



**WESTERN REGION  
HAZARDOUS SUBSTANCE RESEARCH CENTER**

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# 1995 ANNUAL REPORT

Stanford University  
Oregon State University

December 1995

# **DRAFT**

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**WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER  
1995 ANNUAL REPORT**

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## **WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER 1995 ANNUAL REPORT**

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### **THE CENTER AT A GLANCE**

The Western Region Hazardous Substance Research Center (WRHSRC) is a cooperative activity between Stanford University and Oregon State University that was established in February 1989 to address critical hazardous substance problems in EPA Regions 9 and 10. The Regions include the states of Alaska, Arizona, California, Hawaii, Idaho, Nevada, Oregon, and Washington, and Guam. The Center receives its base financial support from the U.S. Environmental Protection Agency, but also is supported through grants, contracts, and gifts from other federal agencies, states, municipalities, consultants, and industry.

The objectives of the Center are:

1. To promote through fundamental and applied research the development of alternative and advanced physical, chemical, and biological processes for treatment of hazardous substances in the surface and subsurface environments.
2. To disseminate the results of research to the industrial and regulatory communities, to foster exchange of information with these communities, and to promote a better understanding of the scientific capability to detect, assess, and mitigate risks associated with hazardous substance usage and disposal.

Groundwater cleanup and site remediation, with a strong emphasis on biological approaches, represent the major focus of Center activities. The research and training functions of the Center address the major hazardous substance problems in EPA Regions 9 and 10, including chlorinated and nonchlorinated solvents, petroleum products, pesticides, and toxic inorganic compounds including heavy metals. Environmental problems from these substances, which often occur in mixtures, result largely from the production of electronic equipment, chemicals, forestry products, and food, as well as mining and military activities, all of which are important in the region pair.

The 23 faculty and 5 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. The Center has two Assistant Directors, Martin Reinhard, who is in charge of the Center's analytical program, and Mark N. Goltz, who assists in both the research and the training and technology transfer activities of the Center. 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. David L. Freyberg	Mrs. Shia Barnett
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 1994 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 Hoeppel	Govt/U.S. Navy	Microbiology
Michael C. Kavanaugh*	ENVIRON	Physical/Chemical Processes
Richard G. Luthy	Carnegie Mellon University	Chemical/Biological Processes
James M. Tiedje	Michigan State University	Microbiology
John L. Wilson†	New Mexico Technical University	Hydrology
John Wise	Govt/EPA, Region 9	Planning
*Chairman		
†Vice Chairman		

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
Mary Masters	Hewlett-Packard	Treatment Technology
James McNabb	Govt/EPA, Kerr Lab.	Groundwater Remediation
Gregory Peterson	CH2M-HILL	Treatment Technology
Mary Peterson	Battelle Pacific Northwest Lab.	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	\$950,000	\$7,893,015
EPA: Other	\$1,190,000	\$2,825,816
Other Govt: Federal†	\$3,395,000	\$7,130,195
Other Govt: State@	0	\$48,252
Consortium	\$1,233,539	\$2,966,604
Private Sector#	<u>\$150,211</u>	<u>\$2,032,452</u>
TOTAL	\$6,918,750	\$22,896,334

\*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.	35	\$963,777

Ph.D.	100	\$4,800,802
Post Doctoral	<u>24</u>	<u>\$1,506,203</u>
TOTAL	172	\$7,366,659

\*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 37 separate research projects. Twelve of these projects were completed this year, and 20 represent new starts. Twelve of the new projects are being supported through the Research and Re-Education for Department of Defense (R2D2) program. Also, an additional four 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 a year is the Technical Outreach Services for Communities (TOSC) program to aid communities impacted by hazardous waste problems. There are also four MAI projects within this category to help train faculty and students hazardous waste activities. 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 geohydrology 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 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 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, 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**

Two national awards for research conducted with support from the WRHSRC were won by Ph.D. students at Stanford University, James Farrell and James Anderson. James Farrell won the CH2M-HILL and the Association of Environmental Engineering Professors Award for best Ph.D. dissertation of the year in environmental engineering. His dissertation is titled, "Desorption Equilibrium and Kinetics of Chlorinated Solvents on Model Solids, Aquifer Sediments, and Soil." It is concerned with a most important process that affects the rate at which soils can be remediated by processes such as soil-vapor extraction, that is, the slow rate of diffusion of volatile contaminants out of pore spaces within soil particles. These studies add greatly to the understanding of this important phenomenon. The publications resulting from his dissertation are already being referenced widely. He together with his advisor, Martin Reinhard, are to be congratulated for this fine piece of research. Farrell is the second WRHSRC researcher to win this prestigious award. Lisa Alvarez-Cohen was a recipient just two years earlier for her dissertation, "Cometabolic Biotransformation of Trichloroethylene and Chloroform by Methanotrophs--Experimental Studies and Modeling of Toxicity and Sorption Effects."

Anderson won the J. James R. Croes Medal of the American Society of Civil Engineers for his paper entitled, "Model for Treatment of Trichloroethylene by Methanotrophic Biofilms," which is the first in a series of papers from his Ph.D. dissertation. In this paper he presented a numerical model which illustrates the effect of the complex of physical, chemical, and biological processes on the rate at which chlorinated solvents can be biodegraded through cometabolism by methane-oxidizing biofilms. This model was used by Anderson for hypothesis testing to determine important areas of uncertainty that are in need of resolution that might be addressed in his subsequent experimental research. The Croes Medal is given for a civil engineering paper that significantly advances engineering science. This paper and those by Farrell and Alvarez-Cohen are contributions to the long series of papers emanating from the WRHSRC concerning processes affecting the movement, fate, and treatment of chlorinated solvents that form the basis for field demonstrations of in-situ biodegradation now being undertaken by the WRHSRC.

Several other WRHSRC researchers have made significant contributions to the understanding of the movement and fate of chlorinated solvents in soils and groundwaters. Using a multi-rate model, WRHSRC researchers Roy Haggerty and Steven Gorelick [*Haggerty and Gorelick, 1995*], examined and quantified the effects of multiple, simultaneous mass transfer processes on soil column experiments for which Farrell and Reinhard received the best dissertation awards noted above *Farrell and Reinhard* [1994]. They found that within relatively homogeneous materials, such as a single size fraction of the Borden sand, the rates of diffusion are well-characterized by a lognormal distribution of diffusion rate coefficients. They also found that extreme variability, exceeding six orders of magnitude, in diffusion rate coefficients must be invoked to represent mass transfer in many experiments. As a consequence of these findings and the findings of previous years, they suggest that any accurate prediction of aquifer remediation time must consider a distribution of mass transfer rates rather than a single rate, as is conventionally assumed. In other words, the pore-scale heterogeneity of an aquifer is tremendously important to the time required for contaminant removal.

Abdul Matin and his co-workers are exploring the potential for increasing TCE cometabolism rates by placing the genes that code for the TCE-oxidizing enzymes under the control of starvation promoters. As such, the rate of enzyme expression is expected to increase significantly. They have now found that the nutrient demand and biomass formation required for the bioconversion of a unit of TCE or phenol by the enzyme when expressed under the control of *E. coli* starvation promoters were reduced by 60 to 90% compared with that of wild type *Pseudomonas*, leading to a 100-fold increase in TMO bioconversion efficacy. Studies by Daniel Arp, Michael Hyman, Kenneth Williamson, and student Roger Ely at Oregon State University formulated a mathematical model describing TCE cometabolism by a nitrifying microorganism, that includes their recent findings on the nature of the toxic effect of TCE degradation to the organisms themselves. This toxicity can have a significant effect on the rates of the process.

Further reflecting the broad interest of the WRHSRC in helping to solve the significant problems with soil and groundwater contamination from chlorinated solvents, a team of WRHSRC researchers, that includes faculty members Martin Reinhard and Paul V. Roberts, research associate Werner Haag, and graduate students Cindy G. Schreier and Naoko Munakata, have been investigating novel dehalogenation reactions using zero-valent metals and catalysts. They found the use of hydrogen gas and palladium as a catalyst for the hydrodehalogenation of chlorinated contaminants in water to be especially promising since reaction times are on the order of minutes. The group is now evaluating the use of Pd based reactors for the treatment of groundwaters contaminated with chlorinated solvents.

Reinhard together with Paul G. Tratnyek of the Oregon Graduate Institute organized the first symposium devoted to the science and engineering of contaminant remediation, especially chlorinated solvents, with zero-valent metals. This symposium took place as part of the 209th American Chemical Society National Meeting in Anaheim, California in April 1995. At the time the symposium was scheduled, the utility of zero-valent metals in remediation had just begun to attract broad interest. The symposium attracted a capacity audience throughout its two-days. A total of 40 papers were presented by 30 different research groups from the US and abroad. The meeting received wide attention and was featured in *C&E News* and the national press.

Kenneth Williamson, Associate Director of the WRHSRC, was responsible for organizing the 1995 Five Centers' Research Conference, which was held July 23 to 26 at Glneden Beach, Oregon. The Conference focused on the collective experiences within the five HSRCs about how to successfully conduct field research related to hazardous site remediation. Terry Hazen from the Westinghouse Savannah River Company, contractor to the U.S. Department of Energy, was the keynote speaker and began the conference with a description of their experiences with the Savannah River remediation project. Ten active field projects within the HSRCs were also presented and served as focal points for discussions of possible cooperative activities between the Centers. Total attendance at the Conference was 216 individuals, including 53 faculty members from the HSRCs.

Three new programs of importance were begun this year by the five HSRCs, the Minority Academic Institution (MAI) program, the Technical Outreach Services for Communities (TOSC) program, and the Research and Re-education for Department of Defense (R2D2)

program. At the WRHSRC with the MAI program, six research and technology transfer initiatives were undertaken with minority institutions. These were coupled with student and faculty visitations to Stanford and Oregon State Universities. Through the WRHSRC TOSC initiative, programs of advice were provided to the citizens represented by the Gold Country Coalition in Sutter Creek, California, and with the Concerned People of Onalaska in Chahelis, Washington. The Sutter Creek site resulted from arsenic in mine tailings that has been under a Superfund emergency response. The Chahelis site contains contamination from an old wood treating mill that is on the Superfund National Priority List. The R2D2 program has helped support the college education in the environmental field for several students from Stanford and Oregon State Universities, as well as providing significant funding for research projects in areas of vital interest to the Department of Defense site cleanup effort.

