sites contaminated by NAPLs immobile NAPL blobs can serve as a long-term source of contamination as more soluble species partition into the aqueous phase. NAPL dissolution can be a rate-limited process dependent on dynamic pore- and field-scale phenomena. Few numerical models developed for hydrogeological applications exist that are multiphase, multicomponent and three-dimensional, and none of these account for rate-limited dissolution. Furthermore, in model applications involving nonequilibrium dissolution, dissolution rate coefficients are needed as input. The traditional approach has been to estimate these rates using correlation expressions developed from laboratory data. Traditional correlation expressions do not expressly account for dynamic processes, are not transferable to other data, and may not be applicable to field settings. Consequently, the validity of local equilibrium in NAPL dissolution in a realistic field setting is not well-understood.

Approach: This study involves 5 stages: (1) development of a pore network model representative of a real porous media; (2) development of a physically-based NAPL dissolution relation using the pore network model; (3) modification of a 3-dimensional compositional simulator to represent nonequilibrium NAPL dissolution; (4) hypothetical and field-data-based modeling studies investigating the limitations of the local equilibrium assumption on field-scale NAPL dissolution; and (5) an investigation of the feasibility of intrinsic bioremediation in the presence of field-scale factors limiting NAPL dissolution

Status: We have developed an initial pore network flow model. Results suggest the network model does a good job of predicting observed experimental flow relationships at the column scale as well as the observed residual NAPL distribution. Discrepancies between the simulated and experimental relationships of NAPL concentration versus Darcy velocity have led us to investigate the significance of the complete mixing assumption used in calculating concentrations in the network model. Specifically, the simulated concentration profile does not reproduce observed concentrations at high flow rates, and the slope of the simulated concentration profile at the low-velocity diffusion-controlled end is too steep. We have gotten away from the complete-mixing model and developed a proportional routing approach. It is a method that represents chamber concentrations as a function of the preferential contribution of solute flux by those tubes with higher flow rates. Two versions of proportional routing are being incorporated into the network model: a purely advective version, and a version that accounts for diffusion within the network tubes. The model involving advective proportional routing is complete and results show a constant concentration profile similar to the complete mixing case. The model representing the diffusion-controlled regime is currently under development.

Modeling VOC Emissions from Hazardous Waste Sites: Lynn M. Hildemann, Stanford University (Supported by R2D2 Program)

Goal: Many potentially toxic volatile organic compounds (VOCs) have been identified in the air downwind of waste disposal sites. An important potential impact of these hazardous emissions is the long-term inhalation exposures experienced by downwind residents. The goal of this project is to develop an integrated emission-dispersion model to evaluate the extent to which these emissions pose a hazard to human health.

Rationale: Because of the high cost of field experiments and the lack of standardized sampling methods, field measurements of airborne emissions from contaminated sites are difficult to obtain. Modeling approaches provide a less-costly alternative to evaluating the potential impacts of VOC emissions from contaminated sites. However, the emissions models currently existing can only be utilized for "screening-level" purposes due to the many fundamental oversimplifications made, which may seriously compromise the accuracy of the predictions. A more accurate approach is needed to reliably estimate the source emissions and to evaluate the risk posed to humans in the near vicinity of the source, as well as further downwind.

Approach: An integrated emission-dispersion model is being developed to predict concentrations of VOCs downwind of a contaminated site, as a function of site conditions and meteorological parameters. The performance of the model will first be evaluated by comparing its predictions with available field measurements. The model will then be modified and utilized to predict concentrations downwind of a particular site. The major factors that influence VOC emissions from the site and the subsequent dispersion in the atmosphere will be examined through sensitivity analysis. The predicted concentrations will also be compared with typical ambient levels of VOCs and concentration levels for which health effects are known, thereby providing a measure of the extent to which the added emissions contribute to the total airborne burden, and the extent to which these emissions pose a significant hazard to humans living downwind.

Status: A more complete analytical emission model has been developed that can predict VOC emissions from hazardous waste sites. The new model removes the major assumptions/simplifications inherent in the screening-level models, and incorporates most of the important mechanisms occurring in unsaturated subsurface landfill environments. An analytical dispersion model previously developed under WRHSRC sponsorship that accurately represents the dispersion phenomena within the atmospheric boundary layer has undergone further modifications to account for inversions aloft and partial absorption by the ground. An assessment of the performance of this model has recently been completed, in which model predictions in both the downwind and vertical directions were found to compare well with measurements from a published, comprehensive field study.

Upscaling Pore-Scale Hydrodynamics and the Transport of Reactive Solutes: Peter K. Kitanidis, Stanford University

Goal: The goals are: (1) to improve the understanding of hydrodynamics, mass transport, and biochemical reactions at the pore scale and (2) to derive physically valid mathematical

representations of processes at the Darcy scale, where the pore details are not resolved. This research project focuses on mathematical analysis but is guided by experiments conducted on the project concerned with aquifer clogging. The objectives include describing the mechanisms that control the growth of biofilms, such as shearing of biofilms at pore necks. A potential practical output of this research would be the derivation of relations between biomass and effective conductivity, dispersivity, and reaction rates in a porous medium with dynamically changing biofilms. Also accounting for sorption and desorption with characteristic times comparable to or slower than those of pore-scale diffusion and reaction.

Rationale: Fundamental understanding of the mechanisms and processes that affect in-situ bioremediation is best at the pore scale. The rates at which subsurface microorganisms transform pollutants is controlled to a great extent by the pore-scale physical mechanisms that supply nutrients and electron acceptors. However, in practice, measurements, predictions, and assessments are made at a much larger scale. The most scientific approach is to derive relations among macroscopic variables by scaling up from the pore scale.

Approach: The approach adopted in this project is to study pore-scale hydrodynamics and rates of mass redistribution in detail at the pore scale. Idealized geometrical models of a porous media are used to obtain order of magnitude estimates of variables. Additionally, general methods of scaling up are applied to derive relations among macroscopic variables.

Status: The project started in March 1995 and is expected to be completed in 1997. A finite-element numerical model has been developed for the simulation of flow, transport, and biochemical reactions in pores. The model simulates: The growth of active biomass, the transport and transformation of a soluble electron donor (substrate) and the transport and transformation of a soluble electron acceptor. Using this model the following phenomena have been studied for some two-dimensional pore geometries: pore-scale hydrodynamics; dilution and mixing of solutes at the pore scale; mass transport limitations on reaction rates; and the growth, decay, and possible redistribution of biomass.

Effects of Redox Zones on the Fate and Transport of Contaminants in the Saturated Subsurface; Characterization and Simulation: Keith Loague, Stanford University (Supported by R2D2 Program)

Goal: Redox conditions influence the mobility and toxicity of metal contaminants and the ability to bioremediate organic contaminants. The goal of this project is to establish a physics and chemistry based simulation protocol that will enable us, and future workers, to quantitatively estimate the fate and transport of contaminants that are influenced or controlled by different redox conditions.

Rationale: Groundwater contamination sites are often characterized by a sequence of redox zones. Assessment of many subsurface contaminants depends upon quantitative characterization of the development and fate of redox zones. The ability to simulate these zones will greatly enhance predictive modeling of the fate of many inorganic and organic contaminants at hazardous waste sites.

Approach: The primary research tool in the proposed effort is coupled numerical simulations of saturated subsurface fluid flow, solute transport, and chemical reactions. The approach for model development and testing in this study is (1) development of a coupled saturated subsurface fluid flow, solute transport, and geochemical models and boundary value problems based upon the massive data for the extensively studied field site located on Cape Cod, MA, (2) sensitivity analysis for the chemical components/reactions and hydrologic variables essential to the development of a robust model of field-scale phenomena, and (3) quantitative model performance evaluation based on both statistical and graphical criteria.

Status: The major results from the project, thus far, are (1) the numerical problems associated with using thermodynamics were overcome by developing a model that considers only those reactions that contribute significantly to a given redox zone, and (2) the demonstration that the development of steady-state concentrations of key chemical parameters does not necessarily imply thermodynamic equilibrium. This was accomplished by using a kinetic formulation that is based upon the mass action of a chemical reaction (i.e., not limited to first or second order expressions). We are currently working on the second phase of this study: coupling the reaction network to a one-dimensional solute transport model.

The “Bubble Wall”: A Passive In Situ System for Treatment and/or Containment of Contaminated Groundwater: Jonathan D. Istok, Virginia A. Fry, John S. Selker, Oregon State University, and Steven M. Gorelick, Stanford University

Goal: The objectives of this project are to investigate the use of a “bubble wall” (a zone of trapped gas emplaced within the saturated zone of a groundwater aquifer) (1) to serve as a source of dissolved gas for use in in situ bioremediation or (2) to serve as a flow barrier for use in hydraulic containment.

Rationale: The rate of many microbially mediated reactions of interest in bioremediation is frequently limited by low dissolved gas concentrations (e.g. low dissolved oxygen concentrations often limit the rate of aerobic transformation of petroleum hydrocarbons). For this reason, a wide variety of methods have been proposed for increasing dissolved gas concentrations in contaminant plumes. Unfortunately, most existing methods (e.g., air sparging or the injection of gas amended water) are inefficient because of (1) the small solubility of most gases in groundwater, and (2) the limited degree of mixing that occurs between injected gas or gas enriched water and the contaminated groundwater. The “bubble wall” technology attempts to overcome these difficulties by using a zone or “wall” of trapped gas bubbles as a passive source of dissolved gas to contaminated groundwater flowing through the wall. The presence of trapped gas in the pore space of a bubble wall also reduces the hydraulic conductivity of the aquifer within the wall. This means that it may be possible to use a “bubble wall” as a flow barrier for use in hydraulic containment (e.g., in place of a slurry wall or sheet pile structure).

Approach: The investigation of the “bubble wall” technology is being performed using a combination of theoretical analysis, numerical modeling, and laboratory experiments. Theoretical analysis is used to develop continuum models to describe the mass transfer of gas from the trapped gas to the mobile aqueous phases and pore-scale models to describe the effect of trapped gas on the hydraulic conductivity and dispersivity of the porous media. Numerical models are used to determine the sensitivity of mass transfer to model parameters (e.g. particle

size distribution, trapped gas volume, solubility and vapor pressure of dissolved gas, and pore water velocity) and to determine the effect of hydraulic conductivity reduction within a bubble wall on the regional groundwater flow field. Laboratory experiments are performed in one-dimensional columns and two-dimensional, vertical and horizontal physical aquifer models using a variety of porous media. The purpose of the laboratory experiments is to investigate methods for emplacement of trapped gas into saturated porous media to create a bubble wall, to validate models for mass transfer of gas from the bubble wall to groundwater, to determine the effect of trapped gas on hydraulic conductivity, and to validate pore-scale models.

Status: A series of experiments were performed to explore the gas entrapment potential of injecting compressed gas, gas supersaturated water, and a hydrogen peroxide laden solution. Results were analyzed with respect to bond and capillary numbers. Experimental and theoretical analyses are in progress to describe the geometry of entrapment as a function of media characteristics, injection rate, and heterogeneity. A kinetic model for dissolved gas transport from the trapped gas bubbles to groundwater has been developed and tested for a wide range of groundwater velocities, amount and composition of trapped gas, and dissolved gases in both small-scale column experiments and intermediate-scale physical aquifer model experiments. The model has also been used in combination with single-well, "push-pull" tests (1) to quantify trapped gas volumes below the water table in two unconfined aquifers, and (2) to estimate mass transfer coefficients for gas transfer from the trapped gas bubbles to groundwater. Additional field experiments are planned in an attempt to quantify the effectiveness of various methods for emplacing trapped gas bubbles.

Redox Transformations of Organic and Inorganic Contaminants in the Subsurface Environment: John C. Westall and James D. Ingle, Oregon State University (Supported by R2D2 Program)

Goal: The long-range goals of this project are: (1) to determine the "best" way to measure the redox conditions of the subsurface environment and (2) to determine how the redox transformations of selected target contaminants and redox sensors couple to the biogeochemical matrix under specific redox conditions.

Rationale: A clear understanding of the redox state and speciation of chemicals is required in virtually all aspects of hazardous waste management: (1) evaluation of disposal options, (2) risk assessments of contaminated sites, and (3) evaluation of clean-up options for contaminated sites. Specifically, the redox state of a contaminant affects its speciation and thereby its transport, fate and biological effects.

Approach: In characterizing redox transformations of contaminants, three components are considered: the redox-active contaminant, the biogeochemical matrix, and the redox sensor. The coupling between all components is being investigated on a species-by-species basis. The focus of this study is on transformations of major biogeochemical redox couples such as Fe(II)/Fe(III) and priority pollutant couples such as Cr(III)/Cr(VI) and As(III)/As(V) in "real" soil systems due to microbial and chemical processes. In parallel experiments, different methods of determining the redox status of the biogeochemical matrix are being evaluated, such as the redox state of immobilized redox indicators, platinum electrode potential, concentrations of soluble matrix components (e.g., Fe(II)), and H₂ concentration.

Status: A bioreactor system developed previously is being used to monitor redox conditions in biologically-active soil slurries. Pt electrode potentials and the absorbance of immobilized organic redox indicators are monitored as the system becomes more reducing. Five indicators encompassing a range of redox conditions (+53 to -286 mV vs. SHE) have been covalently immobilized on affinity chromatography beads (50- μm diameter) which are packed in a spectrophotometric flow cell. The flow cell is placed in an external loop filter/flow system which allows filtered soil water from the slurry to interact with the indicator. The absorbance (and hence speciation) is measured with a spectrophotometer. When the soil slurry becomes more reducing, the immobilized redox indicator thionine is reduced and other experimental evidence supports the hypothesis that Fe(II) in solution is responsible for the indicator reduction. With a buffer test solution in the bioreactor (no soil), four of the immobilized indicators (monitored simultaneously) were titrated with Fe(II). The concentration of Fe(II) necessary to reduce a given fraction of an indicator increased as the formal potential of the indicator became lower. The indicator/Fe(II) reactions exhibit reversibility as evidenced by the re-oxidation of a reduced indicator if the Fe(II) level decreases (by dilution of the reactor solution). The results suggest that several different indicators can be used to define different redox windows and to estimate Fe(II) concentrations as low as 50 μM to as high as 0.1 M. In a bioreactor study with a mixture of soil and mine tailings (Sutter Creek) containing As, reduction of thionine began as the concentration of Fe(II) approached 100 μM and significant As(III) was released into solution. Therefore, it may be possible to develop redox sensors to predict the onset of Fe(III)- and As(V)- reducing conditions and the prevalent form of As in solution. Most recently, sol-gel technology has been used to immobilize indicators to the tips of glass rods (for fiber optic sensors) and to immobilize indicators along with a finely divided palladium catalyst which reacts with H_2 .

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 is being constructed. This apparatus will be seeded initially with a mixed bacterial culture consuming acetate as a primary

substrate. From the understanding that is obtained, pure 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: This project was begun on April 1, 1995. A test system was constructed and operated with a mixed bacterial culture fed 10 mg/l acetate at a superficial velocity of 1.6 m/d. Two types of growth were observed, one was a uniform biofilm around the simulated aquifer media and the other was filamentous aggregates that sometimes spanned the pore spaces between media. The design has been improved considerably for better visualization and for more automation in data collection so more data on the process can be obtained for evaluation. This project is closely connected with the modeling studies of the project "Upscaling pore-scale hydrodynamics and the transport of reactive solutes."

CHLORINATED SOLVENTS

Reductive Transformation of Chlorinated Hydrocarbons by Reduced Ethenes Catalyzed by Vitamin B₁₂ — Mechanistic and Kinetic Studies: Martin Reinhard, Stanford University

Goal: This project aims to (1) determine products and pathways of chlorinated ethene transformation by vitamin B₁₂ under different reducing conditions, (2) develop kinetic models describing the transformation, and (3) elucidate the mechanism.

Rationale: Transition-metal coenzymes such as vitamin B₁₂ have been shown to mediate the cometabolic dehalogenation of chlorinated hydrocarbon compounds in microbial systems. A detailed mechanistic understanding of this reaction may help in optimizing biological detoxification processes and the design of novel remediation approaches.

Approach: In this project, we are focusing on TCE and its metabolites as the substrates. The reaction is studied in aqueous solution containing Ti(III) as a reductant, vitamin B₁₂, and trace concentrations of substrate. To follow the reaction head space samples are taken and the disappearance of substrate and the formation of intermediates and products are measured over time using GC/MS, GC/FID, and GC/ECD. The (transient) products formed include dichlorinated ethylene isomers, vinyl chloride, chloroacetylene, acetylene, ethene, and ethane. Solutions were prepared in the glove box, and then spiked with a micromolar amount of saturated aqueous solution of the chlorinated substrate under light-excluded, anaerobic, and abiotic conditions. Rate constants and product yields are measured over relatively long reaction times so that the formation and decay of stable intermediates can be observed.

Status: The project started in March 1996. Since then, a GC method has been developed to analyze the full range of products and intermediates, and to obtain mass balance data for the transformation of TCE. The intermediates of the TCE transformation were studied in separate experiments under identical conditions. Chloroacetylene, which was commercially not available, was synthesized. Currently, we are in the process of analyzing the data.

Inhibition, Inactivation and Recovery: A Universal Model for Aerobic Cometabolic Degradation of Aliphatic Compounds: Daniel Arp, Michael Hyman, and Ken Williamson, Oregon State University

Goals: The aims of this project are two-fold. First, the further development and corroboration will be done of a mathematical model previously developed which describes the kinetics of aerobic cometabolism of trichloroethylene (TCE). This model was originally developed for the soil nitrifying bacterium, *Nitrosomonas europaea* and describes TCE cometabolism in terms of an inhibition, an inactivation and a recovery process. The present study aims to extend these earlier studies to other organisms including toluene- and methane-oxidizing species. The second aim is to further investigate the physiological responses of *N. europaea* to the toxicity associated with the cometabolism of trichloroethylene.

Rationale: In situ bioremediation schemes have a considerable need for mathematical models that can accurately describe the kinetics of pollutant degradation. In particular, accurate models can provide important information for process design and for the interpretation of degradation studies. At a more fundamental level, there is also a considerable need to understand the molecular basis for the toxicity of various pollutants towards degradative organisms. In addition, it is important to understand the responses of the affected organisms to these toxic effects because these responses in turn affect the kinetics and modeling of the degradation process. In combination these large and small scale approaches should provide a comprehensive description of the kinetics and molecular consequences of aerobic cometabolism of chlorinated solvents.

Approach: The current approach for extending the modeling efforts is based on the quasi-steady-state reactor system which was previously developed for studies with *N. europaea*. In summary, a 1.7 gas-tight reactor vessel containing 0.5 L of reaction mixture is rapidly stirred using a magnetic stir bar. Primary substrates (e.g. ammonium) are supplied at a constant rate. Once steady state rates of activity have been established, an alternative pollutant substrate is added to the reactor and the kinetics of both the primary and secondary substrate degradation are monitored over a period of several hours. In addition, assays of residual enzyme activities are determined for small (< 2ml) samples removed from the reactor at regular intervals. Experiments investigating the mechanism and response of *N. europaea* to the toxicity associated with TCE and chlorinated solvent degradation are conducted on a smaller scale and over shorter times. The effects of these exposures are correlated with the losses of various enzyme activities and the response of the cells is subsequently monitored using radiolabeled $^{14}\text{CO}_2$ to monitor de novo protein synthesis.

