



**WESTERN REGION
HAZARDOUS SUBSTANCE RESEARCH CENTER**

Stanford University
Oregon State University

FINAL REPORT

for the period

1989 to 2003

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**WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER
FINAL REPORT FOR THE PERIOD 1989-2003**

TABLE OF CONTENTS

	Page
INTRODUCTION	1
ADMINISTRATION	1
THE CENTER AT A GLANCE	1
RESEARCH AND DEMONSTRATION PROJECT SUMMARY	8
RESEARCH AND DEMONSTRATION PROJECT DESCRIPTIONS	16
TRAINING AND TECHNOLOGY TRANSFER	213
PUBLICATIONS	226

WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER FINAL REPORT (1989-2003)

INTRODUCTION

The Western Region Hazardous Substance Research Center was formed competitively in 1989 by the U.S. Environmental Protection Agency as one of five national hazardous substance research centers to conduct research and training on hazardous chemicals in the environment. The WRHSRC has been comprised of researchers from Stanford University as the lead university and Oregon State University. A recompetition for the establishment of a new set of HSRCs was completed in 2001 by EPA, and the WRHSRC has again been selected to continue its research and training activities, but Oregon State University is now the lead university. This report represents a summary of all the research, demonstration projects, and training and technology transfer activities undertaken since the WRHSRC was established in 1989 through July 2003, with Stanford University as the lead university. During this time there was a total of 96 research and demonstration projects conducted under the direction of 24 faculty members from the two universities who served as principal investigators since the Center's inception, with 21 serving over the last six years of the current grant. Over the fourteen plus years at since it began with Stanford University as the lead, the WRHSRC has been supported through three different grants from EPA, the last grant was awarded in August 1998. This report covers all activities conducted under all three grants, but serves as the final report for the last grant.

This report begins with a brief overview of the WRHSRC. This is followed by a listing of the different research and demonstration projects conducted since the Centers inception. Each project is then briefly summarized. This summary includes the names of the principal investigators, the project period and funding level, and the goals, rationale, approach, status of each project, and a summary of project publications. This is followed by a summary of activities under the training and technnology transfer function of the Center. The last item in this report is a listing of the project publications , including those still in press.

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THE CENTER AT A GLANCE

The Western Region Hazardous Substance Research Center (WRHSRC) is a cooperative activity between Stanford University and Oregon State University that was established in February 1989 to address critical hazardous substance problems in EPA Regions 9 and 10. The Regions include the states of Alaska, Arizona, California, Hawaii, Idaho, Nevada, Oregon, and Washington, and

Guam. The Center receives its base financial support from the U.S. Environmental Protection Agency, but also is supported through grants, contracts, and gifts from other federal agencies, states, municipalities, consultants, and industry.

The objectives of the Center are:

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

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

The 21 faculty and 3 staff who have directed the Center's research, training, and technology transfer activities over the last 5 years are listed in Table 1. They collectively represent an integrated research team representing five different schools (civil engineering, engineering, science, earth sciences, medicine, and veterinary medicine), and many different disciplines (chemical engineering, chemistry, hydrogeology, hydrology, medicine, microbiology, and petroleum engineering). Perry L. McCarty has been Director of the overall Center and of the research program. Kenneth J. Williamson has served as Associate Director in charge of training and technology transfer and coordinator of the Center's overall activities in Oregon. Martin Reinhard, the Assistant Director, has been in charge of the Center's analytical program.

The Center has had two major advisory groups to guide its activities. The Science Advisory Committee (SAC) with oversight for all Center research activities, and the Training and Technology Transfer Advisory Committee (TTTAC) with oversight of the Center's training and technology transfer activities. The members of the SAC and TTTAC who have advised the WRHSRC are listed in Tables 2 and 3, respectively. They represent federal and state governments, industry, consulting firms, and universities. The Center overall budget since the Center's inception is listed by category of support in Table 4. Of the \$31,731,334 spent over fourteen years of activity, only 40% has come from the core EPA funds provided for Center support. The other 60% came as a result of leveraging the core funds to win matching funds from other organizations and agencies. The education of students interested in careers directed toward finding solutions to environmental problems is another important goal. The number of students supported through WRHSRC funds is listed in Table 5.

Since 1989 the WRHSRC has conducted 95 separate research and demonstration projects. As a result of these activities, the WRHSRC has produced 212 peer-reviewed journal articles, 4 books, 33 book chapters, 33 project reports, 23 extended abstracts, 74 theses and dissertations, and has had 5 patents issued to its researchers. Through its training and technology transfer program, the Center has sponsored and/or organized sessions at 39 different conferences, has provided outreach services for 29 communities with hazardous waste problem concerns, and has aided 4 communities with their brownfields programs. The WRHSRC has thus been an active and highly productive organization.

Table 1. KEY PERSONNEL AT THE WRHSRC

<u>Stanford University</u>	<u>Oregon State University</u>
Prof. Martin Blunt	Prof. Daniel J. Arp
Prof. Mark N. Goltz ^a	Prof. Lynda Ciuffetti
Prof. Steven M. Gorelick	Prof. A. Morrie Craig
Prof. Lynn M. Hildemann	Prof. Michael R. Hyman ^b
Mr. Gary D. Hopkins	Prof. James D. Ingle
Prof. Peter K. Kitanidis	Prof. Jonathan D. Istok
Prof. James O. Leckie	Prof. Peter O. Nelson
Prof. Keith Loague	Prof. John S. Selker
Prof. Abdul Matin	Prof. Lewis Semprini
Prof. Perry L. McCarty	Prof. John C. Westall
Prof. Martin Reinhard	Prof. Kenneth J. Williamson
Prof. Paul V. Roberts	Prof. Sandra L. Woods
Prof. Alfred M. Spormann	

^aCurrently with the Air Force Institute of Technology, Dayton, Ohio, but has remained active with the WRHSRC

^bCurrently with North Carolina State University, but remained active with the WRHSRC.

Table 2. SCIENCE ADVISORY COMMITTEE MEMBERS SINCE 1989

<u>Member</u>	<u>Affiliation</u>	<u>Expertise</u>
Linda M. Abriola	University of Michigan	Physical Processes, Hydrology
John J. Barich	Govt/EPA, Region 10	Environmental Engineering
Douglas R. Christensen	Jacobs Engineering	Engineering
John Conomos	Govt/U.S. Geological Survey	Hydrology
Frank Deaver	Industry, Tektronix	Electronics
David E. Ellis	Du Pont Chemicals	Chemical/Biological Processes
John F. Ferguson	University of Washington	Biological Processes
David Frank	Govt/EPA, Region 10	Hydrogeology
John Glaser	Govt/EPA, Cincinnati Lab	Physical/Chemical Processes
Ronald Hoeppel	Govt/U.S. Navy	Microbiology
Michael C. Kavanaugh*	Malcolm Pirnie	Physical/Chemical Processes
Richard G. Luthy	Carnegie Mellon University	Chemical/Biological Processes
Stephen Schmelling	Govt/EPA, Kerr Laboratory	Groundwater Remediation
Garrison Sposito	University of California, Berkeley	Soil Science
James M. Tiedje	Michigan State University	Microbiology
William A. Wallace	CH2M-Hill, Consulting	Design
John L. Wilson†	New Mexico Technical University	Hydrology

John T. Wilson
John Wise

Govt/EPA, Kerr Laboratory
Govt/EPA, Region 9

Microbiology
Planning

*Current Chairman

†Current Vice Chairman

Table 3. TRAINING AND TECHNOLOGY TRANSFER ADVISORY COMMITTEE MEMBERS SINCE 1989

<u>Member</u>	<u>Affiliation</u>	<u>Expertise</u>
James T. Allen	Govt/ California	Treatment Technology
Michael Anderson	Govt/Oregon	Hydrology
Harry A. Ball	Govt/EPA, Region 9	Environmental Engineering
John J. Barich	Govt/EPA, Region 10	Regulations
Kenneth Bigos	Govt/EPA, Region 9	Air Pollution
Douglas R. Christensen	Jacobs Engineering	Engineering
Lynn Coleman	Govt/Washington	Regulations
Robert Courson	Govt/EPA, Region 10	Planning
Sandy Gurkewitz	Govt/Oregon	Environmental Quality
Ethelwyn Hoffman	Govt/EPA, Region 9	Training
Jerry Jones	Govt/EPA, Kerr Laboratory	Groundwater Remediation
David Kennedy*	Kennedy/Jenks Consultants	Treatment Technology
Jon Kindschy	Univ. California Ext. Service	Hazardous Substances Training
Mary Masters	Industry, Hewlett-Packard	Environmental Engineering
James McNabb	Govt/EPA, Kerr Laboratory	Groundwater Remediation
Gregory Peterson	Peterson Process Engineering	Treatment Technology
Mary E. Peterson	Govt/DOE-Industry	Treatment Technology
David Rozell	Govt/Oregon DEQ	Environmental Quality
M. R. Scalf	Govt/EPA, Kerr Laboratory	Groundwater Remediation
Jack Stanton	Govt/EPA, Washington D.C.	Training/Tech Transfer
Kenneth Sutherland	Industry, Hewlett-Packard	Treatment Technology
Winona Victory	Govt/EPA, Region 9	Science Advisory

*Current Chairman

Table 4. CENTER FUNDING FROM 1989 TO 2003

<u>Funding Sources</u>	<u>Funds</u>
EPA: Centers Program	\$12,652,015
EPA: Other	\$4,477,816
Other Govt: Federal†	\$8,261,195
Other Govt: State@	\$48,252
Consortium	\$3,747,240
Private Sector#	<u>\$2,544,816</u>
TOTAL	\$31,731,334

† Department of Energy; Department of Navy, U.S. Air Force, Strategic Environmental Research and Development Program

@ Oregon Department of Transportation

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

TABLE 5. STUDENT SUPPORT SINCE 1989

<u>Student Support</u>	<u>Number*</u>	<u>Funds †</u>
B.S.	13	\$95,877
M.S.	38	\$1,037,718
Ph.D.	128	\$6,257,086
Post Doctoral	<u>27</u>	<u>\$1,591,748</u>
TOTAL	204	\$8,982,429

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

† Includes tuition, travel, supplies, etc.

WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER RESEARCH AND DEMONSTRATION PROGRAM SUMMARY

The table below contains summaries of research and demonstration projects supported by the WRHSRC from the time of its inception in 1989 through July 2003. Following the summary is a description of each project and its goals, rationale, approach, and accomplishments.

RESEARCH PROGRAM SUMMARY

Project Number and Title	Principal Investigators	Project Dates	Total Budget
OS89-01. Interactions Between Electron Acceptors in the Treatment of Wastewaters Containing Sulfate, Chlorophenols, and Acetate	Sandra L. Woods	1989-1992	\$138,920
OS89-02. Enhancing Biodegradation with Sorption and Alternating Aerobic/Anaerobic Environments	Kenneth J. Williamson, Peter O. Nelson	1989-1992	\$167,825
OS89-03. Development and Verification of a Numerical Model to Predict the Fate and Transport of Chlorinated Phenols in Groundwater	Jonathan D. Istok, Sandra L. Woods	1989-1992	\$165,838
OS90-01. Redox Transformations of Inorganic Pollutants: Coupling to the Biogeochemical Matrix	John Westall James Ingle	1992-1995	\$197,455
OS90-04. Hexavalent Chromium Sorption and Desorption in Natural Soils and Subsoils	Peter O. Nelson, Jonathan D. Istok	1990-1992	\$165,838
OS90-05. Biotransformation of Ordnance Wastes Using Unique Consortia of Anaerobic Bacteria	A. Morrie Craig	1990-1994	\$244,296
OS91-02. The Effect of Environmental Conditions on Reductive Dechlorination Rates	Sandra Woods	1992-1995	\$194,248
OS91-03. Evaluation of Effect of In-Situ Biodegradation for BTEX Isomers in Large-Scale Physical Aquifer Models	Jonathan Istok	1992-1993	\$20,459
OS91-04. Lead Sorption, Transport, and Remediation in Natural Soils and Subsoils	Peter O. Nelson	1992-1995	\$101,260
OS91-07. Degradation of Chlorinated Aliphatic Compounds by Nitrifying Bacteria	Daniel Arp Kenneth Williamson Michael Hyman	1992-1995	\$249,786
OS93-01. Remediation of Contaminated Soil from the Baldock Station Maintenance Facility	Kenneth J. Williamson Sandra Woods	1994	\$40,200

OS94-01. The Effect of Apparent EH, Compound Structure, and Electron Donor on Anaerobic Biotransformation of Trinitrotoluene and its Metabolites	Sandra Woods A. Morrie Craig Kenneth J. Williamson	1995-1997	\$126,434
OS94-02. The "Bubble Wall": A Passive In Situ System for Treatment and/or Containment of Contaminated Groundwater	J.D. Istok V.A. Fry J.S. Selker S.M. Gorelick	1995-1997	\$297,685
OS94-03. Inhibition, Inactivation and Recovery: A Universal Model for Aerobic Cometabolic Degradation of Aliphatic Compounds	Daniel Arp Michael Hyman Kenneth J. Williamson	1995-1997	\$168,097
OS94-04. Development, Characterization, and Performance Evaluation of Ferrous-Ferric Oxide Adsorbents for Metal Removal from Contaminated Groundwater	Peter O. Nelson	1995-1997	\$111,668
OS94-06. Redox Transformations of Organic and Inorganic Contaminants in the Subsurface Environment	John C. Westall and James D. Ingle	1995-1997	\$117,804
OS95-01. Demonstration of an Interceptor Trench Technology for the Bioremediation of a Pentachlorophenol-Contaminated Ground Water	Sandra Woods Kenneth J. Williamson	1997-2001	\$412,377
OS95-04. Development of a Vitamin B ₁₂ -Amended Bioremediation Process for the Reductive Dechlorination of Chlorobiphenyls at all Chlorine Positions	Sandra Woods	1995-1997	\$144,357
OS95-07. An Investigation of the Chlorinated Hydrocarbon Substrate Range of the Filamentous Fungus, <i>Graphium</i> sp.	Michael Hyman Lynda Ciuffetti	1995-1997	\$24,045
OS95-08. Aerobic Cometabolism of Chloroform, 1,1,1-trichloroethane, 1,1-dichloroethylene, and Other Chlorinated Aliphatic Hydrocarbons by Microbes Grown on Butane and Propane	Lewis Semprini Daniel J. Arp	1995-2001	\$408,690
OS97-03. Cytochrome P-450: An Emerging Catalyst for the Co-metabolism of Chlorinated Aliphatic Hydrocarbons and Methyl tery-Butyl Ether?	Michael Hyman Lynda Ciuffetti	1997-2000	\$82,342
OS97-04. In Situ Measurement of TCE Degradation Using a Single-Well, "Push-Pull" Test	Jack Istok, Michael Hyman, Lewis Semprini	1997-2000	\$39,037
OS97-06. Development and Characterization of Redox Sensors for Environmental Monitoring	James Ingle	1997-2000	\$131,865

OS97-09. Assessing Metal Speciation in the Subsurface Environment	John C. Westall	1997-2000	\$107,574
OS97-10. Simultaneous Removal of the Adsorbable and Electroactive Metals from Contaminated Soils and Groundwater	Peter O. Nelson	1997-2000	\$116,591
OS99-01. Multisolute Sorption and Transport Model for Copper, Chromium, and Arsenic Sorption on an Iron-Coated Sand, Synthetic Groundwater System	Peter O. Nelson	1999-2001	\$114,725
OS99-03. Development of Alkoxysilanes as Slow Release Substrates for the Anaerobic and Anaerobic/Aerobic Transformation of Chlorinated Solvents	Lewis Semprini	1999-2001	\$104,903
OS99-04. Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbons by Toluene-Oxidizing Bacteria: Physiological Consequences and Adaptive Responses	Daniel J. Arp Peter J. Bottomley	1999-2001	\$86,455
OS99-05. Development and Characterization of Sensors and Field Instrumentation for the Monitoring of Environmental Redox Conditions	James Ingle	1999-2001	\$114,130
OS99-06. Aerobic Cometabolism of Methyl tert-butyl Ether by Microorganisms Grown on Aliphatic Hydrocarbons	Kenneth J. Williamson Lynda Ciuffetti	1999-2001	\$93,545
OS99-07. Assessing Metal Speciation in the Subsurface Environment - Effect of Wet-Dry Cycles in the Vadose Zone	John C. Westall	1999-2001	
R95-04. Biotransformation of Lead and Chromate by Bacteria	Robert C. Blake Xavier University	1995-1997	\$115,000
R95-11. Magnetic Resonance Studies of Heavy Metals in Clays, Zeolites and Ceramics	Cynthia J. Hartzell Michael P. Eastman Northern Arizona University	1995-1997	\$79,658
R95-14. Probing the Redox Properties of Environmental Systems: Natural Phenolic Materials	Robin Helburn Northern Arizona University	1995-1997	\$70,357
R95-15. Reductive Dehalogenation at Carbon and Derivatized Carbon Electrodes	Merritt Helvenston New Mexico Highlands University	1995-1997	\$149,394

R95-24. Detection of Microorganisms Capable of Anaerobic Degradation of Hazardous Substances in Natural Environments	Sara E. Silverstone California St. Univ., Bakersfield/State U. of New York	1995-1997	\$111,405
SU89-01. Treatment of Complex Mixtures	Martin Reinhard Paul Roberts	1989-1993	\$285,178
SU89-02. Oxidation of Chlorinated Solvents by Methanotrophs	Perry L. McCarty Paul V. Roberts	1989-1993	\$344,816
SU89-03. Detection and Assessment of Subsurface Contamination	Peter K. Kitanidis	1989-1992	\$195,187
SU89-04. Design of Reliable and Cost-Effective Mitigation Schemes	Steven M. Gorelick	1989-1992	\$181,088
SU89-06. Gaseous Stripping of Nonaqueous Phase Liquids from the Vadose Zone	Martin Reinhard Paul Roberts	1989-1992	\$205,280
SU89-07. Anaerobic Microbial Transformation of Homocyclic & Heterocyclic Polynuclear Aromatic Hydrocarbons	Dunja Gribic-Galic	1989-1992	\$189,936
SU89-08. Effects of Sorption on Biodegradation of Halogenated Organics	Perry L. McCarty & Paul V. Roberts	1989-1992	\$210,789
SU89-09. Trace Metal Removal Processes	James O. Leckie	1989-1992	\$211,915
SU89-13. FASTCHEM, Applications and Sensitivity Analysis	Kitanidis Freyberg	1989-1991	\$259,816
SU89-14. Long-term Chemical Transformation of 1,1,1-Trichloroethane (TCA) and Freon 113 under Aquifer Conditions	Martin Reinhard and Perry McCarty	1989-1993	\$180,691
SU89-15. In-Situ Anaerobic Biological Treatment of Aromatics in Groundwater	Martin Reinhard Gary D. Hopkins Alfred M. Spormann	1989-1996	\$1,021,875
SU90-01. Use of Starvation and Stress Promoters for Biodegradation of Hazardous Wastes	A. C. Matin Dunja Gribic-Galic	1990-1992	\$155,619
SU90-02. Determining and Modeling Diffusion-Limited Sorption and Desorption Rates of Organic Contaminants in Heterogeneous Soils	Paul V. Roberts	1990-1992	\$154,823
SU90-03. Dispersion Modeling of Volatile Organic Emissions from Ground-Level Treatment Systems	Lynn M. Hildemann Paul Roberts	1990-1993	\$177,829
SU90-05. Subsurface Mixing of Nutrients and Groundwater for in-Situ Bioremediation	Perry L. McCarty Peter K. Kitanidis Paul V. Roberts Lewis Semprini	1990-1994	\$583,873

SU90-06. Test-Bed Evaluation of In-Situ Bioremediation of Chlorinated Aliphatic Compounds by Toluene Oxygenase Microorganisms	Perry L. McCarty Gary D. Hopkins Lewis Semprini	1990-1994	\$583,873
SU90-07. Demonstration of In-Situ Bioremediation of Chlorinated Aliphatics by Methanotrophs at St. Joseph	Perry L. McCarty Paul V. Roberts Steven M. Gorelick Peter K. Kitanidis Lewis Semprini	1990-1992	\$200,000
SU91-02. Aquifer Remediation Design in the Presence of Kinetic Limitations	Steven Gorelick	1992-1996	\$266,903
SU91-03. Determination of Macroscopic Transport Parameters for Biologically Reacting Solutes in Aquifers	Peter K. Kitanidis	1992-1995	\$251,711
SU91-04. Transformation of Chlorinated Hydrocarbons by Reduced Metallocoenzymes—Kinetic Model Development and Applications to Environmental Systems	Martin Reinhard	1992-1995	\$222,414
SU91-05. Microbial Degradation of Toluene under Sulfate-Reducing Conditions - The Role of Iron	Dunja Gribic-Galic Martin Reinhard	1992-1995	\$256,438
SU91-07. Transformation of TCE by Methanotrophic Biofilms	Perry L. McCarty	1992-1995	\$123,272
SU91-09. Heavy Metals in Ceramic Matrix: Heavy Metal/Clay Interactions in Ceramic Processing	James O. Leckie	1993-1998	\$294,450
SU91-11. Radon-222 Method for Locating and Quantifying Contamination by Residual Non-Aqueous Phase Liquids in the Subsurface	Lewis Semprini	1992-1995	\$143,079
SU92-01. Process Submodel Formulation and Parameter Estimation for Simulation of Bioremediation	Paul V. Roberts	1992-1994	\$199,625
SU92-02. Enhancement of Biodegradation Through the Use of Substituted Porphyrins to Treat Groundwater Contaminated with Halogenated Aliphatics	Martin Reinhard Kerry Sublette Michael McInerney Joseph Suflita Ralph Tanner	1993-1995	\$299,999
SU92-04. Field Test of In-Situ Vapor Stripping for Removal of VOCs from Groundwater	Steven M. Gorelick	1993-1998	\$641,709
SU92-05. System Design for Enhanced In-Situ Biotransformation of Carbon Tetrachloride: Application to DOE's Arid Site Integrated Demonstration	Peter K. Kitanidis Paul Roberts Lewis Semprini	1993-1995	\$318,291

SU92-07. Modeling Strategies for Optimizing In-Situ Bioremediation	Peter K. Kitanidis Paul Roberts Lewis Semprini	1993-1994	\$243,001
SU92-08. Anaerobic Treatment of Chlorinated Solvent Contaminated Groundwater	Perry L. McCarty	1992-1994	\$125,000
SU93-01. In Situ Treatment of Chlorinated Solvents	Perry L. McCarty	1993-1996	\$225,000
SU93-06. Moffett Field In-Situ Bioremediation Study in Support of Full-Scale Evaluation Application	Perry L. McCarty Gary Hopkins Mark Goltz	1993-1995	\$394,834
SU93-07. Full-Scale Evaluation of <i>In Situ</i> Bioremediation of Chlorinated Solvent Groundwater Contamination	Perry L. McCarty Mark N. Goltz Gary D. Hopkins	1995-1998	\$611,061
SU94-02. Upscaling Pore-Scale Hydrodynamics and the Transport of Reactive Solutes	Peter K. Kitanidis	1995-1998	\$159,709
SU94-03. Pathways of Anaerobic Toluene Metabolism by a Sulfate-Reducing Bacterium, Strain PRTOL1	Alfred M. Spormann	1995-1998	\$189,616
SU94-04. Anaerobic Ethylbenzene Oxidation in Denitrifying Strain EB1	Alfred M. Spormann	1995-1998	\$151,585
SU94-05. Molecular Approaches to Optimize Starvation Promoter Driven TCE Bioremediation in <i>Pseudomonas</i>	A. C. Matin	1995-1997	\$114,721
SU94-06. Modeling VOC Emissions from Hazardous Waste Sites	Lynn M. Hildemann	1995-1998	\$166,507
SU94-07. Reductive Transformation of Chlorinated Hydrocarbons by Reduced Ethenes Catalyzed by Vitamin B ₁₂ -- Mechanistic and Kinetic Studies	Martin Reinhard	1995-2000	\$252,142
SU94-08. Evaluation of Strategies for Full Scale Bioremediation of the Seal Beach Site Using Anaerobic Microbial Processes	Martin Reinhard Peter K. Kitanidis	1994-1998	\$106,250
SU94-09. Trace Element Adsorption in Porous Particle Packed Beds	James O. Leckie	1995-2000	\$108,070
SU94-10. Hydrologic and Biological Factors Affecting Aquifer Clogging During In-Situ Bioremediation	Perry L. McCarty	1995-1998	\$190,883
SU95-01. Full-Scale Evaluation of an Apparatus for Down-well Oxygen Transfer to Implement <i>In situ</i> Bioremediation at Edwards AFB	Perry L. McCarty Mark N. Goltz	1995-1998	\$128,274
SU95-02. Field Testing of Palladium-Catalyzed Hydrodehalogenation for Chlorinated Hydrocarbon Removal from Groundwater	Martin Reinhard Paul V. Roberts	1995-2000	\$407,427

SU95-03. Physics of Dissolution of Nonaqueous Phase Liquids: Pore Networks and Field Simulations	Steven M. Gorelick	1995-1998	\$159,610
SU95-04. Three-Phase Flow in Fractured Media	Martin Blunt Paul Roberts	1995-1998	\$270,742
SU95-05. Effects of Redox Zones on the Fate and Transport of Contaminants in the Saturated Subsurface; Characterization and Simulation	Keith Loague	1995-1998	\$121,422
SU95-07. Biochemical Mechanisms of PCE Dehalogenation by Strain MS-1, and its Potential for In-Situ Bioaugmentation	Pramod K. Sharma Perry L. McCarty	1995-1998	\$270,300
SU96-01. A Large Scale Model for Anaerobic Bioremediation at the Seal Beach Site	Martin Reinhard Peter K. Kitanidis	1995-1998	\$225,000
SU96-02. Mechanisms, Chemistry, and Kinetics of Anaerobic Biodegradation of cDCE and Vinyl Chloride	Perry L. McCarty Alfred Spormann	1996-2003	\$860,000
SU97-03. Bioenhanced In-Well Vapor Stripping to Treat Trichloroethylene (TCE)	Mark N. Goltz Perry L. McCarty Steve M. Gorelick Gary D. Hopkins	1997-2002	\$950,000
SU97-04. Effect of Chemical Structure on the Biodegradability of Halogenated Hydrocarbons	Paul V. Roberts	1997-2001	\$71,879
SU97-07. Trace Element Adsorption in Porous Particle Packed Beds	James O. Leckie	1997-2000	\$108,070
SU99-05. Arsenic Removal in High Capacity Porous Alumina Packed-Bed Reactors	James O. Leckie	1999-2001	\$143,770
SU99-06. Measurement of Interfacial Areas and Mass Transfer Coefficients between residual PCE and Water During Surfactant Enhanced Aquifer Remediation: Laboratory Studies and Models	Paul Roberts	1999-2001	\$127,169
SU99-07. Proof of Gene Expression During Bioaugmentation	Craig C. Criddle	1999-2001	\$143,770
SU99-09. Experimental and Mathematical Study of Biomass Growth in Pore Networks and its Consequences in Bioremediation	Peter Kitanidis Perry McCarty	1999-2001	\$143,770
SU99-11. Gene Probes for Detecting Anaerobic Alkylbenzene-Degrading Bacteria	Alfred M. Spormann	1999-2001	\$143,770

SU99-13. Investigation of Palladium Catalyzed Hydrodehalogenation for the Removal of Chlorinated Groundwater Contaminants: Surface Chemistry of Catalyst Deactivation and Regeneration	Martin Reinhard	1999-2001	\$143,770
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**WESTERN REGION HAZARDOUS SUBSTANCE RESEARCH CENTER
RESEARCH AND DEMONSTRATION PROJECT DESCRIPTIONS
FROM 1989 TO 2003**

**Western Region Hazardous Substance Research Center Project
OS89-01. Interactions between Electron Acceptors in the Treatment of Wastewaters
Containing Sulfate, Chlorophenols and Acetate: Sandra L. Woods, Oregon State
University**

EPA Project Officer: Dale Manty

Project Period: 1989-1992

Project Amount: \$138,920

Research Category: Groundwater, chlorinated organics, PCP, biodegradation

Goal: The goal is to develop and verify a mathematical model for anaerobic biotransformations in the presence of competing electron acceptors, and to measure process kinetic constants. Electron acceptor interactions between chlorophenols and their dechlorinated metabolites and interactions between chlorophenols and sulfate are examined.

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 is evaluated in the presence of varying quantities of alternate electron acceptors (metabolic products and sulfate).

Approach: Organisms acclimated to pentachlorophenol, or pentachlorophenol and sulfate, are grown in continuous-flow anaerobic reactors. The reactors are allowed to reach steady state and then used as a source of organisms for subsequent batch experiments. Progress curves for chlorophenol, sulfate, and acetate degradation are generated from the batch experiments. From the results the effect of alternate electron acceptors on the rate of chlorophenol biotransformation are determined.

Status: With acclimation pentachlorophenol is biotransformed by concurrent reductive dechlorination reactions occurring at the ortho, meta, and para positions. Each of the three possible tetrachlorophenols appears as an intermediate, as well as four trichlorophenols and three dichlorophenols. The rate of reductive dechlorination is fastest for the more highly chlorinated congeners. Although reductive dechlorination at the ortho position is observed most frequently, para dechlorination is often more rapid. Additional studies have shown the dependence of reductive dechlorination rates on the apparent oxidation/reduction potential of the system. Rates increase with decreasing oxidation/reduction potential, and reductive dechlorination of pentachlorophenol has been shown to occur at high sulfate concentrations when the oxidation/reduction potential is controlled.

Project Publications:

Cozza, C.L. and S.L. Woods, "Reductive Dechlorination Pathways for Substituted Benzenes: a Correlation with Electronic Properties," *Biodegradation*, **2**, 265-278 (1992).

- Cozza, C.L., *A Model for the Prediction of Biologically Mediated Reductive Dechlorination Pathways*, M.S. Thesis, Oregon State University, Corvallis, OR (1990).
- Nicholson, D.K., *Measurement of the Rates of Reductive Dechlorination of Chlorinated Phenols*, M.S. Thesis, Oregon State University, Corvallis, OR (1990).
- Nicholson, D.K., Woods, S.L., Istok, J.D., and Peek, D.C., "Reductive Dechlorination of Chlorophenols by a Pentachlorophenol-Acclimated Methanogenic Consortium," *Applied and Environmental Microbiology*, **58**(7), 2280-2286 (1992).
- Saberian, A., *Kinetic Model of Pentachlorophenol Degradation by Microorganisms*, Engineering Project, Oregon State University (1991).
- Smith, M., *Reductive Dechlorination of Chlorophenols by Vitamin B₁₂*, Ph.D. Dissertation, Department of Civil Engineering, Oregon State University, Corvallis, OR (1993).

**Western Region Hazardous Substance Research Center Project
OS89-02. Enhancing Biodegradation with Sorption and Alternating Aerobic/Anaerobic
Environments: Kenneth J. Williamson, Peter O. Nelson, Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1989-1992

Project Amount: \$167,825

Research Category: Groundwater, chlorinated organics, treatment, PCP, biodegradation

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 to occur of toxic compounds by the 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, complete and rapid degradation may be possible.

Approach: Anaerobic and aerobic biofilms will be developed on GAC maintained in upflow expanded beds with liquid recycle. Acetate is used as the electron donor for the anaerobic reactor and the primary electron donor for the aerobic reactor. Pentachlorophenol is 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 will be moved from the anaerobic column to the aerobic column with a solids retention time of 30 days. The tri- and di-chlorophenols slowly desorb in the aerobic reactor and should undergo degradation by the aerobic biofilms.

Status: Experiments have been conducted to determine the degradation rates of pentachlorophenol and its degradative products under aerobic and anaerobic conditions. Adsorption isotherms and rates of adsorption and desorption have been determined for these same compounds. Near steady-state removals of pentachlorophenol and its metabolic products have been determined in the anaerobic and aerobic GAC columns under the 30-day solids retention time and 3-day hydraulic detention times in both the anaerobic and aerobic columns. 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. Project completed.

Project Publications:

Hindagolla, W., *A Mathematical Model to Predict Concentrations of Chlorophenols in a Biofilm Activated Carbon Reactor*, M.S. Thesis, Oregon State University, Corvallis, OR (1991).

- Lo, H., *Predicative Modeling of Dual-Solute Competitive Adsorption in Completely Stirred GAC Reactor*, M.S. Thesis, Oregon State University, Corvallis, OR (1991).
- Nelson, P.O., "Equilibrium Adsorption of Chlorophenols on Granular Activated Carbon," *Water Environment Research*, **67**, 892-898 (1995).
- Peters, D., *Anaerobic and Aerobic Treatment of Chlorinated Phenols with Granular Activated Carbon Expanded Bed Reactors*, M.S. Thesis, Oregon State University, Corvallis, OR (1991).
- Wang, G.M., *Aerobic Degradation of Chlorophenols*, M.S. Thesis, Department of Civil Engineering, Oregon State University, Corvallis, OR (1991).
- Yang, M., *Adsorption of Chlorophenols on Granular Activated Carbon*, Ph.D. Dissertation, Department of Civil Engineering, Oregon State University, Corvallis, OR (1994).

**Western Region Hazardous Substance Research Center Project
OS89-03. 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**

EPA Project Officer: Dale Manty

Project Period: 1989-1992

Project Amount: \$165,838

Research Category: Groundwater, chlorinated organics. Modeling, PCP

Goal: The objectives are to develop a numerical model to predict the fate and transport of pentachlorophenol and its primary anaerobic degradation products in field soils and groundwater aquifers, and to verify the model using laboratory and field experiments.

Rationale: Predictive models of pollutant transport are needed for site characterization and to design effective biological remediation strategies.

Approach: A mathematical model was developed based on the processes of advection, dispersion, diffusion, sorption, and anaerobic degradation for pentachlorophenol and its metabolites, an electron donor (acetate), and biomass. Monod kinetics and a "macroscopic bulk concentration" concept are used to describe degradation and growth. The resulting set of nonlinear differential equations are solved by the finite-difference method. Experiments are conducted using a Chehalis soil in batch reactors, columns, and in large soil tanks consisting of an aluminum box (2 m wide by 4 m long by 20 cm deep) supported by a steel framework. Dispersion coefficients and sorption parameters were measured in miscible displacement experiments in packed soil columns (30.0 cm long by 5.38 cm in diameter). Comparisons between predicted and measured effluent concentrations will be made during miscible displacement experiments in inoculated soil columns.

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. Project completed.

Project Publications:

Dawson, K.J. and J.D. Istok, *Aquifer Testing: Design and Analysis of Pumping and Slug Tests*, Lewis Publishers, Inc., Chelsea, Michigan, (1991).

