



**WESTERN REGION  
HAZARDOUS SUBSTANCE RESEARCH CENTER**

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# 1992 ANNUAL REPORT

Stanford University  
Oregon State University

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## TABLE OF CONTENTS

<b>ADMINISTRATION</b> .....	1
<b>THE CENTER AT A GLANCE</b> .....	1
<b>CENTER DIRECTOR'S REPORT</b> .....	5
<b>HIGHLIGHTS FOR 1992</b>	
RESEARCH ACTIVITIES .....	7
Groundwater Management .....	7
Chlorinated Solvents .....	8
Aromatic Compounds .....	10
TRAINING AND TECHNOLOGY TRANSFER .....	11
RESEARCH AND TRAINING AND TECHNOLOGY TRANSFER	
PROGRAM SUMMARY .....	12
Research Program Summary .....	12
Training and Technology Transfer Program Summary .....	15
<b>RESEARCH PROJECT DESCRIPTIONS</b> .....	16
CHEMICAL MOVEMENT, FATE, AND TREATMENT .....	16
CHLORINATED SOLVENTS .....	23
AROMATICS .....	31
HEAVY METALS .....	40
TRAINING AND TECHNOLOGY TRANSFER .....	42
<b>1992 WRHSRC PUBLICATIONS</b> .....	44
<b>PREVIOUS WRHSRC PUBLICATIONS</b> .....	49

## WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER

### ADMINISTRATION

Perry L. McCarty  
Director, WRHSRC  
Department of Civil Engineering  
Stanford University  
Stanford, CA 94305-4020  
(415) 723-4131

Kenneth J. Williamson  
Director, Training and Technology Transfer  
Department of Civil Engineering  
Oregon State University  
Corvallis, OR 97331  
(503) 737-2751

### THE CENTER AT A GLANCE

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

The objectives of the Center are:

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

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

This past year, the Center completed its first project period of three years and began a new project period of five years. The end of the first project period was accompanied by the completion of 19 research projects. Five research projects are continuing into the second

project period, and 11 new research projects had their beginning with the start of the new project period. There are also six newly approved research projects which soon should be funded. The projects continuing into or starting with the second project period continue to focus on groundwater cleanup and remediation of sites contaminated with chlorinated solvents, halogenated aromatic compounds such as pentachlorophenol and PCBs, nonhalogenated aromatics, including petroleum derivatives such as gasoline, and heavy metals. Several new projects 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. Technology transfer and training activities are broad and include sponsorship of sessions at several major conferences, technical workshops directed toward technology transfer, development of a major training program in Oregon, and numerous presentations of research information and technology transfer at conferences, workshops, and seminars for regulators, industry, consulting firms, and university faculty and students.

The faculty and staff who are directing the Center's research, training, and technology transfer activities are listed in Table 1. They collectively represent an integrated research team representing five different schools (engineering, science, earth sciences, medicine, and veterinary medicine), and many different disciplines (microbiology, chemistry, hydrogeology, hydrology, chemical engineering, civil engineering, and medicine). 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. Lewis Semprini is an Assistant Director who coordinates technology transfer and research activities in California. Martin Reinhard is Assistant Director in charge of the Center's analytical program. Marilyn C. King is the Center's Administrative Assistant.

The Center's Science Advisory Committee and Training and Technology Transfer Advisory Committee members during this past year are listed in Tables 2A and 2B, respectively, and represent federal and state governments, industry, consulting firms, and universities. The budgets for the past year and the total since the Center's inception are summarized in Table 3. The education of students interested in careers directed toward finding solutions to environmental problems is another important goal. The number of students supported through WRHSRC funds is also listed in Table 3.

Table 1. KEY PERSONNEL AT THE CENTER

<u>Stanford University</u>	<u>Oregon State University</u>
Prof. David L. Freyberg	Prof. Daniel J. Arp
Prof. Steven M. Gorelick	Prof. A. Morrie Craig
Prof. Dunja Grbić -Galić	Dr. Michael R. Hyman
Prof. Lynn M. Hildemann	Prof. James D. Ingle
Mr. Gary D. Hopkins	Prof. Jonathan D. Istok
Mrs. Marilyn C. King	Prof. Peter O. Nelson
Prof. Peter K. Kitanidis	Prof. John C. Westall
Prof. James O. Leckie	Prof. Kenneth J. Williamson
Prof. Abdul Matin	Prof. Sandra L. Woods

Prof. Perry L. McCarty  
Prof. Martin Reinhard  
Prof. Paul V. Roberts  
Dr. Lewis Semprini

Table 2A. SCIENCE ADVISORY COMMITTEE

<u>Member</u>	<u>Affiliation</u>	<u>Expertise</u>
Randy Bruins	Govt/EPA	Regulations
John Conomos	Govt/USGS	Hydrology
John F. Ferguson	University	Biological Processes
John Glaser	Govt/EPA	Physical/Chemical Processes
Ronald Hoeppel	Govt/DOD	Microbiology
Michael C. Kavanaugh*	Consulting Engineer	Physical/Chemical Processes
James M. Tiedje	University	Microbiology
William A. Wallace	Consulting Engineer	Design
John L. Wilson†	University	Hydrology
John Wise	Govt/EPA	Planning

\*Chairman

†Vice Chairman

Table 2B. TRAINING AND TECHNOLOGY TRANSFER ADVISOR COMMITTEE

<u>Member</u>	<u>Affiliation</u>	<u>Expertise</u>
James T. Allen	Govt/ California	Treatment Technology
John J. Barich	Govt/EPA	Regulations
Kenneth Bigos	Govt/EPA	Air Pollution
Sandy Gurkewitz	Govt/Oregon	Environmental Quality
Ethelwyn Hoffman	Govt/Washington	Training
David Kennedy†	Consulting Engineer	Treatment Technology
Jon Kindschy	University	Hazardous Substances Training
Gregory Peterson	Consulting Engineer	Treatment Technology
M. R. Scalf	Govt/EPA	Groundwater Remediation
Kenneth Sutherland*	Industry	Treatment Technology
Winona Victory	Govt/EPA	Training/Tech Transfer

\*Chairman

†Vice Chairman

Table 3. CENTER FUNDING

<u>Funding Sources</u>	<u>FY 1992*</u>	<u>Funds to Date</u>
EPA: Centers Program	\$1,150	\$5,030
EPA: Other	\$110	\$580
Other Govt: Federal†	\$100	\$1,070
Other Govt: State		
Consortium	\$250	\$810
Private Sector#	\$60	\$1,440
TOTAL	\$1,670	\$8,940

\*Oct. 1, 1991 - Sept. 30, 1992

†Department of Energy; Department of Defense

#Allied Signal Corporation; Brown and Caldwell; CH2M HILL; DuPont Corporation; Electric Power Research Institute; Gas Research Institute; Hewlett-Packard Company; James M. Montgo Consulting Engineers Inc.; Kennedy/Jenks; Kleinfelder, Inc.; MBT Environmental Engineering, Ltd.; McLaren/Hart; Monsanto; Orange County Water District; Schlumberger Technologies; Shell Development Corporation

<u>Student Support</u>	<u>Number*</u>	<u>Funds to Date†</u>
M.S.	15	\$380
Ph.D.	26	\$2,030
Post Doctoral	8	\$440
TOTAL	49	2,860

\*Total numbers working on Center Project

†Includes tuition, travel, supplies, etc.

## CENTER DIRECTOR'S REPORT

As it moves into the second project period of five years, the WRHSRC has 18 faculty members representing two universities and five disciplines (chemistry, engineering, hydrogeology, medicine, and microbiology). This transition between two major project periods took place this past year with the completion of 19 projects, many of which begin in 1989 when the Center started. While there is some continuity with the carryover of five projects, most of the research activity in the second period will be the result of the 11 projects that were started in the second period and the five others that are about to start. These new projects continue to emphasize the theme that the Center began with. That is to focus the Center's limited resources on the major hazardous problems resulting from subsurface contamination. The Center's main focus is on groundwater, and the most prevalent and difficult chemical contaminants in groundwater, which include chlorinated solvents and their transformation products, aromatic hydrocarbons, halogenated hydrocarbons, and heavy metals. A major focus with the organic contaminants is on chemical and biological transformations. The interest here is in determining whether or not natural processes can reduce the hazards associated with subsurface contamination, and whether through engineering approaches the transformation processes can be enhanced in a way that will more rapidly reduce or eliminate the hazards posed in a cost effective manner.

The results of the Center's research over the past three and one-half years have been disseminated broadly. To date there have been 99 publications from work supported by the WRHSRC. These include 33 journal publications, with 15 more submitted and under review; one bound proceedings; 23 chapters in books or articles in bound conference proceedings, ten project reports, seven Ph.D. dissertations, and 10 M.S. theses. There have been numerous oral or poster presentations on research findings at international, national, and regional conferences, and many more that represent broad summaries of research for technology transfer. The WRHSRC has sponsored or cosponsored 18 conferences, seminars, or workshops on subjects as diverse as research findings, environmental engineering education, hazardous substance chemistry and management, and the training and technology transfer process.

One of the significant outcomes of a Five Centers' Conference on Technology Transfer hosted by the WRHSRC through the efforts of Prof. Kenneth Williamson, Director of Training and Technology Transfer, was a realization that true transfer of technology to a user community does not result from oral and written presentations alone. There is a need to work closely with organizations interested in implementing new technology so that the knowledge gained through research can be better communicated and put into practice. In addition, there is much to be learned from the act of implementation itself. Areas where implementation would benefit from additional research can thus be better identified, providing a highly beneficial feedback into the research process itself. Such feed back from the Center's activities with several field pilot and full-scale investigations has resulted in new research projects for the Center.

Most of the WRHSRC's budget is represented by core funding from EPA. However, other federal funds have come to the Center from other branches within EPA, and from the Department of Defense and the Department of Energy, because of the interest these agencies have in the Center's research. In addition, several industrial organizations and consulting engineering firms have contributed support as well in research contracts and gifts. The latter are important to the Center, not only because they represent financial support and cooperative

activity with the users of the Center's research, but they also help achieve the minimum of 20 percent non-federal support that is required for the EPA-sponsored HSRCs.

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 this problem is directing its efforts toward finding engineering methods for the subsurface mixing of chemicals that can help enhance these natural processes with the contaminants of concern. The desire is to produce harmless end products. This is closely associated with the related effort to determine how best to remove polluted groundwater and contaminants from the subsurface environment. The major efforts underway here will continue both through active involvement with those applying technologies pioneered through Center activity, and through additional research to improve the understanding of the processes involved.

The thrust group studying aromatic compounds has focused on anaerobic processes. A great deal of evidence has been obtained that aromatic compounds are often transformed in groundwater naturally by microorganisms living under anaerobic conditions. The anaerobic conditions result when the quantity of contaminants present exceed the available oxygen supply, which is often the case. The basic research ongoing here is directed toward predictions of transformation rates, the determination of transformation products, and the development of engineering methods that might enhance the rates of desired anaerobic transformations. It may be that in some cases, the most environmentally sound and least expensive alternative is the no-treatment alternative. However, in order to justify this, we must first learn how to make sound judgments about transformation potential at any given location. Research on these major issues is continuing.

A third associated thrust area is the modeling of transport and fate processes and groundwater-management strategies. 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 models and engineering methods being developed here have direct impact on the activities of the other two thrust groups described above so that good interactions between the three thrust groups is necessary.

A fourth 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. Research here is being conducted by Center faculty partly through Center funding, but perhaps on a larger scale with direct support from federal and private sources. Highlights of accomplishments this past year in our research thrust groups and our training and technology transfer program are given in the following.

## HIGHLIGHTS FOR 1992

During this past year of WRHSRC activity, many research tasks have been completed, resulting in numerous publications and presentations on research findings. Some of the most significant activities in the three major thrust areas, that address groundwater management and groundwater contamination with chlorinated solvents and aromatic compounds, are singled out for highlighting this year. Unless otherwise indicated references in the text refer to reports listed under Center Publications.

### RESEARCH ACTIVITIES

#### Groundwater Management

The Pump and Treat strategy is often used for removing contaminants from aquifers. Important insights into this process were gained through a study titled, "Aquifer Remediation Design in the Presence of Kinetic Limitations," that was supervised by Prof. Steven Gorelick. The study was concerned with pump-and-treat remediation involving multiple contaminant plumes and removal of these contaminants from groundwater when rate-limited mass transfer occurs. Comparison of extraction well locations and pumping rates led to four conclusions which should interest engineers, hydrogeologists, and regulators. First, the presence of multiple contaminant plumes, each with its own sorptive properties, has a major influence on extraction well placement and pumping rates. Second, the classic method of capturing groundwater contaminants, by placing wells at the down-gradient edge of a contaminant site, is generally an inefficient and risky design for remediation. This extraction well scheme generally requires much higher pumping rates than other remediation options, and has the additional disadvantage of spreading the slowest-moving, most difficult-to-remove contaminant plume. Third, if contaminant transport is strongly rate-limited, rapid groundwater restoration is impossible. If contaminant mass transfer is slow between immobile and mobile phases, aquifer remediation is severely hindered. Fourth, "competition" between extraction wells may significantly reduce the efficiency of multiple-well cleanup designs. For example, a small, but imprudent pumping rate increase at one well can result in groundwater remediation actually requiring more time, despite the higher total pumping rate for all wells.

With contamination of the unsaturated or "vadose" zone above the groundwater table, vapor extraction is commonly used to remove volatile contaminants such as gasoline and chlorinated solvents. In a study directed by Profs. Martin Reinhard and Paul Roberts entitled, "Gaseous Stripping of Nonaqueous Liquids from the Vadose Zone," sorption equilibrium and kinetics were found to be major determinants of the effectiveness of vapor stripping as a remediation technique. Sorption isotherms and rates of volatile organics removal were measured using different aquifer materials, soils, clays and model solids and a novel batch-column desorption technique. Desorption isotherm shape was the only correlating parameter with rates of vapor stripping. Rates did not correlate as expected with the soil properties commonly implicated for slow contaminant removal. Soil properties, including organic matter content, internal porosity, and particle size, were not useful for predicting rates of volatile organic compound removal. Significant hysteresis was observed even after 6 month equilibration periods. The amount of slow desorbing residual on a soil was found to depend not on the equilibrium vapor concentration, nor on the initial solids concentration, but on the highest contaminant concentration to which the solids were exposed. Pore diffusion models were not entirely

adequate to describe the rates of organic removal. Desorption was found to proceed at two widely different rates. The majority of sorbed contaminant was removed within ten minutes, but on some soils, periods greater than two years are required to reach solid concentrations below 1 mg/g.