Status: In previous studies, a methodology for conducting reactor-based studies with *N. europaea* was developed, and the mathematical model describing TCE cometabolism by this organism was formulated. Experiments have now been initiated with *Burkholderia cepacia* G4 to examine whether the model accurately describes the cometabolism of TCE by other organisms. Conditions for quasi-steady state have been established and experiments are now being performed to look at the effect of TCE on the reactor system.

Short term, small scale assays examining inhibition, inactivation, and recovery associated with TCE cometabolism by the toluene oxidizer *B. cepacia* G4 have been initiated. Examination of

these processes in G4 will provide the framework for a comparative study of these features of TCE cometabolism amongst nine other TCE degrading, toluene oxidizers (six uncharacterized bacteria which degrade TCE at varying rates relative to toluene oxidation, *Pseudomonas mendocina* KR1, *Pseudomonas pucheria* PK01, and *Pseudomonas putida* F1).

The mechanism of TCE toxicity for *N. europaea* has been further investigated using short-term incubations. TCE cometabolism has significant effects on both AMO and HAO activities. Inhibitor treatments which specifically inactivate either AMO or HAO do not mimic the effects of TCE. These results lead us to conclude the site of action is not directly related to these enzymes. We are currently investigating our current hypothesis that TCE cometabolism exerts a significant effect on the electron transport in *N. europaea* cells.

An Investigation of the Chlorinated Hydrocarbon Substrate Range of the Filamentous Fungus, *Graphium* sp.: Michael Hyman and Lynda Ciuffetti, Oregon State University (Supported by R2D2 Program)

Goals: There are two main goals of this project. The first is to examine the ability of the filamentous fungus *Graphium* to cometabolically degrade a range of chlorinated C1 and C2 hydrocarbons. The second is to investigate the ability of this organism to degrade a series of alkyl ether compounds, either through cometabolism or through conventional metabolism where the compound is used as a carbon and energy source to support growth.

Rationale: *Graphium* sp. is one of the few fungi known to utilize gaseous n-alkanes as carbon and energy sources to support growth. Aerobic, gaseous hydrocarbon-utilizing microorganisms typically utilize monooxygenase type enzymes to initiate the oxidation of their growth supporting substrates. In many instances these monooxygenase enzymes exhibit relaxed substrate specificity's. The ability of monooxygenase-bearing bacteria to degrade non-growth supporting substrates through the process of cometabolism represents one of the most promising avenues for the bioremediation of simple chlorinated hydrocarbons. While most previous studies have concentrated on bacterial systems, the purpose of this present investigation is to determine whether a similar activity can be observed in hydrocarbon-grown fungal systems. As a prelude to these degradation studies, we are also interested in defining the basic physiology and biochemistry of hydrocarbon oxidation by *Graphium*. A fundamental understanding of these areas is of considerable importance to successfully control the activities of this organism for sustainable degradation purposes.

Approach: The experimental approach has been divided into two areas. First, the overall pathway of gaseous hydrocarbon oxidation by *Graphium* is to be defined. The general approach has involved determining the range of hydrocarbons and potential oxidation intermediates which support the growth of this organism. These quantitative studies have emphasized the yields of mycelia obtained from each substrate and the effects of inhibitors of alkane oxidation on these yields. The second approach involves examining the ability of *Graphium* to degrade chlorinated solvents through the process of cometabolism. The experimental approach involves growing *Graphium* on gaseous n-alkanes and then conducting short term (< 1h) incubations in the presence of suitable concentrations of each chlorinated hydrocarbon. Chlorocarbon degradation is then detected by gas chromatography.

Status: These studies have demonstrated n-alkane-grown *Graphium* can degrade a variety of non-growth supporting substrates including chloromethane, dichloromethane, chloroform chloroethane, 1,2-dichloroethane and 1,1,2,2-tetrachloroethane. The products of these cometabolic reactions and the potential degradation of chlorinated ethylenes have not been identified. *Graphium* was demonstrated to grow on the unusual substrate diethyl ether and can cometabolically degrade the gasoline oxygenate methyl *tert* -butyl ether after growth on gaseous n-alkanes. The products of diethyl ether oxidation have been identified as ethanol and acetaldehyde. For methyl *tert*-butyl ether the predominant pathway of degradation apparently involves the formation of *tert*-butyl formate which subsequently undergoes hydrolysis to yield *tert*-butyl alcohol.

***Pseudomonas* starvation promoter-driven in situ bioremediation: A. Matin, Stanford University**

Goal: Construction of a recombinant strain of *Pseudomonas* species with high efficiency trichloroethylene biodegradation activity *in situ*.

Rationale: Trichloroethylene (TCE) is considered one of the most common pollutants and a potential carcinogen. Toluene-4-monooxygenase (TMO) enzyme complex of *Pseudomonas mendocina* KR-1 degrades TCE into compounds that can be completely mineralized by a mixed microbial population in natural environment. However, bacteria indigenous to polluted environment exhibits low metabolic activity which is most likely due to slow growth as a result of nutrient scarcity. Therefore current *in situ* bioremediation efforts rely on the addition of substantial amount of nutrients to stimulate bacterial growth and induce biodegradation activity. This technology is costly and can be complicated by growth of competing microbial populations, and clogging of subsurface pores with biomass.

On the other hand it is now well established that under various stress conditions a certain class of bacterial genes, called starvation genes, switch on selectively, exhibiting high levels of expression. Expression of appropriate gene(s) under the control of bacterial starvation promoters would allow the uncoupling of expression of the desired metabolic activity from bacterial growth and facilitate bioremediation.

Approach: As a first step towards achieving the final goal, the model system of *Escherichia coli* was used to test the TCE bioconversion efficacy (i.e. the amount of TCE degraded per unit of biomass synthesized) under the conditions of nutrient limitation. TMO was expressed under the control of *E. coli* starvation promoters *cstC* (cloned and characterized in the Matin lab) and *groEL*. In these experiments the nutrient demand and biomass formation required for the bioconversion of a unit of TCE or phenol were reduced by 60 to 90% compared to that of wild type *Pseudomonas*, showing a 100-fold increase of TMO bioconversion efficacy (Matin et al., 1995).

Since *E. coli* is not indigenous to polluted environments and cannot be utilized in bioremediation *in situ*, a *Pseudomonas* strain is now being constructed with high bioconversion efficiency for TCE biodegradation; *Pseudomonas* species are indigenous to nearly all contaminated environments.

Status: A strong starvation promoter from *P. putida* was recently cloned (Kim et al., 1995), and has now been successfully spliced to the *tmo* gene cluster, using a broad host range plasmid (pMMB67) (Hahm and Matin. 1996). *P. putida* strains bearing the recombinant plasmid, but not control strains without the plasmid, carried out rapid and substantial conversion of indole into indigo. This conversion occurred only in the slow-growth/stationary phase, and not in the exponential growth at μ_{max} . Indole to indigo conversion is carried out by TMO and is a convenient assay system because of the blue color of indigo. Ongoing experiments indicate that the recombinant plasmid strain also carry out TCE removal only in the slow-growth/stationary phase, as determined by gas chromatography. Optimization of expression of TCE degradation activity in the slow-growing cells and the efficacy of

trichloroethylene degradation will be followed by the insertion of the expression construct into *Pseudomonas* chromosome and determining conversion efficiencies in model lab. systems.

Aerobic Cometabolism of Chloroform, 1,1,1-trichloroethane, 1,1-dichloroethylene, and Other Chlorinated Aliphatic Hydrocarbons by Microbes Grown on Butane and Propane: Lewis Semprini and Daniel J. Arp, Oregon State University (Supported by R2D2 Program)

Goal: The objectives of this study are: (1) to evaluate the ability of microorganisms grown on butane or propane to degrade a broad range of chlorinated aliphatic hydrocarbons (CAHs), (2) to obtain a better understanding of factors effecting the rates and extents of transformations of these compounds by these microorganisms, (3) to learn more about of biochemistry of the microorganisms and the associated cometabolic processes.

Rationale: In previously funded Center Research butane and propane were discovered as good cometabolic growth substrates for aerobic treatment of chloroform. Chloroform had previously been shown to be a fairly difficult substrate to degrade via aerobic cometabolism. Other contaminants such as 1,1,1-trichloroethane (TCA) and 1,1-dichloroethylene (1,1-DCE) have also been fairly resistant to aerobic cometabolism. Trichloroethylene (TCE) can be effectively degraded by microbes grown on phenol or toluene, however, these growth substrates are regulated chemicals that may be difficult to add for subsurface remediation. This research therefore focuses on evaluating the potential of butane and propane as substrates for CAH cometabolism.

Status: Cultures enriched from the subsurface of Hanford DOE site are currently being evaluated in microcosm studies. Butane-grown mixed cultures are very effective at degrading a broad range of chlorinated ethanes and several chlorinated ethenes. The degradation of all the CAHs was inactivated by acetylene. Relative transformation rates and transformation capacities have been determined. Transformation rates rank as follows: 1,2-dichloroethane > 1,1,-dichloroethane (1,1-DCA) > 1,1,2-trichloroethane > 1,1,1-trichloroethane, and vinyl chloride > 1,1-dichloroethene > cis-dichloroethene > trichloroethene > trans-dichloroethene. Resting cell transformation capacities for the chlorinated ethanes, on a total suspended solids (TSS) cell basis range from 0.07 mg 1,1,1-TCA/mg TSS to 0.40 mg 1,1-DCA/mg TSS. The mixed culture appears well suited for the cometabolism of TCA and it's abiotic and anaerobic transformation products, 1,1-DCE and 1,1-DCA. The propane-grown mixed culture is capable of transforming the very broad range of chlorinated ethanes and ethenes mentioned above. It is more effective at degrading the chlorinated ethenes than the butane-grown culture. An enrichment culture has been obtained from the butane-fed microcosms of Hanford core material. This culture, named CF8, was compared with two butane-grown bacteria, *Pseudomonas butanovora* and *Mycobacterium vaccae*, obtained from culture collections. All three cultures degraded chloroform at similar rates. Chloroform degradation resulted in toxicity to all three bacteria, but *P. butanovora* was more sensitive than CF8 or *M. vaccae*. Chloroform degradation by all three bacteria was inactivated by acetylene and inhibited by ethylene. Ethylene inactivated chloroform degradation by *P. butanovora* (suggesting a P450 prosthetic group), but not *M. vaccae* and CF8. *Allylthiourea* inhibited chloroform degradation by CF8 (suggesting a copper prosthetic group) but not *P. butanovora* or *M. vaccae*. CF8 and *P. butanovora* degraded TCE, 1,1,2-TCA, 1,2-cis-DCE and vinyl chloride, but not 1,1,1-TCA or

1,2-trans-DCE. The cultures are dominated by a single type of bacterium and grow with butane as the sole carbon and energy source.

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, an individual or group of organisms responsible for the complete dehalogenation process from tetrachloroethylene (PCE) to ethene has not yet been identified. Whether or not the complete dehalogenation or 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 the process better so that it can be more widely applied, its suitability for application at a given location can be better determined, and the economics of process implementation can be improved, definitive studies to better understand the nature of the organisms involved and factors affecting transformation rates are needed.

Approach: Anaerobic aquifer material from a contaminated site in Victoria, Texas, was obtained by DuPont Chemicals for this study. Microcosms were constructed of 125 mL bottles containing aquifer material and groundwater from the site. A small portion of the fluid is removed from the bottle periodically and replaced with groundwater amended with various primary substrates and PCE. Samples are analyzed for a range of CAHs and ethene, as well as for primary substrate, sulfate, and methane. The relationships between time and amount of primary substrate on CAH transformation is being evaluated. In separate studies, enrichment cultures are being developed using various electron donors, PCE, sulfate, and other nutrients, and serial dilutions of the cultures are being made as a first step in organism isolation. If satisfactory transformation of PCE is obtained by highly enriched cultures, then attempts will be made to isolate colonies from agar plates or roll tubes. Once isolated, the organisms will be characterized, and factors affecting PCE transformation will be studied in greater detail.

Status: The original project was completed last year, but additional funding was provided last year to extend the project, but with similar objectives. Funding also was received this year from the U.S. Department of Energy to extend this work. Benzoate, acetate, and formate were all found to stimulate dehalogenation, although benzoate appears to be the better of the three. Enrichment cultures have been developed that retain their ability to reduce PCE to ethene. A facultative pure bacterial strain (MS-1) has been isolated that transfers PCE to cis-1,2-dichloroethylene (c-DCE) while growing on a variety of substrates. This is the first facultative bacterium identified with this dehalogenating ability. A new Center grant has been funded to characterize the physiology and biochemistry of this organism and to evaluate its potential for

bioaugmentation for degradation of chlorinated solvents. This overall study clearly demonstrated that there are two separate groups involved in the overall dehalogenation of PCE to ethene, the first is the group that converts PCE to c-DCE as represented by strain MS-1, and the second is the group that converts c-DCE to vinyl chloride and ethene. Rates of transformation were evaluated by a mixed culture growing on yeast extract. The rates of transformation of PCE and TCE to cDCE were about four times faster than for the conversion of cDCE to vinyl chloride and vinyl chloride to ethene. The pH optimum for the culture studied was 6.5 and the temperature optimum was 35° C. Organisms appear to obtain energy from the dehalogenation reaction while using the chlorinated organics as electron acceptors. Monod kinetics of the reactions involved are being evaluated through the extension of the project. Also, enzymatic studies are being conducted in order to better understand the biochemistry of the reactions involved.

Biochemical Mechanisms of PCE Dehalogenation by Strain MS-1, and its Potential for In-situ Bioaugmentation: Pramod K. Sharma and Perry L. McCarty, Stanford University

Goals: The goals of this project are to (1) determine the biochemical mechanisms involved in reductive dehalogenation of tetrachloroethene (PCE) to cis-1,2-dichloroethene (cDCE) by bacterial strain MS-1, which was isolated from Victoria, Texas, aquifer material, and is the first identified facultative bacteria with this reductive ability, (2) to determine the phylogeny and to develop molecular probes for strain MS-1, and (3) to evaluate at laboratory scale the potential for bioaugmentation with strain MS-1.

Rationale: Chlorinated solvents, the most frequently found organic groundwater contaminants at hazardous waste sites, are difficult and expensive to remove by physical or chemical means. Biological methods such as in situ bioremediation or bioaugmentation are an attractive alternative, but microorganisms that can rapidly transform highly chlorinated solvents such as PCE have proven to be difficult to isolate and grow. The few identified organisms that convert PCE to cDCE are all strict anaerobes. Strain MS-1, however, is a facultative bacteria that can grow rapidly under either aerobic conditions or fermentatively on a variety of substrates. Thus, it is a particularly useful organism for studying the biochemistry of this important process, about which little is currently known, and for potential use in bioremediation, especially for the first phase of a two-stage anaerobic/aerobic process where PCE is first converted to cDCE anaerobically, and then cDCE is oxidized aerobically by cometabolism with bacteria that grow on substrates such as phenol or toluene. If used for bioaugmentation, then it will be useful to have molecular probes available to monitor the activity and survival of strain MS-1 in an in-situ or above ground treatment system.

Approach: There are various mechanisms now proposed by which PCE is reductively converted to cDCE, such as CO dehydrogenase and Vitamin B₁₂. Through evaluation of cell-free extracts and then the purified enzyme system, the factors affecting PCE dehalogenation by strain MS-1 will be studied. Also to be determined is whether or not MS-1 can obtain energy for growth from PCE dehalogenation. Biochemical testing, cellular fatty acid fingerprinting, and 16s rRNA analyses will be used to determine how MS-1 is related to other microorganisms. Approaches based upon REP-PCR will be used to develop molecular probes specific for MS-1. A laboratory anaerobic bioreactor will be operated with PCE addition and MS-1 will be

introduced into the reactor to determine its capability to dehalogenate PCE and to survive in a mixed culture system.

Status: This project was begun in March 1995. Strain MS-1 was found to be an enteric bacteria closely related to other enteric microorganisms. REP-PCR analysis indicated that none of the known closely related strains was identical to MS-1, but one of them, *Microbacterium agglomerans*, Group 5, from the American Type Culture Collection, was also found capable of dehalogenating PCE to cDCE. Two additional facultative strains with this ability, both pseudomonads, were also found this past year. MS-1 was introduced successfully into a 39 liter anaerobic fixed-film bioreactor following three months of methanogenic treatment of a mixture of yeast extract (500 mg/l), benzoate (500 mg/l), but without PCE (1 mg/l) conversion. PCE dehalogenation to cDCE began immediately after MS-1 introduction, indicating this organism can successfully carry out the observed dehalogenation in mixed cultures. Biochemical studies are nearing completion. MS-1 and the other isolated strains gain energy and carbon for growth through oxidation of acetate and reduction of PCE to cDCE.

AROMATIC COMPOUNDS

The Effect of Environmental Conditions on Reductive Dechlorination Rates: Sandra L. Woods, Oregon State University

Goal: The goal of this project was to develop an understanding of the effect of environmental conditions on the rates of reductive dechlorination reactions.

Rationale: Reductive dechlorination is a common anaerobic biodegradation mechanism for chlorinated aromatic compounds. This research tested the hypothesis that the rate of reductive dechlorination reactions depend upon the apparent redox potential, or E_H , of the reaction medium.

Approach: The project has two phases. In the first phase, a reactor system was developed to allow measurement of biodegradation rates under constant conditions of pH, E_H , sulfate, sulfide, and acetate concentrations. In the second phase, batch experiments were conducted to measure degradation rates under various, controlled environmental conditions. A model anaerobic system fed acetate, pentachlorophenol (PCP), and nutrients was selected for study.

Status: In 6- to 12-day experiments, a computer-monitored/feed-back-controlled bioreactor was used to hold temperature, pH, acetate concentration, redox potential and H_2 concentration constant at desired levels while transformation of multiple PCP additions was monitored. Transformation of PCP yielded 3,4,5-trichlorophenol (3,4,5-TCP) via 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP). Below an initial PCP concentration of 0.5 mM, PCP was transformed to 3,4,5-TCP within three to six hours. Biomass concentration changes were small, and PCP and 2,3,4,5-TeCP transformations were modeled as pseudo-first order reactions. Growth of a bacterial subpopulation that used PCP and 2,3,4,5-TeCP as terminal electron acceptors was suggested by increases in pseudo-first order rate coefficients that were directly related to the amount of PCP transformed to 3,4,5-TCP, and were independent of the acetate consumed, overall biomass concentration changes, and experimental duration. An average rate coefficient

doubling time, assumed to be equivalent to a subpopulation doubling time, of 1.7 days (1.4 to 2.3 days) was estimated.

The addition of H₂ sustained reductive dechlorination activity for a prolonged period without methanogenesis, and purging H₂ from the reactor decreased rates of reductive dechlorination. H₂ was identified as the primary electron donor for the reductive dechlorination reaction, and a theoretical 2:1, H₂:PCP stoichiometry was observed. However, increases in pseudo-first order rate coefficients were smaller during continuous addition of H₂ than when H₂ was endogenously supplied during acetoclastic methanogenesis. During periods of elevated apparent E_H to -0.1 V, methanogenesis stopped, the H₂ concentration decreased, and transformation of PCP and 2,3,4,5-TeCP continued at progressively slower rates. H₂ added while the apparent E_H was maintained at -0.1 V, caused reductive dechlorination rates to increase, suggesting that decreased transformation rates at elevated apparent E_H is caused by a deficiency of H₂.