Dawson, K.J., *Aquifer Testing: A Manual for the Design and Analysis of Pumping Tests*, M.S. Thesis, Department of Civil Engineering, Oregon State University, Corvallis, OR (1990).

- Fry, V.A., *Mass Transfer Constraints on the Feasibility of In Situ Bioremediation of Contaminated Groundwater*, Ph.D. Dissertation, Department of Civil Engineering, Oregon State University, Corvallis, OR (1994).
- Fry, V.A., J.D. Istok, and R.B. Guenther, "An analytical solution to the solute transport equation with rate-limited desorption and decay," *Water Resources Research*, **29**(9), 3201-3208 (1993).
- Lee, S.B., *A Numerical Model for the Fate and Transport of Chlorinated Phenols in Groundwater*, Ph.D. Dissertation, Department of Civil Engineering, Oregon State University, Corvallis, OR (1990).
- Peda, A., *Electron Data Retrieval: A Sourcebook for Environmental Professionals*, M.S. Project, Oregon State University, Corvallis, OR (1991).
- Schroth, M., *Multiphase Flow in Homogeneous and Bedded Porous Media*, Ph.D. Dissertation, Department of Civil, Construction, and Environmental Engineering, Oregon State University (1996).

**Western Region Hazardous Substance Research Center Project
OS90-01. Redox Transformations of Inorganic Pollutants: Coupling to the
Biogeochemical Matrix: John C. Westall and James D. Ingle, Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1992-1995

Project Amount: \$197,455

Research Category: Groundwater, chemistry, redox

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

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

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

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

similar to the "free" dissolved indicator and is reduced by components of a soil solution in the reactor with the potential measured at a Pt electrode falls below about 0 V and the concentration of Fe(II) is relatively high. Currently, the immobilized indicator is being studied in soil matrices in the laboratory in preparation for field applications.

Project Publications:

- Bos, M.C., *Part I: Development and Application of an Arsenic Speciation Technique Using Ion-Exchange Solid Phase Extraction Coupled with GFAAS; Part II: Investigation of Zing Amalgam as a Reductant*, M.S. Thesis, Department of Chemistry, Oregon State University, Corvallis, OR (1996).
- Lemmon, T.C., J.C. Westall, and J.D. Ingle, Jr., "Development of Redox Sensors for Environmental Applications Based on Immobilized Redox Indicators," *Analytical Chemistry*, **68**(6), 947-953 (1996).
- Lemmon, T.L., *Development of Chemostats and Use of Redox Indicators for Studying Redox Transformations in Biogeochemical Matrices*, Ph.D. Dissertation, Department of Chemistry, Oregon State University, Corvallis, OR (1995).
- Scherer, M.M., J.C. Westall, M. Ziomek-Moroz, and P.G. Tratnyek, "Kinetics of Carbon Tetrachloride Reduction at an Oxide-Free Iron Electrode," submitted 1996.

**Western Region Hazardous Substance Research Center Project
OS90-04. Hexavalent Chromium Sorption and Desorption in Natural Soils and Subsoils: Peter O. Nelson, Jonathan D. Istok, Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1990-1992

Project Amount: \$165,808

Research Category: Groundwater, chemistry, metals, chromium

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 (Fe_3O_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 CrO_4^{2-} and 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 (CrO_4^{2-}) and bichromate (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(III), or by desorption and extraction, which can be enhanced chemically by the addition of competing oxyanions.

Project Publications:

- Azizian, M. F., and Nelson, P. O., "Hexavalent Chromium Adsorption Kinetics and Equilibrium in a Natural Soil," *Iranian Journal of Science and Technology*, Vol. 17, No.2 pp. 81-89, 1993.
- Azizian, M.F., "Experimental Evaluation and Chemical Modeling of Hexavalent Chromium Adsorption, Desorption, and Reduction in a Natural Soil," Ph.D. Thesis, Department of Civil Engineering, Oregon State University: Corvallis, OR, 1993.
- Azizian, M.F., Nelson, P.O., "Chromium Adsorption and Reduction in Natural Soils," Center for Bioengineering and Pollution Control, University of Notre Dame, Notre Dame, Indiana, September 1-4, 1992.
- Azizian, M. F. and Nelson, P. O., "Hexavalent Chromium Adsorption and Reduction in Natural Soils," *Water Environmental Federation*, 65th Annual Conference, pp. 241-248, 1992.
- Nelson, P.O. and M.F. Azizian, "Hexavalent Chromium Adsorption and Desorption in Natural Soils and Subsoils," *Proceedings, Third International Iranian Congress of Civil Engineering*, Shiraz, Iran, 1990.

**Western Region Hazardous Substance Research Center Project
OS90-05. Biotransformation of Ordnance Wastes Using Unique Consortia of Anaerobic
Bacteria: A. Morrie Craig, Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1990-1994

Project Amount: \$244,296

Research Category: Groundwater, biodegradation, TNT

Goal: Trinitrotoluene (TNT) is a toxic munitions pollutant which contaminates more than 1400 U.S. military facilities. We would like to develop a rapid, complete biological method to remediate TNT-contaminated soils employing anaerobic microbes to be used in slurry bioreactors or for *in situ* addition of exogenous microbes derived from animal gut flora.

Rationale: Recent GC/MS experiments have identified aniline and other deaminated compounds as TNT biotransformation products. This evidence indicates that our biological systems rapidly degrade TNT and are able to remove nitrogen atoms off of the aromatic ring. The whole rumen fluid as well as isolate G.8 is able to reductively deaminate some aromatic compounds, and under some circumstances to de-methylate them. This suggests the presence of a reductive deaminase type enzyme.

Approach: Based on previous research results, our recent focus has been on two principal areas of investigation: 1) identification of the major metabolites of TNT produced in a whole rumen fluid incubation and 2) construction and operation of a laboratory-scale, flow-through bioreactor using ruminal and aerobic bacteria. Using the rumen from sheep and goats as a source of anaerobic microbes, we have discovered a consortium of bacteria, as well as a pure isolate (G.8), which are transforming TNT. Uniformly ring labelled ^{14}C -TNT has been used in incubations, metabolites of which were monitored using HPLC, TLC, and GC/MS. The main thrust has been to determine mass balance of TNT degradation and also to determine that no toxic intermediates are formed.

Status: The G.8 isolate was capable of reducing the TNT nitro group to an amino group and was also involved in deamination as a co-metabolite, resulting in nitrogen free compounds such as toluene or o-cresol. Our results support co-metabolic transformation. The reduction (deamination) and oxidation (hydroxylation) reactions took place simultaneously when the amino group was present. The patterns of TNT metabolite transformation were dependent upon the type of electron acceptors. The presence of nitrates in the medium stimulated the reduction of para-positioned nitro groups, and nitrites stimulated the deamination and hydroxylation processes. The absence of such primary energy sources as nitrates or nitrites stimulated the reduction of the ortho-positioned nitro groups. An appropriately designed sequencing reactor system, or other alternatives to control such energy sources as nitrates, or nitrites in the TNT degradation system, might result in full transformation of TNT to o-cresol or toluene. Though the full transformation of TNT was not achieved from the experimental processes described, it is promising that clear pathways and limiting factors were identified for the degradation of TNT.

Project Publications:

- Craig, A.M. and L.L. Blythe, "Review of Ruminant Microbes Relative to Detoxification of Plant Toxins and Environmental Pollutants," *Proceedings of the Fourth International Symposium on Poisonous Plants*, Perth, Australia, September 28-October 1, 1993.
- Craig, A.M., C.J. Latham, L.L. Blythe, W.B. Schmotzer, and O.A. O'Connor, "Metabolism of Toxic Pyrrolizidine Alkaloids from Tansy Ragwort, *Senecio jacobaea*, in Bovine Ruminant Fluid Under Anaerobic Conditions," *Applied and Environmental Microbiology*, **58**(9), 2730-2736 (1992).
- Iyer, R., Aerobic Degradation of Trinitrotoluene (TNT) Metabolites using Phenol Degrading Bacteria, M.S. Project, Department of Civil Engineering, Oregon State University, 1996.
- Lee, T.J., *In Vitro Trinitrotoluene (TNT) Biotransformation with Rumen Fluid and an Isolate G.8*, Ph.D. Dissertation, Department of Civil Engineering, Oregon State University, Corvallis, OR (1995).
- Wachenheim, D.E., Blythe, L. L., and Craig, A. M., "Effects of antibacterial agents on in vitro ovine ruminal biodegradation of the hepatotoxic pyrrolizidine alkaloid, jacobine," *Applied and Environmental Microbiology*, **58**(8), 2559-2564 (1992).
- Wachenheim, D.E., Blythe, L.L., and Craig, A.M., "Characterization of rumen bacterial pyrrolizidine alkaloid biotransformation in ruminants of various species," *Vet. and Human Toxicology*, **34**(6), 513-517 (1992).

**Western Region Hazardous Substance Research Center Project
OS91-02. The Effect of Environmental Conditions on Reductive Dechlorination Rates:
Sandra L. Woods, Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1992-1995

Project Amount: \$194,248

Research Category: Groundwater, chlorinated organics, biodegradation, PCP

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

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

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

Status: In 6- to 12-day experiments, a computer-monitored/feed-back-controlled bioreactor was used to hold temperature, pH, acetate concentration, redox potential and H_2 concentration constant at desired levels while transformation of multiple PCP additions was monitored. Transformation of PCP yielded 3,4,5-trichlorophenol (3,4,5-TCP) via 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP). Below an initial PCP concentration of 0.5 mM, PCP was transformed to 3,4,5-TCP within three to six hours. Biomass concentration changes were small, and PCP and 2,3,4,5-TeCP transformations were modeled as pseudo-first order reactions. Growth of a bacterial subpopulation that used PCP and 2,3,4,5-TeCP as terminal electron acceptors was suggested by increases in pseudo-first order rate coefficients that were directly related to the amount of PCP transformed to 3,4,5-TCP, and were independent of the acetate consumed, overall biomass concentration changes, and experimental duration. An average rate coefficient doubling time, assumed to be equivalent to a subpopulation doubling time, of 1.7 days (1.4 to 2.3 days) was estimated.

The addition of H_2 sustained reductive dechlorination activity for a prolonged period without methanogenesis, and purging H_2 from the reactor decreased rates of reductive dechlorination. H_2 was identified as the primary electron donor for the reductive dechlorination reaction, and a theoretical 2:1, H_2 :PCP stoichiometry was observed. However, increases in pseudo-first order rate coefficients were smaller during continuous addition of H_2 than when H_2 was endogenously supplied during acetoclastic methanogenesis. During periods of elevated apparent E_H to -0.1 V, methanogenesis stopped, the H_2 concentration decreased, and transformation of PCP and 2,3,4,5-

TeCP continued at progressively slower rates. H_2 added while the apparent E_H was maintained at -0.1 V, caused reductive dechlorination rates to increase, suggesting that decreased transformation rates at elevated apparent E_H is caused by a deficiency of H_2 . Project completed.

Project Publications:

- Stuart, S. L., *The Effect of Environmental Conditions on the Reductive Dechlorination of Pentachlorophenol by a Mixed, Methanogenic Culture*, Ph.D. Dissertation, Oregon State University, Corvallis, OR (1996).
- Stuart, S.L., S.L. Woods, T.L. Lemmon, J.D. Ingle, Jr., "The Effect of Redox Potential Changes on Reductive Dechlorination of Pentachlorophenol and the Degradation of Acetate by a Mixed, Methanogenic Culture," *Biotechnology and Bioengineering*, **63**(1), 69-77, (1999).
- Stuart, S.L. and S.L. Woods, "Apparent Pentachlorophenol-Dependent Growth of a Subpopulation in a Mixed, Methanogenic Culture," In *Situ and On-Site Bioremediation: Volume 2, Fourth International In Situ and On-Site Bioremediation Symposium*, New Orleans, Louisiana, April 28-May 1, 1997, Symposium Chairs Bruce C. Alleman and Andrea Leeson, Battelle Press, Columbus, Richland, 475-480 (1997).
- Stuart, S.L. and S.L. Woods, "Kinetic Evidence for Pentachlorophenol-dependent Growth of a Dehalogenating Population in a Pentachlorophenol- and Acetate-fed, Methanogenic Culture," *Biotechnology and Bioengineering*, **57**(94), 420-429 (1998).
- Wang, G.M., *Cometabolic Degradation of Chlorophenolic Compounds*, Ph.D. Dissertation, Department of Civil, Construction, and Environmental Engineering, Oregon State University, (1996).

**Western Region Hazardous Substance Research Center Project
OS91-04. Lead Sorption, Transport, and Remediation in Natural Soils and Subsoils: Peter
O. Nelson, Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1992-1995

Project Amount: \$101,260

Research Category: Groundwater, metals, lead

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

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

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

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

simulate lead chemical behavior in contaminated soils and groundwater. Soil column studies of Pb adsorption on IOCS are consistent with results from batch studies.

Project Publications:

Azizian, M.F., and Nelson, P.O., "Lead Adsorption, Chemically-Enhanced Desorption, and Equilibrium Modeling in an Iron-Oxide-Coated Sand and Synthetic Groundwater System", *Adsorption of Metals by Geomedia*, E.A. Jenne, Ed., American Chemical Society Symposium Series, chapter 6, 165-180, 1998.

Azizian, M.F., Nelson, P.O., "Experimental Evaluation and Chemical Modeling of Lead Adsorption on Iron-Oxide-Coated Sand," *Soil Science Society of America, Annual Meeting*, Seattle, Washington, November 13-18, 1994.

**Western Region Hazardous Substance Research Center Project
OS91-07. Degradation of Chlorinated Aliphatic Compounds by Nitrifying Bacteria:
Daniel J. Arp, Kenneth J. Williamson and Michael R. Hyman, Oregon State University
(Partially Supported by the U. S. Department of Energy)**

EPA Project Officer: Dale Manty

Project Period: 1992-1995

Project Amount: \$249,786

Research Category: Groundwater, chlorinated organics, biodegradation, TCE

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

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

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

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

for TCE. $^{14}\text{CO}_2$ uptake experiments have been used to monitor protein synthesis during cometabolism. These studies aim to determine what proportion of cometabolism is due to preexisting and newly synthesized AMO. An investigation of how cometabolism affects the expression of AMO has recently been initiated. TCE-dependent inactivation of AMO activity does not directly correlate with an equivalent loss of catalytically active AMO. This loss of AMO activity profoundly alters the number and type of polypeptides synthesized by the cells in the presence of ammonia. These preliminary studies suggest that the cellular response to TCE toxicity is more complex than the simple replacement of lost enzyme activity.

Project Publications:

- Arp, D. J., "Understanding the Diversity of Trichloroethylene Co-Oxidations," *Current Opinion in Biotechnology*, **6**, 352-358 (1995).
- Ely, R., K. J. Williamson, M. R. Hyman and D. J. Arp, "Cometabolic Degradation of Chlorinated Solvents," *Bioremediation of Chlorinated Solvents*, Eds., R. E. Hinchee, A. Leeson and L. Semprini, Battelle Press, Columbus, OH 187-196 (1995).
- Ely, R.L., K.J. Williamson, R.B. Guenther, M.R. Hyman, and D.J. Arp, "A Cometabolic Kinetics Model Incorporating Enzyme Inhibition, Inactivation, and Recovery: 1. Model Development, Analysis, and Testing," *Biotechnology and Bioengineering*, **46**, 218-231 (1995).
- Ely, R.L., M.R. Hyman, D.J. Arp, R.B. Guenther, and K.J. Williamson, "A Cometabolic Kinetics Model Incorporating Enzyme Inhibition, Inactivation, and Recovery: 2. Trichloroethylene Degradation Experiments," *Biotechnology and Bioengineering*, **46**, 232-245 (1995).
- Hyman, M.R., Page, C.L., and Arp, D.J., "Oxidation of Methyl Fluoride and Dimethyl Ether by Ammonia Monooxygenase in *Nitrosomonas europaea*," *Applied and Environmental Microbiology*, **60**, 3033-3035 (1994).
- Hyman, M.R., S.A. Russell, R.L. Ely, K.J. Williamson, and D.J. Arp, "Inhibition, Inactivation, and Recovery of Ammonia-Oxidizing Activity in Cometabolism of Trichloroethylene by *Nitrosomas europaea*," *Applied and Environmental Microbiology*, **61**(4), 1480-1487 (1995).

**Western Region Hazardous Substance Research Center Project
OS93-01. Remediation of Contaminated Soil from the Baldock Station Maintenance
Facility: Kenneth J. Williamson and Sandra Woods, Oregon State University (Supported
by Oregon Department of Transportation)**

EPA Project Officer: Dale Manty

Project Period: 1994

Project Amount: \$40,200

Research Category: Chlorinated organics, soils, biodegradation

Goal: The goal of this project is to demonstrate the successful bioremediation of a variety of chlorinated herbicides using sequential anaerobic/aerobic treatment.

Rationale: Sequential anaerobic/aerobic biological treatment has been successfully used to degrade a variety of chlorinated aromatic compounds under laboratory conditions. This project is an extension of laboratory results to a controlled field demonstration.

Approach: Currently, approximately 155 cubic yards of contaminated soil are stored at the Baldock Maintenance Facility, Portland, Oregon. The soils are contaminated with a variety of herbicides; known contaminants include 2,4-D, 2,4,5-T, Silvex, and Dichloroprop. The bioremediation procedure will involve an initial anaerobic phase followed by an aerobic phase. Under anaerobic conditions, the phenoxy herbicides are expected to be reductively dechlorinated. The resulting phenolic compounds are expected to degrade to carbon dioxide and water in the presence of oxygen. In the field portion of the study, soil in one of the containers will be treated by recirculating nutrient-containing water under anaerobic conditions. The water will be sampled periodically to determine degradation rates and, when dechlorination is complete, pure oxygen will be introduced into the recirculation stream. Following the aerobic incubation, the soil will be assayed for residual herbicide concentrations.

Status: Laboratory studies using contaminated soils have been completed. The studies demonstrated that elevated temperatures of above 30° C would be required to obtain remediation of the stored soils. Carbon additions of about 200 mg C/L would be required to maintain anaerobic conditions. Under fully anaerobic conditions, the laboratory results suggest that all four of the herbicides will degrade. Field studies have been initiated using sucrose (250 mg/L) as the carbon source. One field container has been fully flooded and the degradation of the herbicides is being monitored versus time. If the carbon is depleted, a feed solution will be recirculated.

Project Publications:

Degher, A.B., K.G. Wang, K.J. Williamson, S.L. Woods, and R. Strauss, "Bioremediation of Soils Contaminated with 2,4-D, 2,4,5-T, Dichloroprop, and Silvex," In *Situ and On-Site Bioremediation: Volume 2, Fourth International In Situ and On-Site Bioremediation Symposium*, New Orleans, Louisiana, April 28-May 1, 1997, Symposium Chairs Bruce C. Alleman and Andrea Leeson, Battelle Press, Columbus, Richland, 279 (1997).

- Strauss, R., *Anaerobic Bioremediation of Phenoxy-herbicide Contaminated Soils from the Baldock Station Maintenance Facility*, M.S. Project, Department of Civil Engineering, Oregon State University, 1995.
- Wang, K. G., *Aerobic Bioremediation of Contaminated Soils from the Baldock Station Maintenance Facility*, M.S. Project, Department of Civil Engineering, Oregon State University, 1995.

**Western Region Hazardous Substance Research Center Project
OS94-01. The Effect of Apparent E_H , Compound Structure, and Electron Donor on
Anaerobic Biotransformation of Trinitrotoluene and its Metabolites: A. Morrie Craig,
Kenneth J. Williamson, Sandra Woods, Oregon State University (Supported by R2D2
Program)**

EPA Project Officer: Dale Manty

Project Period: 1995-1997

Project Amount: \$126,434

Research Category: Groundwater, soils, biodegradation, TNT

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

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

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

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

Project Publications:

Lotrario, J.B, S.L. Woods, A.M. Craig, and K.J. Williamson, "The Anaerobic Transformation of TNT and other Nitroaromatic Compounds," In *Situ and On-Site Bioremediation: Volume 2, Fourth International In Situ and On-Site Bioremediation Symposium*, New Orleans, Louisiana, April 28-May 1, 1997, Symposium Chairs Bruce C. Alleman and Andrea Leeson, Battelle Press, Columbus, Richland, pgs. 41-46 (1997).

- Lotrario, J.B. and S.L. Woods, "Comparison of the Correlation of MEP and the Free Energies of Reaction with Nitro Group Reduction of Nitrotoluene Congeners," *Bioremediation*, **1**(2), 115-122 (1997).
- Lotrario, Joseph, "Nitro Group Reduction of Five Aromatic Compounds by the Denitrifying Bacteria G.8," M.S. Project Report, Department of Civil Engineering, Oregon State University, Corvallis, Oregon, 1995.
- Stuart, S.L., S.L. Woods, T.L. Lemmon, J.D. Ingle, "The Effect of Redox Potential Changes on Reductive Dechlorination of Pentachlorophenol and the Degradation of Acetate by a Mixed, Methanogenic Culture," *Biotechnology and Bioengineering*, **63**(1), 69-77 (1999).

**Western Region Hazardous Substance Research Center Project
OS94-02. The “Bubble Wall”: A Passive In Situ System for Treatment and/or
Containment of Contaminated Groundwater: Jonathan D. Istok, Virginia A. Fry, John S.
Selker, Oregon State University, and Steven M. Gorelick, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1995-1997

Project Amount: \$297,685

Research Category: Groundwater, model, treatment

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

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

Approach: The investigation of the “bubble wall” technology is being performed using a combination of theoretical analysis, numerical modeling, and laboratory experiments. Theoretical analysis is used to develop continuum models to describe the mass transfer of gas from the trapped gas to the mobile aqueous phases and pore-scale models to describe the effect of trapped gas on the hydraulic conductivity and dispersivity of the porous media. Numerical models are used to determine the sensitivity of mass transfer to model parameters (e.g. particle size distribution, trapped gas volume, solubility and vapor pressure of dissolved gas, and pore water velocity) and to determine the effect of hydraulic conductivity reduction within a bubble wall on the regional groundwater flow field. Laboratory experiments are performed in one-dimensional columns and two-dimensional, vertical and horizontal physical aquifer models using a variety of porous media. The purpose of the laboratory experiments is to investigate methods for emplacement of trapped gas into saturated porous media to create a bubble wall, to validate models for mass transfer of gas from the bubble wall to groundwater, to determine the effect of trapped gas on hydraulic conductivity, and to validate pore-scale models.

Status: Bubble entrapment was found to be a strong function of the method of entrapment, but can be well understood when viewed in the context of dimensionless variables. Up to 20% of the pore space can be filled with stable gas, reducing permeability by a factor of more than two. The geometry of gas entrapment in homogeneous media is parabolic with an error-function gas flow density in lateral sections. These properties can be explained by considering the gas travel to consist of independent streams of bubbles with upward trending Brownian motion. In addition, lateral flow at textural interfaces was modeled using a Bousinesq approach, demonstrating that the extent of lateral bubble movement is linearly related to the slope of the interface, and increases with the inverse square root of the rate of flow. This result supports the field observation of ever smaller lateral gas movement at increasing distance from injection in stratified environments. The study is now complete.

Project Publications:

- Donaldson, J.H., J.D. Istok, and K.T. O'Reilly, "Dissolved Gas Transport in the Presence of a Trapped Gas Phase: Development and Laboratory Testing of a Two-Dimensional Kinetic Model", *Ground Water*, **36**(1), 133-142 (1998).
- Donaldson, J.H., J.D. Istok, M.D. Humphrey, K.T. O'Reilly, C.A. Hawelka, and D.H. Mohr, "Development and Laboratory Testing of a Kinetic Model for Dissolved Oxygen Partitioning and Transport in Porous Media in the Presence of Trapped Gas," *Ground Water*, **40**(1), 113-119 (1997).
- Fry, V. A., J. S. Selker, and S.M. Gorelick, "Experimental Investigations for Trapping Oxygen Gas in Saturated Porous Media for in situ Bioremediation," *Water Resources Research*, **33**(12), 2687-2696 (1997).
- Fry, V.A., J.D. Istok, and K.T. O'Reilly, "Effect of Trapped Gas on Dissolved Oxygen Transport - Implications for In Situ Bioremediation," *Ground Water*, **34**(2), 200-210 (1996).

**Western Region Hazardous Substance Research Center Project
OS94-03. Inhibition, Inactivation and Recovery: A Universal Model for Aerobic
Cometabolic Degradation of Aliphatic Compounds: Daniel Arp, Michael Hyman, and
Kenneth J. Williamson, Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1996-1997

Project Amount: \$168,097

Research Category: Groundwater, chlorinated organics, biodegradation, TCE

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

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

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

Status: In previous studies, a methodology for conducting reactor-based studies with *N. europaea* was developed, and the mathematical model describing TCE cometabolism by this organism was formulated. Experiments were conducted with *Burkholderia cepacia* G4 to examine whether the model accurately describes the cometabolism of TCE by other organisms. The model does not accurately describe cometabolism in *B. cepacia* G4. Factors such as growth and substrate inhibition need to be further examined.

Short term, small scale assays examining inhibition, inactivation, and recovery associated with TCE cometabolism by the toluene oxidizer *B. cepacia* G4 have been initiated. These results reveal additional complexity in whole cell kinetics of toluene and TCE oxidation by *B. cepacia* G4 and further support the idea that mathematical models employing competitive inhibition may not adequately describe the kinetics of cometabolism. The effects of alkynes on toluene-2-monooxygenase (TOM) activity are under investigation. Although acetylene is a weak inactivator, longer chain alkynes are potent and specific inactivators of TOM.

The mechanism of TCE toxicity for *N. europaea* has been further investigated using short-term incubations. TCE cometabolism has significant effects on both AMO and HAO activities. Inhibitor treatments which specifically inactivate either AMO or HAO were used to compare effects seen in TCE cometabolism and several other chlorinated hydrocarbons. The results of TCE and chloroform cometabolism show that HAO is the probable target at low concentrations. In contrast, AMO is inactivated in cis-1,2-dichloroethylene experiments. These results lead us to conclude the site of action is not directly related to these enzymes. One hypothesis is that TCE cometabolism exerts a significant effect on the electron transport in *N. europaea* cells. Project completed.

Project Publications:

- Ely, R. L., *Effects of Substrate Interactions, Toxicity, and Bacterial Response During Cometabolism of Chlorinated Solvents by Nitrifying Bacteria*, Ph.D. Dissertation, Department of Civil, Construction, and Environmental Engineering, Oregon State University, Corvallis, OR (1996).
- Ely, R. L., K.J. Williamson, M.R. Hyman, and D.J. Arp, "Cometabolism of Chlorinated Solvents by Nitrifying Bacteria: Kinetics, Substrate Interactions, Toxicity Effects, and Bacterial Response," *Biotechnology and Bioengineering*, **54**(6), 520-534 (1997).
- Yeager, C. M., P. J. Bottomley, D. J. Arp, and M. R. Hyman. Mechanism-based inactivation of toluene 2-monooxygenase in *Burkholderia cepacia* G4 by alkynes. *Appl. Environ. Microbiol.* **65**, 632-637 (1999).

**Western Region Hazardous Substance Research Center Project
OS94-04. Development, Characterization, and Performance Evaluation of Ferrous-Ferric
Oxide Adsorbents for Metal Removal from Contaminated Groundwater: Peter O. Nelson,
Oregon State University (Supported by R2D2 Program)**

EPA Project Officer: Dale Manty

Project Period: 1995-1997

Project Amount: \$111,668

Research Category: Groundwater, metals

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

Rationale: Remediation of metals-contaminated groundwater focused on ex-situ or in-situ treatment processes. Both methods are often limited by their expense and extended extraction time. An alternative to these approaches is in-situ treatment by interception of the contaminant plume by passive trench that removes or transforms contaminants in place. This study will increase our understanding of mixed oxidation state (ferrous-ferric) iron oxide for removal of adsorbable and electroactive metals in in-situ treatment in an interceptor trench.

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

Status: Results from Fe(II) dissolution experiments indicated that the amount of Fe(II) present in the bulk solution could not have reduced as much Cr(VI) as was experimentally observed, and the Fe(II) dissolution rate was slower than the Cr(VI) reduction rate. The reaction became independent of initial [Cr(VI)] when $[\text{Cr}(\text{VI})]/\{\text{MCS}\}$ ratio was greater than 0.38 mmole Cr(VI)/g MCS, indicating a saturation of surface sites. Cr(VI) reduction was hypothesized to occur in three steps, transport from the bulk solution to the MCS surface, adsorption to the surface either as an outer-sphere complex or as an inner-sphere ternary complex with adsorbed Fe(II), and reduction by Fe(II). Fe(II) oxidation similarly was hypothesized to follow a multistep pathway that includes dissolution from magnetite, inner-sphere adsorption to the ferric oxide component of magnetite (either binary or as ternary complex with Cr(VI)), and oxidation by adsorbed Cr(VI). Initial Cr(VI) concentration, solution pH, and MCS concentration are directly

related to the reduction reaction, and a Cr(VI) reduction rate equation was developed based upon these three parameters, as follows:

$$d[\text{Cr(VI)}]/dt = -k[\text{Cr(VI)}][\text{H}]^{0.2}\{\text{MCS}\}^{1.8}$$

The rate equation successfully predicted kinetics of Cr(VI) reduction by MCS. Project complete.

Project Publications:

Azizian, M.F., P.O. Nelson, and G. Siddiqui, "Development, Characterization, and Performance Evaluation of Ferrous-Ferric Oxide Adsorbents for Metal Removal from Contaminated Groundwater," American Chemical Society, Environmental Chemistry Division, Vol. **37**, No. 1, p200, (1997).

Khaodhiar, S., "*Removal of Chromium, Copper, and Arsenic from Contaminated Groundwater Using Iron-Oxide Composite Adsorbents*", Ph.D. Thesis, Oregon State University, Corvallis, OR (1997).

**Western Region Hazardous Substance Research Center Project
OS94-06. Redox Transformations of Organic and Inorganic Contaminants in the
Subsurface Environment: John C. Westall and James D. Ingle, Oregon State University
(Supported by R2D2 Program)**

EPA Project Officer: Dale Manty

Project Period: 1995-1997

Project Amount: \$117,804

Research Category: Groundwater, chemistry, redox

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

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

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

Status: Organic redox indicators have been covalently immobilized on affinity chromatography beads (50- μ m diameter) which are packed in a spectrophotometric flow cell to allow contact with filtered reactor solution (biologically-active soil slurries, sludges, and ground water) and monitoring of the absorbance (and hence speciation) of the indicators. Two redox indicators, thionine ($E'^7 = +53$ mV vs SHE) and cresyl violet ($E'^7 = -81$ mV vs SHE), have been found to be particularly useful for delineating between Fe(III)-reducing conditions and sulfate-reducing conditions in soil and waste water slurries. They are reduced by quite different levels of Fe(II) and sulfide (HS⁻). Project complete.

Project Publications:

Sule, P.A., and J.D. Ingle, Jr., "Determination of the Speciation of Chromium with an Automated Two-column Ion-exchange System," *Analytica Chimica Acta*, **326**, 85-93 (1996).

**Western Region Hazardous Substance Research Center Project
OS95-01. Demonstration of a Permeable Barrier Technology for the Bioremediation of
Ground Water Contaminated with Waste Mixtures: Sandra Woods and Kenneth J.
Williamson, Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1997-2001

Project Amount: \$412,377

Research Category: Groundwater, chlorinated organics, demonstration, biodegradation

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

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

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

Status:

The construction of a 23" cylindrical down-borehole reactor (basket) was completed. The reactor was installed in the 24" diameter borehole during mid-June, 1997. Work focused on testing and validation of the reactor's sampling and nutrient supply systems. In the absence of an inoculated biomass, several conservative tracer studies were conducted to characterize hydraulic conditions within the well and reactor. The observed retention time was approximately 24 hours.

Chlorophenol ground water concentrations were analyzed on a weekly basis beginning mid February, 1997. Maximum pentachlorophenol concentrations in the groundwater were measured in February at a level of approximately 1 mg/l. The spring and summer months marked a slow decline in aqueous concentrations. Minimum PCP concentration values of 0.2 mg/l were observed in July. Late summer and fall marked the increase in aqueous PCP concentrations and current levels are approximately 2.5 mg/L. Due to the variability in PCP concentrations, measurement of local ground water elevations and rainfall history was instituted to help predict seasonal chlorophenol variations. Observation of ground water elevations in the aquifer system show strong influence by the facility's pumping wells and regional rainfall.

The reactor system was removed and inoculated with cells during the first week of December, 1997. The reactor was modified to include two recirculating flow cells for the characterization of the environmental conditions within the anaerobic and aerobic zones. Redox and pH measurements are coupled to a real time data acquisition system. Chlorophenols and sulfate present in the system are monitored by GC/ECD or IC analyses with space and time.

Mineralization of pentachlorophenol was verified in laboratory scale batch bioassays. Experiments were conducted in 250 mL serum bottles under sequential anaerobic/aerobic conditions using imitation vanilla flavoring as the electron donor/primary substrate. Progress curves were developed for replicate reactors using ^{14}C -labelled PCP or unlabelled PCP. In reactors containing unlabelled PCP, transient production of 2,3,4,5-tetrachlorophenol and accumulation of 3,4,5-trichlorophenol was observed during the initial anaerobic phase. There was no significant removal of ^{14}C from the aqueous phase in the replicates containing ^{14}C -PCP under anaerobic conditions. Upon injection of O_2 , there was immediate removal of 3,4,5-trichlorophenol in the unlabelled replicates concomitant with a decrease in ^{14}C in the aqueous phase and a stoichiometric increase of $^{14}\text{CO}_2$.