## **Chlorinated Solvents**

Several of the Center's projects are concerned with chlorinated solvents, the most common chemicals at contaminated sites. Of interest here are the major solvents: carbon tetrachloride (CT), tetrachloroethylene (PCE), trichloroethylene (TCE), and 1,1,1-trichloroethane (TCA). Other CAHs of interest include natural degradation products of these major solvents, including the three isomers of dichloroethylene (1,1-DCE, c-DCE, t-DCE), 1,1-dichloroethane (DCA), and vinyl chloride (VC). The research on CAHs range from basic studies on the kinetics and reaction products from both chemical and biological transformations as well as methods for introduction and mixing of remediation chemicals with groundwater contaminants, to pilot studies at the Center's Moffett Naval Air Station field site (Moffett Field), to studies directed towards full-scale application at Superfund and other sites with groundwater contamination. Of particular interest for highlighting this year are the results of characterization studies at the St. Joseph, Michigan, Superfund site, and the results of Moffett Field studies on use of phenol for induction of microorganisms for CAH degradation.

### **St. Joseph Superfund Site**

In-situ methanotrophic groundwater treatment of chlorinated aliphatic compounds was proposed by the WRHSRC for evaluation at the St. Joseph, Michigan, Superfund site, and a process design for conducting this was carried out. Additional basic modeling studies were conducted this past year as an aid in designing a system for chemical introduction, and laboratory studies were conducted to determine the effectiveness of methane addition on vinyl chloride decomposition. At the St. Joseph site, the original TCE contamination has been transformed naturally by anaerobic (methanogenic) processes to cis- and trans-DCE, and VC, each of which is present in mg/L concentrations. Last year a detailed characterization at the location of a proposed full-scale pilot demonstration of in-situ bioremediation was carried out by the industry involved, their consulting engineering firm, and the EPA Robert S. Kerr Environmental Research Laboratory. Concentrations into the tens of mg/l were found for these CAHs, which was significantly higher than originally believed. Significant reduction of VC to ethylene was confirmed in this investigation. These results suggest that natural anaerobic in-situ bioremediation to harmless end products is being carried out. The possibility exists that this natural anaerobic process can be further enhanced, and this possibility is now being explored through joint efforts with EPA's Robert S. Kerr Environmental Research Laboratory in Ada, Oklahoma. The WRHSRC has also suggested that in-situ aerobic methanotrophic treatment be used as a polishing step to transform remaining VC at this site (McCarty et al., 1991). The site is relatively simple, hydrogeologically. The groundwater is about 10 m below the surface in a relatively uniform fine-sand aquifer that is between 10 m and 18 m deep.

The St. Joseph study is being carried out cooperatively with Allied-Signal Corporation, Engineering-Science, the Michigan Department of Natural Resources, EPA Region V, the EPA Robert S. Kerr Laboratory, and the WRHSRC. Aseptic core samples obtained by the Kerr Laboratory were examined using protocols developed by the WRHSRC, and found to contain a small population of methane-using (methanotrophic) bacteria that carried out transformations of CAHs at rates similar to the Moffett Field microorganisms. These studies suggest that TCE and c-TCE would not be effectively removed by methanotrophic bacteria, but t-DCE and VC would be. The interest in methanotrophic treatment for this site is primarily because of the high concentration of VC that persists downgradient from the site of contamination. Sorption of contaminants to the sand was relatively small. The site where in-situ treatment is proposed lies on property owned by others, and for this reason there is a need to employ a nonintrusive treatment system. In addition, VC does not sorb well onto activated carbon and cannot be discharged to the atmosphere. These constraints has led to the design of an underground recirculation system where contaminated groundwater, methane, and oxygen are brought together, mixed, and then recirculated back into the aquifer for biological degradation. Several methods for introduction of methane and oxygen have been evaluated, and laboratory studies on a subsurface system for gas introduction have been completed. Mathematical models of the mixing process and the resulting distribution of biological growth have been developed and were used to develop the process design.

Previous studies have indicated that high concentrations of chlorinated solvents (in the range of 20 mg/l) are not toxic to the methanotrophic bacteria. However, the recent finding of TCE transformation product toxicity suggests that growth of bacteria may be hindered when TCE is present. Additional laboratory studies using core material from the St. Joseph aquifer were conducted this past year in order to evaluate the nature of this problem. The studies indicated that mg/l concentrations of TCE did not interfere with ability of methanotrophic bacteria to degrade concentrations of VC up to 20 mg/l. Thus, we believe that in-situ bioremediation of VC contamination is still a viable alternative for the St. Joseph Superfund site, and perhaps others as well.

The St. Joseph site has presented a challenge to the WRHSRC, and has helped define important research questions for further pursuit. It has also offered the opportunity to bring together individuals from the various disciplines within the Center, and the various interested parties associated with the St. Joseph site. Such cooperative activity is required in order to help solve the new and difficult problems posed. We believe that the technologies for subsurface mixing developed here will be useful for remediation at other sites and with other contaminants, where the addition of chemicals for enhancing contaminant mobility as well as transformation may be required. We expect to work towards this end with grants expected soon from the Department of Energy.

#### Field Demonstration with Toluene Oxygenase

The first controlled field demonstration of in-situ cometabolic bioremediation of chlorinated aliphatic compounds by methane-oxidizing microorganisms was conducted at the Moffett Field Naval Air Station in Mountain View, California by WRHSRC staff [P. V. Roberts et al., *In-Situ Aquifer Restoration of Chlorinated Aliphatics by Methanotrophic Bacteria*, EPA/600/2-89/033, U.S. EPA Center for Environmental Information, Cincinnati, OH (July 1989)]. During this past year, a new study was continued to evaluate the effectiveness of a different enzyme system, the

toluene oxygenase (TO) system. The potential for this system was first demonstrated by M. J. K. Nelson et al., *Applied and Environmental Microbiology*, 53, 949-954 (1987). This Moffett Field evaluation is being supported through the WRHSRC by a combination of core funds, the U.S. EPA Biosystems Technology Initiative, and the U.S. Department of Energy. This study is headed by G. D. Hopkins and L. Semprini. Phenol was injected into the Moffett Field aquifer in order to stimulate growth of bacteria containing TO. The thickness of the confined aquifer used is about 1.5 m, and lies at a depth below ground surface of 4.5 to 6 m. The test site consists of an injection well, where phenol, oxygen, and the chlorinated aliphatic hydrocarbons were introduced, and an extraction well located 7 m away. In line between the injection and extraction wells are three monitoring wells. The time of water travel between the injection and extraction wells is about two days. An automated data acquisition system permitted semi-continuous concentration measurements for bromide tracer, dissolved oxygen, phenol, pH, TCE, cis-DCE, and trans-DCE.

With the methane-oxidizing bacteria in the past, CAH degradation at Moffett Field were approximately: TCE- 20%; cis-DCE, 45%; trans-DCE, 90%; and VC, 95%. However, with the TO system, transformations found last year were quite different: TCE-90%; cis-DCE-95%; and trans-DCE, 15%. VC was not evaluated in the latter. The much greater transformation of TCE and cis-DCE by the TO system was most encouraging, and so additional studies were conducted this year. In previous years, only low concentrations (<100 µg/l) of CAHs were evaluated. This year concentrations of TCE up to 1 mg/l were studied. It was found that with 12 mg/l phenol and 30 mg/l dissolved oxygen, that over 90% TCE removal was obtained. Also, by optimizing the system of phenol addition, 99% TCE removal was obtained in a single pass through the groundwater aquifer. Limited studies indicated that greater than 95% removal of VC and c-DCE were also obtained by this system, but that t-DCE removal was only about 40 to 60%. The concentration of phenol remaining in the aquifer was determine to be below detection limit, which was about 20 µg/l. These results indicate that the use of phenol or a related alternative compound that can stimulate a similar oxygenase has great promise for in-situ remediation. Here too we are now working with consulting firms and industry, seeking contaminated sites where this technology might be applied.

### **Aromatic Compounds**

One of the major interests of the WRHSRC thrust group, who are studying biotransformations of halogenated and nonhalogenated aromatic compounds, is on anaerobic transformations. Aquifers heavily contaminated with organic materials tend to be anaerobic, and transformations, if they are to occur, must then be through the action of anaerobic consortia of microorganisms. Research in this area has been pioneered by WRHSRC staff. Studies are being conducted on anaerobic transformations of PCBs, pentachlorophenol (PCP), the soluble components of gasoline (benzene, ethylbenzene, toluene, and xylenes, which are collectively termed BTEX), polycyclic aromatic hydrocarbons (PAHs), and heterocyclic compounds containing oxygen, sulfur and nitrogen.

An interesting and unexpected finding this past year resulted from studies supervised by Profs. Martin Reinhard and Dunja Grbić -Galic. This involved the search for metabolic intermediates of anaerobic toluene degradation by sulfate-reducing enrichment cultures. While the cultures mineralized over 80% of the toluene carbon to carbon dioxide (as demonstrated using <sup>14</sup>C), they

concurrently transformed 5 to 10 percent of the carbon to two dead-end metabolites that were larger than the toluene molecule itself. The identities of the two dead-end metabolites, benzylsuccinic acid and benzylfumaric acid, were confirmed by using gas chromatography/mass spectrometry (GC/MS). Use of stable isotope-labeled toluene and GC/MS confirmed that the compounds resulted from toluene metabolism. This finding is relevant for several reasons: (1) reports of both productive and dead-end metabolites of anaerobic toluene degradation are rare, (2) the finding is largely in agreement with recent observations by P.J. Evans, L.Y. Young and co-workers at New York University obtained with a denitrifying pure culture, which suggests that the pathway applies to disparate anaerobic bacteria, (3) the accumulation of benzylsuccinic acid may have health implications, as it is a potent inhibitor of carboxypeptidase A, and (4) these accumulating dead-end metabolites could be useful for providing evidence of in-situ biodegradation of toluene, if the native bacteria utilize this pathway. Benzoic acid was also found as a transient intermediate of toluene degradation, as has been reported by other research groups for denitrifying and fermentative-methanogenic conditions.

## TRAINING AND TECHNOLOGY TRANSFER

During the last year of the Center's activity, training and technology transfer activities have included numerous conferences, workshops, and classes. In addition, the Center has begun a strong emphasis upon field application of technologies developed through its research efforts.

In relation to training, the Center in consortium with the University of California Extension program was selected as the Western Lead Training Institute. The Institute will begin presenting courses about the management of lead paint removal in 1993.

The Center has fully developed a certificate program in hazardous substance management for professionals for the Portland, Oregon, metropolitan area. The program consists of six courses taught on a two year rotation. Enrollment has grown to about 50 students.

Professional development for regulatory personnel, consulting engineers, and senior environmental managers is provided by Center through a series of five one- to two-day workshops. The workshops are directly related to research being conducted by the Center's faculty. The workshops are typically offered in cooperation with other agencies such as the University of California Extension Program.

Several conferences have been sponsored by the Center to promote hazardous waste education and to give a forum for presentations by the Center's faculty. Conferences have been sponsored throughout EPA Regions 9 and 10 and include the 1992 Idaho Regional Conference on Hazardous Materials, Pocatello, Idaho; the 1992 Responsible Hazardous Materials Management Conference, Portland, Oregon; the *In situ* and On-Site Bioreclamation Conference, San Diego, California; Association of Environmental Engineering Professors Educational Conference, Corvallis, Oregon; and the Subsurface Restoration Conference, Dallas, Texas.

The Center also sponsored the first Five Centers' Training and Technology Transfer Conference at Fallen Leaf Lake, California. One result of that conference was a commitment to pursuing field applications of innovative technologies as a technology transfer activity. Two such projects have been developed within the Center to be conducted at Hanford under DOE funding. Those projects concern field testing of *in-situ* vapor stripping, and design of mixing systems for enhanced *in-situ* biotransformation of TCE. Center faculty are presently attempting to develop several other projects involving field demonstrations and applications.

**RESEARCH AND TRAINING AND TECHNOLOGY TRANSFER  
PROGRAM SUMMARY**

**RESEARCH PROGRAM SUMMARY**

## Chemical Movement, Fate, and Treatment

Reinhard, 1989-1992 Grbić -Galić , Leckie, McCarty, Roberts (\$274k total/\$93k current year)	Treatment of Complex Mixtures
Gorelick 1989-1992 (\$174k total/\$58k current year)	Design of Reliable and Cost-Effective Mitigation Schemes
Reinhard, 1989-1992 Roberts (\$191k total/\$63k current year)	Gaseous Stripping of Nonaqueous Liquids from the Vadose Zone
Kitanidis, 1989-1992 (\$188k total/\$71k current year)	Detection and Assessment of Subsurface Contamination
Kitanidis, 1991 Freyberg (\$53k total/\$53k current year)	FASTCHEM, Applications and Sensitivity Analysis
Hildemann, 1990-1993 Roberts (\$178k total/\$78k current year)	Dispersion Modeling of Volative Organic Emission from Ground-Level Treatment Systems
Gorelick, 1992-1995 (\$267k total/\$84k current year)	Aquifer Remediation Design in the Presence of Kinetic Limitations
Kitanidis, 1992-1995 (\$252k total/\$79k current year)	Determination of Macroscopic Transport Parameters for Biologically Reacting Solutes in Aquifers
Semprini, 1992-94 (\$114k total/\$64k current year)	Radon-222 Method for Locating and Quantifying Contamination by Residual Non-Aqueous Phase Liquids in the Subsurface
Westall, 1992-95 Ingle (\$197k total/\$66k current year)	Redox Transformations of Inorganic Pollutants: Coupling to the Biogeochemical Matrix
Roberts, 1992-94 Semprini (\$200k total/\$100k current year)	Process Submodel Formulation and Parameter Estimation for Simulation of Bioremediation

## Chlorinated Solvents

McCarty, 1989-1993 Roberts (\$345k total/\$80k current year)	Oxidation of Chlorinated Solvents by Methanotrophs
McCarty, 1989-1992 Roberts (\$230k total/\$75k current year)	Effects of Sorption on Biodegradation of Halogenated Orga
Reinhard, 1989-1993 McCarty (\$109k total/\$24k current year)	Long-Term Chemical Transformation of 1,1,1-Trichloroe (TCA) and Freon 113 Under Aquifer Conditions
Matin, 1990-1992 Grbić -Galić (\$149k total/\$81k current year)	Use of Starvation and Stress Promoters for Biodegradati Hazardous Wastes
McCarty, 1992 Kitanidis, Roberts, Semprini (\$90k total/\$90k current year)	Subsurface Mixing of Nutrients and Groundwater for In Bioremediation
McCarty, 1990-94 Roberts, Semprini (\$584k total/\$200k current year)	Test-Bed Evaluation of In-Situ Bioremediation of Chlorin Aliphatic Compounds by Toluene
McCarty, 1992 Roberts, Gorelick, Kitanidis, Semprini (\$200k total/\$200k current year)	Demonstration of In-Situ Bioremediation of Chlorin Aliphatics by Methanotrophs at St. Joseph
Roberts 1990-1992 (\$145 k total/\$72k current year)	Determining and Modeling Diffusion-Limited Sorption Desorption Rates of Organic Contaminants in Heteroger Soils
Reinhard 1992-1995 (\$205k total/\$64k current year)	Transformation of Chlorinated Hydrocarbons by Rec Metallocoenzymes—Kinetic Model Development Applications to Environmental Systems
McCarty 1992-1994 (\$124k total/\$60k current year)	Transformation of TCE by Methanotrophic Biofilms

