



**WESTERN REGION
HAZARDOUS SUBSTANCE RESEARCH CENTER**

1999 ANNUAL REPORT

Stanford University
Oregon State University

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**WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER
1999 ANNUAL REPORT**

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THE CENTER AT A GLANCE

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

The objectives of the Center are:

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

Groundwater cleanup and site remediation, with a strong emphasis on biological approaches, 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 22 faculty and 3 staff who are directing the Center's research, training, and technology transfer activities are listed in Table 1. They collectively represent an integrated research team representing five different schools (civil engineering, engineering, science, earth sciences,

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

Table 1. KEY PERSONNEL AT THE WRHSRC

| <u>Stanford University</u> | <u>Oregon State University</u> |
|----------------------------------|-----------------------------------|
| Prof. Craig C. Criddle | Prof. Daniel J. Arp |
| Prof. Mark N. Goltz ^a | Professor Lynda Ciuffetti |
| Prof. Steven M. Gorelick | Dr. 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 1998 fiscal year and since the Center's inception are listed by category of support in Table 4. The education of students interested in careers directed toward finding solutions to environmental problems is another important goal. The number of students supported through WRHSRC funds is listed in Table 5.

Table 2. SCIENCE ADVISORY COMMITTEE

| <u>Member</u> | <u>Affiliation</u> | <u>Expertise</u> |
|-----------------------|---------------------------------|-------------------------------|
| Linda M. Abriola | University of Michigan | Physical Processes, Hydrology |
| David E. Ellis | Du Pont Chemicals | Chemical/Biological Processes |
| John Glaser | Govt/EPA, Cincinnati Lab | Physical/Chemical Processes |
| Ronald Hoeppe | Govt/U.S. Navy | Microbiology |
| Michael C. Kavanaugh* | Malcolm Pirnie | Physical/Chemical Processes |
| Richard G. Luthy | Carnegie Mellon University | Chemical/Biological Processes |
| Stephen Schmelling | Govt/EPA, Ada Lab | Groundwater Remediation |
| James M. Tiedje | Michigan State University | Microbiology |
| John L. Wilson† | New Mexico Technical University | Hydrology |

John Wise

Govt/EPA, Region 9

Planning

* Chairman

† Vice Chairman

Table 3. TRAINING AND TECHNOLOGY TRANSFER ADVISORY COMMITTEE

| <u>Member</u> | <u>Affiliation</u> | <u>Expertise</u> |
|------------------------|---------------------------|---------------------------|
| James T. Allen | Govt/ California | Treatment Technology |
| Michael Anderson | Govt/Oregon | Hydrology |
| 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 |

* Chairman

Table 4. CENTER FUNDING

| <u>Funding Sources</u> | <u>FY 1999*</u> | <u>Funds to Date</u> |
|------------------------|------------------|----------------------|
| EPA: Centers Program | \$948,000 | \$11,726,015 |
| EPA: Other | \$365,000 | \$3,981,816 |
| Other Govt: Federal† | \$208,000 | \$8,077,195 |
| Other Govt: State@ | 0 | \$48,252 |
| Consortium | \$136,818 | \$3,715,922 |
| Private Sector# | <u>\$100,182</u> | <u>\$2,344,634</u> |
| TOTAL | \$1,758,000 | \$29,893,834 |

* Oct. 1, 1998 - Sept. 30, 1999

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

@ Oregon Department of Transportation

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

TABLE 5. STUDENT SUPPORT

| <u>Student Support</u> | <u>Number*</u> | <u>Funds to Date†</u> |
|------------------------|----------------|-----------------------|
| B.S. | 13 | \$95,877 |
| M.S. | 37 | \$1,013,071 |
| Ph.D. | 128 | \$5,771,658 |

| | | |
|---------------|-----------|--------------------|
| Post Doctoral | <u>26</u> | <u>\$1,563,233</u> |
| TOTAL | 204 | \$8,443,839 |

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

† Includes tuition, travel, supplies, etc.

DIRECTOR'S REPORT

The year 1999 represents the completion of the first decade of activity for the Western Region Hazardous Substance Research Center. Thus, this annual report will present a review of the Center's accomplishments since its inception, rather than the usual annual report alone. The Center faculty and research staff essentially completed 68 separate research projects undertaken since the Center began in 1989. Eleven of these projects were supported through the Research and Re-Education for Department of Defense (R2D2) program. An additional five research projects were funded at minority academic institutions (MAI) through sponsorship by the WRHSRC with financial support for this purpose from the U.S. EPA. As in the past, all WRHSRC projects have a focus on groundwater cleanup and remediation of sites contaminated with chlorinated solvents, halogenated aromatic compounds such as pentachlorophenol, nonhalogenated aromatics, including petroleum derivatives such as gasoline, and heavy metals. Other projects specifically address the evaluation of factors affecting movement and fate of the above contaminants in the environment and design and management issues for site cleanup in general.

Research supported through the WRHSRC resulted in about 170 peer reviewed publications, four patents, and numerous chapters in books, conference reports, extended abstracts, project reports, and both M.S. theses and Doctoral dissertations. The titles of these publications are summarized at the end of this report. The peer-reviewed manuscripts have been published in leading journals. For example, about one-half have been published in the three major applied environmental science journals in microbiology, chemistry, and hydrogeology (*Applied and Environmental Microbiology*, *Environmental Science & Technology*, *Water Resources Research*, respectively). There have been well over 1,000 citations among various journals to the research conducted by the WRHSRC, indicating the research has had significant impact in furthering the research of others.

An additional highly important activity of the WRHSRC is in the education of engineers and scientists in hazardous waste remediation. Toward this end, 39 students have received their Ph.D. dissertations through research funded by the Center. About two-thirds of these are now teaching at other universities and continuing research and education in hazardous waste remediation. Others are working at federal laboratories, with regulatory agencies, and with consulting firms, bringing their expertise in hazardous substances with them. The quality of the doctoral research is indicated by the fact that three of Center-supported doctoral scholars have received awards from the Association of Environmental Engineering and Science Professors for producing the outstanding environmental engineering and science Ph.D. dissertation of the year.

The other significant phase of WRHSRC activities is the Training and Technology Transfer (TTT) program. This program fosters transfer of basic research ideas into practice through field demonstrations, which generally involve working with practitioners, industry, and federal agencies. Through these efforts, eight field demonstration projects have been completed. Two of these at Edwards Air Force Base (in-situ cometabolic degradation of TCE and in-situ vapor stripping of TCE) were supported by the U.S. Air Force with additional support for the vapor stripping project from the U.S. DOE. Another project on in-situ biodegradation of petroleum hydrocarbon at Seal Beach Naval Weapons Laboratory received support from the U.S. Department of Navy. This project is continuing, but with funds received outside of the Center. Thus, it is no longer considered a Center project. This is true of several projects now being

conducted by Center faculty. For example, Center faculty are conducting field demonstration projects at Moffett Federal Air Field (two), the Lawrence-Livermore National Laboratory, and McLellan Air Force Base. These are funded through the U.S. Department of Energy, the U.S. Department of Defense, the Strategic Environmental Research and Demonstration Program (SERDP), and the Environmental Strategic Technology Certification Program (ESTCP). The integrated team approach to solution of complex environmental problems that was fostered by the Center is providing benefits to the Center faculty and researchers who are now able to receive funding for hazardous substance research outside of the Center itself. A current Center Demonstration project on TCE removal from contaminated water is ongoing at Edwards Air Force Base with SERDP and U.S. Air Force financial support.