During 1999, the reactor installed within the permeable barrier at the L.D. McFarland facility was operated under a variety of environmental conditions. Concentrations of the electron donor and electron acceptors (sulfate and oxygen) were varied resulting in different EH regimes within the reactor over time. Pentachlorophenol removal was evaluated under anaerobic conditions and in the presence of imitation vanilla flavoring as the electron donor. Intermediates included 2,3,4,5-tetrachlorophenol, 3,4,5-trichlorophenol, 3,4-dichlorophenol and 3,5-dichlorophenol. Complete removal of pentachlorophenol as well as these intermediates was observed. Parallel laboratory and field experiments were conducted to evaluate the effect of supplemental electron donor and electron acceptor concentrations on chlorophenol removal. Complete pentachlorophenol removal was observed in the laboratory and in the field at imitation vanilla flavoring concentrations of 10 mg/L as COD. Sulfate also was injected into the permeable barrier reactor. At influent concentrations of 100 mg/L, sulfate had no apparent effect on pentachlorophenol removal. A series of coordinated laboratory and field studies were conducted to evaluate the simultaneous removal of pentachlorophenol and naphthalene under sequential anaerobic (for pentachlorophenol reductive dechlorination) and aerobic (for naphthalene oxidation) conditions. Laboratory studies were conducted in serum bottles containing naphthalene, groundwater, and associated biomass from the permeable barrier reactor. Complete naphthalene removal was observed when aerobic serum bottles were amended with 2 to 3 mg/L naphthalene (removal required 100 - 500 hours). Naphthalene removal was evaluated in the field by adding hydrogen peroxide to the mixing wells prior to aerobic biological zones in the permeable barrier reactor. Removal of naphthalene in the aerobic treatment zones was 86% within 12 days and 90% within three weeks of treatment (based upon a comparison of influent

and effluent concentrations). Continued complete removal of pentachlorophenol was observed (PCP continued to be present at levels below our detection limit). The simultaneous removal of pentachlorophenol and naphthalene under sequential anaerobic and aerobic conditions indicates that potential usefulness of a permeable barrier technology for the removal of mixtures of contaminants in groundwater. Project complete.

Project Publications:

- Brown, James, "Down-Hole Permeable Barrier Reactor: Physical Development," M.S. Project Report, Department of Civil Engineering, Oregon State University, Corvallis, Oregon, (1995).
- Cole, J., S. Woods, K. Williamson, and D. Roberts, "Anaerobic Treatment of a Pentachlorophenol-Contaminated Aquifer using a Borehole Reactor," 1998 Joint Conference on the Environment, WERC-WRHSRC-NMHWMS, Crowne Plaza Pyramid, Albuquerque, New Mexico, 57-62 (1998).
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- Havighorst, M. "Bioremediation of Low-Permeability Pentachlorophenol-Contaminated Soil by Laboratory and Scale Processes," M.S. Thesis, Oregon State University, (1998).
- Havighorst, M.B., S.L. Woods, and K.J. Williamson, "Bioremediation of Pentachlorophenol-Contaminated Soil in Laboratory and Full-Scale Processes," In Situ and On-Site Bioremediation: Volume 2, Fourth International In Situ and On-Site Bioremediation Symposium, New Orleans, Louisiana, April 28-May 1, 1997, Symposium Chairs Bruce C. Alleman and Andrea Leeson, Battelle Press, Columbus, Richland, pg. 455, 1997.
- Jason Cole, "Demonstration of a Permeable Barrier Technology for the In-Situ Bioremediation of Pentachlorophenol Contaminated Groundwater," Ph.D. Dissertation, Oregon State University, (2000).
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- Nelson, S. "Down-Borehole Permeable Barrier Reactor: Aerobic Degradation of Naphthalene," M.S. Thesis, Oregon State University, (1999).
- Roberts, D., *Down-Borehole Permeable Barrier Reactor: Verification of Complete Mineralization of Pentachlorophenol in a Sequential Anaerobic-Aerobic Process*, M.S. Thesis, Department of Civil, Construction, and Environmental Engineering, Oregon State University, Corvallis, OR (1997).

**Western Region Hazardous Substance Research Center Project
OS95-04. Development of a Vitamin B₁₂-Amended Bioremediation Process for the
Reductive Dechlorination of Chlorobiphenyls at all Chlorine Positions: Sandra Woods,
Oregon State University (Supported by R2D2 Program)**

EPA Project Officer: Dale Manty

Project Period: 1995-1997

Project Amount: \$144,357

Research Category: Groundwater, chlorinated organics, PCBs,

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

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

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

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

Biological reduction of vitamin B₁₂. This work was completed and reported in the most recent progress report and in Workman et al. (1997).

Vitamin B₁₂-catalyzed transformation of chlorinated methanes. The fully reduced vitamin B_{12s} rapidly dechlorinates chlorobiphenyls at all positions (Woods et al., 199x). However unlike vitamin B_{12s}, vitamin B_{12r} (produced in the *Shewanella alga* system) does not result in rapid dechlorination of 2,3,4,5,6-PCB. For comparison, the transformation of readily dechlorinated methanes [carbon tetrachloride (CT), chloroform (CF), and dichloromethane (DCM)] was examined in batch systems containing vitamin B₁₂, the metal-reducing non-dechlorinating bacterium *Shewanella alga* strain BrY, and an electron donor. Further investigation examined factors that affect the rate of CT transformation. Both cell concentration and vitamin B₁₂

concentration influenced the rate at which CT was transformed in this system. Rate dependence was seen only when vitamin B₁₂ concentrations were below 35 mM for high cell concentrations.

The transformation of a variety of halogenated methanes was examined. Bromoform, dibromomethane, dibromochloromethane, and chlorodibromomethane were transformed in the presence of BrY, lactate and vitamin B₁₂. The initial first-order rates determined for these transformations had the following pattern: bromoform >> carbon tetrachloride > dibromomethane > dibromochloromethane > bromodichloromethane >> chloroform.

Vitamin B₁₂-catalyzed transformation of chlorinated biphenyls. Previous work demonstrated rapid dechlorination of 2,3,4,5,6-PCB in a titanium citrate-reduced vitamin B₁₂s system. The reductive dechlorination pathway for 2,3,4,5,6-PCB indicated that chlorines are removed from all positions. Current work is focused on the factors affecting reductive dechlorination of PCBs by vitamin B₁₂s in a sediment/water system. 2,3,4,5,6-PCB was equilibrated with Willamette River sediment for approximately 6 months. Rapid removal of 2,3,4,5,6-pentachlorobiphenyl was observed in systems containing 333 mM vitamin B₁₂ and excess titanium citrate. The tetra-, tri-, di- and mono-chlorobiphenyls that have been observed are consistent with the intermediates observed for the aqueous system and with thermodynamic predictions based upon distribution coefficients estimated from free energies of formation.

Project Publications:

- Trobaugh, D. "Reductive Dechlorination of Sediment-Sorbed Polychlorinated Biphenyls by Vitamin B₁₂s," M.S. Thesis, Oregon State University, (1998).
- Trobaugh, D.J., S.L. Woods, K.J. Carter, and D. Workman, "Development of a Vitamin B₁₂-Amended PCB Bioremediation Strategy," In *Situ and On-Site Bioremediation: Volume 2, Fourth International In Situ and On-Site Bioremediation Symposium*, New Orleans, Louisiana, April 28-May 1, 1997, Symposium Chairs Bruce C. Alleman and Andrea Leeson, Battelle Press, Columbus, Richland, 413-418 (1997).
- Woods, S.L., D.J. Trobaugh, and K.J. Carter, "Polychlorinated Biphenyl Reductive Dechlorination by Vitamin B₁₂s: Thermodynamics and Regiospecificity," *Environmental Science and Technology*, **33**(6), 857-863 (1999).

**Western Region Hazardous Substance Research Center Project
OS95-07. An Investigation of the Chlorinated Hydrocarbon Substrate Range of the
Filamentous Fungus, *Graphium* sp.: Michael Hyman and Lynda Ciuffetti, Oregon State
University (Supported by R2D2 Program)**

EPA Project Officer: Dale Manty

Project Period: 1995-1997

Project Amount: \$24,045

Research Category: Groundwater, chlorinated organics, biodegradation, MTBE

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

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

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

Status: We have demonstrated n-alkane-grown *Graphium* can degrade numerous CAHs including all 4 trihalomethanes, HC 21, HC 22, chloromethane, dichloromethane, chloroethane, 1,2-dichloroethane and 1,1,2,2-tetrachloroethane. This fungus can also reductively dechlorinated

CT to CF in the absence of oxygen and then consume CF when oxygen is introduced into the reaction. We have also demonstrated that *Graphium* can grow on the unusual substrate diethyl ether (DEE) and can degrade the gasoline oxygenate methyl *tert* butyl ether after growth on gaseous *n*-alkanes. Our recent research has focused on defining the growth substrates that will support this activity and we have demonstrated that *Graphium* will degrade all of the major gasoline oxygenates (MTBE, TAME and ETBE) after growth on propane, butane and isobutane. Project complete.

Project Publications:

OS95-7a Curry, S., L. Ciuffetti, and M Hyman, "Inhibition of growth of a *Graphium* sp. on gaseous *n*-alkanes by gaseous *n*-alkynes and *n*-alkenes," *Applied and Environmental Microbiology*, **62**, 2198-2200 (1996).

**Western Region Hazardous Substance Research Center Project
OS95-08. Aerobic Cometabolism of Chloroform, 1,1,1-trichloroethane, 1,1-dichloroethylene, and Other Chlorinated Aliphatic Hydrocarbons by Microbes Grown on Butane and Propane: Lewis Semprini and Daniel J. Arp, Oregon State University
(Supported by R2D2 Program)**

EPA Project Officer: Dale Manty

Project Period: 1995-2001

Project Amount: \$408,690

Research Category: Groundwater, chlorinated organics, TCA, TCE, biodegradation

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

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

Status: Transformation and kinetic studies evaluated the cometabolism of chlorinated methanes, chlorinated ethanes, and chlorinated ethenes by our butane grown enrichment obtained from the Hanford DOE site. Resting cell studies showed very high transformation capacities ($\mu\text{mole CAH/mg TSS cells}$) in the absence of an endogenous energy source. The chlorinated methanes transformation capacities were: chloromethane (CM), 23; dichloromethane (DCM), 5.8; and chloroform (CF), 0.6; showing transformation was more for effective for the less chlorinated methanes. The transformation of CF, however, promoted the most inactivation of butane-utilizing activity. The chlorinated ethanes transformation capacities ranged from 7.0 for chloroethane (CA) to 0.29 for 1,1,2-trichloroethane (1,1,2-TCA). The transformation and the resulting inactivation were strongly affected by the position of the chlorine substitution. 1,1-dichloroethane and 1,1,1-trichloroethane were more effectively transformed and caused less inactivation than their corresponding isomers with chlorine on both carbons, 1,2-dichloroethane and 1,1,2-trichloroethane. We suspect that the mechanism of cometabolism and the transformation products are causing these differences. The chlorinated ethenes had transformation capacities ranging from 2.7 for vinyl chloride to 0.1 for TCE. The dichloroethylene (DCE) isomers transformation capacities were: trans-DCE, 0.0; cis-DCE, 1.4; 1,1-DCE, 0.9, again showing large variations based on the position of the chlorine substitution. Butane-utilizers effectively transformed 1,1-DCE, however the transformation caused the greatest inactivation of all the compounds tested. Chloride release studies showed the

chlorinated methanes and ethenes were the most completely dechlorinated (70 to 100%), while the chlorinated ethanes were the least dechlorinated (40 to 60 %).

Characterization of butane and chlorinated aliphatic degradation by three bacterial isolates was continued. *Mycobacterium vaccae*, *Pseudomonas butanovora*, and CF8 (an isolate from Hanford core material) all degrade chloroform when grown on butane. Studies with inactivators and inhibitors of CF degradation led to the hypothesis that each of these three bacteria produces a distinct butane monooxygenase. When cultures of each bacterium are exposed to ^{14}C acetylene, activity is lost. When proteins from these bacteria were examined by SDS-PAGE and autoradiography, different proteins were found to be labeled. Butane oxidation is inhibited or inactivated by the same compounds which affect CF oxidation which provides further evidence that butane monooxygenase catalyzes the transformation of CF. A mixed culture (derived from Hanford core material) was grown in the presence and absence of CF. Cultures grew more slowly in the presence of chloroform. Chloroform degradation potential of resting cells taken from these cultures revealed a greater chloroform specific activity in the cultures not exposed to CF than those which were exposed to chloroform. PCR products (using a variety of primers) were similar whether the culture was grown in the presence or absence of CF, although distinct differences were also noted. These results reveal that the presence of CF during growth on butane does influence the microbial population.

In batch kinetic studies with the butane-grown enrichment the maximum degradation/transformation rates (k), half-saturation coefficient (Ks), inhibition types (competitive, non-competitive, and mixed inhibition), and inhibition coefficients (KI and KI') of 1,1,1-TCA, 1,1-DCE, 1,1-DCA, and butane have been determined. A direct linear plot method was used to identify the types of inhibition. 1,1,1-TCA, 1,1-DCE, and 1,1-DCA all competitively inhibited each other. Competitive inhibitions kinetics were found to accurately represent the inhibition observed when all three compounds were present. However, butane (growth substrate) showed different inhibition types, that is, non-competitive inhibition on 1,1,1-TCA and 1,1-DCA and mixed inhibition on 1,1-DCE transformation. Inhibition constants were determined using a linearization method that was developed and by non-linear-least-squares-regression of the inhibition data. Good agreement was obtained for the parameters determined by both methods of analysis. When butane and two or more CAHs were present, a model, which combined both competitive and mixed inhibition kinetics, less accurately simulated our experimental results.

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

Project Publications:

- Hamamura, N. and D.J. Arp, "Isolation and characterization of alkane-utilizing *Nocardiodes* sp. Strain CF8," *FEMS Microbiology Lett.* **186**, 21-26 (2000).
- Hamamura, N., C. Page, T. Long, L. Semprini, and D.J. Arp, "Chloroform Cometabolism by Butane-Grown CF8, *Pseudomonas butanovora*, *Mycobacterium vaccae* JOB 5, and Methane-Grown *Methylosinus trichosporium* OB3b," *Applied and Environmental Microbiology*, **63**(9), 3607-3613 (1997).

- Hamamura, N., R.T. Storfa, L. Semprini, and D.J. Arp, "Diversity in butane monooxygenases among butane-grown bacteria," *Appl. Environ. Microbiol.* **65**, 4586-4593 (1999).
- Kim, Y., D.A. Arp and L. Semprini. "Chlorinated Solvent Cometabolism by Butane-Grown Mixed Culture," *J. Environ. Engr., ASCE*, **126**(1), 934-942 (2000).
- Kim, Y., L. Semprini, and D.J. Arp, "Aerobic cometabolism of chloroform 1,1,1-trichloroethane, 1,1-dichloroethylene, and the other chlorinated aliphatic hydrocarbons by butane-utilizing microorganisms," *Proceedings of the Fourth International Symposium on In Situ and On-Site Bioremediation* (1997).
- Kim, Y., L. Semprini, and D.J. Arp, "Aerobic Cometabolism of Chloroform and 1,1,1-Trichloroethane by Butane Grown Microorganisms," *Bioremediation Journal*, **1**(2), 135-148 (1997).
- Kim, Y., L. Semprini, and D. J. Arp. "Kinetic Studies of Aerobic Transformations of Chlorinated Solvents by Butane-Utilizing Microorganisms," *Proceedings of Joint Conference on the Environment, Albuquerque, March 31 – April 1*, 77-80 (1998).
- Semprini, L., "Strategies for the aerobic co-metabolism of chlorinated solvents," *Current Opinion in Biotechnology*, **8**, 296-308 (1997).
- Semprini, L., R. L. Ely, and M.M. Lang. "Modeling of Cometabolism for the In-situ Biodegradation and Trichloroethylene and Other Chlorinated Aliphatic Hydrocarbons," In: *Bioremediation: Principles and Practice Vol. (1) Fundamentals and Applications*: Editors: S. K. Sikdar and R. L. Irvine, Technomic Publishing Co. Lancaster, PA. pp.89-134 (1998).
- Young, K. "Aerobic cometabolism of chlorinated aliphatic hydrocarbons by a butane-grown mixed culture: transformation abilities, kinetics and inhibition." Ph.D. Dissertation, Oregon State University (2000).
- Kim, Y, D.J. Arp, and L. Semprini, "A Combined Method for Determining Inhibition Type, Kinetic Parameters, and Inhibition Coefficients for Aerobic Cometabolism of 1,1,1-Trichloroethane by a Butane-Grown Mixed Culture". *Biotechnology and Bioengineering*, **77**(5), 564-576 (2002).
- Kim, Y. D.J. Arp, and L. Semprini. "Kinetic and Inhibition Studies for the Aerobic Cometabolism of 1,1,1-Trichloroethane, 1,1-Dichloroethylene, and 1,1-Dichloroethane by a Butane-Grown Mixed Culture." *Biotechnology and Bioengineering*, **80**(5), 498-508 (2002).

**Western Region Hazardous Substance Research Center Project
OS97-03. Cytochrome P-450: An Emerging Catalyst for the Cometabolism of Chlorinated Aliphatic Hydrocarbons and Methyl *tert*-butyl Ether? Michael Hyman and Lynda Ciuffetti, Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1997-2000

Project Amount: \$82,342

Research Category: Groundwater, chlorinated organics, MTBE, biodegradation

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

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

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

Status: This project was completed in 2001. Our studies under Objective #1 demonstrated that alkane-grown *Graphium* rapidly degrades chloroform and a variety of other chlorinated alkane substrates. These reactions are all inhibited by the presence of acetylene and ethylene and are compatible with reactions catalyzed by an alkane-induced cytochrome P-450. We also characterized the ability of alkane-grown *Graphium* to reductively dechlorinate carbon tetrachloride under anaerobic conditions. The introduction of oxygen into these reactions then led to the oxidation of the chloroform generated during the anaerobic reaction phase. Our evidence suggests that both the reductive dehalogenation of carbon tetrachloride and the oxidation of chloroform are both catalytic features of the alkane-induced cytochrome P-450 in this organism. Our studies under Objective #2 have largely focused on the degradation of MTBE by a bacterial strain, *Mycobacterium vaccae* JOB5. Our evidence suggests that this bacterium does not express a cytochrome P-450 enzyme that serves a major catabolic role during growth on short chain alkanes. However, our evidence does suggest that an alkane-inducible monooxygenase activity plays several important roles in the cometabolic degradation of MTBE. For example, cells of *M. vaccae* grown on C₃ to C₈ n-alkanes all oxidize MTBE. In all cases

tertiary butyl formate (TBF) is generated as the first stable oxidation product of MTBE oxidation. A second hydrolytic enzyme is then responsible for hydrolyzing TBF to tertiary butyl alcohol (TBA), which itself undergoes further oxidation by the alkane-induced monooxygenase. Our current evidence suggests TBA is oxidized initially to 2-methyl, 1,2, propanediol which is further oxidized by alcohol and aldehyde dehydrogenases to yield 2-hydroxyisobutyric acid. The further oxidation of 2HIBA has not been confirmed in *M. vaccae* but postulated intermediates such as 2-propanol, acetone and acetol are oxidized by alkane-grown cells of *M. vaccae*. Our results demonstrate that the alkane-induced alkane monooxygenase in *M. vaccae* can also oxidize acetone and acetol while an alkane-induced alcohol dehydrogenase is responsible for the oxidation of 2-propanol to acetone. Our main focus in Objective #3 has been the characterization of the oxygenase responsible for MTBE oxidation in *Graphium*. We have a full length clone of an inducible cytochrome P-450 and have attempted to confirm that this is the enzyme responsible for MTBE-oxidizing activity. Our most recent efforts have been directed at verifying whether oxygenase activity (as measured by naphthalene oxidation) corresponds with the expression pattern we have observed using propane, propanol and propionate as substrate. Once this has been confirmed we hope to conduct expression studies in a strain of *Verticillium* that we have already established does not have either the ability to grow on propane nor the ability to oxidize MTBE.

Project Publications:

- Hardison, L.K., S.S. Curry, L.M. Ciuffetti, and M.R. Hyman, "Metabolism of Diethyl Ether and Cometabolism of Methyltert -Butyl Ether by a Filamentous Fungus, a *Graphium* sp.," *Applied and Environmental Microbiology*, **63**(8), 3059-3067 (1997).
- Hyman, M.R., T. Musaffika-Jeknic, P. Kwon, K. Williamson and K. O'Reilly, "Cometabolism of MTBE by Alkane-Utilizing Microorganisms; In: Proceedings Joint Conference on the Environment" WERC, New Mexico State University 197-201 (1998).
- Hyman, M. R., and K. O'Reilly, Physiological and enzymatic features of MTBE-degrading bacteria , *In: (Alleman, B. C., and A. Leeson, [eds.]), In Situ Bioremediation of Petroleum Hydrocarbons and Other Organic Compounds, pp: 7-12, Battelle Press, Columbus OH (1999).*

**Western Region Hazardous Substance Research Center Project
OS97-04. In-Situ Measurement of TCE Degradation Using a Single-Well "Push-Pull"
Test: Jonathan D. Istok, Lew Semprini, Jennifer Field, Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1997-2000

Project Amount: \$39,037

Research Category: Groundwater, chlorinated organics, demonstration, TCE

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

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

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

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

transformation studies. In a second series of field tests, the use of fumarate to probe for conditions suitable for TCFE reductive dechlorination and as a substrate to stimulate TCFE transformations was investigated. Fumarate reduction to succinate only occurred in portions of the aquifer where dechlorination was also observed. In addition, successive injections of fumarate resulted in increased transformation rates and the appearance of reductive dechlorination in portions of the aquifer where this activity had not previously been detected.

Project Publications:

- Haggerty, R., M.H. Schroth, and J.D. Istok, "Simplified Method of "Push-Pull" Test Data Analysis for Determining *In Situ* Reaction Rate Coefficients," *Ground Water*, **36**(2), 314-324 (1998).
- Istok, J.D., M.D. Humphrey, M.H. Schroth, M.R. Hyman, and K.T. O'Reilly, "Single-Well, "Push- Pull" Test for In Situ Determination of Microbial Metabolic Activities", *Ground Water*, **35**(4), 619-631 (1997).
- Schroth, M.H., J.D. Istok, G.T. Conner, M.R. Hyman, R. Haggerty and K.T. O'Reilly, "Spatial Variability in Aerobic Respiration and Denitrification Rates in a Petroleum Contaminated Aquifer," *Ground Water*, **36**(6), 924-937 (1998).

**Western Region Hazardous Substance Research Center Project
OS97-06. Development and Characterization of Redox Sensors for Environmental
Monitoring. James D. Ingle, Jr., Oregon State University.**

EPA Project Officer: Dale Manty

Project Period: 1997-2000

Project Amount: \$131,865

Research Category: Groundwater, chemistry, redox

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

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

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

Status: Three redox indicators, thionine ($E_7^{0i} = +53$ mV), cresyl violet ($E_7^{0i} = -75$ mV), and phenosafranine ($E_7^{0i} = -267$ mV), which have very different formal potentials (E_7^{0i}), have been evaluated in detail. They can be immobilized by covalent bonding to agarose beads, cellulose filter paper, and transparent films made from materials such as agarose. The agarose films are nearly transparent and are highly hydrophilic allowing for rapid diffusion of reductants (e.g., Fe(II)) and oxidants (e.g., O₂) into the structure. Although not transparent, filter paper is suitable for visual inspection or reflectance measurements. A unique spectrophotometric flow cell with removable windows was constructed to accommodate films and membranes (75- μ L volume) and can be used for field measurements with minimal filtering at flow rates as high as 50 mL/min without clogging. The indicators can be used to differentiate between Fe(III)-reducing, sulfate-reducing, and methanogenic redox levels, respectively. Results obtained in environmental samples strongly support the notion that Fe(II) and S(-II) are the primary reductants of thionine

and cresyl violet produced under these Fe(III)-reducing and sulfate-reducing conditions, respectively. The identity of the reductant(s) of phenosafranine under methanogenic conditions is unknown. Also a simple technique to preconcentrate and determine Se(IV) and Se(VI) below 1 ng/mL (1 ppb) was developed and is based on an anion-exchange filter, sequential elution, and ICP emission spectrometry.

Project Publications:

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

**Western Region Hazardous Substance Research Center Project
OS97-09. Assessing Metal Speciation in the Subsurface Environment: John C. Westall,
Oregon State University.**

EPA Project Officer: Dale Manty

Project Period: 1997-2000

Project Amount: \$107,574

Research Category: Groundwater, metals

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

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

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

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

optimum means of discretizing the affinity spectrum, relating the affinity spectrum properties to systematically varying properties of the sorbent, and coupling of the affinity spectrum models to transport models.

Project Publications:

**Western Region Hazardous Substance Research Center Project
OS97-10. Simultaneous Removal of the Adsorbable and Electroactive Metals from
Contaminated Soils and Groundwater: Peter O. Nelson, Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1997-2000

Project Amount: \$116,591

Research Category: Groundwater, metals, sorption

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

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

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

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

Project Publications:

- Azizian, M.F., K., Osathaphan, and Peter O. Nelson, "Simultaneous removal of Cu(II), Cr(VI), and As(V) metals from contaminated soils and groundwater. American Chemical Society, Environmental Chemistry Division, Vol. **40**, No. 1, 2000.
- Azizian, M.F., K., Osathaphan, and Peter O. Nelson, "Sorption and transport model for copper, chromium, and arsenic In an iron-oxide-coated sand, synthetic groundwater system," American Chemical Society, Environmental Chemistry Division, Vol. 42, No. 1, April 7-11 (2002).
- Khaodhiar, S., M. F. Azizian, and P. O. Nelson, "Copper, Chromium, and Arsenic Adsorption and Equilibrium Modeling in an Iron-Oxide-Coated Sand, Background Electrolyte System," *Water Air and Soil Pollution*, **119**(1-4), 105-120 (2000).
- Osathaphan, K., M.F. Azizian, and P. O. Nelson, "Simultaneous Removal of the Adsorbable and Electroactive Metals from Contaminated Soils and Groundwater." Proceedings, AIHM National Meeting, San Francisco, CA, November 10, 1999.
- Osathaphan, K., Azizian, M.F., and Nelson, P.O., "Chromated copper arsenate, or CCA Adsorption on an Iron-Oxide Adsorbent," Accepted for publication in American Chemical Society, Environmental Chemistry Division, Vol. **41**, No. 1, 2001.

**Western Region Hazardous Substance Research Center Project
OS99-01. Multisolute Sorption and Transport Model for Copper, Chromium, and Arsenic
Sorption on an Iron-Coated Sand, Synthetic Groundwater System: Peter O. Nelson,
Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1999-2001

Project Amount: \$114,725

Research Category: Groundwater, metals, sorption

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

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

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

Status: This project was completed in 2001. Copper, chromate, and arsenate were all significantly retarded due to the adsorption reaction during transport in the iron-oxide coated sand (IOCS) packed column. With minimal effects of flow interruption with a tracer and a small

degree of asymmetrical shape in tracer breakthrough curve, physical non-equilibrium processes were not significant. However, flow interruption experiments performed on the metals showed significant effects of chemical non-equilibrium sorption during transport for each metal. Both the TLM and EIM were successfully used to describe adsorption of copper, chromate and arsenate in both single-metal and multi-metal systems. Nonlinear Langmuir-type sorption isotherms were derived for each metal from the EIM surface complexation model. A two-site sorption model was partially successful in predicting transport in multi-metal systems. With one set of surface complexation constants from the EIM model, sorption isotherm was then incorporated into the transport model. Modeling the transport of the metals using the local equilibrium assumption (LEA) using nonlinear sorption parameters failed significantly to describe the behavior of copper and arsenate transport (R^2 values < 0.10). However, the LEA model predicted the transport of chromate fairly well.

Project Publications:

- Azizian, M.F., K., Osathaphan, and Peter O. Nelson, "Sorption and transport model for copper, chromium, and arsenic In an iron-oxide-coated sand, synthetic groundwater system," American Chemical Society, Environmental Chemistry Division, Vol. 42, No. 1, April 7-11 (2002).
- Azizian, M.F., K., Osathaphan, and Peter O. Nelson, "Simultaneous removal of Cu(II), Cr(VI), and As(V) metals from contaminated soils and groundwater. American Chemical Society, Environmental Chemistry Division, Vol. 40 No. 1, March 26-30th (2000).
- Khaodhiar, S., M.F. Azizian, and P.O. Nelson, "Equilibrium Modeling of Arsenic, Chromium, and Copper Adsorption on an Iron-Oxide-Coated Sand," Extended Abstracts, Vol. 38, No. 1. American Chemical Society, Environmental, Chemistry Division, 215th ACS National Meeting, Dallas, Texas (1998).
- Osathaphan, K., "Adsorption of Arsenate on Iron-Oxide-Coated Sand and Magnetite-Coated Sand", MS Report, Oregon State University, Corvallis, OR, (1997).
- Osathaphan, K., Azizian, M.F., and Nelson, P.O., "Simultaneous Removal of the Absorbable and Electroactive Metals from contaminated Soils and Groundwater, presented at the AIHM meeting, San Francisco, CA, November 10th (1999).
- Osathaphan, K., Azizian, M. F., and Nelson, P.O., "Copper, Chromate, and Arsenate Adsorption on an Iron-Oxide Adsorbent: (1) Adsorption Behavior," submitted (2000).
- Osathaphan, K., Azizian, M. F., and Nelson, P.O., "Copper, Chromate, and Arsenate Adsorption on an Iron-Oxide Adsorbent: (2) Adsorption Modeling," submitted (2000).
- Osathaphan, K., Azizian, M.F., and Nelson, P.O., "Chromated copper arsenate, or CCA Adsorption on an Iron-Oxide Adsorbent," American Chemical Society, Environmental Chemistry Division, Vol. 41, No. 1, pp. 436-440 (2001).
- Osathaphan K., "Multi-metal Equilibrium Sorption and Transport Modeling for Copper, Chromium, and Arsenic in an Iron Oxide-Coated Sand, Synthetic Groundwater System," Civil, Construction, and Environmental Engineering, Oregon Sate University, Ph.D. Dissertation (2001).

**Western Region Hazardous Substance Research Center Project
OS99-03. Development of Alkoxysilanes as Slow Release Substrates for the
Anaerobic/Aerobic Transformation of Chlorinated Solvents: Lewis Semprini, Oregon State
University**

EPA Project Officer: Dale Manty

Project Period: 1999-2001

Project Amount: \$104,903

Research Category: Groundwater, chlorinated organics, TCE, DCE, biodegradation

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

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

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

Status: Batch kinetic microcosm studies investigated the effectiveness of a combined mixed culture compared to the separate cultures for the reductive dehalogenation of PCE and TCE and enhanced DNAPL dissolution. The combined mixed culture was constructed by combining two different enrichment cultures, one from Point Mugu Naval Weapon Facility, CA and another from the Evanite site in Corvallis, OR. The Point Mugu (PM) culture showed reductive dehalogenation of TCE up to a concentration of 3.4 mM, but no ability to dechlorinate PCE. The PM culture rapidly dechlorinates TCE to vinyl chloride (VC), but slowly transforms VC to ETH. The Evanite (EV) enrichment is capable of reductively dechlorinating PCE at its solubility limit (0.9 mM) and completely dechlorinates PCE to ETH, but slowly transforms cis-dichloroethene (c-DCE) to VC.

In the microcosms, TBOS (69.2 mM) was mixed with PCE (8.50 mM) and TCE (8.50 mM) to yield PCE/TBOS and TCE/TBOS NAPL mixtures with a mol fraction of 0.11 PCE or TCE/NAPL. As a result of mixing with TBOS, the aqueous saturation concentrations of PCE and TCE were lowered to 0.1 mM (17 mg/L) of PCE and 0.84 mM (110 mg/L) of TCE, consistent with the expected values by Raoult's Law. Microcosms were constructed with the PM culture, the EV culture, and the combined culture, and duplicates were operated for each PCE/TBOS and TCE/TBOS mixture. As expected in the PM microcosms, PCE was not dechlorinated. VC was observed to accumulate in TCE added microcosms. In the EV microcosms, PCE and TCE were biotransformed to ETH, but c-DCE accumulated to relatively high concentrations (up to 2.89 mM = 280 mg/L) during the dehalogenation processes, which resulted in the slow dehalogenation of PCE and TCE to ETH. Microcosms with the combined mixed culture were observed to reductively dechlorinate both PCE and TCE, and showed lower concentrations of c-DCE and VC compared to EV and PM cultures, respectively. The rate of ETH production by the combined mixed culture with PCE added were much higher than PM and EV cultures alone. In all the systems, dehalogenation occurred in the absence of methanogenesis.

Microcosm experiments were also performed to study the effectiveness of TBOS for bioremediation of PCE DNAPLs using the combined mixed culture. The mixing of PCE DNAPL with TBOS lowered effective aqueous concentrations of PCE. Three different PCE mol fractions: 0.02 (PCE 1.46 mM : TBOS 69.25 mM), 0.11 (PCE 8.47 mM : TBOS 69.25 mM), and 0.50 (PCE 69.25 mM : TBOS 69.25 mM) were studied. The highest rate of ETH production was observed in the 0.02 mol fraction microcosms. A microcosm with (8.47 mM) and 1-butanol as a substrate, showed no reductive dehalogenation at a PCE mol fraction of 0.50. Lowering aqueous PCE concentrations obtained by mixing NAPL PCE with TBOS appears to reduce the toxicity or inhibition effects of chlorinated ethylenes, making conditions more favorable for dehalogenation.

The results thus far show the potential for the biologically treating PCE and TCE NAPL phases by adding TBOS to the NAPLs. The combining of two different mixed cultures has resulted in more effective transformation. The reduction of chlorinated ethylene concentrations by mixing NAPLs with TBOS has enhanced rates of reductive dehalogenation.

In our aerobic studies, an enrichment culture has been isolated that is able to cometabolize c-DCE and VC when grown on butyrate. This result is significant, since butyrate is produced during the fermentation of 1-butanol, and is present in the batch incubations since it is a slowly fermenting anaerobic substrate. Thus, residual concentrations of c-DCE and VC might be cometabolized under aerobic conditions using microorganisms grown on the butyrate that is

formed from TBOS transformation under anaerobic conditions. The results indicate the potential for sequential anaerobic/aerobic treatment through TBOS addition.

Our future studies will evaluate TBOS as a slow release substrate in flow through column tests. The combined anaerobic culture will be used in these tests. If complete transformation to ETH is not achieved, an aerobic polishing step will be used where only oxygen is provided, with the remaining organic acids used as cometabolic substrates.