In-Situ Anaerobic Biological Treatment of Aromatics in Groundwater: Martin Reinhard, Peter Kitanidis, Gary D. Hopkins, and Alfred Spormann, Stanford University

Goal: The goal of this project is to (1) demonstrate anaerobic BTEX transformation under nitrate- and sulfate-reducing conditions and methanogenic conditions at the field scale, and (2) quantify the factors which govern the rate of such transformations.

Rationale: Many regulatory agencies now consider intrinsic bioremediation as an option for the remediation of sites contaminated with hydrocarbon fuels. Since anaerobic processes contribute significantly to intrinsic biotransformation processes, understanding the factors that affect anaerobic biotransformation of BTEX compounds is important.

Approach: A slug of water augmented with BTEX compounds, bromide as a conservative tracer, and an appropriate electron acceptor is injected into a test zone instrumented with multi-level observation wells connected to an automated sampling and analysis system. After incubation from days to months, water samples are withdrawn from the test zone and analyzed for BTEX compounds and electron acceptors to evaluate biotransformation of the BTEX compounds. Laboratory studies using mixed and pure cultures are conducted to gain insight into the factors which control BTEX biotransformation and to compare laboratory with field data. Results of laboratory also serve to validate findings made in the field and to direct the field investigations. Two pure cultures are used to research the kinetics of single and multi-substrate utilization of monoaromatic hydrocarbons. By gaining a better understanding of the complex reaction mechanisms and kinetics of multi-substrate utilization one may be able to optimize bioremediation strategies, model the fate and transport of biologically degradable compounds in greater detail, and predict the time required to remediate contaminated sites more accurately.

Status: During 1996, a new well field has been installed and instrumented for simultaneously conducting three large-scale controlled release experiments. This expanded test site consists of three test zones each equipped with a multi-level injection well, two pairs of down-gradient multi-level observation wells and one up-gradient observation well. Hydraulic characterization of the well field and installation of the automatic sampling and analysis platform is in progress and expected to be completed by spring 1997.

The Effect of Apparent E_H , Compound Structure, and Electron Donor on Anaerobic Biotransformation of Trinitrotoluene and its Metabolites: A. Morrie Craig, Kenneth J. Williamson, Sandra Woods, Oregon State University (Supported by R2D2 Program)

Goal: The goal of this project is to develop an understanding of the effect of compound structure and environmental conditions on the rates of nitro group removal and deamination of nitrotoluene congeners. This process will be applied to develop remediation strategies for trinitrotoluene (TNT).

Rationale: The reduction of the nitro groups of trinitrotoluene and other nitro-aromatic congeners is a common microbiological process. In most instances, however, nitro group reduction yields triaminotoluene (TAT). Our group has been working with a pure culture (G.8) that reduces and deaminates a single nitro group at a time, significantly decreasing the degree of polymerization. An understanding of the factors that affect the pathway and rates will allow development of improved bioremediation strategies.

Approach: This research will examine the relative rates of reduction as they are dependent on compound structure, E_H , and electron donor. The project has two phases: (1) development of a reactor system to allow measurement of degradation kinetics under controlled environmental conditions and (2) evaluation of biotransformation pathways and kinetics for TNT and its metabolites under varying E_H conditions, electron donors, nitrate, and nitrite concentrations.

Status: A feedback controlled reactor was created to study the TNT/nitrate/lactate system. HPLC methods were developed to measure the concentrations of TNT and its metabolites. Preliminary serum bottle studies were performed. The relative rates of nitro-group reduction based on parent compound structure were measured and rates of reduction of the nitro group of TNT and 2,4-dinitrotoluene were nearly identical. The rate of reduction of nitro groups in the ortho position and also 4-nitrotoluene are negligible. Environmental conditions are being modified in an attempt to improve reduction rates.

Pathways of Anaerobic Toluene Metabolism by a Sulfate-Reducing Bacterium, Strain PRTOL1: Alfred M. Spormann, Stanford University

Goal: The objectives of this study are to determine the metabolic pathways involved in anaerobic toluene degradation in sulfate-reducing strain PRTOL1, and to identify metabolic intermediates that can be used as indicators for anaerobic bioremediation of BTEX compounds.

Rationale: Although some information is available about anaerobic mineralization of toluene in one denitrifying strain, it is not clear if all anaerobes that degrade toluene utilize the same biochemical pathway. Concomitant to toluene metabolism in all toluene metabolizing denitrifying cultures that have been investigated as well as in PRTOL1, is the formation of the metabolic by-products, benzylsuccinate and benzylfumarate. The by-products are not further metabolized by pure cultures. Recently, we found that these compounds can be demonstrated to be present in field sites where BTEX compounds were released. We focus on experiments investigating the connection between by-product formation and toluene metabolism.

Approach: Studies using PRTOL1 cells that metabolize toluene with sulfate as the electron acceptor involve cell suspensions and cell extracts. Both sets of experiments are designed to identify the initial biochemical steps involved in toluene oxidation and by-product formation. By manipulating the metabolic performance of PRTOL1 cells in suspensions, intermediates are expected to be transiently formed and consumed. Results of these cell suspension experiments will then be used to design assays to demonstrate specific enzyme activities in cell extracts. In cell extract studies, we expect to specifically investigate the biochemical reactions involved in the formation of benzylsuccinate and benzylfumarate.

Status: For experimental reasons, concentration first was on anaerobic toluene oxidation in denitrifying strain T. Studies in permeabilized cells of this organism demonstrated high specific activities of enzymatic addition of toluene to fumarate to form benzylsuccinate. Permeabilized cells also contained enzymatic activities to oxidize benzylsuccinate via *E*-phenylitaconate (a closely related isomer of benzylfumarate) to benzoate. These results represent strong experimental evidence that, rather than being dead-end products, benzylsuccinate and *E*-phenylitaconate are true intermediates in the anaerobic degradation pathway of toluene. Therefore, demonstrating the presence of these compounds in fuel hydrocarbon contaminated sites indicates directly ongoing oxidation of toluene under anaerobic conditions. A manuscript summarizing these findings was recently accepted for publication (Beller and Spormann 1997, J. Bacteriol. 179, 3)

Anaerobic Ethylbenzene Oxidation in Denitrifying Strain EB1: Alfred M. Spormann, Stanford University

Goal: The objectives of this study are to provide knowledge on the metabolic reactions and intermediates involved in anaerobic ethylbenzene oxidation, and to get a better understanding of the factors that control expression of the degradative enzyme activities.

Rationale: BTEX (benzene, toluene, ethylbenzene, and xylenes) are the most water soluble components of fuel. Leaking underground storage tanks and surface spills often contaminate soils and aquifers. Anaerobic degradation of BTEX has been observed to occur under natural conditions opening the potential to use the degradative activity of microorganisms to remediate contaminated sites. Only recently, pure cultures of toluene or xylene degrading bacteria have been described. At the time this project was started, EB1 which was isolated at Stanford's Environmental Engineering and Science program, was the only anaerobic organism capable of complete ethylbenzene oxidation to CO₂ under anoxic conditions. To predict when and to what extent intrinsic bioremediation of fuel components in general, and ethylbenzene in particular, occur and how these processes can be enhanced, a better understanding of the biochemical pathways and reactions involved is necessary.

Approach: Studies with strain EB1 metabolizing ethylbenzene under denitrifying conditions were conducted. Cells grown anaerobically with ethylbenzene and nitrate as substrates are harvested anaerobically, concentrated, and subjected to cell suspension experiments. Suspensions are amended with ethylbenzene and nitrate, and the concentrations of hydrocarbons and nitrate are followed by GC and IC respectively. Potential intermediates during anaerobic degradation of ethylbenzene are detected using HPLC and the chemical nature of the compounds is identified by GC-MS. Based on the finding of intermediates isotope

exchange studies will be employed to substantiate the proposed pathway. Results from the cell suspension studies will then be used to design enzyme assays with cell-free extracts.

Status: Experiments with anaerobic, ethylbenzene metabolizing cell suspensions of strain EB1 suggested that oxidation of ethylbenzene to 1-phenylethanol represents the first reaction in anaerobic degradation of this aromatic hydrocarbon. Results from further cell suspension studies demonstrated that 1-phenylethanol is oxidized to acetophenone. Acetophenone is then carboxylated to benzoyl acetate as supported by UV-Vis spectral data and a CO₂-dependence of further acetophenone degradation to benzoate. These results were recently published (Ball, Johnson, Reinhard, and Spormann, 1996, J. Bacteriol. 178, 19, 5755-5761). Recently, we demonstrated anaerobic ethylbenzene oxidation to 1-phenylethanol in cell-free extracts of strain EB1. This activity could be observed with benzoquinone, but not with naphthoquinone as electron acceptor.

Development of a Vitamin B₁₂-Amended Bioremediation Process for the Reductive Dechlorination of Chlorobiphenyls at all Chlorine Positions: Sandra Woods, Oregon State University (Supported by R2D2 Program)

Goal: The goal of this project is to develop a vitamin B₁₂-amended anaerobic process that will result in the complete reductive dechlorination of highly chlorinated biphenyls, especially those heavily substituted at the ortho position. Our objectives are (1) to determine the effect of parent compound structure on biotransformation pathways and kinetics, and (2) to demonstrate and optimize PCB reductive dechlorination in a laboratory-scale anaerobic process.

Rationale: Vitamin B_{12s} is a nucleophile with the ability to mediate reductive dechlorination of chlorobiphenyls at all positions. By coupling vitamin B₁₂ with a biological reductant, reductive dechlorination can be enhanced. A microorganism was selected for study due to its ability to reduce cobalt, and potentially, the cobalt center of vitamin B₁₂.

Approach: Reducing systems include a microbial consortium, chemically-reduced vitamin B₁₂, biologically-reduced vitamin B₁₂, and a consortium amended with vitamin B₁₂. The reduction of the catalyst and the transformations of chlorinated organic compounds are being evaluated. 2,3,4,5,6-Pentachlorobiphenyl (2,3,4,5,6-PCB) was selected as the model chlorobiphenyl for study because one ring is fully chlorinated. Other model compounds (for comparison) include the chlorinated phenols and chlorinated methanes.

Status: The project is being conducted in two concurrent parts: (1) evaluation of the microbial reduction of vitamin B₁₂ and (2) evaluation of the transformations mediated by vitamin B₁₂.

Biological reduction of vitamin B₁₂. When incubated in the presence of *Shewanella alga* strain BrY and either lactate or hydrogen, the microbial reduction of vitamin B_{12a} to B_{12r} was observed directly as a spectrophotometric shift in the vitamin B₁₂ spectrum. In treatments containing vitamin B₁₂ and an electron donor without BrY, over 93% remained present as vitamin B_{12a}. Upon the introduction of BrY, the proportion of vitamin B_{12a} present decreased to approximately 15% of the initial concentration. When BrY and vitamin B₁₂ were incubated together without an electron donor, 14% was reduced to vitamin B_{12r}.

Vitamin B₁₂-catalyzed transformation of chlorinated methanes. Unlike vitamin B_{12s}, vitamin B_{12r} does not result in rapid dechlorination of 2,3,4,5,6-PCB. For comparison, the transformation of readily dechlorinated methanes [carbon tetrachloride (CT), chloroform (CF), and dichloromethane (DCM)] was examined in batch systems containing vitamin B₁₂, the metal-reducing non-dechlorinating bacterium *Shewanella alga* strain BrY, and an electron donor. Transformation of both CT and CF was observed, while no significant change was observed for DCM over six weeks. Carbon monoxide was the major product of CT transformation. This work demonstrates that a metal-reducing bacterium, with no apparent ability to dechlorinate directly, transforms both CT and CF when supplied with vitamin B₁₂. The mechanism is the microbial reduction of vitamin B₁₂ which in turn catalyzes the transformation of CT.

Vitamin B₁₂-catalyzed transformation of chlorinated biphenyls. Previous work demonstrated rapid dechlorination of 2,3,4,5,6-PCB in a titanium citrate-reduced vitamin B_{12s} system. The chlorines are removed from all positions. Current work is focused on the factors affecting reductive dechlorination of PCBs by vitamin B_{12s} in a sediment/water system. Rapid vitamin B_{12s}-catalyzed dechlorination of 2,3,4,5,6-PCB was demonstrated with Willamette River sediment. The system contains 333 (M vitamin B₁₂ and excess titanium citrate. Removal appears to follow first order kinetics with a half life of about 1.5 days. The tetrachlorobiphenyls and trichlorobiphenyls have been observed in approximately the ratios expected from studies with the aqueous system. Future work will focus on measuring reductive dechlorination kinetics for PCBs.

HEAVY METALS

Incorporation of Nickel in a Ceramic Matrix - A Method for Treatment and Disposal of Heavy Metal Containing Wastes: George Redden and James O. Leckie, Stanford University

Goal: Ceramic material is proposed as a method for incorporating toxic heavy metals in a solid form that has structural integrity and is resistant to chemical degradation and leaching. This project is exploring the conditions under which nickel can be bound in a ceramic matrix using pure and industrial grade kaolinite. The intent is to determine optimal conditions for ceramic production from nickel contaminated clay that effectively resists leaching of the metal under acidic conditions, and to determine the mechanism by which the nickel is bound in the solid material.

Rationale: Although the cost of materials and energy is high when compared to other treatment methods, there are several distinct compensating advantages to this approach: 1) it is possible to treat metal containing wastes on site and produce a material that is not classified as a hazardous waste, thus reducing regulatory problems and off-site transportation/disposal costs, 2) the ceramic product is significantly lighter than the liquid containing wastes (further reducing transportation costs), 3) the ceramic product is marketable for a number of applications such as light weight filler for concrete, and 4) the incorporated heavy metals may be more resistant to leaching than in other matrices thus making release to the environment following disposal less

likely. The mechanism by which the ceramic matrix isolates heavy metals is not known. Two possibilities are chemical bonding in the alumino-silicate matrix, and encapsulation of metal oxides, carbonates, etc. Finding optimum conditions for producing heavy metal containing ceramics is dependent on this information.

Approach: Using a bench top furnace, kaolinite clay mixed with nickel containing solutions are being sintered under a range of relevant conditions to produce ceramic pellets. The pellets are characterized for mineral and chemical content, ground, and exposed to acidic leaching conditions (consistent with EPA standard tests) to determine the rate and extent of nickel extraction as functions of surface parameters. Solution and solid data are collected to determine the extent of solid dissolution and changes in surface chemical composition, and to distinguish between solute adsorption and leaching from solid phase(s). Scanning Electron Microscopy, X-ray Photoelectron Spectroscopy and X-ray Absorption Spectroscopy are used to verify or analyze changes in surface structure, composition, and coordination around a particular atom.

Status: Most of the data collected verified that sintering industrial kaolinite spiked with either nickel or copper resulted in a phase change which dramatically reduced the metal's extractability in acidic environments. Sintered metal-spiked samples retained 43-57% more metal than their unsintered counterparts. XRD analyses verified structural changes occur upon sintering. Structural differences found in non-spiked and nickel-spiked, sintered samples provide new evidence to support the usefulness of this technique for waste management. Microprobe analysis showed nickel in several phases, the most common being as an impurity in ilmenite or magnetite. The presence of nickel in magnetite (a spinel group mineral) strongly supports the hypothesis that the nickel becomes incorporated into a spinel-type structure during sintering. Nickel, was observed in several other phases as well, most often in an alumino-silicate matrix. The phases detected were all alumina deficient compared with the expected mullite (Si:3Al). The ratios were closer to Si:Al. This result was unexpected since mullite is known to form from kaolinite at temperatures above 900°C. The fused nature of the sintered sample results in an increase in material strength which renders the treatment end product effective as a lightweight building material.

Development, Characterization, and Performance Evaluation of Ferrous-Ferric Oxide Adsorbents for Metal Removal from Contaminated Groundwater: Peter O. Nelson, Oregon State University (Supported by R2D2 Program)

Goal: The goal of this research is the development and characterization of a mixed oxidation state (ferrous-ferric) iron oxide composite adsorbent for simultaneous removal of adsorbable and electroactive metals from contaminated groundwater. The intended application of the sorbent material is in a permeable barrier interceptor trench. As such, desirable properties of the sorbent material are high permeability, rapid reactivity with target metals, high capacity for metals removal, and regenerability.

Rationale: Remediation of metals-contaminated groundwater focused on ex-situ or in-situ treatment processes. Both methods are often limited by their expense and extended extraction time. An alternative to these approaches is in-situ treatment by interception of the contaminant plume by a passive barrier that removes or transforms contaminants in place. This study will

increase our understanding of mixed oxidation state (ferrous-ferric) iron oxide for removal of adsorbable and electroactive metals in in-situ treatment in an interceptor trench.

Approach: Several approaches will be followed in preparation of the ferrous-ferric iron oxide adsorbent. Ferrous-ferric iron oxide adsorbent will be prepared by coating magnetite on silica sand or by coating commercially available magnetite with $\text{Fe}(\text{OH})_3(\text{s})$ precipitate (ferrihydrite). The coated sand will be characterized to determine kinetics and equilibrium removal capacity of the sorbable and electroactive metals. Cr(VI) and Pb(II) are selected as the model metals to test the performance of the mixed oxidation state (ferrous-ferric) sorbent material. Batch reactor experiments will be used to determine sorption kinetics and equilibrium constants. Packed-bed column experiments will be used to more closely simulate field conditions of porous media flow.

Status: Magnetite-coated sand (MCS) was prepared by precipitation of iron(III)-hydroxide on 0.60-0.85 mm Ottawa sand (99.8% quartz (SiO_2)), rinsed with 5% ammonium acetate solution, oven-dried, and heated under nitrogen at 400°C which yields magnetite. The product was characterized by chemical analysis, X-ray diffraction (XRD), and BET surface area. Batch and column studies are in progress to assess the performance of MCS for removal of metals. pH adsorption edge data for various initial lead concentrations show that Pb removal increased with increasing pH from less than 20% at pH=3 to nearly 100% at pH = 6. Chromate removal by MCS at pH values 3 to 6 showed both adsorption and reduction, with adsorption equilibrium achieved within 24 hours while the reduction continued at a decreasing rate for up to several weeks, apparently limited by the rate of Fe(II) dissolution from MCS. Cr(VI) removal by MCS increased with decreasing pH. This can be explained by increasing MCS dissolution at lower pH values, consequently increasing Fe(II) release and Cr(VI) reduction, and also by increasing Cr(VI) adsorption at lower pH values.

Trace Element Adsorption in Porous Particle Packed Beds: James O. Leckie, Stanford University (Supported by R2D2 Program)

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: Six commercially available alumina adsorbents (CP-100, DD-6, DD-660, DD-431 and S-400 (all from ALCOA, PA) and Metal::X (from Solmetex, MA)) were selected on the basis of their surface area and macro- and mesopore content. The adsorbents were ground and sized into four size fractions. Scanning Electron Micrographs and X-ray diffraction patterns have been used to characterize the adsorbents. Equilibrium and time-dependent studies of cadmium sorption have been carried out with these adsorbents as a function of pH, pore-structure, adsorbent size and presence of ligands like chloride. The rate data is being analyzed with a batch reactor model. Additionally, the potential use of the model KINEQL is being investigated for modeling the effects of variable solution compositions on the rate of adsorption. Additional batch and column experiments are underway to investigate the effect of other ligands like thiosulfate and cyanide on cadmium sorption.

MINORITY ACADEMIC INSTITUTION PROJECT DESCRIPTIONS

Biotransformation of Lead and Chromate by Bacteria: Robert C. Blake, Xavier University

Goal: The goal is to study and purposely exploit the various mechanisms whereby toxic cations and anions are transformed by bacteria that live in the soil to bioremediate toxic metal wastes from selected, heavily contaminated groundwaters and soils.