Arp, 1992-1995  
Williamson  
(\$250k total/\$80k current year)

Degradation of Chlorinated Aliphatic Compounds by Nitri  
Bacteria

## **Aromatic Compounds**

Woods 1989-1992 (\$139k total/\$49k current year)	Interactions between Electron Acceptors in the Treatment of Wastewaters Containing Sulfate, Chlorophenols and Acetate
Williamson, 1989-1992 Nelson (\$168k total/\$58k current year)	Enhancing Biodegradation with Sorption with Alternating Aerobic/Anaerobic Environments
Istok, 1989-1992 Woods (\$166k total/\$62k current year)	Development and Verification of a Numerical Model to Predict the Fate and Transport of Chlorinated Phenols in Groundwater
Grbić -Galić 1989-1992 (190k total/\$68k current year)	Anaerobic Microbial Transformation of Homocyclic and Heterocyclic Polynuclear Aromatic Hydrocarbons
McCarty 1992 Grbić -Galić (\$102k total/\$102k current year)	PAH Degradation by Methanotrophic Bacteria
Reinhard, 1991-93 McCarty (\$410k total/\$150k current year)	In-Situ Biological Treatment of Aromatics in Groundwater
Grbić -Galić , 1992-95 Reinhard (\$256k total/\$80k current year)	Microbial Degradation of Toluene Under Sulfate-Reducing Conditions - - The Role of Iron
Istok, 1992 \$20k total/\$20k current year)	An Evaluation of the Effectiveness of In Situ Biodegradation for Benzene, Toluene and Xylene Isomers in Large-Scale Physical Aquifer Models
Grbić -Galić 1992 (\$134k total/\$69k current year)	Aerobic Methanotrophic and Anaerobic Transformation of Biphenyl, Monochlorobiphenyls, and Dichlorobiphenyls
Craig 1990-1992 Woods (\$230k total/\$75k current year)	Biotransformation of Ordnance Wastes Using Unique Consortia of Anaerobic Bacteria

Woods 1992-1993  
(\$194k total/\$68k current year)

The Effect of Environmental Conditions on Redu  
Dechlorination Rates

**Heavy Metals**

Leckie 1989-1992  
(\$204k total/\$77k current year)

Trace Metal Removal Processes

Nelson, 1989-1992  
Istok  
(\$100k total/\$52k current year)

Hexavalent Chromium Sorption and Desorption in Na  
Soils and Subsoils

## **TRAINING AND TECHNOLOGY TRANSFER PROGRAM SUMMARY**

Williamson 1989-1993 (\$60k total/\$15k current year)	Hazardous Waste Training
Semprini 1989-1993 (\$60k total/\$15k current year)	Advanced Topic Workshops
Williamson 1989-1993 (\$60k total/\$15k current year)	Continuing Education Program
Williamson, Semprini 1989-1993 (\$45k total/\$15k current year)	Conference Sponsorship
McCarty, Williamson 1992-1993 (\$10k total/\$10k current year)	Field Application of Remediation Technologies

## RESEARCH PROJECT DESCRIPTIONS

### CHEMICAL, MOVEMENT, FATE, AND TREATMENT

#### **Treatment Of Complex Mixtures: Martin Reinhard and Paul V. Roberts, Stanford University**

Goal: A computer-aided information system was developed for estimating physico-chemical properties of organic compounds needed for the design of treatment processes and assessing the risk of hazardous waste sites.

Rationale: Relevant physico-chemical properties are unknown for many waste constituents. In some cases, such properties can be estimated using estimation techniques.

Approach: A structure-based data evaluation system for organic compounds (DESOC) has been developed for storage, retrieval, evaluation and estimation of physico-chemical properties including octanol-water partition coefficients, aqueous solubility, vapor pressure, the acid dissociation constant, molar volume and refraction, cohesion parameters (Hildebrand values, HBA), Henry's coefficient, and connectivity indices.

Status: Property estimation is based on quantitative structure-property or property-property relationships (QSPR or QPPR), and novel models based on structural similarity (SIMOC) and group interchange (GIMOC). DESOC has been employed to assess air stripping for volatiles removal (evaluation of the relationship structure/air-water partition coefficient) and activated carbon treatment (evaluation of the relationship physico-chemical property/Freundlich parameter). Activated carbon adsorption data of organic compounds have been analyzed with respect to the relationship between Freundlich equation parameters and physico-chemical properties. The properties which have been considered include molar refraction, molar volume, aqueous solubility and octanol-water partition coefficient. Significant correlation was found between Freundlich parameters and molar refraction and group parameters.

Client/Users: (1) Environmental consultants and engineers interested in treatment plant design for hazardous wastes, and (2) risk analysts and government regulators who need to store, retrieve, estimate physico-chemical data for evaluating fate and transport of organic chemicals.

#### **Design of Reliable and Cost-Effective Mitigation Schemes: Steven M. Gorelick, Stanford University**

Goal: The aim of this project is to develop and test methods for design of pump-and-treat aquifer remediation systems. These methods can target reliable strategies that identify the best well locations and pumping rates to ensure capture of contaminant plumes. Reliable designs must be based on reliable predictive models. Quantifying both model parameter uncertainty and consequent prediction uncertainty is a major research goal.

Rationale: Simulation models can be powerful tools for designing aquifer remediation schemes. Unfortunately, there is tremendous uncertainty associated with our predictive models of subsurface contaminant transport, even for substances whose chemical behavior is well

understood. Given this uncertainty, one must over design any pump-and-treat system. Knowledge of the nature of simulation model uncertainty and the development of risk-based design strategies are therefore essential.

Approach: The project is being conducted in cooperation with Environment Canada, and focuses on the Gloucester Special Waste Compound in Ontario, Canada. In 1989 design began from pump-and-treat remediation to remove hazardous organic solvents. The best remediation design requires optimal well selection and the determination of optimal pumping rates. The problem is being approached by developing stochastic simulation models based upon available field data. Then these models are combined with nonlinear optimization methods in order to identify reliable design strategies. Model parameter uncertainty is considered to be a key indicator of design reliability. In this approach, model parameters are first described statistically. This quantification of uncertainty in model input is then translated into confidence bounds on model predictions of contaminant transport. The reliability indicated by the predictive model is then used to over design the remediation system to the extent necessary to insure success.

Status: This project was completed in March of 1992. Publications describe a suite of methods applied to the Gloucester Landfill, Canada contamination problem. The first method determines transport model parameters and their uncertainties. The second method uses the uncertainties in parameter values to evaluate the range of likely predicted contaminant concentrations over time and space. The third method determines optimal and reliable remedial strategies for contaminant removal. Overdesign through enhanced pumping was determined for remediation taking 6, 12, and 18 years. Overdesign factors translated into pumping at enhanced rates of between 18 and 27 percent in order to achieve 90 percent reliable remedial designs.

Client/Users: Engineers and hydrogeologists involved in aquifer remediation design. Groundwater modellers interested in parameter estimation and optimization methods.

**Gaseous Stripping of Nonaqueous Liquids from the Vadose Zone: P.I.: Martin Reinhard and Paul V. Roberts, Stanford University**

Goal: The goal of this project is to gain an understanding of the factors affecting vapor stripping of organic solvent contamination in the unsaturated zone. By measuring the equilibrium and kinetics of organic vapor sorption for a variety of soil and organic types, we hope to gain an understanding of how soil and contaminant properties control the vapor stripping process.

Rationale: Sorption equilibrium and kinetics are major determinants of the effectiveness of vapor stripping as a remediation technique. There is presently little information on the adsorption and desorption behavior of organic solvents in unsaturated soil systems. Information from this research will be useful in designing and evaluating vapor stripping programs based on properties of the soil and the contaminants.

Approach: This investigation can be divided into three areas. The first area is the measurement of organic vapor sorption isotherms on a range of different solids. The second area involves measuring the rate at which the organic solvents desorb from the soils under vapor stripping conditions. The third area encompasses computer modeling of the stripping process using the isotherm and kinetic data obtained in the other two phases of the project.

Status: All experimental work has been completed and the final report is finished. An experimental procedure has been developed and published to measure extremely slow desorption rates from columns packed with aquifer material and soils. Contaminant removal rates were found to be independent of the purge gas flow after 10 minutes, indicating that particle scale kinetics were controlling desorption rates. Significant hysteresis between adsorption and desorption was observed. The fraction of slowly released contaminant on a soil could not be predicted by any measured soil properties. A pore diffusion mechanism was not adequate to describe the observed rates of desorption.

Client/Users: This research is useful to consultants and regulators for designing and evaluating vapor stripping systems, and for assessing transport of VOCs through the unsaturated zone.

**Detection and Assessment of Subsurface Contamination: Peter K. Kitanidis, Stanford University**

Goal: The objective of this project was the improvement of data-analysis methods for the detection and assessment of groundwater contamination and the determination of the mechanisms and parameters which govern the transport and fate of pollutants at field scales.

Rationale: There is seldom enough information to determine with certainty the precise values of all parameters, especially at small scales. Measurements of some critical transport parameters, such as hydraulic conductivity, indicate variability over orders of magnitude over short distances. Thermodynamic constants and rate coefficients needed in models are quite variable. In many cases, one is interested in the "effective" parameters of the heterogeneous formation, i.e., those which govern the net or "macroscopic" rate of advection, dispersion, and chemical attenuation, rather than the highly variable local rates.

Approach: The developed approach combines measurements, mechanistic models describing the transport and fate of pollutants, and statistical methods. Recognizing the spatial variability of geologic formations and the paucity of data about them, the parameters which determine the transport and fate of solutes, such as conductivity, retardation, and reaction coefficients, are described in statistical terms as "random functions". These moments of these functions are obtained from data and other (such as geological) information using geostatistical techniques. Then, through the governing flow and mass transport equations, the statistics of solute concentration are determined. These methods were applied to two problems of practical interest: (a) The evaluation of the macroscopic, or field-scale, conductivity, transmissivity, and dispersivity and (b) the conditioning of predictions on measurements.

Status: The project was completed in 1992. Significant progress was achieved in shedding light on the meaning of effective (field-scale) conductivities, transmissivities, and dispersivities and in providing methods for their computation; this research advanced a novel volume-averaging approach based on the Taylor-Aris-Brenner method of moments and efficient computational methods. It also produced new geostatistical data-analysis methods for the estimation of contaminant mass and contouring concentrations in hazardous-wastes sites.

Clients/Users: Groundwater modelers; engineers working in the characterization of hazardous-waste sites; regulators; and policy makers interested in the worth of data.

**FASTCHEM Applications and Sensitivity Analysis: Peter K. Kitanidis and David L. Freyberg, Stanford University (Supported by the Electric Power Research Institute)**

Goal: FASTCHEM is a collection of computer programs which can be used to predict the advection, dispersion, and geochemical transformation of chemicals emanating from utility waste disposal sites. The objectives of this research were: (1) To quantify the sensitivity and uncertainty of predicted chemical distributions (in time and space) to variations in input parameters; and (2) to develop a set of computer programs which apply state-of-the art estimation methods for the interpolation of parameters from sparse measurements; (3) to evaluate the literature and to develop new optimization/simulation techniques for decontamination strategies at utility hazardous waste sites under uncertainty.

Rationale: Amendments to Resource Conservation and Recovery Act in 1984 and reauthorization of the Safe Drinking Water Act in 1986 have prompted the formulation of several new regulations to protect groundwater quality. In response to new regulations and to develop a capability to assess the potential success of remedial actions, the Electric Power Research Institute (EPRI) has supported the development of the interim hydrogeochemical modeling package FASTCHEM. This package of codes can be used to simulate the advection, dispersion, and chemical attenuation of inorganic chemicals that may be leached from electric utility waste disposal sites. EPRI, the sponsor of this research, was interested in determining the applicability of the model and in incorporating subroutines for the utilization of data.

Approach: The usefulness of the model was evaluated through application to specific case studies. Methods for incorporating available measurements were based on linear estimation methods.

Status: The project was completed in December 1991. A number of changes in the computations and input-output subroutines were made and others were recommended for the improvement of the model. The model was applied to six case studies which represent typical problems of ash disposal from coal-fired plants. Among other features, these case studies served to illustrate the use of areal-view depth-averaged models in combination with vertical cross-section saturated and unsaturated flow models; the combined use of hydrogeologic and geochemical modeling; the use of models in showing regulatory compliance and in improving the design for either ash pond or landfill; and strengths and limitations of models.

Clients/Users: Utility industry; regulators; policy makers.

**Dispersion Modeling of Volatile Organic Emissions from Ground-Level Treatment Systems: Lynn M. Hildemann and Paul V. Roberts, Stanford University (Partially Supported by the U.S. Department of Energy)**

Goal: Treatment of contaminated groundwater can result in the emission of hazardous volatile organics into the atmosphere. The goal of this project is to develop a detailed model for the

dispersion of emissions from a ground-level area source into the atmosphere that will accurately predict concentration levels in the vicinity of the source as well as further downwind.

Rationale: The potential toxicity of air emissions resulting from the treatment of wastewater and groundwater has been the focus of increasing concern over the past decade. However, published attempts at estimating the potential impact of such treatment processes on the local atmosphere have utilized simple dispersion models which are only reliable at significant distances downwind for an elevated point source. A more accurate approach is needed to evaluate the risk posed to humans in the near vicinity of a ground-level treatment process due to atmospheric emissions.

Approach: A detailed model capable of predicting the near-source dispersion of emissions from a ground-level area source will be developed. In order to accurately model dispersion in the surface layer regime, this model will incorporate accurate expressions for the variation of wind speed and eddy diffusivity with vertical height, and also will include dry deposition as a removal mechanism. The dispersion model will be linked to an existing volatilization model which predicts emission rates resulting from aerobic biological treatment based on the influent concentrations and operating parameters of the system. The predictions of this combined modeling system will be compared with predictions obtained using simpler models, and also with published measurements of concentrations downwind of treatment sites.

Status: Development of a dispersion model which accurately represents conditions near ground level has been completed, and the model has been linked to a second model used to estimate volatile organic carbon emission rates from a surface aerobic treatment unit based on influent concentrations and operating conditions. The predictions of the dispersion model have been compared with those obtained using other "popular" models under various conditions, revealing that these popular models often are under-predicting downwind concentrations. Currently, the model is being refined to better reflect the interaction between the type of terrain and the variation of turbulence with height. Published experimental measurements of airborne concentrations downwind of ground level sources are also being sought for model verification.