Project Publications:

- Semprini, L., Vancheeswaran, S., Yu, S., Min-Ying Chu, M-Y, and Halden, R.U., "Tetraalkoxysilanes as slow release substrates to promote aerobic and anaerobic dehalogenation reactions in the subsurface," Extended Abstracts of American Chemical Society, Division of Environmental Chemistry, 220th ACS National Meeting, Washington, D.C. August 20-24, Vol. 40 No. 2. page 350-351 (2000).
- Semprini, L. and S. Vancheeswaran U.S. Patent Application Serial No. 09/331,992: "Slow Release Substrates for Driving Microbial Transformations of Environmental Contaminants," April (2002).
- Vancheeswaran, S., R.U. Halden, K.J. Williamson, J.D. Ingle, and L. Semprini. "Abiotic and Biological Transformation of Tetraalkoxysilanes and Trichloroethene/cis-1,2-Dichloroethene Cometabolism Driven by Tetrabutoxysilane-Degrading Microorganisms," *Environ. Sci. Technol.* **33**(7), 1077-1085 (1999).
- Yu, S., and Semprini, L.. "Comparison of Trichloroethylene Reductive Dehalogenation by Microbial Communities Stimulated on Silicon-based Organic Compounds as Slow-release Anaerobic Substrates." *Water Research*, **36**(20), 4985-4996 (2002).
- Yu, S. and L. Semprini, "Dechlorination of PCE DNAPL with TBOS Using a Binary Mixed Culture", The 3rd International Conference on Remediation of Chlorinated and Recalcitrant Compounds, May 20-23, Monterey, California (2002).

**Western Region Hazardous Substance Research Center Project
OS99-04. Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbons by Toluene-Oxidizing Bacteria: Physiological Consequences and Adaptive Responses: Peter J. Bottomley and Daniel J. Arp, Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1999-2001

Project Amount: \$86,455

Research Category: Groundwater, chlorinated organics, TCE, biodegradation

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

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

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

Status: This project was completed in 2001. Toluene-grown cells of *B. cepacia* G4 oxidized ethylene and propylene to their respective epoxides with no observable effect on cell culturability or general respiratory activity. In contrast, TCE oxidation, which produces TCE epoxide as an initial product, produces a variety of cytotoxic effects. Although toluene 2-monooxygenase activity was lost during TCE oxidation, accumulation of general cellular damage was more rapid. For example, loss of cell culturability and acetate-dependent O₂ consumption were rapid relative to loss of oxygenase activity. However, loss of cell viability was

dependent on the method of assessment. TCE-injured cells were sensitive to peroxide generated during autoclaving of agar. Most-probable-number dilution series were the most reliable method of determining cell viability. Tn5 mutants of *B. cepacia* were generated and screened for increased sensitivity to TCE oxidation. Genes putatively encoding enzymes involved in DNA repair were disrupted (UvrB, RuvB, RecA, RecG). A smaller group of genes involved in carbon metabolism were also disrupted. The work with toluene oxidizers was completed with a study of induction of toluene oxygenases in three bacteria when grown on various carbon sources. For example, *B. cepacia* grown on fructose induces substantial levels of toluene monooxygenase in the presence of TCE.

Project Publications:

- Yeager, C. M., P. J. Bottomley, D. J. Arp, and M. R. Hyman. Mechanism-based inactivation of toluene 2-monooxygenase in *Burkholderia cepacia* G4 by alkynes. *Appl. Environ. Microbiol.* 65, 632-637 (1999).
- Yeager, C.M., P.J. Bottomley, and D.J. Arp. Cytotoxicity associated with trichloroethylene oxidation in *Burkholderia cepacia* G4. *Appl. Environ. Microbiol.* In press (2001)
- Yeager, C. M., P. J. Bottomley, and D. J. Arp. Requirement of DNA repair mechanisms for survival of *Burkholderia cepacia* G4 upon degradation of trichloroethylene. *Appl. Environ. Microbiol.* 67, 5384-5391 (2001).
- Arp, D. J., C.M. Yeager and M. R. Hyman. Molecular and Cellular Fundamentals of Aerobic Cometabolism of Trichloroethylene, *Biodegradation*. 12: 81-103, 2001 (2001)
- Chris Yeager, Ph.D. Dissertation, "Physiological consequences of trichloroethylene degradation by the toluene-oxidizing bacterium *Burkholderia cepacia* G4" (2001).

**Western Region Hazardous Substance Research Center Project
OS99-05. Development and Characterization of Sensors and Field Instrumentation for
Monitoring of Environmental Redox Conditions. James D. Ingle, Jr., Oregon State
University**

EPA Project Officer: Dale Manty

Project Period: 1999-2001

Project Amount: \$114,130

Research Category: Groundwater, chemistry, redox

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

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

Approach: The major focus of this study is determining how redox sensors based on immobilized, colored redox indicators can be constructed and used to determine when certain biogeochemical redox processes are occurring. These redox processes include microbially-mediated transformations of organic (e.g., TCE) and inorganic (e.g., As, Se, Cr) contaminants and of major matrix components which sustain an environmental system at a given redox level (e.g., Fe(III)-reducing, sulfate-reducing, methanogenic, or dechlorinating conditions). The response of the indicators in a variety of samples will be correlated to specific types of microbial activity, the appearance or disappearance of specific matrix species (e.g., sulfide, Fe(II), CH₄, and H₂), or the transformation of a particular organic or inorganic contaminants. The immobilized indicators will be incorporated into simple, miniature spectrochemical and electrochemical devices to make them attractive for monitoring redox conditions in laboratory vessels and in field situations.

Status: We have demonstrated that the redox indicators thionine (formal potential or $E_7^{0'}$ = +53 mV), cresyl violet ($E_7^{0'}$ = -75 mV), and phenosafranine ($E_7^{0'}$ = -267 mV) respond to environmental reductants and oxidants and are useful for delineating between Fe(III)-reducing conditions, sulfate-reducing conditions, and methanogenic conditions in reduced soil, waste water slurries, and ground water with an active dechlorinating culture. These indicators are immobilized in robust, thin, transparent polymer films that are incorporated into small and portable devices (flow cells and spectrometers) that allow the sample solution to be in contact with the indicators. This arrangement allows monitoring of anaerobic redox conditions of samples in numerous types of closed systems in both laboratory and field settings. These devices have been shown to provide a response due to changes in the color of the indicator that

indicates the onset of dechlorinating conditions in laboratory microcosm bottles and of the dependence of Fe(III)-reducing conditions on time and depth in sub-surface soil. The indicators and devices can be used for continuous monitoring of redox conditions over weeks because the color change of the indicator is reversible.

Project Publications:

Jones, B. D. and Ingle, Jr., J. D. "Evaluation of Immobilized Redox Indicators as Reversible, In Situ Redox Sensors for Determining Fe(III)-Reducing Conditions in Environmental Samples," *Talanta*, in press (2001).

**Western Region Hazardous Substance Research Center Project
OS99-06. Aerobic Cometabolism of Methyl tert-butyl Ether by Microorganisms Grown on
Aliphatic Hydrocarbons: Kenneth J. Williamson and Linda Ciuffetti, Oregon State
Univeristy**

EPA Project Officer: Dale Manty

Project Period: 1999-2000

Project Amount: \$93,545

Research Category: Groundwater, MTBE, biodegradation

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

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

Approach: The approach will include:

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

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

Status: *Graphium* sp., a fungus and a bacterial strain, *Mycobacterium vaccae* JOB5, were tested for their ability to use both propane and isopentane as growth substrates. While *n*-alkane grown cultures were able to aerobically cometabolize MTBE, no growth of either strain was observed when MTBE was used as the sole source of energy and carbon. The transformation capacity of these cultures was experimentally determined. In bacterial cultures, the rate of MTBE consumption is faster when isopentane is used as the initial growth substrate in comparison to propane. Propane grown *Graphium* cultures showed low MTBE oxidation rates in liquid suspension.. To better quantitate fungal biomass, filter-attached cultures were used in later experiments. GC analysis confirmed that *Tert*-butyl formate and *tert*-butyl alcohol (TBF & TBA) are initial breakdown products of MTBE oxidation. In both *M. vaccae* and *Graphium*, the degradation rates went from high to low in the following order: TBF, MTBE and TBA. TBF is

hydrolyzed both biotically and abiotically to TBA. The biotic TBF hydrolysis rate in both organisms was determined through a series of experiments with different initial concentrations of TBF. Aerobic cometabolism of MTBE was investigated in both *Graphium* and *M. vaccae* cultures. The kinetics of this reaction were established. In the bacterial system, the K_{max} and the K_s of this reaction were determined for isopentane and propane grown cultures. In the fungal system, the K_{max} and K_s were established only for propane grown fungal cultures. Isopentane-grown *Graphium* cultures were not tested for MTBE oxidation activity because of their very slow growth rate and low mycelial yield on this substrate, even at a five-fold inoculum increase. K_s and K_{max} were experimentally determined by adding varying concentrations of MTBE, TBF and TBA to both fungal and bacterial cultures. Consumption of these substrates was followed using gas chromatography assays. Similar experiments were carried out to determine the inhibitory effects of each growth substrate on the rate of MTBE, TBF and TBA degradation. Inhibition type (competitive) and inhibition coefficient (K_{ic}) was determined for the bacterial system by resting cell experiments in batch reactors. Non-linear least squares regression (NLSR), reciprocal plot, and the direct linear plot methods were used to determine the kinetic parameters. Resting cell experiments under anaerobic conditions were also conducted. Oxidation of propane, isopentane, MTBE and TBA was not observed under these conditions. However, TBF degradation occurred at a considerable rate. These results imply that a different enzyme is responsible for the hydrolysis of TBF. Blocking experiments were conducted in bacterial and fungal cultures. In both systems, the addition of acetylene inhibited the oxidation of propane, isopentane, MTBE and TBA but not TBF. In the bacterial system, studies were performed with acetylene from two different sources (a) commercial cylinder and (b) calcium carbide generated, and with two different solutions (a) growth media and (b) phosphate buffer solution. Experiments to determine the rates of MTBE and iso-pentane oxidation (present at the same time) by *M. vaccae* were conducted. Complete consumption of isopentane, oxygen, MTBE and its breakdown products (TBF & TBA) in triplicate growth batch reactors were followed by gas chromatography (head- space and liquid injection samples). At each sampling time, the biomass produced was monitored by optical density measurement and protein analysis. A mathematical expression will be proposed to model the results obtained, where the kinetic constants from the single-compound experiments will be used.

Project Publications:

**Western Region Hazardous Substance Research Center Project
OS99-07. Assessing Metal Speciation in the Subsurface Environment - Effect of Wet-Dry
Cycles in the Vadose Zone: John C. Westall, Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1999-2002

Project Amount:

Research Category: Groundwater, inorganic, speciation, sorption

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

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

Approach: Three fundamental barriers to successful description of the association of metal ions with heterogeneous environmental sorbents under varying water content are addressed: (i) the paucity of multidimensional datasets (i.e., datasets with variations in many solution chemistry properties such as pH, salt concentration, total metal concentration, etc.) for sorption of inorganic contaminants; (ii) the paucity of data for sorption of inorganic contaminants in media that are subject to variations in water content; and (iii) the inadequacy of detailed mechanistic models in dealing with these data. First, for water-saturated media, data for binding of inorganic contaminants to heterogeneous environmental sorbents will be collected; the initial focus will be on inorganic priority pollutants common to groundwater pollution problems. Then the "discrete log K spectrum" or "affinity spectrum" approach to modeling the interactions in these systems will be further developed and applied. This modeling approach might appear to be more empirical and less mechanistic than the traditional "surface complexation - electrostatic" approach, but is the method of choice for most real, complex, heterogeneous environmental materials. Finally, the work with saturated soils will be extended to include the effect of wet-dry

cycles in unsaturated soils. Because of the variation in water content during these cycles, the binding of inorganic contaminants to soil surfaces may be described more accurately as precipitation-dissolution than as adsorption-desorption. Binding data will be collected will be collected from soils that are exposed to metal ions in a saturated condition, subsequently allowed to dry, and then re-wetted to allow the bound metal to be extracted; laboratory soil column experiments will be used extensively in this phase of the experiment.

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

Publications:

**Western Region Hazardous Substance Research Center Project
R95-04. Biotransformation of Lead and Chromate by Bacteria: Robert C. Blake, Xavier University**

EPA Project Officer: Dale Manty

Project Period: 1995-1997

Project Amount: \$115,000

Research Category: Groundwater, metals, lead, chromium, biotransformation

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

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

Approach: The specific aims are two-fold: (1) To perform detailed kinetic studies on the transformation and immobilization of soluble lead and chromium as catalyzed by strain OR-02 and related organisms. The purpose of these experiments is two-fold: (i) to identify the optimum solution conditions necessary to achieve maximal and timely removal of each soluble metal species; and (ii) to identify and document potential sources of interference for each metal transformation activity. The latter experiments focus on the possible inhibition of metal transformations by metal-complexing agents and/or other metal ions. (2) To investigate various means for immobilization of strain OR-02 and to determine the efficacy of the immobilized bacterium for the removal of lead and chromium from contaminated waters. Immobilization strategies include, but are not limited to, entrapment within calcium-alginate beads and adsorption of the live bacteria onto a hydrophobic, porous polyurethane.

Status: When native cells of strain OR-02 were cultured in the presence of 1.0 mM Pb(II), growth of the organism was accompanied by the quantitative disappearance of the soluble Pb(II) from solution. The missing lead was all contained in small brown-black biocolloids from 50 to 150 nanometers in diameter (by contrast, the intact bacterial cells were rod-shaped with a diameter of 0.5 to 1.0 micrometers and a length of approximately 3 to 5 micrometers). The exact formulation of the culture medium under otherwise optimal conditions had no quantifiable influence on the disappearance of soluble lead. Thus the timely bacterial-dependent immobilization of soluble lead occurred equally well in both minimal (glucose plus mineral salts) and complex (tryptone plus yeast extract) media, even though components of the latter formed tight complexes with the lead cation. Growth of strain OR-02 in the presence of chromate was accompanied by the gradual (24 to 48 hr) electrochemical reduction of the yellow Cr(VI) (chromate) to the faint blue-green trivalent Cr (III). Strain OR-02 grew after a 2-hr lag period when inoculated into rich media containing up to 1.0 mM chromate. Optimal culture

conditions for the bacterial-dependent reduction of Cr(VI) were identical to those for the corresponding immobilization of lead: rich media (Luria broth); pH 7.5; and 25° C. Although bacterial growth was severely inhibited at 5.0 mM chromate, biological reduction of the chromate was still observed. Indeed, effective reduction of up to 30 mM chromate was readily observed with cell suspensions of strain OR-02, although the bacterium did not grow under such conditions. Project complete

Project Publications:

**Western Region Hazardous Substance Research Center Project
R95-11. Magnetic Resonance Studies of Heavy Metals in Clays, Zeolites and Ceramics:
Cynthia J. Hartzell and Michael P. Eastman, Department of Chemistry, Northern Arizona
University**

EPA Project Officer: Dale Manty

Project Period: 1995-1997

Project Amount: \$79,658

Research Category: Groundwater, metals

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

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

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

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

Project Publications:

**Western Region Hazardous Substance Research Center Project
R95-14. Probing the Redox Properties of Environmental Systems: Natural Phenolic
Materials: Robin Helburn, Department of Chemistry, Northern Arizona University**

EPA Project Officer: Dale Manty

Project Period: 1995-1997

Project Amount: \$70,357

Research Category: Groundwater, chemistry, organics

Goal: The long term goals of this project have been to: (1) use cyclic voltammetry to study the redox properties of some natural polyphenolic materials, and (2) investigate the redox properties and environmental applications of a colored water soluble organic free radical. We have proposed that the latter dye(s) (verdazyls) may be used to probe the free radical scavenging behavior or natural polyphenols in environmental and biological systems; we have found such hypothesis to be untenable, but are still characterizing the electrochemical properties of these interesting indicators.

In addition to the above projects, we have pursued a new goal that overlaps directly with the interests of our HSRC collaborator (i.e. sensor development). In the new project, a student has developed a simple method for interfacing probe-type sensors to portable calculators (i.e. for use in the field).

Rationale: The success of environmental remediation depends on our ability to obtain a scientific understanding of chemical processes occurring at a given site. An understanding of redox processes as well as methods for developing portable analytical tools constitutes useful and related research.

Approach: Standard cyclic voltammetry and step potential methods have been used to characterize the polyphenols and verdazyls. The sensor-calculator interfacing was performed using a Texas Instruments TI-85 calculator. The interface design was based on I²C technology.

Status: Project completed.

Project Publications:

Hickman, A. B., W. Delinger, R. Helburn. "Calculator -Based Instrumentation: The Design of a Digital Interface Based on (IC)-C-2 Technology". *J. Chem. Ed.* **77**(2), 255-257 (2000).
Hickman, A,B., "Electrochemical Characterization of 1,3,5-Triphenylverdazyls; Design of a Digital Interface Between Calculators and Sensors Based on I²C Technology." (masters thesis). R. Helburn, M.P. Eastman, A. Mayer. "Electrochemical Studies of a Series of Triphenylverdazyls". *Pacific Conference on Chemistry and Spectroscopy*. Irvine, CA October 21-25 1997.

Yardy, N., R.W. Zoellner, R. Helburn. A Combined Electrochemical and Computational Study of Quercetin and Redox Homologs. *Proceedings of the National Council on Undergraduate Research*. 1996. Vol. 3, 1668-1671.

**Western Region Hazardous Substance Research Center Project
R95-15. Reductive Dehalogenation at Carbon and Derivatized Carbon Electrodes:
Merritt Helvenston, New Mexico Highlands University**

EPA Project Officer: Dale Manty

Project Period: 1995-1997

Project Amount: \$149,394

Research Category: Groundwater, chlorinated organics, electrochemistry

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

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

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

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

Project Publications:

Western Region Hazardous Substance Research Center Project
R95-24. Detection of Microorganisms Capable of Anaerobic Degradation of Hazardous Substances in Natural Environments: Sara E. Silverstone, State University of New York, College at Brockport

EPA Project Officer: Dale Manty

Project Period: 1995-1997

Project Amount: \$111,405

Research Category: Groundwater, chlorinated organics, BTEX

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

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

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

Status: Two 16S rRNA probes specific to the anaerobic toluene-degrader PRTOL1 were developed and used for monitoring the growth of PRTOL1 in a soil slurry amended with various carbon sources, and also capable of enumerating PRTOL1 added to Seal Beach soil to a minimum concentration of 10×10^5 cells/ gram. Three highly specific probes were identified for the anaerobic ethylbenzene-degrader EB1 and hybridization conditions optimized for use in Seal Beach and Moffett Field soils. The 16S rRNA sequence was determined for the anaerobic PCE-degrader MS-1 and two highly specific probes were identified. These probes are currently being used in microcosm experiments using Moffett Field aquifer material. Results indicate that the Moffett Field aquifer contains no indigenous MS-1 that MS-1 can survive in Moffett Field aquifer microcosms for up to 21 days, after which time populations decline below 10×10^5 cells/ml. Project complete.

Project Publications:

**Western Region Hazardous Substance Research Center Project
SU89-01. Treatment of Complex Mixtures: Martin Reinhard and Paul V. Roberts,
Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1989-1993

Project Amount: \$285,178

Research Category: Groundwater, organic contaminants, treatment, model

Goal: A computer-aided information system was developed for estimating physico-chemical properties of organic compounds needed for the design of treatment processes and assigning 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, evaluation, retrieval, and estimation of physico-chemical properties, including octanol-water partition coefficient, aqueous solubility, vapor pressure, molar volume and refraction, and Henry's coefficient.

Status: With the completion of a user interface and a manual, DESOC has been released. The capabilities and applicability of DESOC for air water partitioning and complex treatment problems has been demonstrated. A novel algorithm (GIMOC) for estimating compound properties was developed and is incorporated in DESOC. GIMOC is a combination of the group contribution model and similarity based approaches. Project complete.

Project Publications:

Drefahl, A. and M. Reinhard, "Similarity-Based Search and Evaluation of Environmentally Relevant Properties for Organic Compounds in Combination with the Group Contribution Approach," *J. Chem. Info. Comp. Sci.*, **33**, 886-895 (1993).

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

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

**Western Region Hazardous Substance Research Center Project
SU89-02. Oxidation of Chlorinated Solvents by Methanotrophs: Perry L. McCarty,
Stanford University (Supported by the Gas Research Institute)**

EPA Project Officer: Dale Manty

Project Period: 1989-1993

Project Amount: \$344,816

Research Category: Groundwater, chlorinated organics, biodegradation, aerobic, methane

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 they are used in combination are then being predicted and measured in order to test the hypothesis. The effect of reactor operational parameters such as detention time and nitrogen supply on the quantity of methane required to effect a given amount of TCE transformation are being evaluated. 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. The effects of reactor detention time and nitrogen nutrient concentration on transformation capacities and yields have been evaluated. 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. Also, reduced detention time and reduced nitrogen supply changes culture characteristics, but does not result in significant changes in the transformation yield (ratio of TCE transformed to methane consumed). Transformation yield was found to change more radically with time for a given mixed culture than with the variables explored. This study is now completed.

Project Publications:

- Henry, S.M. and D. Grbić -Galić , “Influence of Endogenous and Exogenous Electron Donors and Trichloroethylene Oxidation Toxicity on Trichloroethylene Oxidation by Methanotrophic Cultures from a Ground Water Aquifer,” *Applied and Environmental Microbiology*, **57**(1), 236-244 (1991).
- Henry, S.M. and D. Grbić -Galić , “Inhibition of Trichloroethylene Oxidation by the Transformation Intermediate Carbon Monoxide,” *Applied and Environmental Microbiology*, **57**, 1770-1776 (1991).
- Henry, S.M., *Trichloroethylene Transformation by Methanotrophs from a Groundwater Aquifer*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1991).
- Henrysson, T. and P.L. McCarty, “Influence of the Endogenous Storage Lipid Poly- β -Hydroxybutyrate on the Reducing Power Availability During Cometabolism of Trichloroethylene and Naphthalene by Resting Methanotrophic Mixed Cultures,” *Applied and Environmental Microbiology*, **59**(5), 1602-1606 (1993).
- McCarty, P. L., “Transferability of Biotreatment from Site to Site,” *Wider Application and Diffusion of Bioremediation Technologies, The Amsterdam '95 Workshop*, OECD, Paris, 201-210 (1996).
- McCarty, P.L., “Engineering Concepts for In-Situ Bioremediation,” Proceedings, *Bioremediation Fundamentals & Effective Applications*, 3rd Annual Symposium, Gulf Coast Hazardous Substance Research Center, 3-13 (1991).
- McCarty, P.L., “Factors Affecting Biotransformation Rates in Groundwater” *Groundwater Contamination: Application of Research to Management Problems in Asia and the Pacific*, Proceedings of the 2nd SCOPE Regional Workshop, Chiang Mai, Thailand, 261-263 (1995).
- McCarty, P.L., “In Situ Bioremediation of Chlorinated Solvents,” *Current Opinion in Biotechnology*, **4**(3), 323-330 (1993).
- McCarty, P.L., “Transport, Fate, and In-Situ Bioremediation of Chlorinated Solvents in Groundwater,” *Proceedings of the Japanese Association of Groundwater Hydrology*, Yokohama, 116-135 (1992).
- Smith, L. H., P. L., McCarty, and P. K. Kitanidis, "Spreadsheet Method for Evaluation of Biochemical Reaction Rate Coefficients and Their Uncertainties by Weighted Nonlinear Least-Squares Analysis of the Integrated Monod Equation," *Applied and Environmental Microbiology*, **64**(6), 2044-2050 (1998).
- Smith, L. H., P.K. Kitanidis, and P.L. McCarty, “Numerical Modeling and Uncertainties in Rate Coefficients for Methane Utilization and TCE Cometabolism by a Methane Oxidizing Mixed Culture,” *Biotechnology and Bioengineering*, **53**(3), 320-331 (1997).
- Smith, L.H. and P.L. McCarty, “Laboratory Evaluation of a Two-Stage Treatment System for Cometabolism by a Methane-Oxidizing Mixed Culture,” *Biotechnology and Bioengineering*, **55**(4), 650-659 (1997).

Smith, L.H., *Performance of a Two-Stage Process for Cometabolic Treatment of Trichloroethylene-Contaminated Water by Methane-Oxidizing Mixed Cultures*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1996).

**Western Region Hazardous Substance Research Center Project
SU89-03. Detection and Assessment of Subsurface Contamination: Peter K. Kitanidis,
Stanford University.**

EPA Project Officer: Dale Manty

Project Period: 1989-1992

Project Amount: \$195,187

Research Category: Groundwater, characterization

Goal: The thrust of this project is the development of better techniques for characterizing contaminated sites. Objectives include the development of better computational 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 for characterizing a contaminated site, especially at the local scale. Measurements of some critical transport parameters, such as hydraulic conductivity, indicate variability over orders of magnitude over short distances. Other parameters, such as thermodynamic constants and rate coefficients, are quite variable too. In many cases, however, determination of 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, is sufficient and is what is sought by this study.

Approach: The developed approach combines measurements, mechanistic models describing the transport and fate of pollutants, and statistical methods. In recognition of the spatial variability of geologic formations and incomplete knowledge of their properties, the parameters which determine the transport and fate of solutes, such as conductivity, retardation, and reaction coefficients, are characterized in statistical terms. The mathematical formalism of random functions is used to describe spatially variable quantities through statistical moments, such as mean and covariance functions. These moments 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 are applied to two problems of practical interest: the evaluation of the macroscopic, or field-scale, flow and transport parameters and the conditioning of predictions on measurements.

Status: Initial efforts focused on the determination of the relation between the measurable but highly erratic local parameters and the effective parameters which govern the flow and solute transport at macroscopic scales of interest. The second year produced a numerical spectral method for carrying out computations of interest. Project complete.

Project Publications:

- Chrysikopoulos, C., *Solute Transport in Porous Media: Investigation of Well-to-Well Circulation and Analysis of Spatially Variable Retardation*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1991).
- Chrysikopoulos, C.V., P.K. Kitanidis, and P.V. Roberts, "Analysis of One-Dimensional Solute Transport through Porous Media with Spatially Variable Retardation Factor," *Water Resources Research*, **26**(3), 437-446 (1990).
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- Dykaar, B.B. and P.K. Kitanidis, "Determination of Effective Hydraulic Conductivity for Heterogeneous Porous Media Using a Numerical Spectral Approach: 1. Method," *Water Resources Research*, **28**(4), 1155-1166 (1992).
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**Western Region Hazardous Substance Research Center Project
SU89-04. Design of Reliable and Cost-Effective Mitigation Schemes: Steven M. Gorelick,
Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1989-1992

Project Amount: \$181,088

Research Category: Groundwater, treatment, optimization modeling

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 for 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 study is complete. A simulation - optimization model, which accounts for parameter uncertainty, was constructed and run for remediation of a single plume at the Gloucester site. Parameter estimates and uncertainties were obtained using the coupled-process approach. It was found that achieving 90% reliability, versus 50% reliability, of remediation required about 30% over-design of the pumping system. A model was also developed for optimal remediation of three plumes that each travel at different retarded velocities. Pumping from two wells proved to be optimal for the multiple-plume system and required about three times the pumping compared to removal of a single plume. Finally, work was initiated to consider plume migration and potential remediation when transport is mediated by mass-transfer kinetics.

Project Publications:

- Gailey, R.M. and S.M. Gorelick, "Design of Optimal, Reliable Plume Capture Schemes: Application to the Gloucester Landfill Groundwater Contamination Problem," *Ground Water*, **31**(1), 107-114 (1993).
- Gailey, R.M., A.S. Crowe, and S.M. Gorelick, "Coupled Process Parameter Estimation and Prediction Uncertainty Using Hydraulic Head and Concentration Data," *Advances in Water Resources*, **14**(5), 301-314 (1991).
- Gailey, R.M., *Design of Cost-Effective, Reliable Remedial Pumping Schemes: Application to the Gloucester Landfill Groundwater Contamination Problem*, M.S. Thesis, Stanford University, Stanford, CA (1990).
- Gorelick, S.M., "Large Scale Nonlinear Deterministic and Stochastic Optimization: Formulations Involving Simulation of Subsurface Contamination," *Mathematical Programming*, **48**, 19-39 (1990).
- Haggerty, R., *Design of Multiple Contaminant Remediation in the Presence of Rate Limitations*, M.S. Thesis, Department of Applied Earth Sciences, Stanford University, Stanford, CA (1992).

**Western Region Hazardous Substance Research Center Project
SU89-06. Gaseous Stripping of Nonaqueous Phase Liquids from the Vadose Zone: Martin
Reinhard and Paul V. Roberts, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1989-1992

Project Amount: \$205,280

Research Category: Groundwater, vadose, contaminant transport

Goal: The objective of this research was to investigate the rates of organic contaminant desorption from soils and sediments and elucidate the mechanisms responsible for slow desorption rates.

Rationale: Because soil-air-venting, pump-and-treat, and bioremediation are all predicated on desorption of sorbed contaminants, remediation of soils and sediments contaminated by organic solvents is often limited by the release of contaminants sorbed within porous soil or sediment grains. Assessment of desorption rates and an understanding the mechanisms responsible for contaminant release will yield: better appraisals of when conditions are favorable for a particular remediation strategy, better estimates of remediation times, and the development of strategies for enhancing desorption rates

Approach: The methods used in this study allowed both early and late time measurement of desorption rates. The improved methodology was capable of resolving desorption rates over more than seven orders of magnitude, and was used to measure both desorption isotherms and desorption kinetics for chloroform, trichloroethylene, and tetrachloroethylene from model solids, aquifer sediments, and soil. The use of model solids with selected and controlled properties facilitated investigation of both the mechanisms responsible for organic vapor sorption, and those controlling desorption rates.

Status: The study resulted in the following major conclusions. The improved experimental techniques revealed that, contrary to prior investigations over more limited concentration ranges, the pore diffusion model was not adequate to describe both the fast and slow desorbing fractions. For the experiments with contaminant vapor concentrations near saturation, the pore diffusion model was able to describe desorption of the fast desorbing fraction, but was not able to describe the secondary, slowly released fraction. The pore diffusion model considers diffusion in mesopores to be rate limiting. Experiments performed at different initial concentrations showed that the pore diffusion model may be erroneously fit to desorption rate data over limited concentration ranges. Consequently, the pore diffusion model is severely limited for predictive purposes beyond measured concentration ranges, or for extrapolation to other systems. Measurable solid properties including internal porosity, organic matter content, internal surface area, and pore and particle size showed no correlation with the fraction of slow desorbing contaminant. Only the slopes of the desorption isotherms correlated with the fractions of slowly released contaminants. However, decreasing pore diffusion rates resulting from isotherm nonlinearity were not sufficient to account for the decreasing desorption rates. The kinetic and

isotherm data implicate intragranular micropores of mineral solids as responsible for both the isotherm nonlinearity, and the slowly released fraction. This is in contrast to previous investigations implicating soil organic matter as responsible for the slow release of sorbed contaminants. Results from this research indicate that current models for predicting desorption rates are not adequate over large concentration ranges, or for assessing rates *a priori* based on the measured solid and contaminant physical properties. Without an adequate mechanistic model for contaminant desorption, it appears that actual measurements of desorption rates must be made to provide engineers and regulators with the decontamination rates to be expected under environmental conditions. Project complete.

Project Publications:

- Farrell, J. and M. Reinhard, "Desorption of Halogenated Organics from Model Solids, Sediments, and Soil Under Unsaturated Conditions: 1. Isotherms," *Environmental Science and Technology*, **28**(1), 53-62 (1994).
- Farrell, J. and M. Reinhard, "Desorption of Halogenated Organics from Model Solids, Sediments, and Soil Under Unsaturated Conditions: 2. Kinetics," *Environmental Science and Technology*, **28**(1), 63-72 (1994).
- Farrell, J. and M. Reinhard, "Measurement of Organic Vapor Isotherms on Wet Soils and Aquifer Materials," *Current Practices in Ground Water and Vadose Zone Investigations, ASTM STP 1118*, Eds., D.M. Nielsen and M.N. Sara, American Society for Testing and Materials, Philadelphia (1991).
- Farrell, J., *Desorption Equilibrium and Kinetics of Chlorinated Solvents on Model Solids, Aquifer Sediments, and Soil*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1993).
- Grathwohl, P. and M. Reinhard, "Desorption of Trichloroethylene in Aquifer Materials: Rate Limitation at the Grain Scale," *Environmental Science and Technology*, **27**(12), 2360-2366 (1993).
- Grathwohl, P. and M. Reinhard, *Sorption and Desorption Kinetics of Trichloroethene in Aquifer Material under Saturated Conditions*, Technical Report No. WR-2, WRHSRC, Department of Civil Engineering, Stanford, CA (1991).

**Western Region Hazardous Substance Research Center Project
SU89-07. Anaerobic Microbial Transformation of Homocyclic and Heterocyclic
Polynuclear Aromatic Hydrocarbons: Dunja Grbić -Galić , Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1989-1992

Project Amount: \$189,936

Research Category: PAH, groundwater, biodegradation, aromatic compounds

Goal: Monoaromatic hydrocarbons, such as toluene and benzene, have been shown to undergo microbial degradation in the absence of oxygen, and even to serve as microbial growth substrates under those conditions. However, reports on anaerobic biodegradability of polynuclear aromatic hydrocarbons (PAH) are scarce. The goal of this project is to seek anaerobic microbial activity toward PAH and complex nitrogen, sulfur, and oxygen heterocycles (NSO) under sulfate-reducing and methanogenic conditions. The primary emphasis is on evaluation of biotransformation routes once the activity is established.

Rationale: PAH and NSO are chemically stable; some of them are potential carcinogens. They are widespread environmental contaminants. Frequently, oxygen is not present in the contaminated habitats, so that the anaerobic transformation is the only possibility for detoxification or destruction of these compounds. Thus, it is important to know whether such transformations can occur, and if so, what are the microbial physiological groups involved, what is the degree of transformation, what intermediates and products are formed, and what interactions occur in mixtures of PAH and NSO.

Approach: Saturated batch microcosms with aquifer solids from the anoxic zone of the Pensacola, Florida, creosote-contaminated aquifer have been amended with defined prerduced mineral medium, and with either single PAH (indene, naphthalene, acenaphthene) and NSO compounds (indole, quinoline, isoquinoline, benzothiphenene, benzofuran), or with mixtures of some of these chemicals. In some cases lactate, benzoate, or mixtures of benzoate and fatty acids have been added to examine the possibility of cometabolic transformation. In some microcosms, sulfate has been added as a potential electron acceptor. Once transformations are observed, attempts of culture enrichment and isolation will be started. In addition to aquifer-derived microcosms, mixed methanogenic cultures (originally obtained from sewage sludge), shown to degrade toluene, benzene, naphthalene, and acenaphthene, will be evaluated.

Status: Indene, naphthalene, indole, quinoline, isoquinoline, benzothiphenene, and benzofuran have been completely degraded (to CO₂ and CH₄) as single substrates under methanogenic conditions, within 1-2 months of incubation at 35°C. With quinoline and isoquinoline, transformation intermediates have been detected. Degradation occurred when the compounds were present as sole carbon and energy sources, but degradation was accelerated in the presence of additional organic substrates (benzoate, fatty acids). In addition, transformation of acenaphthene (a PAH) to 1-acenaphthenol and quinoline to 2-quinolinol have been observed under sulfate-reducing conditions. Further degradation of 2-quinolinol to unknown products has

also been noted. The sulfate reduction occurs concomitant with acenaphthene and quinoline transformation, and these transformations are dependent on sulfate reduction (and vice versa), indicating participation of sulfate reducers in the degradation process. Project complete.