## **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 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**

<b><u>Project Description</u></b>	<b><u>Principal Investigators</u></b>	<b><u>End Date</u></b>	<b><u>Total Budget</u></b>
<b>Chemical Movement, Fate, and Treatment</b>			
Aquifer Remediation Design in the Presence of Kinetic Limitations	Steven Gorelick	1995	\$235,769
Determination of Macroscopic Transport Parameters for Biologically Reacting Solutes in Aquifers	Peter Kitanidis	1995	\$ 218,774
Redox Transformations of Inorganic Pollutants: Coupling to the Biogeochemical Matrix	John Westall James Ingle	1995	\$ 158,151
Radon-222 Method for Locating and Quantifying Contamination by Residual Non-Aqueous Phase Liquids in the Subsurface	Lewis Semprini	1995	\$143,079
Three-phase Flow in Fractured Media	Martin Blunt and Paul Roberts	1997	\$383,545
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
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<b><u>Project Description</u></b>	<b><u>Principal Investigators</u></b>	<b><u>End Date</u></b>	<b><u>Total Budget</u></b>
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
<b>Chlorinated Solvents</b>			
Transformation of Chlorinated Hydrocarbons by Reduced Metallocoenzymes—Kinetic Model Development and Applications to Environmental Systems	Martin Reinhard	1995	\$222,414
Reductive Transformation of Chlorinated Hydrocarbons by Reduced Ethenes Catalyzed by Vitamin B12--Mechanistic and Kinetic Studies	Martin Reinhard	1997	\$178,567
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	\$114,720

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
Transformation of TCE by Methanotrophic Biofilms	Perry L. McCarty	1995	\$123,272
Degradation of Chlorinated Aliphatic Compounds by Nitrifying Bacteria	Daniel Arp Kenneth Williamson Michael Hyman	1995	\$ 216,515
In Situ Treatment of Chlorinated Solvents	Perry L. McCarty	1996	\$225,000

<b><u>Project Description</u></b>	<b><u>Principal Investigators</u></b>	<b><u>End Date</u></b>	<b><u>Total Budget</u></b>
Moffett Field In-Situ Bioremediation Study in Support of Full-Scale Evaluation Application	Perry McCarty Gary Hopkins Mark Goltz	1995	\$ 394,834
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
<b>Aromatic Compounds</b>			
Microbial Degradation of Toluene under Sulfate-Reducing Conditions - The Role of Iron	Martin Reinhard	1995	\$ 233,075
The Effect of Environmental Conditions on Reductive Dechlorination Rates	Sandra Woods	1995	\$ 154,818
In-Situ Anaerobic Biological Treatment of Aromatics in Groundwater	Martin Reinhard, Gary D. Hopkins, and Alfred M. Spormann	1996	\$456,250

A Large Scale Model for Anaerobic Bioremediation of the Seal Beach Site	Martin Reinhard and Peter K. Kitanidis	1995	\$85,000
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
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

<b><u>Project Description</u></b>	<b><u>Principal Investigators</u></b>	<b><u>End Date</u></b>	<b><u>Total Budget</u></b>
<b>Heavy Metals</b>			
Lead Sorption, Transport, and Remediation in Natural Soils and Subsoils	Peter O. Nelson	1995	\$101,260
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	\$169,870

### 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	\$63,315
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	\$111,404

## TRAINING AND TECHNOLOGY TRANSFER PROGRAM SUMMARY

<u>Project Description</u>	<u>Principal Investigators</u>	<u>End Date</u>	<u>Total Budget</u>
Hazardous Waste Training	Kenneth J. Williamson Peter O. Nelson	1996	\$120,000
Continuing Education	Kenneth J. Williamson	1996	\$120,000
1995 Five Centers' Research Conference	Kenneth J. Williamson Perry L. McCarty	1995	\$100,000
Conference Sponsorship	Kenneth J. Williamson	1996	\$120,000
Evaluation of Strategies for Full Scale Bioremediation of the Seal Beach Site Using Anaerobic Microbial Processes	Martin Reinhard Gary Hopkins Peter Kitanidis	1995	\$ 106,250
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	\$149,849
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
Technical Outreach Services for Communities	Kenneth J. Williamson	1997	\$562,500

<b><u>Project Description</u></b>	<b><u>Principal Investigators</u></b>	<b><u>End Date</u></b>	<b><u>Total Budget</u></b>
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**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
Environmental Education Enrichment Project (EEEP)	R. Barry King Albuquerque Technical- Vocational Institute	1995	\$10,762
Bioremediation Technology Transfer Conferences at Albuquerque Technical-Vocational Institute	R. Barry King Albuquerque Technical- Vocational Institute	1996	\$34,375

## RESEARCH PROJECT DESCRIPTIONS

### CHEMICAL MOVEMENT, FATE, AND TREATMENT

#### **Aquifer Remediation Design in the Presence of Kinetic Limitations: Steven M. Gorelick, Stanford University**

Goal: The objectives of this study are (1) to recognize and quantify field-scale rate limitations on pump-and-treat aquifer remediation; (2) to predict the effects of contaminant mass transfer rate limitations during remediation; and (3) to develop practical guidelines for conventional and unconventional remediation strategies, such as pulsed-pumping, that efficiently remove rate-limited contaminants.

Rationale: In recent years, it has become clear that the cleanup of polluted groundwater has been less successful than originally anticipated. Conventional pump-and-treat methods have failed to remove the amount of contaminant mass that was once predicted. Although pumping tends first to remove high concentrations from an aquifer, it soon removes much lower concentrations, which are generally far above water quality standards, and can remain so for decades. Contaminants dissolved in mobile water move quickly toward the well, but slow desorption and diffusion of immobile contaminant limits the rate at which groundwater can be remediated. These rate-limiting diffusion and desorption processes have been extensively documented and are frequently cited as a fundamental reason for the failure of conventional remediation strategies. However, these processes have not been incorporated into the design of aquifer remediation, and the parameters governing mass transfer have not been estimated at the field-scale.

Approach: This study involves five stages: (1) development of a two-dimensional, advective-dispersive groundwater contaminant transport model that includes linear and nonlinear rate-limited mass transfer; (2) comparison of contaminant removal by continuous- and pulsed-pumping; (3) sensitivity analysis with different nonequilibrium models and rate parameters to develop efficient pump/rest cycles for pulsed-pumping; (4) development of a nonequilibrium rate parameter estimation method from standard field observations; and (5) formulation and development of an optimization procedure for design of pulsed-pumping remediation.

Status: This project is now complete. It was shown that pulsed pumping does not remove more contaminant mass than continuous pumping at the average rate, but that when properly engineered, pulsed pumping can remove as much contaminant as continuous pumping. A new model was developed of mass transfer that is shown to be a general form of the family of diffusion models and first-order mass transfer models. This "multirate" model is able to represent diffusional mass transfer within many physical and statistical combinations of immobile zones. The model was applied (1) to estimate statistical distributions of mass transfer rate coefficients in column experiments; (2) to investigate the effects on aquifer remediation in the presence of a heterogeneous mixture of clay layers, grain aggregates, and surface reactions; (3) and to develop methods for estimating mass transfer rate coefficients from field and laboratory data. In the first application, extreme variability must be invoked (exceeding six orders of magnitude) in diffusion rate coefficients to represent mass transfer within a relatively homogeneous porous medium. In the second application, it was shown that understanding the

variations in rates of mass transfer is likely the key to making accurate predictions of remediation times.

**Determination of Macroscopic Transport Parameters for Biologically Reacting Solutes in Aquifers: Peter K. Kitanidis, Stanford University**

Goal: This research aims to improve our understanding of scale effects on the transport and fate of biologically reacting chemicals in heterogeneous formations. Our objectives include: (a) to develop guidelines on how to relate field and laboratory data; (b) to explain how heterogeneity and diffusional rate limitations at a sub-scale may determine the observed rates of transport and transformation; and (c) to suggest reasonable macroscopic models.

Rationale: Soils and aquifers are composed of materials that are heterogeneous at all scales. Several studies have indicated the need for a quantitative understanding of the effect of spatial variability and sub-scale processes on the mechanism and the parameters that describe the transport and fate of reactive solutes at a certain scale. For example, it has been found that the dispersion coefficient obtained from a conservative nonreacting tracer test in the field can be considerably larger than the dispersion coefficient that applies to the transport of reacting solutes.

Approach: Macroscopic parameters are computed from microscopic ones using a volume averaging approach based on the Taylor-Aris-Brenner method of moments. We intend to examine some cases of upscaling from the pore scale to the laboratory scale and from the laboratory scale to the field.

Status: This project was completed in early 1995. The equations which govern the transport of a reactive solute at the macroscale have been derived using the Taylor-Aris-Brenner method of moments. The analysis provides the macroscopic coefficients of reaction, velocity, and dispersion which describe the transport of the volume averaged concentration. The results are not limited to any particular scale, but can be used to move between a small and large domain whenever there exists a disparity in spatial scales, such as from pore to Darcy scales, or from laboratory to field scales. As a particular application of the method, a two-dimensional pore scale model of a porous medium was created. The model captures some of the essential features of pore scale flow by having converging and diverging flow in pore throats and chambers. A numerical solution scheme was developed which solves for all the relevant macroscopic parameters. The solution technique is based on a new iterative solver and is reasonable fast and efficient allowing for many trial runs. Graphs have been made which show how the macroscale parameters are controlled by the underlying microscale mechanisms.

**Redox Transformations of Inorganic Pollutants: Coupling to the Biogeochemical Matrix: John C. Westall and James D. Ingle, Oregon State University**

Goal: The long-range goals of this project are: (i) to understand under what conditions redox transformations of inorganic pollutant species will take place and (ii) to develop sensors that can be deployed in-situ to indicate when these conditions prevail.

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

Approach: In characterizing redox transformations of inorganic contaminants, we consider three components: the redox-active inorganic contaminant, the geochemical matrix, and the redox sensor. Coupling of redox processes must be understood at three linkages -- contaminant and matrix, sensor and matrix, and contaminant and sensor. Each linkage must be investigated on a species-by-species basis, without the a priori assumption of equilibrium. The focus of this study is on transformations of Cr(III)/Cr(VI) in biogeochemical matrices of Fe(II), Fe(II)-minerals, Mn(IV)-minerals, and soils. In parallel experiments, transformations of redox indicators are investigated spectrochemically and electrochemically, toward the goal of developing alternative redox sensors that can be deployed in-situ.