Client/Users: Industries and regulators involved in the treatment of contaminated groundwater, and researchers concerned with assessing the risks associated with such treatments.

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

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

Rationale: Nonequilibrium, or rate-limited transport is frequently described in both lab and field experiments, and imposes a fundamental limit to pump-and-treat groundwater remediation. Under natural conditions local equilibrium may be a valid approximation because groundwater velocities are very slow, and the effects of chemical or physical rate limitations on solute migration and distribution may be important and unrecognized. Since site characterization is

usually conducted under non-pumping conditions while the contaminants are moving in a natural flow system, the false assumption of local equilibrium may be adopted. When contaminant removal is attempted through pumping, groundwater velocities increase dramatically and mass transfer rate limitations become extremely important. Consequently, contaminant removal efficiency will be drastically overestimated when local equilibrium is assumed.

Approach: This study involves five stages: (1) development of a two-dimensional, advective-dispersive groundwater contaminant transport model which assumes first-order mobile-immobile domain nonequilibrium; (2) comparison of contaminant removal under continuous- and pulsed-pumping; (3) sensitivity analysis with different nonequilibrium rate parameters to develop efficient pump/rest cycles for pulsed-pumping; (4) development of a nonequilibrium rate parameter estimation method from standard field observations; and (5) formulation and development of an optimization procedure for design of pulsed-pumping remediation of rate-limited contaminants.

Status: The two-dimensional solute transport code SUTRA has been modified to handle first-order mobile-immobile nonequilibrium for multiple contaminants. In work which combined a previous WRHSRC project and the current study, optimal extraction well locations and pumping rates are compared for multiple contaminant plumes that have been chromatographically separated and which exhibit rate-limited transport. Preparation of a manuscript is in its final stages and will be submitted for publication by December 1992. Key results from the paper include the following: (1) classic contaminant capture at down-gradient wells is generally a poor design for contaminant cleanup involving multiple plumes; and (2) competition between pumping wells may significantly affect the feasibility and efficiency of specific multiple-well cleanup designs.

Client/Users: Engineers, hydrogeologists, and regulators, involved in remediation design and contaminant transport modelling where rate-limited transport is of concern or multiple plumes are present, will be interested in this technology.

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

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

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

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

Status: The project started in Spring 1992.

Clients/Users: Environmental modelers; developers of in-situ bioremediation methods.

**Radon-222 Method for Locating and Quantifying Contamination by Residual Non-Aqueous Phase Liquids in the Subsurface: Lewis Semprini, Stanford University (Partially Supported by the U. S. Department of Energy)**

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

Rationale: Locating and describing quantitatively the residual or pools associated with source areas of NAPL contamination in the subsurface remains is one of the major obstacles to implementing thorough site monitoring and effective remedial programs. Geochemical methods may provide an inexpensive means of locating and quantifying NAPL contamination, and monitoring the progress of remediation. Radon-222 concentrations in subsurface fluids may provide a means of locating and quantifying NAPL contamination in the subsurface. The method is based on radon's unique properties including: its production from the decay of radium-226 in subsurface materials, its radioactive decay with a half-life of 3.83 days, and its preferential partitioning into the NAPL.

Approach: Equilibrium model calculations indicate that radon should become depleted during transport through a NAPL zone. Thus, changes in the radon concentration in an aquifer might be used to locate and quantify NAPL contamination. Laboratory and field studies will be used to verify this concept and to develop this method for finding sources of NAPL.

Status: Laboratory studies have been initiated with saturated soil columns and batch systems using aquifer solids obtained from the CFC Borden groundwater test site. Radon emanations measured with saturated Borden solids (in the absence of NAPLs) agree closely with measured values of groundwater from the Borden aquifer. Studies are now being initiated in soil columns with NAPL present. Field studies have also been initiated as a cooperative project with the Solvents Research Program at the Waterloo Centre for Groundwater Research. Two field experiments are being investigated at the CFB Borden test site: the natural-gradient emplaced homogeneous source experiment which was initiated in 1989 by Rivett and Feenstra, and the controlled gradient dissolution experiment of Broholm and Feenstra, which was initiated in the summer of 1992. Initial measurements from the natural-gradient emplaced source experiment indicate radon is depleted as it flows through the NAPL source area.

Client/Users: Consultants, industries and regulators, and researchers interested in locating and quantifying NAPL contamination, and monitoring the remediation process.

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

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

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

Approach: In characterizing redox transformations of inorganic pollutants, we consider three components: the redox-active inorganic pollutant, the geochemical matrix, and the redox sensor. Coupling of redox processes must be understood at three linkages -- pollutant and matrix, sensor and matrix, and pollutant and sensor. Each linkage must be investigated on a species-by-species basis, without the a priori assumption of equilibrium. The initial focus of this study is on transformations of Cr(III) / Cr(VI) in geochemical matrices of Fe(II), Fe(II)-minerals, Mn(IV)-minerals, natural organic matter, and aquifer material from a Cr-contaminated site. In parallel experiments, transformations of redox indicators will be investigated spectrochemically and electrochemically, towards the goal of developing optrodes and electrodes that can be deployed in-situ.

Status: This project began in March, 1992. Thermodynamic models for redox conditions in geochemical matrices have been developed to establish equilibrium boundary conditions. Initial electrochemical and spectrochemical studies of redox indicators have been completed. A chemostat for maintaining a dynamic redox buffer for control of experimental redox conditions is nearing completion.

Client/User: Consultants, regulators, geochemists, and modelers concerned with all aspects of the speciation of redox-active pollutants.

**Process Submodel Formulation and Parameter Estimation for Simulation of Bioremediation: Paul Roberts, Lewis Semprini, and Margaret Lang, Stanford University**

Goal: To improve mathematical simulation and parameter estimation methods for bioremediation based on aerobic cometabolic transformation.

Rationale: Previous and ongoing work sponsored by the Center has demonstrated the efficacy of aerobic cometabolic transformations in degrading halogenated aliphatic contaminants such as vinyl chloride. However, our ability to design such systems is limited by gaps in understanding the effects of complications such as aquifer heterogeneity, mass transfer rate limitations, and toxicity or inhibition.

Approach: Field and laboratory data are first being reviewed to refine analysis of conservative tracer transport data in order to better characterize ground water flow. Then, vertical variations

in velocity will be quantified, the dimensionality of the transport models will be increased, and parameter uncertainty will be analyzed. Reacting solute behavior will be considered more rigorously by incorporating new insights into rate-limited sorption/desorption and cometabolism. The aim is to attain an integrated understanding of solute transport, distribution, and reaction in heterogeneous media, which will facilitate evaluation and design, including the proper approach for delivery of substrate and electron acceptor.

Status: This two-year project was initiated in September 1992. To date, the Moffett multi-level tracer data have been analyzed to gain quantitative understanding of the degree of vertical variability in hydraulic conductivity. The multi-level tracer data were used to obtain vertically variable transport parameters by independently fitting the data to a one-dimensional conservative solute transport model. A pseudo-two-dimensional solute-transport model which assumes perfectly stratified flow has also been developed for the Moffett Field test zone. This model has been applied to reanalyze bromide breakthrough curves available from previous tracer tests to demonstrate that inclusion of the vertical variation in transport better explains the observed behavior. Currently, more complete models incorporating sorption and reactions are being developed to simulate the behavior of cometabolites, substrates, and electron acceptors.

Clients/Users: Researchers, regulatory agencies, and consultants who evaluate, optimize, and design bioremediation technologies.

## **CHLORINATED SOLVENTS**

### **Oxidation of Chlorinated Solvents by Methanotrophs: Perry L. McCarty and Paul V. Roberts, Stanford University (Supported by the Gas Research Institute)**

Goal: Methanotrophic bacteria, which oxidize methane for energy, have been found capable of oxidizing chlorinated solvents such as TCE by cometabolism. The goals of this project are: (1) to evaluate the effect of competitive inhibition between methane and chlorinated compounds on the overall chlorinated solvent degradation rate; (2) to assess the importance of mass transfer limitations on treatment-reactor design; and (3) to use the knowledge gained to optimize the design for an above-ground treatment system for chlorinated solvent destruction.

Rationale: There have been several studies of treatment systems for degrading chlorinated solvents and related compounds. However, the knowledge of reaction kinetics associated with cometabolism are poorly understood so that little scientific basis currently exists for optimizing treatment reactor design. In addition, since the primary substrate, methane, the required electron acceptor, oxygen, and the chlorinated contaminants are all poorly soluble in water, mass transfer properties of treatment systems become of great significance and must be considered.

Approach: It is hypothesized that oxidation rates for methane and chlorinated contaminants can be described by a competitive inhibition model. Here, the rate-limiting step is the oxidation of either methane or TCE by methane monooxygenase (MMO). To evaluate this hypothesis, reaction coefficients for methane and TCE alone are being evaluated using a mixed methanotrophic culture derived from the Moffett Field aquifer. Reaction rates when methane and TCE are present together are then being predicted and measured in order to test the hypothesis. In mass transfer studies, model calculations are being made for a variety of reactor

configurations in order to determine flow patterns that are realistic and optimal for given treatment objectives. These calculations consider mass transfer effects and biological reaction kinetics. The model results are expected to guide reactor design as well as help to determine important knowledge gaps that may be in need of further research.

Status: Laboratory studies have indicated basic reaction coefficients for methane and trichloroethylene utilization alone by methanotrophs. Initial studies with the two compounds together in various combinations have been conducted. Model calculations have been carried out. These studies have indicated that the presence of methane at high concentration enhances rather than retards TCE degradation, but at low concentrations competitive inhibition becomes significant. Thus competitive inhibition kinetics alone does not appear appropriate in models for predicting transformation rates. The nature of this phenomenon continues to be explored.

Client/Users: Researchers interested in cometabolism for halogenated aliphatic compound destruction; and industry, consulting engineers, and state and EPA regulators who are evaluating treatment options for chlorinated solvents.

**Effects of Sorption on Biodegradation of Halogenated Organics: Perry L. McCarty and Paul V. Roberts, Stanford University**

Goal: The goal of this project is to determine how compound sorption onto solid surfaces such as soil and sorption media (e.g., activated carbon) affects the rates of degradation of chlorinated solvents by methanotrophic bacteria.

Rationale: Rates of biodegradation of organic compounds are known to be affected by sorption onto surfaces, but the nature of this effect is not well understood. Whether it enhances or reduces transformation rates appears to depend upon properties of the sorbed compound, the surface to which it is sorbed, and the biological process involved. Greater knowledge of the effect of sorption is needed in order to better predict fate of chemicals in the environment, and to devise treatment schemes where sorption is involved.

Approach: The availability of sorbed trichloroethylene (TCE) to biological degradation by methanotrophic bacteria, which use methane as primary substrate for growth, is being evaluated. First, biotransformation kinetics for oxidation of TCE by non-fed (resting) methanotrophic bacteria are being determined, using a mixed culture derived from the Moffett Field site, and parameters associated with sorption kinetics of TCE on a well-defined synthetic media (silicalite) as well as aquifer solids are being determined. A numerical model is also being developed to relate desorption and biotransformation kinetics, using the hypothesis that transformation rates are directly related to solution concentration of TCE. In addition, transformation rates of chloroform (CF), in the presence and absence of TCE, are being conducted.

Status: This study of the kinetics of TCE and CF transformation by resting cells and the effect of sorption on TCE transformation rates is now complete. Five manuscripts and a book chapter resulting from this work have been published. Significant findings of this research are that TCE transformation products are toxic to methanotrophic bacteria, and that resting cells have a finite capacity for TCE and CF transformation. A new cometabolic transformation model was

developed that incorporates the phenomena of Monod transformation kinetics, product toxicity, competitive inhibition, and transformation capacity. A model for a two-stage treatment system comprised of a growth reactor and a transformation reactor was developed.

Client/Users: Researchers interested in cometabolism of halogenated aliphatic compounds; and industry, consulting engineers, and state and EPA regulators who are evaluating treatment options for chlorinated solvents.

**Long-Term Chemical Transformation of 1,1,1-Trichloroethane (TCA) and Freon 113 Under Aquifer Conditions: Martin Reinhard and Perry L. McCarty, Stanford University (Supported by Schlumberger)**

Goal: The goals of this project are to (1) determine the rates and pathways of chemical transformation of 1,1,1-trichloroethane (TCA) and Freon 113 under conditions representative of those encountered in an aerobic aquifer, and (2) determine the extent to which sorbing aquifer materials, different solution composition and bacteria may influence transformation rates and pathways.

Rationale: Previous data obtained at high temperature have demonstrated that TCA undergoes abiotic dehalogenation in aqueous solution. However, extrapolation of high-temperature data obtained in distilled water to groundwater temperature is fraught with uncertainty. Moreover, the influences exerted by the presence of aquifer solids and groundwater solutes on transformation rates are not well understood.

Approach: This work involves a multi-year study of the rates and pathways of reaction of TCA and Freon 113 in aqueous solution in the presence, as well as absence of aquifer material. Because under ambient conditions, the half-life of TCA is about a year and several different products may form, substrate disappearance and product formation are being monitored over several years to obtain mass balances.

Status: During the three years that this study has been underway, 73% of the TCA has transformed in the sterilized aqueous solutions, 78% in sterilized and 70% in non-sterilized sediment slurries. The half-lives of TCA in the three systems investigated, 508 days, 447 days, and 540 days, respectively, are not significantly different indicating that 1) sorption does not appear to affect the TCA degradation rate, and 2) microorganisms present do not appear to contribute to the observed breakdown of TCA. In the sterilized aqueous solutions, 1,1-dichloroethylene (DCE) and carbon dioxide now represent about 7% and 38%, respectively, of the initial TCA concentration. With sediment present, the amount of DCE detected has been less (1 to 2%), probably due to sorption. Carbon dioxide production with non-sterilized sediment (28%) is roughly twice that in the sterilized sediment slurries (15%). Acetate concentration has at times reached 20% of the initial TCA concentration in all systems, with the highest amounts detected in the sterilized sediment slurries. 1,1-Dichloroethane has been detected sporadically. Current efforts are being directed toward identifying the reason for an apparent mass imbalance. Freon 113 has remained stable since an initial 40% decrease in the first four months. The study is expected to be completed in 1993.

Client/Users: Consultants, regulators, and researchers interested in natural processes affecting the long-term fate of TCA and Freon 113 in the subsurface environment.

**Use of Starvation and Stress Promoters for Biodegradation of Hazardous Wastes: Abdul Matin, Dunja Grbić -Galić , Stanford University**

Goal: The goal of this project is to construct and characterize recombinant bacteria in which the expression of an enzyme that catalyzes the degradation of hazardous subsurface contaminants is regulated by genetic elements (promoters) which are induced under typical environmental stress conditions, e.g., starvation.