Rationale: This project focuses on a strain (OR-02) of *Xanthomonas maltophilia* isolated from mercury-contaminated soil at Oak Ridge, TN. Growth of this organism promoted the transformation of a variety of toxic cations and anions, including Pb(II) and Cr(VI). The lead- and chromium-transformation activities of this organism will be studied and quantified with the aim of exploiting strain OR-02 for the bioremediation of contaminated waste waters or soils.

Approach: The specific aims are two-fold: (1) To perform detailed kinetic studies on the transformation and immobilization of soluble lead and chromium as catalyzed by strain OR-02 and related organisms. The purpose of these experiments is two-fold: (i) to identify the optimum solution conditions necessary to achieve maximal and timely removal of each soluble metal species; and (ii) to identify and document potential sources of interference for each metal transformation activity. The latter experiments focus on the possible inhibition of metal transformations by metal-complexing agents and/or other metal ions. (2) To investigate

various means for immobilization of strain OR-02 and to determine the efficacy of the immobilized bacterium for the removal of lead and chromium from contaminated waters. Immobilization strategies include, but are not limited to, entrapment within calcium-alginate beads and adsorption of the live bacteria onto a hydrophobic, porous polyurethane.

Status: When native cells of strain OR-02 were cultured in the presence of 1.0 mM Pb(II), growth of the organism was accompanied by the quantitative disappearance of the soluble Pb(II) from solution. The missing lead was all contained in small brown-black biocolloids from 50 to 150 nanometers in diameter (by contrast, the intact bacterial cells were rod-shaped with a diameter of 0.5 to 1.0 micrometers and a length of approximately 3 to 5 micrometers). The exact formulation of the culture medium under otherwise optimal conditions had no quantifiable influence on the disappearance of soluble lead. Thus the timely bacterial-dependent immobilization of soluble lead occurred equally well in both minimal (glucose plus mineral salts) and complex (tryptone plus yeast extract) media, even though components of the latter formed tight complexes with the lead cation. Growth of strain OR-02 in the presence of chromate was accompanied by the gradual (24 to 48 hr) electrochemical reduction of the yellow Cr(VI) (chromate) to the faint blue-green trivalent Cr (III). Strain OR-02 grew after a 2-hr lag period when inoculated into rich media containing up to 1.0 mM chromate. Optimal culture conditions for the bacterial-dependent reduction of Cr(VI) were identical to those for the corresponding immobilization of lead: rich media (Luria broth); pH 7.5; and 25° C. Although bacterial growth was severely inhibited at 5.0 mM chromate, biological reduction of the chromate was still observed. Indeed, effective reduction of up to 30 mM chromate was readily observed with cell suspensions of strain OR-02, although the bacterium did not grow under such conditions.

Magnetic Resonance Studies of Heavy Metals in Clays, Zeolites and Ceramics: Cynthia J. Hartzell and Michael P. Eastman, Department of Chemistry, Northern Arizona University

Goal: The overall research goal is to gain a scientific basis for dealing with the problems of sub-surface heavy metal contamination and of mixed radionuclide-organic co-contamination which results from PUREX and related actinide processing activities.

Rationale: The success of environmental remediation depends on the development of a scientific basis for understanding the mutual interactions of contaminants within the subsurface environment. The studies will provide a means of determining the *in situ* speciation of these ions in clays and ceramics. It is necessary to know the speciation before remediation procedures can be determined. Such information will be valuable in evaluating the ceramics resulting from the vitrification of clays.

Approach: This study targets the metal ions Cs, Cd, Pb and Ni as well as the actinides Am and U. The project will apply the techniques of solid state NMR and EPR to the elucidation of the interaction of metal cations with clays. The advantage of NMR is that discrete peaks are obtained for nuclei in different environments in a sample. Both NMR and EPR yield information on ion mobility. EPR will be used to study the behavior of paramagnetic ions in clays and ceramics. NMR studies will be used to determine the speciation of Cs, Cd and Pb in the clay environment and to probe the behavior of the actinides Am and U in clays and ceramics by studying the lanthanides Eu and Gd as analogs. EPR will be used to determine the

coordination of Ni in clays at varying stages of vitrification and to determine the extent of hydration of Gd in clays using EPR.

Status: Over the last year, ^{133}Cs NMR studies have been carried out on Cs^+ -exchanged hectorite heated to temperatures of 500, 800, and 1200°C . A nonheat-treated sample shows a major sharp peak at -33ppm with a broad shoulder at 20 ppm (the downfield peak), indicating two Cs environments in the clays. As the temperature of heat-treatment is increased, the spectra display changes in the relative intensities of these peaks as well as changes in the chemical shift. The downfield peak becomes progressively larger. Current studies focus on the nature of the two environments and on probing for mobility between these two environments. Studies of Ni^{+2} show evidence of EPR signal only at 70 K. NMR studies indicate a paramagnetic Ni^{+2} species in solution.

**Probing the Redox Properties of Environmental Systems: Natural Phenolic Materials:
Robin Helburn, Department of Chemistry, Northern Arizona University**

Goal: The long term goals of this research are to: (1) use cyclic voltammetry to study the redox properties of tannins, and other natural organic materials that contain quinone and phenol components; (2) study the quinone and phenol constituents in natural organic material using chronoamperometry; and (3) investigate the properties and potential environmental applications of a colored water soluble organic free radical. We propose that the latter "dye" may be used to probe the free radical scavenging behavior of natural polyphenols in environmental and biological systems.

Rationale: The success of environmental remediation depends on our ability to obtain a scientific understanding of the natural chemical processes occurring at a given site. It is known that redox processes play an important role in the overall chemistry of environmental systems. We are examining the redox properties of some natural organic components which may occur in these systems.

Approach: Standard voltammetry and chronoamperometric techniques are being used to study the redox properties of model tannins and phenols under controlled anaerobic conditions. The "probe-like" properties of the water soluble organic free radical are being evaluated using uv-visible absorbance measurements at 750 nm. The uv-visible measurements allow us to monitor the disappearance of the colored free radical in the presence and absence of natural polyphenols. The uv-visible studies are being followed up with electron spin resonance measurements. ^1H NMR is being used to probe the mechanism of interaction between the free radical scavenging polyphenol and the colored water soluble free radical.

Status: With respect to the voltammetry portion of our project, a combined electrochemical/computational approach is currently being developed for interpreting cyclic voltammograms of complex polyphenolic systems. We have used the molecule quercetin to test this method. Preliminary results suggest that semi-empirical MNDO calculations may be used as an aid in assigning individual cyclic voltammetric peaks to specific structural moieties on the quercetin molecule. A *new* offshoot of the electrochemical studies (i.e., not related to the initial research plan) involves the interfacing of a temperature/pH probe to a TI-85 calculator using an I²C (Inter-Integrated Circuit) protocol. The goal of this latter project is to create a small hand held device that will enable real time plotting of variables and monitored data in a field setting.

Reductive Dehalogenation at Carbon and Derivatized Carbon Electrodes: Merritt Helvenston, New Mexico Highlands University

Goal: The goal is to evaluate the utility of reduction of halocarbons such as chlorinated methanes, ethylenes and benzenes at carbon electrodes. Kinetics and reactant fate will be monitored.

Rationale: Few detailed studies of the environmental utility of electrolytic reduction of halocarbons have been published. The high activation barrier to electrolysis of halocarbons requires that very negative electrode potentials be used. Carbon appears desirable because its high overpotential to electrolysis of water permits the use of higher potentials at carbon than at other electrodes materials. Also catalysts expected to react slowly with water but rapidly with halocarbons will be studied.

Approach: Benchtop batch reactors using graphitic and glassy carbon cathodes are being used with aqueous solutions of carbon tetrachloride (CT), perchloroethylene and chlorinated benzenes under anaerobic conditions. Products are analyzed by GC-FID/ELCD for the organics and a chloride selective electrode for inorganic chloride.

Status: Carbon tetrachloride was reduced at both graphite and palladized graphite electrodes, with the latter performing better. Reduction of CCl_4 (200 ppm) at palladized electrodes shows 80% conversion in 6 hours affording a 23% chloroform and 50% methane with a mass balance of about 90%. Reduction of 75 ppm gave 87% conversion to 1.7% chloroform and 87% methane. Dechlorination accounted for 40% of the electrical consumption at the electrode. At graphite electrodes 99.7% of the CCl_4 was consumed in 24 hours. The major products were chloroform (60%) and methylene chloride (20%) while only 10% methane was formed. Chlorobenzene and 2-chloro-1-propanol also undergo reduction at the palladized electrode.

Detection of Microorganisms Capable of Anaerobic Degradation of Hazardous Substances in Natural Environments: Sara E. Silverstone, State University of New York, College at Brockport

Goal: The goals of this project are to develop a quantitative method for in situ monitoring of anaerobic toluene, PCE, and ethylbenzene- degrading microbes using fluorescence-labelled oligonucleotide probes and to apply this method to field and laboratory studies of contaminated soils.

Rationale: The Stanford laboratories participating in the WRHSRC have isolated a number of novel microorganisms capable of degrading a variety of hazardous organic substances. The use of specific microorganisms in bioremediation requires knowledge about the competitive behavior of the strains and ways to stimulate their growth. In order to identify and study these organisms in soil microcosms and assess the feasibility of using them in bioaugmentation experiments, it is necessary to have a rapid and efficient method of detecting and enumerating the bacteria. To the extent that such organisms can be utilized in bioremediation, this research will be an important contribution to the design of hazardous waste cleanup strategies.

Approach: The approach is to construct fluorescent and digoxigenin-labelled, strain-specific 16S rRNA-directed oligonucleotide probes. Probe target sites are selected based on database searches for unique sequences. Hybridization conditions for each probe are optimized and strain-specificity of the probes is assessed. Protocols are being developed for the use of these probes in whole-cell hybridization in soils. Probes will be used to monitor the effects of environmental and nutritional parameters upon the relative population densities, metabolic state and spatial distribution of target organisms in soil microcosms and at contaminated field sites.

Status: Two 16S rRNA probes have been developed for the anaerobic toluene-degrader PRTOL1. These have been tested in dot-blot and whole-cell hybridization assays against closely related sulfate reducing bacteria and have been found to be specific for PRTOL1. One probe has been selected for the ethylbenzene-degrader EB1. Determination of the specificity of this probe is in progress. We are currently waiting to receive the PCE-degrading strain MS-1 from our collaborators, at which time we will proceed to sequence the 16S rRNA in order to select a target sequence for a probe.

TRAINING & TECHNOLOGY TRANSFER PROJECT DESCRIPTIONS

DEMONSTRATION PROJECTS

Field Test of In-Situ Vapor Stripping for Removal of VOCS from Groundwater: Steven M. Gorelick, Stanford University (Supported by the U. S. Department of Energy)

Goal: This project is aimed at demonstrating an in-situ method for removing VOCs from groundwater. Through simple gas injection into a well, this method enables removal of VOCs from contaminated groundwater without bringing the water to the ground surface. Recirculation of groundwater is accomplished via air-lift pumping. Theoretical and simulation studies have been carried out previously. Current effort is focused upon pilot scale field demonstrations at two locations: Edwards Air Force Base (AFB) near Lancaster, California, and the Savannah River Site, a Department of Energy facility near Aiken, South Carolina. At both places the principal groundwater contaminant is trichloroethylene. The demonstrations will provide information regarding the efficiency of the in-situ VOC removal system at these and potentially other sites that are contaminated with VOCs.

Rationale: Many contaminated sites contain groundwater with high concentrations of dissolved VOCs. The in-well VOC removal system is an in-situ alternative to pump and treat. It creates an in-well air stripper which volatilizes the VOCs contained in the groundwater, and removes these contaminants as a vapor. The stripped VOC vapor is contained within the well, extracted under a vacuum, and treated at the ground surface. By conversion of a groundwater contamination problem into a simple vapor extraction system, the method has the advantage of not requiring removal, handling, treatment, storage, and disposal of contaminated groundwater. The expense of lifting the water to the surface for treatment is avoided. There is no need for a surface air-stripping tower when using this method. If the water is co-contaminated with tritium, the tritium can be left in the groundwater to decay while the VOCs are stripped and removed.

Approach: The in-situ VOC removal system is being demonstrated at Edwards Air Force Base and the Savannah River Site. The demonstration unit at each site consists of a single VOC-removal well and several monitoring wells. The overall approach to the demonstrations is to conduct 3 month initial tests of the system. Based on the results of those tests, additional sensitivity studies will be performed to assess design parameters such as gas injection rate, gas type, gas temperature, gas-line submergence depth, and controls on gas bubble size.

Status: The in-well vapor stripping demonstration commenced at Edwards AFB in mid-January and ran continuously for over 7 months. Stripping ratios of approximately 90 percent were maintained throughout the demonstration. Monitoring wells were sampled at approximately 1 week intervals during the demonstration, and then sampled periodically in the 2 months following the demonstration. These data are now being incorporated into an enhanced hydrogeologic model for the site. A report assessing the performance of the in-well vapor stripping system was prepared in collaboration with researchers at Pacific Northwest National Laboratory. A 2-week pilot demonstration was conducted at the Savannah River site in early September. The results from this test are inconclusive, and testing of the system for a longer period of time is being planned for 1997.

Full-Scale Evaluation of an Apparatus for Down-well Oxygen Transfer to Implement *In situ* Bioremediation at Edwards AFB: Perry L. McCarty and Mark N. Goltz, Stanford University (Supported by R2D2 Program)

Goal: Evaluate the efficiency and effectiveness of various down-well oxygen transfer devices in supplying oxygen to indigenous microorganisms as a component of *in situ* bioremediation of trichloroethylene at the Edwards AFB demonstration site.

Rationale: For aerobic bacterial activity, the addition of oxygen is necessary to serve as an electron acceptor. Oxygen addition is an expensive item in aerobic *in situ* bioremediation. Attempts to reduce the costs for oxygen addition are thus warranted. In addition, little attention has been paid to the difficulties of adding oxygen or other gases to groundwater contaminated with volatile compounds, which can inadvertently be transferred to the atmosphere by air sparging directly into an aquifer or within a well casing. One possible method to avoid contaminant volatilization is to use hydrogen peroxide as an oxygen source. Unfortunately, hydrogen peroxide is an especially expensive source of oxygen. An alternative method to avoid volatilization is to add pure oxygen gas directly into a well where groundwater is being recirculated. This research will evaluate in the laboratory and the field the potential of an apparatus for transferring oxygen into contaminated groundwater that is caused to circulate through a well in order to prevent contaminant volatilization or dilution. This work is also applicable to other gases (methane, for instance) which also may need to be added to groundwater to support *in situ* bioremediation.

Approach: The research is to be carried out in two phases. In the first phase of testing, various down-well oxygen transfer devices (the ADGT or apparatus for down-well gas transfer, the Membran bubbleless gas transfer module, a gas infusion device, and static mixers), will be tested in the laboratory under conditions that attempt to mimic the subsurface environment at Edwards. In Phase II of the research one of the devices will be installed in a treatment well at Edwards and its full-scale performance evaluated.

Status: The four oxygen transfer devices were evaluated in the laboratory under a range of conditions. The static mixer, which had been installed at Edwards based on practical considerations (mainly ease of installation and operation) was demonstrated to operate effectively in the field (see Full-Scale Evaluation of *In Situ* Bioremediation of Chlorinated Solvent Groundwater Contamination). Currently, the data from the laboratory work are being analyzed, to determine if the laboratory tests were predictive of the field performance of the static mixers and if the other oxygen transfer devices may be practical and effective for employment in the field.

Field Testing of Palladium-Catalyzed Hydrodehalogenation for Chlorinated Hydrocarbon Removal from Groundwater: Martin Reinhard and Paul V. Roberts, Stanford University (Supported by R2D2 Program)

Goal: This project aims to (1) establish the viability of hydrogen/palladium treatment for halogenated hydrocarbon removal by determining the catalyst lifetime and reaction kinetics in a continuous-flow packed bed reactor; (2) identify competitors/inhibitors in the process and

minimize their effects; (3) quantify hydrogen consumption and determine effective supply methods; (4) scale up, optimize and implement the process at field-scale.

Rationale: Palladium has been demonstrated to catalyze the reduction of halogenated hydrocarbons to alkanes by hydrogen. Batch studies with palladium indicate a rate of reaction several orders of magnitude higher than in the zero-valent iron system (another method currently under study). Chlorinated ethenes, including PCE and vinyl chloride, were completely removed from tap water within ten minutes at room temperature by 0.5% palladium on alumina at 0.1 atm of hydrogen pressure. (Schreier and Reinhard, *Chemosphere*, 31(6) pp 3475-3487, 1995.) This quick reaction time demonstrates palladium's great potential for application in pump-and-treat groundwater remediation. Although palladium has been used as a reduction catalyst for years in organic chemistry applications, the system has not yet been well characterized for groundwater treatment.

Approach: This project entails construction and testing of bench-scale continuous-flow reactors. For testing, we have been using artificially contaminated deionized water, groundwater from Livermore, California, and groundwater from the California Central Valley. Two bench-scale systems have been constructed, one which is operated in the flow-through mode and one which is operated in a rotating basket-mode. Base-line tests with deionized water have indicated 99% TCE removal at inlet concentrations ranging from 1.5 to 25 mg/L with no noticeable decrease in catalyst activity over 5 months. When actual ground waters is used, the catalyst is deactivated at rates that depend on water quality. The approach to identify deactivating water quality parameters has been to correlate the water quality with catalyst deactivation time and then to study the effect of the hypothesized parameters in separate experiments.

Status: Current efforts are directed towards getting a better understanding of the catalyst performance under treatment conditions, specifically (1) quantifying the competing effects of oxygen and TCE reduction, (2) improving the methodology to characterize catalyst deactivation using spectroscopic methods, and (3) characterizing the effects of the water matrix on the process with special consideration of the bicarbonate and carbonate ions. A field-scale test is ongoing at the Lawrence Livermore National Laboratory to treat TCE and tritium contaminated groundwater in a treatment well using a submersed Pd reactor.

Full-Scale Evaluation of *In Situ* Bioremediation of Chlorinated Solvent Groundwater Contamination: Perry L. McCarty, Mark N. Goltz, and Gary D. Hopkins, Stanford University (Supported by the U. S. Air Force)

Goal: To evaluate at full scale the efficiency and effectiveness of *in situ* aerobic cometabolic bioremediation of chlorinated aliphatic hydrocarbon compounds at an actual hazardous waste site.

Rationale: Full-scale implementation of an innovative technology, working with organizations in the user community, is an ideal way to communicate knowledge gained by research and put it into practice. A full-scale application of *in situ* aerobic biodegradation of chlorinated solvents is justified based upon research at Stanford University and elsewhere that has shown that the process can operate efficiently with indigenous microorganisms. This project is a cooperative

activity between the U. S. Air Force, the U. S. Environmental Protection Agency, and other parties with interest in this activity.

Approach: Studies conducted by Stanford University researchers at the Moffett Field pilot site have demonstrated that trichloroethylene (TCE) can be effectively biodegraded cometabolically through the introduction into the subsurface of a primary substrate (such as toluene or phenol) and oxygen to support the growth and energy requirements of a native population of microorganisms. To demonstrate the technology at full-scale, a remediation system was installed at a TCE contaminated site at Edwards AFB. The remediation system consists of two wells, each screened at two depths. Each well connects two aquifers separated by an aquitard, the upper unconfined aquifer is 8 m deep and the lower confined aquifer is 6 m deep. In operation, a submersible pump, installed between the two screens, draws water at rates of 25 to 37 liters/min into the well at one screened interval in one aquifer and discharges the water out of the second screened interval in the second aquifer. The well has feed lines which introduce toluene, oxygen gas, and hydrogen peroxide (as a source of oxygen) into the discharge water. A biotransformation zone develops near the discharge side of each well, and the water circulates between the wells to clean the aquifer. An extensive automated sampling and analysis system monitors system operation.