Another major TTT project that has been ongoing for four years is the Technical Outreach Services for Communities (TOSC) program to aid communities impacted by hazardous waste problems. Many communities within the EPA Region 9 and 10 states have benefited from the independent review and assistance that the WRHSRC has provided when requested to do so. In addition, the TTT function of the Center continues to include sponsorship of conferences and sessions concerned with hazardous waste remediation.

One of the major findings of recent years is that chlorinated solvents are transformed in groundwater by both chemical and biological processes. The thrust group associated with the problem of chlorinated solvent contamination is multidisciplinary with active research at both Stanford and Oregon State and combined expertise in microbiology, chemistry, hydrogeology, hydrology, and engineering. The group is directing its combined efforts towards obtaining better fundamental understanding of the chemistry, microbiology, and hydrogeology involved. The projects seek to find engineering methods for in situ treatment, including the subsurface mixing of chemicals that can help enhance natural processes for destroying the contaminants of concern or for physically removing them from the subsurface. Projects emphasize both aerobic and anaerobic biological transformations of carbon tetrachloride, tetrachloroethylene, trichloroethylene, dichloroethylenes, and vinyl chloride, as well as reductive dehalogenations of the same compounds biotically and also abiotically by heavy metals and substituted porphyrins. A continuum of projects from basic laboratory studies through analytical simulation model development, to field demonstrations have provided an integrated whole from conceptualization to implementation. Feedback between the different activities has helped insure that the major technical problems in technology development and implementation were appropriately addressed.

The thrust group studying aromatic compounds and a portion of that studying chlorinated solvents have focused on anaerobic processes. A great deal of evidence has been obtained that aromatic compounds, such as the BTEX group from gasoline, and chlorinated aromatic compounds, such as pentachlorophenol, are often transformed in groundwater naturally (intrinsic bioremediation or natural attenuation) by microorganisms living under anaerobic conditions. The anaerobic conditions result when the quantity of contaminants present exceed the available oxygen supply, which is often the case. The basic research ongoing here at Stanford (with focus on BTEX compounds) and Oregon State (with focus on pentachlorophenol and PCBs) is directed toward predictions of transformation potential and transformation rates, the determination of transformation products, the biochemistry and genetics of the processes involved, and the development of engineering methods to enhance the intrinsic rates of anaerobic transformations. It may be that in some cases, the most environmentally sound and

least expensive alternative is the no-treatment alternative, an approach that relies on intrinsic bioremediation. However, in order to justify this, we must first learn how to make sound judgments about transformation potential at any given location and to successfully monitor the process. Research on these major issues is continuing. As with the chlorinated solvent studies, both laboratory and field investigations and their integration are underway.

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

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

HIGHLIGHTS OF THE 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.

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.

Petroleum Hydrocarbon Treatment Demonstrations

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

Nevertheless, the story is not felt by WRHSRC researchers to be complete. Students advised by Prof. Reinhard and Prof. Alfred Spormann are addressing several important questions of how anaerobic degradation of BTEX compounds occurs. 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. A new outreach program started this year is the Technical Assistance to Brownfield Communities (TAB) Program. It is similar in scope to the TOSC Program and also is 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.

Table 6. WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER PROGRAM SUMMARY

RESEARCH PROGRAM SUMMARY

| <u>Project Description</u> | <u>Principal Investigators</u> | <u>End Date</u> | <u>Total Budget</u> |
|---|---------------------------------------|------------------------|----------------------------|
| Chemical Movement, Fate, and Treatment | | | |
| Development and Characterization of Sensors and Field Instrumentation for the Monitoring of Environmental Redox Conditions | James Ingle | 2000 | \$55,444 |
| Experimental and Mathematical Study of Biomass Growth in Pore Networks and its Consequences in Bioremediation | Peter Kitanidis Perry McCarty | 2001 | \$143,770 |
| Development and Characterization of Redox Sensors for Environmental Monitoring | James Ingle | 2000 | \$131,865 |
| Hydrological and Biology Factors Affecting Aquifer Clogging During In-Situ Bioremediation | Perry L. McCarty | 1999 | \$152,706 |
| 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 |

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| Cytochrome P-450: An Emerging Catalyst for the Co-metabolism of Chlorinated Aliphatic Hydrocarbons and Methyl tery-Butyl Ether? | Michael Hyman Lynda Ciuffetti | 2000 | \$157,968 |
| Aerobic Cometabolism of Mixtures of Chlorinated Aliphatic Hydrocarbons by Microorganisms Grown on Butane: Kinetic, Biochemical, and Modeling Studies | Lewis Semprini Daniel J. Arp | 2000 | \$174,188 |
| Effect of Chemical Structure on the Biodegradability of Halogenated Hydrocarbons | Paul V. Roberts | 2000 | \$71,879 |
| Reductive Transformation of Chlorinated Ethenes Catalyzed by Vitamin B12 – Mechanistic and Kinetic Studies | Martin Reinhard | 2000 | \$73,575 |
| Mechanisms, Chemistry, and Kinetics of Anaerobic Degradation of cDCE and Vinyl Chloride | Perry L. McCarty Alfred Spormann | 2002 | \$745,852 |
| Proof of Gene Expression During Bioaugmentation | Craig C. Criddle | 2001 | \$143,770 |

Aromatic Compounds

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| 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 Lynda Ciuffetti | 2001 | \$96,516 |
| Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbons by Toluene-Oxidizing Bacteria: Physiological Consequences and Adaptive Responses | Daniel J. Arp Peter J. Bottomley | 2001 | \$86,455 |

Heavy Metals

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| 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 |

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| Multisolute Sorption and Transport Model for Copper, Chromium, and Arsenic Sorption on an Iron-Coated Sand, Synthetic Groundwater System | Peter O. Nelson Mohammad F. Azizian | 2001 | \$114,725 |
| Trace Element Adsorption in Porous Particle Packed Beds | James O. Leckie | 2000 | \$108,070 |
| Assessing Metal Speciation in the Subsurface Environment | John C. Westall | 2000 | \$107,574 |
| Simultaneous Removal of the Adsorbable and Electroactive Metals from Contaminated Soils and Groundwater | Peter O. Nelson Mohammad F. Azizian | 2000 | 116,591 |

