WESTERN REGION
HAZARDOUS SUBSTANCE RESEARCH CENTER

# 2000 ANNUAL REPORT

Stanford University
Oregon State University

December 2000

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# WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER
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## ADMINISTRATION


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## THE CENTER AT A GLANCE

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

The objectives of the Center are:

1. To promote through fundamental and applied research the development of alternative and advanced physical, chemical, and biological processes for treatment of hazardous substances in the surface and subsurface environments.

2. To disseminate the results of research to the industrial and regulatory communities, to foster exchange of information with these communities, and to promote a better understanding of the scientific capability to detect, assess, and mitigate risks associated with hazardous substance usage and disposal.

Groundwater cleanup and site remediation, with a strong emphasis on biological approaches, represents 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 20 faculty and 2 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 (engineering, science, earth sciences, medicine, and veterinary medicine), and many different disciplines (environmental engineering, 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, technology transfer and community outreach, as well as coordinates the Center's overall activities in Oregon. Martin Reinhard, the Assistant Director, is in charge of the Center's analytical program. Sharon Parkinson is the Center's Administrative Assistant.

Table 1. KEY PERSONNEL AT THE WRHSRC

| Stanford University | Oregon State University |
|---|---|
| Prof. Craig C. Criddle | Prof. Daniel J. Arp |
| Prof. Mark N. Goltz[a] | Prof. Lynda Ciuffetti |
| Prof. Steven M. Gorelick | Prof. Michael R. Hyman[b] |
| Mr. Gary D. Hopkins | Prof. James D. Ingle |
| Prof. Peter K. Kitanidis | Prof. Jonathan D. Istok |
| Prof. James O. Leckie | Prof. Peter O. Nelson |
| Prof. Keith Loague | Prof. Lewis Semprini |
| Prof. Perry L. McCarty | Prof. John C. Westall |
| Ms. Sharon E. Parkinson | Prof. Kenneth J. Williamson |
| Prof. Martin Reinhard | Prof. Sandra L. Woods |
| Prof. Paul V. Roberts | |
| Prof. Alfred M. Spormann | |

[a] Currently with the Air Force Institute of Technology, Dayton, Ohio, but still active with the WRHSRC
[b] Currently with North Carolina State University

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 2000 fiscal year and since the Center's inception are listed by category of support in Table 4. The education of students interested in careers directed toward finding solutions to environmental problems is another important goal. The number of students supported through WRHSRC funds is listed in Table 5.

Table 2. SCIENCE ADVISORY COMMITTEE

| Member | Affiliation | Expertise |
|---|---|---|
| Linda M. Abriola | University of Michigan | Physical Processes, Hydrology |
| David E. Ellis | Du Pont Chemicals | Chemical/Biological Processes |
| John Glaser | Govt/EPA, Cincinnati Lab | Physical/Chemical Processes |
| Ronald Hoeppel | Govt/U.S. Navy | Microbiology |
| Michael C. Kavanaugh* | Malcolm Pirnie | Physical/Chemical Processes |
| Stephen Schmelling | Govt/EPA, Ada Lab | Groundwater Remediation |
| 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

| Member | Affiliation | Expertise |
|---|---|---|
| James T. Allen | Govt/ California | Treatment Technology |
| Michael Anderson | Govt/Oregon | Hydrology |
| Harry A. Ball | Govt/EPA, Region 9 | Environmental Engineering |
| John J. Barich | Govt/EPA, Region 10 | Regulations |
| Kenneth Bigos | Govt/EPA, Region 9 | Air Pollution |
| Douglas R. Christensen | Independent Consultant | Engineering |
| Lynn Coleman | Govt/Washington | Regulations |
| Jerry Jones | Govt/EPA, Kerr Lab. | Groundwater Remediation |
| David Kennedy* | Kennedy/Jenks Consultants | Treatment Technology |
| Gregory Peterson | Cascade Earth Sciences | Treatment Technology |
| JohnWesnousky | Govt/ California | Treatment Technology |

* Chairman

Table 4. CENTER FUNDING

| Funding Sources | FY 2000* | Funds to Date |
|---|---|---|
| EPA: Centers Program | $926,000 | $12,652,015 |
| EPA: Other | $496,000 | $4,477,816 |
| Other Govt: Federal† | $184,000 | $8,261,195 |
| Other Govt: State@ | 0 | $48,252 |
| Consortium | $131,318 | $3,847,240 |
| Private Sector# | $100,182 | $2,444,816 |
| TOTAL | $1,837,500 | $31,731,334 |

* Oct. 1, 1999 - Sept. 30, 2000

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

@ Oregon Department of Transportation

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

TABLE 5. STUDENT SUPPORT

| Student Support | Number* | Funds to Date† |
|---|---|---|
| B.S. | 13 | $95,877 |
| M.S. | 38 | $1,037,718 |

| | | |
|---|---|---|
| Ph.D. | 128 | $6,257,086 |
| Post Doctoral | 27 | $1,591,748 |
| TOTAL | 204 | $8,982,429 |

* Total numbers in researcher-years participating on Center Projects since 1989
† Includes tuition, travel, supplies, etc.

## DIRECTOR'S REPORT

The year 2000 represents the eleventh year of Center activity. During this year, eight research projects and one demonstration project were completed. Also, 13 new research projects and one new demonstration project got underway, giving a total of 17 research projects and three demonstration projects that will continue through the coming year. The year 2001 will mark the last year of full activity for the current Center. The U.S. Environmental Protection Agency has solicited proposals for five new hazardous substance research centers (HSRCs) to replace this Center and the four other HSRCs that began in 1989. Prof. Lewis Semprini, one of the WRHSRCs principal investigators at Oregon State University, has submitted a proposal for continuation of this Center with OSU being the lead university instead of Stanford University. At the time of this writing, EPA had not yet announced the winners of the new competition, and so we do not know whether the WRHSRC will continue its activities under new leadership, or will discontinue. In any event, the faculty and staff associated with this Center have made many important contributions to the hazardous substance literature, and the outreach program has assisted many communities with their hazardous substance problems.

One of the major growth elements for the WRHSRC has been in it community outreach program under the direction of Professor Kenneth Williamson of Oregon State University. In addition to the Technical Outreach Services for Communities (TOSC) program that has been ongoing for several years, the WRHSRC has expanded with a new Technical Assistance to Brownfields (TAB) program and a new Technical Outreach Services for Native American Communities (TOSNAC) program. During the current year, the WRHSRC provided TOSC assistance to 17 communities, located throughout EPA Regions 9 and 10, with their hazardous waste problems. In addition, three brownfield's initiatives were begun, one with a native American community. It is unlikely that this highly successful outreach program will be discontinued if the WRHSRC loses out in the current competition, but would be integrated over the next two years into a new HSRC. We certainly hope, however, that the WRHSRC will continue to function, and with it, the current outreach programs as well.

One of the major activities of the WRHSRC over the past year was its sponsorship of the Five Hazardous Substance Research Center's Research Symposium at Asilomar Conference Center in Pacific Grove, California, July 9-12, 2000. This represented the third five-Centers research symposium held since the HSRCs were formed in 1989. The first was sponsored by the Great Lakes Mid-Atlantic HSRC at Gull Lake, Michigan, just after the HSRCs began operation, and the second by the WRHSRC at Gleneden Beach, Oregon, in 1995. The main objective of the Asilomar Symposium was to learn the state of the art in various hazardous substance research areas covered by the HSRCs. This Symposium celebrated ten years of HSRC activities. The aim was to highlight what was known about an important hazardous substance problem when the five Centers began, what is now known about each problem and its resolution, what the HSRCs contributions have been, and what yet needs to be done. Ten oral presentations, two by Principal Investigators from each HSRC, provided the overviews of important research areas. A view of the Center's accomplishments from the consulting engineering community was provided by Dr. Michael C. Kavanaugh, Malcolm-Pirnie, and from industry by Dr. David Ellis, DuPont. A discussion of the HSRCs evolution towards successful training and technology transfer was provided by Dr. Kenneth Williamson, Oregon State University. In addition, there were 84 posters highlighting current research activities given in two sessions by HSRC students and principal investigators. The 192 attendees included HSRC researchers and Principal Investigators, and members of both the Training and Technology Transfer Committees and the Science Advisory Commitees of each HSRC. The results of the Asilomar Symposium will be summarized soon in the HSRCs newsletter, *CENTERPOINT*.

## HIGHLIGHTS OF THE PAST DECADE

The major focus of research activities for the WRHSRC, and indeed its major mission, has been the conduct of basic research that will help resolve problems with hazardous waste cleanup. Proof of the success of our efforts relies to some extent on the degree to which the results of this basic research find its way into cleanup practice. There is a big step between discovery of a principle and its use in solving a practical problem. For this reason, the WRHSRC has taken on the mission, within its training and technology transfer responsibilities, to demonstrate technologies that have grown out of the basic research program. We believe that our involvement in such demonstrations will help hasten application. It also involves our research team in the practical side of the problems they study. Technical barriers that are uncovered by attempts at practical application then tend to feed back rapidly into the basic research program. We are pleased that with the above benefits in mind, several field demonstrations have been undertaken and completed by the WRHSRC.

### Chlorinated Solvent Treatment Demonstrations

During previous efforts to implement full-scale application of methanotrophic cometabolic biodegradation of chlorinated solvents at the St. Joseph, Michigan, Superfund site, the Center investigators found that they were challenged by many new questions that required new research. Among these were how to insure that indigenous microorganisms at the St. Joseph site would perform similarly to those present in groundwater at our Moffett field research station at the Moffett Federal Air Station, where the pilot scale field studies were conducted. How can the chemicals of interest, for example methane and oxygen, be introduced into the groundwater in such a way that they would mix with the contaminants of interest and the bacteria that would effect their biodegradation? What chemical introduction strategies would be optimal for bringing about efficient biodegradation of contaminants? How would microbial growth and biodegradation activity be distributed within the treatment zone? What type of monitoring protocol should be used to evaluate the possible effectiveness of in-situ bioremediation? These questions are being addressed within several of the ongoing WRHSRC research projects. But, in addition, we realized that new questions of importance are likely to arise when full-scale implementation itself was carried out, and partially for this reason, efforts to undertake full-scale implementation were begun.

The research questions raised by efforts at St. Joseph, Michigan, were further explored through additional field studies at Moffett field. The successful results here led to a decision to seek funds to demonstrate in-situ cometabolic biodegradation of trichloroethylene at full scale. A major recent highlight was the completion of this first full-scale evaluation of in situ cometabolic biodegradation of trichloroethylene (TCE) in groundwater. This was accomplished through the injection into groundwater of toluene, hydrogen peroxide, and oxygen at a

Superfund site at Edward's Air Force Base in southern California. Organisms that oxidize the toluene produce an enzyme (toluene ortho monooxygenase) that fortuitously degrades TCE. The novel system used does not require that any groundwater be brought to the surface, TCE is biodegraded *in situ*. This rather simple treatment system involves two wells with pumps that cause the groundwater to circulate between them so that the chemicals required can be added, mixed with the contaminants, and then introduced back into the aquifer for in situ biodegradation. This led to the degradation of 97 to 98% of the 1200 μg/l of TCE in the 60 to 80 m wide plume of regional flow that was caused to pass through this treatment system. Detailed results of the Edwards' demonstration can be found in: McCarty, P. L., Goltz, M. N., Hopkins, G. D., Dolan, M. E., Allan, J. P., Kawakami, B. T., and Carrothers, T. J., "Full-Scale Evaluation of *In Situ* Cometabolic Degradation of Trichloroethylene in Groundwater through Toluene Injection," *Environmental Science and Technology,* **32**(1), 88-100 (1998).