Rationale: Microbial biodegradative activity in the environment is predicated upon the growth of microorganisms with the concomitant expression of catabolic enzymes. However, the oligotrophic nature of most subsurface environments means that microorganisms exist in a state of nutrient-limited stress and grow very slowly. Recombinant bacteria which regulate the expression of a catabolic enzyme of interest by starvation stress-controlled promoters should be useful in the remediation of subsurface sites contaminated by hazardous substances.

Approach: A library of *E. coli* stress promoters has been previously constructed in Dr. Matin's laboratory. These are genetic elements (DNA) which regulate the synthesis of proteins at the level of transcription. They are activated by a signal produced at the onset of specific stress conditions, e.g., starvation. The catabolic enzyme of interest is toluene-4-monooxygenase (T-4-MO) which degrades trichloroethylene (TCE) and catalyzes the initial degradation of a variety of hazardous compounds. The gene cluster encoding this enzyme has been cloned from *Pseudomonas mendocina*, sequenced, and partially characterized; it was made available to us by Amgen, Inc. Our approach is to splice the cluster under the regulation of these stress (particularly starvation) promoters and to characterize the degradative activities of the resultant strains.

Status: The T-4-MO genes have been placed under the regulation of two carbon starvation promoters and under a more generalized stress promoter in *E. coli*. Batch and continuous culture (chemostat) experiments to characterize these constructs with respect to TCE and phenol degradation have begun. Organisms with the more generalized stress promoter are able to maintain TCE or phenol degradative activity for several hours after the onset of starvation. Additionally, attempts are being made to optimize the regulation by one of the carbon starvation promoters. The polymerase chain reaction technique has been used to alter specific DNA sequences in the promoter to *E. coli* consensus sequences and to remove intervening extraneous DNA between the promoter and the T-4-MO genes. The construction of this strain has recently been completed.

Clients/Users: Researchers, consultants, and regulators interested in understanding the process of biotransformation at a fundamental level and applying this understanding to increasing the efficiency of biotransformation under common subsurface environmental conditions.

**Subsurface Mixing of Nutrients and Groundwater for In-Situ Bioremediation: P. L. McCarty, P. K. Kitanidis, P. V. Roberts, and L. Semprini, Stanford University (Supported by the Gas Research Institute and the U.S. EPA Biosystems Program)**

Goal: In-situ bioremediation of groundwater generally requires that gaseous substances such as methane and oxygen be mixed with contaminated groundwater. A scheme by which the gases are introduced directly into the groundwater for mixing with the contaminants is proposed for evaluation in order to avoid bringing contaminants to the surface for this purpose.

Rationale: In-situ bioremediation of chlorinated solvents in groundwater with methanotrophic bacteria requires that the primary substrate, methane, and oxygen for its oxidation be introduced into the groundwater and mixed with the contaminants. This is difficult to accomplish, especially when the contaminants do not sorb strongly to the soil. Bringing contaminated groundwater to the surface for this purpose poses a health hazard, and so a method for accomplishing the introduction and mixing of gases without removing groundwater is desirable.

Approach: A subsurface mixing system is proposed which consists of a series of submerged wells with intake screens at the bottom and top. A pump permits drawing groundwater into the well through the bottom screen and pumping it back into the aquifer through the top screen, or vice versa. In the well oxygen and methane are introduced and mixed with the groundwater. An analysis is being conducted to determine the effect of well depth, well placement, and pumping rate and direction on the degree of mixing of dissolved gases with contaminated groundwater, and to predict the effect of these operating parameters on the degree of in-situ bioremediation that can be achieved. Alternative methods for introduction of gases into the submerged wells are also being evaluated.

Status: This project began in October 1990 and is essentially complete. A combined analytical and numerical model for mixing has been developed and used for the design of the pilot study proposed for the St. Joseph, Michigan, Superfund site. In a parallel study, the growth of microorganisms along stream tubes, the recycle of nutrients to a single, submerged mixing unit, and the treatment of chlorinated solvents with time have been calculated numerically. A method for subsurface introduction of methane and oxygen into a recirculating groundwater stream was developed and tested in the laboratory. A manuscript resulting from this research is now under review.

Client/Users: Researchers studying in-situ bioremediation; and state and EPA regulators, industries, and consulting engineers with interests in biotreatment systems for organically contaminated groundwaters.

**Test-Bed Evaluation of In-Situ Bioremediation of Chlorinated Aliphatic Compounds by Toluene Oxygenase Microorganisms: P.L. McCarty, G.D. Hopkins, L. Semprini, and P. V. Roberts, Stanford University (Partially Supported by the U.S. Department of Energy and the U.S. EPA Biosystems Program)**

Goal: The goal is to evaluate in the field and the laboratory the rate and extent of degradation of chlorinated aliphatic hydrocarbons (CAHs) through cometabolism using phenol as a primary substrate.

Rationale: Recent research has indicated that microorganisms that oxidize phenol can oxidize CAHs such as trichloroethylene (TCE) by cometabolism, similar to biodegradation by

methanotrophs with methane monooxygenase (MMO). In order to obtain a comparison between the relative advantages and disadvantages of phenol- and methane-oxidizing microorganisms, a field evaluation is being conducted at the Moffett Naval Air Station in Mountain View California.

Approach: The field study with phenol oxidizing bacteria is being conducted similar to the previous study in which methane was used as a primary substrate so that the results can be directly compared. Here phenol and oxygen are dissolved in the recycled groundwater along with TCE, cis-and-trans-1,2-dichloroethylene (DCE), and vinyl chloride for introduction into a 1 meter thick confined aquifer located four meters below the ground surface. The growth of a native phenol degrading population and the rate and extent to which the introduced chlorinated compounds are removed is being monitored and compared with conservative tracers as a reference to determine the extent of transformation. In conjunction with the field studies laboratory microcosm studies are being performed under conditions that mimic the field site.

Status: Results of the 1991 field tests demonstrated that indigenous phenol-utilizers could effectively degrade TCE and cis-DCE at concentrations of 40 and 50 mg/L, respectively. Over 90% of the cis-DCE and 85% of the TCE were degraded in the 2 meter biostimulated zone through the continuous addition of 12 mg/L of phenol. Thus, phenol oxidizers were found to be much more effective than methane oxidizers for TCE and cis-DCE removal. During the 1992 field season better than 95% TCE removal was obtained with up to 1 mg/L TCE. Greater than 95% vinyl chloride removal was also obtained. These results are highly encouraging, and possibilities for full-scale application are now being sought.

Clients/Users: State and EPA regulators, industries, and consulting engineers concerned with chlorinated solvents and related compounds present in groundwater.

**Demonstration of In-Situ Bioremediation of Chlorinated Aliphatics by Methanotrophs at St. Joseph: P. L. McCarty, P. V. Roberts, S. M. Gorelick, P. K. Kitanidis, and L. Semprini, Stanford University (Supported by the Gas Research Institute and the U.S. EPA Biosystems Program)**

Goal: Laboratory and field studies recently completed at Stanford University demonstrated that in-situ cometabolic biodegradation of chlorinated solvents and related compounds by methanotrophic bacteria is feasible. This project is for the evaluation of alternative technologies and design of a system for conducting such treatment at full scale.

Rationale: Bioremediation of groundwaters offers great promise because it results in destruction of contaminants. However, application of promising techniques requires research and demonstration at full scale. Field and laboratory studies have helped develop a basic understanding of the processes involved in methanotrophic treatment of chlorinated aliphatic compounds; the next step toward application is an evaluation of potential technologies for the process through research and demonstration at full scale.

Approach: The St. Joseph, Michigan, Superfund site has contamination of a relatively homogeneous fine-sand aquifer with mg/L concentrations of trichloroethylene, dichloroethylene, and vinyl chloride. Laboratory, field, and modeling studies conducted by the WRHSRC indicated

that conditions were ideal for evaluating in-situ bioremediation at this site. In order to develop an appropriate treatment system design, additional site characterization is required, alternative technologies need to be researched, modeling studies for alternatives need to be evaluated, and a system design needs to be developed for evaluation by the responsible industries, their engineers, and state and EPA regulators. These tasks will be carried out by a team of researchers at Stanford University in cooperation with the interested parties.

Status: Initial funding for this two-year study was received from EPA and the Gas Research Institute in September 1990. Model development and design for the St. Joseph pilot study are completed. Detailed characterization of the site has also been completed in a cooperative study by the industry involved and the EPA Robert S. Kerr Environmental Research Laboratory, Ada, Oklahoma. These studies indicated that the concentration of chlorinated solvents and their degradation products are much higher than formerly believed. Also, complete anaerobic transformation to ethene has been found. These results have shifted the priority towards attempts to enhance the anaerobic process, and then to use the in-situ aerobic methanotrophic process for polishing of groundwater. The research under this project is now complete, and manuscripts on results are in preparation.

Client/Users: State and EPA regulators, industries with halogenated solvent groundwater problems, and consulting engineers responsible for design of treatment systems to remove and perhaps degrade chlorinated solvents and related compounds present in groundwaters.

**Determining and Modeling Diffusion-limited Sorption and Desorption Rates of Organic Contaminants in Heterogeneous Soils: Paul V. Roberts, Stanford University**

Goal: This project aimed to advance the understanding of the basic processes governing the uptake and release of aqueous-phase organic contaminants in a heterogeneous porous medium

Rationale: Aquifer remediation strategies, such as pump-and-treat or bioremediation, can be greatly prolonged by diffusional rate limitations on sorption and desorption from the soil matrix. Equilibrium models, which are the most commonly used solute transport models, do not account for these effects. There is a need to improve the basis for assessing when mass transfer conditions are favorable for pump-and-treat and/or bioremediation strategies, and to develop methodologies for predicting the required duration or remediation efforts.

Approach: Long-term batch experiments for measuring the uptake and release rates of several contaminants from solids obtained from an experimental aquifer restoration site have been conducted and interpreted to evaluate and parameterize grain-scale diffusion models. A bench-scale experimental protocol was developed for studying desorption rates that enables long-term measurement with real aquifer solids. The method consisted of a combination of periodic purging into scintillation fluid, followed by direct extraction of the solids into scintillation fluid. Experiments were conducted with core samples from the Borden and Moffett sites. Exploratory experiments also have been conducted with clay layers.

Status: This project was completed in the summer of 1992. The rate data obtained from this study were interpreted using a pore diffusion model that simulates either sorption or desorption, allowing for nonlinear equilibrium and accounting for the periodically changing boundary

condition where necessary. Extremely long equilibration times (up to hundreds of days) are observed. In some cases, desorption occurs considerably more slowly than does sorption; these differences can be accounted for in part by failure to fully equilibrate during sorption. The desorption rate conformed approximately to the pore-diffusion model, although deviations in the form of excessively long tailing are encountered. The apparent activation energy is within the range anticipated for aqueous diffusion. Preliminary experiments with clay layers demonstrated the significance of an additional diffusional resistance at the layer scale. The results can be used to establish a lower bound for diffusional resistances to desorption rate, to be incorporated into transport and fate models. Application of the laboratory-estimated rate parameters substantially improves simulations of solute behavior at the Moffett Field site.

Clients/Users: Researchers interested in the effect of mass transfer limitations on the transport or biodegradation of organic contaminants; also state and EPA regulators, industry, and consultants concerned with site remediation.

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

Goal: This project is aimed to (1) elucidate the role of the reducing agent in coenzyme-catalyzed transformation of chlorinated hydrocarbons, and (2) develop appropriate kinetic models.

Rationale: Transition-metal coenzymes have been known to catalyze reductive transformation of halogenated organic contaminants in biological as well as abiotic systems. The chemistry of these reactions is important for understanding the fates of these pollutants *in vivo* and/or the detoxification processes in microbial systems and, potentially, for the development of novel remediation technologies. The type and strength of the reductant have been shown to affect the reaction rates as well as the product distribution patterns; however, the effects of reducing agent in these catalytic processes involving cofactors are not well characterized in the literature.

Approach: The transformation of carbon tetrachloride (CT) is studied in the presence of a coenzyme, which is reduced either by a thio-reductant (cysteine or dithiothreitol) or titanium(III) citrate. Experiments are conducted in homogeneous aqueous solutions containing a reductant and a cofactor, either hematin or vitamin B<sub>12</sub>, at room temperature under light-excluded, anaerobic, and abiotic conditions over a wide pH range. The disappearance of CT and the appearance of products and intermediates is measured over time to obtain rate constants. Reaction products are quantified to obtain mass balances. Attempts will also be made to identify intermediates to help establish transformation pathways. Other chlorinated substrates (e.g., tetrachloroethene) may be studied once the chemistry of CT transformation is understood.

Status: Rate constants of several reductant-coenzyme combinations were obtained over a broad pH range. Two products of CT transformation, chloroform and carbon monoxide, have been identified. Currently, mass balances are being sought using radio-labeled <sup>14</sup>CCl<sub>4</sub>. This project is expected to be completed in March 1995.

Client/Users: Environmental consultants, engineers, and industry who are looking for decontamination alternatives, and researchers interested in the reductive dehalogenation of haloaliphatic compounds by metallocoenzymes.

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

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

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

Approach: A biofilm model that considers cometabolism of CAHs in the presence of the growth substrate, methane, is under development and evaluation to examine the relative significance of the different biofilm processes and to predict their likely impact on CAH transformation rates. For this study, the CAH used for model simulations and experimental studies is trichloroethylene (TCE). The model is general and so can be applied to cometabolism of other CAHs and to bacteria which grow on other substrates and have oxygenase enzymes capable of CAH cometabolism. The model is being used for hypothesis testing, and to indicate the processes of importance that are in need of further evaluation. Preliminary results from the model have

indicated the importance of the minimum substrate concentration required for bacterial growth ( $S_{min}$ ), and the rate of decay of active biomass. Factors affecting these two process parameters will be experimentally evaluated.

Status: A methanotrophic biofilm model has been developed in which Monod kinetics with competitive inhibition is used to describe the oxidation of methane and TCE by the organisms in the biofilm. Diffusion within the biofilm is modeled by Fick's second law and mass transfer resistance to the biofilm is accounted for by using mass transfer coefficients. The model includes active biomass growth, inactivation, decay, and CAH transformation product toxicity. Simulations suggest that a maximum TCE flux exists with respect to methane concentration. At low methane concentrations (approaching  $S_{min}$ ), there is insufficient methane for net growth of the biofilm. At high methane concentrations, competitive inhibition limits TCE transformation. A manuscript for the biofilm has been submitted. An experimental procedure for determining  $S_{min}$  has been developed, and consists of monitoring the methane concentration in bottles that have been seeded with a small methanotrophic biomass inoculum. Preliminary results are available. During the coming year, the model will be extended to consider use of phenol as a primary substrate, and the experimental determination of factors affecting  $S_{min}$  should be completed.