Project Publications:

- Edwards, E.A. and D. Grbić -Galić , “Anaerobic Degradation of Toluene and o-Xylene by a Methanogenic Consortium,” *Applied and Environmental Microbiology*, **60**(1), 313-322 (1994).
- Edwards, E.A., A.M. Edwards, and D. Grbić -Galić , “A Method for Detection of Aromatic Metabolites at Very Low Concentrations,” *Applied and Environmental Microbiology*, **60**, 323-327 (1994).
- Grbić -Galić , D., “Anaerobic Microbial Degradation of Aromatic Hydrocarbons,” *Microbial Enhancement of Oil Recovery--Recent Advances*, Ed. E.C. Donaldson, Elsevier, Amsterdam, 145-161 (1991).
- Grbić -Galić , D., “Anaerobic Transformation of Aromatic Hydrocarbon Pollutants under Fermentative/Methanogenic Conditions,” *Periodicum Biologorum*, **93**(4), 533-546 (1991).
- Grbić -Galić , D., “Methanogenic Transformation of Aromatic Hydrocarbons and Phenols in Groundwater Aquifers,” *Geomicrobiol. J.*, **8**, 167-200 (1991).
- Grbić -Galić , D., Henry, S.M., Godsy, E.M., Edwards, E., and Mayer, K.P., “Anaerobic Degradation of Aromatic Hydrocarbons and Aerobic Degradation of Trichloroethylene by Subsurface Microorganisms,” *Organic Substances and Sediments in Water, Vol. 3: Biological*, Ed. R.A. Baker, Lewis Publ., Inc., Chelsea, 239-266 (1991).
- Mraković , I., *Sulfidogenic Degradation of Polycyclic Aromatic Hydrocarbons by Aquifer Derived Microorganisms*, Engineer Degree Thesis, Department of Civil Engineering, Stanford University, Stanford, CA (1993).

**Western Region Hazardous Substance Research Center Project
SU89-08. Effects of Sorption on Biodegradation of Halogenated Organics: Perry L. McCarty and Paul V. Roberts, Stanford University (funded by EPA).**

EPA Project Officer: Dale Manty

Project Period: 1989-1992

Project Amount: \$210,789

Research Category: Groundwater, chlorinated organics, biodegradation, TCE, sorption

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: Studies of the kinetics of TCE and CF transformation by resting cells and the effect of sorption on TCE transformation rates have been completed. 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.

Project Publications:

Alvarez-Cohen, L. and P.L. McCarty, "A Cometabolic Biotransformation Model for Halogenated Aliphatic Compounds Exhibiting Product Toxicity," *Environmental Science and Technology*, **25**(8), 1381-1387 (1991).

- Alvarez-Cohen, L. and P.L. McCarty, "Product Toxicity and Cometabolic Competitive Inhibition Modeling of Chloroform and Trichloroethylene by Methanotrophic Resting Cells," *Applied and Environmental Microbiology*, **57**(4), 1031-1037 (1991).
- Alvarez-Cohen, L. and P.L. McCarty, "Two-Stage Dispersed-Growth Treatment of Halogenated Aliphatic Compounds by Cometabolism," *Environmental Science and Technology*, **25**(8), 1387-1393 (1991).
- Alvarez-Cohen, L., *Cometabolic Biotransformation of Trichloroethylene and Chloroform by Methanotrophs--Experimental Studies and Modeling of Toxicity and Sorption Effects*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1991).
- Alvarez-Cohen, L., P.L. McCarty, and P.V. Roberts, "Sorption of Trichloroethylene onto a Zeolite, Accompanied by Methanotrophic Biotransformation," *Environmental Science and Technology*, **27**(10), 2141-2148 (1993).
- Alvarez-Cohen, L., P.L. McCarty, E. Boulygina, R.S. Hanson, G.A. Brusseau, and H.C. Tsien, "Characterization of a Methane-Utilizing Bacterium from a Bacterial Consortium that Rapidly Degrades Trichloroethylene and Chloroform," *Applied and Environmental Microbiology*, **85**(6), 1886-1893 (1992).
- Alvarez-Cohen, L.M. and P.L. McCarty, "Effects of Toxicity, Aeration, and Reductant Supply on Trichloroethylene Transformation by a Mixed Methanotrophic Culture," *Applied and Environmental Microbiology*, **57**(1), 228-235 (1991).
- Criddle, C.S., Alvarez, L.M., and McCarty, P.L., "Microbial Processes in Porous Media," *Transport Processes in Porous Media*, Eds., J. Bear and M.Y. Corapcioglu, Kluwer Academic Publishers, Dordrecht, The Netherlands, 639-691 (1991).
- McCarty, P.L. and L. Alvarez-Cohen, "Zeolite Enhanced Organic Biotransformation," U.S. Patent No. 5,139,682 (August 18, 1992).

**Western Region Hazardous Substance Research Center Project
SU89-09. Trace Metal Removal Processes: James O. Leckie, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1989-1992

Project Amount: \$211,915

Research Category: Groundwater, metals, treatment

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 a 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. Project complete.

Project Publications:

- Papelis, C., "X-ray Photoelectron Spectroscopic Studies of Cadmium and Selenite Adsorption on Aluminum Oxides," *Environmental Science & Technology*, **29**(6), 1526-1533 (1995).
- Papelis, C., *Cadmium and Selenite Adsorption on Porous Aluminum Oxides: Equilibrium, Rate of Uptake and Spectroscopic Studies*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1992).
- Papelis, C., G.E. Brown, G.A. Parks, J.O. Leckie, "X-ray Absorption Spectroscopic Studies of Cadmium and Selenite Adsorption on Aluminum Oxides," *Langmuir*, **11**(6), 2041-2048 (1995).
- Papelis, C., P.V. Roberts, J.O. Leckie, "Modeling the Rate of Cadmium and Selenite Adsorption on Micro- and Mesoporous Transition Aluminas," *Environmental Science & Technology*, **29**(4), 1099-1108 (1995).

**Western Region Hazardous Substance Research Center Project
SU89-13. FASTCHEM Applications and Sensitivity Analysis: Peter K. Kitanidis and
David L. Freyberg, Stanford University.**

EPA Project Officer: Dale Manty

Project Period: 1989-1991

Project Amount: \$259,816

Research Category: Groundwater, models

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 the work include: (1) quantifying the sensitivity and uncertainty of predicted chemical distributions (in time and space) to variations in input parameters; (2) developing a set of computer programs which apply state-of-the art estimation methods for the interpolation of parameters from sparse measurements; and (3) evaluating the literature and developing new techniques for optimal decontamination strategies at utility hazardous waste sites under uncertainty.

Rationale: Amendments to the 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. There is a need now to determine the applicability of the model and for incorporating subroutines for the utilization of data.

Approach: The model's sensitivity and uncertainty is evaluated through application to specific case studies. Methods for incorporating available measurements are based on linear estimation methods.

Status: The computer codes have been installed and tested and recommendations for model improvement have been made. The model has been applied to six case studies representing typical problems of ash disposal from coal-fired plants. The sensitivity of the results to the input parameters has been evaluated. An applications manual has been developed. Project complete.

Project Publications:

Angamma, K., *SITE: An interactive toolkit for estimation*, Engineer Degree Project Thesis, Department of Civil Engineering, Stanford University, Stanford, CA (1995).
Kitanidis, P.K., D. L. Freyberg, A. Dudek-Ronan, S. Itagaki, N. Chan, S. I. and Lee, *Applications Handbook for FASTchem™*, EPRI Report No. TR-101218, Electric Power Research Institute, Palo Alto, CA, (1992).

**Western Region Hazardous Substance Research Center Project
SU89-14. Long-term Chemical Transformation of 1,1,1-Trichloroethane (TCA) and Freon
113 under Aquifer Conditions: Martin Reinhard and Perry McCarty, Stanford University
(Supported by Schlumberger)**

EPA Project Officer: Dale Manty

Project Period: 1989-1993

Project Amount: \$180,691

Research Category: Groundwater, chlorinated organics, TCA

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

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

Approach: Transformation rates of 1,1,1-trichloroethane (TCA) and 1,1,2-trichloro-1,2,2-trifluoroethane (CFC 113) were determined at ambient temperature for three aerobic systems: 0.2 µm-filtered groundwater, autoclaved and non-autoclaved sediment slurries for four years.

Status: The 95% confidence intervals for the half-lives of TCA (753 days for filtered groundwater, 603 days for autoclaved sediment slurries, and 770 days for non-autoclaved sediment slurries) overlapped, suggesting that there were no effects of sorption or microbial activity on initial TCA transformation in aerobic systems. Products of TCA transformation in all systems were 1,1-dichloroethylene (DCE), carbon dioxide (CO₂), and acetate. Because CO₂ was produced in all three systems, microbial contamination of supposedly sterile systems was measured. Sorption of TCA and transformation products was measured in non-autoclaved sediment slurries. A TCA transformation experiment was also run at 50°. The half-lives from the 50°C TCA transformation study were not significantly different (9.32 days for Milli-Q water, 9.19 days for groundwater alone, 9.50 days for groundwater and ground ampoule glass, and 9.21 for groundwater spiked with ¹⁴C-labeled TCA). Products of TCA transformation at 50°C were DCE (40%) and acetate (57-60%). ¹⁴CO₂ accounted for 4% of ¹⁴C-labeled TCA transformation products at 50°C. The percentage of DCE formation appeared to increase with temperature. CFC 113 did not transform during the four-year study. Project completed.

Project Publications:

McCarty, P.L. and M. Reinhard, "Biological and Chemical Transformations of Halogenated Aliphatic Compounds in Aquatic and Terrestrial Environments," *Biogeochemistry of*

Global Change: Radiatively Active Trace Gases, Ed. R.S. Oremland, Chapman and Hall, Inc., New York, 839-852 (1993).

**Western Region Hazardous Substance Research Center Project
SU89-15. In-Situ Anaerobic Biological Treatment of Aromatics in Groundwater: Martin Reinhard, Peter Kitanidis, Gary D. Hopkins, and Alfred Spormann, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1989-1996

Project Amount: \$1,021,875

Research Category: Groundwater, BTEX, biodegradation, demonstration

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

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

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

Status: During 1996, a new well field has been installed and instrumented for simultaneously conducting three large-scale controlled release experiments. This expanded test site consists of three test zones each equipped with a multi-level injection well, two pairs of down-gradient multi-level observation wells and one up-gradient observation well. Hydraulic characterization of the well field and installation of the automatic sampling and analysis platform is in progress and expected to be completed by spring 1997. Evaluation of BTEX transformation at this field site was conducted with funds from the Department of Defense Environmental Security Technology Certification Program (ESTCP).

Project Publications:

- Ball, H.A. and M. Reinhard, "Monoaromatic Hydrocarbon Transformation Under Anaerobic Conditions at Seal Beach, California: Laboratory Studies," *Environmental Toxicology and Chemistry*, **15**(2), 114-122, (1996).
- Ball, H.A., M. Reinhard, and E. Orwin, *Factors Influencing the Anaerobic Biodegradation of Gasoline Hydrocarbons by Groundwater Bacteria at Seal Beach, CA.*, Technical Report No. WRC-5, WRHSRC, Department of Civil Engineering, Stanford, CA (1993).
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- Beller, H., *Anaerobic Metabolism of Toluene and Other Aromatic Compounds by Sulfate-Reducing Soil Bacteria*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1995).
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**Western Region Hazardous Substance Research Center Project
SU90-01. Use of Starvation and Stress Promoters for Biodegradation of Hazardous
Wastes: A. C. Matin, Dunja Grbić -Galić , Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1990-1992

Project Amount: \$155,619

Research Category: Groundwater, chlorinated organics, biodegradation, TCE

Goal: The goal of this project is to use bacterial-stress (especially starvation) promoters to create recombinant strains that exhibit high biodegradative activities under typical environmental stress conditions.

Rationale: Microbial degradative activities in the environment can be limited by stresses such as nutrient deficiencies for carbon, nitrogen, or phosphorus. Bacterial strains that are capable of high degradative activities under such environmental stresses should be useful in the remediation of hazardous waste sites.

Approach: Stress promoters are genetic elements that are activated in response to specific stress conditions. Bacterial promoters that are switched on under carbon starvation have been isolated and characterized. Our approach is to splice the toluene-4-monooxygenase (T-4-MO) gene which degrades hazardous compounds such as trichloroethylene (TCE), under the regulation of these promoters and to characterize the degradative activities of the resultant strains.

Status: T-4-MO has been placed under the regulation of two carbon starvation promoters and under a more generalized stress promoter in *E. coli*. Experiments have begun to characterize these constructs. Attempts were made to optimize the regulation of one of the carbon starvation promoters by removing intervening DNA sequences between the promoter and T-4-MO. Organisms with the more generalized stress promoter are able to maintain TCE degradative activity for several hours after the onset of starvation. Project complete

Project Publications:

Little, C.D., Fraley, C.D., McCann, M.P., and Matin, A., "Use of Bacterial Stress Promoters to Induce Biodegradation Under Conditions of Environmental Stress," *On-Site Bioreclamation, Processes for Xenobiotic and Hydrocarbon Treatment*, Eds., R.E. Hinchee and R.F. Olfenbuttel, Butterworth-Heinemann, Boston, 493-498 (1991).

**Western Region Hazardous Substance Research Center Project
SU90-02. Determining and Modeling Diffusion-Limited Sorption and Desorption Rates of
Organic Contaminants in Heterogeneous Soils: Paul V. Roberts, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1990-1992

Project Amount: \$154,823

Research Category: Groundwater, sorption, organics

Goal: The overall goal of this project is 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 the diffusion 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 presently little basis for assessing when mass transfer conditions are favorable for pump-and-treat and/or bioremediation strategies, nor is there an accepted method for predicting the required duration of 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. The data are used to evaluate and parameterize existing transport models.

Status: A purge-and-trap method for studying desorption rates has been developed that enables the conduct of long-term experiments; mass balances confirm the efficacy of the experimental method. Batch sorption rate data have been interpreted within the framework of a pore-diffusion model that simulates either sorption or desorption. A mathematical model has been developed to interpret the rate data, permitting estimation of the effective diffusivity; either linear or Freundlich isotherm data can be accommodated. Desorption rate behavior conforms to the grain-scale mathematical model. Application of the laboratory-determined parameters as inputs to a transport model simulates the transport behavior at the Moffett Field site, more successfully than does an equilibrium model. Work is continuing on diffusion into clay layers and on temperature effects. Project complete.

Project Publications:

Dawson, H.E. and P.V. Roberts, "Influence of Viscous, Gravitational, and Capillary Forces on DNAPL Saturation," *Ground Water*, **35**(2), 261-269 (1997).

Dawson, H.E., *Entrapment and Mobilization of Residual Halogenated Organic Liquids in Saturated Aquifer Material*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1992).

Gvirtzman, H. and P. Roberts, "Pore Scale Spatial Analysis of Two Immiscible Fluids in Porous Media," *Water Resources Research*, **27**(6), 1165-1176 (1991).

- Harmon, T.C. and P.V. Roberts, "Comparison of Intraparticle Sorption and Desorption Rates for a Halogenated Alkene in a Sandy Aquifer Material," *Environmental Science and Technology*, **28**(9), 1650-1660 (1994).
- Harmon, T.C. and P.V. Roberts, "Determining and Modeling Mass Transfer Rate Limitations in Heterogeneous Aquifers," *Water Science and Technology*, **26**(1-2), 71-77 (1992).
- Harmon, T.C. and P.V. Roberts, "The Effect of Equilibration Time on Desorption Rate Measurements with Chlorinated Alkenes and Aquifer Particles," *Environmental Progress*, **13**(1), 1-8 (1994).
- Harmon, T.C., *Determining and Modeling Diffusion-Limited Desorption Rates in Heterogeneous Aquifer Solids*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1992).
- Harmon, T.C., Semprini, L., and Roberts, P.V., "Simulating Groundwater Solute Transport Using Laboratory-based Determined Sorption Parameters," *J. Environmental Engineering*, **118**(5), 666-689 (1992).

**Western Region Hazardous Substance Research Center Project
SU90-03. 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)**

EPA Project Officer: Dale Manty

Project Period: 1990-1993

Project Amount: \$177,829

Research Category: Air pollution, hazardous chemicals

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 has been developed. In order to accurately model dispersion in the surface layer regime, this model incorporates accurate expressions for the variation of wind speed and eddy diffusivity with vertical height, and also includes dry deposition as a removal mechanism. The dispersion model has been linked to an existing volatilization model which predicts emission rates resulting from aerobic biological treatment based on the influent concentrations and the operating parameters of the system. The predictions of this combined modeling system have been compared with predictions obtained using simpler, more commonly-used models.

Status: This project was completed in August of 1993. A dispersion model was developed which accurately represents conditions near groundlevel, and the model was linked to a second model used to estimate VOC emission rates from a surface aerobic treatment unit based on influent concentrations and operating conditions. The predictions of the dispersion model were compared with those obtained using other "popular" models under various conditions, revealing that these popular models often are under-predicting downwind concentrations by as much as an order of magnitude. The model has been refined to better reflect the interaction between the type of terrain and the variation of turbulence with height. The literature also has been examined to characterize emission rates from landfill sites, in the interests of utilizing this source emission data as input to the model.

Project Publications:

- Chrysikopoulos, C.V., Hildemann, L.M., and Roberts, P.V., "Modeling Emissions from Aerobic Treatment Systems," in *Environmental Engineering: Proceedings of the 1991 Specialty Conference*, American Society of Civil Engineers, New York, NY, 414-418, (1991).
- 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).
- 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).
- Lin, J.S. and L. Hildemann, "Analytical Solutions of the Atmospheric Diffusion Equation with Multiple Sources and Height-Dependent Wind Speed and Eddy Diffusivities," *Atmospheric Environment*, **30**(2), 239-254 (1996).
- Lin, J.S. and L.M. Hildemann, "Air Emission Models for Hazardous Waste Landfills," in *Joint CSCE--ASCE Conference on Environmental Engineering*, Eds., R.N. Yong, J. Hadjinicolaou, and A.M.O. Mohamed, Geotechnical Research Centre of McGill University, 75-82 (1993).
- Lin, J.S. and L.M. Hildemann, "Comparison of Dispersion Models for Wastewater Treatment Emissions," *Environmental Engineering: Proceedings of the Environmental Engineering Sessions at Water Forum '92*, American Society of Civil Engineers, New York, NY, 7-11 (1992).
- Lin, J.S. and L.M. Hildemann, "Emissions, Ambient Concentrations, and Potential Health Hazards of VOCs from Wastewater Treatment," *ASCE Conference on Environmental Engineering*, 580-584 (1994).

**Western Region Hazardous Substance Research Center Project
SU90-05. Subsurface Mixing of Nutrients and Groundwater for in-Situ Bioremediation:
P. L. McCarty, P. K. Kitanidis, P. V. Roberts, and L. Semprini, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1990-1994

Project Amount: \$583,873

Research Category: Groundwater, treatment, mixing

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: A combined analytical and numerical model for mixing has been developed and is being 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 were determined numerically.

Project Publications:

Lang, M.M., Design and Optimization of In Situ Bioremediation Systems Relying on Cometary Degradation, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1995)

- Lang, M.M., P.V. Roberts, and L. Semprini, "Model Simulations in Support of Field Scale Design and Operation of Bioremediation Based on Cometary Degradation," *Ground Water*, **35**(4), 565-573 (1997).
- MacDonald, T.R., *Flow from a Recirculation Well for Enhanced In-Situ Bioremediation*, Ph. D. Dissertation, Stanford University, Stanford, CA (1995).
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- MacDonald, T.R., P.K. Kitanidis, P.L. McCarty, P.V. Roberts, "Mass Transfer Limitations for Macroscale Bioremediation Modeling and Implications on Aquifer Clogging," *Ground Water*, **37**(4), 523-531 (1999).
- McCarty, P. L. and L. Semprini, "Engineering and Hydrogeological Problems Associated with *In Situ* Treatment," *Hydrological Sciences*, **38**(4), 261-272 (1993).
- McCarty, P.L., "Factors Affecting Biotransformation Rates in Groundwater" *Groundwater Contamination: Application of Research to Management Problems in Asia and the Pacific*, Proceedings of the 2nd SCOPE Regional Workshop, Chiang Mai, Thailand, 261-263 (1995).

**Western Region Hazardous Substance Research Center Project
SU90-06. Test-Bed Evaluation of In-Situ Bioremediation of Chlorinated Aliphatic
Compounds by Toluene Oxygenase Microorganisms: P.L. McCarty, G.D. Hopkins, L.
Semprini, Stanford University (Partially Supported by the U. S. Department of Energy and
the U. S. EPA Biosystems Program)**

EPA Project Officer: Dale Manty

Project Period: 1990-1994

Project Amount: \$583,873

Research Category: Groundwater, chlorinated organics, TCE, demonstration

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 c-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. This project is now completed.

Project Publications:

- Hopkins, G.D. and P.L. McCarty, "Field Evaluation of *in Situ* Aerobic Cometabolism of Trichloroethylene and Three Dichloroethylene Isomers Using Phenol and Toluene as the Primary Substrates," *Environmental Science & Technology*, **29**(6), 1628-1637 (1995).
- McCarty, P.L. and G. Hopkins, "Field-Scale Study of In Situ Bioremediation of TCE-Contaminated Ground Water and Planned Bioaugmentation," *Symposium on Bioremediation of Hazardous Wastes: Research, Development, and Field Evaluations*, EPA/600/R-94/075, 65-68 (1994).
- McCarty, P.L. and L. Semprini, "Ground-Water Treatment for Chlorinated Solvents," in *Handbook of Bioremediation*, Ed. Norris et al., Lewis Publishers, Boca Raton, 87-116 (1994).
- McCarty, P.L. and M. Reinhard, "Biological and Chemical Transformations of Halogenated Aliphatic Compounds in Aquatic and Terrestrial Environments," *Biogeochemistry of Global Change: Radiatively Active Trace Gases*, Ed. R.S. Oremland, Chapman and Hall, Inc., New York, 839-852 (1993).
- McCarty, P.L. and P.V. Roberts, editors, "Contaminants in the Subsurface Environment," *Water Science and Technology*, **22**(6), 110 pp. (1990).
- McCarty, P.L., "Processes Governing the Movement and Fate of Contaminants in Groundwater," *Groundwater Contamination: Application of Research to Management Problems in Asia and the Pacific*, Proceedings of the 2nd SCOPE Regional Workshop, Chiang Mai, Thailand, 38-45 (1995).
- McCarty, P.L., "Processes Governing the Movement and Fate of Contaminants in Groundwater," *Groundwater Contamination: Application of Research to Management Problems in Asia and the Pacific*, Proceedings of the 2nd SCOPE Regional Workshop, Chiang Mai, Thailand, 38-45 (1995).
- Munakata-Marr, J., *The Effects of Bioaugmentation on Trichloroethylene Cometabolism in Aquifer Microcosms*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1996).

**Western Region Hazardous Substance Research Center Project
SU90-07. 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**

EPA Project Officer: Dale Manty

Project Period: 1990-1992

Project Amount: \$200,000

Research Category: Groundwater, chlorinated organics, TCE, demonstration, biodegradation

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 were completed. Detailed characterization of the site has been completed in a cooperative study by the industry involved and the EPA Robert S. Kerr Environmental Research Laboratory, Ada, Oklahoma. The field study results indicated that chlorinated organic concentrations were much higher than indicated in previous engineering reports of the study (concentrations in the hundreds of milligrams per liter), much too high for aerobic cometabolism. Extensive dehalogenation to ethene was also present, suggesting that natural attenuation or engineered anaerobic dehalogenation were better alternatives than aerobic treatment, or that a combined anaerobic/aerobic system would be another alternative worthy of exploration. Project complete.

Project Publications:

- Dolan, M. and McCarty, P. L., "Methanotrophic Chloroethene Transformation Capacities and 1,1-Dichloroethene Transformation Product Toxicity," *Environmental Science and Technology*, **29**(11), 2741-2747 (1995).
- Dolan, M. E. and McCarty, P. L., "Small-Column Microcosm for Assessing Methane-Stimulated Vinyl Chloride Transformation in Aquifer Samples," *Environmental Science & Technology*, **29**(8), 1892-1897 (1995).
- Dolan, M. E. and P.L. McCarty, "Factors Affecting Transformations of Chlorinated Aliphatic Hydrocarbons by Methanotrophs," *Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds*, Eds., R.E. Hinchee, A. Leeson, L. Semprini, and S.K. Ong, Lewis Publishers, Boca Raton, 303-308 (1994).
- Dolan, M. E., *Methanotrophic Cometabolic Transformation of Vinyl Chloride and Other Chlorinated Ethenes in Aquifer Microcosms*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1996).
- McCarty, P.L., "Engineering Concepts for *In Situ* Bioremediation," *Journal of Hazardous Materials*, **28**, 1-11 (1991).
- McCarty, P.L., L. Semprini, M.E Dolan, T.C.. Harmon, S. Just, C. Tiedeman, S.M. Gorelick, and P.V. Roberts, *Evaluation of In-Situ Methanotrophic Bioremediation for Contaminated Groundwater, St. Joseph, Michigan*, Technical Report No. WR-1, WRHSRC, Department of Civil Engineering, Stanford University, Stanford, CA (1990).
- McCarty, P.L., Semprini, L., Dolan, M.E., Harmon, T.C., Tiedeman, C., and Gorelick, S.M., "*In-Situ* Methanotrophic Bioremediation for Contaminated Groundwater at St. Joseph, Michigan," *On-Site Bioreclamation, Processes for Xenobiotic and Hydrocarbon Treatment*, Eds., R.E. Hinchee and R.F. Olfenbuttel, Butterworth-Heinemann, Boston, 16-40 (1991).
- Roberts, P.V., G.D. Hopkins, L. Semprini, and P.L. McCarty, "Pulsing for Electron Donor and Electron Acceptor for Enhanced Biotransformation of Chemicals," U.S. Patent No. 5,006,250 (April 9, 1991).
- Semprini, L., G.D. Hopkins, D. Grbić -Galić , P.L. McCarty, and P.V. Roberts, "A Laboratory and Field Evaluation of Enhanced *In Situ* Bioremediation of Trichloroethylene, cis- and trans-Dichloroethylene, and Vinyl Chloride by Methanotrophic Bacteria," *Bioremediation Field Experience*, Eds., P.E. Flathman, D.E. Jerger, and J.H. Exner, Lewis Publishers, Boca Raton, 383-412 (1994).
- Semprini, L., Hopkins, G.D., McCarty, P.L., and Roberts, P.V., "In-Situ Transformation of Carbon Tetrachloride and Other Halogenated Compounds Resulting from Biostimulation Under Anoxic Conditions," *Environmental Science and Technology*, **26**(12), 2454-2461 (1992).
- Semprini, L., McCarty, P., Dolan, M., Lang, M., McDonald, T., Bae, J., and Kitanidis, P., "Design and Treatability Study of *In Situ* Bioioremiation of Chlorinated Aliphatics by Methanotrophs at St. Joseph, Michigan," *Bioremediation of Hazardous Wastes*, EPA/600/R-92/126, U.S. EPA Center For Environmental Research Information, Cincinnati, 43-45 (1992).
- Tiedeman, C. and S.M. Gorelick, "Analysis of Uncertainty in Optimal Groundwater Contaminant Capture Design," *Water Resources Research*, **29**(7), 2139-2154 (1993).
- Tiedeman, C., *Optimal Hydraulic Containment Designs Under Parameter Uncertainty for a Vinyl Chloride Plume in Southwest Michigan*, M.S. Thesis, Department of Applied Earth Sciences, Stanford University, Stanford, CA (1992).

**Western Region Hazardous Substance Research Center Project
SU91-02. Aquifer Remediation Design in the Presence of Kinetic Limitations: Steven M. Gorelick, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1992-1996

Project Amount: \$266,903

Research Category: Groundwater, treatment, optimization modeling

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

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

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

Status: This project is now complete. It was shown that pulsed pumping does not remove more contaminant mass than pumping continuously at the average rate over the same time frame. However, when properly engineered, pulsed pumping can remove as much contaminant as continuous pumping. A new model was developed of mass transfer that is shown to be a general form of the family of diffusion models and first-order mass transfer models. This "multirate" model is able to represent diffusional mass transfer within many physical and statistical combinations of immobile zones. The model was applied (1) to estimate statistical distributions of mass transfer rate coefficients in column experiments; (2) to investigate the effects on aquifer

remediation in the presence of a heterogeneous mixture of clay layers, grain aggregates, and surface reactions; (3) and to develop methods for estimating mass transfer rate coefficients from field and laboratory data. In the first application, extreme variability must be invoked (exceeding six orders of magnitude) in diffusion rate coefficients to represent mass transfer within a relatively homogeneous porous medium. In the second application, it was shown that understanding the variations in rates of mass transfer is likely the key to making accurate predictions of remediation times.

Project Publications:

- Haggerty, R. and S.M. Gorelick, "Modeling Mass Transfer Processes in Soil Columns with Pore-Scale Heterogeneity," *Soil Science Society of America Journal*, **62**(1), 62-74 (1998).
- Haggerty, R., and S.M. Gorelick, "Multiple-Rate Mass Transfer for Modeling Diffusion and Surface Reactions in Media with Pore-Scale Heterogeneity," *Water Resources Research*, **31**(10), 2383-2400 (1995).
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- Haggerty, R., and S.M. Gorelick, "Design of Multiple Contaminant Remediation in the Presence of Rate Limitations," *Eos*, **73**(43), (1992).
- Haggerty, R., *Aquifer Remediation in the Presence of Rate-Limited Mass Transfer*, Ph. D. Dissertation, Stanford University, Stanford, CA (1995).
- Haggerty, R., C.F. Harvey and S.M. Gorelick, "An Evaluation of Pulsed Pumping for Aquifer Remediation," *Eos*, **74**(43), (1993).
- Harvey, C.F., Haggerty, R., and Gorelick, S.M., "Aquifer remediation: A method for estimating mass transfer rate coefficients and an evaluation of pulsed pumping," *Water Resources Research*, **30**(7), 1979-1991 (1994).

**Western Region Hazardous Substance Research Center Project
SU91-03. Determination of Macroscopic Transport Parameters for Biologically Reacting
Solutes in Aquifers: Peter K. Kitanidis, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1992-1995

Project Amount: \$251,711

Research Category: Groundwater, optimization modeling, scaling effects

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

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

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

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

Project Publications:

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- Dykaar, B.B. and P.K. Kitanidis, "Macrotransport of a biologically reacting solute through porous media," *Water Resources Research*, **32**(2), 307-320 (1996).
- Dykaar, B.B. and P.K. Kitanidis, "Transmissivity of a Heterogeneous Formation," *Water Resources Research*, **31**(5), 985-1001 (1993).
- Kitanidis, P.K. and B.B. Dykaar, "Stokes Flow in a Slowly Varying Two-Dimensional Periodic Pore," submitted 1996.
- Kitanidis, P.K., "Quasi-linear geostatistical theory for inverting," *Water Resources Research*, **30**(10), 2411-2419 (1995).
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- Shen, K.-F., *Optimal parameter estimation in geostatistics*, Engineer Degree Thesis, Department of Civil Engineering, Stanford University, Stanford, CA (1994).
- Thierrin, J. and P.K. Kitanidis, "Solute dilution at the Borden and Cape Cod groundwater tracer tests," *Water Resources Research*, **30**(11), 2883-2890 (1994).
- Van Lent, T., and P. K. Kitanidis, "Effects of first-order approximations on head and specific discharge covariances in high contrast log-conductivity," *Water Resources Research*, **32**(5), 1197-1207 (1996).

**Western Region Hazardous Substance Research Center Project
SU91-04. Transformation of Chlorinated Hydrocarbons by Reduced Metallocoenzymes--
Kinetic Model Development and Applications to Environmental Systems: Martin
Reinhard, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1992-1995

Project Amount: \$222,414

Research Category: Groundwater, chlorinated ethylenes, chemical transformation

Goal: This project is aimed at (1) revealing the role of the reducing agent in coenzyme-catalyzed transformation of chlorinated hydrocarbons, (2) developing kinetic models describing the systems, (3) elucidating the reaction pathways, and (4) evaluating transformation intermediates and products.

Rationale: Transition-metal coenzymes have been known to catalyze reductive transformation of halogenated organic contaminants in biological systems as well as *in vitro*. The chemistry of this type of reactions is important in both respects: it may (1) shed light on the fate of these pollutants *in vivo* and/or the cometabolic processes in microbial systems; (2) provide information for the development of novel remediation technologies. The mechanisms of organohalide transformation involving metallocofactors in media containing an excess reducing agent are not yet resolved. In addition, the type and strength of the reductant have been shown to significantly impact cofactor reactivity, pollutant degradation rate, and product distribution, but the effects and causes are not well-characterized.

Approach: This project entails kinetic studies of the transformation of carbon tetrachloride (CTET) by metallocoenzymes which are reduced either by a thio-reductant (cysteine or dithiothreitol) or titanium(III) citrate. Experiments are conducted in homogeneous aqueous solutions containing reductant (in excess) and a cofactor, either hematin or vitamin B₁₂. Kinetic experiments are conducted at room temperature under light-excluded, anaerobic, and abiotic conditions. The disappearance of CTET and the appearance of products, primarily chloroform and carbon monoxide, are measured as a function of pH. Radiolabeled ¹⁴CCl₄ was used to evaluate mass balances.

Status: The transformation of CTET by reduced vitamin B₁₂ or hematin in cysteine solution has been studied over a wide range of pH. Rapid transformation was observed at pH ≥ 8 with the highest transformation rates occurring at pH 11.5 and 9.5 with B₁₂ and hematin, respectively. Most of the transformation products were water-soluble and only two volatile products, CHCl₃ and CO, were identified. In all cases, the total volatile products accounted for less than 30% of the mass balance. In cysteine solution, a kinetic transition due to deactivation of the cofactor was observed in both coenzyme systems. As in the case of titanium(III) citrate, hematin exhibited a more significant deactivation than vitamin B₁₂. The reduction of B₁₂ to B_{12r} was found to be the rate-limiting step and the pentacoordinated cob(II)alamin-cysteine thiolate complex is hypothesized to be the species reacting with CTET. This project is complete.