TRAINING AND TECHNOLOGY TRANSFER PROGRAM SUMMARY

Demonstration Projects

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| In Situ Measurement of TCE Degradation Using a Single-Well, "Push-Pull" Test | Jack Istok Lewis Semprini Jennifer A. Field | 2001 | \$213,606 |
| Field Testing of Palladium Catalyzed Hydrogenation for Chlorinated Hydrocarbon Removal: Evaluation of Catalyst Degrading Mechanism | Martin Reinhard Paul V. Roberts | 2000 | \$86,270 |
| Demonstration of a Permeable Barrier Technology for the Bioremediation of Ground Water Contamination with Waste Mixtures | Sandra Woods Kenneth J. Williamson | 2000 | \$121,577 |
| Bioenhanced In-Well Vapor Stripping to Treat Trichloroethylene (TCE) | Perry L. McCarty Mark N. Goltz Steven M. Gorelick Gary D. Hopkins | 2001 | \$805,000 |

Training and Technology Transfer Projects

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| Hazardous Waste Training | Peter O. Nelson Ann Kimerling Ken Williamson | 2001 | |
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| 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

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

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

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

Approach: Various components and processes are considered in characterizing redox status and transformations in groundwater. The three components are the intrinsic redox-active species or contaminants, the biogeochemical matrix, and the redox sensor. The processes are the microbial TEA processes (e.g., Fe(III)-reduction, methanogenesis) that are ultimately responsible for redox transformations of the component species. The focus has been directed to understanding 1) how redox sensors (e.g., Pt electrode, redox indicators) couple to components in environmental and microbial systems (e.g., groundwater, soil and wastewater slurries and microbial cultures) and 2) what information redox sensors provide about the redox status of the system in terms of the redox state of various contaminants (e.g., As, Cr) or transformations of contaminants (e.g., TCE to DCE). In parallel experiments, novel redox sensors based on *immobilized* redox indicators have been developed and characterized, and the development of a field sensor for low-level H₂ analysis has been pursued.

Status: Three redox indicators, thionine ($E_7^{0'} = +53$ mV), cresyl violet ($E_7^{0'} = -75$ mV), and phenosafranine ($E_7^{0'} = -267$ mV), which have very different formal potentials ($E_7^{0'}$), have been evaluated in detail. They can be immobilized by covalent bonding to agarose beads, agarose films, cellulose filter paper, and dialysis membranes. The agarose films are nearly transparent and are highly hydrophilic allowing rapid diffusion of reductants (e.g., Fe(II)) and oxidants (e.g., O₂) into the structure. Although not transparent, filter paper is suitable for visual inspection or reflectance measurements. Dialysis membranes are clear (without indicator) and more robust than the other forms. A unique spectrophotometric flow cell with removable windows was constructed to accommodate films and membranes (75- μ L volume) and can be used for field measurements with minimal filtering at flow rates as high as 50 mL/min without clogging. The indicators can be used to differentiate between Fe(III)-reducing, sulfate-reducing, and methanogenic redox levels, respectively. Results obtained in environmental samples strongly support the notion that Fe(II) and S(-II) are the primary reductants of thionine and cresyl violet produced under these Fe(III)-reducing and sulfate-reducing conditions, respectively. The identity of the reductant(s) of phenosafranine under methanogenic conditions is unknown. Also a simple technique to preconcentrate and determine Se(IV) and Se(VI) below

1 ng/mL (1 ppb) was developed and is based on an anion-exchange filter, sequential elution, and ICP emission spectrometry.

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 observations and direct measurements 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 support 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. Engineered bioremediation generally necessitates that nutrients required for biological growth and at times necessary 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. Although many researchers have been working on bioremediation problems, the focus of most research projects has been on the identification of microbes and conditions that are required for biodegradation. 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 will consist of integrated experimental studies of biological growth in porous media and mathematical modeling of the physical, chemical, and biological processes involved. The experimental studies will 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. The experimental plan is thus an iterative one that will evolve as experimental observations and modeling studies progress.

Status: The project has just been approved for funding, research is about to begin.

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), CH₄, and H₂), 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: This project has just been approved for funding and research has just begun.

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

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

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

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

Status: Direct visualization of microbial growth in porous media has helped better understand clogging phenomena. Patterns of microbial growth in aquifer material were found to vary considerably depending upon microorganisms and environmental conditions. Aggregate forming colonies tend to promote clogging with minimum pore size reduction compared with biofilm forming colonies. Continuous disinfection was found necessary to maintain open channels for flow between areas of high bacterial density. This study is now complete.

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 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: 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 for analysis were obtained. These samples consist of fresh catalyst; catalyst exposed to DI waters containing carbonate, bicarbonate, carbon dioxide, and sulfite, and groundwater with high-sulfate concentrations; and deactivated catalyst regenerated by exposure to air, air at 100°C, or to Clorox bleach. These samples will be analyzed with XRD, XPS and SEM. This study commenced 1 October 1999 and is authorized for a two-year period terminating 30 September 2001.

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 PCE-water interfacial area and mass transfer coefficient, better predictions of PCE dissolution, 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: Experiments to measure PCE solubility enhancement due to surfactant addition (below the cmc), as well as measurement of PCE-water interfacial tension, have been completed. Several other preliminary experiments have also been completed. An adsorption isotherm was carried out to ensure that surfactant adsorption to the sand was negligible. Surfactant partitioning into the bulk PCE phase was also shown to be negligible. These two experiments will ensure that all the surfactant in our column experiments will either be in the aqueous phase or sorbed at the PCE-water interface. Methods to analyze the surfactant using the ultra-violet absorption have also been developed. Currently, experiments are underway to determine PCE saturation as a function of column length and surfactant flood duration. We have been able to show that uniform PCE residual saturations within the sand column may be obtained. Measurement of interfacial area and mass transfer coefficients will begin after PCE saturation characterization in the sand columns is completed.

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 tetraalkoxysilanes 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: Currently we are determining on which contaminated sites we will focus our laboratory studies. The potential of tetrabutoxysilane (TBOS) to drive the anaerobic transformation of TCE is being evaluated in microcosm studies for three sites: 1) Site-300, Lawrence Livermore National Laboratory (LLNL), where TBOS drives the transformation of TCE to c-DCE; 2) the Pt. Mugu Naval Weapons Station, CA, where TBOS transforms TCE to vinyl chloride and ethene, with the last step of VC to ethene being very slow; and 3) the Evanite Site in Corvallis, OR, where TCE is being rapidly transformed to ethene. Thus, TBOS has been demonstrated to promote TCE transformation to varying extents, which are site specific.

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. Furthermore, the use of short-term studies using resting cells typically allows us to minimize the effects of new enzyme synthesis or pathway induction on the part of the test organism in response to new substrate, cosubstrate and inhibitors.