Client/Users: Researchers considering the important factors affecting biofilm processes for cometabolism of CAHs, and consultants who are interested in factors that affect rates achieved in biofilm reactors to aid in design and optimization of reactor operation.

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

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

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

Approach: The rates of substrate (ammonia) and cosubstrate (chlorinated aliphatic) degradation will be determined using kinetic models which will allow (a) maximal specific rates and (b) most sustainable rates of cosubstrate oxidation to be determined. The commonly encountered

metabolic toxicity which results from cometabolic oxidation of chlorinated aliphatics will be investigated by quantifying the relationship which exists between the amount of cosubstrate oxidation and the extent of inactivation of individual enzyme activities. The ability of nitrifiers to recover from these toxic effects will also be determined. Using results from these physiological experiments various reactor designs will be studied and compared so as to determine an optimal configuration.

Status: Studies were initiated for the first two objectives. Physiological studies with nitrifying bacteria have concentrated on the compound trichloroethylene (TCE). The relationship between the amount of TCE oxidation and the extent of cellular toxicity were determined. The rates of cellular recovery following exposure to TCE were also determined as a function of the extent of TCE-dependent inactivation. These experiments provided a quantitative approach which will be subsequently used to assess the consequences of TCE oxidation by other bacterial types. Similar experiments are now being extended to consider other common chlorinated aliphatic compounds and other bacterial types. Gas-tight, laboratory-scale reactors were developed and tested. Initial kinetic studies were conducted following the effects of carbon tetrachloride on long-term (>3h) incubations using nitrifying bacteria. Subsequent studies will examine additional chlorinated aliphatics. A further study was initiated to examine whether the expression of epoxide hydratase and isomerase enzymes in alkene-oxidizing bacteria protects these organisms from the toxicity associated with the cometabolism of chlorinated ethylenes such as TCE.

Client/Users: Industrial/consulting engineers and state/federal regulators who are evaluating treatment options for chlorinated aliphatic hydrocarbons. Industrial and academic researchers in the field of cometabolism.

## **AROMATICICS**

### **Interactions between Electron Acceptors in the Treatment of Wastewaters Containing Sulfate, Chlorophenols and Acetate: Sandra L.Woods, Oregon State University**

Goal: The goal of this project is to develop and verify a mathematical model for anaerobic biotransformations of chlorophenols in the presence of competing electron acceptors, and to measure process kinetic constants.

Rationale: Reductive dechlorination of chlorinated aromatic compounds appears to progress such that parent compounds are almost completely removed before degradation of metabolic products begins. To evaluate this hypothesis, the kinetics of pentachlorophenol biotransformation and each of its metabolic products was evaluated. Additional experiments were conducted in the presence of high concentrations of sulfate.

Approach: Organisms acclimated to pentachlorophenol or pentachlorophenol and sulfate were grown in continuous flow anaerobic reactors. The reactors were allowed to reach steady-state and then used as a source of organisms for subsequent batch experiments. Progress curves for chlorophenol, sulfate (if present) and acetate degradation were generated from the batch experiments. Biodegradation rates were calculated from the resulting progress curves.

Status: Individual batch degradation experiments were conducted in the absence of sulfate for pentachlorophenol and each of its metabolic products. These progress curves allowed determination of a complex pentachlorophenol-biodegradation pathway. All three tetrachlorophenol isomers were observed, as well as 7 additional tri- and di-chlorophenols. While the consortium had the ability to dechlorinate at the ortho, meta, and para positions, ortho dechlorination was observed most frequently. Dechlorination at the ortho or para positions was most rapid. Dechlorination at the meta position was very slow or not observed. Because degradation rates were observed to decrease with decreasing chlorination, metabolic products would be expected to accumulate in pentachlorophenol-contaminated environments. Pentachlorophenol dechlorination rates were measured similarly in the presence of varying initial sulfate concentrations (0.6 and 8 mM). The degradation rate for pentachlorophenol was slow and equal in both systems (the apparent Eh rose to -250mV). When the apparent Eh was held below -400 mV by the addition of sodium sulfide, the rate of pentachlorophenol dechlorination increased fourfold and was approximately equal regardless of whether the initial sulfate concentration was 0.6 or 8 mM.

Client/User: Consultants and regulators concerned with site characterization for bioremediation or above-ground biological treatment of chlorophenols, and researchers interested in biotransformations in a complex system.

**Enhancing Biodegradation with Sorption and Alternating Aerobic/Anaerobic Environments: Kenneth J. Williamson and Peter O. Nelson, Oregon State University**

Goal: The goal is to develop and verify a mass transport and biokinetics model of the sorption and biological degradation of pentachlorophenol and its degradative products by anaerobic and aerobic biofilms on activated carbon.

Rationale: Granular activated carbon (GAC) has been shown useful for enhancing biological treatment of toxic organic compounds by adsorption, resulting in reduced aqueous concentrations to below inhibitory levels. This enables biological degradation of toxic compounds to occur by attached biofilms. Haloaromatic compounds undergo different biodegradation pathways under anaerobic and aerobic conditions. Anaerobic pathways typically result in metabolic products that cannot undergo further metabolism in that environment. By sorbing these compounds to GAC and then moving the GAC to an aerobic environment, improved rates of degradation may be possible.

Approach: Anaerobic and aerobic biofilms have been developed on GAC maintained in upflow expanded beds with liquid recycle. Acetate was used as the electron donor for the anaerobic reactor and the primary electron donor for the aerobic reactor. Pentachlorophenol was fed to the anaerobic column, and should rapidly dechlorinate to a series of tri- and di-chlorophenols. These metabolic products should sorb strongly to the GAC. The GAC was moved from the anaerobic column to the aerobic column with a solids retention time of 30 days. The tri- and di-chlorophenols will slowly desorb in the aerobic reactor and should undergo aerobic degradation.

Status: Project completed. Project has shown that removal of chlorinated phenols by biodegradation in GAC columns will be difficult because of the strong adsorption coupled with slow desorption rates.

Client/Users: Practitioners developing new technologies for pump-and-treat remediation.

**Development and Verification of a Numerical Model to Predict the Fate and Transport of Chlorinated Phenols in Groundwater: Jonathan D. Istok and Sandra L. Woods, Oregon State University**

Goal: The goals are (1) to develop a numerical model to predict the fate and transport of pentachlorophenol and its primary degradation products in field soils and groundwater aquifers, (2) to verify the model using laboratory and field experiments, and (3) to evaluate the effectiveness of alternate in situ biological remediation strategies for chlorinated phenols.

Rationale: Predictive models for the fate and transport of organic compounds are needed for site characterization and to design effective biological remediation strategies. Pentachlorophenol (PCP) was selected for this research because (1) it is a common groundwater contaminant in the western United States due to its widespread use in the wood products industry, and (2) numerical models developed to predict PCP fate and transport are potentially applicable to a large class of halogenated compounds.

Approach: The governing equations are developed for the case of one-dimensional steady-state, groundwater flow for two classes of reactive species, those that are transported (e.g., chlorinated phenols and electron donor) and those that are stationary (e.g., sorbed species and the microbial populations). The applicability of these equations was evaluated for a system consisting of a specific soil (Chehalis silt loam), electron acceptors (selected chlorinated phenols), and electron donor (acetate). The Chehalis soil was selected to be representative of PCP-contaminated sites in the northwestern U.S. The chlorinated phenols (2- and 4-CP, 2,4-DCP, 2,4,6- and 3,4,5-TCP, 2,3,4,5-TeCP, and PCP) were selected from the observed anaerobic degradation pathway for PCP.

Status: Model parameters were measured individually in a series of experiments in batch and column reactors. Additional batch experiments were conducted with a mixed culture (acclimated anaerobic digester sludge) to identify the degradation pathway and measure degradation rates for each chlorinated phenol in the degradation pathway. Sorption is described using diffusion-limited desorption models. Monod kinetics were used to describe the degradation reactions (reductive dechlorination) and the growth of two microbial populations, one population performs reductive dechlorination of PCP and metabolites, the other performs fermentation of the electron donor.

Client/User: The results should be of interest to those developing groundwater remediation systems for PCP contaminated sites, regulatory agencies, and other researchers.

**Anaerobic Microbial Transformation of Homocyclic and Heterocyclic Polynuclear Aromatic Hydrocarbons: Dunja Grbić -Galić, Stanford University**

Goal: Studies were performed to assess anaerobic biodegradability of complex ring compounds, such as polynuclear aromatic hydrocarbons (PAH) and heterocyclic compounds containing nitrogen, sulfur, or oxygen heteroatoms (NSO). These chemicals are constituents of complex

pollutant mixtures such as petroleum, tar, and creosote. Three types of anaerobic conditions were addressed: denitrifying, sulfate-reducing, and fermentative/methanogenic.

Rationale: PAH and NSO are chemically stable compounds; some have carcinogenic and mutagenic properties. Their widespread occurrence as environmental pollutants, as well as lack of knowledge of their fate (especially in the absence of molecular oxygen), are of concern. It is important to understand the anaerobic microbial processes that can affect PAH and NSO either in the presence of exogenous electron acceptors such as nitrate or sulfate (conditions of anaerobic respiration), or in their absence (conditions of fermentation/methanogenesis). The rates and extent of transformation of PAH and NSO, intermediates and products of the transformation, and microorganisms involved are of interest.

Approach: Saturated batch microcosms were established with anaerobic aquifer solids from several different sites: Norman, OK (landfill leachate contamination; sulfate-reducing site); Traverse City, MI (JP-4 jet fuel contamination; methanogenic site); Pensacola, FL (creosote contamination; methanogenic site); Patuxent River, MD (gasoline contamination; sulfate-reducing site); and an oil refinery (CA), (denitrifying activity towards aromatic hydrocarbons). The PAHs and NSOs tested included indene, naphthalene, acenaphthene (PAH), indole, quinoline, isoquinoline, acridine (N compounds), thiophene, benzothiophene, dibenzothiophene (S compounds), furan, benzofuran, and dibenzofuran (O compounds). The transformation of substrates, utilization of electron acceptors, and product formation were monitored by GC, HPLC, IC, and GC/MS. Attempts were made to isolate pure cultures of active microorganisms.

Status: All the aquifer inocula except from the Norman site exhibited transforming activity towards some of the test substrates. The inoculum from the creosote-contaminated Pensacola site was the most versatile. Methanogenic microcosms derived from this site mineralized indene, naphthalene, indole, quinoline, isoquinoline, benzothiophene, and benzofuran. The addition of accessory organic substrates (benzoate, fatty acids) accelerated the transformation of some compounds (naphthalene, benzothiophene, benzofuran). Early oxidation intermediates were observed in some cases: oxindole from indole, 2-quinolinol from quinoline, and isoquinolinol from isoquinoline. The lag periods before the onset of transformation varied from 10 days (indene) to 5 months (benzothiophene). When mixtures of NSO compounds were used as a complex substrate, no effects on the rates or patterns of transformation of single substrates were observed.

Sulfate-reducing conditions supported transformation of indole and quinoline (Patuxent River and Pensacola sites), as well as acenaphthene (Pensacola microcosms only). Oxindole, 2-quinolinol, and 1-acenaphthenol, respectively, were observed as early oxidation intermediates. Some intermediates (2-quinolinol, 1-acenaphthenol) persisted for longer than a hundred days. Several attempts have been made to isolate pure cultures from these microcosms, with only partial success.

Denitrifying microcosms derived from the oil refinery pond sludge transformed only indole and quinoline, with transient appearance of an unidentified oxidation intermediate (from indole) or 2-quinolinol (from quinoline). Mineralization of both substrates was completed in one (indole) to three months (quinoline).

Client/Users: EPA regulators, industries, and consultants concerned with clean-up of sites contaminated by petroleum or creosote.

**PAH Degradation by Methanotrophic Bacteria: Perry L. McCarty and Dunja Grbić-Galić, Stanford University (Supported by Kerr-McGee Corporation)**

Goals: Polycyclic aromatic hydrocarbons (PAHs) are hazardous compounds found associated with wood preservatives (creosote), coal gasification sites, and petroleum product wastes. The goal of this project is to explore the ability of a methanotrophic mixed culture developed at Stanford to initiate the oxidation of four characteristic PAHs, thereby rendering the compounds more susceptible to further degradation by other microorganisms.

Rationale: The compounds being studied are naphthalene, phenanthrene, pyrene, and benzo(a)pyrene, which increase in complexity from two to five rings. The initial step in oxidation of PAHs generally involves the activities of monooxygenases or dioxygenases. The methane monooxygenase (MMO) present in methanotrophs is quite nonspecific and so offers the potential for initiating PAH oxidation. The Stanford mixed culture has been well characterized and is being used for studies of chlorinated solvent degradation. It possesses the soluble MMO which is believed to be the form with broad substrate specificity.

Approach: The initial step is to evaluate the ability of the methanotrophic culture to initiate oxidation of the four PAHs. HPLC is being used to measure the concentration of the four compounds and to detect intermediates in PAH oxidation. Initially, the four compounds will be added individually to aqueous solutions containing growth nutrients for the methanotrophs, a culture (about 250 mg/L) will be added, and the change in concentration of the PAH with time will be monitored. If transformation indeed occurs, then a broader study will be initiated to determine the effect of sorption to soil on PAH oxidation by methanotrophs. At that time, <sup>14</sup>C-labeled PAHs will be used to make better mass balances because of the analytical difficulties in making mass balances when soils are used. If oxidation is obtained, then the study will be broadened to evaluate rates and extents of transformations of soil bound PAH by methanotrophic bacteria.

Status: This one-year exploratory project is now complete and a report to the client has been submitted. The rapid oxidation of naphthalene to naphthanol within a few hours was found both by methane and phenol oxidizing consortia. Phenanthrene was found to be slowly oxidized by methane oxidizers, but not by phenol oxidizers. Pyrene and benzo(a) pyrene were not oxidized by either culture. Thus, the potential of oxygenases from these two consortia for PAH oxidation appears quite limited. No further research in this area is contemplated.

Client/Users: Industry and regulatory authorities concerned with PAH contamination of soil from creosote sites and sites where consumer gas was produced.

**In-Situ Biological Treatment of Aromatics in Groundwater: Martin Reinhard and Perry L. McCarty, Stanford University (Supported by the U. S. Navy)**

Goals: The objective of this study is to 1) develop and characterize microbial consortia capable of transforming aromatic hydrocarbon compounds under anaerobic conditions and to understand

the effect of environmental factors on the transformation processes, and 2) evaluate potential in-situ application of anaerobic processes for bioremediation of a gasoline contaminated field site.