Project Publications:

- Chiu, P.C. *Transformation of Carbon Tetrachloride by Metallocoenzymes*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1995).
- Chiu, P.C. and M. Reinhard, "Metallocoenzyme-Mediated Reductive Transformation of Carbon Tetrachloride in Titanium(III) Citrate Aqueous Solution," *Environmental Science and Technology*, **29**, 595-603 (1995).
- Schreier, C., *The Destructive Removal of Chlorinated Organic Compounds From Water using Zero-Valent Metals of Hydrogen and Supported Palladium*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1995).
- Schreier, C.G. and M. Reinhard, "Catalytic Hydrodehalogenation of Chlorinated Ethylenes Using Palladium and Hydrogen for the Treatment of Contaminated Water," *Chemosphere*, **31**, 3475-3487 (1995).
- Schreier, C.G. and M. Reinhard, "Transformation of Chlorinated Organic Compounds by Iron and Manganese Powders in Buffered Water and in Landfill Leachate," *Chemosphere*, **29**(8), 1743-1753 (1994).
- Siantar, D.P., C.G. Schreier, and M. Reinhard, "Transformation of the Pesticide 1,2-Dibromo-3-Chloropropane (DBCP) and Nitrate by Iron Powder and by H₂/Pd/Al₂O₃," presented before the Division of Environmental Chemistry, American Chemical Society (1995).
- Siantar, D.P., C.G. Schreier, C.S. Chou, and M. Reinhard, "Treatment of 1,2-Dibromo-3-Chloropropane and Nitrate-Contaminated Water with Zero-Valent Iron or Hydrogen/Palladium Catalysts," *Water Research*, **30**, 2315-2322 (1996).

**Western Region Hazardous Substance Research Center Project
SU91-05. Microbial Degradation of Toluene Under Sulfate-Reducing Conditions--The
Role of Iron: Martin Reinhard, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1992-1995

Project Amount: \$256,438

Research Category: Groundwater, BTEX, biodegradation

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

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

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

Status: This project was completed in early 1995. Experiments with mixed enrichment cultures that compared the effects of adding ferrous sulfate vs. zinc sulfate demonstrated that sulfide toxicity, not a nutritional iron limitation, is the factor being ameliorated by the presence of iron in the cultures. This result applies both to toluene and to benzoate, a transient intermediate of toluene degradation. A novel sulfate-reducing, toluene-degrading organism, strain PRTOL1, was isolated from the enrichment cultures. Characterization of this organism was conducted. Mineralization of toluene to CO₂ by strain PRTOL1 has been demonstrated with radiolabeled toluene, and the stoichiometry of sulfate consumed relative to toluene consumed has been shown

to be consistent with theoretical predictions. Benzylsuccinic acid and benzylfumaric acid, two dead-end metabolites that were identified as minor products of toluene degradation in the enrichment cultures, are also produced by strain PRTOL1. The ability of strain PRTOL1 to utilize a range of electron donors and acceptors has been tested using cell suspensions. The 16S rRNA gene sequence of strain PRTOL1, which was analyzed at Michigan State University, has shown that PRTOL1 is indeed a novel bacterium.

Project Publications:

- Beller, H.R., *Anaerobic Metabolism of Toluene and Other Aromatic Compounds by Sulfate-Reducing Soil Bacteria*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1995).
- Beller, H.R. and M. Reinhard, "The Role of Iron in Enhancing Anaerobic Toluene Degradation in Sulfate-Reducing Enrichment Cultures," *Microbial Ecology*, **30**, 105-114 (1995).
- Beller, H.R., A.M. Spormann, P. Sharma, J.R. Cole, and M. Reinhard, "Isolation and Characterization of a Novel Toluene-Degrading, Sulfate-Reducing Bacterium," *Applied and Environmental Microbiology*, **62**(4), 1188-1196, (1996).
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- Beller, H.R., W.H. Ding, and M. Reinhard, "Byproducts of Anaerobic Alkylbenzene Metabolism Useful as Indicators of in Situ Bioremediation," *Environmental Science and Technology*, **29**(11), 2864-2870 (1995).

**Western Region Hazardous Substance Research Center Project
SU91-07. Transformation of TCE by Methanotrophic Biofilms: Perry L. McCarty,
Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1992-1995

Project Amount: \$123,272

Research Category: Groundwater, chlorinated organics, TCE, biodegradation

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

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

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

Status: This project was completed this year. A methanotrophic biofilm model was developed in which Monod kinetics with competitive inhibition was used to describe the oxidation of methane and TCE by the organisms in the biofilm. The model includes diffusion, active biomass growth, inactivation, decay, and CAH transformation product toxicity. Simulations suggest that a maximum TCE flux exists with respect to methane concentration. At low methane concentrations (approaching S_{min}), there is insufficient methane for net growth of the biofilm. At high methane concentrations, competitive inhibition limits TCE transformation. This factors plus others such as transformation product toxicity and availability of reducing power are expected to affect S_{min} and organism growth rate in general. An experimental procedure for determining S_{min} and organism growth rate was developed, and consists of monitoring the

methane concentration changes with time in cultures seeded with a small methanotrophic biomass inoculum. The Monod model was found to provide excellent fits to batch kinetics for methane utilization with organisms expressing particulate methane monooxygenase (MMO_p) and over four orders of magnitude in initial biomass concentration and more than two orders of magnitude of methane concentration. It was found that the presence of chlorinated aliphatic hydrocarbons (CAHs), such as trichloroethylene, *cis*- and *trans*-1,2-dichloroethylene, 1,1-dichloroethylene, and vinyl chloride, increased S_{min} and decreased organism growth rate. The factor affecting these changes was different for the different CAHs, with 1,1-dichloroethylene producing the greatest transformation product toxicity, and both *trans*-1,2-dichloroethylene and vinyl chloride significantly reducing the reductant supply (NADH) for regeneration of MMO activity. Competitive inhibition was also effective with trichloroethylene and some of the other CAHs. Thus, no easy generalizations can be made about which process is affected the greatest by different CAHs, each requires an individual evaluation.

Project Publications:

- Anderson, J. E. and P. L. McCarty, "Transformation Yields of Chlorinated Ethenes by a Methanotrophic Mixed Culture Expressing Particulate Methane Monooxygenase," *Applied and Environmental Microbiology*, **63**(2), 687-693 (1997).
- Anderson, J.E. and P.L. McCarty, "Effect of Three Chlorinated Ethenes on Growth Rates for a Methanotrophic Mixed Culture," *Environmental Science and Technology*, **30**(12), 3517-3524 (1996).
- Anderson, J.E. and P.L. McCarty, "Effect of Chlorinated Ethenes on S_{min} for a Methanotrophic Mixed Culture," *Environmental Science and Technology*, **31**(8), 2204-2210 (1997).
- Anderson, J.E. and P.L. McCarty, "Model for Treatment of Trichloroethylene by Methanotrophic Biofilms," *Journal of Environmental Engineering*, **120**(2), 379-400 (1994).
- Anderson, J.E., *Effect of Chlorinated Ethene Biodegradation on Growth Rates of Methanotrophic Bacteria in Biofilms and Mixed Cultures*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1996).

**Western Region Hazardous Substance Research Center Project
SU91-09. Heavy Metals in Ceramic Matrix: Heavy Metals/Clay Interactions in Ceramic
Processing: James O. Leckie, Stanford University (Partially Supported by the U. S.
Department of Energy)**

EPA Project Officer: Dale Manty

Project Period: 1993-1998

Project Amount: \$294,450

Research Category: Groundwater, soils, metals, treatment, stabilization

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

Rationale: Although the cost of materials and energy is high when compared to other treatment methods, there are several distinct compensating advantages to this approach: 1) it is possible to treat metal containing wastes on site and produce a material that is not classified as a hazardous waste, thus reducing regulatory problems and off-site transportation/disposal costs, 2) the ceramic product is significantly lighter than the liquid containing wastes (further reducing transportation costs), 3) the ceramic product is marketable for a number of applications such as light weight filler for concrete, and 4) the incorporated heavy metals may be more resistant to leaching than in other matrices thus making release to the environment following disposal less likely. The mechanism by which the ceramic matrix isolates heavy metals is not known. Two possibilities are chemical bonding in the alumino-silicate matrix, and encapsulation of metal oxides, carbonates, etc. Finding optimum conditions for producing heavy metal containing ceramics is dependent on this information.

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

Status: Most of the data collected verified that sintering industrial kaolinite spiked with either nickel or copper resulted in a phase change which dramatically reduced the metal's extractability in acidic environments. Sintered metal-spiked samples retained 43-57% more metal than their

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

Project Publications:

Dion, L., *Immobilizing Heavy Metals in a Ceramic Matrix: A Hazardous Waste Treatment Technology*, Engineer Degree Thesis, Department of Civil Engineering, Stanford University, Stanford, CA (1996).

**Western Region Hazardous Substance Research Center Project
SU91-11. Radon-222 Method for Locating and Quantifying Contamination by Residual
Non-Aqueous Phase Liquids in the Subsurface: Lewis Semprini, Oregon State University**

EPA Project Officer: Dale Manty

Project Period: 1992-1995

Project Amount: \$143,079

Research Category: Groundwater, DNAPL, characterization, radon tracer

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

Rationale: Locating and describing quantitatively the residual or pools associated with source areas of NAPL contamination in the subsurface remains as one of the major obstacles to implementing through site monitoring and effective remediation programs. Geochemical methods may provide an inexpensive means of locating and quantifying NAPL contamination, and monitoring the progress of remediation.

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

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

Project Publications:

- Cataloub, M.G., S. Gottipatti, J.F. Higginbotham, O. Hopkins, and L. Semprini, "222Rn as an Indicator of Nonaqueous Phase Liquid Contamination in Groundwater," *Health Physics* 70(6) S36-TPM-A-4 (1996).
- Gottipati, S., *Radon-222 as a Tracer for Performance Assessment of NAPL Remediation Technologies in the Saturated Zone: An Experimental Investigation*, M.S. Thesis, Department of Civil, Construction, and Environmental Engineering, Oregon State University, Corvallis, OR (1996).
- Hopkins, O.S., *Radon-222 as an Indicator for Nonaqueous Phase Liquids in the Saturated Zone: Developing a Detection Technology*, M.S. Thesis, Department of Civil Engineering, Oregon State University, Corvallis, OR (1994).
- Semprini, L., K. Broholm, and M.E. McDonald, "Radon-222 Deficit Method for Locating and Quantifying NAPL Contamination in the Subsurface," *Eos*, 74(43), (1993).
- Semprini, L., M. Cantaloub, S. Gottipati, O. Hopkins, and J. Istok. "Radon-222 as a Tracer for Quantifying and Monitoring NAPL Remediation," in *Nonaqueous Phase Liquids: Chlorinated and Recalcitrant Compounds*, Eds: G.B. Wickramanayake and R.E. Hinchee, Battelle Press, Columbus, OH, pp: 137-142 (1998).
- Semprini, L., O.S. Hopkins, and B.R. Tasker. "Laboratory, Field and Modeling Studies of Radon-222 as a Natural Tracer for Monitoring NAPL Contamination" *Journal of Transport in Porous Media*, 38(1 / 2) 223-240 (2000).
- Tasker, B.R., *Radon-222 as an Indicator for Non-Aqueous Phase Liquids in the Saturated Zone: A Numerical Methods Analysis*, M.S. Engineering Report, Department of Civil Engineering, Oregon State University, Corvallis, OR (1995).
- Cantaloub, M.G., *Aqueous-Organic Partition Coefficients for Radon-222 and their Application to Radon Analysis by Liquid Scintillation Methods*, M.S. Thesis, Department of Civil Engineering, Oregon State University, Corvallis, OR (2001).

**Western Region Hazardous Substance Research Center Project
SU92-01. Process Submodel Formulation and Parameter Estimation for Simulation of
Bioremediation: Paul Roberts, Stanford University and Lewis Semprini, Oregon State
University**

EPA Project Officer: Dale Manty

Project Period: 1992-1994

Project Amount: \$199,625

Research Category: Groundwater, simulation modeling, biodegradation, chlorinated organics

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. To evaluate and circumvent these difficulties, advanced control methods are needed.

Approach: Previous work encompassed 1) review of field and laboratory data, 2) quantification of vertical variations in velocity, 3) assessment of model dimensionality, and 4) incorporation of sorption and competitive inhibition phenomena. Work in the past year assessed the applicability of adaptive control engineering to in situ bioremediation systems.

Status: This two year project, which was initiated in September 1992, concluded in September 1994. Advances in 1993-94 resulted in improved understanding of the dependence of contaminant removal by methanotrophic bacteria on oxygen and methane delivery schedules, evaluation of different system configurations, and an exploration of adaptive control technologies. Simulation experiments of full-scale in situ bioremediation systems using methanotrophic organisms revealed that the contaminant removal rates varied greatly with the delivery schedule of oxygen and methane. These simulation experiments also show that steady-state oxygen and methane delivery schedules did not perform well and in some cases caused system failure. Consequently, methods of adapting oxygen and methane delivery based on the state and performance of the system were explored. The investigation encompassed both the two-well and single vertical well circulation systems. The optimal substrate delivery policy entails introducing the substrates at the rate at which they are required for net microbial growth. A control method for monitoring and adjusting the substrate delivery rates is proposed.

Project Publications:

Staudinger, J., and P.V. Roberts, " A Critical Review of Henry's Law Constants for Environmental Applications," *Critical Reviews, Environmental Science and Technology*, **26**(3), 205-297 (1996).

Staudinger, J. and P.V. Roberts, "A Critical Review of Henry's Law Constants for Environmental Applications," *Critical Reviews, Environmental Science and Technology*, **26**(3), 205-297 (1996).

**Western Region Hazardous Substance Research Center Project
SU92-02. Enhancement of Biodegradation through the Use of Substituted Porphyrins to
Treat Groundwater Contaminated with Halogenated Aliphatics: Martin Reinhard,
Stanford University; Kerry Sublette, Tulsa University; Michael McInerney, Joseph M.
Sufлита and Ralph S. Tanner, University of Oklahoma (Supported by the U. S. Department
of Energy)**

EPA Project Officer: Dale Manty

Project Period: 1993-1995

Project Amount: \$299,999

Research Category: Groundwater, chlorinated solvents, biodegradation

Goal: This project is a collaborative effort of three laboratories, Stanford University, Tulsa University and the University of Oklahoma. The objectives are three fold: (1) assess whether porphyrin augmentation improves the performance of dehalogenating cultures, especially for the treatment of chlorinated ethanes and ethenes (University of Oklahoma), (2) assess whether immobilized porphyrins can be used for treating waste streams containing chlorinated ethanes and ethenes (Tulsa), and (3) evaluate different reductant/metallocoenzyme systems with respect to their ability to dehalogenate halogenated aliphatics.

Rationale: Both microbial and abiotic dehalogenation methods can be used to degrade chloroaliphatics. This project examined whether the two methods could be combined to obtain an overall superior process.

Dehalogenation by Metallocoenzymes and Zero-Valent Iron Powder (Stanford University):

Approach: Transformation by iron(0) powder was one of the abiotic dehalogenation methods that was evaluated. Iron(0) was chosen because it is reactive towards PCE as well as a range of substrates without the involvement of porphyrin. The reaction variables that were studied included temperature and pH. Once the PCE system is understood, other chlorinated substrates will be tested. Metallocoenzymes studied include Vitamin B₁₂, which has a cobalt center, and hemein, which has an iron center. Soluble bulk reductants used were titanium (III) citrate, dithiothreitol, and cysteine. The most promising reaction systems will be further evaluated in combination with immobilized porphyrins (U. of Tulsa) and in biological cultures (U. of Oklahoma).

Status: Efforts are ongoing to understand the role of iron, a potential bulk reducing agent for porphyrin systems, in the transformation of PCE. PCE is converted by iron to ethene and ethane which typically account for about 15-30% of the mass balance. Trichloroethylene is observed as a reactive intermediate whose maximum concentration is less than about 2% of the initial PCE concentration. The possibility that the reactivity of iron towards PCE is due to the nature of the buffer and the type of water used needs further investigation. The project was suspended in October 1994 due to a lack of funds.

Transformation Reactions of Immobilized Porphyrins (Tulsa University):

Approach: After conducting batch experiments to evaluate reaction conditions with different reductants, fixed-bed reactors with immobilized cyanocobalamin were tested for their efficacy to remove PCE from water.

Status: By using vitamin B₁₂, PCE was converted to trichloroethylene and a mixture of cis- and trans-isomers of 1,2-dichloroethylene. The following reductants were investigated (in order of effectiveness): cysteine < 2-mercaptoethanol < HS⁻ < dithiothreitol (DTT) < Ti(III) citrate. Only DTT and Ti(III) citrate were selected for further study. It was found that a reaction temperature of 45°C and a pH of 9 were optimum. With Ti(III) citrate as the reducing agent, 96% PCE was converted in 19 h, with DTT, 92% PCE were converted in 70 h. TCE was the predominant product (95/5 ratio). B₁₂ has been immobilized in an investigation of PCE dechlorination in a packed-bed reactor using Duolite S-761 as the solid support. With citrate as the reductant, PCE (0.18 mM) conversion was 55% at residence times of 1.75 and 3.5 h. The product distribution was 94% TCE and 6% DCE at the lower residence time. DCE formation increased to 45% at the higher residence time. The subcontract was completed October 1994.

Effect of Reduced Porphyrins on Anaerobic Culture Performance (University of Oklahoma):

Approach: Whole cell suspensions of *Desulfomonile tidjie*, a PCE-dehalogenating bacterium, were incubated with and without vitamin B₁₂ amendments under a range of conditions. Different sulfur reductants including cysteine, hydrogen sulfide and DTT were used.

Status: The rates of dehalogenation in the cell suspensions were the same as those without B₁₂. This was true with many different experimental conditions, including different experimental conditions, the presence of different reductants, and different concentrations of cysteine-sulfide. Abiotic dehalogenation of PCE in the presence of B₁₂ was not observed. The preliminary data indicate that *D. tidjie* was dehalogenating chlorinated alkenes at a faster rates than organisms that contain high levels of corrin factors. Possible failure to stimulate dehalogenation may be related to membrane permeability problems associated with the use of whole cells, the kind of reductant used, and the concentrations of B₁₂ reductants used. This subcontract was completed October 1994.

Overall Status: The project was completed March 1995. Kinetic and mechanistic models for the dechlorination of carbon tetrachloride were developed for both metallocoenzymes and both reductants. Mechanisms for the deactivation of the metallocoenzymes were proposed. Significant findings are: (1) the Michaelis-Menten kinetic model applies only if deactivation is insignificant; (2) the type of reducing agent significantly affects product distribution, rate and mode of deactivation; (3) B₁₂ thiolate type complexes are likely involved in microbial cometabolic microbial dechlorination; (4) application of metallocofactors for treatment of carbon tetrachloride is limited by the significant inactivation by CTET metabolites. These models serve as an essential knowledge base for future studies to understand cometabolic reductive dechlorination.

Project Publications:

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- Chiu, P.C. and M. Reinhard, "Metallocoenzyme-Mediated Reductive Transformation of Carbon Tetrachloride in Titanium(III) Citrate Aqueous Solution," *Environmental Science & Technology*, **29**, 595-603 (1995).
- Habeck, D.B. and K.L. Sublette, "Reductive Dechlorination of Tetrachloride (PCE) Catalyzed by Cyanocobalamin," Center for Environmental Research and Technology, University of Tulsa, *Applied Biochemistry and Biotechnology*, **51/52**, 747-759 (1995).
- Schreier, C., *The Destructive Removal of Chlorinated Organic Compounds From Water using Zero-Valent Metals of Hydrogen and Supported Palladium*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1995).
- Schreier, C.G. and M. Reinhard, "Catalytic Hydrodehalogenation of Chlorinated Ethylenes Using Palladium and Hydrogen for the Treatment of Contaminated Water," *Chemosphere*, **31**, 3475-3487 (1995).
- Schreier, C.G. and M. Reinhard, "Transformation of Chlorinated Organic Compounds by Iron and Manganese Powders in Buffered Water and in Landfill Leachate," *Chemosphere*, **29**(8), 1743-1753 (1994).
- Siantar, D.P., C.G. Schreier, and M. Reinhard, "Transformation of the Pesticide 1,2-Dibromo-3-Chloropropane (DBCP) and Nitrate by Iron Powder and by H₂/Pd/Al₂O₃," presented before the Division of Environmental Chemistry, American Chemical Society (1995).
- Smith, R., G.T. Townsend, M.J. McInerney, J.M. Suflita, and R.S. Tanner, "Enhanced Reductive Biodegradation of Chlorinated Alkenes by Cobalt-Centered Porphyrins and Corrins," University of Oklahoma, Final Report, 1994.

**Western Region Hazardous Substance Research Center Project
SU92-04. Field Test of In-Situ Vapor Stripping for Removal of VOCS from Groundwater:
Steven M. Gorelick, Stanford University (Supported by the U. S. Department of Energy)**

EPA Project Officer: Dale Manty

Project Period: 1993-1998

Project Amount: \$641,709

Research Category: Groundwater, treatment, vapor stripping, volatile organics

Goal: This project is aimed at demonstrating an in-situ method for removing VOCs from groundwater known as in-well vapor stripping. Through simple gas injection into a well, this method enables removal of VOCs from contaminated groundwater without bringing the water to the ground surface. Recirculation of groundwater is accomplished via air-lift pumping. Theoretical and simulation studies have been carried out previously. This effort focused upon pilot scale field demonstrations at location Edwards Air Force Base (AFB) near Lancaster, California. The principal groundwater contaminant is trichloroethylene. The demonstration should provide information regarding the efficiency of in-well vapor stripping for VOC removal at a complex field site contaminated with VOCs.

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

Approach: The in-situ VOC removal system was demonstrated at Edwards Air Force Base. The demonstration unit consists of a single VOC-removal well and several monitoring wells. The overall approach to the demonstration is to run the system for 6 months and analyze the results using simulation.

Status: The field demonstration of in-well vapor stripping at Edwards AFB and analysis of the are complete. The in-well vapor stripping demonstration commenced at Edwards AFB in mid-January 1996 and ran continuously for over 7 months. Stripping ratios of approximately 90 percent were maintained throughout the demonstration. Monitoring wells were sampled at approximately 1 week intervals during the demonstration, and then sampled periodically in the 2 months following the demonstration. A comprehensive hydrogeologic model was developed to explain hydraulic head observations and the concentration histories at the observation wells. Maximum likelihood parameter estimation and cokriging were used to estimate spatially variable hydraulic conductivity and effective porosity based on hydraulic heads and estimated advective

travel times. A streamline method was used to calculate breakthrough curves. The data are best explained by advective-dispersive transport involving rate limited mass transfer between a mobile and an immobile domain. Longitudinal dispersivity and the first-order rate coefficients for mass transfer between mobile and immobile domains were determined by matching observed and simulated breakthrough/ recovery curves. The recirculation ratio for water near the well is approximately 70%. Owing to the success of the demonstration, in-well vapor stripping was subsequently used to successfully reduce TCE concentrations at the "hot-spot" area at Edwards AFB.

Project Publications:

- Francois, O., T. Gilmore, M.J. Pinto, and S.M. Gorelick, "A Physically Based Model for Air-Lift Pumping," *Water Resources Research*, **32**(8), 2383-2399 (1996).
- Gorelick, S.M. and M.J. Pinto, "Removing VOCs from Groundwater Using In-Well Vapor Stripping: Lab, Field, and Modelling," Proceedings of International Conference on Groundwater Quality Protection, Taiwan, p. 71-84 (1997).
- Gvirtzman, H. and S.M. Gorelick, "Using Air-lift Pumping As an In-situ Aquifer Remediation Technique," *Water Science Technology*, **27**(7-8), 195-201 (1993).
- Pinto, M.H., *Analysis of In-Well Vapor Stripping: An Integrated Approach*, Ph.D. Dissertation, Stanford University, Stanford, CA (2000).
- Pinto, M., H. Gvirtzman, and S.M. Gorelick, "Aquifer Remediation by In-Well Vapor Stripping: 2. Modelling Results," *Journal of Contaminant Hydrology*, **29**(1), 41-58 (1998).

**Western Region Hazardous Substance Research Center Project
SU92-05. System Design for Enhanced In-Situ Biotransformation of Carbon
Tetrachloride: Application to DOE's Arid Site Integrated Demonstration: Peter K.
Kitanidis and Paul V. Roberts, Stanford University, and Lewis Semprini, Oregon State
University (Supported by the U. S. Department of Energy)**

EPA Project Officer: Dale Manty

Project Period: 1993-1995

Project Amount: \$318,291

Research Category: Groundwater, chlorinated organics, biodegradation, carbon tetrachloride

Goal: To assist in the design of a nutrient delivery and mixing system for the pilot scale evaluation of in-situ bioremediation of carbon tetrachloride at the Hanford DOE site; to participate in the experimental design of the in-situ bioremediation tests; and to help in the interpretation of the results of the pilot-scale field experiments.

Rationale: The project is part of the larger Arid Site Integrated Demonstration that is being conducted at the Hanford DOE Facility. The specific application is bioremediation of carbon tetrachloride contamination at the 200-West area using mixing wells and chemical injection.

Approach: Specific tasks include: (a) Interpretation of field data and data from well and tracer tests, (b) development and application of hydrodynamic and transport models, (c) improvement of the understanding of the kinetic rates for the carbon tetrachloride transformation process, by conducting laboratory experiments and incorporating results into models, (d) utilization of results from models to assist in the design of the nutrient delivery system, (e) evaluation of the ability of indigenous microcosms from the Hanford subsurface to aerobically cometabolically degrade chloroform.

Status: Models for design of the delivery system have been applied. The well has been drilled. Field data are being interpreted. Batch soil microcosms and continuous flow soil microcosms packed with Hanford core material have been conducted. A stainless steel column reactor has been used for conducting laboratory experiments to mimic the recirculation process at the site. Project complete.

Project Publications

DeMenibus, M.-A., *Mixing Strategies for In-Situ Remediation*, Engineer Degree Thesis, Department of Civil Engineering, Stanford University, Stanford, CA (1994).

Kitanidis, P.K., "The concept of the dilution index," *Water Resources Research*, **30**(7), 2011-2026 (1994).

MacDonald, T.R. and P.K. Kitanidis, "Modeling the Free Surface of an Unconfined Aquifer Near a Recirculation Well," *Ground Water*, **31**(5), 774-788 (1993).

MacDonald, T.R., *Flow from a Recirculation Well for Enhanced In-Situ Bioremediation*, Ph. D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1995).

- Niemit, M.R., *Continuous-Flow and Batch Column Studies of Anaerobic Carbon Tetrachloride Biotransformation on Hanford Soil*, M.S Thesis, Department of Civil Engineering, Oregon State University, Corvallis, OR (1995).
- Semprini, L., G.D. Hopkins, P.V. Roberts, and P.L. McCarty, "In Situ Biotransformation of Carbon Tetrachloride, Freon 113, Freon 11, and 1,1,1-TCA under Anoxic Conditions," *On Site Bioreclamation*, Eds., R.E. Hinchee and R.F. Offenbuttel, Butterworth-Heinemann, Boston, 41-58 (1991).
- Thierrin, J. and P.K. Kitanidis, "Solute dilution at the Borden and Cape Cod groundwater tracer tests," *Water Resources Research*, **30**(11), 2883-2890 (1994).
- Workman, D., *Transformation of Halogenated Methanes by Microbially-Reduced Vitamin B₁₂*, Ph.D. Dissertation, Department of Civil, Construction, and Environmental Engineering, Oregon State University, Corvallis, OR (1997).

**Western Region Hazardous Substance Research Center Project
SU92-07. Modeling Strategies for Optimizing In-Situ Bioremediation: Peter K. Kitanidis
and Paul V. Roberts, Stanford University, and Lewis Semprini, Oregon State University
(Supported by the U. S. Department of Energy)**

EPA Project Officer: Dale Manty

Project Period: 1993-1994

Project Amount: \$243,001

Research Category: Groundwater, treatment, biodegradation, optimization modeling

Goal: First, to develop, calibrate, and apply mathematical models of subsurface flow and transport and fate of halogenated aliphatic compounds. Second, to develop conceptual designs for enhanced in-situ bioremediation. The research is guided by our understanding of conditions at two DOE sites: the Hanford site and the Rocky Flats site. The primary objectives are (a) to improve the understanding of in-situ mixing of nonreactive and reactive solutes, and (b) to recommend effective designs for in-situ mixing and strategies for improving the conditions for bioremediation.

Rationale: In-situ bioremediation typically requires the injection into the formation and mixing of electron acceptors, donors, or nutrients. Other innovative remediation methods, such as chemically enhanced pump-and treat remediation, require controlled mixing of chemicals. It is important to improve the grasp of dilution and mixing processes in the subsurface and to devise effective methods for their control.

Approach: The hydrodynamic parts of the model(s) account for three-dimensional flow and transport. The effects of heterogeneity on dilution and mixing are being studied. The biochemical parts (kinetics of biotransformation) incorporate current understanding of processes based on recent laboratory and field experiments. The research focuses on the incorporation of appropriate kinetic formulations for the cometabolic transformations that account for toxicity effects and deactivation of the microbial population.

Status: During the first year, the processes of in-situ dilution and mixing were studied at a more fundamental level; a report is being prepared that describes these processes and provides guidelines for practitioners of in-situ bioremediation. Different ways to represent cometabolic transformations are being evaluated. Preliminary simulations of the transient in-situ experiments at the Moffett field site, using microbes grown on phenol, indicate that a deactivation cometabolic transformation model yields results consistent with the field observations. This project is now completed.

Project Publications:

Harmon, T.C. and P.V. Roberts, "The Effect of Equilibration Time on Desorption Rate Measurements with Chlorinated Alkenes and Aquifer Particles," *Environmental Progress*, **13**(1), 1-8 (1994).

Keller, A.A., P.V. Roberts, and P.K. Kitanidis, "Prediction of Single Phase Transport Parameters in a Variable Aperture Fracture," *Geophysical Research Letters*, **22**(11) 1425-1428 (1995).

- Kim, Y., *Aerobic Cometabolism of Chloroform by Butane and Propane Grown Microorganisms from the Hanford Subsurface*, M.S. Thesis, Department of Civil, Construction, and Environmental Engineering, Oregon State University, Corvallis, OR (1996).
- Niemi, M.R., *Continuous-Flow and Batch Column Studies of Anaerobic Carbon Tetrachloride Biotransformation on Hanford Soil*, M.S. Thesis, Department of Civil Engineering, Oregon State University, Corvallis, OR (1995).

**Western Region Hazardous Substance Research Center Project
SU92-08. Anaerobic Treatment of Chlorinated Solvent Contaminated Groundwater: Perry L. McCarty, Stanford University (Supported by EPA Biosystems Program)**

EPA Project Officer: Dale Manty

Project Period: 1992-1994

Project Amount: \$125,000

Research Category: Groundwater, chlorinated organics, biodegradation, anaerobic

Goal: The objectives of this study are to determine the factors affecting the rate and extent of anaerobic trichloroethylene (TCE) transformation to ethene in groundwater, and to identify the microbial communities responsible for the transformation.

Rationale: The complete anaerobic dechlorination of TCE to ethene has recently been reported both in laboratory cultures and at field sites. Little is known, however, about the microbial community responsible for this transformation. The electron donors capable of stimulating dechlorination and the factors that affect the rate and extent of this transformation have not yet been studied.

Approach: Aquifer material was obtained by personnel from the U. S. EPA Robert S. Kerr Environmental Research Laboratory from a site contaminated by TCE in St. Joseph, Michigan, which has shown natural anaerobic dechlorination. This material was provided to the WRHSRC and was anaerobically transferred into 25 mL test tubes which act as small laboratory columns. The fluid in the test tubes is periodically exchanged with filter-sterilized groundwater from the site and amended with TCE and a series of different primary substrates. The effluent fluid from the columns following incubation is analyzed for dechlorination, substrate consumption, and electron acceptor usage.

Status: Several different substrates have been tested for their ability to stimulate dechlorination in the columns. Dechlorination to ethene has been observed with all substrates tested, although at varying rates and to varying extents. This transformation has been observed under both sulfate-reducing and methanogenic conditions. This study is now complete.

Project Publications:

- Haston, Z.C., Sharma, P.K., Black, J.N.P., and McCarty P.L., "Enhanced Reductive Dechlorination of Chlorinated Ethenes," *Symposium on Bioremediation of Hazardous Wastes: Research, Development, and Field Evaluations*, EPA/600/R-94/075, 11-14 (1994).
- McCarty, P.L., "An Overview of Anaerobic Transformation of Chlorinated Solvents," *Symposium on Intrinsic Bioremediation of Ground Water*, EPA/540/R-94/515, EPA Office of Research and Development, Washington, D.C., 135-142 (1994).
- McCarty, P.L. and J.T. Wilson, "Natural Anaerobic Treatment of a TCE Plume, St. Joseph, Michigan, NPL Site," *Bioremediation of Hazardous Wastes*, EPA/600/R-92/126, U.S. EPA Center For Environmental Research Information, Cincinnati, 47-50 (1992).
- Semprini, L., P.K. Kitanidis, D. Kampell, and J.T. Wilson, "Chemical Distributions and Anaerobic Transformation of Chlorinated Aliphatic Hydrocarbons in a Sand Aquifer,"

Emerging Technologies in Hazardous Waste Management VI, *American Chemical Society, Book of Abstracts*, Ed. D.W. Tedder (1994).

Semprini, L., P.K. Kitanidis, D.H. Kampbell, and J.T. Wilson, "Anaerobic Transformation of Chlorinated Aliphatic Hydrocarbons in a Sand Aquifer Based on Spatial Chemical Distributions," *Water Resources Research*, **31**(4), 1051-1062 (1995).

**Western Region Hazardous Substance Research Center Project
SU93-01. In Situ Treatment of Chlorinated Solvents: Perry L. McCarty, Stanford
University (Supported by DuPont Chemicals)**

EPA Project Officer: Dale Manty

Project Period: 1993-1996

Project Amount: \$225,000

Research Category: Groundwater, chlorinated organics, biodegradation, anaerobic

Goal: The objectives of this study are to describe the bacterium or groups of bacteria that are responsible for conversion of tetrachloroethene (PCE) to ethene in aquifer samples taken from a contaminated site in Victoria, Texas, and to examine the factors affecting the rate and extent of transformation.