Status: Progress has been made on all three of the research objectives. Our studies of the degradation of chloroform by *Graphium* (Objective #1) have been completed. Most progress with Objective #2 has been made with our bacterial MTBE degraders. Our results suggest the short chain alkane oxygenase expressed by *Mycobacterium vaccae* JOB5 participates in four reactions in the degradation of MTBE: the initial oxidation of MTBE to TBA (*via* TBF), the further oxidation of TBA to a diol, the oxidation of acetone to hydroxyacetone and the further oxidation of hydroxyacetone. Our studies related to Objective #3 suggest *M. vaccae* can express at least two alkane-oxidizing oxygenases. One oxygenase is expressed in response to short chain alkanes (C2-C8) while the second enzyme is expressed in response to longer chain alkanes (C10 and above). Only the short chain alkane monooxygenase is capable of oxidizing MTBE. Selective inhibition experiments suggests that neither of these enzymes are cytochrome P-450 type oxygenases. Our most recent studies with *Graphium* have attempted to identify the propane- and MTBE-oxidizing oxygenase at the gene level. Using conserved sequences found among eukaryotic alkane-oxidizing cytochrome P-450s have used PCR to generate probes for Northern blots designed to follow the selective expression of cytochrome P-450. We have observed strong and selective hybridization with transcripts produced during growth on propane, propanol and propionate. Our immediate efforts are directed at verifying whether oxygenase activity corresponds with this expression pattern.

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

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

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

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

Status: In batch kinetic studies with the butane-grown enrichment the maximum degradation/ transformation rates (k), half-saturation coefficient (Ks), inhibition types (competitive, non-competitive, and mixed inhibition), and inhibition coefficients (KI and KI') of 1,1,1-TCA, 1,1-DCE, 1,1-DCA, and butane have been determined. A direct linear plot method was used to identify the types of inhibition. 1,1,1-TCA, 1,1-DCE, and 1,1-DCA all competitively inhibited each other. Competitive inhibitions kinetics were found to accurately represent the inhibition observed when all three compounds were present. However, butane (growth substrate) showed different inhibition types, that is, non-competitive inhibition on 1,1,1-TCA and 1,1-DCA and mixed inhibition on 1,1-DCE transformation. When butane and two or more CAHs were

present, a model, which combined both competitive and mixed inhibition kinetics, did not accurately simulate our experimental results.

Strain CF8, originally isolated from a microcosm of Hanford aquifer solids, has now been brought into pure culture. Identification by 16s rDNA indicates that the bacterium is of the *Nocardiodes* family, the first example of an alkane oxidizer in this genus. Light sensitivity of butane oxidation and thermal aggregation of the polypeptide that labels with $^{14}\text{C}_2\text{H}_2$ further support a relatedness to ammonia monooxygenase and particulate methane monooxygenase.

Effect of Chemical Structure on the Biodegradability of Halogenated Hydro-carbons: Paul V. Roberts, Stanford University

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

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

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

Status: A series of haloalkane structures have been docked into the crystal structure active site of the haloalkane dehalogenase enzyme using AUTODOCK - a computational docking algorithm. Docking analysis can determine whether a given compound can be positioned within the active site to interact favorably with the catalytic amino acid sidechains. By analyzing a wide range of haloalkane structures, the possible substrate range of haloalkane dehalogenase can be defined. Using a set of distance criteria derived from knowledge of the catalytic mechanism, 19 compounds out of 66 haloalkanes were identified that docked according to the distance criteria and were labeled as potential substrates for the enzyme.

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

Goal: In this project, we aim to develop a better understanding of the role of vitamin B₁₂ in biological dehalogenation reactions.

Rationale: Transition-metal coenzymes such as vitamin B₁₂ have been shown to mediate the dehalogenation of chlorinated hydrocarbon compounds in microbial systems. The mechanism of abiotic dehalogenation by vitamin B was studied in aqueous solutions in the presence of bulk reductants (TI(III) or thio-reductants). These studies indicate the formation of relatively stable Co-C bonds and the formation of radical intermediates. The significance of these findings for biological remediation is not yet understood. Biological systems differ from abiotic systems in several respects, including (1) the absence of chloroacetylene formation, (2) generally no trans-DCE or 1,1-DCE formation, (3) dehalogenation stops at the at cis-DCE in pure cultures except for *D. ethenogenes.*, (4) the dehalogenation rates of PCE, TCE, and cis-DCE are relatively close, but in abiotic systems tend to decrease in the order PCE, TCE and cis-DCE, and (5) catalyst deactivation is observed in abiotic experiments. By gaining a mechanistic understanding of these differences it will be possible to optimize biological detoxification processes and, eventually, to promote the design of novel remediation approaches.

Approach: The kinetic and product distribution data from microbial, enzymatic, and abiotic dehalogenation will be compared using chlorinated ethylenes as the substrates. Specifically, the factors that affect the stability of the various C0-(Cl-ethylene) complexes will be determined in abiotic studies, and the existence of these complexes in enzymatic and biological systems will be investigated. Cometabolically dehalogenating organisms and enrichment cultures will be investigated using UV-spectroscopy.

Status: Microcosms exhibiting dehalogenating activity have been developed from a site where fuel hydrocarbon and chlorinated ethylenes were present in a commingled plume. The microcosms were amended with toluene and PCE and substrate and electron acceptor utilization was monitored over a period of nearly a year. It was observed that PCE is the only known electron acceptor that is consumed and that PCE is sequentially dehalogenated to vinyl chloride indicates the presence of an anaerobic respiring consortium in the enrichment culture. No ethene, trans-TCE, or 1,1-DCE has been detected. Aliquots of this enrichment have been transferred to methanogenic media for further purification. The ability of electron donors to promote the dehalogenation process will be investigated in a combined field and laboratory study with funding from different sources.

**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 aquifer samples, and to examine the factors affecting the rate and extent of transformation.

Rationale: Several species of bacteria have been isolated and identified by others that have the ability to reductively dehalogenate chlorinated aliphatic hydrocarbons (CAHs). However, little is known about individual or groups of organisms responsible for the complete dehalogenation process from tetrachloroethylene (PCE) to ethene. Whether or not the complete dehalogenation or all of the individual dehalogenation steps are the result of cometabolism or of energy metabolism is also not known. In addition, the various factors that affect the rates of dehalogenation have not been adequately evaluated. Such factors may include the electron donor used, the presence or absence of alternative electron acceptors 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. An enrichment culture has also been developed on vinyl chloride for biochemical and enzymatic studies.

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

Proof of Gene Expression During Bioaugmentation: Craig C. Criddle, Stanford 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: This project was just funded, and we are now initiating work.

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 alkylbenzene-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. Furthermore, knowledge of whether a site has the potential for rapid or slow intrinsic degradation of aromatic hydrocarbons is crucial for taking action to enhance the intrinsic rate. Traditionally, microcosm studies with samples obtained from the contaminated site have been employed to determine the potential and the rate of biodegradation. 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.

The specific objectives of this research proposal are:

- To identify and isolate the genes involved in anaerobic ethylbenzene oxidation to benzoate
- To develop a DNA microarray-based test system to detect genes encoding key enzymes in anaerobic alkylbenzene degradation in field samples

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: No results have been obtained so far because this project just started last month

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

Goal: To advance the use of cometabolic bioremediation for the removal of MTBE from contaminated groundwaters.

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. While unproven, bioremediation by cometabolic organisms appears to be a promising technology to achieve removal of MTBE.