The Edwards' demonstration represents the culmination of basic laboratory and field experimental work conducted during the nine years of WRHSRC activity. Twenty-three faculty and staff members, not only from Stanford and Oregon State, but also from Michigan State University, the University of Western Florida, and the University of Minnesota, together with 30 graduate students participated in this research. To date there have been 50 WRHSRC sponsored journal publications, 14 conference reports, 7 book chapters, 13 doctoral dissertations, and 3 patents issued that are related to this technology. The full scale demonstration required support and administrative approvals from a variety of agencies, including the U.S. EPA Region IX, California EPA, the Lahonton Regional Water Quality Control Board, and the U.S. Air Force. The site demonstration itself was financially supported by the Armstrong Laboratory Environics Directorate, Tyndall Air Force Base, the Headquarters U.S. Air Force Environmental Quality Division, and Woodward-Clyde Consultants. Technical support for construction and operation of the demonstration site was provided by Earth Tech, and support for all phases was provided by the Edwards Air Force Base.

Another WRHSRC-sponsored field demonstration for TCE removal from groundwater is one led by Prof. Steven Gorelick. This is an *in situ* air stripping process for removal of volatile organic compounds from groundwater for which he and a former postdoctoral scholar, H. Gvirtzman, hold a patent. The system used involves a well screened at two intervals, one deep in the aquifer and the other spanning between the aquifer and the vadose zone above. Air is injected into the well in such a manner that the volatile compounds are transferred to the rising air stream which leaves the water and is taken to the surface for removal of the contaminants from the air stream, if necessary. Thus, there is no need to bring the water to the ground surface. The treated water discharges back into the aquifer through the upper screen. In this manner, the groundwater is caused to circulate throughout the aquifer bringing back untreated groundwater to enter the lower screen and the air-stripping system. More can be found about this process through the publication: Gvirtzman, H. and S. M. Gorelick, "Using air-lift pumping as an in-situ aquifer remediation technique,: *Water Science Technology*, **27** (7-8), 195-201 (1993). The demonstration at Edwards was very successful such that the Air Force decided to evaluate its use further for removal of TCE from the concentrated source area, rather than just from the plume, and has successfully done so. Also, the WRHSRC received funding from the Department of Defense's Strategic Environmental Research and Development Program (SERDP) for a demonstration of a combination of the in-situ air stripping and the in-situ cometabolic processes for removal of the TCE source at Edwards. This project is being conducted in conjunction with Dr. Mark Goltz at the Air Force Institute of Technology in Dayton, Ohio.

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

Oregon State University researchers are also exploring the potential for other novel microorganisms for cometabolism of troublesome chlorinated solvents. Profs. Lewis Semprini and Daniel J. Arp are exploring bacteria that use n-alkanes such as butane and propane for energy and growth, perhaps with some similarities to the fungus described above. They also found that chloroform was cometabolized through n-alkane oxidation, this time using butane. Also of interest is that both 1,1,1-trichloroethane (TCA) and its anaerobic degradation product, 1,1-dichloroethane (1,1-DCA) are readily degraded by these microorganisms. Neither TCA nor 1,1-DCA are cometabolized well by most organisms studied to date, and thus use of butane when TCA and/or 1,1-DCA are present is of significant interest. Field studies using bioaugmentation with both *Graphium* sp. and the butane-oxidizing bacteria represent the next step in evaluation of the potential of these organisms. Prof. Semprini has such a study now under way and funded by the Strategic Environmental Research and Development Program, Department of Defense. The field portion of this study will make use of the Stanford Field Demonstration Pilot Plant at Moffett Federal Air Field, California.

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, Naoko Munakata, and Gregory Lowry 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. This group has ongoing laboratory studies to improve on this important process and have completed with the Lawrence Livermore National Laboratory the first field-scale demonstration of this process. It is undergoing field tests in Germany, and recently funding from the Environmental Strategic Technology Certification Program has provided funding to provide important data for commercialization of this effective process. This highly successful project has continuing studies ongoing through the WRHSRC.

**<u>Petroleum Hydrocarbon Treatment Demonstrations</u>**

A major field demonstration by the WRHSRC has been that of natural attenuation of gasoline contamination at the Naval Weapons Laboratory, Seal Beach, California, a study directed by Prof. Martin Reinhard, Stanford University. The interest here is in the fate of aromatic hydrocarbons resulting from the soluble components of gasoline that contaminate groundwater. These contaminates are generally termed the BTEX compounds (benzene, toluene, ethylbenzene, and xylenes). Prof. Reinhard and coworkers reported upon a noted disappearance of xylenes in a landfill leachate plume in 1984 (*Environmental Science & Technology*, 18: 953-961) and concluded, "The selective removal of the xylenes was hypothesized to be due to biotransformation." This greatly disputed hypothesis was convincingly confirmed by the late Stanford Professor Dunja Grbic-Galic and Dr. T. Vogel in 1986 (*Applied and Environmental Microbiology,* 5:200-202). They noted oxygen was incorporated into the aromatic ring under anaerobic conditions from water, rather than from molecular oxygen, the then believed only route by which this group of compounds could be transformed. Now, many microorganisms have been isolated that are capable of anaerobic decomposition of BTEX compounds. The scientific underpinning these studies provided together with frequently observed natural shrinkage of hydrocarbon plumes with time once the contamination source has been removed, has now led to a major change in the thinking by regulatory agencies. In many instances, remediation of BTEX plumes is no longer required as natural attenuation itself is believed to be sufficiently protective. This change in practice resulting from this good science is now saving billions of dollars.

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

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

**Pesticide Removal Studies**

In 1994, a field effort was undertaken with the Oregon Department of Transportation for biodegradation of chlorinated pesticides (i.e., Silvex, Dichloroprop, 2,4,5-T and 2,4-D) in contaminated soil. Here, Profs. Kenneth Williamson and Sandra Woods of Oregon State University brought expertise gained through several years of research on anaerobic and aerobic transformation of chlorinated aromatic compounds towards solution of a practical problem in Oregon. Anaerobic biological treatment as a first stage of treatment to remove chlorine atoms from the pesticide molecule was undertaken, with a second stage planned of aerobic treatment for further degradation of the pesticides. Anaerobic treatment as a first stage for dehalogenation of industrial chemicals that are otherwise highly resistant to aerobic treatment has been a major theme of research within the WRHSRC. Such field demonstrations will help speed the practical application of this innovative process and may save the Oregon Department of Transportation significant disposal costs.

**TOSC, TAB and Other WRHSRC Programs**

Three programs of importance were begun in 1995 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. 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. Through the WRHSRC TOSC initiative, programs of advice were provided to citizens throughout the region-pair. Detailed activities of this important program are summarized in the body of this report. New outreach programs starting this year are the Technical Assistance to Brownfield Communities (TAB) Program and the Technical Outreach Services to Native American Communities (TOSNAC) Program . These are similar in scope to the TOSC Program and also are summarized in the body of this report. These important programs now occupy a significant portion of the WRHSRC award budget.

## Awards

In 1993, Lisa Alvarez-Cohen was a recipient of the Environmental Science Research Award of the Association of Environmental Engineering and Science Professors (AEESP) for her WRHSRC supported Ph.D. dissertation, "Cometabolic Biotransformation of Trichloroethylene and Chloroform by Methanotrophs--Experimental Studies and Modeling of Toxicity and Sorption Effects." In 1995, James Farrell won the CH2M-HILL Award from AEESP for his WRHSRC supported Ph.D. dissertation, "Desorption Equilibrium and Kinetics of Chlorinated Solvents on Model Solids, Aquifer Sediments, and Soil." Finally, James Anderson first 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," and then went on to win the CH2M- Hill Research Award from AEESP for his Ph.D. dissertation. Thus, over the span of ten years, three doctoral students supported through the WRHSRC have won awards for best environmental engineering and science dissertation of the year.

**CENTER PROGRAM SUMMARY FOR 2000**

TABLE 6. RESEARCH PROGRAM SUMMARY

| Project Description | Principal Investigators | End Date | Total Budget |
|---|---|---|---|
| **Chemical Movement, Fate, and Treatment** | | | |
| Experimental and Mathematical Study of Biomass Growth in Pore Networks and its Consequences in Bioremediation | Peter Kitanidis<br>Perry McCarty | 2001 | $143,770 |
| Development and Characterization of Redox Sensors for Environmental Monitoring | James Ingle | 2000 | $131,865 |
| **Chlorinated Solvents** | | | |
| Investigation of Palladium Catalyzed Hydrodehalogenation for the Removal of Chlorinated Groundwater Contaminants: Surface Chemistry of Catalyst Deactivation and Regeneration | Martin Reinhard | 2001 | $143,770 |
| Measurement of Interfacial Areas and Mass Transfer Coefficients between residual PCE and Water During Surfactant Enhanced Aquifer Remediation: Laboratory Studies and Models | Paul Roberts | 2001 | $127,169 |
| Development of Alkoxysilanes as Slow Release Substrates for the Anaerobic and Anaerobic/Aerobic Transformation of Chlorinated Solvents | Lewis Semprini | 2001 | $104,903 |
| Cytochrome P-450: An Emerging Catalyst for the Co-metabolism of Chlorinated Aliphatic Hydrocarbons and Methyl tery-Butyl Ether? | Michael Hyman<br>Lynda Ciuffetti | 2001 | $157,968 |
| Mechanisms, Chemistry, and Kinetics of Anaerobic Degradation of cDCE and Vinyl Chloride | Perry L. McCarty<br>Alfred Spormann | 2001 | $745,852 |
| Proof of Gene Expression During Bioaugmentation | Craig C. Criddle | 2001 | $143,770 |

**Aromatic Compounds**

| | | | |
|---|---|---|---|
| Gene Probes for Detecting Anaerobic Alkylbenzene-Degrading Bacteria | Alfred M. Spormann | 2001 | $143,770 |
| Aerobic Cometabolism of Methyl tert-butyl Ether by Microorganisms Grown on Aliphatic Hydrocarbons | Kenneth J. Williamson<br>Lynda Ciuffetti | 2001 | $96,516 |
| Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbons by Toluene-Oxidizing Bacteria: Physiological Consequences and Adaptive Responses | Daniel J. Arp<br>Peter J. Bottomley | 2001 | $86,455 |

**Heavy Metals**

| | | | |
|---|---|---|---|
| Arsenic Removal in High Capacity Porous Alumina Packed-Bed Reactors | James O. Leckie | 2001 | $143,770 |
| Assessing Metal Speciation in the Subsurface Environment - Effect of Wet-Dry Cycles in the Vadose Zone | John Westall | 2001 | $107,177 |
| Multisolute Sorption and Transport Model for Copper, Chromium, and Arsenic Sorption on an Iron-Coated Sand, Synthetic Groundwater System | Peter O. Nelson<br>Mohammad F. Azizian | 2001 | $114,725 |

TABLE 7. TRAINING AND TECHNOLOGY TRANSFER PROGRAM SUMMARY

**Demonstration Projects**

| | | | |
|---|---|---|---|
| In Situ Measurement of TCE Degradation Using a Single-Well, "Push-Pull" Test | Jack Istok<br>Lewis Semprini<br>Jennifer A. Field | 2001 | $213,606 |
| Field Testing of Palladium Catalyzed Hydrogenation for Chlorinated Hydrocarbon Removal: Evaluation of Catalyst Degrading Mechanism | Martin Reinhard<br>Paul V. Roberts | 2000 | $86,270 |
| Bioenhanced In-Well Vapor Stripping to Treat Trichloroethylene (TCE) | Perry L. McCarty<br>Mark N. Goltz<br>Steven M. Gorelick<br>Gary D. Hopkins | 2001 | $805,000 |

**Training and Technology Transfer Projects**

| | | | |
|---|---|---|---|
| Hazardous Waste Training | Peter O. Nelson<br>Ann Kimerling<br>Ken Williamson | 2001 | |
| Conference Sponsorship | Ken Williamson | 2001 | |
| Technical Outreach Services for Communities (TOSC) | Kenneth J. Williamson | 2001 | $485,558 |
| Technical Assistance to Brownfield Communities (TAB) Program | Kenneth J. Williamson | 2001 | $135,000 |

## RESEARCH PROJECT DESCRIPTIONS

## CHEMICAL MOVEMENT, FATE, AND TREATMENT

### Experimental and Mathematical Study of Biomass Growth in Pore Networks and its Consequences in Bioremediation: Peter K. Kitanidis and Perry L. McCarty, Stanford University

Goal: The objectives of this research are: (1) To advance methods for the observation and direct measurement of pore-scale processes that include flow, mass transport, and biomass growth. (2) To perfect methods for the mathematical modeling of the same pore-scale processes. (3) To conduct integrated experimental and modeling studies that will shed light on the processes that promote or control biomass growth, the geometry of biomass distribution, and the shearing and sloughing of biomass due to stresses. (4) To evaluate the effects of pore-scale reactive transport processes on Darcy-scale behavior. Thus, to determine the equations that numerical models should solve in engineering practice. (5) To devise better methods for the control of biofouling and the improvement of the efficiency of in-situ bioremediation.