Status: The four month evaluation has been completed and *in situ* cometabolic degradation of 100 to 1000 µg/l TCE through toluene, oxygen, and hydrogen peroxide injection was demonstrated at the Edwards site. TCE removals on the order of 75%-85% were achieved with each pass of groundwater through the biotransformation zones around the treatment wells. An eight month follow-on project is currently underway to implement operational changes to improve performance and to extend the time of steady-state operation.

Demonstration of an Interceptor Trench Technology for the Bioremediation of a Pentachlorophenol-Contaminated Ground Water: Sandra Woods, Oregon State University (Supported by R2D2 Program)

Goal: The goal of this project is to demonstrate the applicability of a permeable barrier technology for the bioremediation of groundwaters contaminated with pentachlorophenol (PCP).

Rationale: Pentachlorophenol (PCP) is a fully chlorinated aromatic compound that is biotransformed by reductive dechlorination, a common anaerobic biodegradation mechanism. The rate of PCP reductive dechlorination is rapid and results in the production of lesser chlorinated phenols. As the degree of chlorination decreases, reductive dechlorination rates also decrease. Under aerobic conditions, however, transformation of the lower chlorinated phenolic compounds is rapid and may result in complete mineralization. This project will demonstrate a passive, in-situ sequential anaerobic/aerobic biological treatment technique for PCP-contaminated ground water.

Approach: To allow for the physical, hydrogeological, biological, and regulatory conditions present, a diverse project team was assembled. Teams focused on the following: (1) design considerations for the physical development of the permeable barrier technology, (2) interactions of subsurface flow and mixing, (3) biological process design including evaluation

of electron donors and carbon sources, (4) reactor construction, (5) sampling design, and (6) regulatory and legal constraints.

Status: A 23" cylindrical down-borehole reactor (basket) has been constructed. The basket contains three mixing wells for introduction of electron donors, acceptors, and nutrients. Current work focuses on final design and testing of nutrient delivery and mixing systems, sample collection systems, and a system to raise and lower the basket and monitor and adjust reactor orientation within the borehole. Changing the basket's orientation should result in changes in the groundwater retention time within the reactor.

A 24" diameter borehole was constructed during March, 1996. The depth of the well is 30 feet with the screened interval between 18 and 21 feet. The well cuttings have been transferred to a steel container at OSU for evaluation of a sequential anaerobic/aerobic strategy for their bioremediation. At the site, PCP groundwater concentrations range from 0 to 3 mg/L; measured dissolved oxygen concentrations are less than 1.5 mg/L; the groundwater temperature is 15°C; and no other contaminants are present at significant concentrations.

Process design has focused on identifying an electron donor to support reductive dechlorination and primary carbon source for the subsequent oxidation of PCP's metabolites. Imitation vanilla flavoring has been selected for several reasons: (1) it is generally recognized as safe by the Food and Drug Administration; (2) it is acceptable to the site owner and regulatory agencies; (3) it is composed of several aromatic compounds (guaiacol, ethyl vanillin, and sodium benzoate) and supports the reductive dechlorination of pentachlorophenol; and (4) it supports the oxidation of dichlorophenols under aerobic conditions. In batch bioassays in the laboratory, imitation vanilla flavoring supports PCP reductive dechlorination at the *ortho* and *para* positions yielding 2,3,4,5-TeCP and 2,3,5,6-TeCP. Further dechlorination yields 3,5-DCP. 3-Chlorophenol was the terminal metabolic product measured in the batch bottle study. Aerobic batch bioassays conducted with imitation vanilla flavoring indicate rapid removal of 3,4-DCP and 3,5-DCP. Thus, this electron donor and carbon source will be employed in the field. Mineralization of pentachlorophenol under sequential anaerobic/aerobic conditions is being verified in the laboratory using ¹⁴C-labelled pentachlorophenol.

The basket will be lowered into the borehole in January, 1997. Initial tracer studies will be conducted to characterize groundwater flow to the basket and measure retention times within the anaerobic and aerobic zones. Subsequent experiments will evaluate aerobic and anaerobic transformations separately and in sequence.

MINORITY ACADEMIC INSTITUTION TRAINING AND TECHNOLOGY PROJECTS

Faculty Enhancement on Hazardous Contaminant Research with Specific Emphasis On In-Situ Anaerobic Biodegradation of BTEX Hydrocarbon Contaminant: Aregai Teclé, Northern Arizona University in collaboration with Peter K. Kitanidis and Martin Reinhard, Stanford University

Goals: The goal of the project is to enhance the principal investigators research capability in hazardous contaminant remediation by working on a particular research project with researchers

at the Western Region Hazardous Substances Research Center (WRHSRC) at Stanford University. The specific research goal of the project is to determine the movement and anaerobic biodegradability of BTEX contaminants in the field.

Rationale: Conventional methods of restoring gasoline contaminated aquifers usually involve either pump-and-treat, or in-situ biostimulation of aerobic bacteria by injecting adequate oxygen and nutrients into a contaminated zone. But, because such methods are expensive, and cumbersome in the first case and limiting in the latter (because of oxygen's low solubility in water), an anaerobic bioremediation approach is being studied as an alternative. The applicability and reliability of the anaerobic method to a real problem in the field is being evaluated using a suitable contaminant movement and degradation model.

Approach: Many relevant data types collected from the contaminated site during the period 1987-1993 are available for evaluation. The data includes concentrations of BTEX compounds, carbon dioxide and many inorganic substances, physical aquifer characteristics, redox potentials, and microbial biomass accumulation. The behavior of each one of these elements in the field with time and space are analyzed separately. Then, a two-dimensional contaminant transport model is being used to solve the problem. Once the computational analysis is completed, the possible influence of the above aquifer characteristics on the degradability of the HC contaminants will be described.

Status: Microbial enrichments derived from Seal Beach sediments have been shown to anaerobically degrade BTEX compounds elsewhere. This project is designed as a complement to such studies to determine the anaerobic biodegradability of the BTEX contaminants under natural field conditions. To date, the necessary parameters and boundary conditions for the transport and biodegradation of each BTEX compound are determined and used in BIOPLUME II to assess the biodegradability of the BTEX compounds. The preliminary results were presented as a poster paper at the 1996 Western Hazardous Substance Research Center's Annual Conference at Stanford University on July 24, 1996.

Bioremediation Technology Transfer Conferences at Albuquerque Technical-Vocational Institute: R. Barry King, Albuquerque Technical-Vocational Institute

Goal: The goal is to present current HSRC research findings and field-ready applications of bioremediation technologies through presentations by qualified individuals. The format is a series of formal conferences held at Albuquerque Technical Vocational Institute that are nationally advertised. The intended audience is the public and interested minority populations.

Rationale: Recent advances in accepted EPA site remediation technologies have directed attention to the ease and cost-effectiveness of bioremediation. However, public perception of this emerging technique is often distorted or lacking entirely. These Conferences are directed toward populations that have the least opportunity for exposure to the scientists and practitioners who work in this field.

Approach: Conferences are planned that will address mine waste, surface soils, vadose zone soils, aquifers, pit-pond-lagoon closure, chlorinated solvent degradation, mixed waste, and modeling performance prediction.

Status: The initial TT Conference was held on September 29, 1995 at TVI. The topics were MINE WASTE AND SOILS. Presenters came from Colorado School of Mines, Oregon State University, International Technology Corp. and TVI. Other conferences were as follows:

Subsurface Soils & In Situ Aquifers - Feb. 16, 1996
Pits, Lagoons & Chlorinated Solvents - July 12, 1996
Mixed Waste & Modeling Performance Prediction - Sept. 27, 1996

TRAINING AND TECHNOLOGY TRANSFER

Hazardous Waste Training: Kenneth J. Williamson and Peter O. Nelson, 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 14 workshops within Oregon, Washington, Idaho, and Alaska. Funding for the Center was extended for 1996-97.

Continuing Education: Kenneth J. Williamson, Oregon State University

Goal: To maintain a program in hazardous substance management for professionals in the State of Oregon.

Rationale: Continuing education is needed to support professionals seeking training and education in the State of Oregon.

Approach: A certificate of completion program is offered in the Portland area which involves a curriculum of six courses taught on a two-year cycle.

Status: Two courses were offered during 1995-96. The program is presently being modified to include more community college participation.

Conference Sponsorship: 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 1996 Idaho Regional Conference on Hazardous Materials, April 1996.

The 1996 Responsible Hazardous Materials Management Conference (REMCON), Portland, Oregon, September, 1996.

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.

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 who do not qualify for a technical Assistant Grant (TAG) from the US Environmental Protection Agency.

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.

Status: The Western Region's Technical Outreach Services for Communities program continues to solicit its services to communities throughout EPA regions 9 and 10. Currently, the team is involved with the following community groups: (1) the Donit Waste Arizona Group in South Phoenix, Arizona, which is concerned with impacts upon the community from a fire at a printed board site, (2) a Citizen group in Ketchikan, Alaska, that is concerned with hazardous material releases to the bay from a pulp mill, (3) the Citizen's Advisory Committee in Sitka, Alaska, which sought advice concerning hazardous material releases from a pulp mill, (4) the Community Advisory Board in Galena, Alaska, which has concerns over hazardous material releases from an Air Force solid waste facility, (5) the Citizen's Advisory Committee in Oakland, California, which wanted help in providing a soil cleanup matrix for use with Oakland Brownsfield sites, (6) the Citizen's Advisory Committee in Casmalia, California, which sought advice about the Casmalia Resources Hazardous Waste facility, and (7) the North Island Naval Station Restoration Advisory Board, which wanted technical assistance on restoration work at the Naval facilities.

Research and Re-education for Department of Defense (R2D2) Program

Goal: The R2D2 Program seeks to provide education for careers in environmental engineering and science to individuals displaced from the military due to downsizing of the nation's military activities.

Rationale: Training of displaced personnel assisting in the transition from military service to civilian life.

Approach: At Oregon State University, the Research and Re-education for Department of Defense (R2D2) program offers students an opportunity to complete undergraduate and graduate studies in civil and environmental engineering, and chemical engineering. At Stanford University, the R2D2 program is offering advanced M.S., Engineer, or Ph.D. degrees in various areas of environmental engineering and science.

Status: The R2D2 program funded four graduate students at Oregon State University.

Michael Cantaloub- M.S., Environmental Engineering
Research Project: Use of Radon-222 to Quantify NAPL Contamination in the Subsurface

Greg Connor- M.S., Environmental Engineering
Research Project: Push-Pull Test for IN-Situ Determination of Microbial Metabolic Activities in the Subsurface

Mark Havighorst- M.S., Environmental Engineering
Project: Use of a Permeable Barrier for In-Situ Remediation of Pentachlorophenol

Paul Kwon- M.S., Environmental Engineering
Project: In-situ Remediation of MTBE use Graphium sp.

At Stanford University three graduate R2D2 students were engaged in research projects and four others will participate in internships with the government or private companies prior to receiving their graduate degrees.

Brett Kawakami - Engineer Degree, Environmental Engineering and Science
Research Project: Full-Scale Evaluation of *In Situ* Bioremediation of Chlorinated Solvent Groundwater Contamination

Scott Newberg - M.S., Environmental Engineering and Science
Research Project: Laboratory-scale Bioaugmentation with Strain MS-1 to Achieve Reductive Dehalogenation of PCE and TCE to cDCE

Russ Philbrick - M. S., Environmental Fluid Mechanics and Hydrology
Research Project: Optimal Control of Stochastic, Multi-purpose, Multi-reservoir Systems

John Frech - M.S., Environmental Engineering and Science

John Hicks - M.S., Engineering and Economic Systems

Alan Leung - M.S., Environmental Engineering and Science

Catheryne Nicholson - M.S., Environmental Engineering and Science

1996 WRHSRC PUBLICATIONS

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

A. Refereed Journal Articles

- Anderson, J.E. and P.L. McCarty, "Effect of Three Chlorinated Ethenes on Growth Rates for a Methanotrophic Mixed Culture," *Environmental Science and Technology*, **30**(12), 3517-3524 (1996).
- Ball, H.A., H.A. Johnson, M. Reinhard, and A. M. Spormann, "Initial Reactions in Anaerobic Ethylbenzene Oxidation by a Denitrifying Bacterium, Strain EB1," *Journal of Bacteriology*, **178**(19), 5755-5761 (1996).
- Ball, H.A. and M. Reinhard, "Monoaromatic Hydrocarbon Transformation Under Anaerobic Conditions at Seal Beach, California: Laboratory Studies," *Environmental Toxicology and Chemistry*, **15**(2), 114-122, (1996).
- Beller, H.R., A.M. Spormann, P. Sharma, J.R. Cole, and M. Reinhard, "Isolation and Characterization of a Novel Toluene-Degrading, Sulfate-Reducing Bacterium," *Applied and Environmental Microbiology*, **62**(4), 1188-1196, (1996).
- Chiu, P.C. and M. Reinhard, "Transformation of Carbon Tetrachloride by Reduced Vitamin B12 in Aqueous Cysteine Solution," *Environmental Science and Technology*, **30**(6), 1882-1889 (1996).
- Cole, J.D., S.L. Woods, and T.R. Bricker, "Pentachlorophenol Reductive Dehalogenation in an Interceptor Trench: Temperature Effects," *Ground Water*, **34**(5), 784-790 (1996).
- Curry, S., L. Ciuffetti, and M. Hyman, "Inhibition of growth of a Graphium sp. on gaseous *n*-alkanes by gaseous *n*-alkynes and *n*-alkenes," *Applied and Environmental Microbiology*, **62**, 2198-2200 (1996).
- Dykaar, B.B. and P.K. Kitanidis, "Macrotransport of a biologically reacting solute through porous media," *Water Resources Research*, **32**(2), 307-320 (1996).
- Francois, O., T. Gilmore, M.J. Pinto, and S.M. Gorelick, "A Physically Based Model for Air-Lift Pumping," *Water Resources Research*, **32**(8), 2383-2399 (1996).
- Fry, V.A., J.D. Istok, and K.T. O'Reilly, "Effect of Trapped Gas on Dissolved Oxygen Transport - Implications for In Situ Bioremediation," *Ground Water*, **34**(2), 200-210 (1996).
- Kitanidis, P.K., "On the geostatistical approach to the inverse problem," *Advances in Water Resources*, **19**(6), 333-342 (1996).

- Kitanidis, P.K., "Analytical expressions of conditional means, covariance, and sample functions in Geostatistics," *Journal of Stochastic Hydrology and Hydraulics*, **10**(4), 279-294 (1996).
- Kitanidis, P.K. and K.-F. Shen, Geostatistical interpolation of chemical concentration, *Advances in Water Resources*, **19**(6), 369-378 (1996).
- Lemmon, T.C., J.C. Westall, and J.D. Ingle, Jr., "Development of Redox Sensors for Environmental Applications Based on Immobilized Redox Indicators," *Analytical Chemistry*, **68**(6), 947-953 (1996).
- Lin, J.S. and L. Hildemann, "Analytical Solutions of the Atmospheric Diffusion Equation with Multiple Sources and Height-Dependent Wind Speed and Eddy Diffusivities," *Atmospheric Environment*, **30**(2), 239-254 (1996).
- Sharma, P. and P. L. McCarty, "Isolation and Characterization of a Facultative Bacterium that Reductively Dehalogenates Tetrachloroethene to cis-1,2-dichloroethene," *Applied and Environmental Microbiology*, **62**(3), 761-765 (1996).
- Siantar, D.P., C.G. Schreier, C.S. Chou, and M. Reinhard, "Treatment of 1,2-Dibromo-3-Chloropropane and Nitrate-Contaminated Water with Zero-Valent Iron or Hydrogen/Palladium Catalysts," *Water Research*, **30**, 2315-2322 (1996).
- Staudinger, J., and P.V. Roberts, "A Critical Review of Henry's Law Constants for Environmental Applications," *Critical Reviews, Environmental Science and Technology*, **26**(3), 205-297 (1996).
- Sule, P.A., and J.D. Ingle, Jr., "Determination of the Speciation of Chromium with an Automated Two-column Ion-exchange System," *Analytica Chimica Acta*, **326**, 85-93 (1996).
- Van Lent, T., and P. K. Kitanidis, "Effects of first-order approximations on head and specific discharge covariances in high contrast log-conductivity," *Water Resources Research*, **32**(5), 1197-1207 (1996).

B. Articles Submitted or in Press

- Anderson, J. E. and P. L. McCarty, "Transformation Yields of Chlorinated Ethenes by a Methanotrophic Mixed Culture Expressing Particulate Methane Monooxygenase," *Applied and Environmental Microbiology*, in press.
- Anderson, J. E. and P. L. McCarty, "Effect of Chlorinated Ethenes on S_{min} for a Methanotrophic Mixed Culture," *Environmental Science and Technology*, in press.
- Beller, H.R. and A.M. Spormann, "Anaerobic Activation of Toluene and *o*-Xylene by Addition to Fumarate in Denitrifying Strain T," *Journal of Bacteriology*, in press.
- Dawson, H.E. and P.V. Roberts, "Influence of Viscous, Gravitational, and Capillary Forces on DNAPL Saturation," *Ground Water*, in press.

- Donaldson, J.H., J.D. Istok, and K.T. O'Reilly, "Dissolved Gas Transport in the Presence of a Trapped Gas Phase: Experimental Evaluation of a Two-Dimensional Kinetic Model," submitted 1996.
- 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*, in press.
- Ely, R. L., K. J. Williamson, M. R. Hyman, and D. J. Arp, "Cometabolism of Chlorinated Solvents by Nitrifying Bacteria: Kinetics, Substrate Interactions, Toxicity Effects, and Bacterial Response," *Biotechnology and Bioengineering*, in press.
- Fry, V.A., J.S. Selker, and S.M. Gorelick, "Experimental Investigations for Trapping Oxygen Gas in Saturated Porous Media for In Situ Bioremediation," submitted 1996.
- Haggerty, R., and S.M. Gorelick, "Identifying Mass Transfer Processes Using Measurements of Mass Fraction Remaining from Column Experiments," *Journal of Contaminant Hydrology*, submitted 1996.
- Haggerty, R., and S. M. Gorelick, "Modeling Mass Transfer Processes in Soil Columns with Pore-Scale Heterogeneity," submitted 1996.
- Hamamura, N., C. Page, T. Long, L. Semprini, and D.J. Arp, "Chloroform Cometabolism by Butane-Grown CF8, *Pseudomonas butanovora*, *Mycobacterium vaccae* JOB 5, and Methane-Grown *Methylosinus trichosporium* OB3b," submitted 1996.
- Hedberg, S.A., S.M. Gorelick, and M.J. Pinto, "The Effects of Hydraulic Conductivity Heterogeneity on the Remediation Performance of a Vertical Recirculating In-Well VOC Vapor Stripping System," submitted 1996.
- Keller, A.A., M.J. Blunt, and P.V. Roberts, "Micromodel Observation of the Role of Oil Layers in Three Phase Flow," *Transport in Porous Media*, in press.
- Kim, Y., L. Semprini, and D.J. Arp, "Aerobic Cometabolism of Chloroform and 1,1,1-Trichloroethane by Butane Grown Microorganisms," submitted 1996.
- Kitanidis, P.K. and B.B. Dykaar, "Stokes Flow in a Slowly Varying Two-Dimensional Periodic Pore," *Transport in Porous Media*, submitted 1996.
- Lang, M.M., P.V. Roberts, and L. Semprini, "Model Simulations in Support of Field Scale Design and Operation of Bioremediation Based on Cometabolic Degradation," *Ground Water*, in press.
- Lin, J.-S. and L.M. Hildemann, "Modeling Vertical Spread of Airborne Pollutants from Sources Near Groundlevel--A Comparison Study," submitted 1996.