Approach: The approach will include:

- Measurement of biodegradation rates of MTBE for aquifer materials stimulated by either propane and isopentane in batch microcosms
- Measurement of relative in-situ biodegradation rates of pure-cultures of MTBE degrading fungus (*Graphium* sp.) and a MTBE degrading bacteria (*Mycobacterium vaccae*) in aquifer material
- Determination of the removal of MTBE in laboratory columns for aquifer materials with and without bioaugmentation with *Graphium* or *M. vaccae*.

All experiment will include mass balances of MTBE, the primary substrate (propane or isopentane), cell protein, and dissolved oxygen.

Status: Work has just been initiated on the microcosms using propane and isopentane. Analytical measurements have been established for all the chemical species to be measured. Pure cultures of *Graphium* and *M. vaccae* have been established and are routinely cultured.

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: The effects of TCE cometabolism on resting cell suspensions of *B. cepacia* G4 have been examined. A slow loss of toluene 2-monooxygenase activity is observed upon TCE degradation; however, the cells exhibit an exponential loss of viability, as determined by LB plate counts. Neither oxygenase activity nor cell viability is lost upon ethylene consumption. Preliminary evidence suggests that many *B. cepacia* G4 cells are sub-lethally injured while degrading TCE, and can eventually divide if incubated under suitable conditions. Also, it appears that *B. cepacia* G4 suffers DNA damage during TCE cometabolism.

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 is expected to reduce the maximum contaminant level for arsenic in drinking water to less than 10 ppb (currently 50 ppb) on account of the increased cancer risk now associated with arsenic exposure. Such a downward revision would render most US surface and groundwaters to be out of compliance with the standard, thus requiring some form of treatment before these water resources can be deemed fit for human consumption. Conventional solubility controls are incapable of achieving 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: The first set of column experiments for arsenic sorption on the alumina (DD660) is being initiated currently. Influent characteristics for these experiments are: Arsenic (V) concentrations of 25 ppb to 1000 ppb, solution open to CO₂ and pH 6.3. Concurrently, equilibrium batch sorption experiments are being setup to investigate the equilibrium sorption of arsenic (V) onto DD660 in the presence of CO₂, sulfate and phosphate. The experimental results will be modeled with the Triple-Layer Model (utilizing the surface complexation approach). The feasibility of integrating advection-dispersion into KINEQL (a model incorporating surface complexation and mass-transfer in batch sorption systems) to develop the packed-bed model is being discussed with the author of KINEQL, Sotira Yiacoumi (Georgia Institute of Technology).

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 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: This project is new with a start date of October 1, 1999.

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

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

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

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

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

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

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

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

Status: Most of the proposed experimental work for the project is complete. We are in the process of concluding column experiments that evaluate sorption performance as a function of the influent concentration and pH. Such experiments have been conducted for both selenite and cadmium. Equilibrium sorption results have been successfully modeled using the Triple-Layer Model (TLM). The modeling of the rate of uptake data of cadmium (in the absence and presence of ligands) and selenite in batch systems is currently being done using the KINEQL approach. Model development for describing column breakthrough characteristics is also being conducted concurrently in our research group as part of another project.

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

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

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

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

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

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

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

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

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

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

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: Laboratory and microcosm experiments have been completed to identify appropriate cometabolic substrates for use in field push-pull test measurements of reductive transformations of TCE. Laboratory microcosm experiments and field push-pull tests have been conducted to study the transport and transformation behavior of a fluorinated analog for TCE, trichlorofluoroethene (TCFE). In situ transformation rates of TCE and TCFE have been quantified with and without the addition of exogenous electron donors. Laboratory microcosm and field push-pull tests have been used to quantify spatial variability in glucosidase activity and lipid-bound phosphate as indicators of microbial biomass and to develop correlations between these parameters and measured rates of TCE and TCFE transformations.

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), trichloroethylene (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 results have been reported by McNab et al. In *ES&T*, Vol. 34, No. 1, 2000, p. 149-153. 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.

Demonstration of a Permeable Barrier Technology for the Bioremediation of Ground Water Contaminated with Waste Mixtures: Sandra Woods and Kenneth J. Williamson, Oregon State University

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

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

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

Status: During 1999, the reactor installed within the permeable barrier at the L.D. McFarland facility was operated under a variety of environmental conditions. Concentrations of the electron donor and electron acceptors (sulfate and oxygen) were varied resulting in different EH regimes within the reactor over time. Pentachlorophenol removal was evaluated under anaerobic conditions and in the presence of imitation vanilla flavoring as the electron donor. Intermediates included 2,3,4,5-tetrachlorophenol, 3,4,5-trichlorophenol, 3,4-dichlorophenol and 3,5-dichlorophenol. Complete removal of pentachlorophenol as well as these intermediates was observed. Parallel laboratory and field experiments were conducted to evaluate the effect of supplemental electron donor and electron acceptor concentrations on chlorophenol removal. Complete pentachlorophenol removal was observed in the laboratory and in the field at imitation vanilla flavoring concentrations of 10 mg/L as COD. Sulfate also was injected into the permeable barrier reactor. At influent concentrations of 100 mg/L, sulfate had no apparent effect on pentachlorophenol removal. A series of coordinated laboratory and field studies were conducted to evaluate the simultaneous removal of pentachlorophenol and naphthalene under sequential anaerobic (for pentachlorophenol reductive dechlorination) and aerobic (for naphthalene oxidation) conditions. Laboratory studies were conducted in serum bottles containing naphthalene, groundwater, and associated biomass from the permeable barrier reactor. Complete

naphthalene removal was observed when aerobic serum bottles were amended with 2 to 3 mg/L naphthalene (removal required 100 - 500 hours). Naphthalene removal was evaluated in the field by adding hydrogen peroxide to the mixing wells prior to aerobic biological zones in the permeable barrier reactor. Removal of naphthalene in the aerobic treatment zones was 86% within 12 days and 90% within three weeks of treatment (based upon a comparison of influent and effluent concentrations). Continued complete removal of pentachlorophenol was observed (PCP continued to be present at levels below our detection limit). The simultaneous removal of pentachlorophenol and naphthalene under sequential anaerobic and aerobic conditions indicates that potential usefulness of a permeable barrier technology for the removal of mixtures of contaminants in groundwater.

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 least at some sites. The efficacy of each of the two innovative remediation technologies that comprise BEHIVS were successfully demonstrated during field trials for TCE removal at Edwards AFB. Both technologies make use of groundwater recirculation wells, which may be defined as wells with injection and extraction screens that create groundwater circulation cells in the surrounding aquifer. The in-well vapor stripper established a vertical circulation cell in the aquifer, whereas the aerobic cometabolism system employed a pair of vertical recirculation wells, one operated in an upflow mode, the other in a downflow mode, to establish two horizontal cells. By combining the two in situ treatment systems, the advantages of each can be captured in a way that enhances the performance of the other.