Rationale: Many of the contaminants found in groundwater can be destroyed or immobilized through in-situ biological processes. In engineered bioremediation, nutrients necessary for biological growth and, at times, selected microorganisms be injected into an aquifer or delivered to the vadose zone to stimulate the desired biochemical reactions. Excessive clogging of the aquifer due to biological growth near the injection well has been identified as one of the most costly and difficult aspects of such bioremediation. The difficulties associated with bioclogging in aquifers are well known, yet basic studies to address this important issue are rare. For the successful implementation of in-situ bioremediation methods, one must be able to predict field-scale reaction rates and to devise practical methods for the addition and mixing of required nutrients and the control of biological clogging.

Approach: This research study consists of integrated experimental studies of biological growth in porous media and mathematical modeling of the physical, chemical, and biological processes involved. The experimental studies utilize a unique experimental facility and will allow us to evaluate modeling hypotheses on reduction in porosity and hydraulic conductivity as well as biological growth distribution and substrate transport and consumption. The experimental studies will also guide the formulation of new modeling hypotheses that are more realistic of the overall processes observed. The modeling studies in turn will generate hypotheses that can be evaluated experimentally.

Status: Microorganisms strain MS1, which is capable of transforming PCE to *cis*-DCE anaerobically, has been cultured in batch experiments, and the pore-network micromodel has been modified and tested for anaerobic conditions. In a control experiment the dissolution processes of PCE, TCE in a pore-network micromodel was evaluated. Dissolution experiments with PCE, TCE, and hexadecane have shown that TCE dissolution was followed by TCE partitioning into the hexadecane pool, which then increased in size. One-dimensional and two-dimensional models were developed to examine the growth of biomass for biologically enhanced PCE DNAPL dissolution, using a finite-difference method. Preliminary simulations indicate that the biomass living very close to PCE DNAPL source zones (within 1 cm) could effectively enhance the DNAPL dissolution. The degree of enhancement depends on several factors, such as the length of PCE DNAPL zones, the supply of the substrate, and the toxicity of PCE to the dechlorinating bacteria.

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

Goal: Long-range goals: (i) to understand the conditions under which redox transformations of environmental species (inorganic and organic components, contaminants) take place and (ii) to develop, evaluate, and deploy field methodology to determine when these conditions occur.

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

Approach: The major focus of this study is determining how redox sensors based on immobilized, colored redox indicators can be constructed and used to determine when certain biogeochemical redox processes are occurring. These redox processes include microbially-mediated transformations of organic (e.g., TCE) and inorganic (e.g., As, Se, Cr) contaminants and of major matrix components which sustain an environmental system at a given redox level (e.g., Fe(III)-reducing, sulfate-reducing, methanogenic, or dechlorinating conditions). The response of the indicators in a variety of samples will be correlated to specific types of microbial activity, the appearance or disappearance of specific matrix species (e.g., sulfide, Fe(II), $CH_4$, and $H_2$ ), or the transformation of a particular organic or inorganic contaminants. The immobilized indicators will be incorporated into simple, miniature spectrochemical and electrochemical devices to make them attractive for monitoring redox conditions in laboratory vessels and in field situations.

Status: We have demonstrated that the redox indicators thionine (formal potential or $E_7^{0'}$ = +53 mV), cresyl violet ($E_7^{0'}$ = -75 mV), and phenosafranine ($E_7^{0'}$ = -267 mV) respond to environmental reductants and oxidants and are useful for delineating between Fe(III)-reducing conditions, sulfate-reducing conditions, and methanogenic conditions in reduced soil and waste water slurries. Methods have been continually refined to immobilize these indicators in robust, thin, transparent films that are in a form suitable for field monitoring of redox conditions. The first models of a number of small and portable devices (flow cells and spectrometers) have been developed to use these "redox films" for monitoring anaerobic samples in closed systems in both laboratory and field settings. These devices have been evaluated for monitoring dechlorinating conditions in laboratory containers such as microcosm bottles and effluents from columns packed with aquifer material and for sub-surface depth profiling of redox conditions such as Fe(II) levels in soil.

## CHLORINATED SOLVENTS

### Investigation of Palladium Catalyzed Hydrodehalogenation for the Removal of Chlorinated Groundwater Contaminants: Surface Chemistry of Catalyst Deactivation and Regeneration: Martin Reinhard, Stanford University

Goal: This project aims to (1) evaluate surface chemical reactions of commonly found natural groundwater solutes at the surface of supported palladium catalysts, particularly the effects of carbonate, nitrate, sulfate, and pH; (2) elucidate the deactivation and reactivation mechanisms, chemical and physical, for these solutes; (3) investigate the potential biofouling and catalyst deactivation that may result from biological activity expected in long-term treatment applications; (4) develop custom catalyst supports to circumvent chemical catalyst deactivation and fouling through ion exclusion or repulsion; (5) develop convenient and economical methods to regenerate catalysts *in situ*.

Rationale: Batch studies with supported palladium catalysts have demonstrated virtually complete reductive dehalogenation of chlorinated ethylenes to ethane at room temperature in short contact times, with reaction rates that are orders of magnitude higher than zero-valent iron. Other batch studies have shown the ability of palladium to catalyze the reaction of a range of compounds: all 6 species of chlorinated ethylenes, carbon tetrachloride, chloroform, 1,2-dibromo-3-chloropropane, Freon 113, chlorobenzene, naphthalene and lindane. However, laboratory column studies and field tests have indicated that catalyst activity may decline under some conditions, thereby potentially affecting the economic competitiveness of this process. Research is needed to optimize the catalyst and operating parameters for the field, by determining the causes of activity loss and preventing or minimizing such effects.

Approach: Advanced surface spectroscopic techniques will be used for characterizing processes that occur at the catalyst surface. The surface of the fresh catalyst will be compared to that of samples taken throughout the duration of column tests run with natural and synthetic groundwaters. The surface of catalyst that was subjected to different treatments will be characterized by chemical and crystal analysis, and the long-term effects of biological growth on catalyst activity will be evaluated. With this understanding of catalyst deactivation mechanisms, custom catalyst supports will be designed to circumvent the competition, inhibition, fouling, and poisoning effects of naturally found groundwater solutes. Finally, convenient methods for regenerating the catalyst beds *in situ* will be developed and evaluated.

Status: Samples of dispersed Pd/alumina were exposed to synthetic and natural groundwater in column experiments and were then analyzed using XPS (x-ray photoelectron spectroscopy). All water-exposed catalysts showed elevated levels of carbon and reduced nitrogen; this indicates an accumulation of organics or biofilms. In addition, the Pd metal was found to oxidize from its zero-valent state to a Pd(II) state, with larger percentages of Pd(II) correlating to lower catalytic activity. No effect was seen with pH changes (among carbonate, bicarbonate and carbonic acid). In addition to the work with dispersed catalysts, a model catalyst was developed and consists of a 0.5” X 0.5” square, flat, polycrystalline, α-alumina plate coated with approximately 500 Å of Pd. The catalyst showed activity for TCE removal from water. A continuously stirred tank reactor (CSTR) was designed and constructed to hold the model catalyst plates. A bromide tracer test indicated that the flow characteristics and residence time distribution match theoretical expectations. TCE sorption to the Pd will be investigated, then the catalyst will be exposed to a continuous flow of water and the surface will be periodically spectroscopically analyzed for changes.

**Measurement of Interfacial Areas and Mass Transfer Coefficients Between Residual PCE and Water During Surfactant Enhanced Aquifer Remediation: Laboratory Studies and Models: Paul Roberts, Stanford University**

Goal: The overall goal of this research is to improve the prediction of residual DNAPL (specifically, PCE) dissolution rates in saturated aquifers via surfactant enhanced aquifer remediation (SEAR). By simultaneous measurement of the PCE-water interfacial area and the mass transfer coefficient, better predictions of PCE dissolution rate as a function of saturation will be attained. These predictions will lead to better cost estimates and feasibility studies of SEAR, as well as other dissolution-based remediation strategies.

Rationale: Due to relatively high water flow rates during SEAR treatment, DNAPL dissolution in high permeability (sandy) aquifers is generally a non-equilibrium process controlled by interfacial mass transfer. Two of the parameters governing this interfacial mass transport are the interfacial mass transfer coefficient (k) and fluid-fluid interfacial area ($a_i$). In order to predict DNAPL-water interfacial mass transfer, as well as overall DNAPL dissolution rates, these parameters must be determined. Hitherto, no studies have been carried out which independently measure both DNAPL dissolution rates and DNAPL-water interfacial areas in porous media.

Approach: A series of measurements in saturated sand columns will be carried out to measure the PCE-water interfacial area at PCE residual saturation. Additional interfacial area measurements will be taken as the residual PCE begins to slowly dissolve due to surfactant flooding. Thus, the relationship between immobile PCE saturation and $a_i$ will be determined. In addition, steady-state PCE dissolution experiments will be simultaneously carried out. Mass transfer coefficients (k) will be calculated from the data. Effects of sand type, velocity, area, surfactant concentration, and PCE saturation on k will be examined.

Status: Data has been collected measuring the PCE-water interfacial area versus residual PCE saturation. Three PCE residual saturations have been analyzed during the dissolution process. Experiments carried out on duplicate columns have shown that both the interfacial area and the saturation data are reproducible. The data also suggest that the residual PCE dissolution does not behave according to 'shrinking sphere' models. Steady state dissolution data as a function of pore water velocity have also been obtained for the same residual PCE saturations. However, more experiments need to be conducted to reduce uncertainty of the data and to determine mass transfer correlations based on the PCE-water interfacial area and pore water velocity. These experiments are currently in progress.