- Pinto, M.J., H. Gvirtzman, and S.M. Gorelick, "Laboratory Scale Analysis of Aquifer Remediation by In-Well Vapor Stripping: 2. Modelling Results," *Journal of Contaminant Hydrology*, in press.
- Reinhard, M., S.B. Shang, P.K. Kitanidis, E. Orwin, and G.D. Hopkins, (Stanford University), C. A. LeBron (NFESC), "In Situ BTEX Biotransformation under Enhanced Nitrate- and Sulfate-Reducing Conditions," *Environmental Science & Technology*, in press.
- Scherer, M.M., J.C. Westall, M. Ziomek-Moroz, and P.G. Tratnyek, "Kinetics of Carbon Tetrachloride Reduction at an Oxide-Free Iron Electrode," submitted 1996.
- Smith, L.H. and P.L. McCarty, "Laboratory Evaluation of a Two-Stage Treatment System for Cometabolism by a Methane-Oxidizing Mixed Culture," *Biotechnology and Bioengineering*, in press.
- 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 Square Analysis of the Integrated Monod Equation," *Biotechnology and Bioengineering*, in press.
- Snodgrass, M.F. and P.K. Kitanidis, "A geostatistical approach to contaminant source identification," *Water Resources Research*, in press.
- Workman, D., S. L. Wood, Y. A. Gorby, J. K. Frederickson, and M. J. Truex, "Microbial Reduction of Vitamin B₁₂ by *Shewanella alga* strain BrY with Subsequent Transformation of Carbon Tetrachloride," submitted 1996.

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 *Sorption of Metals by Earth Materials*, Ed. E. A. Jenne, American Chemical Society, in press.
- McCarty, P. L., "Transferability of Biotreatment from Site to Site," *Wider Application and Diffusion of Bioremediation Technologies, The Amsterdam '95 Workshop*, OECD, Paris, 201-210 (1996).
- McCarty, P.L., "Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbons," *Subsurface Restoration Handbook*, Ed. C. H. Ward J. D. Cherry, M. R. Scalf, Ann Arbor Press, Inc., Chelsea, MI, in press.
- McCarty, P. L., M. N. Goltz, G. D. Hopkins, J. P. Allan, "In Situ Biodegradation of Chlorinated Solvent Contaminants in Groundwater, " Proceedings WEFTEC '96, Vol 3., Part I: *Remediation of Soil and Groundwater*, Water Environment Federation, Washington D.C., 217-223 (1996).

D. Project Reports

- Coston, J. A., R. H. Abrams, D. B. Kent, "Chemical analysis of selected inorganic solutes by ICPAES," U.S. Geological Survey Data Report (1996).
- Iyer, R., Aerobic Degradation of Trinitrotoluene (TNT) Metabolites using Phenol Degrading Bacteria, M.S. Project, Department of Civil Engineering, Oregon State University, 1996.
- Lin, D-C., Model of Chlorophenol Degradation on Granular-Activated-Carbon-Supported Biofilm in Sequential Anaerobic-Aerobic Reactor, M.S. Project, Department of Civil Engineering, Oregon State University, 1996.
- Reinhard, M., S. Shang, P.K. Kitanidis, E. Orwin, H.R. Beller, M. Snodgrass, V. Warikoo, and G.D. Hopkins, *In Situ BTEX Biotransformation Under Unamended and Enhanced Nitrate-Reducing, Sulfate-Reducing, and Methanogenic Conditions, Vol. I: Data Evaluation, Vol. II: Appendices*, Technical Report No. WRC-6, WRHSRC, Department of Civil Engineering, Stanford University, Stanford, CA, 1996.

E. Extended Abstracts

- Abrams, R. H., D. B. Kent, and K. Loague, "Development and fate of redox zones in aquifers: Determination of the reaction network, *EOS Transactions*, 77(17) (1996).
- Abrams, R. H., D. B. Kent, and K. Loague, "Redox zones in contaminated aquifers: Implications of the reaction network on solute transport modeling," 213th American Chemical Society National Meeting, Division of Environmental Chemistry Preprints of Extended Abstracts, 37(1) in press.
- Cohen, A.J.B., "Hydrogeologic Characterization of Fractured Rock Formations: A Guide for Groundwater Remediators," EPA/600/S-96/001, National Risk Management Research Laboratory, United States Environmental Protection Agency, May 1996.

F. Theses/Dissertations

- Anderson, J.E., *Effect of Chlorinated Ethene Biodegradation on Growth Rates of Methanotrophic Bacteria in Biofilms and Mixed Cultures*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1996).
- Ball, H.A., *Microbial Transformation of Aromatic Hydrocarbons Under Anaerobic Conditions*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1996).
- Bos, M.C., *Part I: Development and Application of an Arsenic Speciation Technique Using Ion-Exchange Solid Phase Extraction Coupled with GFAAS; Part II: Investigation of Zing Amalgam as a Reductant*, M.S. Thesis, Department of Chemistry, Oregon State University, Corvallis, OR (1996).
- Dion, L., *Immobilizing Heavy Metals in a Ceramic Matrix: A Hazardous Waste Treatment Technology*, Engineer Degree Thesis, Department of Civil Engineering, Stanford University, Stanford, CA (1996).
- Dolan, M. E., *Methanotrophic Cometabolic Transformation of Vinyl Chloride and Other Chlorinated Ethenes in Aquifer Microcosms*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1996).
- Ely, R. L., *Effects of Substrate Interactions, Toxicity, and Bacterial Response During Cometabolism of Chlorinated Solvents by Nitrifying Bacteria*, Ph.D. Dissertation, Department of Civil, Construction, and Environmental Engineering, Oregon State University, Corvallis, OR (1996).
- Gottipati, S., *Radon-222 as a Tracer for Performance Assessment of NAPL Remediation Technologies in the Saturated Zone: An Experimental Investigation*, M.S. Thesis, Department of Civil, Construction, and Environmental Engineering, Oregon State University, Corvallis, OR (1996).

- Kaslik, P., *Down-Borehole Permeable Barrier Reactor: Primary Substrate Selection for Aerobic Dichlorophenol Degradation*, M.S. Thesis, Department of Civil, Construction and Environmental Engineering, Oregon State University, Corvallis, OR (1996).
- Keller, A.A., *Single and Multiphase Flow and Transport in Fractured Porous Media*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1996).
- Kim, Y., *Aerobic Cometabolism of Chloroform by Butane and Propane Grown Microorganisms from the Hanford Subsurface*, M.S. Thesis, Department of Civil, Construction, and Environmental Engineering, Oregon State University, Corvallis, OR (1996).
- Munakata-Marr, J., *The Effects of Bioaugmentation on Trichloroethylene Cometabolism in Aquifer Microcosms*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1996).
- Schroth, M., *Multiphase Flow in Homogeneous and Bedded Porous Media*, Ph.D. Dissertation, Department of Civil, Construction, and Environmental Engineering, Oregon State University (1996).
- Smith, L.H., *Performance of a Two-Stage Process for Cometabolic Treatment of Trichloroethylene-Contaminated Water by Methane-Oxidizing Mixed Cultures*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1996).
- Stuart, S. L., *The Effect of Environmental Conditions on the Reductive Dechlorination of Pentachlorophenol by a Mixed, Methanogenic Culture*, Ph.D. Dissertation, Oregon State University, Corvallis, OR (1996).
- Wang, G. M., *Cometabolic Degradation of Chlorophenolic Compounds*, Ph.D. Dissertation, Department of Civil Engineering, Oregon State University, (1996).

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).
- Alvarez-Cohen, L. and P.L. McCarty, "A Cometabolic Biotransformation Model for Halogenated Aliphatic Compounds Exhibiting Product Toxicity," *Environmental Science and Technology*, **25**(8), 1381-1387 (1991).
- Alvarez-Cohen, L.M. and P.L. McCarty, "Effects of Toxicity, Aeration, and Reductant Supply on Trichloroethylene Transformation by a Mixed Methanotrophic Culture," *Applied and Environmental Microbiology*, **57**(1), 228-235 (1991).
- Alvarez-Cohen, L. and P.L. McCarty, "Product Toxicity and Cometabolic Competitive Inhibition Modeling of Chloroform and Trichloroethylene by Methanotrophic Resting Cells," *Applied and Environmental Microbiology*, **57**(4), 1031-1037 (1991).
- Alvarez-Cohen, L. and P.L. McCarty, "Two-Stage Dispersed-Growth Treatment of Halogenated Aliphatic Compounds by Cometabolism," *Environmental Science and Technology*, **25**(8), 1387-1393 (1991).
- Alvarez-Cohen, L., P.L. McCarty, E. Boulygina, R.S. Hanson, G.A. Brusseau, and H.C. Tsien, "Characterization of a Methane-Utilizing Bacterium from a Bacterial Consortium that Rapidly Degrades Trichloroethylene and Chloroform," *Applied and Environmental Microbiology*, **85**(6), 1886-1893 (1992).
- Alvarez-Cohen, L., P.L. McCarty, and P.V. Roberts, "Sorption of Trichloroethylene onto a Zeolite, Accompanied by Methanotrophic Biotransformation," *Environmental Science and Technology*, **27**(10), 2141-2148 (1993).
- Anderson, J.E. and P.L. McCarty, "Model for Treatment of Trichloroethylene by Methanotrophic Biofilms," *Journal of Environmental Engineering*, **120**(2), 379-400 (1994).
- Arp, D. J., "Understanding the Diversity of Trichloroethylene Co-Oxidations," *Current Opinion in Biotechnology*, **6**, 352-358 (1995).
- Bae, J., L. Semprini, and P.L. McCarty, "Apparatus for Down-Well Oxygen Transfer into Contaminated Aquifers," *Journal of Environmental Engineering*, **121**(8), 565-570 (1995).

- Beller, H.R. and M. Reinhard, "The Role of Iron in Enhancing Anaerobic Toluene Degradation in Sulfate-Reducing Enrichment Cultures," *Microbial Ecology*, **30**, 105-114 (1995).
- Beller, H.R., W.H. Ding, and M. Reinhard, "By-Products of Anaerobic Alkylbenzene Metabolism Useful as Indicators of in situ Bioremediation," *Environmental Science & Technology*, **29**(11), 2864-2870 (1995).
- Beller, H.R., M. Reinhard, and D. Grbić -Galić , "Metabolic By-Products of Anaerobic Toluene Degradation by Sulfate-Reducing Enrichment Cultures," *Applied and Environmental Microbiology*, **58**(9), 3192-3195 (1992).
- Chiu, P.C. and M. Reinhard, "Metallocoenzyme-Mediated Reductive Transformation of Carbon Tetrachloride in Titanium(III) Citrate Aqueous Solution," *Environmental Science & Technology*, **29**, 595-603 (1995).
- Chrysiopoulos, C.V., L.M. Hildemann, and P.V. Roberts, "Modeling the Emission and Dispersion of Volatile Organics from Surface Aeration Water Treatment Facilities," *Water Research*, **26**(8), 1045-1052 (1992).
- Chrysiopoulos, C.V., L.M. Hildemann, and P.V. Roberts, "A Three-Dimensional Atmospheric Dispersion-Deposition Model for Emissions from a Ground-Level Area Source," *Atmospheric Environment*, **26A**(5), 747-757 (1992).
- Chrysiopoulos, C.V., P.K. Kitanidis, and P.V. Roberts, "Analysis of One-Dimensional Solute Transport through Porous Media with Spatially Variable Retardation Factor," *Water Resources Research*, **26**(3), 437-446 (1990).
- Chrysiopoulos, C.V., P.K. Kitanidis, and P.V. Roberts, "Generalized Taylor-Aris Moment Analysis of the Transport of Sorbing Solutes Through Porous Media with Spatially Periodic Retardation Factor," *Transport in Porous Media*, **7**, 163-185 (1992).
- Chrysiopoulos, C.V., P.K. Kitanidis, and P.V. Roberts, "Macrodispersion of Sorbing Solutes in Heterogeneous Porous Formations in Spatially-Periodic Retardation and Velocity Field," *Water Resources Research*, **28**(6), 1517-1529 (1992).
- Cozza, C.L. and S.L. Woods, "Reductive Dechlorination Pathways for Substituted Benzenes: a Correlation with Electronic Properties," *Biodegradation*, **2**, 265-278 (1992).
- Craig, A.M., C.J. Latham, L.L. Blythe, W.B. Schmotzer, and O.A. O'Connor, "Metabolism of Toxic Pyrrolizidine Alkaloids from Tansy Ragwort, *Senecio jacobaea*, in Bovine Ruminant Fluid Under Anaerobic Conditions," *Applied and Environmental Microbiology*, **58**(9), 2730-2736 (1992).
- Dolan, M. and McCarty, P. L., "Methanotrophic Chloroethene Transformation Capacities and 1,1-Dichloroethene Transformation Product Toxicity," *Environmental Science and Technology*, **29**(11), 2741-2747 (1995).

- Dolan, M. E. and McCarty, P. L., "Small-Column Microcosm for Assessing Methane-Stimulated Vinyl Chloride Transformation in Aquifer Samples," *Environmental Science & Technology*, **29**(8), 1892-1897 (1995).
- Drefahl, A. and M. Reinhard, "Similarity-Based Search and Evaluation of Environmentally Relevant Properties for Organic Compounds in Combination with the Group Contribution Approach," *J. Chem. Info. Comp. Sci.*, **33**, 886-895 (1993).
- Dykaar, B.B. and P.K. Kitanidis, "Determination of Effective Hydraulic Conductivity for Heterogeneous Porous Media Using a Numerical Spectral Approach: 1. Method," *Water Resources Research*, **28**(4), 1155-1166 (1992).
- Dykaar, B.B. and P.K. Kitanidis, "Determination of Effective Hydraulic Conductivity for Heterogeneous Porous Media Using a Numerical Spectral Approach: 2. Results," *Water Resources Research*, **28**(4), 1167-1178 (1992).
- Dykaar, B.B. and P.K. Kitanidis, "Transmissivity of a Heterogeneous Formation," *Water Resources Research*, **31**(5), 985-1001 (1993).
- Edwards, E.A., A.M. Edwards, and D. Grbić -Galić , "A Method for Detection of Aromatic Metabolites at Very Low Concentrations," *Applied and Environmental Microbiology*, **60**, 323-327 (1994).
- Edwards, E.A. and D. Grbić -Galić , "Anaerobic Degradation of Toluene and o-Xylene by a Methanogenic Consortium," *Applied and Environmental Microbiology*, **60**(1), 313-322 (1994).
- Edwards, E.A., and D. Grbić -Galić , "Complete Mineralization of Benzene by Aquifer Microorganisms under Strictly Anaerobic Conditions," *Applied and Environmental Microbiology*, **58**(8), 2663-2666 (1992).
- Edwards, E.A., L.E. Wills, M. Reinhard, and D.Grbić -Galić , "Anaerobic Degradation of Toluene and Xylene by Aquifer Microorganisms under Sulfate-Reducing Conditions," *Applied and Environmental Microbiology*, **58**(3), 794-800 (1992).
- Ely, R.L., K.J. Williamson, R.B. Guenther, M.R. Hyman, and D.J. Arp, "A Cometabolic Kinetics Model Incorporating Enzyme Inhibition, Inactivation, and Recovery: 1. Model Development, Analysis, and Testing," *Biotechnology and Bioengineering*, **46**, 218-231 (1995).
- Ely, R.L., M.R. Hyman, D.J. Arp, R.B. Guenther, and K.J. Williamson, "A Cometabolic Kinetics Model Incorporating Enzyme Inhibition, Inactivation, and Recovery: 2. Trichloroethylene Degradation Experiments," *Biotechnology and Bioengineering*, **46**, 232-245 (1995).

- Farrell, J. and M. Reinhard, "Desorption of Halogenated Organics from Model Solids, Sediments, and Soil Under Unsaturated Conditions: 1. Isotherms," *Environmental Science and Technology*, **28**(1), 53-62 (1994).
- Farrell, J. and M. Reinhard, "Desorption of Halogenated Organics from Model Solids, Sediments, and Soil Under Unsaturated Conditions: 2. Kinetics," *Environmental Science and Technology*, **28**(1), 63-72 (1994).
- Fleming F.F., J.T. Hovermale, J.F. White, and A.M. Craig, "Improved Labelling Methods for C⁹⁻²H-Retronecine," *Heterocycles*, **38**(1), 135-139 (1993).
- Fry, V.A., J.D. Istok, and R.B. Guenther, "An analytical solution to the solute transport equation with rate-limited desorption and decay," *Water Resources Research*, **29**(9), 3201-3208 (1993).
- Gailey, R.M., A.S. Crowe, and S.M. Gorelick, "Coupled Process Parameter Estimation and Prediction Uncertainty Using Hydraulic Head and Concentration Data," *Advances in Water Resources*, **14**(5), 301-314 (1991).
- Gailey, R.M. and S.M. Gorelick, "Design of Optimal, Reliable Plume Capture Schemes: Application to the Gloucester Landfill Groundwater Contamination Problem," *Ground Water*, **31**(1), 107-114 (1993).
- Gorelick, S.M., "Large Scale Nonlinear Deterministic and Stochastic Optimization: Formulations Involving Simulation of Subsurface Contamination," *Mathematical Programming*, **48**, 19-39 (1990).
- Grathwohl, P. and M. Reinhard, "Desorption of Trichloroethylene in Aquifer Materials: Rate Limitation at the Grain Scale," *Environmental Science and Technology*, **27**(12), 2360-2366 (1993).
- Grbić -Galić , D., "Anaerobic Transformation of Aromatic Hydrocarbon Pollutants under Fermentative/Methanogenic Conditions," *Periodicum Biologorum*, **93**(4), 533-546 (1991).
- Grbić -Galić , D., "Methanogenic Transformation of Aromatic Hydrocarbons and Phenols in Groundwater Aquifers," *Geomicrobiol. J.*, **8**, 167-200 (1991).
- Gvirtzman, H. and S.M. Gorelick, "Using air-lift pumping as an in-situ aquifer remediation technique," *Water Science Technology*, **27**(7-8), 195-201 (1993).
- Gvirtzman, H. and P. Roberts, "Pore Scale Spatial Analysis of Two Immiscible Fluids in Porous Media," *Water Resources Research*, **27**(6), 1165-1176 (1991).
- Haag, F., Reinhard, M., and McCarty, P.L., "Degradation of Toluene and p-Xylene in Anaerobic Microcosms: Evidence for Sulfate as a Terminal Electron Acceptor," *Env. Tox. Chem.*, **10**, 1379-1389 (1991).