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

Status: A numerical fate and transport model of the technology is substantially complete and has been used to design the treatment wells and monitoring network for this demonstration. Extensive site characterization was accomplished in order to localize the TCE source location to help position the treatment wells and monitoring system. The characterization work involved application of 2-D refractive and 3-D reflective geophysical surveys. Based upon the results of the surveys, direct-push sampling at the site, and model simulations, the treatment well locations were determined. A work plan was submitted to Federal, California, and regional regulators, who gave preliminary approval to construct the site. Site construction has commenced.

TRAINING AND TECHNOLOGY TRANSFER

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

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

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

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

Status: The Center conducted 14 workshops within Oregon, Washington, Alaska and Idaho in 1999. Funding for the Center has been extended through 2000. 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

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:

In Situ and On-site Bioremediation, The Fifth International Symposium, April 19-22, 1999, San Diego, California. WRHSRC researchers presented 14 papers.

Technical Innovations Session, 1999 Annual Meeting of American Institute of Hydrology and the Fourth USACIS Joint Conference on Environmental Hydrology and Hydrogeology, November 7-10, 1999, San Francisco, CA. WRHSRC researchers presented 6 papers.

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

Goal: The Technical Outreach Services for Communities Program is a community assistance program designed to aid community groups confronted with environmental contamination from hazardous waste sites. TOSC provides interested community groups with technical information and assistance that allows for early and meaningful public participation in decisions which affect their health and welfare. The TOSC program also provides a viable alternative for communities that are affected by Superfund sites but do not qualify for a Technical Assistance Grant (TAG) from the U. S. Environmental Protection Agency.

The TOSC program conducts its activities 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
- Alexandra Degher, Research Assistant,
Ph. (541) 737-4026, FAX (541) 737-2735, Email: degher@enr.orst.edu
- Andrea Ferro, Research Assistant,
Ph. (650) 723-0315, FAX (650) 725-9474, Email: aferro@stanford.edu
- Janet Gillaspie, Consultant, President Environmental Strategies, LLC
Ph. (503) 233-3980, FAX (503) 230-2892, Email: envstrat@teleport.com

The staff provides expertise in the following areas:

- remediation technologies
- health effects of hazardous materials exposure
- risk characterization and assessment
- hazardous waste regulations and legislation

Status: Activities during 1999 were as follows:

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 has included:

- Mapping community mortalities to perform cluster analysis
- Reviewing mortality report by Arizona Dept. of Health Services
- Tabulating and analyzing questionnaires from existing health study
- Obtaining 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 incidence of health symptoms with increased proximity to the fire. 1996 EPA sample results for 35 households were analyzed by TOSC personnel and showed that all chemicals were below Health Based Comparison Levels.

Mapped 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 a cluster effect exists within the community. Mapped annual number of deaths before and after the 1992 fire to see if mortality rate increased after the fire.

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 odors and respiratory health problems in the community.

Work has included:

- Review of air emissions sampling plan and report.
- Review of initial air emissions Health Risk Assessment results
- Review of Final Draft Operational Air Permit, with presentation of findings to the community.
- Review of the emissions sampling to be conducted by the facility as a condition of the operational permit.

Progress to Date: Reviewed facility emissions data and risk assessment reports from 1994 and earlier years. Reviewed multiple drafts of the proposed air permit. Attended meetings of local agency, facility, and community representatives to negotiate air permit provisions. Assisted in reaching tentative 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) remains unknown following Arizona Dept. of Environmental Quality and U.S. EPA investigations.

Work has included:

- Providing information regarding chemical sensitivities, and names of experts in this field to community leaders.
- Reviewing ADEQ, ADHS, and EPA investigation documents.

- Preparing a report summarizing the findings from the review of the agencies' investigation documents and recommending further action by the agencies

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. The final report will be sent to the state environmental and health agencies, as well as to Region 9 EPA and ATSDR after the community group's comments are submitted and incorporated.

Sitka, Alaska: Currently in the process of closing out TOSC's work with Citizen's Advisory Committee, Sitka, Alaska (Bill Janes, DEC), related to hazardous material releases from Alaska Pulp Corporation pulp mill.

Work has included:

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

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

Alameda Naval Air Station, San Francisco, California: Providing assistance to the Restoration Advisory Board (RAB) regarding issues related to the cleanup of Operable Units 1 and 3 (OU-1 and OU-3) at this former Naval Air Station.

Work has included:

- 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.
- Review and comment on Feasibility Study for OU-3.

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 its behalf, and is awaiting a revised response to OU-1 comments. Submitted comments on OU-3 FS on October 21, 1999. TOSC noted several deficiencies in the FS and anticipates review and comment on revised documents from the Navy.

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

Work has included:

- Review and evaluation of soil and groundwater sampling results.
- Review and comment on Remedial Investigation and Feasibility Study (RI/FS) documents.

Progress to Date: Completed historical document review. Comments provided to the California Dept. of Toxic Substance Control regarding a proposed neighborhood soil removal plan. Comments on recent groundwater monitoring results provided to SAEJ. TOSC anticipates review and comment on the next Draft RI/FS.

Bayview-Hunters Point, California: Assisting the Southeast Alliance for Environmental Justice (SAEJ) community advocacy group regarding issues of concern relating to planned Brownfields redevelopment of Hunters Point Naval Base near San Francisco.

Work has included: Review of Phase I site assessment data being used to direct redevelopment plans.

Progress to Date: TOSC completed its review of Phase I assessment data and submitted comments to SAEJ. TOSC anticipates closure of the project in the near future.

Casmalia, California: [Community Closed] TOSC had been working with Citizen's Advisory Committee (CAC) associated with the Casmalia Resources Hazardous Waste facility.

Work has included:

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

Progress to Date: Reviewed background material. Assisted the community in review of consent decree and presented comments at public meeting. Provided information to the community on the necessary steps to become incorporated as a non-profit organization. TOSC offered assistance to community group regarding their application for a Technical Support Grant. Community group declined to apply for the grant. TOSC services formally closed out on January 11, 1999.

Garden Valley, CA: Initiated work with this El Dorado County community related to airborne asbestos.

Work has included:

- Review of California Regional Air Resources Board sampling plan
- Review of El Dorado County draft ordinance on allowable asbestos content of road surface material
- Participating in September 2, 1999 public meeting

Progress to Date: TOSC has reviewed and commented on the sampling plan and ordinance. Participated in the September 2, 1999 public meeting. TOSC will provide additional document review and comment as needed.

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

Work has included:

- Review of Navy contractor reports and documents associated with soil and groundwater contamination at two operable units (sites 9 and 11).
- Attendance at RAB meetings to comment on proposed investigation, treatability study, and remediation plans.

Progress to Date: Attended several RAB meetings. Reviewed background information. Provided technical assistance related to incineration options, risk of worker exposure to TCE, and management of migration of contaminants to San Diego Bay. Reviewed and commented on Site 9 Draft Feasibility Study. TOSC will continue to serve on RAB Technical Workgroup to address Site 9 RI/FS issues.

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.