**Development of Alkoxysilanes as Slow Release Substrates for the Anaerobic/Aerobic Transformation of Chlorinated Solvents: Lewis Semprini, Oregon State University**

Goal: The goal of this research is to investigate the use of silicon based organic compounds as slow release substrates to promote both the anaerobic and aerobic transformation of chlorinated aliphatic hydrocarbons (CAHs). The silicon based organic compounds (tetraalkoxysilanes) slowly hydrolyze to generate organic compounds that undergo fermentation reactions to drive anaerobic transformations of CAHs. Recently we have found that these same compounds can serve as cometabolic substrates to drive the aerobic oxidation of trichloroethene (TCE) and 1,2-cis-dichloroethene (cis-DCE). Thus there is potential for sequential anaerobic/aerobic treatment. The objectives of the laboratory studies are to determine how passive biological reactive barriers can be created by the injection of these compounds into the subsurface. The specific objectives are: 1) to study the physical transport of tetralkoxysilanes in porous media and to determine their abiotic hydrolysis rates; 2) to conduct anaerobic transformation studies in continuous flow columns; 3) to conduct anaerobic/aerobic transformation studies in continuous flow columns having an anaerobic zone followed by an aerobic zone.

Rational: Effective methods for the bioremediation of subsurface CAH contamination requires passive, simple, and low cost treatment systems. The proposed research will investigate novel system(s) for driving these enhanced microbial reactions. Silicon based organic compounds will serve as slow release substrates to drive both anaerobic and aerobic transformations. Passive biological barrier systems might therefore be created through the injection of silicon based organic compounds into the subsurface. This research will focus on transport, chemical, and biological processes of importance for the development this passive treatment system.

Approach: Transport and hydrolysis studies will be performed in two laboratory systems: 1) small laboratory columns operated in a batch exchange mode and 2) larger laboratory columns operated in a continuous flow mode. The studies will evaluate the rate of hydrolysis dependence on 1) the tetraalkoxysilanes structure; 2) the loading of the compound on the aquifer materials upon filtration, and 3) the flowrate through the system. Biological transformation studies will then be performed with columns used for transport and hydrolysis studies. The biostimulation of the anaerobic community and transformation of the CAHs will be evaluated in a well-controlled 1-D flow geometry that simulates a passive barrier. The columns will also be bioaugmented with an effective culture that completely transforms TCE to ethene. We will then progress to sequential aerobic/anaerobic column studies.

Status: Microcosms were constructed to evaluate the effectiveness of TBOS as a slow-release substrate for the anaerobic reductive dechlorination of TCE in groundwater from the LLNL Site-300, CA, the Pt. Mugu Naval Weapon Station, CA, and the Evanite site in Corvallis, OR. In these microcosn studies TBOS slowly hydrolyzed to 1-butanol which was fermented to butyrate and/or acetate. Hydrogen produced in the fermentation process likely served as an electron donor for the anaerobic dechlorination. The results show TBOS's potential as a slow-release anaerobic substrate for in-situ bioremediation at different TCE-contaminated sites. Batch and semi-continuous column tests were carried out to investigate the abiotic hydrolysis rate of TBOS to 1-butanol. In batch tests, the abiotic hydrolysis of TBOS followed first order kinetics, with respect to TBOS concentration on the solids, over a broad range of concentrations up to 13 mg/g of soils. The hydrolysis rates in column tests were much lower than those achieved in batch tests. This likely resulted from difficulties of spreading of TBOS on the solid surfaces in the column. The results suggest that the delivery system to create a passive barrier using TBOS as a slow release substrate is a key issue that needs to be addressed in future research.

**Cytochrome P-450: An Emerging Catalyst for the Cometabolism of Chlorinated Aliphatic Hydrocarbons and Methyl *tert*-butyl Ether?**
**Michael Hyman and Lynda Ciuffetti, Oregon State University**

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

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

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

Status: Our studies are currently focussing on Objectives #2 and 3 since Objective #1 has been completed. We have established alkane oxygenase expressed by *M. vaccae* JOB5 participates in multiple reactions in the degradation of MTBE, including the oxidation of late intermediates. Our most recent studies have examined the oxidation of one of these putative intermediates, acetone, in detail. Our latest results suggest there are two reactions involved in acetone consumption by propane-grown *M. vaccae*. A portion (~40%) of acetone uptake activity is insensitive to acetylene. This suggests this activity is independent of oxygenase activity. The remaining acetone uptake activity is fully inactivated by acetylene and can also be inhibited by propane. This suggests this activity is oxyeganse-dependent. We are currently exploring whether the first acetone uptake activity is due to an acetone carboxylase activity similar to that shown in alkene-oxidizing bacteria. If this is activity is involved it may account for the substoichiometric product of $CO_2$ from MTBE. It may also point to a role for $CO_2$ in MTBE oxidation and explain putative intermediates are so elusive during MTBE oxidation assays. Our main focus in Objective #3 continiues to be the characterization of the oxygenase responsible for MTBE oxidation in *Graphium*. We now have a full length clone of an inducible cyctochrome P-450 and are now trying to confirm that this is the enzyme responsible for MTBE-oxidizing activity. Our immediate efforts are directed at verifying whether oxygenase activity (naphthalene oxidation) corresponds with the expression pattern we have observed using propane, propanol and propionate as substrate. Once this has been confirmed we hope to conduct expression studies in a strain of *Verticillium* that we have already established does not have either the ability to grow on propane nor the ability to oxidize MTBE.

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

Goal: The objectives of this study are to describe the bacterium or groups of bacteria that are responsible for conversion of tetrachloroethene (PCE) to ethene in aquifers, and to examine the factors affecting the rate and extent of transformation.

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

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

Status: The original project was funded only by DuPont, but additional support has now been received from the U.S. Department of Energy. This overall study has demonstrated that there are two separate groups involved in the overall dehalogenation of PCE to ethene in our culture, the first group converts PCE to cDCE, and the second converts cDCE to vinyl chloride and ethene. The rates of transformation of PCE and TCE to cDCE were higher than for the conversion of cDCE to vinyl chloride and vinyl chloride to ethene. Organisms appear to obtain energy from the dehalogenation reaction while using the chlorinated organics as electron acceptors. Kinetic studies have indicated the threshold level of hydrogen for dehalogenation, and the effects of CAH concentration on the reaction rates. Batch and column studies have demonstrated that enhanced dissolution of PCE DNAPLs is possible. The advantages and disadvantages of various electron donors, including propionate, benzoate, pentanol, oleate, biomass, and vegetable oil have been demonstrated. Enzymatic studies are continuing on VC dehalogenation in order to better understand the biochemistry of the reactions involved.

**Proof of Gene Expression During Bioaugmentation: Craig C. Criddle, Stanford University, and Syed Hashsham, Michigan State University**

Goal: The overall objective of this work is to develop tools for the evaluation of gene expression in microbial communities. In this proposal, we investigate gene expression for the bioremediation of carbon tetrachloride (CT) by *Pseudomonas* sp. strain KC.

Rationale: Experimental justification for bioaugmentation is typically obtained by comparing the bioremediation of inoculated and uninoculated samples. This approach is adequate for bench-scale studies. At full scale, however, design and operation of uninoculated controls is difficult and expensive. Inadvertent inoculation of "uninoculated" regions must be avoided, and the inoculated and uninoculated regions must initially be geologically, chemically, and biologically similar. Other methods, besides the use of uninoculated control regions, are needed to establish that added organisms are in fact mediating the desired transformations. A logical approach is to prove expression of the genes required for the desired transformation. Gene expression occurs at different levels as the synthesis of mRNA (transcription), the formation of polypeptides (translation), and the biochemical reaction itself. Proof of gene expression is best obtained at each level, because each piece of evidence strengthens the conclusion that gene expression is occurring as intended.

Approach: Transcription and translation have traditionally been established by gel electrophoresis, using Northern blots for mRNA and Western blots and 2-D gels for proteins. These methods are not suitable for microbial communities containing large numbers of diverse biomolecules because they do not provide sufficient discrimination and sensitivity. In this proposal, we focus on the use and development of new tools to permit analysis of gene expression within microbial communities. The tools to be evaluated are cDNA microarrays for mRNA transcripts and Surface Enhanced Laser Desorption Ionization Mass Spectrometry (SELDI-MS) for proteins. We will focus on *Pseudomonas* sp. strain KC and on CT transformation by this strain. To identify mRNA transcripts and proteins for the genes that encode CT transformation, we will use mutants that we have previously generated and characterized. We will then search for diagnostic mRNA transcripts and proteins in mixtures of strain KC and Schoolcraft aquifer flora. The proposed research will pioneer testing and development of both microarray technology and SELDI-MS as diagnostic tools for environmental applications.

Status: We have assembled and tested a microarray printer, we have tested and a microarray reader for use on this work. We have also isolated a DNA template of the 17 known open reading frames in Pseudomonas KC for PCR amplification. Multiple primers have been designed to obtain PCR products of ~100bp length for each ORF. Isolation and purification of total RNA from the pure cultures was used to preparing labeled cDNA targets. Work on PCR amplifications of the probes and slide printing is in progress. We have also completed multiple SELDI-MS analyses of protein extracts and found that this method is not sufficiently sensitive. Accordingly, in the coming year we will focus our efforts on the microarray technology, both at Stanford and at Michigan State University.

## AROMATIC COMPOUNDS

### Gene probes for detecting anaerobic alkylbenzene-degrading bacteria: Alfred M. Spormann, Stanford University

Goal: The objective of this project is to develop molecular tools for detecting anaerobic alkybenzene-degrading bacteria in natural samples. This includes the identification of the genes involved, as well as the fabrication of a DNA microarray carrying probes for the key enzymes involved in anaerobic alkylbenzene degradation. We and others have cloned the genes encoding the key enzyme for anaerobic toluene mineralization, benzylsuccinate synthase, however, the genes facilitating anaerobic ethylbenzene degradation to benzoate are unknown.

Rationale: Remediation of the many fuel hydrocarbon-contaminated sites by traditional physical-chemical processes amounts to enormous costs to the industry and society. Because aromatic hydrocarbons can be degraded biologically, it is in principle possible to predict whether or not these compounds can be mineralized at a given site and what the estimated rate would be. Traditionally, microcosm studies with samples obtained from the contaminated site have been employed for this purspose. These studies are time and labor intensive. With knowledge of the biochemical pathways and the genes involved, more rapid and less expensive tests can be developed. The principle of a molecular test that we envision is based on whether the genes encoding the key enzymes involved in these processes are present in contaminated environments. Using those genes that encode the key enzymes will provide highly specific probes, which unequivocally will query for the presence of the first unique biochemical steps involved in anaerobic degradation of the alkylbenzenes.

Approach: We are studying anaerobic toluene, *m*-xylene, and ethylbenzene-degrading denitrifying bacteria (*Azoarcus* strain T and *Azoarcus* strain EB1, respectively), and have so far identified the genes encoding the key enzymes involved in anaerobic toluene and, most likely, in *m*-xylene mineralization. These genes will provide the basis for developing probes for detecting the presence of these enzymes. However, the genes encoding the key enzymes of ethylbenzene degradation are unknown. We therefore will isolate those genes. Preliminary data obtained from 2-D protein gel electrophoresis indicated several polypeptides that are specifically expressed in cells of *Azoarcus* strain EB1 when grown on ethylbenzene but not when grown on benzoate. Using reverse genetics, the corresponding genes will be cloned, and probes derived from these genes will be printed on a DNA microarray together with the benzylsuccinate synthase genes.