Rationale: Although most gasoline constituents are readily degraded in aerobic surface water systems, the groundwater environment associated with hydrocarbon spills is typically anaerobic, thus precluding aerobic degradation pathways. In the absence of oxygen, degradation of gasoline components can take place only with the utilization of alternate electron acceptors such as nitrate, sulfate, carbon dioxide, and possibly ferric iron. Neither the potential for transformation of these compounds under field conditions using alternate electron acceptors, nor the rate of such transformations has yet been assessed.

Approach: Anaerobic transformation of various monoaromatic hydrocarbons is being evaluated in the laboratory and a field bioreactor experiment. In the laboratory, microbial consortia from aquifer solids and other sources are being evaluated for anaerobic biotransformation of benzene and selected alkyl-substituted monoaromatic compounds. Potential biotransformation intermediates, biotransformation kinetics, and the effect of oxygen on a denitrifying pure culture that transforms ethylbenzene are being determined. Anaerobic degradation of gasoline hydrocarbons is being tested in large scale bioreactors placed in the ground at a gasoline-contaminated aquifer at the Seal Beach site in Southern California. The field research is being developed and carried out in cooperation with research staff of the Orange County Water District.

Status: Toluene, ethylbenzene, and m-xylene have been biotransformed in the laboratory under anoxic, denitrifying conditions. Evaluation of the denitrifying pure culture that degrades ethylbenzene is ongoing. Under sulfate reducing conditions, transformation of toluene, p-xylene, o-xylene, and benzene has been confirmed. At the Seal Beach site, results from the bioreactor experiments indicate that toluene biodegradation was enhanced by nitrate addition. It is likely that several electron acceptors played a role in the overall activity observed in the bioreactors. Results from laboratory microcosm studies were consistent with those from the bioreactors. The microcosm study also indicated that reduction of sulfate occurred readily but only after nitrate had been completely utilized.

Client/Users: Researchers interested in anaerobic biotransformation of hydrocarbons and aquifer bioremediation; and state and EPA regulators and industry concerned with gasoline and hydrocarbon remediation.

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

Goal: The goal of this project is to explore two phenomena that are potentially important to remediation of gasoline-contaminated aquifers but are not well-studied: sulfidogenic toluene degradation and the stimulation of this process by iron. These phenomena have been observed in bacterial enrichment cultures originally inoculated with fuel-contaminated soil collected near Patuxent River (Maryland). The emphasis is on elucidating degradation pathways and the nature of iron's effect (for example, whether iron reduces sulfide toxicity or is an essential nutritional requirement, and whether iron stimulates only the first step in toluene degradation or multiple steps).

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

Approach: Experiments are being performed to explore the importance of sulfide removal in the absence of iron; for example, zinc has been substituted for iron in enrichment cultures to see whether toluene degradation would be stimulated. In addition, studies are being made to determine whether iron stimulates the degradation of metabolic intermediates of toluene degradation. To accomplish this task, stable isotope-labeled toluene and gas chromatography/mass spectrometry were first used to identify metabolites of toluene degradation. Enrichments were then constructed using intermediates as sole, primary substrates. Attempts are also being made to isolate the one or more species in the enrichment culture that engage in toluene degradation.

Status: Recent experiments with enrichment cultures have confirmed that Fe(II) is effective at stimulating toluene degradation [this was previously demonstrated for Fe(III)]. Experiments substituting zinc carbonate for ferrous iron have shown that zinc did not enhance toluene degradation and that the lack of stimulation was not a result of zinc toxicity. However, the effectiveness of zinc carbonate at removing sulfide from solution remains to be demonstrated with direct sulfide measurements. Benzoate, found to be a transient intermediate of toluene degradation in these cultures, is now being used as a primary substrate in some enrichment cultures. Further attempts will be made to isolate other transient intermediates. Two dead-end metabolites, benzylsuccinic acid and benzylfumaric acid, have also been identified as minor products of toluene degradation. Preliminary attempts at isolating sulfate-reducing bacteria have been unsuccessful.

Client/Users: State and EPA regulators, industry, and consultants concerned with cleanup or natural bioremediation of sites contaminated by gasoline or aviation fuel; researchers interested in geochemical factors affecting the anaerobic degradation of monoaromatic hydrocarbons.

### **An Evaluation of the Effectiveness of In Situ Biodegradation for Benzene, Toluene and Xylene Isomers in Large-Scale Physical Aquifer Models: Jonathan D. Istok, Oregon State University**

Goals: The goals of this research are: (1) to obtain a detailed description of in situ biodegradation processes, including the spatial distribution of microbial growth rates and activities, in an experimental aquifer system, and (2) to determine the effectiveness of hydrogen peroxide injection as a supplemental oxygen source for the degradation of BETX compounds.

Approach: Experiments are conducted in large-scale physical aquifer models (2m x 4m x 0.2m) which contain an array of wells used for injection, extraction, and sampling. Additional samples can be obtained from any location in the sand pack using syringe needles. A two-well system is being evaluated, wherein BTEX and hydrogen peroxide are injected in one well and extracted from another. The biostimulated zone is approximately 2 m long, with residence times of 2 to 8 days. Detailed measurements of BTEX, oxygen and carbon dioxide concentrations are made on a three-dimensional sampling array. Rates of microbial transformation are computed from small-scale in situ bioassays performed at selected locations throughout the sandpack. The experiments are being designed and interpreted using a numerical fate and transport model.

Status: Two physical models have been constructed, instrumented, and packed with sand selected to be representative of alluvial valley aquifers in Oregon. Preliminary hydrologic characterization (hydraulic conductivity, dispersion coefficients) has been completed. Batch experiments to design feed mixtures for the BTEX transport experiments are in progress. A two-dimensional numerical model has been written and tested, and is being used to determine injection rates and concentrations. Preliminary testing of in situ bioassays to measure rates of BTEX degrading activity is being tested in a small-scale model.

Preliminary experiments are being conducted with toluene in aqueous solution at a concentration of 10 mg/L. A portion of the sandpack has been "contaminated" with 3.0 L of toluene solution and is being remediated by pump and treat. Data from this experiment will serve as the control to a sequence of enhanced bioremediation experiments based on increasing the dissolved oxygen concentration in the sand pack. Air sparging, pure oxygen amendment, and injection of hydrogen peroxide and permanganate will each be investigated. Remediation experiments are based on a two-well system with no recycle.

Clients/Users: The results will be useful for designing in situ remediation strategies, and will provide the first comprehensive description of the bioremediation process for regulators, site engineers, and researchers.

**Aerobic Methanotrophic Transformation of Biphenyl, Monochlorobiphenyls, and Dichlorobiphenyls: Dunja Grbić -Galić , Stanford University (Supported by the General Electric Corporation)**

Goal: To assess the abilities of mixed and pure methanotrophic cultures, enriched from Moffett Field aquifer (Moffett Naval Air Station, Moffett Field, CA) and previously shown to oxygenate trichloroethylene (TCE), to transform ortho-chlorinated or ortho-hydroxylated biphenyls. These congeners had been found to accumulate in anaerobic methanogenic sediments and cultures transforming polychlorinated biphenyls (PCBs). The ortho-substituted PCB congeners of most interest are monochlorobiphenyls (1- and 4-MCB) and dichlorobiphenyls (2,3-, 2,6-, 2,2'-, and 2,4'-DCBs).

Rationale: The methanotrophic cultures tested include two mixed cultures harboring a Type II methanotroph (mixed culture MM1, and consortium CSC-1), a mixed culture harboring both Type I and Type II methanotrophs (culture MM2), and a pure culture of Type I methanotroph (*Methylomonas* sp. MM2). Type II methanotrophs are known to harbor a soluble methane monooxygenase (sMMO) which has been shown to exhibit a broad substrate specificity (including monohalogenated arylhalides and polyaromatic substrates). Type I methanotrophs express only particulate MMO which is believed to have a narrower substrate specificity. It is of interest to understand how these two groups contribute to the fate of PCBs in the environment.

Approach: The cultures were grown in a batch system on methane/oxygen mixture (60:40). To fully induce the soluble MMO, the nutrient medium contained no copper. The 30-ml aliquots of the suspended cultures were incubated with a range of chloro- and hydroxy-biphenyls in Erlenmeyer flasks, and alternately fed with methane and air over a 20-day period. Samples from the cultures were extracted, processed, and analyzed spectrophotometrically by GC/MS, and <sup>1</sup>H-NMR.

Status: A range of MCBs and DCBs were transformed by all the test cultures. All cultures generated precursors to yellow absorbing products found in alkaline extracts. These compounds, with a characteristic absorbance of a dienone-structure, are now hypothesized to be chemically-mediated ring-fission products, since they were observed in alkaline extracts only and not in the culture fluid. Further characterization of these compounds is ongoing. The mixed Type II methanotrophic cultures expressing soluble MMO (MM1 and CSC-1) were shown to mediate hydroxylation of 2- and 4-MCB and of 2-chloro-4-hydroxybiphenyl when maintained on methane under copper-limiting conditions. The hydroxy-group was inserted on the nonchlorinated ring, but the stereospecificity still needs to be elucidated. Neither chloride release nor presence of dechlorinated intermediates were observed. *Methylomonas* sp. MM2, the Type I methanotroph, expressing only a particulate MMO, was not shown to produce hydroxylated products from these compounds.

Client/Users: This project will contribute to our knowledge of the fate of PCBs in the environment. As such, it will interest industries and agencies concerned with in-situ bioremediation processes for PCB-contaminated sites.

**Biotransformation of Ordnance Wastes Using Unique Consortia of Anaerobic Bacteria: A. Morrie Craig and Sandra Woods, Oregon State University (Supported by the U. S. Navy)**

Goals: The objectives are to determine trinitrotoluene (TNT) degradation rates and transformation products with anaerobic metabolism by sheep ruminal microorganisms utilizing <sup>14</sup>C radiolabel.

Rationale: Pilot trials have shown that a new source of bacteria capable of degrading aromatic compounds has been found in sheep's rumen. The uniqueness of these microorganisms is the time rate in which total degradation of the toxic molecules occur. Few anaerobes from sediments can degrade TNT; however, preliminary evidence has shown that sheep microorganisms can degrade 100 mg/mL within 96 hours. Commercialization of these bacteria in bioremediation of munitions sites for the Navy and other branches of the armed forces would be highly desirable.

Approach: A three-fold approach is used: (1) the ruminal microorganisms are enriched for TNT degradation, and a partially purified bacterial consortium is defined; (2) degradation of radiolabeled TNT will be used to identify the metabolic transition and end products of TNT metabolism; and (3) optimum growth parameters and degradation rates utilizing this consortium will be determined.

Status: This study is completed. The ruminal consortium was found to reduce the nitro-groups on TNT to ammino groups as has been reported by others. Subsequently, however, and within a matter of hours, the aromatic compound was further degraded and converted to several fatty acid components as confirmed through mass balance with <sup>14</sup>C tracer. Such opening of the aromatic ring under anaerobic conditions is a new finding. Enrichments of the microbes which are degrading TNT have led us to identification of two different bacteria. The biotransformation engendered by these enrichments mimics that found in whole rumen fluid. The results of this work are nearing completion.

Client/Users: The U.S. Navy and other DOD units, their consultants, and regulatory agencies who are interested in the biotransformation of munitions; and researchers who are exploring new microbial consortia for aromatic compound degradation.

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

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

Rationale: Reductive dechlorination is a common anaerobic biodegradation mechanism for chlorinated aromatic compounds. This research will test the hypothesis that the rate of reductive dechlorination reactions increase with decreasing apparent Eh. The sulfate/sulfide system will be studied to better understand the oxidation/reduction conditions within the system.

Approach: The project has two phases. In the first phase, a reactor system is being developed to allow measurement of biodegradation rates under constant conditions of biomass, pH, sulfate, sulfide and acetate concentrations. In the second phase, batch experiments will be conducted to

measure degradation rates under different environmental conditions. A model anaerobic system fed acetate, pentachlorophenol, and nutrients has been selected for study.

Status: Phase 1 - A batch reactor system that can be used to control the major species in the model system (sulfate, sulfide, acetate,...) has been constructed. Work is continuing to develop a computer program to control the addition of solutions to the batch reactor and collect data from pH and platinum electrodes. Phase 2 - A steady-state, acclimated methanogenic consortium is being maintained on acetate and pentachlorophenol for use in subsequent batch biodegradation experiments. The purpose is to provide similar initial conditions for each biodegradation rate determination. A suggested batch experiment was conducted in the reactor system to evaluate the ability to maintain a constant pH and acetate concentration by adding acetic acid at a constant rate. Future experiments will focus on controlling other major species such as sulfate and sulfide, following which, biodegradation rates will be measured.

Client/User: Consultants and regulators concerned with site characterization for bioremediation or above-ground biological treatment of chlorophenols, and researchers interested in biotransformations in a complex system.

## **HEAVY METALS**

### **Trace Metal Removal Processes: James O. Leckie, Stanford University**

Goal: The goal is development of an experimental data base and mathematical model for mass-transfer limited adsorption of trace metals in porous particles. Experimental results and the mathematical model will facilitate the design of removal process utilizing porous metal oxide particles.

Rationale: Previous experimental work with porous, high-surface-area oxide particles has demonstrated a dramatic increase in adsorption capacity of the porous material relative to nonporous particles. From an engineering perspective, porous particles with high internal surface area are appropriate for treating large volumes of dilute metal bearing wastewaters and allowing regeneration and recycling of the adsorbent. Reactions within porous particles can be severely limited by intraparticle diffusion. Investigation of diffusional limitations is an objective of this project. Choice of particles with a suitable pore size distribution will provide the high surface area desired without imposing severe pore diffusion limitations.

Approach: The approach involves the design and implementation of a parametric study of adsorption of an oxyanion (selenite) and a cation (cadmium) on porous, amorphous alumina particles. Samples of three different porous aluminum oxide particles were obtained from ALCOA for the study. The first phase of the experimental work involves the physical-chemical characterization of the adsorbent (particle morphology, particle size distribution, pore size distribution, surface area, solid structure, surface site density, acidity constants, electrolyte binding constants, and trace-element-binding constants). The second and main phase of the experimental work explores the role of mass transfer on trace element adsorption in the porous alumina particles. The parametric study includes experiments at variable pH values, solid and trace element concentrations, solid/solution ratios, and ionic strengths. Based on particle characterization and data developed in the second experimental phase, a diffusion model will be used to test the hypothesis of diffusion limited adsorption.

Status: The physical-chemical characterization of the adsorbent has been completed. Equilibrium experiments with selenite and cadmium have confirmed the high adsorptive capacity of the porous alumina. Rate experiments with cadmium and selenite indicate that the approach to equilibrium depends on particle size and solid structure (porosity, tortuosity) as expected for diffusion-limited adsorption. Equilibrium data were modeled with the triple-layer surface complexation model and the results are satisfactory considering the wide range of solid/solution ratios. The rate of uptake experiments were modeled assuming diffusion of solute in a sphere from limited volume. The last phase of the project will be focused on model refinement.