Work has included:

- Review of past and current data from groundwater, soil, and air sampling.
- Review of the Record of Decision
- Documenting health-related concerns of residents living next to the site
- Providing an updated list of contaminants affecting groundwater, soil, and air both at the site and in the adjacent community.
- Review of data from several new groundwater and soil gas sampling events conducted in Fall 1998 due to the re-opening of the ROD.
- 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. Facilitated community effort to have the California Department of Health Services (DHS) conduct a survey of health symptoms and concerns of local residents. DHS agreed to conduct a survey of community health concerns as part of its Public Health Assessment process. TOSC assisted in administering the health survey. TOSC will submit comments to DHS on the Draft Public Health Assessment on behalf of the PONC.

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).

Work has included:

- Review and comment on RI/FS and Draft and Final ROD documents at OU-3.
- Review and comment on Draft RI/FS at OU-1.
- Ongoing educational programs for RAB members related to remediation plans and activities.

Progress to Date: Review of final OU-3 documents completed and comments submitted to the RAB provided. Review on draft OU-1 RI/FS completed and comments submitted to the RAB. TOSC made a presentation on the OU-1 FS at a January 1998 RAB meeting and has attended additional regularly scheduled RAB meetings. TOSC will review and comment on the revised draft OU-1 RI/FS in January 2000.

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.

Work has included: Review and evaluation of the Human Health Risk Assessment and the draft Ecological Risk Assessment.

Progress to Date: Completed review and gave a presentation on Risk Assessments at an August 1998 RAB meeting. TOSC anticipates additional review and comment on revised draft Risk assessment which are still under development by the Navy.

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.

Work has included:

- Review and comment on documents related to the potential closure of a former Open-burn Open-detonation (OBOD) site on this base.
- Review and comment on Army's sampling plan and investigation results associated with area groundwater and near-shore water contamination.

Progress to Date: Provided contact information for Unexploded Ordinance (UXO) expertise. TOSC attended a meeting of all stakeholders and toured the site in December 1998. Document review and comment completed. Army has agreed to conduct additional investigation activities at MMR and TOSC will review the results of their work in 2000.

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

Work has included: Review of RI/FS documents and proposed remediation actions.

Progress to Date: Document review completed. Assessment of EPA airborne contaminant sampling and analysis complete, assessment report drafted, final version forthcoming. Graduate thesis project investigating local radiation exposure risks from off-site surface soils ongoing. Provided review and comment on Proposed Ruling to address PM-10 emissions.

Bunker Hill NPL Site, Kellogg, Idaho: Have initiated assistance to the Silver Valley People's Action Coalition/CRC Co. (SVPAC) regarding the adjacent Bunker Hill Superfund site.

Work has included:

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

Progress to Date: Attended meeting with SVPAC, EPA Region 10 staff, and EPA Superfund Ombudsman. Attended public meeting in which EPA described its plan for the five year review of the Record of Decision. Reviewed Hirschhorn report as well consultant report on study of lead dust cleanup in Kellogg homes. Provided cost estimate for professional cleaning of homes to remove lead dust contamination.

Concerned Blair Area Neighbors, Eugene, OR: Initiated assistance to the Community related to severe health effects that community members believe are related to environmental contamination in the neighborhood.

Work has included:

- Review of Oregon DEQ records to identify potential contaminant sources in the neighborhood.
- Administering health effects survey to document symptoms experienced by residents
- Presenting findings of survey and records review to DEQ on behalf of the community to persuade the agency to initiate site assessment activities.

Progress to Date: Reviewed DEQ documents and identified two potential contaminant sources. Will administer a health survey to community members in late November 1999.

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 PCE and TCE groundwater contamination and cleanup.

Work has included:

- 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.
- Provide information on potential effect(s) that the contaminated groundwater plume might have on the local water district drinking water supply.
- Evaluate air quality concerns in local residential basements.
- Evaluate possible exposures through 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. Attending monthly community meetings. Presented detail on alternative bioremediation protocols, environmental partitioning of P/TCE, and fabrication of activated charcoal filters for air strippers. Currently facilitating the possible establishment of a local Health Registry through the ATSDR, and a possible microcosm study to assess the feasibility of bioremediation options.

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

Work has included:

- 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: Contacted WA Department of Agriculture to discuss pesticide application practices of local orchard owners, and most effective means of reporting pesticide exposures. Awaiting their report. Obtained agreement from Region 10 ATSDR representative to assist as needed following report review.

Quincy, WA: Providing assistance to community affected by contamination from a former agricultural products facility. Contaminants on the site include pesticides and heavy metals.

Work has included:

- Review of Washington Department of Ecology investigation reports
- Providing information on the health effects of selected site contaminants

Progress to Date: Met with community leader at Department of Health office to begin document review. Will visit community in December 1999.

**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 22 active research projects. Of these, 13 are new projects that are just getting underway, and the remainder are research projects that should be completed during the year 2000. Additionally, WRHSRC researchers have four 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 22 WRHSRC research projects and four demonstration projects is provided in the following for the projects listed in Table 3. Each is discussed in the order listed in that table.

Development and Characterization of Sensors and Field Instrumentation for the Monitoring of Environmental Redox Conditions, James Ingle.

This project seeks to develop cheaper and more reliable indicators of redox environments in groundwater as necessary to understand transformation potential of hazardous chemicals. Commercialization of the success methods developed is being pursued.

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.

Hydrological and Biology Factors Affecting Aquifer Clogging During In-Situ Bioremediation, Perry L. McCarty.

This is the original study in this area, with the above similar studying being a continuation of this topic. Technology transfer approach will be similar.

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.

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

WRHSRC basic studies have demonstrated that some organisms that grow on butane or propane can aerobically cometabolize 1,1,1-trichloroethane. This has led to two major field demonstrations supported by the U.S. Department of Defense. Because of the importance to better implementation, these basic studies are being conducted. Technology transfer will be through application of knowledge in ongoing field demonstrations, as well as through the normal presentations of basic knowledge at national meetings and publication in peer-reviewed journals.

Effect of Chemical Structure on the Biodegradability of Halogenated Hydrocarbons, Paul V. Roberts.

This goal of this basic research study is to determine the extent to which one can predict susceptibility and rates of degradation of compounds by cometabolism through a better understanding of enzyme structure and function. Technology transfer will be through presentations of basic knowledge at national meetings and publication in peer-reviewed journals.

Reductive Transformation of Chlorinated Ethenes Catalyzed by Vitamin B12 – Mechanistic and Kinetic Studies, Martin Reinhard.

Vitamin B12 has been implicated in the reductive dehalogenation of chlorinated solvents. This basic study is to generate understanding of the chemical processes involved in this highly unusual use of vitamin B12 by microorganisms. 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 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. The 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 through 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 through 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.

Trace Element Adsorption in Porous Particle Packed Beds, James O. Leckie.

This is an ongoing basic research study about completed to determine the effectiveness of alumina for removal of trace levels of inorganic materials from groundwater. The success of this approach led to the above study on arsenic removal. Technology transfer will be through presentations of basic knowledge at national meetings and publication in peer-reviewed journals.