Status: This project was completed in early 1995. A reactor system ("redox-stat") was developed, which can monitor redox conditions and control the redox status of a biologically active reactor at any selected level, making it possible to carry out a wide range of experiments under controlled conditions. A unique external loop allows for continuous filtering prior to on-line spectrophotometric measurement or measurement of dissolved species with other sensors. Numerous redox indicators have been electrochemically and spectrochemically characterized in simple solution experiments. Ten redox indicators which react reversibly with several reductants and oxidants were further studied. The selected indicators couple with a platinum electrode to "poise" the redox potential of the system over the EH range -450 to +300 mV at pH 7. The absorbance of the oxidized form of most indicators can be used to estimate the "redox" potential and predict the prevalent redox forms of Fe and Cr. In more complex systems with minerals or soil, adsorption of the indicators on the surface of the solids precludes spectrophotometric measurements. To alleviate this problem, two indicators have been immobilized on an affinity chromatography gel and packed in a flow cell for spectrophotometric monitoring in the external loop. The immobilized indicator thionine has redox characteristics similar to the "free" dissolved indicator and is reduced by components of a soil solution in the reactor with the potential measured at a Pt electrode falls below about 0 V and the concentration of Fe(II) is relatively high. Currently, the immobilized indicator is being studied in soil matrices in the laboratory in preparation for field applications.

### **Radon-222 Method for Locating and Quantifying Contamination by Residual Non-Aqueous Phase Liquids in the Subsurface: Lewis Semprini, Oregon State University**

Goal: The goal of this project is to develop a geochemical method for locating and quantifying Non-Aqueous Phase Liquid (NAPL) contamination in the subsurface. The method is based on the concentration of radon-222 in subsurface fluids.

Rationale: Locating and describing quantitatively the residual or pools associated with source areas of NAPL contamination in the subsurface remains as one of the major obstacles to implementing through site monitoring and effective remediation programs. Geochemical

methods may provide an inexpensive means of locating and quantifying NAPL contamination, and monitoring the progress of remediation.

Approach: Radon-222 concentrations in subsurface fluids may provide a means of locating and quantifying NAPL contamination in the subsurface. The method is based on radon's unique properties including: its production from the decay of radium-226 in subsurface materials, its radioactive decay with a half-life of 3.83 days, and its preferential partitioning into organics compared to water. Equilibrium model calculations indicate that radon would become depleted in the aqueous phase during transport through a NAPL zone. As the NAPL saturation increases the decrease in radon concentration compared to the background concentration would also decrease. Thus, changes in the radon concentration in an aquifer might be used to locate and quantify NAPL contamination.

Status: The method has been evaluated in field, laboratory, and modeling studies. The equilibrium partitioning model has been confirmed in the laboratory studies. Results from field studies have shown that the degree of residual saturation can be determined using the radon method, when the NAPL is fairly uniformly mixed in the subsurface. When NAPLs are distributed heterogeneously in the subsurface, radon measurements in the mobile fluid indicate NAPL presence over short spatial scales. A main limitation of the method is the reequilibration of radon in the mobile fluid once the fluid is no longer in contact with the NAPL. This has been confirmed in the field and in the modeling studies. Thus the radon method is useful close to the source of NAPL contamination. Our recent work therefore has focused on using radon to monitor the progress of NAPL remediation. Laboratory column studies have shown that increases in radon concentration and decreases in the retardation of injected water lacking radon, accompany surfactant induced NAPL remediation. Thus, once a NAPL zone has been located and is being remediated, monitoring the changes in radon concentration in mobile fluid as remediation progresses is a promising, non intrusive means, of determining the progress of remediation.

### **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 are under way, with three fractured cores prepared and preliminary scans already performed to determine optimal scanning conditions. The numerical modeling of the experiments will begin early in 1996, according to the original plan.

**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: Stage 1 of the study has been completed. The preliminary network model was fine tuned to better simulate capillary pressure curves. Flow is now included in the network model and the model can represent absolute and relative permeabilities. Next, NAPL dissolution will be studied as a function of capillary pressure, saturation, interfacial area, flow rate, permeability, and relative permeability. To represent NAPL concentration in the network model, a new analytic solution is being developed for solute transport in a tube of finite length that is applicable over a wide range of flow rates. This analytic solution will be applied to represent solute transport in the network model tubes. This, together with a set of rules based on an assumption of complete mixing to govern solute partitioning at chamber intersections, will represent solute transport in the entire network model. A residual NAPL blob will then be established in the network model and its dissolution will be monitored over time as a function of concentration and the other phenomena mentioned above.

**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 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 will be 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. The analytical dispersion model previously developed that accurately represents the dispersion phenomena within the atmospheric boundary layer has recently undergone further modifications to account for inversions and partial absorption by the ground.

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

Goal: We want: (a) to improve our understanding of hydrodynamics, mass transport, and biochemical reactions at the pore scale and (b) 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 by Professor McCarty and his co-workers in another project. Our 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: Our fundamental understanding of the mechanisms and processes affecting 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, we make measurements and wish to make predictions and assessments at a much larger scale. The most scientific approach is to derive relations among macroscopic variables by scaling up from the pore scale.

Approach: Our approach is to study pore-scale hydrodynamics and rates of mass redistribution in detail at the pore scale. Our research includes using idealized geometrical models of a porous media to obtain order of magnitude estimates of variables and general methods of scaling up to derive relations among macroscopic variables.

Status: The project started in March 1995 and during the first six months focus was on studying pore-scale hydrodynamics and developing solutions for velocity and pressure distribution in the medium used in the laboratory experiments. Despite the relative simplicity of the geometry, an accurate analytical solution has not yet been derived. Currently numerical methods are being applied and tested.

**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 our 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 (i) 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, (ii) sensitivity analysis for the chemical components/reactions and hydrologic variables essential to the development of a robust model of field-scale phenomena, and (iii) quantitative model performance evaluation based on both statistical and graphical criteria.

Status: Simulations of Zn transport at the Cape Cod site have set a foundation for the Fe transport simulations by (i) providing an analysis of the numerical stability of potential finite-element meshes; especially the limitations geochemical gradients place on spatial and temporal discretization, (ii) providing an analysis of the appropriateness of boundary conditions, and (iii) providing needed experience in analyzing highly nonlinear coupled solute transport/geochemical simulations. Development of the complete reaction set that controls the distribution of dissolved Fe in the anoxic/Fe zone is currently underway.

**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 numerical model has been developed to describe mass transfer between trapped gas and dissolved gas in groundwater flowing through the wall. The model includes processes of advection, dispersion, and diffusion-limited mass transfer and has been verified using a poorly graded sand and several trapped and dissolved gas pairs for a wide range of trapped gas volumes and pore water velocities. Important findings were that mass transfer is extremely rapid at low velocities, mass transfer increases and porous media dispersivity decreases linearly with increasing trapped gas. The laboratory experiments have shown that all three methods tested for emplacing trapped gas into a saturated porous media (injection of gas, injection of water supersaturated with gas and injection of a hydrogen peroxide solution) have been effective in trapping a significant volume of gas (14-27% of the pore space) into fine to coarse sand. The volume of gas trapped is greater when the gas exsolves out of solution as occurs with the injection of water supersaturated with gas and injection of a hydrogen peroxide solution. The reduction in hydraulic conductivity,  $K$  due to the trapped gas ranges between 1.6 to a maximum of 2.8 ( $K_{sat}/K_{trapgas}$ ) and follows the van Genuchten-Mualem model for hydraulic conductivity as a function of moisture content.

**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: (i) to determine the "best" way to measure the redox conditions of the subsurface environment and (ii) 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: (i) evaluation of disposal options, (ii) risk assessments of contaminated sites, and (iii) 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, we consider three components: 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, we are evaluating different methods of determining the redox status of the biogeochemical matrix such as the redox state of immobilized redox indicators, platinum electrode potential, concentrations of soluble matrix components (e.g., Fe(II)), and H<sub>2</sub> concentration.

Status: A sophisticated reactor system ("redox-stat") developed previously is being used to monitor redox conditions and control the redox status of biologically-active slurries in the reactor. A unique external flow loop provides a continuous flow of filtered sample for other measurements. This allows on-line monitoring of the absorbance (and hence fraction reduced) of organic redox indicators immobilized on affinity chromatography gel beads (50- $\mu$ m diameter) which are packed into a spectrophotometric flow cell. Two redox indicators, thionine and phenosafranine, retain their redox activity when immobilized and react reversibly with important oxidizing and reducing agents such as O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>, Cr(VI), and Fe(II) which do not couple well with a platinum (Pt) electrode. Hence, the absorbance of the oxidized form of the indicator can be used to estimate the "redox" potential and predict the prevalent forms of Fe, Cr and As. When applied to soil slurries, immobilized thionine is reversibly reduced and oxidized when the potential at a Pt electrode is cycled between about 0 and 100 mV (vs. SHE) at pH 7. In addition, the increase in Fe(II) concentration and reduction of the indicator at lower redox potential were correlated to an increase in the phosphate concentration. Experimental data strongly support the hypothesis that the indicator is reduced by Fe(II) in solution even though Fe(II) does not appear to poison the Pt electrode. Most recently, we have been able to immobilize thionine on larger-diameter gel beads (200-1000  $\mu$ m) which will help to decrease the pressure build up in flow cells and advance our development of field useable redox probes and fiber optic redox sensors. In addition, four new, reversible redox indicators have been successfully immobilized on the gel beads, increasing the range of redox conditions we can evaluate.

### **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 two-year 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 is being improved for better visualization and for more automation in data collection so more data on the process can be obtained for evaluation.

## **CHLORINATED SOLVENTS**

### **Transformation of Chlorinated Hydrocarbons by Reduced Metallocoenzymes—Kinetic Model Development and Applications to Environmental Systems: Martin Reinhard, Stanford University**

Goal: This project was aimed at improving our understanding of the chemical reactions occurring between chlorinated hydrocarbons and metallocoenzymes and to assess the possibility of using metallocoenzymes as catalysts in treatment systems.

Rationale: Transition-metal coenzymes such as vitamin B<sub>12</sub> occur at high levels in some anaerobic microorganism which are often found to reductively dechlorinate CAHs. They have been hypothesized to catalyze the reductive transformation of halogenated organic contaminants in biological systems. Knowing the chemistry of these reactions is therefore important for understanding and optimizing the biological detoxification/activation processes. Metallocoenzymes have also been proposed as catalysts for treatment systems.