Status: The anaerobic degradation of ethylbenzene in strain EB1 is initiated by a novel enzyme, ethylbenzene dehydrogenase, that converts ethylbenzene to (S)-(-)-1-phenylethanol (Johnson and Spormann 1999 J.Bacteriol. **181**). The ethylbenzene dehydrogenase activity has recently been purified and characterized in our laboratory. Biochemical data indicates the enzyme contains molybdenum, iron and sulfur. The N-terminal amino acid sequences of this heterotrimeric enzyme was used to identify and clone the three structural genes of ethylbenzene dehydrogenase. A 6 kb region of the EB1 chromosome was cloned and sequenced. The nucleotide sequence contains three predicted open reading frames (*ebdA*, *ebdB* and *ebdC*). The predicted EbdA polypeptide (100 kDa) has homology to several polypeptides known to bind a molybdopterin cofactor, selenate reductase (SerA), nitrate reductase(NarG) and DMSO reductase (DmsA). The EbdB subunit (35 kDa) contains a number of proposed 4Fe-4S binding motifs. The EbdC subunit (23 kDa) has homology to only one sequence in the protein data bases, SerC of selenate reductase. SerC has been proposed to be a cytoplasmic membrane anchor subunit, suggesting a role in anchoring the soluble A and B subunits to the cell membrane. These results are all consistant with the biochemical data obtained with purified ethylbenzene dehydrogenase. Further experiments are in progress to identify the other genes facilitating ethylbenzene degradation to benzoate.

**Aerobic Cometabolism of Methyl tert-butyl Ether by Microorganisms Grown on Aliphatic Hydrocarbons: Kenneth J. Williamson and Linda Ciuffetti, Oregon State Univeristy**

Goal: To determine the kinetic coefficients and substrate and metabolite inhibition of MTBE degradation by Mcycobacterium vaccae and Graphium sp.

Rationale: MTBE has been found to be a wide contaminant in groundwater as a result of gasoline spills and leaking underground storage tanks. Remediation technologies are desperately needed by regulatory personnel to facilitate the cleanup of contaminated groundwater at reasonable costs. Unfortunately, both conventional pump-and-treat technologies and soil vapor extraction lack effectiveness for removal of MTBE. Bioremediation by cometabolic organisms appears to be a promising technology to achieve removal of MTBE.

Approach: A fungal strain, Graphium sp., and a bacterial strain, *Mycobacterium vaccae*, were tested for their potential to grow on both propane and isopentane as primary substrates and to aerobically cometabolize MTBE. Cell suspensions were used for the tests with *M. vaccae*; Graphium sp. was tested with filter-attached organisms because of observed low growth rates. Varying concentrations of primary substrates and MTBE were independently added for both cultures and the rate of primary substrate and MTBE consumption was determined by gas chromatography. Similar experiments were conducted to determine the inhibitory effects of each growth substrate on the rate of MTBE degradation by adding the substrate and cometabolite concurrently. Resting cell experiments in batch reactors were performed and the direct linear plot method will be used to determine the kinetic parameters.

Status: Both organisms were found to grow on propane and isopentane. No growth of either strain was observed when MTBE used as the sole source of energy and carbon. The transformation capacities of the cultures were determined. The isopentane-grown fungal strain was found to grow only slowly with low mycelia yield. The rate for MTBE consumption was faster for the isopentane grown bacterial culture relative to the propane grown-culture. Tert butyl -formate and tert butyl-alcohol (TBF & TBA) had been confirmed by GC analysis as the breakdown products of MTBE. Separate experiments were conducted to determine abiotic TBF hydrolysis rates. Degradation curves of MTBE were used to determine $K_{max}$ and $K_s$ values to characterize the interactions between the substrates and the oxygenase enzyme. Determination of inhibition type and inhibition coefficients ($K_{ic}$ & $K_{iu}$) are presently being determined for the two substrates and MTBE, TBF, and TBA. Experiments are being conducted to stimulate bacterial or fungal mixed cultures present in aquifer solids to degrade MTBE. Tests are being conducted in microcosms and in 5.5 x 30 cm columns, both with aquifer solids from an active test site. Experiments will be conducted with bioaugmentation of Graphium sp. and *Mycobacterium vaccae* by addition to aquifer solids in some microcosms.

**Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbons by Toluene-Oxidizing Bacteria: Physiological Consequences and Adaptive Responses: Peter J. Bottomley and Daniel J. Arp, Oregon State University**

Goal: The objectives of this study are to systematically characterize the toxicity and energy costs associated with chlorinated aliphatic hydrocarbon (CAH) cometabolism in *B. cepacia* G4 and other representative toluene-oxidizing bacteria, and to identify cellular mechanisms and growth conditions that minimize these deleterious effects.

Rationale: From the biological standpoint, degradation of CAHs by aerobic cometabolism is largely dependent on two factors: 1) cellular energy requirements and 2) the toxicity often associated with CAH oxidation. Data from previous studies have implied that loss of oxygenase activity, cell viability, or reductant stores may ultimately limit the capacity of individual bacterial strains or consortia to oxidize CAHs. However, the extent that these or other factors interact to limit CAH cometabolism at the cellular level is largely unknown. Furthermore, cellular factors that ultimately limit CAH oxidation have rarely been compared among different bacteria. Certainly, it is not known what physiological or genetic determinants distinguish the proficient CAH-degrading strains. By identifying the common biochemical and molecular mechanisms that limit CAH cometabolism, better control and application of this important degradative process should be realized.

Approach: The effects of CAH cometabolism at the cellular level will first be examined in *B. cepacia* G4. The effects of short-term incubations with trichloroethylene (TCE), 1,1-dichloroethylene (1,1-DCE), and ethylene on cell viability, oxygenase activity, and cellular energy reserves will be assessed in G4. Growth conditions that limit toxicity or energy depletion in G4, thus maximizing its TCE-degrading potential, will also be investigated. In addition, the pattern of gene or protein expression in B. cepacia G4 cells exposed to TCE, 1,1-DCE, ethylene, and other general environmental stresses will be analyzed. Using the results obtained with G4 as a framework, the factors that limit CAH cometabolism by other toluene-oxidizing bacteria will be investigated.

Status: TCE cometabolism results in a loss of cell viability. However, quantification of this loss depends upon the method used to measure viability. Plate counts provide a drastic underestimate compared to LPN counts, unless pyruvate or peroxidase are included in the solid medium. Apparently, cells damaged by TCE are particularly sensitive to the oxygen radicals that are produced in agar media during autoclaving. Transposon mutagenesis of B. cepacia G4 was carried out and mutants debilitated in their ability to recover from TCE exposure were isolated. These mutants fall into two classes, DNA repair genes and carbon metabolism genes. The former suggests that DNA damage occurs upon treatment with TCE thereby requiring the DNA repair systems of the bacteria. The significance of the carbon metabolism mutants is not yet understood.

## HEAVY METALS

**Arsenic Removal in High Capacity Porous Alumina Packed-Bed Reactors: J.O. Leckie. Stanford University.**

Goals: The objective of this project is to utilize high sorption capacity porous alumina particles in continuous-flow packed bed systems for removal of arsenic from contaminated waters. The specific goals are: (1) to study the batch and column sorption behavior of arsenic onto porous alumina particles as a function of solution chemistry (pH, ionic strength, solid-solution ratio, redox state of arsenic (III or V) and presence of co-contaminants), (2) to develop a model that incorporates the effects of solution chemistry, mass-transfer and advection-dispersion to describe arsenic column breakthrough curves and (3) to study the potential for regeneration of the sorbent.

Rationale: EPA recently reduced the maximum contaminant level for arsenic in drinking water to 10 ppb (from 50 ppb) on account of the increased cancer risk now associated with arsenic exposure. Many US surface and groundwaters are out of compliance with this standard, thus requiring some form of treatment before these water resources can used for human consumption. Conventional solubility controls cannot achieve such concentration reductions. Sorption onto packed-beds of porous alumina particles is a promising technology that offers significant advantages over currently available alternative methods (precipitation, ion-exchange and reverse osmosis). The technology could also benefit groundwater remediation efforts in countries like Bangladesh, where the absence of an adequate distribution system has caused millions of people to be dependent on arsenic-contaminated groundwater for their drinking water needs.

Approach: The proposed technology will require the development of a mathematical model for simulating processes relevant to sorption in packed-beds. We are employing a mechanistic surface complexation approach for the sorption behavior along with the pore-diffusion model for the rate-limited diffusion of arsenic into the porous particles. The packed-bed model will be developed (and calibrated) in a series of sequential steps by investigating increasingly complex systems: (a) equilibrium batch sorption, (b) rate-limited sorption in batch systems and (c)transport and rate-limited sorption in column systems. These steps will be used to isolate the effects of the individual phenomena (chemistry, mass-transfer and advection-dispersion) on the arsenic breakthrough curve. Suitable batch and column experiments have been designed to complement model development. Experimental conditions have been selected to incorporate a wide-range of solution chemistries to test the versatility of the packed-bed model.

Status: Equilibrium batch experiments of As (V and III) sorption to DD660 porous alumina particles have been conducted over a range of As concentrations, ionic strengths, and for solutions both open and closed to atmospheric $CO_2$. Equilibrium batch sorption experiments have also been completed for As (V and III) sorption onto DD660 in the presence of varying concentrations of $CO_2$, sulfate, phosphate and silicate. Equilibrium batch sorption experiments for the sorption of sulfate, phosphate and silicate as a function of anion concentration, pH and ionic strength have also been completed. We are currently modeling the equilibrium sorption behavior using the Triple-Layer Model (utilizing the surface complexation approach). Rate of uptake experiments of As (V and III) under selected conditions (pH, ionic strength and presence of competing ions) are near completion. Preliminary batch regeneration studies of the alumina particles have been completed using pH and carbonate concentration to increase As desorption from the surface. We are currently preparing more column experiments to investigate As (V and III) sorption in the presence of competing ligands. We have begun the esperimental design for mixed oxidation state removals in sorptive reactions wth the DD660 alumina.

**Assessing Metal Speciation in the Subsurface Environment - Effect of Wet-Dry Cycles in the Vadose Zone: John C. Westall, Oregon State University**

Goals: The long-range goal of this project is to develop and apply a modeling approach that is suitable for describing the binding of inorganic contaminants to heterogeneous subsurface materials, under water-saturated and water-unsaturated conditions, over a wide range of variations in solution chemistry. The final product is anticipated to be an easy, automated procedure for determining affinity spectra for heterogeneous sorbents, which would then be the basis for modeling metal speciation at a site.

Rationale: Speciation models are needed in virtually all aspects of management of metal contamination of the subsurface environment, including risk assessment, site remediation, and waste disposal. Three of the greatest obstacles in the prediction of metal speciation in field systems are (i) the heterogeneity of environmental materials, such as humic substances or the surfaces of rocks and minerals, (ii) slow kinetics of chemical reactions, such as phase transitions (precipitation-dissolution reactions), and (iii) chemical changes that accompany the variation in water content in the unsaturated zone. In this project, metal speciation models are to be developed that are much better suited to real, complex, heterogeneous materials from the field under conditions of varying water content. Better knowledge of metal speciation will lead to cheaper and better decisions about disposal options, risk assessment, and clean-up procedures.

Approach: Three fundamental barriers to successful description of the association of metal ions with heterogeneous environmental sorbents under varying water content are addressed: (i) the paucity of multidimensional datasets (i.e., datasets with variations in many solution chemistry properties such as pH, salt concentration, total metal concentration, etc.) for sorption of inorganic contaminants; (ii) the paucity of data for sorption of inorganic contaminants in media that are subject to variations in water content; and (iii) the inadequacy of detailed mechanistic models in dealing with these data. First, for water-saturated media, data for binding of inorganic contaminants to heterogeneous environmental sorbents will be collected; the initial focus will be on inorganic priority pollutants common to groundwater pollution problems. Then the "discrete log K spectrum" or "affinity spectrum" approach to modeling the interactions in these systems will be further developed and applied. This modeling approach might appear to be more empirical and less mechanistic than the traditional "surface complexation - electrostatic" approach, but is the method of choice for most real, complex, heterogeneous environmental materials. Finally, the work with saturated soils will be extended to include the effect of wet-dry cycles in unsaturated soils. Because of the variation in water content during these cycles, the binding of inorganic contaminants to soil surfaces may be described more accurately as precipitation-dissolution than as adsorption-desorption. Binding data will be collected will be collected from soils that are exposed to metal ions in a saturated condition, subsequently allowed to dry, and then re-wetted to allow the bound metal to be extracted; laboratory soil column experiments will be used extensively in this phase of the experiment.