Rationale: Several species of bacteria have been isolated and identified by others that have the ability to reductively dehalogenate chlorinated aliphatic hydrocarbons (CAHs). However, an individual or group of organisms responsible for the complete dehalogenation process from tetrachloroethylene (PCE) to ethene has not yet been identified. Whether or not the complete dehalogenation or the individual dehalogenation steps are the result of cometabolism or of energy metabolism is also not known. In addition, the various factors that affect the rates of dehalogenation have not been adequately evaluated. Such factors may include the electron donor used, the presence or absence of alternative electron acceptors, pH, and temperature. In order to understand the process better so that it can be more widely applied, its suitability for application at a given location can be better determined, and the economics of process implementation can be improved, definitive studies to better understand the nature of the organisms involved and factors affecting transformation rates are needed.

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

Status: This project is completed, and the sponsor has approved funding for additional study with similar objectives, but under a separate project number and title. Benzoate, acetate, and formate were all found to stimulate dehalogenation, although benzoate appears to be the better of the three. Enrichment cultures have been developed that retain their ability to reduce PCE to

ethene. A facultative pure bacterial strain (MS-1) has been isolated that transfers PCE to cis-1,2-dichloroethylene (c-DCE) while growing on a variety of substrates. This is the first facultative bacterium identified with this dehalogenating ability. This overall study clearly demonstrated that there are two separate groups involved in the overall dehalogenation of PCE to ethene in our culture, the first group that converts PCE to c-DCE as represented by strain MS-1, and the second group that converts c-DCE to vinyl chloride and ethene. Rates of transformation of PCE and TCE to cDCE were about four times faster than for the conversion of cDCE to vinyl chloride and vinyl chloride to ethene. The pH optimum for the culture studied was 6.5 and the temperature optimum was 35° C. Monod kinetics of the reactions involved are being evaluated through the extension of the project.

Project Publications:

- Haston, Z.C., P.K. Sharma, J.N.P. Black, and P.L. McCarty, "Enhanced Reductive Dechlorination of Chlorinated Ethenes," *Symposium on Bioremediation of Hazardous Wastes: Research, Development, and Field Evaluations*, EPA/600/R-94/075, 11-14 (1994).
- Sharma, P. and P.L. McCarty, "Isolation and Characterization of a Facultative Bacterium that Reductively Dehalogenates Tetrachloroethene to cis-1,2-dichloroethene," *Applied and Environmental Microbiology*, **62**(3), 761-765 (1996).

**Western Region Hazardous Substance Research Center Project
SU93-06. Moffett Field In-Situ Bioremediation Study in Support of Full Scale Application:
Perry L. McCarty, Gary D. Hopkins, and Mark N. Goltz, Stanford University (Supported
by the U. S. Air Force)**

EPA Project Officer: Dale Manty

Project Period: 1993-1995

Project Amount: \$394,834

Research Category: Groundwater, chlorinated organics, demonstration, TCE, biodegradation

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

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

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

Status: This project was completed in early 1995. Laboratory microcosm studies of cores taken from the demonstration site at Edwards AFB (see the companion project titled Full-Scale Evaluation of In-Situ Bioremediation of Chlorinated Solvent Groundwater Contamination) have shown that the microorganisms at the site are capable of aerobically cometabolizing TCE in the presence of either phenol or toluene. From studies at Moffett Field, it was seen that the presence of 1,1-DCE seriously reduced the effectiveness of oxidative biodegradation of TCE, probably due to 1,1-DCE transformation product toxicity. Therefore, one of the selection criteria that was used in choosing Edwards as the demonstration site was that 1,1-DCE not be present as a co-contaminant. Toluene was shown to be as effective as phenol for cometabolism

of CAHs, and due to regulatory considerations, will be used for the full-scale demonstration. Hydrogen peroxide, which was found to be a suitable alternative to pure oxygen as a source of oxygen for in-situ biodegradation, will also be used in the full-scale demonstration. Based on the Moffett Field results, the plan at Edwards to demonstrate in-situ aerobic cometabolism of TCE is to pulse in a primary substrate, toluene, while continuously adding hydrogen peroxide as an oxygen source.

Project Publications:

- Hopkins, G.D. and P.L. McCarty, "Field Evaluation of *in Situ* Aerobic Cometabolism of Trichloroethylene and Three Dichloroethylene Isomers Using Phenol and Toluene as the Primary Substrates," *Environmental Science & Technology*, **29**(6), 1628-1637 (1995).
- Hopkins, G.D., Munakata, J., Semprini, L., and McCarty, P.L., "Trichloroethylene Concentration Effects on Pilot Field Scale *In-Situ* Groundwater Bioremediation by Phenol-Oxidizing Bacteria," *Environmental Science and Technology*, **27**, 2542-2547 (1993).
- Hopkins, G.D., Semprini, L., and McCarty, P.L., "Evaluation of Enhanced *In Situ* Aerobic Biodegradation of cis- and trans-1-Trichloroethylene and cis- and trans-2, 2-Dichloroethylene by Phenol-Utilizing Bacteria," *Bioremediation of Hazardous Wastes*, EPA/600/R-92/126, U.S. EPA Center For Environmental Research Information, Cincinnati, 71-73 (1992).
- Hopkins, G.D., Semprini, L., and McCarty, P.L., "Microcosm and In Situ Field Studies of Enhanced Biotransformation of Trichloroethylene by Phenol-Utilizing Microorganisms," *Applied and Environmental Microbiology*, **59**, 2277-2285 (1993).
- Jenal-Wanner, U. and P.L. McCarty, "Development and Evaluation of Semicontinuous Slurry Microcosms to Simulate *In Situ* Biodegradation of Trichloroethylene in Contaminated Aquifers," *Environmental Science and Technology*, **31**(10), 2915-2922 (1997).
- McCarty, P.L., "Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbons," Chap. 23, *Subsurface Restoration*, Eds., C.H. Ward, J.A. Cherry, M.R. Scalf, Ann Arbor Press, Inc., Chelsea, MI, 373-395 (1997).
- Munakata-Marr, J., *The Effects of Bioaugmentation on Trichloroethylene Cometabolism in Aquifer Microcosms*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1996).
- Semprini, L., G.D. Hopkins, and P.L. McCarty, "A Field and Modeling Comparison of *In Situ* Transformation of Trichloroethylene by Methane Utilizers and Phenol Utilizers," *Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds*, Eds., R.E. Hinchee, A. Leeson, L. Semprini, and S.K. Ong, Lewis Publishers, Boca Raton, 248-254 (1994).
- Semprini, L., G.D. Hopkins, D. Grbić -Galić , P.L. McCarty, and P.V. Roberts, "A Laboratory and Field Evaluation of Enhanced *In Situ* Bioremediation of Trichloroethylene, cis- and trans-Dichloroethylene, and Vinyl Chloride by Methanotrophic Bacteria," *Bioremediation Field Experience*, Eds., P.E. Flathman, D.E. Jerger, and J.H. Exner, Lewis Publishers, Boca Raton, 383-412 (1994).
- 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).
- McCarty, P.L. and G. Hopkins, "Field-Scale Study of In Situ Bioremediation of TCE-Contaminated Ground Water and Planned Bioaugmentation," *Symposium on Bioremediation of Hazardous Wastes: Research, Development, and Field Evaluations*, EPA/600/R-94/075, 65-68 (1994).

**Western Region Hazardous Substance Research Center Project
SU93-07. Full-Scale Evaluation of *In Situ* Bioremediation of Chlorinated Solvent
Groundwater Contamination: Perry L. McCarty, Mark N. Goltz, and Gary D. Hopkins,
Stanford University (Supported by the U. S. Air Force)**

EPA Project Officer: Dale Manty

Project Period: 1995-1998

Project Amount: \$611,061

Research Category: Groundwater, chlorinated organics, demonstration, TCE, biodegradation

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

Rationale: Full-scale implementation of an innovative technology, working with organizations in the user community, is an ideal way to communicate knowledge gained by research and put it into practice. A full-scale application of *in situ* aerobic biodegradation of chlorinated solvents was justified based upon research at Stanford University and elsewhere that has shown that the process can operate efficiently with indigenous microorganisms. This project is a cooperative activity between the U. S. Air Force, the U. S. Environmental Protection Agency, Woodward-Clyde Corporation, and other parties with interest in this activity.

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

Status: This one year successful evaluation was completed in March 1997. With pumping at 25 liters per minute at each well, and the introduction of 9 mg/l toluene, 30 mg/l dissolved oxygen, and 41 mg/l hydrogen peroxide for fouling control and additional oxygen, 83 to 85 percent TCE biodegradation was achieved with each pass through a treatment well. An estimated 60-m width of the TCE contaminated plume was treated with this system, reducing its upgradient TCE by

about 98 percent from 1200 µg/l to 25 µg/l. Toluene concentration was reduced to 1.4 ± 0.6 µg/l at the 22m x 22m boundary of the steady zone. Potential clogging was successfully controlled.

Project Publications:

- Gandhi, R. K., Hopkins, G. D., Goltz, M. N., Gorelick, S. M., and McCarty, P. L., "Full-Scale Demonstration of In Situ Cometabolic Biodegradation of Trichloroethylene in Groundwater, 1: Dynamics of a Recirculating Well System," *Water Resources Research*, 38(4), 10.1- 10.16 (2002).
- Gandhi, R. K., Hopkins, G. D., Goltz, M. N., Gorelick, S. M., and McCarty, P. L., "Full-Scale Demonstration of In Situ Cometabolic Biodegradation of Trichloroethylene in Groundwater, 2: Comprehensive Analysis of Field Data Using Reactive Transport Modeling," *Water Resources Research*, 38 (2002).
- Goltz, M.N., G.C. Mandalas, G.D. Hopkins, and P.L. McCarty, "Technology Transfer of an Innovative Remediation Technology from the Laboratory to the Field: A Case Study of *In Situ* Aerobic Cometabolic Bioremediation," *Environmental Engineering and Policy*, 1 117-124 (1998).
- Goltz, M.N., G.D. Hopkins, and P.L. McCarty, "Field Studies: Elicitation of Fate and Transport Processes and Application to Full-scale Remediation," *Soil and Groundwater Pollution*, Ed. A.J.B. Zehnder, Kluwer Academic Publishers, Dordrecht, 110-116 (1995).
- Goltz, M.N. and K.J. Williamson, "Transfer and Commercialisation of Contaminated Groundwater Remediation Technologies," *International Journal of Technology Transfer and Commercialisation*, 1(4):329-346 (2002).
- Hopkins, G. D., M.N. Goltz, J.P. Allan, M.E. Dolan, and P.L. McCarty, "Full-Scale In-Situ Cometabolic Biodegradation of Trichloroethene-Contaminated Groundwater through Toluene Injection," Extended Abstract, American Chemical Society, Div. Environmental Chemistry, San Francisco, 233-235 (1997).
- Kawakami, B.T., J.A. Christ, M.N. Goltz, and P.L. McCarty, "Hydraulic Design of an In Situ Injection/Extraction Bioremediation System," *Designing and Applying Treatment Technologies—Remediation of Chlorinated and Recalcitrant Compounds*, Wickramanayake, G.B. and R.E. Hinchee, Eds., pp. 33-38, Battelle Press, Columbus, OH, (1998).
- Mandalas, G.C., J.A. Christ, G.D. Hopkins, P.L. McCarty, and M.N. Goltz, "Remediation Technology Transfer from Full-scale Demonstration to Implementation: A Case Study of Trichloroethylene Bioremediation," *Risk, Resource, and Regulatory Issues--Remediation of Chlorinated and Recalcitrant Compounds*, Wickramanayake, G.B. and R.E. Hinchee, Eds., pp. 235-240, Battelle Press, Columbus, OH (1998).
- McCarty, P. L., "Bioremediation of Chlorinated Solvents in Groundwater," *Groundwater Contamination and Its Control in China*, Ed. R. Fu, Y. Qian, and C. A. Shoemaker, Tsinghua University Press, Beijing, China, 83-94 (2000).
- McCarty, P. L., "Novel Biological Removal of Hazardous Chemicals at Trace Levels," Proceedings, International Symposium on Development of Innovative Water and Wastewater Treatment Technologies for the 21st Century, submitted (1999).
- McCarty, P. L., M. N. Goltz, G. D. Hopkins, J. P. Allan, "In Situ Biodegradation of Chlorinated Solvent Contaminants in Groundwater," Proceedings WEFTEC '96, Vol 3., Part I: *Remediation of Soil and Groundwater*, Water Environment Federation, Washington D.C., 217-223 (1996).

- McCarty, P. L., M. N. Goltz, G. D. Hopkins, M. E. Dolan, J. P. Allan, B. T. Kawakami, and Carrothers, T. J., "Full-Scale Evaluation of *In Situ* Comatabolic Degradation of Trichloroethylene in Groundwater through Toluene Injection," *Environmental Science and Technology*, **32**(1), 88-100 (1998).
- McCarty, P.L., M.N. Goltz, G.D. Hopkins, and J.P. Allan, "In Situ Biodegradation of Chlorinated Solvent Contaminants in Groundwater, " Proceedings WEFTEC '96, Vol 3., Part I: *Remediation of Soil and Groundwater*, Water Environment Federation, Washington D.C., 217-223 (1996).

**Western Region Hazardous Substance Research Center Project
SU94-02. Upscaling Pore-Scale Hydrodynamics and the Transport of Reactive Solutes:
Peter K. Kitanidis, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1995-1998

Project Amount: \$159,709

Research Category: Groundwater, optimization modeling, fate and transport, contaminants

Goal: (a) to improve understanding of hydrodynamics, mass transport, and biochemical reactions at the pore scale and (b) to derive physically valid mathematical representations of processes at the Darcy scale, where the pore details are not resolved. This research project focuses on mathematical analysis but is guided by experiments conducted by Professor McCarty and his co-workers in another project. Among the objectives of this project is to describe the mechanisms that control the growth of biofilms, such as shearing of biofilms at pore necks. A potential practical output of this research would be the derivation of relations between biomass and effective conductivity, dispersivity, and reaction rates in a porous medium with dynamically changing biofilms. Also accounting for sorption and desorption with characteristic times comparable to or slower than those of pore-scale diffusion and reaction.

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

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

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

Project Publications:

Cao, J., and P. K. Kitanidis, "Adaptive-Grid Simulation of GroundWater Flow in Heterogeneous Aquifers." *Adv. in Water Resources*, **22**(1), 17-31 (1998).

- Cao, J., and P. K. Kitanidis, "Pore-scale Dilution of Conservative Solutes: An Example," *Water Resources Research*, **34**(8), 1941-1949, (1998).
- Cao, J., and P.K. Kitanidis, "Adaptive Finite-Element Simulation of Stokes Flow in Porous Media," *Adv. in Water Resour.*, **22**(7), 681-696, (1999).
- Kitanidis, P. K., "How Observations and Structure Affect the Geostatistical Solution to the Steady-State Inverse Problem", *Ground Water*, **36**(5), 754-763 (1998).
- Kitanidis, P.K. and B.B. Dykaar, "Stokes flow in a slowly varying two-dimensional periodic pore," *Transport in Porous Media*, **26**(1), 89-98 (1997).
- Kitanidis, P.K., "A Variance-Ratio Test for Supporting a Variable Mean in Kriging," *Mathematical Ecology*, **29**(3), 335-348 (1997).
- Kitanidis, P.K., "The minimum structure solution to the inverse problem," *Water Resources Research*, **33**(10), 2263-2272 (1997).
- Snodgrass, M. F., and P. K. Kitanidis, "A Method to Infer In-Situ Reaction Rates From Push-Pull Experiments," *Ground Water*, **36**(4), 645-650, (1998).
- Snodgrass, M.F. and P.K. Kitanidis, "A geostatistical approach to contaminant source identification," *Water Resources Research*, **33**(4), 537-546 (1997).

**Western Region Hazardous Substance Research Center Project
SU94-03. Pathways of Anaerobic Toluene Metabolism by a Sulfate-Reducing Bacterium,
Strain PRTOL1: Alfred M. Spormann, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1995-1998

Project Amount: \$ 189,616

Research Category: Groundwater, BTEX, biochemistry, anaerobic biodegradation

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

Rationale: Although some information is available about anaerobic mineralization of toluene in one denitrifying strain, it is not clear if all anaerobes that degrade toluene utilize the same biochemical pathway. Concomitant to toluene mineralization in all toluene-degrading denitrifying cultures that have been investigated as well as in PRTOL1 is the formation of two metabolites, benzylsuccinate and *E*-phenylitaconate (or benzylfumarate). These metabolites are not further metabolized by whole cells of the pure toluene-degrading cultures studied to date and have been assumed to be dead-end by-products. The relationship between these metabolites and toluene mineralization is not resolved. Recently, we detected these compounds at field sites where BTEX compounds were consumed under anaerobic conditions (Beller et al. 1995). We focus on experiments investigating the connection between benzylsuccinate formation and alkylbenzene metabolism.

Approach: Permeabilized cell assay and cell extract experiments are being used to investigate the initial biochemical steps involved in toluene degradation and benzylsuccinate formation. Denitrifying strain T (Dolfing et al. 1990), which grows anaerobically on toluene and *m*-xylene and forms benzylsuccinate and phenylitaconate during toluene metabolism, is being used in addition to strain PRTOL1 because it is more amenable to laboratory cultivation. In cell extract studies, we specifically investigate the biochemical reactions involved in the formation of benzylsuccinate and phenylitaconate. Recently, we demonstrated that benzylsuccinate formation is the first step in anaerobic toluene mineralization in denitrifying strain T (Beller and Spormann 1997, *J. Bacteriol.*, 179 (3) p.670-676).

Status: Benzylsuccinate synthase is a novel, unique enzyme that catalyzes the formation of a new carbon-carbon bond and of a new chiral carbon. Benzylsuccinate formed by permeabilized cells of strain T are being analyzed by chiral HPLC and retention time are compared with authentic standards of each enantiomer. In further experiments, we test whether each enantiomer can be converted to phenylitaconate and benzoate by permeabilized cells. Both sets of experiments will demonstrate conclusively which benzylsuccinate isomer is formed and transformed by cells of strain T, respectively.

We also investigated whether toluene-mineralizing sulfate-reducing strain PRTOL1, a member of the delta subclass of proteobacteria, can activate toluene by addition to fumarate. Using permeabilized suspensions of toluene-grown PRTOL1 cells, we showed that *in vitro* benzylsuccinate formation from toluene plus fumarate was catalyzed at a rate that accounted for about 7% of the *in vivo* toluene transformation rate. This result, together with mass spectral data suggests that also in this organism anaerobic toluene is activated by benzylsuccinate synthase. Thus, toluene addition to fumarate may be a general mode of anaerobic toluene activation in phylogenetically disparate groups of toluene-mineralizing bacteria. The project is complete.

Project Publications:

- Beller, H.R. and A. M. Spormann, "Substrate Range of Benzylsuccinate Synthase from *Azoarcus* Strain T," *FEMS Microbiology Letters*, (**178**) 147-153 (1999).
- Beller, H.R. and A.M. Spormann, "Anaerobic Activation of Toluene and *o*-Xylene by Addition to Fumarate in Denitrifying Strain T," *Journal of Bacteriology*, **179**(3), 670-676 (1997).
- Beller, H.R. and A.M. Spormann, "Benzylsuccinate Formation as a Means of Anaerobic Toluene Activation by Sulfate-Reducing Strain PRTOL1," *Applied and Environmental Microbiology*, **63**(9), 3729-3731 (1997).
- Beller, H.R., and A. M. Spormann, "Analysis of the Novel Benzylsuccinate Synthase Reaction for Anaerobic Toluene Activation Based on Structural Studies of the Product," *Journal of Bacteriology* **180**(20), 5454-5457 (1998).
- Heider, J., A.M. Spormann, H.B. Beller, and F. Widdel Anaerobic bacterial metabolism of hydrocarbons, *FEMS Microbiology Review*, **22**(5) 459-473 (1998).

**Western Region Hazardous Substance Research Center Project
SU94-04. Anaerobic Ethylbenzene Oxidation in Denitrifying Strain EB1: Alfred M.
Spormann, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1995-1998

Project Amount: \$151,585

Research Category: Groundwater, BTEX, biochemistry, anaerobic biodegradation

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

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

Approach: Studies were conducted with strain EB1 which can metabolize ethylbenzene under denitrifying conditions. Cells grown anaerobically with ethylbenzene and nitrate as substrates are harvested anaerobically, concentrated, and subjected to cell suspension experiments. Suspensions are amended with ethylbenzene and nitrate, and the concentrations of ethylbenzene and nitrate are followed by GC and IC respectively. Potential intermediates during anaerobic degradation of ethylbenzene are detected using HPLC and the chemical nature of the compounds is identified by GC-MS. Based on the finding of intermediates isotope exchange studies will be employed to substantiate the proposed pathway. Results from the cell suspension studies will then be used to design enzyme assays with cell-free extracts.

Status: In experiments previously published (Ball et al., 1996) we identified the initial reactions in anaerobic ethylbenzene mineralization. Ethylbenzene oxidation is initiated by an anaerobic dehydrogenation reaction that results in 1-phenyl ethanol formation. Subsequent reactions convert 1-phenyl ethanol to benzoate via acetophenone as a transient intermediate. We focused on experiments to characterize the key enzymatic reactions of this pathway in cell free extracts of strain EB1. *In vitro* assays for ethylbenzene dehydrogenase and 1-phenyl ethanol dehydrogenase were developed in which the oxidation of the substrate is coupled to the reduction of *p*-benzoquinone. The specific activity of ethylbenzene dehydrogenase was approximately 10 nmol x min⁻¹ x [mg protein]⁻¹. Both enzymes activities were localized to the

membrane fraction. The stereoselectivity of 1-phenyl ethanol dehydrogenase was investigated. In cell-free extracts, the specific activity of (*S*)-(-)- 1-phenyl ethanol oxidation was 9-fold higher than the rate for acetophenone formation from (*R*)-(+)- 1-phenyl ethanol. In competition experiments, we found that the presence of (*R*) - 1-phenyl ethanol did not effect the rate of *in vitro* (*S*)- 1-phenyl ethanol oxidation. Michaelis-Menten kinetic studies on 1-phenyl ethanol dehydrogenase yielded a K_m and V_{max} for (*S*)- 1-phenyl ethanol of 25 μM and 5 $\text{nmol} \times \text{min}^{-1} \times [\text{mg protein}]^{-1}$. In an effort to develop an understanding of the ethylbenzene dehydrogenase reaction mechanism, transformation of halogenated ethylbenzenes, ethyltoluenes, styrene, and stable isotope labeled ethylbenzene was investigated. The project has been completed.

Project Publications:

- Ball, H.A., H.A. Johnson, M. Reinhard, and A. M. Spormann, "Initial Reactions in Anaerobic Ethylbenzene Oxidation by a Denitrifying Bacterium, Strain EB1," *Journal of Bacteriology*, **178**(19), 5755-5761 (1996).
- Ball, H.A., *Microbial Transformation of Aromatic Hydrocarbons Under Anaerobic Conditions*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1996).
- Johnson, H. A. and A. M. Spormann, "*In vitro* Studies on the Initial Reactions of Anaerobic Ethylbenzene Mineralization," *J. Bacteriology*, (**181**), 5662-5668 (1999).

**Western Region Hazardous Substance Research Center Project
SU94-05. Molecular Approaches to Optimize Starvation Promoter Driven TCE
Bioremediation in *Pseudomonas*: A. Matin, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1995-1997

Project Amount: \$114,721

Research Category: Groundwater, chlorinated organics, biodegradation, TCE

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

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

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

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

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

Status: A strong starvation promoter from *P. putida* was cloned (Kim et al., 1995), and has been successfully spliced to the *tmo* gene cluster, using a broad host range plasmid (pMMB67)

(Hahm and Matin. 1996). *P. putida* strains bearing the recombinant plasmid, but not control strains without the plasmid, carried out rapid and substantial conversion of indole into indigo. This conversion occurred only in the slow-growth/stationary phase, and not in the exponential growth at μ_{\max} . Indole to indigo conversion is carried out by TMO and is a convenient assay system because of the blue color of indigo. The recombinant plasmid strain also carries out TCE removal only in the slow-growth/stationary phase, as determined by gas chromatography. The study has been completed.

Project Publications:

Fraley, C.D., J.H. Kim, M.P. McCann, and A. Matin, "The *Escherichia coli* Starvation Gene, *cstC*, is Involved in Amino Acid Cometabolism," *Journal of Bacteriology*, **180**, 4287 (1998).

Kim, Y., L.S. Watrud, and A. Matin, "A Carbon Starvation Survival Gene Of *Pseudomonas putida* Is Regulated By *s54* ", *Journal of Bacteriology*, **177**(7), 1850-1859 (1995).

Matin, A., C.D. Little, C.D. Fraley, and M. Keyhan, " Use Of Starvation Promoters To Limit Growth And Selectively Express Tichloroethylene And Phenol Transformation Activity in Recombinant *Escherichia coli* ", *Applied and Environmental Microbiology*, **61**(9), 3323-3328 (1995).

**Western Region Hazardous Substance Research Center Project
SU94-06. Modeling VOC Emissions from Hazardous Waste Sites: Lynn M. Hildemann,
Stanford University (Supported by R2D2 Program)**

EPA Project Officer: Dale Manty

Project Period: 1995-1998

Project Amount: \$166,507

Research Category: Air pollution, volatile organics, fate and transport modeling

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

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

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

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

both the downwind and vertical directions were found to compare well with measurements from a published, comprehensive field study. The project has been completed.

Project Publications:

- Lin, J.S. and L. Hildemann, "A Generalized Mathematical Scheme to Analytically Solve the Atmospheric Diffusion Equation with Dry Deposition," *Atmospheric Environment*, **31**(1), 59-71 (1997).
- Lin, J.S. and L.M. Hildemann, "A Nonsteady-State Analytical Model to Predict VOC Emissions from Hazardous Waste Sites," in *Proceedings of the 1995 ASCE - CSCE Environ. Engineering Conference*, American Society of Civil Engineers, New York, 238-245, (1995).
- Lin, J.S. and L.M. Hildemann, "Mathematically Modeling the Transport of Hazardous Air Pollutants from Multiple Sources," in *Proc. 1995 ASCE - CSCE Environ. Eng. Conf.*, American Society of Civil Engineers, New York, 620-627, (1995).
- Lin, J.-S. and L.M. Hildemann, "Modeling Vertical Spread of Airborne Pollutants from Sources Near Ground Level - Comparison with Field Measurements," *Journal of Environmental Engineering*, **123**(12), 1194-1202 (1997).
- Lin, J.S. and L.M. Hildemann, "A Nonsteady-State Analytical Model to Predict VOC Emissions from Landfills," *Journal of Hazardous Materials*, **40**(3), 271-295 (1995).
- Lin, J.S. and L.M. Hildemann, "Analytical Solutions of the Atmospheric Diffusion Equation with Multiple Sources and Height-Dependent Wind Speed and Eddy Diffusivities," *Atmospheric Environment*, **30**(2), 239-254 (1996).
- Lin, J.S., *Derivation and Evaluation of Analytical Pollutant Dispersion-Deposition Models*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1997).

**Western Region Hazardous Substance Research Center Project
SU94-07. Reductive Transformation of Chlorinated Hydrocarbons by Reduced Ethenes
Catalyzed by Vitamin B₁₂ — Mechanistic and Kinetic Studies: Martin Reinhard, Stanford
University**

EPA Project Officer: Dale Manty

Project Period: 1995-2000

Project Amount: \$252,142

Research Category: Groundwater, chlorinated organics, degradation

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

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

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

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

promote the dehalogenation process will be investigated in a combined field and laboratory study with funding from different sources.

We developed kinetic data of TCE transformation and quantified the volatile products and intermediates acetylene, chloroacetylene, ethene, 1,1-, cis- and trans-DCE, vinyl chloride. In separate kinetic experiments under identical conditions we studied the kinetics of the TCE intermediates, notably acetylene, chloroacetylene, and 1,1-DCE. Chloroacetylene, which was commercially not available, was synthesized. From the data, kinetic sub-models were developed for acetylene, chloroacetylene and 1,1-DCE. These kinetic sub-models serve as a basis for modeling the transformation of TCE. The project has been completed.

Project Publications:

- Chiu, P.C. and M. Reinhard, "Transformation of Carbon Tetrachloride by Reduced Vitamin B12 in Aqueous Cysteine Solution," *Environmental Science and Technology*, **30**(6), 1882-1889 (1996).
- Reinhard, M., M. Semadeni, P.K. Sharma, and W.R. Haag, "Dehalogenation of Haloaliphatic Hydrocarbon Compounds in the Aquatic and Terrestrial Environment," Chapter in *American Society of Agronomy Monograph "Bioremediation of Contaminated Soils."* D. Adriano, J.-M. Bollag, Eds., 133-173 (1999).
- Semadeni, M., P.-C. Chiu, and M. Reinhard. "Reductive Transformation of Trichloroethene Catalyzed by Cobalamin: Reactivities of the Intermediates, Acetylene, Chloroacetylene, and the DCE Isomers," *Environmental Science and Technology*, **32**(9), 1207-1213 (1998).

**Western Region Hazardous Substance Research Center Project
SU94-08. Evaluation of Strategies for Full Scale Bioremediation of the Seal Beach Site
Using Anaerobic Microbial Processes: Martin Reinhard, Gary Hopkins, and Peter
Kitanidis, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1994-1998

Project Amount: \$106,250

Research Category: Groundwater, BTEX, demonstration, fate and transport modeling,
biodegradation

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

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

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

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

planned for the 1995/6 project year. For the future, we plan to develop a model more advanced than existing models and well-adjusted to represent the in-situ biodegradation of fuels in groundwater. This project has been completed.

Project Publications:

Snodgrass, M.F. and P.K. Kitanidis. "A Method to Infer in situ Reaction Rates from Push-Pull Experiments." *Ground Water* **36**(4) 645-650 (1998).

**Western Region Hazardous Substance Research Center Project
SU94-09. Trace Element Adsorption in Porous Particle Packed Beds: James O. Leckie,
Stanford University (Supported by R2D2 Program)**

EPA Project Officer: Dale Manty

Project Period: 1995-2000

Project Amount: \$108,070

Research Category: Groundwater, metals, sorption, treatment

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

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

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

Status: On the basis of equilibrium and rate of sorption characteristics of 5 transition alumina solids (from ALCOA) for cadmium sorption, DD660 and DD431 have been selected for further study. The two adsorbents have been characterized using XRD, SEM, BET surface area measurements and potentiometric titrations. With both solids, equilibrium experiments have been conducted where the variables have been: total concentration of cadmium (10^{-3} M to 10^{-6}

M), ionic strength (0.01 M to 0.1 M NaNO₃) and presence of ligands (chloride, thiosulfate and EDTA) at various concentrations. The data is currently being modeled using the surface complexation approach. Since, the effect of EDTA on cadmium sorption is more complex than that of the other ligands, EDTA-alone experiments and cadmium-EDTA ‘mixed-system’ experiments have been conducted at different EDTA concentrations and different Cd:EDTA ratios, respectively, to have a suitable dataset for an unambiguous interpretation of the sorption behavior. Additionally, rate of uptake experiments have been conducted with the selected solids at fixed pH. The variables that have been investigated are: cadmium concentration (10⁻³ M to 10⁻⁶ M), adsorbent particle size (0.1 mm, 0.5 mm and 1.0 mm), pH (7.5, 8 and 8.5) and presence of ligands (chloride, thiosulfate and EDTA) at two different ligand concentrations. The rate of uptake data will be modeled after the equilibrium sorption data has been adequately modeled using the surface complexation approach.

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

Project Publications:

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**Western Region Hazardous Substance Research Center Project
SU94-10. Hydrologic and Biological Factors Affecting Aquifer Clogging During In-Situ
Bioremediation: Perry L. McCarty, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1995-1998

Project Amount: \$190,883

Research Category: Groundwater, biodegradation, in-situ clogging

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

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

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

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

Project Publications:

- Dupin, H.J. "*Biological and Hydrodynamic Factors Affecting Aquifer Clogging During In-Situ Bioremediation*," Ph.D Dissertation, Department of Civil and Environmental Engineering, Stanford University, Stanford, CA (1999).
- Dupin, H.J. and McCarty, P.L., "Mesoscale and Microscale Observations of Biological Growth in a Silicon Pore Imaging Element, *Environmental Science and Technology*, **33**(8), 1230-1236 (1999).
- Dupin, H. J. and McCarty, P. L., "Impact of Colony Morphologies and Disinfection on Biological Clogging in Porous Media," *Environmental Science & Technology* (8) **34**, 1513-1520 (2000).
- Dupin, H. J., Kitanidis, P., and McCarty, P., "Simulations of Two Dimensional Modeling of Biomass Aggregate Growth in Network Models," *Water Resources Research*, **37**(12), 2981-2994 (2001).
- Dupin, H. J., Kitanidis, P. and McCarty, P. L. , "Pore-Scale Modeling of Biological Clogging Due to Aggregate Expansion: A Material Mechanics Approach," *Water Resources Research*, **37**(12), 2965-2979 (2001).

**Western Region Hazardous Substance Research Center Project
SU95-01. Full-Scale Evaluation of an Apparatus for Down-well Oxygen Transfer to
Implement *In situ* Bioremediation at Edwards AFB: Perry L. McCarty and Mark N.
Goltz, Stanford University (Supported by R2D2 Program)**

EPA Project Officer: Dale Manty

Project Period: 1995-1998

Project Amount: \$128,274

Research Category: Groundwater, treatment, gas transfer

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

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

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

Status: The four oxygen transfer devices were evaluated in the laboratory under a range of conditions. The static mixer, which had been installed at Edwards based on practical considerations (mainly ease of installation and operation) was demonstrated to operate effectively in the field (see Full-Scale Evaluation of *In Situ* Bioremediation of Chlorinated Solvent Groundwater Contamination). This project has been completed. Full-scale field studies conducted at Edwards Air Force Base have demonstrated that static mixers are highly effective for transferring oxygen to groundwater.

Project Publications:

Bae, J., L. Semprini, and P. L. McCarty, "Apparatus for Down-Well Oxygen Transfer into Contaminated Aquifers," *Journal of Environmental Engineering*, **121**(8), 565-570 (1995).
Semprini, L., P.L. McCarty, P.K. Kitanidis, and J. Bae, "Method and Apparatus for *In Situ* Groundwater Remediation," U.S. Patent No. 5,302,286 (April 12, 1994).