Approach: This project entails kinetic studies of the transformation of carbon tetrachloride (CTEY) by metallocoenzymes which are reduced either by a thio-reductants (cysteine) or titanium citrate. Experiments are conducted in aqueous solutions containing excess reductant, a

cofactor, either hematin or vitamin B<sub>12</sub> and a pH buffer. The disappearance of CTET and the appearance of products, primarily chloroform and carbon monoxide, are measured as a function of pH. Radiolabeled <sup>14</sup>CCl<sub>4</sub> is used to evaluate mass balances.

Status: The project has been completed March 1995. Kinetic and mechanistic models for the dechlorination of carbon tetrachloride were developed for both metallocoenzymes and both reductants. Mechanisms for the deactivation of the metallocoenzymes were proposed. Significant findings are: (1) the Michaelis-Menten kinetic model applies only if deactivation is insignificant; (2) the type of reducing agent significantly affects product distribution, rate and mode of deactivation; (3) B<sub>12</sub><sub>r</sub> thiolate type complexes are likely involved in microbial cometabolic microbial dechlorination; (4) application of metallocofactors for treatment of carbon tetrachloride is limited by the significant inactivation by CTET metabolites. These models serve as an essential knowledge base for future studies to understand cometabolic reductive dechlorination.

### **Reductive Transformation of Chlorinated Hydrocarbons by Reduced Ethenes Catalyzed by Vitamin B<sub>12</sub> — Mechanistic and Kinetic Studies Martin Reinhard, Stanford University**

Goal: This project aims to (1) determine transformation products and pathways of chlorinated ethene transformation by vitamin B<sub>12</sub> under different reducing conditions, (2) develop kinetic models describing the transformation, and (3) elucidate the mechanism.

Rationale: Transition-metal coenzymes such as vitamin B<sub>12</sub> are presumed to be responsible for the cometabolic dehalogenation of chlorinated ethylenes in microbial systems. Detailed knowledge of chlorinated ethylene transformation with reduced B<sub>12</sub> is important to understand and optimize the biological detoxification processes and to develop novel remediation strategies. To date, detailed kinetic and mechanistic models have not been developed and the mechanism has not been elucidated. Earlier studies have focused on carbon tetrachloride as the substrate and the type and strength of the reductant. In this project, we are focusing on chlorinated ethylenes as the substrates.

Approach: Reactions were studied in homogeneous aqueous solutions containing a pH buffer, a reductant, and a vitamin B<sub>12</sub>. 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. Head space samples were taken and disappearance of substrate (chlorinated ethylenes) and formation of products (ethane, ethene, acetylene) were monitored over time on an HP 5890 GC/ECD and FID. In the future, rate constants and product yields will be evaluated over a broad range of pH. To elucidate the transformation pathways, isotopic experiments will be carried out and the products formed in deuterated media will be identified using a mass spectrometer. UV-visible spectra of vitamin B<sub>12</sub> and its derivatives will be obtained before and during the reaction using an HP 8451A Diode Array Spectrophotometer.

Status: The project started in March 1996. During the first few months, we have developed a GC method to analyze the full range of products and intermediates. Currently, efforts are

underway to obtain a mass balance, develop rate laws under different conditions, and observe the formation of intermediates.

**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 *Nitrosomonas europaea* to the toxicity associated with the cometabolism of trichloroethylene.

Rationale: In situ bioremediation schemes have a considerable need for mathematical models which 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. In summary, cells are exposed to TCE and other chlorinated solvents in small (10 ml) vessels containing 1 ml of medium. 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 to examine whether the model accurately describes the cometabolism of TCE by other organisms. Initial efforts to overcome some of the technical problems associated with conducting similar experiments with methane-

oxidizing bacteria. The challenge is to develop a suitable methane delivery system which will allow similar experiments to be conducted at quasi steady state conditions.

The experiments aimed at examining the responses of *N. europaea* to the toxicity associated with TCE cometabolism have revealed that the loss of ammonia dependent O<sub>2</sub> uptake activity after exposure to TCE does not correlate with an equivalent loss of ammonia monooxygenase activity in the cells. Rather than inactivating ammonia monooxygenase in a similar fashion to a suicide substrate, the toxic effect of TCE appears to be a loss of integrity in the electron transport system. The effects of TCE cometabolism on the rates of electron transport are being examined using non-physiological electron donors such as tri- and tetra-methyl-hydroquinone. The cometabolism of TCE has an effect on hydroxylamine oxidoreductase activity. Also examined was the response of this organism to H<sub>2</sub>O<sub>2</sub> which is a potent inactivator of HAO. The results suggest that there is little similarity between the response of *N. europaea* to these two treatments. The effects of TCE cometabolism are more non-specific than originally thought and add support to the argument that TCE cometabolism directly affects the efficiency of electron transport in *N. europaea*.

**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 C<sub>1</sub> and C<sub>2</sub> 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. The first approach is to define the overall pathway of gaseous hydrocarbon oxidation by *Graphium*. The general approach has involved determining the range of hydrocarbons and potential oxidation intermediates which support the growth of this organism. Also, the ability of *Graphium* to grow on simple ether-bonded compounds such as diethyl ether is being investigated. In addition, the

mode of action of a series of unsaturated hydrocarbons which selectively inhibit growth of this organism on gaseous hydrocarbons is being investigated.

The second approach which involves examining the ability of *Graphium* to degrade chlorinated solvents through cometabolism. The experimental approach will be to cultivate *Graphium* on gaseous n-alkanes and to then conduct short-term (< 1h) incubations in the presence of suitable concentrations of each chlorinated hydrocarbon.

Status: The studies have demonstrated that low concentrations of acetylene, propyne, butyne, ethylene and propylene (0.5% vol/vol gas phase) all completely inhibit the growth of *Graphium* on either ethane, propane, or butane (10% vol/vol gas phase). In contrast, none of these compounds have any affect on the growth of *Graphium* on Potato Dextrose or on likely intermediates in the ethane oxidation pathway such as ethanol or acetate. Acetylene and ethylene (and their close homologs) are all known as mechanism-based inactivators of cytochrome P-450 type monooxygenases. Taken together, these results suggest that *Graphium*, like other fungal species, brings about the oxidation of saturated hydrocarbons through the activity of an n-alkane-induced cytochrome P-450 type enzyme system. *Graphium* is the first organism obtained in pure culture which is capable of growth on diethyl ether as sole carbon and energy source. Acetylene completely prevents growth of *Graphium* on diethyl ether but does not affect growth on the most common contaminants in diethyl ether such as ethanol and acetaldehyde. Preliminary evidence also suggests *Graphium* is capable of growth on other ether-bonded compounds including propyl, ethyl propyl and butyl ether.

### ***Pseudomonas* Starvation Promoter-Driven *In Situ* Bioremediation: Abdul 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 pollutant environment exhibits low metabolic activity which is most likely due to slow growth as a result of stress conditions. 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 TMO bioconversion efficacy (i.e. the amount of contaminant 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 our 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 the 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*, we are now constructing a *Pseudomonas* strain with high bioconversion efficiency for trichloroethylene biodegradation; *Pseudomonas* species are indigenous to nearly all contaminated environments.

Status: A strong starvation promoter from *P. putida* has been recently cloned and characterized in our lab. (Kim, et al., 1995). We are currently working on the construction of expression vectors carrying TMO gene cluster under the control of a *P.putida* starvation promoter. Optimization of expression of active TMO 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 laboratory 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 cultures are very effective at degrading CF and TCA. Cometabolism is associated with butane-utilization and requires oxygen. Essentially complete dechlorination of chloroform have been observed, with yields (mg CF/mg butane) of several percent. Initial observations indicate that TCA is even more effectively transformed. TCE is degraded by butane-utilizers, but at slower rates and to lesser extents than CF and TCA. Propane-utilizers degrade CF and TCA at slower rates and to lesser extents than butane-utilizers, however they more effectively degrade TCE. An enrichment culture has been obtained from

the butane-fed microcosms of Hanford core material. The cultures are dominated by a single type of bacterium and grow with butane as the sole carbon and energy source. The cultures degrade chloroform. Chloroform degradation is inactivated by acetylene, inhibited by butane, and is dependent upon O<sub>2</sub>. Butyrate (a metabolite of butane) stimulates chloroform degradation. The results support a cometabolic mechanism for chloroform degradation. The enrichment culture was also grown on pentane, hexane, butanol, butyric acid, acetate and glucose. Substantial rates of chloroform degradation were only observed with cultures grown on pentane, hexane or butane. A pure culture of *Pseudomonas butanovora*, obtained from ATCC, was grown on butane and examined for its ability to degrade chloroform. The cultures degraded chloroform at rates similar to those observed for the enrichment culture. However, some differences in the apparent affinities for chloroform and butane were observed.

## **Transformation of TCE by Methanotrophic Biofilms: Perry L. McCarty, Stanford University**

Goals: The goals of this project are: (1) to develop and evaluate the overall performance of a biofilm model for cometabolic chlorinated aliphatic hydrocarbon (CAH) biodegradation, (2) to experimentally determine the minimum substrate concentration ( $S_{min}$ ) required for biofilm growth and the factors affecting it, and (3) to experimentally determine the rate at which methanotrophic organisms are inactivated within biofilms.

Rationale: Both in-situ and above ground biotransformation of CAHs by methanotrophs and phenol-oxidizing organisms have been studied for treatment of CAH-contaminated waters. Here, attached microorganisms or biofilms are operative. To date, observed CAH transformation rates by biofilms are orders of magnitude slower than maximum rates determined from dispersed growth studies. Many reasons for this difference have been suggested, including inhibition by methane or phenol, CAH transformation product toxicity, exhaustion of reducing power, diffusional constraints, and the presence in biofilms of only a small active biomass population. Because of the many interacting processes involved, the problem is complex and the major processes of importance are difficult to determine.

Approach: A biofilm model that considers cometabolism of CAHs in the presence of the growth substrate, either methane or phenol, is being evaluated to examine the relative significance of the different biofilm processes and to predict their likely impact on CAH transformation rates. Trichloroethylene (TCE) is being used for model simulations and experimental studies. The model is being used for hypothesis testing, and to indicate the processes of importance that are in need of further evaluation. Simulations have indicated the importance of the minimum substrate concentration required for bacterial growth ( $S_{min}$ ) and the rate of decay of active biomass. Factors affecting these process parameters will be experimentally evaluated.