- Habeck, D.B. and K.L. Sublette, "Reductive Dechlorination of Tetrachloride (PCE) Catalyzed by Cyanocobalamin," Center for Environmental Research and Technology, University of Tulsa, *Applied Biochemistry and Biotechnology*, **51/52**, 747-759 (1995).
- Haggerty, R., and S.M. Gorelick, "Multiple-Rate Mass Transfer for Modeling Diffusion and Surface Reactions in Media with Pore-Scale Heterogeneity," *Water Resources Research*, **31**(10), 2383-2400 (1995).
- Haggerty, R., and S. M. Gorelick, "Design of Multiple Contaminant Remediation: Sensitivity to Rate-Limited Mass Transfer," *Water Resources Research*, **30**(2), 435-446 (1994).
- Haggerty, R., and S.M. Gorelick, "Design of multiple contaminant remediation: Sensitivity to rate-limited mass transfer," *Water Resources Research*, **30**(2), 435-446 (1994).
- Harmon, T.C. and P.V. Roberts, "Comparison of Intraparticle Sorption and Desorption Rates for a Halogenated Alkene in a Sandy Aquifer Material," *Environmental Science and Technology*, **28**(9), 1650-1660 (1994).
- Harmon, T.C. and P.V. Roberts, "The Effect of Equilibration Time on Desorption Rate Measurements with Chlorinated Alkenes and Aquifer Particles," *Environmental Progress*, **13**(1), 1-8 (1994).
- Harmon, T.C. and P.V. Roberts, "Determining and Modeling Mass Transfer Rate Limitations in Heterogeneous Aquifers," *Water Science and Technology*, **26**(1-2), 71-77 (1992).
- Harmon, T.C., Semprini, L., and Roberts, P.V., "Simulating Groundwater Solute Transport Using Laboratory-based Determined Sorption Parameters," *J. Environmental Engineering*, **118**(5), 666-689 (1992).
- Harvey, C.F., Haggerty, R., and Gorelick, S.M., "Aquifer remediation: A method for estimating mass transfer rate coefficients and an evaluation of pulsed pumping," *Water Resources Research*, **30**(7), 1979-1991 (1994).
- Henry, S.M. and D. Grbić -Galić, "Influence of Endogenous and Exogenous Electron Donors and Trichloroethylene Oxidation Toxicity on Trichloroethylene Oxidation by Methanotrophic Cultures from a Ground Water Aquifer," *Applied and Environmental Microbiology*, **57**(1), 236-244 (1991).
- Henry, S.M. and D. Grbić -Galić, "Inhibition of Trichloroethylene Oxidation by the Transformation Intermediate Carbon Monoxide," *Applied and Environmental Microbiology*, **57**, 1770-1776 (1991).
- Henrysson, T. and P.L. McCarty, "Influence of the Endogenous Storage Lipid Poly- β -Hydroxybutyrate on the Reducing Power Availability During Cometabolism of Trichloroethylene and Naphthalene by Resting Methanotrophic Mixed Cultures," *Applied and Environmental Microbiology*, **59**(5), 1602-1606 (1993).

- Hopkins, G.D., Munakata, J., Semprini, L., and McCarty, P.L., "Trichloroethylene Concentration Effects on Pilot Field Scale *In-Situ* Groundwater Bioremediation by Phenol-Oxidizing Bacteria," *Environmental Science and Technology*, **27**, 2542-2547 (1993).
- Hopkins, G.D., Semprini, L., and McCarty, P.L., "Microcosm and In Situ Field Studies of Enhanced Biotransformation of Trichloroethylene by Phenol-Utilizing Microorganisms," *Applied and Environmental Microbiology*, **59**, 2277-2285 (1993).
- Hopkins, G.D. and P.L. McCarty, "Field Evaluation of *in Situ* Aerobic Cometabolism of Trichloroethylene and Three Dichloroethylene Isomers Using Phenol and Toluene as the Primary Substrates," *Environmental Science & Technology*, **29**(6), 1628-1637 (1995).
- Hyman, M.R., S.A. Russell, R.L. Ely, K.J. Williamson, and D.J. Arp, "Inhibition, Inactivation, and Recovery of Ammonia-Oxidizing Activity in Cometabolism of Trichloroethylene by *Nitrosomas europaea*," *Applied and Environmental Microbiology*, **61**(4), 1480-1487 (1995).
- Hyman, M.R., Page, C.L., and Arp, D.J., "Oxidation of Methyl Fluoride and Dimethyl Ether by Ammonia Monooxygenase in *Nitrosomonas europaea*," *Applied and Environmental Microbiology*, **60**, 3033-3035 (1994).
- Keller, A.A., P.V. Roberts, and P.K. Kitanidis, "Prediction of Single Phase Transport Parameters in a Variable Aperture Fracture," *Geophysical Research Letters*, **22**(11) 1425-1428 (1995).
- Kim, Y., L.S. Watrud, and A. Matin, "A Carbon Starvation Survival Gene Of *Pseudomonas putida* Is Regulated By *s54* ", *Journal of Bacteriology*, **177**(7), 1850-1859 (1995).
- Kitanidis, P.K., "Analysis of Macrodispersion through Volume-Averaging: Moment Equations," *Stochastic Hydrology and Hydraulics*, **6**, 5-25 (1992).
- Kitanidis, P.K., "The concept of the dilution index," *Water Resources Research*, **30**(7), 2011-2026 (1994).
- Kitanidis, P.K., "Effective Hydraulic Conductivity for Gradually Varying Flow," *Water Resources Research*, **26**(6), 1197-1208 (1990).
- Kitanidis, P.K., "Generalized Covariance Functions in Estimation," *Mathematical Geology*, **25**(5), 525-540 (1993).
- Kitanidis, P.K., "Orthonormal Residuals in Geostatistics: Model Criticism and Parameter Estimation," *Mathematical Geology*, **23**(5), 741-758 (1991).
- Kitanidis, P.K., "Quasi-linear geostatistical theory for inversing," *Water Resources Research*, **30**(10), 2411-2419 (1995).

- Lin, J.S. and L.M. Hildemann, "Analytical Solutions of the Atmospheric Diffusion Equation with Multiple Sources and Height-Dependent Wind Speed and Eddy Diffusivities," *Atmospheric Environment*, **30**(2), 239-254 (1995).
- Lin, JS. and L.M. Hildemann, "A Nonsteady-State Analytical Model to Predict VOC Emissions from Landfills," *Journal of Hazardous Materials*, **40**(3), 271-295 (1995).
- MacDonald, T.R. and P.K. Kitanidis, "Modeling the Free Surface of an Unconfined Aquifer Near a Recirculation Well," *Ground Water*, **31**(5), 774-788 (1993).
- Matin, A., C.D. Little, C.D. Fraley, and M. Keyhan, "Use Of Starvation Promoters To Limit Growth And Selectively Express Tichloroethylene And Phenol Transformation Activity in Recombinant Escherichia coli ", *Applied and Environmental Microbiology*, **61**(9), 3323-3328 (1995).
- McCarty, P.L., "Engineering Concepts for *In Situ* Bioremediation," *Journal of Hazardous Materials*, **28**, 1-11 (1991).
- McCarty, P.L., "*In Situ* Bioremediation of Chlorinated Solvents," *Current Opinion in Biotechnology*, **4**(3), 323-330 (1993).
- McCarty, P. L. and L. Semprini, "Engineering and Hydrogeological Problems Associated with *In Situ* Treatment," *Hydrological Sciences*, **38**(4), 261-272 (1993).
- Nelson, P.O., "Equilibrium Adsorption of Chlorophenols on Granular Activated Carbon," *Water Environment Research*, **67**, 892-898 (1995).
- Nicholson, D.K., Woods, S.L., Istok, J.D., and Peek, D.C., "Reductive Dechlorination of Chlorophenols by a Pentachlorophenol-Acclimated Methanogenic Consortium," *Applied and Environmental Microbiology*, **58**(7), 2280-2286 (1992).
- Papelis, C., "X-ray Photoelectron Spectroscopic Studies of Cadmium and Selenite Adsorption on Aluminum Oxides," *Environmental Science & Technology*, **29**(6), 1526-1533 (1995).
- Papelis, C., G.E. Brown, G.A. Parks, J.O. Leckie, "X-ray Absorption Spectroscopic Studies of Cadmium and Selenite Adsorption on Aluminum Oxides," *Langmuir*, **11**(6), 2041-2048 (1995).
- Papelis, C., P.V. Roberts, J.O. Leckie, "Modeling the Rate of Cadmium and Selenite Adsorption on Micro- and Mesoporous Transition Aluminas," *Environmental Science & Technology*, **29**(4), 1099-1108 (1995).
- Schreier, C.G. and M. Reinhard, "Transformation of Chlorinated Organic Compounds by Iron and Manganese Powders in Buffered Water and in Landfill Leachate," *Chemosphere*, **29**(8), 1743-1753 (1994).

- Schreier, C.G. and M. Reinhard, "Catalytic Hydrodehalogenation of Chlorinated Ethylenes Using Palladium and Hydrogen for the Treatment of Contaminated Water," *Chemosphere*, **31**, 3475-3487 (1995).
- Semprini, L., P.K. Kitanidis, D.H. Kampbell, and J.T. Wilson, "Anaerobic Transformation of Chlorinated Aliphatic Hydrocarbons in a Sand Aquifer Based on Spatial Chemical Distributions," *Water Resources Research*, **31**(4), 1051-1062 (1995).
- Semprini, L., Hopkins, G.D., McCarty, P.L., and Roberts, P.V., "In-Situ Transformation of Carbon Tetrachloride and Other Halogenated Compounds Resulting from Biostimulation Under Anoxic Conditions," *Environmental Science and Technology*, **26**(12), 2454-2461 (1992).
- Semprini, L., Hopkins, G.D., Roberts, P.V., and McCarty, P.L., "Pilot Scale Field Studies of *In-Situ* Bioremediation of Chlorinated Solvents," *Journal of Hazardous Materials*, **32**, 145-162 (1992).
- Thierrin, J. and P.K. Kitanidis, "Solute dilution at the Borden and Cape Cod groundwater tracer tests," *Water Resources Research*, **30**(11), 2883-2890 (1994).
- Tiedeman, C. and S.M. Gorelick, "Analysis of Uncertainty in Optimal Groundwater Contaminant Capture Design," *Water Resources Research*, **29**(7), 2139-2154 (1993).
- Wachenheim, D.E., Blythe, L.L., and Craig, A.M., "Characterization of rumen bacterial pyrrolizidine alkaloid biotransformation in ruminants of various species," *Vet. and Human Toxicology*, **34**(6), 513-517 (1992).
- Wachenheim, D.E., Blythe, L. L., and Craig, A. M., "Effects of antibacterial agents on in vitro ovine ruminal biodegradation of the hepatotoxic pyrrolizidine alkaloid, jacobine," *Applied and Environmental Microbiology*, **58**(8), 2559-2564 (1992).

B. Books and Book Chapters

- Criddle, C.S., Alvarez, L.M., and McCarty, P.L., "Microbial Processes in Porous Media," *Transport Processes in Porous Media*, Eds., J. Bear and M.Y. Corapcioglu, Kluwer Academic Publishers, Dordrecht, The Netherlands, 639-691 (1991).
- Dawson, K.J. and J.D. Istok, *Aquifer Testing: Design and Analysis of Pumping and Slug Tests*, Lewis Publishers, Inc., Chelsea, Michigan, (1991).
- Drefahl, A. and M. Reinhard, *Handbook for Estimating Physico-Chemical Properties of Organic Compounds*, Stanford Bookstore, Stanford, California (1995).
- Drefahl, A. and M. Reinhard, *Data Evaluation System for Organic Compounds*, Stanford Bookstore, Stanford, California (1995).

- Ely, R., K. J. Williamson, M. R. Hyman and D. J. Arp, "Cometabolic Degradation of Chlorinated Solvents," *Bioremediation of Chlorinated Solvents*, Eds., R. E. Hinchee, A. Leeson and L. Semprini, Battelle Press, Columbus, OH 187-196 (1995).
- Grbić -Galić , D., "Anaerobic Microbial Degradation of Aromatic Hydrocarbons," *Microbial Enhancement of Oil Recovery--Recent Advances*, Ed. E.C. Donaldson, Elsevier, Amsterdam, 145-161 (1991).
- Grbić -Galić , D., Henry, S.M., Godsy, E.M., Edwards, E., and Mayer, K.P., "Anaerobic Degradation of Aromatic Hydrocarbons and Aerobic Degradation of Trichloroethylene by Subsurface Microorganisms," *Organic Substances and Sediments in Water, Vol. 3: Biological*, Ed. R.A. Baker, Lewis Publ., Inc., Chelsea, 239-266 (1991).
- McCarty, P.L. and M. Reinhard, "Biological and Chemical Transformations of Halogenated Aliphatic Compounds in Aquatic and Terrestrial Environments," *Biogeochemistry of Global Change: Radiatively Active Trace Gases*, Ed. R.S. Oremland, Chapman and Hall, Inc., New York, 839-852 (1993).
- McCarty, P.L. and P.V. Roberts, editors, "Contaminants in the Subsurface Environment," *Water Science and Technology*, **22**(6), 110 pp. (1990).
- McCarty, P.L. and L. Semprini, "Ground-Water Treatment for Chlorinated Solvents," in *Handbook of Bioremediation*, Ed. Norris et al., Lewis Publishers, Boca Raton, 87-116 (1994).
- Reinhard, M., "In-Situ Bioremediation Technologies for Petroleum-Derived Hydrocarbons Based on Alternate Electron Acceptors (Other than Molecular Oxygen)," in *Handbook of Bioremediation*, Ed. Norris et al., Lewis Publishers, Boca Raton, 131-147 (1994).
- Semprini, L., G.D. Hopkins, D. Grbić -Galić , P.L. McCarty, and P.V. Roberts, "A Laboratory and Field Evaluation of Enhanced *In Situ* Bioremediation of Trichloroethylene, cis- and trans-Dichloroethylene, and Vinyl Chloride by Methanotrophic Bacteria," *Bioremediation Field Experience*, Eds., P.E. Flathman, D.E. Jerger, and J.H. Exner, Lewis Publishers, Boca Raton, 383-412 (1994).

C. Bound Proceedings

- Azizian, M.F. and P.O. Nelson, "Hexavalent Chromium Adsorption and Reduction in Natural Soils," *Research Symposia Proceedings*, Water Environment Federation 65th Annual Conference, New Orleans, LA, 241-247 (1992).
- Ball, H.A., Reinhard, M., and McCarty, P.L., "Biotransformation of Monoaromatic Hydrocarbons Under Anoxic Conditions," *In Situ Bioreclamation: Applications and Investigations for Hydrocarbon and Contaminated Site Remediation*, Eds., R.E. Hinchee and R.F. Olfenbittel, Butterworth-Heinemann, Boston, 458-462 (1991).

- Chrykopoulos, C.V., Hildemann, L.M., and Roberts, P.V., "Modeling Emissions from Aerobic Treatment Systems," in *Environmental Engineering: Proceedings of the 1991 Specialty Conference*, American Society of Civil Engineers, New York, NY, 414-418, (1991).
- Craig, A.M. and L.L. Blythe, "Review of Ruminant Microbes Relative to Detoxification of Plant Toxins and Environmental Pollutants," *Proceedings of the Fourth International Symposium on Poisonous Plants*, Perth, Australia, September 28-October 1, 1993.
- Dolan, M. E. and P.L. McCarty, "Factors Affecting Transformations of Chlorinated Aliphatic Hydrocarbons by Methanotrophs," *Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds*, Eds., R.E. Hinchee, A. Leeson, L. Semprini, and S.K. Ong, Lewis Publishers, Boca Raton, 303-308 (1994).
- Edwards, E.A., Wills, L.E., Grbić -Galić , D., and Reinhard, M., "Anaerobic Degradation of Toluene and Xylene--Evidence for Sulphate as the Terminal Electron Acceptor," *In Situ Bioreclamation: Applications and Investigations for Hydrocarbon and Contaminated Site Remediation*, Eds., R.E. Hinchee and R.F. Olfenbuttel, Butterworth-Heinemann, Boston, 463-471 (1991).
- Farrell, J. and M. Reinhard, "Measurement of Organic Vapor Isotherms on Wet Soils and Aquifer Materials," *Current Practices in Ground Water and Vadose Zone Investigations, ASTM STP 1118*, Eds., D.M. Nielsen and M.N. Sara, American Society for Testing and Materials, Philadelphia (1991).
- Haston, Z.C., Sharma, P.K., Black, J.N.P., and McCarty P.L., "Enhanced Reductive Dechlorination of Chlorinated Ethenes," *Symposium on Bioremediation of Hazardous Wastes: Research, Development, and Field Evaluations*, EPA/600/R-94/075, 11-14 (1994).
- Hopkins, G.D., Semprini, L., and McCarty, P.L., "Evaluation of Enhanced *In Situ* Aerobic Biodegradation of cis- and trans-1-Trichloroethylene and cis- and trans-2, 2-Dichloroethylene by Phenol-Utilizing Bacteria," *Bioremediation of Hazardous Wastes*, EPA/600/R-92/126, U.S. EPA Center For Environmental Research Information, Cincinnati, 71-73 (1992).
- Kitanidis, P.K., "Recent advances in geostatistical inference on hydrogeological variables," *Reviews of Geophysics*, Supplement to U.S. National Report to International Union of Geodesy and Geophysics 1991-1994, 1103-1109, (1995).
- Lin, J.S. and L.M. Hildemann, "Air Emission Models for Hazardous Waste Landfills," in *Joint CSCE--ASCE Conference on Environmental Engineering*, Eds., R.N. Yong, J. Hadjinicolaou, and A.M.O. Mohamed, Geotechnical Research Centre of McGill University, 75-82 (1993).
- Lin, J.S. and L.M. Hildemann, "Comparison of Dispersion Models for Wastewater Treatment Emissions," *Environmental Engineering: Proceedings of the Environmental Engineering Sessions at Water Forum '92*, American Society of Civil Engineers, New York, NY, 7-11 (1992).

- Lin, J.S. and L.M. Hildemann, "Emissions, Ambient Concentrations, and Potential Health Hazards of VOCs from Wastewater Treatment," *ASCE Conference on Environmental Engineering*, 580-584 (1994).
- Lin, J.S. and L.M. Hildemann, "A Nonsteady-State Analytical Model to Predict VOC Emissions from Hazardous Waste Sites," in *Proc. 1995 ASCE - CSCE Environ. Eng. Conf.*, American Society of Civil Engineers, New York, 238-245, (1995).
- Lin, J.S. and L.M. Hildemann, "Mathematically Modeling the Transport of Hazardous Air Pollutants from Multiple Sources," in *Proc. 1995 ASCE - CSCE Environ. Eng. Conf.*, American Society of Civil Engineers, New York, 620-627, (1995).
- Little, C.D., Fraley, C.D., McCann, M.P., and Matin, A., "Use of Bacterial Stress Promoters to Induce Biodegradation Under Conditions of Environmental Stress," *On-Site Bioreclamation, Processes for Xenobiotic and Hydrocarbon Treatment*, Eds., R.E. Hinchee and R.F. Olfenbuttel, Butterworth-Heinemann, Boston, 493-498 (1991).
- McCarty, P.L., "An Overview of Anaerobic Transformation of Chlorinated Solvents," *Symposium on Intrinsic Bioremediation of Ground Water*, EPA/540/R-94/515, EPA Office of Research and Development, Washington, D.C., 135-142 (1994).
- McCarty, P.L., "Engineering Concepts for In-Situ Bioremediation," *Proceedings, Bioremediation Fundamentals & Effective Applications*, 3rd Annual Symposium, Gulf Coast Hazardous Substance Research Center, 3-13 (1991).
- McCarty, P.L., "Processes Governing the Movement and Fate of Contaminants in Groundwater," *Groundwater Contamination: Application of Research to Management Problems in Asia and the Pacific*, Proceedings of the 2nd SCOPE Regional Workshop, Chiang Mai, Thailand, 38-45 (1995).
- McCarty, P.L., "Factors Affecting Biotransformation Rates in Groundwater" *Groundwater Contamination: Application of Research to Management Problems in Asia and the Pacific*, Proceedings of the 2nd SCOPE Regional Workshop, Chiang Mai, Thailand, 261-263 (1995).
- McCarty, P.L., "Transport, Fate, and In-Situ Bioremediation of Chlorinated Solvents in Groundwater," *Proceedings of the Japanese Association of Groundwater Hydrology*, Yokohama, 116-135 (1992).
- McCarty, P.L. and G. Hopkins, "Field-Scale Study of In Situ Bioremediation of TCE-Contaminated Ground Water and Planned Bioaugmentation," *Symposium on Bioremediation of Hazardous Wastes: Research, Development, and Field Evaluations*, EPA/600/R-94/075, 65-68 (1994).
- McCarty, P.L., Semprini, L., Dolan, M.E., Harmon, T.C., Tiedeman, C., and Gorelick, S.M., "In-Situ Methanotrophic Bioremediation for Contaminated Groundwater at St. Joseph, Michigan," *On-Site Bioreclamation, Processes for Xenobiotic and Hydrocarbon Treatment*, Eds., R.E. Hinchee and R.F. Olfenbuttel, Butterworth-Heinemann, Boston, 16-40 (1991).