Status: The start date of this project was October 1, 1999. The initial work has been focused on model development. The existing "affinity spectrum" approach has been modified in the following ways: (i) the computer program has been converted from Basic to Fortran, which facilitates linkage to external numerical subroutine libraries, and the code itself has been substantially overhauled; (ii) benchmark datasets for the revised code have been developed and tested; (iii) affinity spectrum models for adsorption of target inorganic species, selenate and selenite, under water-saturated conditions, have been  completed.

**Multisolute Sorption and Transport Model for Copper, Chromium, and Arsenic Sorption on an Iron-Coated Sand, Synthetic Groundwater System: Peter O. Nelson, Oregon State University**

Goal: The goal of this proposal is to examine whether the observations and models of metal adsorption on metal oxides can be used to describe the adsorption and transport behavior of a mixture of metals, including both cations and anions, in a heterogeneous porous medium. The objectives of this proposed research are to: (1) determine competitive adsorption equilibria in mixed metals systems using a semiempirical electrostatic implicit adsorption model (EIM); (2) develop an advective-dispersive mixed-solute transport model for the fate and transport of metals in groundwater using the EIM to account for local adsorption equilibria; (3) verify the model by calibration with a CCA-IOCS system; and (4) apply the model to multisolute transport in a real soil.

Rationale: Surface complexation models (SCMs) are capable of simulating the experimentally observed acid-base titration properties of metal oxide minerals. They are also capable of simulating the adsorption of aqueous solution species as a function of pH, solute concentration, and ionic strength. SCMs represent surface chemical reactions with a set of quasi-thermodynamic constants that are independent of changes in solution conditions. The use of parameters from SCMs for modeling the advective-dispersive transport of metals in porous media would be likely to be more flexible and useful for a wider range of environmental conditions than the more empirical adsorption isotherm models (e.g., Freundlich, Langmuir). Recent advances in surface complexation modeling have shown that it is possible to describe adsorption of single solutes to multisite adsorbents using a model without an electrostatic term (electrostatic-implicit model, or EIM; Westall et al., 1995). This model is also applicable to multisolute adsorbate-adsorbent systems, and could be used to describe adsorption of mixed-metals (e.g., CCA) to heterogeneous surfaces (e.g., IOCS).

Approach: The SCM or EIM approach shows great promise in advancing transport modeling for mixed-metals contamination in soils. Batch reactor experiments will be used to develop SCM modeling parameters in single and multisolute systems (copper, chromate, and arsenate) under varying conditions. An advective-dispersive transport model will be adapted to include SCM parameters for adsorption of metals. Column reactor breakthrough experiments will serve as validation of the multisolute transport model.

Status: Both the TLM and the EIM fits to batch experimental data from adsorption of copper, chromate and arsenate in single-metal and multi-metal systems over ranges of concentration and pH have been completed. An advective-dispersive transport model is being developed that incorporates one universal combination of surface complexation reactions and a corresponding set of "conditional" surface complexation equilibrium constants of the EIM for modeling the fate and transport of metals in column breakthrough experiments. Column breakthrough experiments are in progress for single and multisolute systems.

## TRAINING & TECHNOLOGY TRANSFER PROJECT DESCRIPTIONS

## DEMONSTRATION PROJECTS

### In-Situ Measurement of TCE Degradation Using a Single-Well "Push-Pull" Test: Jonathan D. Istok, Lew Semprini, Jennifer Field, Oregon State University

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

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

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

Status: A comprehensive field study was conducted to determine the transport behavior, transformation pathway, and transformation rates of TCFE under defined conditions in a TCE-contaminated aquifer. Single-well, push-pull tests were conducted in two water-bearing zones with different contaminant and biogeochemical environments. Although their transport behavior varied from well to well, TCFE, dichlorofluoroethene (DCFE), and TCE were transported similarly to each other in all wells. In the first water-bearing zone, TCFE was reductively dechlorinated to cis-DCFE, trans-DCFE and (E)-1-chloro-2-fluoroethene (CFE), while co-injected TCE was concurrently transformed to cis-dichloroethene (DCE), trans-DCE, 1,1-DCE, and a trace amount of chloroethene (CE). The TCFE transformation rate was 0.036 μmol/L-day; however, when 2.0 mmol/L formate was added, the rate increased to between 0.053 and 0.30 μmol/L-day. TCE transformation rates with added formate ranged from 0.009 to 0.012 μmol/L-day. TCFE transformation rates were smaller in the second water-bearing zone and were not influenced significantly by the choice of electron donor or the addition of water from the first zone. The results illustrate the potential utility of TCFE as a TCE-analog.

**Field Testing of Palladium Catalyzed Hydrogenation for Chlorinated Hydro-carbon Removal: Martin Reinhard and Paul Roberts, Stanford University**

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

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

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

Status: The first year of the field-scale demonstration employing a reactive well equipped with a Pd-reactor has been completed at LLNL. The demonstration unit utilized a packed-bed column and a microporous hollow fiber membrane hydrogen supply module. Removals were 99% or better for PCE, TCE, and 1,1-DCE, 98% for carbon tetrachloride, 91% for chloroform, and 0% for 1,2-Dichloroethane. These removals are consistent with previously reported laboratory studies. Periodic aeration and shut-downs was necessary for maintaining catalyst activity. On going studies aim to improve the overall efficiency of the reactor and to determine the cost-effectiveness of the approach.

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

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

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

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

Status: The numerical fate and transport model of the technology is complete and has been used to design the treatment wells and monitoring network for this demonstration. Extensive site characterization was accomplished using 2-D refractive and 3-D reflective geophysical survey as well as more traditional methods. A work plan was submitted to Federal, California, and regional regulators, and construction of the treatment system and monitoring wells has been completed. The system is now undergoing site testing, and operation will begin sometime during the first month or two of 2001. Six months of continuous operation is planned in order to reach steady-state operation within the treatment zone. This will be followed with three months of shut down for the treatment system, with continuous monitoring of samples from the many groundwater locations to determine the location and extent of TCE rebound in groundwater that might result.

## TRAINING AND TECHNOLOGY TRANSFER

**Hazardous Waste Training: Peter O. Nelson, Ann Kimlering, and Kenneth Williamson**

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

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

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

**Status:** The Center conducted 26 workshops within Oregon, Washington, Alaska and Idaho in 2000. Funding for the Center has been extended through 2001. The Center has been EPA certified for lead training and is the only certified training program for lead abatement in the Pacific Northwest.

**Conference Sponsorship: Kenneth J. Williamson, Oregon State University and Perry McCarty, Stanford University**

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

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

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

Remediation of Chlorinated and Recalcitrant Compounds, The Second International Conference, May 22-25, Monterey, California.

HSRC Research Symposium, July 9-12, 2000, Asilomar, Pacific Grove, CA

**TECHNICAL OUTREACH SERVICES FOR COMMUNITIES (TOSC) PROGRAM:**
**Kenneth J. Williamson, Oregon State University**

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

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

- Kenneth J. Williamson, TOSC Program Director,
  Professor of Civil, Construction, and Environmental Engineering,
  Ph. (541)737-6836, FAX (541) 737-3099, Email: kenneth.williamson@orst.edu
- Anna Harding, Associate Professor & Chair Department of Public Health,
  Ph. (541) 737-3825, FAX (541) 737-4001, Email: anna.harding@orst.edu
- Mary Masters, Technical Outreach Specialist,
  Ph. (650) 843-0339, FAX (650) 725-9474, Email: mmasters@cive.stanford.edu
- Michael Fernandez, Technical Outreach Specialist,
  Ph. (541) 737-4023, FAX (541) 737-2735, Email: michael.fernandez@orst.edu
- Terry Brock, Graduate student and Program Coordinator,
  Ph. (541) 737-5736, FAX (541) 737-2735, terry.brock@orst.edu
- Andrea Ferro, Research Assistant,
  Graduate Student - Stanford University
  Ph. (650) 723-0315, FAX (650) 725-9474, Email: aferro@stanford.edu
- Janet Gillaspie, Consultant, *Environmental Strategies*,
  Ph. (503) 233-3980, FAX (503) 230-2892, Email: envstrat@teleport.com

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

**Approach:** The Western Region's outreach program is one of five nationally instituted community outreach pilot programs. Centered at Oregon State University, the TOSC team is comprised of university faculty and staff, as well as contracted environmental professionals with specialization in environmental engineering, risk communication, public health, information transfer, and community relations. The TOSC team provides communities with technical assistance related to understanding the effect of hazardous waste sites.

Over the past year, the WR TOSC program has grown significantly to include Technical Outreach to Brownfields (TAB) and Technical Outreach Services to Native American Communities (TOSNAC). In addition, the TAB program has provided conferences devoted to rural brownfields communities in both Oregon and Washington. The TOSC program has successfully obtained EPA and state agency intervention at Union Hills in Arizona, and Waste Disposal Inc., and Eldorado County in California.

**<u>Status:</u>**

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

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

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

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

**Tempe, Arizona:** Assistance is being provided to the Kiwanis Park Neighborhood Association (KPNA) regarding the operational air permitting process for the adjacent ME West Castings foundry. The KPNA is concerned that emissions from the foundry are causing odor, and respiratory health problems in the community.

Chemicals of Concern: Benzene, Hexavalent Chromium, and Other Metals.

**Services to Include: Review of air emissions sampling plan and report**

Review of Final Draft Operational Air Permit, with presentation of findings to the community.
TOSC will monitor and review the emissions sampling to be conducted by the facility prior to the issuance of an operational permit.

Progress to Date:

Reviewed facility emissions data and risk assessment reports from 1994 and earlier years,
Reviewed multiple parts of the proposed air permit,
Attended meetings of local agency, facility, and community representatives to negotiate air permit provisions,
Assisted in reaching agreement on majority of community concerns,
At the request of KPNA, TOSC has developed a draft air sampling plan to monitor emissions in the community.

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

- Provide information regarding chemical sensitivities, and names of experts in this field to community leaders
- Review agency investigation documents
- Solicit EPA or ADEQ intervention to address health concerns and investigate potential contaminant sources.

Progress to Date: Information from chemical sensitivity literature search and review, names of nationally recognized researchers and physicians sent to community. Document review completed. Conducted a health survey of community volunteers on November 11, 1998 to document reported health symptoms and examine any patterns/similarities of symptoms. Prepared a final report and sent it to the state environmental and health agencies, as well as to Region 9 EPA and ATSDR. EPA agreed to meet with the community as a result of the report and subsequently agreed to perform further field work at the site. The field work is expected to begin in early fall.