Status: This project was completed this year. A methanotrophic biofilm model was developed in which Monod kinetics with competitive inhibition was used to describe the oxidation of methane and TCE by the organisms in the biofilm. The model includes diffusion, active biomass growth, inactivation, decay, and CAH transformation product toxicity. Simulations suggest that a maximum TCE flux exists with respect to methane concentration. At low methane concentrations (approaching  $S_{min}$ ), there is insufficient methane for net growth of the biofilm. At high methane concentrations, competitive inhibition limits TCE transformation. This factors plus others such as transformation product toxicity and availability of reducing power are expected to affect  $S_{min}$  and organism growth rate in general. An experimental procedure for determining  $S_{min}$  and organism growth rate was developed, and consists of monitoring the methane concentration changes with time in cultures seeded with a small methanotrophic biomass inoculum. The Monod model was found to provide excellent fits to batch kinetics for methane utilization with organisms expressing particulate methane monooxygenase ( $MMO_p$ ) and over four orders of magnitude in initial biomass concentration and more than two orders of magnitude of methane concentration. It was found that the presence of chlorinated aliphatic hydrocarbons (CAHs), such as trichloroethylene, *cis*- and *trans*-1,2-dichloroethylene, 1,1-dichloroethylene, and vinyl chloride, increased  $S_{min}$  and

decreased organism growth rate. The factor affecting these changes was different for the different CAHs, with 1,1-dichloroethylene producing the greatest transformation product toxicity, and both trans-1,2-dichloroethylene and vinyl chloride significantly reducing the reductant supply (NADH) for regeneration of MMO activity. Competitive inhibition was also effective with trichloroethylene and some of the other CAHs. Thus, no easy generalizations can be made about which process is affected the greatest by different CAHs, each requires an individual evaluation.

**Degradation of Chlorinated Aliphatic Compounds by Nitrifying Bacteria: Daniel J. Arp, Kenneth J. Williamson and Michael R. Hyman, Oregon State University (Partially Supported by the U. S. Department of Energy)**

Goal: The objectives are (1) to determine the rates of cometabolic degradation of chlorinated hydrocarbons by ammonia-oxidizing bacteria and to compare these rates with those obtained with other bacterial types such as methanotrophic bacteria; (2) to characterize the physiological response of nitrifying bacteria to the toxicity associated with cometabolic degradation of chlorinated compounds; and (3) to develop and optimize reactor designs suitable for using nitrifying bacteria in cleanup processes.

Rationale: Autotrophic nitrifying bacteria have several features which make them attractive candidates for use in bioremediation schemes. First, they possess a non-specific monooxygenase enzyme which is capable of oxidizing a large number of non-growth supporting substrates. Second, because ammonia is very water soluble it can be introduced into systems at high concentrations, thereby supporting high rates of cometabolic oxidation. Third, although these bacteria achieve high rates of substrate oxidation they exhibit low rates of biomass accumulation. The cometabolism of chlorinated aliphatic compounds by nitrifying bacteria involves aspects of all three of these features. Characterization of the bioremediation potential of these bacteria will involve a clear understanding of the individual significance and interrelation of these factors.

Approach: The rates of substrate (ammonia) and cosubstrate (chlorinated aliphatic) degradation will be determined using kinetic models that will allow (a) maximal specific rates and (b) most sustainable rates of cosubstrate oxidation. Metabolic toxicity which results from the cometabolic oxidation of chlorinated aliphatics will be investigated by quantifying the relationship which exists between the amount of cosubstrate oxidation and the extent of inactivation of individual enzyme activities. The ability of nitrifiers to recover from these toxic effects will also be determined. Using results from these physiological experiments various reactor designs will be studied and compared so as to determine an optimal configuration.

Status: This study was completed in early 1995. For the first objective listed above both reactor-size (1l) and small scale (1ml) studies have been completed and used to determine the rate of TCE cometabolism by *Nitrosomonas europaea*. These studies have led to the development of a kinetic model which describes the effects of TCE on *N. europaea* in terms of three individual parameters: (1) the inhibition, (2) the inactivation, and (3) the recovery of AMO activity. Other chlorinated substrates including chloroform, 1,1-dichloroethylene and 1,2-dichloroethane have been examined to determine the general applicability of the model derived for TCE.  $^{14}\text{CO}_2$  uptake experiments have been used to monitor protein synthesis during

cometabolism. These studies aim to determine what proportion of cometabolism is due to preexisting and newly synthesized AMO. An investigation of how cometabolism affects the expression of AMO has recently been initiated. TCE-dependent inactivation of AMO activity does not directly correlate with an equivalent loss of catalytically active AMO. This loss of AMO activity profoundly alters the number and type of polypeptides synthesized by the cells in the presence of ammonia. These preliminary studies suggest that the cellular response to TCE toxicity is more complex than the simple replacement of lost enzyme activity.

**In Situ Treatment of Chlorinated Solvents: Perry L. McCarty, Stanford University (Supported by DuPont Chemicals and Monsanto)**

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: This project was completed, but the sponsor has now approved for funding additional study with similar objectives. 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 in our culture, the first group that converts PCE to c-DCE as represented by strain MS-1, and the second group that converts c-DCE to vinyl chloride and ethene. 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. Monod kinetics of the reactions involved are being evaluated through the extension of the project.

**Moffett Field In-Situ Bioremediation Study in Support of Full Scale Application: Perry L. McCarty, Gary D. Hopkins, and Mark N. Goltz, Stanford University (Supported by the U. S. Air Force)**

Goal: To obtain supporting information for the proposed full-scale evaluation of in-situ aerobic biodegradation of chlorinated aliphatic hydrocarbon (CAH) compounds at an Air Force facility with respect to the possible presence of a suitable biodegrading population, the effectiveness for 1,1-dichloroethylene (1,1-DCE) degradation, the appropriate chemicals for addition, and methods for optimizing field operations.

Rationale: In order to conduct a full-scale evaluation of in-situ bioremediation of chlorinated solvent contamination, as discussed in a companion project which applies bioremediation technology to a contaminated Air Force site, supporting information from the laboratory and a controlled field site is required. This project complements the full-scale study by providing these necessary data.

Approach: Recent studies conducted at the Moffett Field site have demonstrated that trichloroethylene (TCE) can be effectively biodegraded cometabolically through the introduction into the subsurface of a primary substrate (such as phenol) and oxygen to support the growth and energy requirements of a native population of microorganisms. However, several tasks remain before the process can be implemented at full scale. These tasks are: (1) to evaluate if a CAH degrading population similar to that found at Moffett Field is present at the site, (2) to evaluate how the presence of 1,1-DCE will impact the efficacy of TCE degradation, (3) to evaluate the effectiveness of alternative substrates, such as toluene, (4) to evaluate the effectiveness of hydrogen peroxide as an oxygen source, and (5) to determine the optimum approach for full-scale field evaluation of in-situ CAH biodegradation for application at the site. Studies in the laboratory and the Moffett Field site will be conducted to answer these questions, prior to and in conjunction with implementation of a full-scale field study.

Status: This project was completed in early 1995. Laboratory microcosm studies of cores taken from the demonstration site at Edwards AFB (see the companion project titled Full-Scale Evaluation of In-Situ Bioremediation of Chlorinated Solvent Groundwater Contamination) have shown that the microorganisms at the site are capable of aerobically cometabolizing TCE in the presence of either phenol or toluene. From studies at Moffett Field, it was seen that the presence of 1,1-DCE seriously reduced the effectiveness of oxidative biodegradation of TCE, probably due to 1,1-DCE transformation product toxicity. Therefore, one of the selection

criteria that was used in choosing Edwards as the demonstration site was that 1,1-DCE not be present as a co-contaminant. Toluene was shown to be as effective as phenol for cometabolism of CAHs, and due to regulatory considerations, will be used for the full-scale demonstration. Hydrogen peroxide, which was found to be a suitable alternative to pure oxygen as a source of oxygen for in-situ biodegradation, will also be used in the full-scale demonstration. Based on the Moffett Field results, the plan at Edwards to demonstrate in-situ aerobic cometabolism of TCE is to pulse in a primary substrate, toluene, while continuously adding hydrogen peroxide as an oxygen source.

**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 (Supported by R2D2 Program)**

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 we 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 B<sub>12</sub>. 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 finger printing, 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 two-year 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. Thus, two facultative strains with this ability are now known. 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 just beginning.

## AROMATIC COMPOUNDS

### **Microbial Degradation of Toluene Under Sulfate-Reducing Conditions--The Role of Iron: Martin Reinhard, Stanford University**

Goal: The goal is to explore two phenomena that are potentially important to remediation of gasoline-contaminated aquifers but are not well-studied: sulfidogenic toluene degradation and the stimulation of this process by iron. These phenomena have been observed in bacterial enrichment cultures originally inoculated with fuel-contaminated soil collected near the Patuxent River (Maryland). The emphasis is on elucidating degradation pathways and the nature of iron's effect. The isolation of a pure sulfidogenic, toluene-degrading culture is an objective that would facilitate the study of these processes.

Rationale: Toluene, a U. S. EPA priority pollutant, is a water-soluble constituent of refined petroleum products (e.g., aviation fuels and gasoline) and is a widespread contaminant of groundwater near leaking underground storage tanks, landfills, and other uncontrolled releases of fossil fuels. The ability of native bacteria at such contaminated sites to degrade toluene has important environmental implications; such organisms are probably the primary determinant of the rate of decontamination in the absence of remediation. If remedial action is taken, such organisms may be an important factor in the remediation scheme. The development of anaerobic conditions and the presence of sulfate in contaminated aquifers could foster sulfidogenic toluene degradation. Iron, the fourth most abundant element in the earth's crust, could have an important effect on the rate of sulfidogenic degradation of hydrocarbons.

Approach: Experiments have been performed to explore the importance of sulfide removal in the absence of iron; for example, zinc was substituted for iron in enrichment cultures to see whether toluene degradation would be stimulated. In addition, studies were made to determine whether iron stimulates the degradation of metabolic intermediates of toluene degradation. This required initial investigations with gas chromatography-mass spectrometry to identify metabolites of toluene degradation. Serial dilutions with toluene in liquid medium were used to isolate a toluene-degrading bacterium from enrichment cultures.