Client/Users: Researchers interested in trace metal removal processes; electronics, electroplating, and power industries; areas with high trace metal concentration problems (e.g. Kesterson reservoir).

### **HEXAVALENT CHROMIUM SORPTION AND DESORPTION IN NATURAL SOILS AND SUBSOILS: Peter O. Nelson, Jonathan D. Istok, Oregon State University**

Goal: The intent of this research is to gain a better understanding of the chemical behavior of hexavalent chromium, Cr(VI), in natural soils. Results will be used to improve transport modeling of the fate of Cr(VI) and to better design remediation schemes for chromium-contaminated soils.

Rationale: Remediation schemes for chromium contamination sites give strong consideration to soil flushing, or pump-and-treat technology. The efficacy of pump-and-treat is highly dependent on chromium sorption and desorption kinetics and on the influence of competing solute anions. Models to predict the transport of Cr(VI) in soils must therefore incorporate these effects.

Approach: A controlled laboratory investigation is used in which Cr(VI) sorption and desorption reactions with natural soils and subsoils are studied. Batch reactor experiments are used to determine equilibrium sorption parameters for chromate and sorbing co-solutes on soil, and to study sorption and desorption kinetics over extended time periods (weeks to months) that are relevant to soil contamination sites. Soil column studies are used to more closely simulate field conditions of porous media flow for investigation of diffusion-limited sorption and desorption kinetics.

Status: Results of this study indicated that adsorption and reduction of Cr(VI) are the major processes that control the rate of transport and mobility of chromium in a natural soil. Cr(VI) removal from solution increased with increasing solute concentration and with decreasing solution pH. Small amounts of ferrous ions in minerals such as magnetite ( $\text{Fe}_3\text{O}_4$ ) contained in the soil caused reduction of Cr(VI) to Cr(III) even at pH above 8. The equilibrium adsorption capacity of the soil was described with the Langmuir model, while surface complexation reactions of the  $\text{CrO}_4^{2-}$  and  $\text{HCrO}_4^-$  ions were predicted by a triple layer model. Outer-sphere surface complexation reactions and two-site (FeOH and AlOH) modeling were used to simulate adsorption of the chromate ( $\text{CrO}_4^{2-}$ ) and bichromate ( $\text{HCrO}_4^-$ ) ions. Chromium breakthrough and desorption curves from soil column experiments showed retardation, suggesting adsorption, and a loss of mass, suggesting reduction of Cr(VI) to Cr(III) and subsequent adsorption or precipitation in the soil. Competing oxyanions increased Cr(VI) desorption by direct competition for adsorption sites, with the order of effectiveness for enhancing Cr(VI) desorption being:  $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^- > \text{HCO}_3^- > \text{SO}_4^{2-} > \text{NO}_3^-$ . The study concluded that remediation of hexavalent chromium-contaminated soils can be achieved by reduction, resulting in the less

mobile and much less toxic Cr(VI), or by desorption and extraction, which can be enhanced chemically by the addition of competing oxyanions.

Client/Users: Results of this research will enhance understanding of Cr(VI) chemical behavior in soils, and will be useful for modeling the fate and clean-up of chromium at contaminated soil sites for the prevention of groundwater contamination. Practitioners selecting remediation processes for sites with hexavalent chromium contamination will benefit from the methodology developed in this research.

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

### **TRAINING AND TECHNOLOGY TRANSFER**

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

Goal: To promote training activities related to hazardous substance management.

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

Approach: The Center in consortium with the University of California Extension Program in Hazardous Materials Management sought support to serve as one of the regional EPA training centers for lead paint abatement. The Center will conduct the training within Oregon, Washington, Idaho, and Alaska.

Status: The Center was successfully chosen for the EPA grant. Training will begin in 1993.

#### **Advanced Topic Workshops: Lewis Semprini, Stanford University**

Goal: To provide workshops on advanced topics to regulators, consultants, industrial personnel, and researchers.

Rationale: A need exists for transfer of results from the basic and applied research conducted at the Center.

Approach: The Center has developed five workshops to be conducted upon demand. These workshops are being offered in conjunction with the University of California Extension Service in Environmental Hazardous Materials Management.

Status: The workshops are offered when adequate demand is generated.

#### **Continuing Education Program: Kenneth J. Williamson, Oregon State University and Donald C. Olcott, Continuing Education, Oregon State University**

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

Rationale: An education program is not available in hazardous substance management and site remediation for persons in the Portland metropolitan area.

Approach: A series of six courses has been developed to be offered through OSU's continuing education program. The courses are transferable for graduate credit and a certificate of completion is offered for those students successfully completing the courses.

Status: The series of courses are on-going and will be offered on a two-year cycle. Attendance has steadily increased and about 50 persons are presently in the program.

**Conference Sponsorship: Kenneth J. Williamson, Oregon State University, and Lewis Semprini, Stanford University**

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

Rationale: Conferences are a highly efficient and cost-effective way to achieve the transfer of technology.

Status: This year the Center sponsored or co-sponsored the following conferences:

The 1992 Idaho Regional Conference on Hazardous Materials, Pocatello, Idaho, April, 1992.

Reinhard, M., One-Day Seminar, "State-of-the-Art Soil Sampling and Analysis for Organics," HAZMACON, Long Beach, California, April 1, 1992.

Westall, J. and M. Reinhard, Two-Day Symposium on Redox Transformations in the Environment, American Chemical Society National Meeting, San Francisco, California, April 5-10, 1992.

The 1992 Responsible Hazardous Materials Management Conference, Portland, Oregon, September, 1992.

Subsurface Restoration Conference, Dallas, Texas, June, 1992.

**Field Application of Remediation Technologies: Perry L. McCarty, Stanford University, and Kenneth J. Williamson, Oregon State University**

Goal: To seek funding to support field applications and demonstrations of innovative remediation technologies developed within the Center.

Rationale: Field application represents one necessary step in the ultimate transfer of technology.

Approach: Funding is being sought to support field applications in relation to several technological developments from the research within the Center.

Status: Two projects should soon be funded by the Department of Energy :

"Field Test of In-situ Vapor Stripping"

"Design of Enhanced In-Situ Biotransformation of TCE"

Several other projects are presently being proposed.

## 1992 WRHSRC PUBLICATIONS

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

### A. Refereed Journal Articles

- Alvarez-Cohen, L., P. L. McCarty, E. Boulygina, R. S. Hanson, G. A. Brusseau, and H. C. Tsien, "Characterization of a Methane-Utilizing Bacterium from a Bacterial Consortium that Rapidly Degrades Trichloroethylene and Chloroform," *Appl. Environ. Microbiol.* **85**(6): 1886-1893 (1992).
- Beller, H.R., M. Reinhard, and D. Grbić -Galić , "Metabolic By-Products of Anaerobic Toluene Degradation by Sulfate-Reducing Enrichment Cultures," *Appl. Environ. Microbiol.* **58**(9): 3192-3195 (1992).
- Chrysikopoulos, C. V., L. M. Hildemann, and P. V. Roberts, "Modeling the Emission and Dispersion of Volatile Organics from Surface Aeration Water Treatment Facilities," *Water Research*, **26**(8), 1045-1052 (1992).
- Chrysikopoulos, C. V., P. K. Kitanidis, and P. V. Roberts, "Generalized Taylor-Aris Moment Analysis of the Transport of Sorbing Solutes Through Porous Media with Spatially Periodic Retardation Factor," *Transport in Porous Media*, **7**, 163-185 (1992).
- Chrysikopoulos, C. V., P. K. Kitanidis, and P. V. Roberts, "Macrodispersion of Sorbing Solutes in Heterogeneous Porous Formations in Spatially-Periodic Retardation and Velocity Field," *Water Resources Research*, **28**(6), 1517-1529 (1992).
- Chrysikopoulos, C.V., L. M. Hildemann, and P. V. Roberts, "A Three-Dimensional Atmospheric Dispersion-Deposition Model for Emissions from a Ground-Level Area Source," *Atmospheric Environment*, **26A**(5), 747-757 (1992).
- Cozza, C.L. and S.L. Woods, "Reductive Dechlorination Pathways for Substituted Benzenes: a Correlation with Electronic Properties," *Biodegradation*, **2**, 265-278 (1992).
- Craig, A.M., C. J., Latham, L. L. Blythe, W. B. Schmotzer, and O. A. O'Connor, "Metabolism of Toxic Pyrrolizidine Alkaloids from Tansy Ragwort, *Senecio jacobaea*, in Ovine Ruminant Fluid Under Anaerobic Conditions," *Appl. Environ. Microbiol.* **58**(9) 2730-2736 (1992).
- Dykaar, C. B., and P. K. Kitanidis, "Determination of Effective Hydraulic Conductivity in Heterogeneous Porous Media by the Numerical Spectral Method: 1. Theory," *Water Resources Research*, **28**(4), 1155-1166 (1992).
- Dykaar, C. B., and P. K. Kitanidis, "Determination of Effective Hydraulic Conductivity in Heterogeneous Porous Media by the Numerical Spectral Method: 2. Applications," *Water Resources Research*, **28**(4), 1167-1178 (1992).

- Edwards, E.A., and D. Grbić -Galić , "Complete Mineralization of Benzene by Aquifer Microorganisms under Strictly Anaerobic Conditions," *Appl. Environ. Microbiol.* **58**(8): 2663-2666 (1992).
- Edwards, E.A., L.E. Wills, M. Reinhard, and D Grbić -Galić , "Anaerobic Degradation of Toluene and Xylene by Aquifer Microorganisms under Sulfate-Reducing Conditions," *Appl. Environ. Microbiol.* **58**(3): 794-800 (1992).
- Gailey, R. M., A. S. Crowe, and S. M. Gorelick, "Coupled Process Parameter Estimation and Prediction Uncertainty Using Hydraulic Head and Concentration Data," *Advances in Water Resources*, **14**(5) (1991).
- Grbić -Galić , D. "Anaerobic Transformation of Aromatic Hydrocarbon Pollutants under Fermentative/Methanogenic Conditions," *Periodicum Biologorum* **93**(4): 533-546 (1991).
- Gvirtzman, H., and P. V. Roberts, "Pore-Scale Spatial Analysis of Two Immiscible Fluids in Porous Media," *Water Resources Research*, **27**(6), 1165-1176 (1991).
- Harmon, T. C., L. Semprini, and P. V. Roberts, "Simulating Groundwater Solute Transport Using Laboratory-based Determined Sorption Parameters," *J. Environmental Engineering*," **118**(5), 666-689 (1992).
- Kitanidis, P. K., "Analysis of Macrodispersion through Volume-Averaging: Moment Equations," *Stochastic Hydrology and Hydraulics*, **6**, 5-25 (1992).
- Nicholson, D.K., S.L. Woods, J.D. Istok, and D.C. Peek, "Reductive Dechlorination of Chlorophenols by a Pentachlorophenol-Acclimated Methanogenic Consortium," *App. Environ. Microbiol.* **58**(7): 2280-2286 (1992).
- Semprini, L., Hopkins, G. D., Roberts, P. V., and McCarty, P. L., "Pilot Scale Field Studies of *In-Situ* Bioremediation of Chlorinated Solvents," *Journal of Hazardous Materials*, **32**, 145-162 (1992).

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

- Alvarez-Cohen, L. and McCarty, P. L., "Sorption of Trichloroethylene onto a Zeolite Accompanied by Methanotrophic Biotransformation," submitted for publication (1991).
- Anderson, J. E. and McCarty, P.L., "A Model of Treatment of Trichloroethylene by Methanotrophic Biofilms," submitted (1992).
- Azizian, M. F. and P. O. Nelson, "Hexavalent Chromium Adsorption and Desorption in Natural Soils," *Iranian Journal of Science* (in press, 1992).
- Bae, J., Semprini, L., and McCarty, P. L., "Down-Well Apparatus for Adding Oxygen and Methane into a Contaminated Aquifer for Bioremediation," submitted (1992).

- Dolan, M. E. and McCarty, P. L., "Chloroethene Transformation Capacities Using A Mixed Methanotrophic Culture," submitted (1992).
- Fry, V. A., Istok, J. D., and Guenther, R. B., "An analytical solution to the solute transport equation with rate-limited desorption and decay," submitted (1992).
- Gailey, R. M., and S. M. Gorelick, "Design of Optimal, Reliable Plume Capture Schemes: Application to the Gloucester Landfill Groundwater Contamination Problem," (accepted for publication, *Ground Water*, (1993).

- Henrysson, T. and McCarty, P. L., "Influence of the Endogenous Storage Lipid Poly- $\beta$ -hydroxybutyrate on the Reducing Power Availability During Cometabolism of Trichloroethylene and Naphthalene by Resting Methanotrophic Mixed Cultures," submitted (1992).
- Hopkins, G. D., Semprini, L., and McCarty, P. L., "Microcosms and In-Situ Field Studies of Enhanced Biotransformation of Trichloroethylene by Phenol-Utilizing Microorganisms," submitted (1992).
- McCarty, P. L. and Reinhard, M., "Biological and Chemical Transformations of Halogenated Aliphatic Compounds in Aquatic and Terrestrial Environments," *The Biogeochemistry of Global Change: Radiative Trace Gases*, Ed. R. S. Oremland, Chapman & Hall, Inc., New York (in press).
- Semprini, L. G. D. Hopkins, P.V. Roberts, and P.L. McCarty, "Pilot Scale Studies of In-situ Bioremediation of Chlorinated Solvents," *Journal of Hazardous Materials* (in press).
- Tiedeman, C. and Gorelick, S. M., "Analysis of Uncertainty in Optimal Groundwater Contaminant Capture Design," submitted (1992).
- Wachenheim, D.E., Blythe, L. L., Craig, A. M. 1992. "Characterization of ruminal pyrrolizidine alkaloid biotransformation in ruminants of various species," *Vet. and Human Toxicology* (in press).
- Wachenheim, D.E., Blythe, L. L., Craig, A. M. 1992. Effects of antibacterial agents on in vitro ovine ruminal biodegradation of the hepatotoxic pyrrolizidine alkaloid, jacobine. *Appl. Environ. Microbiol.* (in press).

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

- Azizian, M. F. and P. O. Nelson, "Hexavalent Chromium Adsorption and Reduction in Natural Soils", *Research Symposia Proceedings*, Water Environment Federation 65th Annual Conference, New Orleans, La., 241-247 (1992).
- Dawson, K. J., and J. D. Istok, *Aquifer Testing: Design and Analysis of Pumping and Slug Tests*, Lewis Publishers, Inc., Chelsea, Michigan, (1991).
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