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

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| Chemicals of Concern: | VOCs, PAHs, Pesticides, PCBs, and Metals identified in soils and groundwater. |
| Services to Include: | Review and comment on the Remedial Investigation, Human Health Risk Assessment, and the Ecological Risk Assessment associated with soil and groundwater contamination at OU-1 |
| Progress to Date: | OU-1 Document review and comment completed. TOSC presented its findings at a November 3, 1998 RAB meeting. The RAB has determined the Navy's response to RAB/TOSC comments to be inadequate. The RAB has asked the agencies to intervene on it's behalf, and is awaiting a revised response to OU-1 comments,<br><br>RAB has submitted an informal request for TOSC assistance re: OU-3 document review. |

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

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| Chemicals of Concern: | PCE, PCBs, Pesticides, Arsenic, and Lead in soils and groundwater. |
| Services to Include: | Review and evaluation of soil and groundwater sampling results, |

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| | Conduct a community educational workshop focused on potential health concerns associated with contaminants found in local residential soils,<br>Review and comment on Remedial Investigation and Feasibility Study (RI/FS) documents.<br>TOSC will review the next Draft RI/FS. |
| Progress to Date: | Completed historical document review.<br>Comments provided to the California Dept. of Toxic Substance Control regarding a proposed neighborhood soil removal plan.<br>Comments on recent groundwater monitoring results provided to SAEJ. |

**California Trade and Commerce Agency:** CTCA received an EPA pilot grant to conduct site assessments at select group of former lumber mill sites in California. Assisted TCA in presenting its workshop in June 1999.

Workshop was targeted to small communities in California interested in redeveloping former mill sites as well as other former commercial/industrial facilities.

Agreed to assist pilot mill community in their assessment, cleanup, and redevelopment efforts on an ongoing basis. Communities assisted and assistance provided include the following:
**North Fork – review of data summary report**
Del Norte County – redevelopment charrette
Foresthill – redevelopment charrette

**Naval Air Station North Island, Coronado, California:** Continue work with the Naval Air Station North Island Restoration Advisory Board (RAB).

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| Chemicals of Concern: | Primarily TCE and Metals |
| Services to Include: | Review of Navy contractor reports and documents associated with soil and groundwater contamination at two operable units (sites 9 and 11).<br>Attend RAB meetings to comment on proposed investigation, treatability study, and remediation plans.<br>TOSC will continue to serve on RAB Technical Workgroup to address Site 9 RI/FS issues. |
| Progress to Date: | Attended several RAB meetings. Reviewed background information.<br>Provided technical assistance related to incineration option, risk of worker exposure to TCE, and management of migration of contaminants to San Diego Bay.<br>Reviewed and commented on Site 9 Draft Feasibility Study. |

**The Presidio of San Francisco, California:** Providing assistance to the Restoration Advisory Board (RAB) regarding issues related to the remediation and reuse of this U.S. Army facility.

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| Services Include: | Research to determine whether proposed institutional control remedies at any DOD remediation sites have been successfully contested by RABs,<br>Research into non-hazardous landfill debris recycling |
| Progress to date: | Attended three RAB meetings; research on both of the above topics is underway. |

**Waste Disposal, Inc. NPL Site, Santa Fe Springs, California:** Assistance is being provided to the Protect Our Neighborhood Committee (PONC) regarding the adequacy of the remedy selected for the WDI Superfund Site, and the potential that exposures to site-related contaminants may be in some way related to community illnesses.

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| Chemicals of Concern: | Metals, VOCs, PAHs, PCBs, and Pesticides in Soils,<br>Methane, Benzene, Vinyl Chloride, TCE and Other VOCs in Soil Gas,<br>VOCs in Groundwater. |
| Services to Include: | Review the Record of Decision,<br>Review past and current data associated with groundwater, soil, and air sampling,<br>Determine health related concerns of residents living adjacent to the site,<br>Provide an updated list of contaminants affecting groundwater, soil, and air both at the site and in the adjacent community,<br>Will review data from several new groundwater and soil gas sampling events conducted in Fall '98 due to the re-opening of the ROD.<br>TOSC will submit comments to the State Dept. of Health Services (DHS) on their Draft Public Health Assessment, on behalf of the PONC. |
| Progress to Date: | Attended several meetings, completed historical document review.<br>Facilitating community effort to have the state Dept. of Health Services (DHS) conduct a survey of health symptoms and concerns of local residents. DHS has agreed to conduct a survey of community health concerns as part of its Public Health Assessment process, scheduled to begin 2/99. |

**Marine Corps Air Facility, Tustin, California:** Providing technical assistance to RAB in review of groundwater remediation activities in two Operable Units at this Marine Corps Air Facility (MCAF).

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| Chemicals of Concern: | TCE and Other VOCs in Groundwater |
| Services to Include: | Review and comment on RI/FS and Draft ROD documents at OU-3,<br>Review and comment on Draft RI/FS at OU-1,<br>Ongoing educational programs for RAB members related to remediation plans and activities |
| Progress to Date: | Document review ongoing, some comment provided. Awaiting revised report from the Army Corps of Engineers regarding |

proposed flood control project along site boundary, and its potential impact on groundwater contaminant migration at OU-3, TOSC conducted a presentation on the OU-1 FS at a January ’98 RAB meeting, and has attended additional regular RAB meetings.

**Mare Island Naval Shipyard, Vallejo, California:** Providing assistance to RAB in review of remediation activities at the Mare Island Naval Shipyard where the RI/FS is underway.

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| Chemicals of Concern: | VOCs, Metals, Pesticides, PAHs, and PCBs in Soils, Groundwater, and Marine Sediments. |
| Services to Include: | Review and evaluation of the Human Health Risk Assessment and the Ecological Risk Assessment |
| Progress to Date: | Completed review and gave a presentation on Risk Assessments at an August '98 RAB meeting. May be asked to conduct additional review and comment, awaiting RAB decision. |

**Makua Military Reservation, Oahu Island, Hawaii:** Providing assistance to the Malama Makua community group regarding investigation and possible closure activities at the U.S. Army's Makua Military Reservation, a live ordinance testing and training facility.

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| Chemicals of Concern: | Lead, Arsenic, 2,4-DNT, and 2,6-DNT in Soils, Groundwater has not yet been detected beneath the investigation area. |
| Services to Include: | Review and comment on documents related to the potential closure of a former Open-Burn Open-Detonation (OBOD) site on this base,<br>Review and comment on Army's investigation of area groundwater and near-shore water contamination. |
| Progress to Date: | Have provided contact information for Unexploded Ordinance (UXO) expertise,<br>TOSC attended a meeting of all stakeholders, and toured the site in December 1998,<br>Document review is in progress, comments to be submitted 2/99. |

**Blair Community, Eugene, OR –** Providing assistance to the Blair Community related to health concerns from presently unknown sources. TOSC services to include:

- Meeting with community to discuss health symptoms and concerns
- Reviewing regulatory agency records to determine if potential contaminant sources exist in the neighborhood
- Prepare a report based on the agency file review with recommendations for further action.

Progress to date: Participated in several community meetings to discuss health concerns and obtain historical information about neighborhood. Conducted health survey to obtain detailed information about residents' health symptoms. Prepared report on survey results and agency file review. Obtained agency commitment to perform site investigation work in order to determine if hazardous substance releases are likely to have occurred. Will assist community with investigation of pesticide exposures as well.

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

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

Progress to date: Review of RI report and modeling of stripping tower air discharge completed. Attended several community meetings. Presented detail on alternative bioremediation protocol, environmental partitioning of P/TCE, and fabrication of activated charcoal filters for air strippers. Facilitated dose reconstruction modeling through ATSDR Involved in discussion of a registry, and/or health study in conjunction with ATSDR. Presented detail on alternative bioremediation and air stripping protocols, environmental partitioning of PCE and TCE, and fabrication of activated charcoal filters for air strippers. Facilitated dose reconstruction modeling through ATSDR. Involved in discussion of a registry, and/or health study in conjunction with ATSDR. Presented detail on alternative bioremediation and air stripping protocols, environmental partitioning of PCE and TCE, and fabrication of activated charcoal filters for air strippers. Facilitated dose reconstruction modeling through ATSDR. Involved in discussion of a registry, and/or health study in conjunction with ATSDR

**Portland, OR –** Providing assistance to the City of Portland Brownfield Showcase Community. Portland was awarded an EPA Brownfields Showcase grant in 1996. The grant is intended to fund a city program to promote and enhance opportunities for redevelopment of brownfields in the city. Work will include assistance with community outreach and education efforts, review of web site and suggestions for improvement, and review of technical reports as necessary.

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

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

Progress to date: Attended community meeting on August 20, 1998. Contacted WA Department of Agriculture to discuss pesticide application practices of local orchard owners, and most effective means of reporting pesticide exposures. Currently reviewing WA Department of Agriculture report on orchard spraying incidents and will make recommendations for further action based on that review. Agreement from Region 10 ATSDR representative to assist as needed following report review.

**Quincy, WA** - Providing assistance to the community the investigation and cleanup of a fumigant release from the Cenex Harvest States fumigant storage facility.

Progress to date: Participating in periodic community meetings. Reviewed and commented on FS.Will review and comment on Department of Ecology remedy selection.

**Other Activities:**

Rural Brownfields Conferences -Working with EPA Region 10 Brownfields Coordinator in developing a series of one to two day workshops to be held in Region 10. Workshops are targeted to rural area municipal and development leaders, and will provide Brownfields basics, a handbook of tools and information resources, as well as consideration of some local/regional brownfield issues, including financing, community outreach, and site assessment and cleanup. Working with the Region 10 Environmental Finance Center (EFC) and Oregon Economic Development Department to assist TAB program in presenting these workshops.

The first workshop was held on November 1 and 2, 1999 in Bend, OR. A second Oregon workshop will take place on September 25 and 26, 2000.

Currently planning conference for Washington rural communities scheduled for November 1 and 2 in Ellensburg, WA.

**TECHNICAL ASSISTANCE TO BROWNFIELDS COMMUNITIES (TAB) PROGRAM: Kenneth J. Williamson, Oregon State University**

Goal: The TAB program provides assistance to communities attempting to address cleanup and redevelopment of properties whose reuse has been prevented by real or perceived contamination. The TAB program makes use of the same faculty, consultant, and research assistant staff as those involved in the TOSC program. TAB attempts to improve involvement of all affected parties in cleanup and redevelopment process through education and training. TAB also attempts to accelerate the redevelopment process through the application of HSRC and other research and through improved community involvement.

Status: Activities during 1999 were as follows:

**City of Portland, OR:** Brownfields Showcase Community: Assisted the community in the screening of potential redevelopment sites. Participated in Technical Committee meetings and developed a draft protocol for soliciting input on historical usage of properties from local residents. Will continue to participate in Technical Committee meetings and provide technical assistance in the form of report and data review as needed.

**California Trade and Commerce Agency (TAC):** Assisted TAC in planning its mill site redevelopment kickoff workshop on June 21, 1999. Provided three speakers for the conference. Will continue to work with TAC to assist mill site communities overcome obstacles to redeveloping the sites. Will also participate in the planning and staging of a follow-up workshop for mill site communities.

**Hoopa Valley Tribe, California**: TAB is currently negotiating an agreement to provide technical assistance to the Tribal Environmental Protection Agency. The tribe is planning for redevelopment of an eighty-three acre former mill-site and has asked for TAB assistance with investigation and cleanup plans as well as with community outreach regarding redevelopment of the site.

Tools for Redeveloping Oregon Rural Brownfields Conference: Planned and staged a conference to assist rural communities in redeveloping local brownfields. The conference took place on November 1 and 2, 1999 and included presentations from twenty-three speakers on topics including environmental assessment and cleanup, managing legal liability, financing cleanup and redevelopment, and actual case studies of brownfields redevelopment projects. Forty representatives of municipal, county, regional, state, and federal government attended the conference.

## TECHNOLOGY TRANSFER PLAN

Webster defines technology as "the science or study of the practical or industrial arts." Contrary to the view of many, technology then is not simply the development of a new thing, but the generation of new knowledge that has practical significance. Technology transfer may involve the transfer of useful knowledge as well as the transfer of a new approach to cleanup. Transfer of newly created knowledge is a major goal of the WRHSRC' technology transfer activities. This is most effectively done by presentations at national meetings and publication in well read and respected peer review journals. In other cases, new approaches to cleanup have evolved from the centers basic studies. Such new approaches generally require laboratory evaluation, pilot studies, and then full-scale demonstrations. This may be done by Center faculty themselves, or through participation in demonstrations by industry, federal laboratories, other universities, or a combination of such organizations. The results of such demonstrations are transferred by involving end users in the demonstration projects, presentations at important national and international meetings, and publication in peer reviewed journals.