Status: This project was completed in early 1995. Experiments with mixed enrichment cultures that compared the effects of adding ferrous sulfate vs. zinc sulfate demonstrated that sulfide toxicity, not a nutritional iron limitation, is the factor being ameliorated by the presence of iron

in the cultures. This result applies both to toluene and to benzoate, a transient intermediate of toluene degradation. A novel sulfate-reducing, toluene-degrading organism, strain PRTOL1, was isolated from the enrichment cultures. Characterization of this organism was conducted. Mineralization of toluene to CO<sub>2</sub> by strain PRTOL1 has been demonstrated with radiolabeled toluene, and the stoichiometry of sulfate consumed relative to toluene consumed has been shown to be consistent with theoretical predictions. Benzylsuccinic acid and benzylfumaric acid, two dead-end metabolites that were identified as minor products of toluene degradation in the enrichment cultures, are also produced by strain PRTOL1. The ability of strain PRTOL1 to utilize a range of electron donors and acceptors has been tested using cell suspensions. The 16S rRNA gene sequence of strain PRTOL1, which was analyzed at Michigan State University, has shown that PRTOL1 is indeed a novel bacterium.

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

Goal: The goal of this project is 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 will test the hypothesis that the rate of reductive dechlorination reactions depend upon the apparent redox potential, or EH, 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 biomass, pH, sulfate, sulfide, and acetate concentrations. In the second phase, batch experiments are being conducted to measure degradation rates under various, controlled environmental conditions. A model anaerobic system fed acetate, pentachlorophenol (PCP), and nutrients has been selected for study.

Status: This project was completed in early 1995. A reactor system was developed to control and/or monitor pH, apparent oxidation-reduction potential, and acetate, biomass, sulfate, and sulfide concentrations. An additional platinum electrode was added to monitor apparent redox measurements and to help identify possible poisoning of the controlling platinum electrode. Both hydrogen peroxide and potassium ferricyanide were used to raise and control the apparent redox potential above the “natural” system potential of -250 to -260 mV, and both titanium citrate and hydrogen were used to lower the apparent redox potential. Pentachlorophenol dechlorination were modeled as first order with respect to PCP concentration. When multiple PCP additions were made, without manipulating redox potential, the rate of dechlorination was observed to increase with the number of PCP additions. This suggests either induction of enzyme(s) or growth of a sub-population responsible for dechlorination. Overall biomass concentration changes relatively little during a typical experiment. The trend of increasing dechlorination rate with multiple PCP additions was shown to persist during “mild” redox potential increases of 50 to 150 mV. However, dramatically decreased dechlorination rates were measured during 250 mV perturbations. PCP dechlorination continued at elevated redox potentials that essentially halted acetate consumption and methane production, lending support to the hypothesis of a separate dechlorinating sub-population.

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

Goal: The goal of this project is to (1) assess the potential for transformation of these compounds using alternate electron acceptors under field conditions, 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 control anaerobic biotransformation of BTEX compounds is important.

Approach: The controlled release experiments entailed slug tests in which a single well was used for the injection of the "slug" (approximately 1000L) containing known concentrations of BTEX compounds and electron acceptors. After incubation from days to months, water samples were withdrawn from the test zone to evaluate biotransformation of the BTEX compounds. The different electron acceptors investigated (e.g., nitrate, sulfate and carbon dioxide) were added as needed. 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 this project year, replicate controlled releases of BTEX compounds under intrinsic, nitrate-reducing, sulfate-reducing and methanogenic conditions have been completed. BTEX removal under intrinsic conditions seem slow and inhibited by unknown factors. The nitrate-reducing controlled release experiments demonstrated rapid removals of toluene, m-xylene and ethylbenzene and slow removal of o-xylene. Benzene was relatively stable. Under sulfate reducing conditions, removals of toluene, m- and o-xylenes were rapid and removals of ethylbenzene was slower. Benzene was removed slowly but did not show a characteristic curve for biological removal. Under methanogenic conditions, removals for toluene, m-xylene and o-xylene were fast whereas removals of benzene and ethylbenzene were slow and gradual. p-Xylene was tested only under methanogenic conditions where its removal was slow but significant. The results of laboratory studies are generally consistent with results from the field. Laboratory studies focused on growth studies to obtain the generation times for two strains grown on a single substrate. Cell suspension studies were conducted to obtain the specific rate of utilization of the single substrate, toluene, by toluene-grown cells. Additional cell suspension studies are in progress to determine the specific rates of utilization of m-xylene by toluene-grown cells and by m-xylene-grown cells. Results from these experiments will help evaluate whether the enzymes for m-xylene degradation are constitutive or induced.

**A Large Scale Model for Anaerobic Bioremediation at the Seal Beach Site: Martin Reinhard and Peter K. Kitanidis, Stanford University**

Goal: The goal of this project is to develop a general transport model applicable to the Seal Beach site, (2) identification of additional hydraulic data needs, (3) incorporation of biotransformation rate expressions into the overall model.

Rationale: Increasingly, intrinsic and anaerobic biotransformation are considered an option or the remediation of fuel-hydrocarbon contaminated sites. Modeling tools are needed which predict the fate of BTEX contaminants under different geochemical conditions and for the design of monitoring programs.

Approach: Computer models are being developed to interpret contaminant behavior in the test zone and to model processes at the regional scale. MODFLOW and BIOPLUME II will be used to model the processes at the regional scale. These model will allow us to organize the available data at the regional scale and to evaluate possible regional response scenarios to the purposed remediation techniques. Two separate computer based models are being developed to model the treatment zone in detail. The first of this models is relatively simple and will be used in the planning and design stages of the scaled up project. The model assumes that the aquifer is homogenous and neglects dispersion and transformation reactions. The model calculates the flow field analytically and then uses this flow field to calculate solute travel times and the zone of injection influence. Although the model is idealized, it will aid in placing the observation wells. A second model considers the transport and reactions of multiple-species including the BTEX compounds, the electron acceptors and sorption onto and desorption from the aquifer solids. This model will be use to evaluate a number of possible response scenarios within the test zone. The model will also aid in estimating the bulk rate of biodegradation and in understanding what factors influence this rate and how this rate differs from those observed in the laboratory or at the small scale slug tests.

Status: The computer model for the evaluation of slug tests with reactive solutes has been completed. It has been used to match reaction rates to the observed response. The two models of the processes occurring within the scaled up test zone are working. Currently reaction parameters derived from the previous tests and model are incorporated in the new models. As more data is gathered from the site and as our ability to describe the processes occurring at the site improves the models will be modified to reflect this.

**The Effect of Apparent EH, 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.

Rationale: The reduction of nitro groups of trinitrotoluene and other nitro-aromatic congeners is a common microbiological process.

Approach: This research will examine the relative rates of reduction as they are dependent on compound structure, EH, and electron donor. The project has two phases. In the first phase, the feedback controlled reactor that was being used to study the effect of the environmental

conditions on reductive dechlorination is being modified to study the TNT/nitrate/lactate system. In the second phase the reactor will be used to evaluate biotransformation pathways and kinetics for TNT, its metabolites, and related compounds under varying conditions of EH, nitrate, and electron donor.

Status: Phase 1- Development of a reactor system. An HPLC method has been developed to measure the concentration of TNT and its metabolites. A serum bottle study has been performed measuring the relative rate of nitro-group reduction based on parent compound structure. EH was lowered using titanium citrate in a serum bottle study to monitor the abiotic transformation of TNT. This information is now being used to develop the reactor system.

**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: Cell growth experiments with PRTOL1 revealed that toluene-metabolizing cells co-metabolically transform o- and p-xylene to the corresponding methyl derivatives of benzylsuccinate and benzylfumarate, although PRTOL1 is unable to grow on o- and p-xylene. In cell extract studies, we found that PRTOL1 contains activities of the key enzymes of the carbon monoxide dehydrogenase pathway for complete acetyl-CoA oxidation rather than activities of the citric acid cycle. This finding is important because it has been proposed that succinyl-CoA, originating from a catabolic citric acid cycle, may be involved in by-product formation.

**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 CO<sub>2</sub> 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: During anaerobic ethylbenzene mineralization by EB1 in cell suspensions 1-phenyl-ethanol and acetophenone were formed. The formation was transient and was paralleled by nitrate and nitrite consumption. In addition, it was found that EB1 is able to grow anaerobically with 1-phenyl-ethanol and acetophenone as substrates under denitrifying conditions. These results suggest that anaerobic degradation of ethylbenzene in strain EB1 may proceed with 1-phenyl-ethanol and acetophenone as intermediates.

### **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 B12 is a nucleophile with the capability of reductively dechlorinating chlorobiphenyls at all positions. By coupling vitamin B12 with a biological reductant, reductive dechlorination can be supported. A microorganism was selected for study due to its ability to reduce cobalt, and potentially, the cobalt center of vitamin B12.

Approach: During the first year of the project, we are concentrating on meeting objective 1 by evaluating several reducing systems. Our model compound is 2,3,4,5,6-pentachlorobiphenyl, selected for study because one ring is fully chlorinated. We will expand the study to include other chlorobiphenyls as the work progresses. Our reducing systems include a PCB-dechlorinated microbial consortium, chemically-reduced vitamin B12, biologically-reduced vitamin B12, and a PCB-dechlorinating consortium amended with vitamin B12. We are separately evaluating the reduction of the catalyst and the reductive dechlorination of the chlorobiphenyl.

Status: We have determined a complete reductive dechlorination pathway for 2,3,4,5,6-pentachlorobiphenyl in a system containing titanium citrate-reduced vitamin. 2,3,4,5,6-Pentachlorobiphenyl is reductively dechlorinated primarily at the meta and para position, but its products are dechlorinated at the ortho, meta, and para positions to yield each of the six possible dichlorobiphenyl intermediates.

Once the ability of vitamin B12s to yield ortho, meta, and para dechlorination was identified, we began to examine the ability to microbially-reduce vitamin B12. Fully oxidized B12 at a concentration of 46mM was incubated with a pure culture of *Shewanella* alga, formerly *Geobacter sulfurreducens*, under anaerobic conditions at pH 7. Lactate was provided as the electron donor. Over a period of 10 days, vitamin B12 was reduced from the fully oxidized form, B12a, to a reduced form, B12r. The rate at which this organism is capable of reducing vitamin B12 is currently under investigation.

The vitamin B12/*Shewanella* alga system was evaluated for its ability to reductively dechlorinate a range of halogenated organic compounds, including carbon tetrachloride, dibromodichloromethane, bromotrifluoromethane, and tetrachloroethylene. All of the halogenated methanes were transformed rapidly except tetrachloroethylene. Further evaluation of transformation rates at an elevated pH are planned since the literature has shown that dechlorination rates using vitamin B12 are pH dependent. The reduction of 2,3,4,5,6-pentachlorobiphenyl in this system has been assessed at pH 7 and pH 8.4. There does not appear to be any significant dechlorination of the 2,3,4,5,6-pentachlorobiphenyl at pH 7. However, when the pH was raised to 8.4 it appeared as though some transformation occurred. Currently the evaluation of extent of apparent transformation is being reexamined. In addition, the use of other possible catalytic systems are being evaluated.