- McCarty, P.L. and J.T. Wilson, "Natural Anaerobic Treatment of a TCE Plume, St. Joseph, Michigan, NPL Site," *Bioremediation of Hazardous Wastes*, EPA/600/R-92/126, U.S. EPA Center For Environmental Research Information, Cincinnati, 47-50 (1992).
- Nelson, P.O. and M.F. Azizian, "Hexavalent Chromium Adsorption and Desorption in Natural Soils and Subsoils," *Proceedings, Third International Iranian Congress of Civil Engineering*, Shiraz, Iran, (May 14-16, 1990).
- Reinhard M., G.D. Hopkins, E. Orwin, S. Shang, and C.A. LeBron, "In Situ Demonstration of Anaerobic BTEX Biodegradation Through Controlled-Release Experiments," *Applied Bioremediation of Petroleum Hydrocarbons*, R.E. Hinchee, J.A. Kittel, H.J. Reisinger, Eds, *Bioremediation* **3**(6), Battelle Press, 263-270 (1995).

- Reinhard, M., Wills, L.E., Ball, H.A., Harmon, T., Phipps, D.W., Ridgway, H.F., and Eisman, M.P., "A Field Experiment for the Anaerobic Biotransformation of Aromatic Hydrocarbon Compounds at Seal Beach, California," *In Situ Bioreclamation: Applications and Investigations for Hydrocarbon and Contaminated Site Remediation*, Eds., R.E. Hincee and R.F. Olfenbuttel, Butterworth-Heinemann, Boston, 487-495 (1991).
- Semprini, L., McCarty, P., Dolan, M., Lang, M., McDonald, T., Bae, J., and Kitanidis, P., "Design and Treatability Study of *In Situ* Bioremediation of Chlorinated Aliphatics by Methanotrophs at St. Joseph, Michigan," *Bioremediation of Hazardous Wastes*, EPA/600/R-92/126, U.S. EPA Center For Environmental Research Information, Cincinnati, 43-45 (1992).
- Semprini, L., G.D. Hopkins, and P.L. McCarty, "A Field and Modeling Comparison of *In Situ* Transformation of Trichloroethylene by Methane Utilizers and Phenol Utilizers," *Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds*, Eds., R.E. Hincee, A. Leeson, L. Semprini, and S.K. Ong, Lewis Publishers, Boca Raton, 248-254 (1994).
- Semprini, L., G.D. Hopkins, P.V. Roberts, and P.L. McCarty, "In Situ Biotransformation of Carbon Tetrachloride, Freon 113, Freon 11, and 1,1,1-TCA under Anoxic Conditions," *On Site Bioreclamation*, Eds., R.E. Hincee and R.F. Offenbuttel, Butterworth-Heinemann, Boston, 41-58 (1991).

D. Project Reports

- Ball, H.A., M. Reinhard, and E. Orwin, *Factors Influencing the Anaerobic Biodegradation of Gasoline Hydrocarbons by Groundwater Bacteria at Seal Beach, CA.*, Technical Report No. WRC-5, WRHSRC, Department of Civil Engineering, Stanford, CA (1993).
- Brown, James, "Down-Hole Permeable Barrier Reactor: Physical Development," M.S. Project Report, Department of Civil Engineering, Oregon State University, Corvallis, Oregon, (1995).
- Dykarr, B.B. and P.K. Kitanidis, *Derivation of the Equations Governing the Transmissivity of a Three-dimensional Stationary Heterogeneous Porous Medium*, Technical Report No. WRC-3, WRHSRC, Department of Civil Engineering, Stanford, CA (1992).
- Grathwohl, P. and M. Reinhard, *Sorption and Desorption Kinetics of Trichloroethene in Aquifer Material under Saturated Conditions*, Technical Report No. WR-2, WRHSRC, Department of Civil Engineering, Stanford, CA (1991).
- Haston, Z.C., J.N.P. Black, P.L. McCarty, and D. Grbić -Galić, *Oxidation of Polycyclic Aromatic Hydrocarbons by Methanotrophic and Phenol-Degrading Mixed Cultures*, Technical Report No. WRC-4, WRHSRC, Department of Civil Engineering, Stanford, CA (1992).

- Kitanidis, P.K., D. L. Freyberg, A. Dudek-Ronan, S. Itagaki, N. Chan, S. I. and Lee, *Applications Handbook for FASTchem™*, EPRI Report No. TR-101218, Electric Power Research Institute, Palo Alto, CA, (1992).
- Lotrario, Joseph, "Nitro Group Reduction of Five Aromatic Compounds by the Denitrifying Bacteria G.8," M.S. Project Report, Department of Civil Engineering, Oregon State University, Corvallis, Oregon, 1995.
- McCarty, P.L., L. Semprini, M.E Dolan, T.C.. Harmon, S. Just, C. Tiedeman, S.M. Gorelick, and P.V. Roberts, *Evaluation of In-Situ Methanotrophic Bioremediation for Contaminated Groundwater, St. Joseph, Michigan*, Technical Report No. WR-1, WRHSRC, Department of Civil Engineering, Stanford University, Stanford, CA (1990).
- Saberiyan, A., *Kinetic Model of Pentachlorophenol Degradation by Microorganisms*, Engineering Project, Department of Civil Engineering, Oregon State University (1991).
- Smith, R., G.T. Townsend, M.J. McInerney, J.M. Suflita, and R.S. Tanner, "Enhanced Reductive Biodegradation of Chlorinated Alkenes by Cobalt-Centered Porphyrins and Corrins," University of Oklahoma, Final Report, 1994.
- Strauss, R., *Anaerobic Bioremediation of Phenoxy-herbicide Contaminated Soils from the Baldock Station Maintenance Facility*, M.S. Project, Department of Civil Engineering, Oregon State University, 1995.
- Tasker, B.R., Radon-222 as an Indicator for Non-Aqueous Phase Liquids in the Saturated Zone: A Numerical Methods Analysis, M.S. Engineering Report, Department of Civil Engineering, Oregon State University, Corvallis, OR (1995).
- Wang, K. G., *Aerobic Bioremediation of Contaminated Soils from the Baldock Station Maintenance Facility*, M.S. Project, Department of Civil Engineering, Oregon State University, 1995.
- WRHSRC, *1989 Annual Report*, Department of Civil Engineering, Stanford University, (October 1989).
- WRHSRC, *1990 Annual Report*, Department of Civil Engineering, Stanford University, (October 1990).
- WRHSRC, *1991 Annual Report*, Department of Civil Engineering, Stanford University, (December 1991).
- WRHSRC, *1992 Annual Report*, Department of Civil Engineering, Stanford University, (December 1992).
- WRHSRC, *1993 Annual Report*, Department of Civil Engineering, Stanford University, (December 1993).
- WRHSRC, *1994 Annual Report*, Department of Civil Engineering, Stanford University, (December 1994).

WRHSRC, *1995 Annual Report*, Department of Civil Engineering, Stanford University, (December 1995).

E. Extended Abstracts

Dillard, L. A., M. J. Blunt, H. I. Essaid, and S. M. Gorelick, "Investigating the physics of dissolution of nonaqueous phase liquids in heterogeneous porous media using pore network modeling," *Eos Transactions*, **76**(46), (1995).

Essaid, H. I., W. N. Herkelrath, and L. A. Dillard, "The influence of spatial variability of hydraulic properties on the flow of water near a subsurface oil body," *Eos Transactions*, **76**(46) (1995).

Haggerty, R., and S.M. Gorelick, "Design of multiple contaminant remediation in the presence of rate limitations," *Eos*, **73**(43), (1992).

Haggerty, R., and S.M. Gorelick, "A new model for rate-limited mass transfer: Multiple rate approach," *Eos*, **75**(44), (1994).

Haggerty, R., R.S. Harvey, C.F. and S.M. Gorelick, "An evaluation of pulsed pumping for aquifer remediation" *Eos*, **74**(43), (1993).

Haggerty, R. and S.M. Gorelick, "A new model for rate-limited mass transfer: Multiple rate approach," *Eos*, **75**(44), (1994).

Haggerty, R., C.F. Harvey and S.M. Gorelick, "An evaluation of pulsed pumping for aquifer remediation," *Eos*, **74**(43), (1993).

Schreier, C.G. and M. Reinhard, "Catalytic Dehydrohalogenation and Hydrogenation using H₂ and Supported Palladium as a Method for the Removal of Tetrachloroethylene from Water," presented before the Division of Environmental Chemistry, American Chemical Society (1995).

Semprini, L., K. Broholm, and M.E. McDonald, "Radon-222 Deficit Method for Locating and Quantifying NAPL Contamination in the Subsurface," *Eos*, **74**(43), (1993).

Semprini, L., P.K. Kitanidis, D. Kampell, and J.T. Wilson, "Chemical Distributions and Anaerobic Transformation of Chlorinated Aliphatic Hydrocarbons in a Sand Aquifer," Emerging Technologies in Hazardous Waste Management VI, *American Chemical Society, Book of Abstracts*, Ed. D.W. Tedder (1994).

Siantar, D.P., C.G. Schreier, and M. Reinhard, "Transformation of the Pesticide 1,2-Dibromo-3-Chloropropane (DBCP) and Nitrate by Iron Powder and by H₂/Pd/Al₂O₃," presented before the Division of Environmental Chemistry, American Chemical Society (1995).

F. Theses/Dissertations

- Alvarez-Cohen, L., *Cometabolic Biotransformation of Trichloroethylene and Chloroform by Methanotrophs--Experimental Studies and Modeling of Toxicity and Sorption Effects*, Ph.D. Dissertation, Stanford University, Stanford, CA (1991).
- Angamma, K., *SITE: An interactive toolkit for estimation*, Engineer Degree Thesis, Department of Civil Engineering, Stanford University, Stanford, CA (1995).
- Beller, H., *Anaerobic Metabolism of Toluene and Other Aromatic Compounds by Sulfate-Reducing Soil Bacteria*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1995).
- Chiu, P., *Transformation of Carbon Tetrachloride by Metallocoenzymes*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1995).
- Chrysikopoulos, C., *Solute Transport in Porous Media: Investigation of Well-to-Well Circulation and Analysis of Spatially Variable Retardation*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1991).
- Cozza, C.L., *A Model for the Prediction of Biologically Mediated Reductive Dechlorination Pathways*, M.S. Thesis, Oregon State University, Corvallis, OR (1990).
- Dawson, H.E., *Entrapment and Mobilization of Residual Halogenated Organic Liquids in Saturated Aquifer Material*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1992).
- Dawson, K.J., *Aquifer Testing: A Manual for the Design and Analysis of Pumping Tests*, M.S. Thesis, Department of Civil Engineering, Oregon State University, Corvallis, OR (1990).
- DeMenibus, M.-A., *Mixing Strategies for In-Situ Remediation*, Engineer Degree Thesis, Department of Civil Engineering, Stanford University, Stanford, CA (1994).
- Dykaar, B., *Macroflow and Macrotransport in Heterogeneous Porous Media*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1994).
- Edwards, E.A., *Transformation of Non-oxygenated, Homocyclic Aromatic Compounds by Aquifer Microorganisms under Methanogenic and Sulfate-reducing Conditions*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1993).
- Farrell, J., *Desorption Equilibrium and Kinetics of Chlorinated Solvents on Model Solids, Aquifer Sediments, and Soil*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1993).
- Fry, V.A., *Mass Transfer Constraints on the Feasibility of In Situ Bioremediation of Contaminated Groundwater*, Ph.D. Dissertation, Oregon State University, Corvallis, OR (1994).

- Gailey, R.M., *Design of Cost-Effective, Reliable Remedial Pumping Schemes: Application to the Gloucester Landfill Groundwater Contamination Problem*, M.S. Thesis, Stanford University, Stanford, CA (1990).
- Haggerty, R., *Design of Multiple Contaminant Remediation in the Presence of Rate Limitations*, M.S. Thesis, Department of Applied Earth Sciences, Stanford University, Stanford, CA (1992).
- Haggerty, R., *Aquifer Remediation in the Presence of Rate-Limited Mass Transfer*, Ph. D. Dissertation, Stanford University, Stanford, CA (1995).
- Harmon, T.C., *Determining and Modeling Diffusion-Limited Desorption Rates in Heterogeneous Aquifer Solids*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1992).
- Henry, S.M., *Trichloroethylene Transformation by Methanotrophs from a Groundwater Aquifer*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1991).
- Hindagolla, W., *A Mathematical Model to Predict Concentrations of Chlorophenols in a Biofilm Activated Carbon Reactor*, M.S. Thesis, Oregon State University, Corvallis, OR (1991).
- Hopkins, S.O., *Radon-222 as an Indicator for Nonaqueous Phase Liquids in the Saturated Zone: Developing a Detection Technology*, M.S. Thesis, Department of Civil Engineering, Oregon State University, Corvallis, OR (1994).
- Lang, M.M., *Design and Optimization of In Situ Bioremediation Systems Relying on Cometabolic Degradation*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1995)
- Lee, S.B., *A Numerical Model for the Fate and Transport of Chlorinated Phenols in Groundwater*, Ph.D. Dissertation, Department of Civil Engineering, Oregon State University, Corvallis, OR (1990).
- Lee, T. J., *In Vitro Trinitrotoluene (TNT) Biotransformation with Rumen Fluid and an Isolate G.8*, Ph.D. Dissertation, Department of Civil Engineering, Oregon State University, Corvallis, OR (1995).
- Lemmon, T.L., *Development of Chemostats and Use of Redox Indicators for Studying Redox Transformations in Biogeochemical Matrices*, Ph.D. Dissertation, Department of Chemistry, Oregon State University, Corvallis, OR (1995).
- Lo, H., *Predicative Modeling of Dual-Solute Competitive Adsorption in Completely Stirred GAC Reactor*, M.S. Thesis, Oregon State University, Corvallis, OR (1991).
- MacDonald, T.R., *Flow from a Recirculation Well for Enhanced In-Situ Bioremediation*, Ph. D. Dissertation, Stanford University, Stanford, CA (1995).

- Mraković, I., *Sulfidogenic Degradation of Polycyclic Aromatic Hydrocarbons by Aquifer Derived Microorganisms*, Engineer Degree Thesis, Department of Civil Engineering, Stanford University, Stanford, CA (1993).
- Niemit, M.R., *Continuous-Flow and Batch Column Studies of Anaerobic Carbon Tetrachloride Biotransformation on Hanford Soil*, M.S. Thesis, Department of Civil Engineering, Oregon State University, Corvallis, OR (1995).
- Gottipati, S., *Radon-222 as a Tracer for Performance Assessment of NAPL Remediation Technologies in the Saturated Zone: An Experimental Investigation*, M.S. Thesis, Department of Civil, Construction, and Environmental Engineering, Oregon State University, Corvallis, OR (1996).
- Kim, Y., *Aerobic Cometabolism of Chloroform by Butane and Propane Grown Microorganisms from the Hanford Subsurface*, M.S. Thesis, Department of Civil, Construction, and Environmental Engineering, Oregon State University, Corvallis, OR (1996).
- Nicholson, D.K., *Measurement of the Rates of Reductive Dechlorination of Chlorinated Phenols*, M.S. Thesis, Oregon State University, Corvallis, OR (1990).
- Papelis, C., *Cadmium and Selenite Adsorption on Porous Aluminum Oxides: Equilibrium, Rate of Uptake and Spectroscopic Studies*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1992).
- Peda, A., *Electron Data Retrieval: A Sourcebook for Environmental Professionals*, M.S. Project, Oregon State University, Corvallis, OR (1991).
- Peters, D., *Anaerobic and Aerobic Treatment of Chlorinated Phenols with Granular Activated Carbon Expanded Bed Reactors*, M.S. Thesis, Oregon State University, Corvallis, OR (1991).
- Schreier, C., *The Destructive Removal of Chlorinated Organic Compounds From Water using Zero-Valent Metals of Hydrogen and Supported Palladium*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1995).
- Shen, K.-F., *Optimal parameter estimation in geostatistics*, Engineer Degree Thesis, Department of Civil Engineering, Stanford University, Stanford, CA (1994).
- Smith, M., *Reductive Dechlorination of Chlorophenols by Vitamin B₁₂*, Ph.D. Dissertation, Department of Civil Engineering, Oregon State University, Corvallis, OR (1993).
- Tiedeman, C., *Optimal Hydraulic Containment Designs Under Parameter Uncertainty for a Vinyl Chloride Plume in Southwest Michigan*, M.S. Thesis, Department of Applied Earth Sciences, Stanford University, Stanford, CA (1992).

Van Lent, T.J., *Numerical Spectral Methods Applied to Flow in Highly Heterogeneous Aquifers*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1992).

Wang, G.M., *Aerobic Degradation of Chlorophenols*, M.S. Thesis, Oregon State University, Corvallis, OR (1991).

Yang, M., *Adsorption of Chlorophenols on Granular Activated Carbon*, Ph.D. Dissertation, Department of Civil Engineering, Oregon State University, Corvallis, OR (1994).

G. Patents

Gorelick, S.M. and H. Gvirtzman, "In-situ Vapor Stripping for Removing Volatile Organic Compounds from Groundwater," U.S. Patent No. 5,180,503 (January 19, 1993).

McCarty, P.L. and L. Alvarez-Cohen, "Zeolite Enhanced Organic Biotransformation," U.S. Patent No. 5,139,682 (August 18, 1992).

Roberts, P.V., G.D. Hopkins, L. Semprini, and P.L. McCarty, "Pulsing for Electron Donor and Electron Acceptor for Enhanced Biotransformation of Chemicals," U.S. Patent No. 5,006,250 (April 9, 1991).

Semprini, L., P.L. McCarty, P.K. Kitanidis, and J. Bae, "Method and Apparatus for *In Situ* Groundwater Remediation," U.S. Patent No. 5,302,286 (April 12, 1994).

H. Software

Drefahl, A., J. Kolenbrander, and M. Reinhard, DESOC, a Data Evaluation System for Organic Compounds, Stanford University, 1995.