Assessing Metal Speciation in the Subsurface Environment, John C. Westall.

This is an older project to the above project by Westall on metal speciation. The basic information developed has been the basis for the new project above on speciation. Technology transfer will be through presentations of basic knowledge at national meetings and publication in peer-reviewed journals.

Simultaneous Removal of the Adsorbable and Electroactive Metals from Contaminated Soils and Groundwater, Peter O. Nelson.

This is a previous project to develop barrier wall technology for removal of heavy metals from contaminated groundwater, that led to the above project by Nelson. 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-pull 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.

Demonstration of a Permeable Barrier Technology for the Bioremediation of Ground Water Contamination with Waste Mixtures, Sandra Woods and Kenneth J. Williamson.

This demonstration is currently nearing completion. Technology transfer will be through 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.

1999 WRHSRC PUBLICATIONS

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

A. Refereed Journal Articles

- Cao, J., and P.K. Kitanidis, "Adaptive Finite-Element Simulation of Stokes Flow in Porous Media," *Adv. in Water Resour.*, **22**(7), 681-696, (1999).
- Dupin, J.J. and McCarty, P.L., "Mesoscale and Microscale Observations of Biological Growth in a Silicon Pore Imaging Element," *Environmental Science and Technology*, **33**(8), 1230-1236 (1999).
- Haston, Z.C. and McCarty, P.L., "Chlorinated Ethene Half-Velocity Coefficients (K_S) for Reductive Dehalogenation," *Environmental Science and Technology*, **33**(2), 223-226 (1999).
- Keller, A.A., M.J. Blunt and P.V. Roberts, "Behavior of Dense Non-Aqueous Phase Liquids in fractured porous media under two-phase flow conditions", *Transport in Porous Media*, in press.
- Keller, A.A., P.V. Roberts and M.J. Blunt, "Effect of Fracture Aperture Variations on Dispersion of Contaminants," *Water Resources Research*, **35**(1) 55-63 (1999).
- Khaodhiar, S., M.F. Azizian, and P.O. Nelson, "Copper, Chromium, and Arsenic Adsorption and Equilibrium Modeling in an Iron-Oxide-Coated Sand, Synthetic Groundwater System," submitted (1998).
- MacDonald, T.R., P.K. Kitanidis, P.L. McCarty, P.V. Roberts, "Mass Transfer Limitations for Macroscale Bioremediation Modeling and Implications on Aquifer Clogging," *Ground Water*, **37**(4), 523-531 (1999).
- MacDonald, T.R., P.K. Kitanidis, P.L. McCarty, P.V. Roberts, "Effects of Shear Detachment on Biomass Growth and In Situ Bioremediation," *Ground Water*, **37**(4), 555-563 (1999).
- Stuart, S.L., S.L. Woods, T.L. Lemmon, J.D. Ingle, Jr, "The Effect of Redox Potential Changes on Reductive Dechlorination of Pentachlorophenol and Degradation of Acetate by a Mixed, Methanogenic Culture", *Biotechnology and Bioengineering*, in press.
- Yang, Y. and McCarty, P.L., "Response to "Comment on Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Mixed Culture," *Environmental Science and Technology*, **33**(12), 2128 (1999).

B. Articles Submitted or in Press

- Dupin, H.J. and McCarty, P.L., "Colony Morphologies from Biological Growth in Porous Media Based on Microscale and Mesoscale Observations, submitted (1999).

Dupin, H.J. and McCarty, P.L., "Effect of Chlorine on Biological Growth in Silicon Pore Imaging Elements," submitted (1999).

Haston, Z.C., Yang, Y., and McCarty, P.L., "Organism Growth and Substrate Utilization Kinetics for the Anaerobic Dehalogenation of cis-Dichloroethene and Vinyl Chloride," submitted (1999).

McCarty, P.L., "Novel Biological Removal of Hazardous Chemicals at Trace Levels," Proceedings, International Symposium on Development of Innovative Water and Wastewater Treatment Technologies for the 21st Century, submitted (1999).

McNab, Jr., W, R. Ruiz, and M. Reinhard, "In-Situ Destruction of Chlorinated Hydrocarbons in Groundwater Using Catalytic Reductive Dehalogenation in a Reactive Well: Testing and Operational Experiences," *Environmental Science and Technology*, submitted (1999).

Yang, Y. and McCarty, P.L., "Biomass, Oleate, and Other Possible Substrates for Chloroethene Reductive Dehalogenation," submitted (1999).

C. Chapters in Books or Bound Proceedings

McCarty, P.L., "Chlorinated Organics," Chap. 4, *Environmental Availability of Chlorinated Organics, Explosives, and Metals in Soils*, Eds. W.C. Anderson, R. C. Loehr, B. P. Smith, American Academy of Environmental Engineers, Annapolis, 35-84 (1999).

Osathaphan, K., M.F. Azizian, and P.O. Nelson, "Simultaneous Removal of Cu(II), Cr(VI), and As(V) Metals from Contaminated Soils and Groundwater," Symposium on Emerging Technologies: Hazardous Waste Management in the 21st Century, I&EC Division, ACS Spring 2000 National Meeting, San Francisco, CA, March 26-30, 2000, submitted (1999).

Osathaphan, K., M.F. Azizian, and Peter O. Nelson, "Simultaneous Removal of the Adsorbable and Electroactive Metals from Contaminated Soils and Groundwater." Accepted in Proceedings, **AIHM** National Meeting, San Francisco, CA, November 10, 1999.

Reinhard, M., M. Semadeni, P.K. Sharma, and W.R. Haag, "Dehalogenation of Haloaliphatic Hydrocarbon Compounds in the Aquatic and Terrestrial Environment," Chapter in American Society of Agronomy Monograph "Bioremediation of Contaminated Soils." D. Adriano, J.-M. Bollag, Eds., 133-173 (1999).

D. Book

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

E. CD ROM

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

F. Graduate student theses supported by the Center:

Abrams, R. H. *"A Compartmentalized Approach To Simulating Redox Zones In Contaminated Aquifers,"* Ph.D Dissertation, Department of Geological and Environmental Sciences, Stanford University, Stanford, CA (1999).

Dupin, H.J. *"Biological and Hydrodynamic Factors Affecting Aquifer Clogging During In-Situ Bioremediation,"* Ph.D Dissertation, Department of Civil and Environmental Engineering, Stanford University, Stanford, CA (1999).

Haston, Z.C., *"Factors Affecting Growth and Utilization in the Anaerobic Dehalogenation of Chlorinated Ethenes,"* Ph.D Dissertation, Department of Civil and Environmental Engineering, Stanford University, Stanford, CA (1999).

Jones, B., *Applications of Redox Indicators for Evaluating Redox Conditions in Environmental Samples,* Ph.D. Dissertation, Oregon State University, Corvallis, OR (1999).

PREVIOUS WRHSRC PUBLICATIONS

A. Refereed Journal Articles

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