## **HEAVY METALS**

### **Lead Sorption, Transport, and Remediation in Natural Soils and Subsoils: Peter O. Nelson, Oregon State University**

Goal: The overall purpose of this research is to investigate conditions that enhance the mobility of lead in contaminated soils for in-situ remediation by a pump-and-treat process, or alternatively, to chemically immobilize lead through sorption or precipitation processes. The study will increase our understanding of lead chemistry in soils and groundwaters through both physico-chemical techniques and computer modeling.

Rationale: Although lead is often considered "immobile" in soils, dissolved lead in groundwater exceeds the EPA drinking water MCL in numerous lead-contaminated soils. Better understanding is needed of processes that control lead mobility in soils, including sorption-desorption, precipitation-dissolution, and complexation; and the effects on these processes of environmental conditions such as pH, soil organic matter content, metal speciation, surface chemical properties of soil mineral phases, and reaction time (kinetics).

Approach: Oxides of iron are ubiquitous subsurface mineral constituents and control the mobility of metal ions in soils and groundwater by adsorptive retardation. In this laboratory study, iron-oxide-coated sand (IOCS) is used to model lead chemical behavior in soil using batch and column reactor experiments. Several parameters are varied in order to ascertain their effects: pH, ionic strength, complexation by organic ligands, competing cations, and reaction time. Batch reactor experiments are used to study sorption and desorption kinetics and to determine equilibrium parameters for lead adsorption. Continuous-flow packed-beds (soil columns) are used to more closely simulate field conditions of porous media flow. Data from both batch and soil column studies is used in computer modeling to simulate the effects of changing environmental conditions on lead mobility.

Status: Lead adsorption kinetics show that equilibrium being reached in about 4 hours in completely-mixed batch systems. Equilibrium adsorption of lead increases dramatically with pH, being almost undetectable at pH 4 and nearly 100% at pH 6, and is affected very little by competition from major electrolyte cations and increases in ionic strength. EDTA decreases lead adsorption to nearly zero at higher EDTA/Pb molar ratios ( $> 2/1$ ) and higher pH values ( $>7$ ), but at lower molar ratios ( $< 2/1$ ) and lower pH values ( $< 5$ ) it promotes lead adsorption on IOCS through a ternary complex. NTA decreases lead adsorption on IOCS in a stoichiometric manner with near complete inhibition of adsorption at molar ratios of NTA/Pb  $> 1.0$ , but adsorption of the NTA-Pb complex is insignificant. A chemical equilibrium computer program (HYDRAQL) using a surface complexation, triple-layer model (TLM) for adsorption was used successfully to simulate lead chemical behavior in contaminated soils and groundwater. Soil column studies of Pb adsorption on IOCS are consistent with results from batch studies.

### **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 is collected to determine the extent of solid dissolution, 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: Data from batch extraction tests, collected from both nickel and copper contaminated samples, strongly suggests a stronger binding environment for the sintered samples under acidic conditions. Sequential extraction tests of the metals under acidic conditions is currently under investigation. Characterization of the sorption data for the greater solids concentration used in the leaching experiments suggests that residual nickel may remain adsorbed to the unfired clay surface under the pH conditions of the leaching experiments (pH 4). Preliminary XPS spectra reveal subtle differences in binding energies of the metal cation between sintered and unsintered samples. XPS spectra was collected for NiO, NiCO<sub>3</sub>, NiSO<sub>4</sub> and NiCl<sub>2</sub> standards representing different chemical environments. Large binding energy differences were not observed upon comparing these standards, however, the results indicate that significantly different coordination environments yield binding energies with small differences between them. Although the small change in binding energy excludes a change in oxidation state of the metal it can represent substantial differences in the chemical environment. Research will continue exploring the surface chemistry using XPS. Future work also includes XAS analysis to further investigate the mechanisms by which the metals are bound or contained.

**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 ( $\text{SiO}_2$ )), rinsed with 5% ammonium acetate solution, oven-dried, and heated under nitrogen in 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.

**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: Analytical methods development for cadmium and selenium analysis on the Atomic Absorption Spectrophotometer at the sub-micromolar in the experimental matrix has been completed to replace the use of radionuclides for analytical purposes. Experimental work on time dependent uptake of cadmium and selenite from simple non-complexing electrolyte solutions over a range of pH values has been completed to provide baseline data for comparison with mass transfer rates for the more complex systems where cadmium will be complexed with chloride, sulfate, carbonate or thiosulfate.. For selenite competitive adsorption with anions such as chloride, sulfate and carbonate will be compared to baseline data. The mass transfer coefficients are being estimated for each experimental condition to evaluate the effect of complexing ligands. Samples of eight different highly porous alumina materials are being characterized for total surface area, total pore volume and pore volume distribution. These materials are being size reduced to allow narrow ranges of particle sizes to be selected from sieved material. The particle size selection for column studies will be based on headloss modeling as a function of particle size and column cross-section and length. A detailed experimental plan is being developed to coordinate the batch and column studies. Preliminary column studies have been initiated as detailed experimental plans for column studies are being completed. Work has been initiated on development of the mathematical construct for mass transfer limited adsorption in the packed bed adsorber.

## **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: Suspensions of OR-02 effectively removed up to 1.0 mM soluble lead from complex media formulations within 48 hours under optimal culture conditions. The lead was all contained in small biocolloids approximately 1/4 the size of the bacterial cells. Increasing concentrations of citrate served to inhibit both the rate and the extent of lead biocolloid formation. Efforts to optimize lead biocolloid formation in the presence of citrate concentrations equimolar with that of the lead are in progress. In the meantime, whether the immobilized bacterium could be exploited to remove soluble lead was investigated. Protocols were developed to entrap and immobilize strain OR-02 in calcium-alginate beads. The efficacy of lead removal by immobilized bacteria was examined in a stirred-tank reactor for the following 3 preparations: (i) bacteria were entrapped in beads in the presence of soluble lead; (ii) beads were formed in the presence of lead followed by the adhesion of bacteria to the preformed beads; and (iii) bacteria were entrapped in beads and then exposed to soluble lead. Only the latter preparation was effective in the removal of lead from solution. The long-term stability of the bacterial-laden calcium-alginate beads is currently under investigation.

**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 vitification and to determine the extent of hydration of Gd in clays using EPR.

Status: This project began in May of 1995. Nuclear magnetic resonance studies have been carried out on the clay hectorite exchanged with Cs (I). The NMR spectra display a peak at -33 ppm, which is characteristic of a hydrated ion. The Cs-hectorite was subsequently heated to 150. Electron paramagnetic resonance studies have been carried out on hectorite exchanged with Ni (II). The expected Ni (II) signal is not obvious in the room temperature sample or in material that has been heated to 150, 300 or 600.

**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. We are using  $^1\text{H}$  NMR to probe the mechanism of interaction between the free radical scavenging polyphenol and the colored water soluble free radical.

Status: Beginning in the summer of 1995, voltammetry studies began by identifying some optimal conditions (*i.e.* solvent, supporting electrolyte and electrode materials) for studying the electrochemical properties of natural tannins. Glassy carbon was found to be a suitable working electrode. Dimethylsulfoxide (DMSO) with tetrabutylammonium perchlorate (TBAP) is being used as the supporting solvent and electrolyte, respectively. For some of the more "reversible" tannins, DMSO can be used to separate the individual one- and two-electron-transfer oxidation and reduction waves. Currently, some of the fundamental aspects of the electrochemical behavior of natural tannins are being addressed in protic vs. aprotic solvents. In applying the above electrode-cell system to a reference humic acid, an unexpected result was obtained. The low concentrations of iron in the humic acid produced a highly reversible set of oxidation and reduction waves. Based on this observation, cyclic voltammetry may be used to compare the properties of redox-active metal humate complexes to those of the uncomplexed metal, or to the properties of other environmentally significant metal-organic complexes. Studies of the colored water soluble organic free radical have progressed to the point where a calibration curve has been obtained showing a linear decrease in absorbance (at 750 nm) with increasing concentration of a free radical scavenging polyphenol.

**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. Product are analyzed by includes GC-FID/ELCD for the organics and a chloride selective electrode for inorganic chloride.

Status: The project was initiated this summer and preliminary results for carbon tetrachloride show sequential dechlorination to methyl chloride using the graphitic electrode. Greater than 95% of the CT was converted in 10 hr affording chloroform and methylene chloride as the major products. We are redesigning the reactor to minimize the headspace, because the alkyl halides partition to the gas phase which greatly slows the reaction. In addition, glassycarbon will be used, which seems to have greater selectivity for reaction with halocarbons than with water.

### **Detection of Microorganisms Capable of Anaerobic Degradation of Hazardous Substances in Natural Environments: Sara E. Silverstone, California State University at Bakersfield**

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 probe target sites have been selected for the anaerobic toluene-degrader PRTOL-1. Optimization of hybridization conditions and assessment of the probes stain-specificity is in progress. These experiments are being conducted using both dot-blot of total nucleic acid and whole cell hybridization.

## **DEMONSTRATION PROJECTS**

### **Evaluation of Strategies for Full Scale Bioremediation of the Seal Beach Site Using Anaerobic Microbial Processes: Martin Reinhard, Gary Hopkins, and Peter Kitanidis, Stanford University**

Goal: The goal of this project is to develop a plan for the remediation of the Seal Beach gasoline site using anaerobic microbial processes. Specifically, we are developing a hydraulic and process model for large-scale implementation of the processes we have been investigating in the laboratory, in pilot reactors and on a small field scale. By the end of this project year (September 1995) we will recommend a strategy for cleaning up large sections of the site based on the results of the field and laboratory studies and hydraulic modeling.

Rationale: Field and laboratory work has indicated that BTEX contamination can be removed biologically under anaerobic conditions using different electron acceptors or acceptor combinations. We have studied in detail nitrate- and sulfate-reducing and methanogenic conditions. All these conditions appear to have specific advantages and disadvantages and it appears that a combination of conditions could yield the best result. It is not clear, however, which of the possible electron acceptor combination should be chosen.

Approach: The approach has been to simulate different treatment options by releasing controlled amounts of BTEX compounds into a small test zone of the aquifer. The BTEX compounds were added to approximately 1000 L of water to which was added nitrate, sulfate, or carbonate salts as the electron acceptor. The water was then removed from the test zone in small batches and analyzed for BTEX compounds and nitrate and sulfate. Laboratory experiments were also conducted to explore the factors that affect BTEX degradation rate under anaerobic conditions.