The WRHSRC currently has 14 active research projects. Additionally, WRHSRC researchers have three demonstrations ongoing funded through the WRHSRC plus several more that are funded outside the WRHSRC. The major goal of the original solicitation for the five HSRCs was to conduct basic research that would help to better understand how hazardous chemicals move and are transformed in the environment, their ultimate fate, and methods for their removal or control. In some instances, the knowledge gained has saved the country billions of dollars because it has allowed us to select more appropriate remedial methods. For example, the observation and then basic studies that proved conclusively that aromatic hydrocarbons such as benzene, toluene, xylene, and ethylbenzene are biodegradable under anaerobic conditions has led to the concept of natural attenuation. The knowledge gained from these basic studies has provided the scientific underpinning for EPA's new protocol on natural attenuation. However, natural attenuation has limitations. For example, MTBE, an additive to gasoline is not readily biodegradable and so persists even when the aromatic hydrocarbons of most concern in the past, disappear. Unfortunately, knowledge of MTBE's persistence, which was available long ago, was not effectively transferred until the problem of its contamination of groundwaters reached crisis proportions and will now cost the country billions of dollars to rectify. This demonstrates the importance of gaining new knowledge and effectively transferring it. Had knowledge of MTBE been transferred effectively early on so that the problem did not develop, how would one estimate the cost savings to the country that would have resulted? This is probably not possible, and probably should not be attempted. One has to have faith that the generation of new knowledge will lead to better decisions that in the long run will be better for all.

EPA and other federal agencies have also been pursuing natural attenuation protocols for chlorinated solvents, compounds of major study by the WRHSRC. Our studies, which are supported by industry as well as the federal government, have been directed towards determining factors affecting the rate and extent of chlorinated solvents degradation. This is a complex process that has involved numerous scientific studies, laboratory bench-scale evaluations, and major field studies, not only by the WRHSRC, but by many others. These studies have had a major impact on knowledge of chlorinated solvent movement, transformation, and fate in the environment that is helping to develop realistic approaches to remediation for the protection of human health and the environment. This knowledge is helping to make the new protocols much sounder.

Often, serendipity plays an important role in the development of new findings of great significance. For example, WRHSRC researchers were among the first to observe that aromatic hydrocarbons and chlorinated solvents were transformed under anaerobic conditions, which is the underpinning for the above protocols. This was the result of field observations and has led to major approaches of importance. Our researchers similarly found an organism that anaerobically biodegrades carbon tetrachloride anaerobically without the production of chloroform. Bioaugmentation with this organism is now ongoing at a field site in Michigan.

In summary, new knowledge of hydrogeology, microbiology, chemistry, and engineering together are required to understand hazardous chemical movement and fate in the environment. This knowledge helps to better evaluate which potential approaches to cleanup may work and which ones may not. Many of our research projects are directed towards obtaining such new knowledge, while others are concerned with bench scale studies of new cleanup approaches that have evolved, eventually leading to field scale demonstrations. With this background, the technology transfer efforts for each of the 14 WRHSRC research projects and three demonstration projects is provided in the following for the projects listed in Table 6. Each is discussed in the order listed in that table.

**Experimental and Mathematical Study of Biomass Growth in Pore Networks and its Consequences in Bioremediation, Peter Kitanidis and Perry McCarty.**

Knowledge of the bioclogging problem in aquifers is a major problem for biological remediation of hazardous organics. Basic knowledge obtained here will be transferred primarily through presentations at national meetings and publication in peer reviewed journals.

**Development and Characterization of Redox Sensors for Environmental Monitoring, James Ingle.**

This is a continuation project of that listed above, with the same approach to technology transfer.

**Investigation of Palladium Catalyzed Hydrodehalogenation for the Removal of Chlorinated Groundwater Contaminants: Surface Chemistry of Catalyst Deactivation and Regeneration, Martin Reinhard.**

This original approach for destroying chlorinated solvents in-situ has been most successful and has already been demonstrated full-scale in the field through cooperative studies with the Lawrence Livermore National Laboratory and with researchers in Germany. This basic study is directed towards improvement of the life of the palladium catalyst used to reduce costs. Direct application to ongoing field studies is the major technology transfer approach, with publication in peer review journals and presentations at national meetings occurring as well.

**Measurement of Interfacial Areas and Mass Transfer Coefficients between residual PCE and Water During Surfactant Enhanced Aquifer Remediation: Laboratory Studies and Models, Paul Roberts.**

This is a basic study directed at better understanding the use of detergents for solubilization of dense non-aqueous phase liquids, such as chlorinated solvents. Technology transfer here will be through presentations of basic knowledge at national meetings and publication in peer-reviewed journals.

**Development of Alkoxysilanes as Slow Release Substrates for the Anaerobic and Anaerobic/Aerobic Transformation of Chlorinated Solvents, Lewis Semprini.**

This study resulted through serendipity from a field study conducted with the Lawrence Livermore National Laboratory. Alkoxysilanes were found to have excellent potential as slow hydrogen release compounds for reductive dehalogenation of chlorinated solvents. The basic studies will indicate their usefulness and limitations in comparison with commercially available compounds now used for this purpose. Technology transfer will be through presentations of basic knowledge at national meetings and publication in peer-reviewed journals.

**Cytochrome P-450: An Emerging Catalyst for the Co-metabolism of Chlorinated Aliphatic Hydrocarbons and Methyl tery-Butyl Ether?, Michael Hyman and Lynda Ciuffetti.**

This basic study is the outgrowth from other basic research and field demonstrations on biodegradation of chlorinated solvents and MTBE, both of which are aerobically biodegradable through cometabolism. This project will help better understand what organisms and transformation processes involved in the transformations of these, the major organic groundwater contaminants in the country. Technology transfer will be through presentations of basic knowledge at national meetings and publication in peer-reviewed journals.

**Mechanisms, Chemistry, and Kinetics of Anaerobic Degradation of cDCE and Vinyl Chloride, Perry L. McCarty and Alfred Spormann**

This broad study of anaerobic biological dehalogenation of chlorinated solvents is supported by DuPont and the U.S. Department of Defense to obtain more basic information of this important process to aid in extending the successful use of its potential in the field. The direct transfer to industry is assured by the close cooperation with DuPont and participation in the Research Technology Development Forum that is concerned with the application of this approach for engineered and natural remediation of chlorinated solvents. Technology transfer is also be through numerous presentations of basic knowledge at national meetings and publication in peer-reviewed journals.

**Proof of Gene Expression During Bioaugmentation, Craig C. Criddle.**

WRHSRC researcher Criddle discovered a unique microorganisms for anaerobically converting carbon tetrachloride to carbon dioxide and chloride without formation of chloroform. He has been involved in successful field-scale demonstrations of bioaugmentation with this organism. This basic study is to develop new molecular biology approach for monitoring the persistence of this microorganism and its activity in the field. Th knowledge from this basic research with much promise may find direct application in the ongoing field application studies, and also will be transferred in the usual way by presentations at national meetings and publication in peer-reviewed journals.

**Gene Probes for Detecting Anaerobic Alkylbenzene-Degrading Bacteria, Alfred M. Spormann**

This study, like the above one, is to make use of new methods of molecular biology for monitoring the presence and effectiveness of key genes for the biodegradation of hazardous compounds in the environment. Technology transfer will be through presentations of basic knowledge at national meetings and publication in peer-reviewed journals.

**Aerobic Cometabolism of Methyl tert-butyl Ether by Microorganisms Grown on Aliphatic Hydrocarbons, Kenneth J. Williamson and Lynda Ciuffetti.**

This basic study evolved from former WRHSRC studies indicating fungi grown on propane could cometabolize MTBE. It has now been found that bacteria grown on propane and other hydrocarbons also cometabolize MTBE. The potential for using aerobic cometabolism for degrading MTBE in situ is large and the subject of this basic study. Technology transfer will be through presentations of basic knowledge at national meetings and publication in peer-reviewed journals. Opportunities for field demonstration are also being pursued.

**Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbons by Toluene-Oxidizing Bacteria: Physiological Consequences and Adaptive Responses, Daniel J. Arp and Peter J. Bottomley.**

Toluene using microorganisms were found through WRHSRC research to be highly effective for in situ cometabolism of TCE through extensive laboratory research and several field demonstrations. Basic research to better understand this process is the goal of this project. Technology transfer will be through presentations of basic knowledge at national meetings and publication in peer-reviewed journals, or thorough direct application in ongoing field demonstrations.

**Arsenic Removal in High Capacity Porous Alumina Packed-Bed Reactors, James O. Leckie.**

The extensive presence of hazardous concentrations of arsenic in groundwater requires inexpensive and effective approaches for removal at very low levels. Extensive studies with alumina packed beds for removal of trace levels of other contaminants such as selenium (see below) led to this new study. Technology transfer will be through presentations of basic knowledge at national meetings and publication in peer-reviewed journals, or thorough direct application in field demonstrations, if possible.

**Assessing Metal Speciation in the Subsurface Environment - Effect of Wet-Dry Cycles in the Vadose Zone, John Westall.**

The toxicity and mobility of heavy metals in the subsurface depends upon the redox state. This basic study is to help better determine factors affecting speciation of metals. Technology transfer will be through presentations of basic knowledge at national meetings and publication in peer-reviewed journals.

**Multisolute Sorption and Transport Model for Copper, Chromium, and Arsenic Sorption on an Iron-Coated Sand, Synthetic Groundwater System, Peter O. Nelson.**

One approach for preventing migration of hazardous inorganics in groundwater is through use of reaction walls. This basic study is to determine the effectiveness of iron coated sand to act as a barrier to migration of hazardous inorganics with different speciation characteristics. Technology transfer will be through presentations of basic knowledge at national meetings and publication in peer-reviewed journals.

**In Situ Measurement of TCE Degradation Using a Single-Well, "Push-Pull" Test, Jack Istok, Lewis Semprini, and Jennifer A. Field.**

The push-full test has been developed through the WRHSRC and has been field evaluated at a number of locations. This basic demonstration is to further illustrate the potential for other applications. Technology transfer is through additional applications at other locations in conjunction with studies by others, as well as through the normal presentations of basic knowledge at national meetings and publication in peer-reviewed journals.

**Field Testing of Palladium Catalyzed Hydrogenation for Chlorinated Hydrocarbon Removal: Evaluation of Catalyst Degrading Mechanism, Martin Reinhard and Paul V. Roberts.**

This is one of the demonstrations conducted together with that of the Lawrence Livermore National Laboratory and in Germany to demonstrate this process for commercialization. Further transfer to the user community will be through normal presentations at national meetings and publication in peer-reviewed journals.

**Bioenhanced In-Well Vapor Stripping to Treat Trichloroethylene (TCE), Mark N. Goltz,** Steven Gorelick, Perry L. McCarty, and Gary D. Hopkins.

This demonstration project of two technologies in combination, each of which was developed through basic studies and field demonstrations by the WRHSRC in conjunction with the U.S. Departments of Defense and Energy, is funded by the U.S. Department of Defense. Through close cooperation with the U.S. Department of Defense and private contractors, technology transfer is assured. Further, transfer will be achieved through the usual route of presentations at national meetings and publication in peer reviewed journals.

## 2000 WRHSRC PUBLICATIONS

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