Status: This project was completed in early 1995. Two sets of replicate release demonstrations under nitrate- (EO4 and EO5) and sulfate-reducing conditions (EO6 and EO7) were completed as was the first under methanogenic conditions (EO8). Under denitrifying-conditions toluene, m-xylene, and ethylbenzene were found to be rapidly degraded (within days) while o-xylene was degraded at a slower rate. The rate of benzene degradation was much slower but appeared to be significant. To evaluate the results of the controlled release demonstrations, a two-dimensional model for solute transport was developed and tested. The model is now being used to evaluate the aromatic degradation rate observed in the controlled release experiments and will serve as a basis for large scale implementation of the technology. Current efforts focus on the development of kinetic models to characterize contaminant transformation under various anaerobic conditions and to incorporate these kinetic models into the large-scale model. Large-scale models are being used for the design and interpretation of intermediate-scale controlled release experiments planned for the 1995/6 project year. For the future, we plan to develop a model more advanced than existing models and well-adjusted to represent the in-situ biodegradation of fuels in groundwater.

### **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 in the group of Professor Gorelick in the School of Earth Sciences at Stanford. 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 valuable 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 all the way 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: Aquifer testing, well installation, and baseline groundwater sampling have been completed at Edwards AFB. The system at Edwards has undergone baseline groundwater sampling and preliminary but brief testing in September. Well installations at the Savannah River Site are nearly complete, and it is hoped that baseline groundwater sampling and system startup will occur before the end of 1995. Extensive numerical simulation of groundwater flow, infiltration capacity, and VOC removal have been completed for the Savannah River site. These simulations will be updated by incorporating recently obtained information from soil and groundwater samples collected during well installation. Also completed is modeling of a laboratory-scale mockup of the in-well vapor stripping system, in collaboration with Dr. Haim Gvritzman at the Hebrew University of Jerusalem. Computer simulations were used to successfully explain the experimental behavior of the laboratory system.

**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 the most 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 being 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, 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, the most promising of the devices (as determined from Phase I) will be installed in a treatment well at Edwards and its full-scale performance evaluated.

Status: The construction of the experimental setup to test the oxygen transfer devices in the laboratory is near completion. Data collection from Phase I is scheduled to commence in late 1995. Upon completion of the bioremediation demonstration at Edwards AFB in April 1996, Phase II testing can commence on site at Edwards.

**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 the hydrogen/palladium system 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 the 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 of several parallel bench-scale continuous-flow reactors. These reactors enable simultaneous study of several water sources: a controlled supply of contaminated deionized water, groundwater from Livermore, California, and groundwater from the California Central Valley. This study seeks to ensure adequate residence time and catalyst life and to determine the effects of any competitors or inhibitors. Initial characterization tests are conducted with nitrate solutions and are followed by more detailed tests using TCE and DBCP solutions. Once the parameters have been quantified at the bench scale, a pilot-scale reactor is constructed and optimized. This design is then scaled up to a field-scale model and tested.

Status: One bench-scale system has been constructed with a 4.5 ml reactor containing 1% palladium on alumina. The reactor is fed by a mixture of two flows: hydrogen-saturated water driven from a reservoir by pneumatic pressure, and a concentrated contaminant solution injected into the water stream using a syringe pump. The system has been demonstrated to degrade nitrate in deionized water at concentrations on the order of 0.1 mM (6 mg/L as NO<sub>3</sub><sup>-</sup>). Initial tests with TCE-contaminated water indicate reduction of the TCE from an inlet concentration of approximately 1 mg/L to less than 3 µg/L (lower detection limit). This study commenced 1 March 1995 and is authorized for a two year period terminating 28 February 1997.

**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 several years of research at Stanford University and elsewhere, and by field demonstrations 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 (to include citizen groups).

Approach: Recent 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. The proposed remediation system will consist of two wells, each screened at two depths. In operation, a submersible pump, installed between the two screens, will draw water into the well at one screened interval and discharge the water out of the

second screened interval. The well will have feed lines to introduce toluene and hydrogen peroxide (as a source of oxygen) into the discharge water. A biotransformation zone will be developed near the discharge side of each well, and the water will circulate between the wells to clean the aquifer.

Status: Initial characterization of a TCE-contaminated site at Edwards AFB has been completed. A work plan for the demonstration has been submitted to regulators and base officials, and approval has been obtained to begin the demonstration. A memorandum of agreement between Stanford University and the U.S. Air Force at Edwards has been finalized, resolving liability and other issues. Construction of the demonstration facility is nearly complete, and the four month demonstration is scheduled to commence in late 1995.

**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 an interceptor trench technology for the bioremediation of groundwaters contaminated with pentachlorophenol (PCP).

Rationale: Pentachlorophenol (PCP) is a fully chlorinated aromatic compound susceptible to reductive dechlorination, a common anaerobic biodegradation mechanism. The degradation of PCP by anaerobic 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, degradation of the lower chlorinated phenolic compounds is rapid and may result in complete mineralization. This project will demonstrate a 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: Great progress has been made during the first six months of this project. An ideal site has been located, the groundwater has been characterized, and the site owner is committed to supporting this demonstration. We have received written permission from the Oregon Department of Environmental Quality for the study.

Numerous design considerations have been addressed and a full scale treatment cell has been constructed and is awaiting field validation tests. Laboratory studies for both anaerobic and aerobic treatment regimes have provided useful information in the selection of a substrate that will serve as electron donor and carbon source. Results of the mixing and nutrient addition systems study has provided a better understanding of the physical interactions occurring in the trench system. Lessons learned were incorporated into the physical design of the treatment cell.

## **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 Tecle, Northern Arizona University in close 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) in 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. Toward this end, the necessary parameters and boundary conditions for the transport and biodegradation of each BTEX compound are being determined to arrive at the needed solutions for each compound individually.

### **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 are scheduled 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

**Environmental Education Enrichment Project (EEEP): R. Barry King, Albuquerque Technical-Vocational Institute**

Goal: The goal is 1) to provide summer intern environmental research opportunity at a full university for graduating community college students who exhibit outstanding achievement and good study skills, 2) to create interaction between TVI and WRHSRC, 3) to motivate students to pursue advanced study and careers in hazardous substance management, and 4) to enhance the ability of the MAI to participate in training and technology transfer.

Rationale: Currently, only 20 percent of our Environmental Technology graduates go on to pursue their educations. As a Minority Academic Institution, TVI was chosen to send two students as summer interns to Oregon State University. The EEEP is seen as an opportunity to attract more AA Degree graduates into four-year institutions .

Approach: TVI sent two student interns to OSU for the summer session. They were Frank Flores and Glenn Salm. Frank worked on a project for Professor Sandra Woods involving design of an Interceptor Trench for Bioremediating PCP in a contaminated site using a basket-type reactor set within the aquifer. Glenn worked on a project for Professor Jack Istok involving a gasoline contaminated aquifer in which testing was being done to determine the rate at which aerobic microbes consume oxygen from various oxygen donors.

Status: Frank and Glenn successfully completed their research at OSU on Friday, August 11, 1995.

## **TRAINING AND TECHNOLOGY TRANSFER PROJECT DESCRIPTIONS**

### **TRAINING AND TECHNOLOGY TRANSFER**

#### **Hazardous Waste Training: Kenneth J. Williamson and Peter O. Nelson**

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 Region 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 8 workshops within Oregon, Washington, Idaho, and Alaska. The Center was successfully chosen for an extension for 1995-96.

#### **Continuing Education: Kenneth J. Williamson**

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

Rationale: An education program was not available in hazardous substance management and site remediation for people in the Portland metropolitan area, but was needed.

Approach: A series of six courses are offered through OSU's Continuing Education program. A certificate of completion is offered for those students successfully completing the courses.

Status: The series of courses are offered on a two-year cycle. Attendance has remained constant at about 18 students per course. Three courses were offered in 1994-95 including "Environmental Chemistry," "Hazardous Waste Legislation," and "Groundwater Remediation."

#### **1995 Five Centers' Research Conference: Kenneth J. Williamson and Perry L. McCarty**

Goal: To promote interaction between researchers in the five HSRC's and field application of research results.

Rationale: A specialty conference provides the best format to promote one-on-one interaction between faculty at the five HSRCs.

Approach: A national conference was organized that involved presentations from all five HSRCs of active projects that demonstrate field applications. Discussions were facilitated to discuss that approaches of each project, successes and failures, and future directions. The discussions allowed active participation of faculty and graduate students.

Status: The conference was conducted on July 23-26 at Gleneden Beach, Oregon. Two hundred and sixteen persons attended including 53 faculty from the five HSRCs.

## **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 efficient and cost-effective way to achieve the transfer of technology.

Status: This year the Center sponsored or organized sessions for the following conferences:

The 1995 Idaho Regional Conference on Hazardous Materials, Pocatello, Idaho, April, 1995.

The 1995 Responsible Hazardous Materials Management Conference (REMCON), Portland, Oregon, September, 1995.

The Third International Symposium on In Situ and On-Site Bioreclamation, San Diego, California, April, 1995.

The 1995 Five Centers' Research Conference, "From Flask to Field", Glenden Beach, Oregon, July, 1995.

Symposium on Science and Engineering of Contaminant Remediation with Zero-Valent Metals, 209th American Chemical Society National Meeting, Anaheim, California, April, 1995.

## **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 communities in Alaska, California, Washington, and Idaho.

## **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 will help make their transition to civilian life easier as well as more beneficial to the country.

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 has successfully funded the following five graduate students in the fields of civil and environmental engineering at Oregon State University.

Jim Brown - M.S., Environmental Engineering

Research Project: Development of Permeable Barrier Reactor that will be Installed in the Screened Interval of a Borehole

Michael Cantaloub - M.S., Environmental Engineering

Research Project: Using Radon-222 to Locate and Qualify NAPL Contamination in the Subsurface

Greg Connor - M.S., Environmental Engineering

Research Project: Push-Pull Test for In-Situ Determination of Microbial Metabolic Activities

Mark Havighorst - M.S., Environmental Engineering

Project: In-Situ Remediation of Pentachlorophenol

Paul Kwon (Spring 1996- Civil Engineering)

The program is currently soliciting and will fund another graduate student along with eight upper level undergraduate students between Winter 1995 and Fall 1996.

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

## 1995 WRHSRC PUBLICATIONS

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

### A. Refereed Journal Articles

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