**Western Region Hazardous Substance Research Center Project
SU95-02. Field Testing of Palladium-Catalyzed Hydrodehalogenation for Chlorinated
Hydrocarbon Removal from Groundwater: Martin Reinhard and Paul V. Roberts,
Stanford University (Supported by R2D2 Program)**

EPA Project Officer: Dale Manty

Project Period: 1995-2000

Project Amount: \$407,427

Research Category: Groundwater, treatment, chlorinated solvents, palladium catalyst

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

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

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

Status: The project has been completed. Laboratory studies have shown that by periodic flushing of the catalyst its activity can be maintained for at least three month without signs of catalyst degradation. Potential problems for operating a palladium catalyzed ground water treatment system include the presence of hydrogen sulfide in groundwater, growth of hydrogen-oxidizing sulfate reducing bacteria in the reactor, and clogging of the hydrogen feed system due to biological fouling. Laboratory experiments have shown that periodic (weekly) flushing of the reactor with hypochlorite (Clorox) prevents these problems. Clorox flushing also restores catalyst activity loss due to unidentified chemical factors. A field-scale demonstration system

employing a Pd-reactor that was installed in a well has been in operation at LLNL since October 1998 (McNab et al., *Env. Sci. Technol.* 2000). The demonstration unit utilizes a packed-bed column and a microporous hollow fiber membrane hydrogen supply module. Removals were 99% or better for PCE, TCE, and 1,1-DCE, 98% for carbon tetrachloride, 91% for chloroform, and 0% for 1,2-Dichloroethane. These removals are consistent with previously reported laboratory studies. Periodic flushing with Clorox has been instituted for maintaining catalyst activity. Second-generation systems have been in operation at LLNL and are under construction at Edwards AFB with DOD support. The objective of the latter study is to assess the economic feasibility of the process.

Project Publications:

- Lowry, G.V., P.V. Roberts, W. McNab, and M. Reinhard, "Catalytic Reduction of Halogenated Ethenes, Ethanes and Methanes Using Hydrogen and a Palladium Catalyst," American Chemical Society National Meeting, San Francisco, CA (1997).
- Lowry G.V. and M. Reinhard, "Pd-Catalyzed TCE Dechlorination in Groundwater: Solute Effects, Biological Control, and Oxidative Catalyst Regeneration." *Environ. Sci. Technol.*, **34**(15), 3217-3223 (2000).
- McNab, Jr., W. R. Ruiz, and M. Reinhard, "In-Situ Destruction of Chlorinated Hydrocarbons in Groundwater Using Catalytic Reductive Dehalogenation in a Reactive Well: Testing and Operational Experiences," *Environmental Science and Technology*, **34**(1) 149-153 (2000).
- Munakata, N., W. McNab, W. Haag, P.V. Roberts, and M. Reinhard, "Effects of Water Matrix on Pd-Catalyzed Hydrodehalogenation," American Chemical Society National Meeting, San Francisco, CA (1997).
- Schreier, C.G. and M. Reinhard, "Catalytic Dehydrohalogenation and Hydrogenation using H₂ and Supported Palladium as a Method for the Removal of Tetrachloroethylene from Water," presented before the Division of Environmental Chemistry, American Chemical Society (1995).
- Schüth, C., S. Disser, F. Schüth, and M. Reinhard. "Tailoring Catalysts for hydrodechlorinating Chlorinated Hydrocarbon Contaminants in Groundwater." *Applied catalysis B: Environmental*, **28** 147-152 (2000).
- Siantar, D.P., C.G. Schreier, C.S. Chou, and M. Reinhard, "Treatment of 1,2-Dibromo-3-Chloropropane and Nitrate-Contaminated Water with Zero-Valent Iron or Hydrogen/Palladium Catalysts," *Water Research*, **30**, 2315-2322 (1996).

**Western Region Hazardous Substance Research Center Project
SU95-03. Physics of Dissolution of Nonaqueous Phase Liquids: Pore Networks and Field
Simulations: Steven M. Gorelick, Stanford University (Supported by R2D2 Program)**

EPA Project Officer: Dale Manty

Project Period: 1995-1998

Project Amount: \$159,610

Research Category: Groundwater, DNAPL, fate and transport modeling

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

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

Approach: This study involves 4 stages: (1) development of a pore network model representative of a real porous media; (2) development of a physically-based NAPL dissolution relation using the pore network model; (3) modification of a 3-dimensional compositional simulator to represent nonequilibrium NAPL dissolution, and (4) hypothetical field-scale modeling studies investigating the limitations of the local equilibrium assumption on field-scale NAPL dissolution.

Status: This study is complete. A pore network model, consisting of a simulated 3D array of pore tubes connected by chambers, was developed to represent the physics of nonequilibrium NAPL dissolution in porous media. Two-phase flow and transport of dissolved NAPL components were represented in the network, as well as local dissolution from the NAPL phase into the surrounding water film and corners. This local dissolution was based on a solution to one-dimensional diffusion equations and is a function of the water velocity in the corner of a

NAPL-filled tube or chamber. The network model successfully matches data for laboratory drainage and imbibition, and predicts experimentally determined NAPL blob-size distribution. Furthermore, simulated results of effluent solute concentration are consistent with steady-state column data. A theoretical result of general interest is the determination of a relationship between the NAPL dissolution rate coefficient and Peclet number. Four distinct regimes of this relationship were identified, representing four different physical process controlling dissolution: pore diffusion, mixing and multiple contact, corner diffusion, and a transition zone. Corner diffusion controls NAPL dissolution in the high-Peclet regime, which is characteristic of nonequilibrium NAPL dissolution. Several relationships between NAPL dissolution rate coefficient and Peclet number were generated for NAPL saturations during drainage and imbibition. These functions can be used to obtain a NAPL dissolution rate coefficients for input in a field-scale simulator for any specified Peclet number and NAPL saturation. The network model was used to investigate the relationship between interfacial area, NAPL saturation, and dissolution rate coefficients.

Project Publications:

- Dillard, L.A., H.I. Essaid, and M.J. Blunt, "A Functional Relation For Field-scale Nonaqueous Phase Liquid Dissolution Developed Using a Pore Network Model," *Journal of Contaminant Hydrology*, (awaiting print) **48**(2), (2001).
- Dillard, L.A., and M.J. Blunt, "Development of a Pore Network Simulation Model to Study Nonaqueous Phase Liquid Dissolution," *Water Resources Research*, **36**(2), 439-454 (2000).
- Dillard, L.A., M.J. Blunt, S.M. Gorelick, and H.I. Essaid, "Development of a constitutive relation between nonaqueous phase liquid dissolution rate and porous media and fluid properties," *EOS*, **78**(17), S152 (1997).
- Dillard, L.A., M.J. Blunt, H.I. Essaid, and S.M. Gorelick, "Investigating the physics of dissolution of nonaqueous phase liquids in heterogeneous porous media using pore network modeling," *EOS*, **76**(46), F257 (1995).
- Essaid, H. I., W. N. Herkelrath, and L. A. Dillard, "The influence of spatial variability of hydraulic properties on the flow of water near a subsurface oil body," *EOS*, **76**(46) (1995).
- Zhou, D., L.A. Dillard, and M.J. Blunt, "A Physically Based Model of Dissolution of Nonaqueous Phase Liquids in the Saturated Zone," *Transport in Porous Media*, **39**(2), 227-255 (2000).
- Zhou, D, L.A. Dillard, and M.J. Blunt, "A mechanistic model for non-aqueous phase liquid dissolution in the saturated zone," *EOS*, **78**(17), S132 (1997).

**Western Region Hazardous Substance Research Center Project
SU95-04. Three-Phase Flow in Fractured Media: Martin Blunt and Paul Roberts,
Stanford University (Supported by R2D2 Program)**

EPA Project Officer: Dale Manty

Project Period: 1995-1998

Project Amount: \$270,742

Research Category: Groundwater, DNAPL, movement and fate

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

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

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

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

- Dillard, L.A., and M.J. Blunt, "Development of a Pore Network Simulation Model to Study Nonaqueous Phase Liquid Dissolution," *Water Resources Research*, **36**(2), 439-454 (2000).
- Dillard, L.A., H.I. Essaid, and M.J. Blunt, A functional relation for field-scale nonaqueous phase liquid dissolution developed using a pore network model, *J. Contaminant Hydrology*, in press.
- Dillard, L.A., M.J. Blunt, H.I. Essaid, and S.M. Gorelick, "Investigating the physics of dissolution of nonaqueous phase liquids in heterogeneous porous media using pore network modeling," *Eos Transactions*, **76**(46) (1995).
- Essaid, H.I., W.N. Herkelrath, and L.A. Dillard, "The influence of spatial variability of hydraulic properties on the flow of water near a subsurface oil body," *Eos Transactions*, **76**(46) (1995).
- Keller, A. A. "Steam Injection to Displace DNAPLs From Fractured Media", in International Association of Hydrological Sciences, Publication No. 250, Oxfordshire, UK (1998).
- Keller, A. A. and P. V. Roberts, "High Resolution, Non-destructive Measurement and Characterization of Fracture Aperture," submitted 1997.
- Keller, A. A., "High Resolution, Non-Destructive Measurement and Characterization of Fracture Apertures," *Int. J. Rock Mech. Min. Sci.*, **35**(8), 1037-1050 (1998).
- Keller, A. A., M. J. Blunt and P. V. Roberts, "Behavior of Non-Aqueous Phase Liquids in fractured porous media under two-phase flow conditions", *Transport in Porous Media*, **38**(1-2), 1135-1137 (2000).
- Keller, A.A., M.J. Blunt, and P.V. Roberts, "Micromodel Observation of the Role of Oil Layers in Three Phase Flow," *Transport in Porous Media*, **26**, 277-297 (1997).
- Keller, A.A., P.V. Roberts and M.J. Blunt, "Effect of Fracture Aperture Variations on Dispersion of Contaminants," *Water Resources Research*, **35**(1) 55-63 (1999).
- Keller, A.A., P.V. Roberts, and P.K. Kitanidis, "Prediction of Single Phase Transport Parameters in a Variable Aperture Fracture," *Geophysical Research Letters*, **22**(11) 1425-1428 (1995).
- Keller, A.A., *Single and Multiphase Flow and Transport in Fractured Porous Media*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1996).
- Zhou, D., L.A. Dillard, and M.J. Blunt, "A Physically Based Model of Dissolution of Nonaqueous Phase Liquids in the Saturated Zone," *Transport in Porous Media*, **39**(2) 227-255 (2000).

**Western Region Hazardous Substance Research Center Project
SU95-05. Effects of Redox Zones on the Fate and Transport of Contaminants in the
Saturated Subsurface; Characterization and Simulation: Keith Loague, Stanford
University (Supported by R2D2 Program)**

EPA Project Officer: Dale Manty

Project Period: 1995-1998

Project Amount: \$121,422

Research Category: Groundwater, chemistry, redox, movement and fate modeling

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

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

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

Status: The reaction network for redox zones was compartmentalized by focusing on the dominant reactions. The compartmentalized approach allows for a complex geochemical system to be solved easily as a function of time and energy yield (i.e., Gibbs free energy). Our method (i) solves the numerical system without evoking a redox parameter, (ii) improves the numerical stability of redox systems by choosing which compartment, and thus which reaction network, to use based upon the concentration ratios of key constituents, (iii) simulates the development of redox zones as a function of time without the use of inhibition factors or switching functions, and (iv) reduces the number of transport equations that need to be solved in space and time. The compartmentalized reaction network model has been coupled with a two-dimensional solute transport code. This project has been completed.

Project Publications:

- Abrams, R. H. "*A Compartmentalized Approach To Simulating Redox Zones In Contaminated Aquifers*," Ph.D. Dissertation, Department of Geological and Environmental Sciences, Stanford University, Stanford, CA (1999).
- Abrams, R.H. and K. Loague, A compartmentalized solute transport model for redox zones in contaminated aquifers. 1. Theory and development. *Water Resources Research*, **36**, 2011-2013 (2000).
- Abrams, R.H. and K. Loague, A compartmentalized solute transport model for redox zones in contaminated aquifers. 2. Field-scale simulations. *Water Resources Research*, **36**, 2015-2029 (2000).
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**Western Region Hazardous Substance Research Center Project
SU95-07. Biochemical Mechanisms of PCE Dehalogenation by Strain MS-1, and its
Potential for In-situ Bioaugmentation: Pramod K. Sharma and Perry L. McCarty,
Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1995-1998

Project Amount: \$270,300

Research Category: Groundwater, chlorinated solvents, biodegradation

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

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

Approach: There are various mechanisms now proposed by which PCE is reductively converted to cDCE, such as CO dehydrogenase and Vitamin B₁₂. Through evaluation of cell-free extracts and then the purified enzyme system, the factors affecting PCE dehalogenation by strain MS-1 will be studied. Also to be determined is whether or not MS-1 can obtain energy for growth from PCE dehalogenation. Biochemical testing, cellular fatty acid finger printing, and 16s rRNA analyses will be used to determine how MS-1 is related to other microorganisms.

Status: This project was begun in March 1995. Strain MS-1 was found to be an enteric bacteria closely related to other enteric microorganisms. REP-PCR analysis indicated that none of the known closely related strains was identical to MS-1, but one of them, *Microbacterium agglomerans*, Group 5, from the American Type Culture Collection, was also found capable of dehalogenating PCE to cDCE. Two additional facultative strains with this ability, both

pseudomonads, were also found this past year. MS-1 was introduced successfully into a 39 liter anaerobic fixed-film bioreactor following three months of methanogenic treatment of a mixture of yeast extract (500 mg/l), benzoate (500 mg/l), but without PCE (1 mg/l) conversion. PCE dehalogenation to cDCE began immediately after MS-1 introduction, indicating this organism can successfully carry out the observed dehalogenation in mixed cultures. MS-1 and the other isolated strains were found to gain energy and carbon for growth through oxidation of acetate and reduction of PCE to cDCE. This study has been completed.

Project Publications:

Sharma, P. and P. L. McCarty, "Isolation and Characterization of a Facultative Bacterium that Reductively Dehalogenates Tetrachloroethene to cis-1,2-dichloroethene," *Applied and Environmental Microbiology*, **62**(3), 761-765 (1996).

**Western Region Hazardous Substance Research Center Project
SU96-01. A Large Scale Model for Anaerobic Bioremediation at the Seal Beach Site:
Martin Reinhard and Peter K. Kitanidis, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1995-1998

Project Amount: \$225,000

Research Category: Groundwater, fate and transport modeling, treatment, BTEX

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

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

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

Status: The computer model for the evaluation of slug tests with reactive solutes has been completed. It has been used to match reaction rates to the observed response. The two models of the processes occurring within the scaled up test zone are working. Reaction parameters derived from the previous tests and model are incorporated in the models. The study is now completed.

Project Publications:

Cunningham, J.A., H. Rahme, G.D. Hopkins, and M. Reinhard. "Enhanced in situ Bioremediation of BTEX-Contaminated Groundwater by Combined Injection of Nitrate and Sulfate," *Environmental Science and Technology*, 35(8) 1663-1670 (2001).

**Western Region Hazardous Substance Research Center Project
SU96-02. Mechanisms, Chemistry, and Kinetics of Anaerobic Degradation of cDCE and
Vinyl Chloride: Perry L. McCarty and Alfred M. Spormann, Stanford University
(Supported by DuPont Chemicals and U.S. Department of Energy)**

EPA Project Officer: Dale Manty

Project Period: 1996-2003

Project Amount: \$860,000

Research Category: Groundwater, chlorinated solvents, anaerobic biodegradation

Goal: The objectives of this study are to describe the bacterium or groups of bacteria that are responsible for conversion of tetrachloroethene (PCE) to ethene in aquifer samples taken from a contaminated site in Victoria, Texas, and to examine the factors affecting the rate and extent of anaerobic transformations of contaminated groundwater and chlorinated solvent DNAPL.

Rationale: Several species of bacteria have been isolated and identified by others that have the ability to reductively dehalogenate chlorinated aliphatic hydrocarbons (CAHs). However, only one organism responsible for the complete dehalogenation from tetrachloroethylene (PCE) to ethene has so far been identified. Available evidence that the individual dehalogenation steps are the result of cometabolism or of energy metabolism was not known when this study began. In addition, the various factors that affect the rates of dehalogenation have not been adequately evaluated. Such factors may include the electron donor used, the presence or absence of alternative electron acceptors, pH, and temperature. In order to understand the process better so that it can be more widely applied, its suitability for application at a given location can be better determined, and the economics of process implementation can be improved, definitive studies to better understand the nature of the organisms involved and factors affecting transformation rates are needed.

Approach: Anaerobic aquifer material from a contaminated site in Victoria, Texas, was obtained by DuPont Chemicals for this study and enrichment cultures capable of the anaerobic reductive dehalogenation of PCE to ethene were maintained for several years in laboratory chemostats. Batch studies using cultures from the chemostats were used to evaluate reaction kinetics. Column studies were used to evaluate the potential for anaerobic biological treatment of DNAPL source areas. Samples are analyzed for a range of CAHs and ethene, as well as for primary substrates, sulfate, and methane. The relationships between time and amount of primary substrate on CAH transformation was evaluated. In separate studies, enrichment cultures were developed using various electron donors and PCE, cis-1,2-dichloroethene (cDCE), or vinyl chloride (VC) as electron acceptors. Further studies were conducted at high chlorinated solvent concentrations and with PCE DNAPL, both in batch cultures and with columns of aquifer material.

Status: Benzoate, acetate, and formate were all found to stimulate dehalogenation, although benzoate appears to be the better of the three. Enrichment cultures have been developed that retain their ability to reduce PCE to ethene. This overall study clearly demonstrated that in the

cultures developed there are two separate groups involved in the overall dehalogenation of PCE to ethene, the first is the group that converts PCE to c-DCE, and the second is the group that converts c-DCE to vinyl chloride and ethene. Rates of transformation were evaluated by a mixed culture growing on yeast extract. The rates of transformation of PCE and TCE to cDCE were about four times faster than for the conversion of cDCE to vinyl chloride and vinyl chloride to ethene. Organisms appear to obtain energy from the dehalogenation reaction while using the chlorinated organics as electron acceptors. Monod kinetics of the reactions when the CAHs are rate limiting have been completed. Preliminary enzymatic studies have been conducted on VC transformation, indicating that it is particulate and not highly oxygen sensitive. Efficient dehalogenation using benzoate as an electron acceptor can be attained by control of molecular hydrogen concentration.

Further studies were conducted with substrates that might be distributed throughout an aquifer to serve as slow-hydrogen release compounds. Included here were precipitated calcium oleate and biomass in the form of bacterial cells. Both were found suitable for this purpose. Studies with high concentrations of PCE indicated that dehalogenated bacteria could achieved such dehalogenation in solutions saturated with PCE. Further, these high concentrations, as well as high concentrations of cDCE and ethene were inhibitory to competing methanogenic bacteria. Thus, dehalogenation of PCE DNAPL has good potential as a remediation alternative. This has been demonstrated column studies containing PCE DNAPL at 2 percent residual pore space saturation. Enhanced PCE dissolution by rapid conversion of PCD to cDCE was demonstrated. Properties of substrates for best enhanced DNAPL dissolution were evaluated with the finding that those that partition strongly into DNAPLs are best. Competitive PCR was found to be a highly useful method for quantification of the dehalogenating microorganism that convert DCE and VC to ethene. Enrichment cultures grown on VC and molecular biological analysis indicated that the Victoria culture organism was a member of the *Dehalococcoides* genus (Strain VS) and has the capability to obtain energy for growth from vinyl chloride. The numerous results of these studies will be useful in assessments of the potential for reductive dehalogenation of chlorinated solvents at contaminated sites.

Project Publications:

- Rosner, B. M., P.L. McCarty, and A.M. Spormann, "In Vitro Studies on Reductive Vinyl Chloride Dehalogenation by an Anaerobic Mixed Culture," *Applied and Environmental Microbiology*, **63**(11), 4139-4144 (1997).
- Yang, Y. and P.L. McCarty, "Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Mixed Culture," *Environmental Science and Technology*, **32**(24), 3591-3597 (1998).
- Haston, Z.C. and McCarty, P.L., "Chlorinated Ethene Half-Velocity Coefficients (K_S) for Reductive Dehalogenation," *Environmental Science and Technology*, **33**(2), 223-226 (1999).
- Haston, Z.C., "*Factors Affecting Growth and Utilization in the Anaerobic Dehalogenation of Chlorinated Ethenes*," Ph.D Dissertation, Department of Civil and Environmental Engineering, Stanford University, Stanford, CA (1999).
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- Yang, Y. and P. L. McCarty, Biologically Enhanced Dissolution of Tetrachloroethene DNAPL, *Environmental Science & Technology*, **34**(14), 2979-2984 (2000).
- McCarty, P. L. and Ellis, D. E., "Natural Attenuation," *Innovative Approaches to the On-Site Assessment and Remediation of Contaminated Sites*, eds. D. Reible and K. Demnerova, Kluwer Academic Publishers, Dordrecht, 141-181 (2002).
- Yang, Y., and McCarty, P. L., "Comparison of Donor Substrates for Biologically Enhanced Tetrachloroethene (PCE) DNAPL Dissolution," *Environmental Science & Technology*, **36**(15), 3400-3404 (2002).
- Chu, M., Kitanidis, P. K. and McCarty, P. L., "Effects of Biomass Accumulation on Biologically Enhanced Dissolution of a PCE Pool: A Numerical Simulation." *Journal of Contaminant Hydrology*, **65**, 75-100 (2003).
- Cupples, A. M., Spormann, A. M., and McCarty, P. L., "Growth of a *Dehalococcoides*-like Microorganism on Vinyl Chloride and *cis*-Dichloroethene as Electron Acceptors as Determined by Competitive PCR," *Applied and Environmental Microbiology*, **69**(2) 953-959 (2003).
- Yang, Y., and McCarty, P. L., "Response to Comment on 'Comparison of Donor Substrates for Biologically Enhanced Tetrachloroethene (PCE) DNAPL Dissolution'," *Environmental Science & Technology*, **37**(11), 2620-2621 (2003).

**Western Region Hazardous Substance Research Center Project
SU97-03. Bioenhanced In-Well Vapor Stripping to Treat Trichloroethylene (TCE): Mark N. Goltz, Department of Engineering and Environmental Management, Air Force Institute of Technology; Perry L. McCarty, Steve M. Gorelick and Gary D. Hopkins, Stanford University (Supported by the U. S. Air Force and the Strategic Environmental Research and Development Program)**

EPA Project Officer: Dale Manty

Project Period: 1997-2002

Project Amount: \$950,000

Research Category: Groundwater, demonstration, chlorinated solvents, vapor stripping, biodegradation

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

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

Approach: Using the BEHIVS concept, an in-well vapor stripper, operating in an upflow mode, was augmented by a biotreatment well that promotes aerobic cometabolic bioremediation, operating in a downflow mode. The well pair was used to establish horizontal circulation cells to remediate the source of TCE in a single contaminated aquifer at Edwards AFB. The upgradient vapor stripping well was used to reduce source concentrations to levels (< 1 mg/L) that would enable the two biotreatment wells, located about 8 m downgradient to remove TCE to near regulatory levels with no need to pump contaminated groundwater to the surface for treatment. Model simulations were used to illustrate how this configuration may be applied to remediate a chlorinated solvent source area under typically encountered conditions of anisotropy. Model simulations were also used for system design.

Status: This study was completed in 2002 through additional funding from Earth Tech Inc. through the U.S. Department of Defense. TCE concentrations within the study area before BEHIVS operation average 4,600 µg/L in the lower portion of the aquifer and 1,240 µg/L in the upper portion. Operation of the BEHIVS system resulted in reducing the lower aquifer zone TCE concentrations by 91% in the treatment area, with 10 of the 14 monitoring wells showing concentration reductions of between 94 and 97 %. Average TCE concentrations in the upper aquifer zone within the treatment area were reduced by 56%. The total TCE mass removal was 8.1 kg, 7.1 kg of which resulted from in-well vapor stripping and 1.0 kg from biotreatment. With an air to water ratio between 73 and 90, TCE removal by single-pass vapor stripping averaged 95.4%. With an injected toluene concentration of 12 mg/L, maximum percentage removal of TCE through biological treatment was about 70 %, and the maximum µg/L removal at higher TCE concentrations was about 400 µg/L. Rebound studies indicated TCE concentrations rose to near the pre-operational level within about 3.5 months after BEHIVS operation was stopped. Periodic operation of the BEHIVS system should permit maintaining low TCE concentrations downgradient from the source area and thus protect downgradient users. Detailed modeling analysis provided valuable information about the location of the TCE source in this aquifer so that future efforts at source removal can be more focused.

Project Publications:

- McCarty, P. L., "Bioremediation of Chlorinated Solvents in Groundwater," *Groundwater Contamination and Its Control in China*, Ed. R. Fu, Y. Qian, and C. A. Shoemaker, Tsinghua University Press, Beijing, China, 83-94 (2000).
- McCarty, P. L., "Strategies for In-Situ Bioremediation of Chlorinated Solvent Contaminated Groundwater," *Groundwater Quality 2001*, University of Sheffield, Sheffield, UK, 12-15 (2001).
- McCarty, P. L., Gorelick, S. M., Goltz, M. N., Hopkins, G. D., and Eisenberg, F., "Operation and Analysis of the BEHIVS System at Edwards Air Force Base," Western Region Hazardous Substance Research Center, Department of Civil and Environmental Engineering, Stanford University, Stanford, CA (June 30, 2002).

**Western Region Hazardous Substance Research Center Project
SU97-04. Effect of Chemical Structure on the Biodegradability of Halogenated Hydrocarbons: Paul V. Roberts, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1997-2000

Project Amount: \$71,879

Research Category: Groundwater, chlorinated solvents, biodegradation

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

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

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

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

Project Publications:

SU97-07. Trace Element Adsorption in Porous Particle Packed Beds: James O. Leckie, Stanford University

EPA Project Officer: Dale Manty

Project Period: 1997-2000

Project Amount: \$108,070

Research Category: Groundwater, heavy metals, treatment, sorption

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

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

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

Status: The proposed experimental work for the project is complete. Column experiments have been conducted to evaluate sorption performance as a function of the influent concentration and pH. Such experiments have been conducted for both selenite and cadmium. Equilibrium sorption results have been successfully modeled using the Triple-Layer Model (TLM). The modeling of the rate of uptake data of cadmium (in the absence and presence of ligands) and selenite in batch

systems was performed using the KINEQL approach. Model development for describing column breakthrough characteristics were conducted as part of another project.

Project Publications:

Papelis, C., "X-ray Photoelectron Spectroscopic Studies of Cadmium and Selenite Adsorption on Aluminum Oxides," *Environmental Science & Technology*, **29**(6), 1526-1533 (1995).

Papelis, C., *Cadmium and Selenite Adsorption on Porous Aluminum Oxides: Equilibrium, Rate of Uptake and Spectroscopic Studies*, Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA (1992).

Papelis, C., G.E. Brown, G.A. Parks, and J.O. Leckie, "X-ray Absorption Spectroscopic Studies of Cadmium and Selenite Adsorption on Aluminum Oxides," *Langmuir*, **11**(6), 2041-2048 (1995).

Papelis, C., P.V. Roberts, and J.O. Leckie, "Modeling the Rate of Cadmium and Selenite Adsorption on Micro- and Mesoporous Transition Aluminas," *Environmental Science & Technology*, **29**(4), 1099-1108 (1995).

**Western Region Hazardous Substance Research Center Project
SU99-05. Arsenic Removal in High Capacity Porous Alumina Packed-Bed Reactors: J.O.
Leckie, Stanford University.**

EPA Project Officer: Dale Manty

Project Period: 1999-2001

Project Amount: \$143,770

Research Category: Groundwater, trace elements, arsenic, treatment, adsorption

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

Rationale: EPA recently reduced the maximum contaminant level for arsenic in drinking water to 10 ppb (from 50 ppb). Many US surface and groundwaters are out of compliance with this standard, thus requiring some form of treatment before these water resources can be used for human consumption. Sorption onto packed-beds of porous alumina particles is a promising technology that offers significant advantages over currently available alternative methods (precipitation, ion-exchange and reverse osmosis). The technology could also benefit groundwater remediation efforts in countries like Bangladesh, where millions of people are dependent on arsenic-contaminated groundwater for their drinking water needs.

Approach: A detailed parametric experimental investigation of the processes governing the fixed-bed performance in treating arsenic contaminated waters was undertaken. This information is necessary for the development of a mathematical model for simulating processes relevant to sorption in packed-beds. This model must incorporate equilibrium sorption, mass-transfer, and advection-dispersion parameters. Three types of experiments were conducted: (a) equilibrium batch sorption experiments to isolate the effect of solution and surface chemistry, (b) rate-limited sorption experiments in batch systems at fixed pH to study the combined effect of surface chemistry and mass transfer processes and (c) column experiments to study the combined effects of all three processes. Experimental conditions were selected to incorporate a wide-range of solution chemistries to test the versatility of this treatment technology.

Status: Equilibrium batch experiments of As (V and III) sorption to DD660 porous alumina particles have been conducted over a range of As concentrations, ionic strengths, and for solutions containing mixed As oxidation states. Equilibrium batch sorption experiments have also been completed for As (V and III) sorption onto DD660 in the presence of varying concentrations of CO₂, sulfate, phosphate and silicate. Equilibrium batch sorption experiments for the sorption of sulfate, phosphate and silicate as a function of anion concentration, pH and ionic strength have also been completed. The Triple-Layer Model (utilizing the surface complexation approach) successfully modeled the adsorption behavior of arsenic under most of the experimental conditions investigated. Rate of uptake experiments of As (V and III) under selected conditions (pH, ionic strength and presence of competing ions) were conducted and the results interpreted using a pore-diffusion model linked with an empirical K_d approach. Batch

regeneration studies of the alumina particles have been completed using pH and carbonate concentration to increase As desorption from the surface. We conducted column experiments to investigate the efficiency of As (V and III) under varying conditions of pH, As concentration and the presence of sulfate, phosphate or silicate.

Project Publications:

**Western Region Hazardous Substance Research Center Project
SU99-06. Measurement of Interfacial Areas and Mass Transfer Coefficients Between
Residual PCE and Water During Surfactant Enhanced Aquifer Remediation: Laboratory
Studies and Models: Paul Roberts, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1999-2001

Project Amount: \$127,169

Research Category: Groundwater, DNAPL, analysis

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

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

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

Status: PCE-water interfacial area versus saturation (PCE volume/void volume) has been obtained for PCE saturations ranging from 0.12 (initial residual saturation) to 0.034 (after dissolution via surfactant flooding). The PCE-water interfacial area decreases with PCE saturation and varies with the PCE saturation to the 1.6 power. The “dissolving sphere” model does not describe the magnitude or trend of the dissolution data. Interfacial area results are in some quantitative agreement with previously developed network models [Dillard *et al.*, 2000]. PCE trapped near rough corners of pore spaces dissolves quickly due to its relatively high interfacial area. As this PCE dissolves, the PCE-water interfacial area quickly decreases as the

“wedges” of water in the corners expand and become more rounded. Rate limited dissolution data has revealed that the overall dissolution coefficient (which is the product of the interfacial mass transfer coefficient and the PCE-water interfacial area) decreases with decreasing PCE saturation and interfacial area. *Thus, the interfacial mass transfer coefficient itself is a function of the PCE saturation.* This is likely due to changes in pore scale configuration of the PCE, changes in porewater flow patterns, and changes in diffusional length scales as the residual PCE dissolves. Further study is needed to fully understand and quantify these effects.

Project Publications:

**Western Region Hazardous Substance Research Center Project
SU99-07. Proof of Gene Expression During Bioaugmentation: Craig C. Criddle, Stanford
University**

EPA Project Officer: Dale Manty

Project Period: 1999-2001

Project Amount: \$143,770

Research Category: Groundwater, chlorinated solvents, chloroform, biodegradation

Goal: The overall objective of this work is to develop tools for the evaluation of gene expression in microbial communities. We have focused our efforts on the development of techniques to monitor the expression of genes enabling biodegradation of carbon tetrachloride (CT) by *Pseudomonas* sp. strain KC.

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

Approach: Transcription and translation have traditionally been established by gel electrophoresis, using Northern blots for mRNA and Western blots and 2-D gels for proteins. These methods are not suitable for microbial communities containing large numbers of diverse biomolecules because they do not provide sufficient discrimination and sensitivity. This work has focused on the use and development of new tools to permit analysis of gene expression within microbial communities. Specifically, we have evaluated use of cDNA microarrays for mRNA transcripts and Surface Enhanced Laser Desorption Ionization Mass Spectrometry (SELDI-MS) for proteins. Our selected model system is *Pseudomonas stutzeri* KC, a bacterium that transforms CT and has been successfully used for CT bioremediation at full-scale. We have also assessed detection of a biomolecule produced by strain KC – pyridine-2,6-bis-thiocarboxylate (PDTC) – as this molecule is required for CT transformation, and it is a direct indicator of gene expression.

Status:

The DNA microarray portions of this project were completed in 2001. A cDNA microarray containing probes for the 17 open reading frames of the *pdt* operon in *Pseudomonas stutzeri*

strain KC was prepared and tested using wild type and mutant *Pseudomonas stutzeri* strain KC under various growth conditions. The following parameters were studied: effect of probe size on signal intensity, cross hybridization of probes in the presence of background microflora from Schoolcraft site and other mixed community samples, effect of carbon source and iron limitation on the mRNA expression ratios for selected ORFs. It was concluded that smaller probes will have decreased signal intensity and may not be appropriate when the amount of genetic material is low. Cross hybridization appeared to be less likely for unique genes such as found on *pdt* operon. Future efforts to enable the use of microarrays in bioremediation should focus on improving the signal intensity and extraction procedures of mRNA.

Experiments with SELDI-MS indicated that this technique is insufficiently sensitive and quantitative for the detection of proteins produced by strain KC. Accordingly, we have refocused our efforts on the monitoring of PDTC, as an alternative indicator of gene expression. A colorimetric technique was tested to monitor PDTC production. An LC-MS based technique is currently under evaluation.

Project Publications:

- Musarrat, J., J. Larabee, C. Criddle, and S.A. Hashsham, Monitoring the abundance of mRNA transcripts associated with carbon tetrachloride dechlorination in *Pseudomonas stutzeri* strain KC under different environmental conditions using DNA microarray. *Proceedings of the 101st General Meeting of the American Society for Microbiology*. Orlando, Florida, May 20-24 (2001).
- Musarrat, J., C. S. Criddle, and S.A. Hashsham, Development of DNA Microarray Technology for Environmental Applications. *Proceedings of the 74th Water Environment Federation (WEF) Technology Conference and Exposition*, Oct 14-17, Atlanta, Georgia (2001).

**Western Region Hazardous Substance Research Center Project
SU99-09. Experimental and Mathematical Study of Biomass Growth in Pore Networks and its
Consequences in Bioremediation: Peter K. Kitanidis and Perry L. McCarty, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1999-2001

Project Amount: \$143,770

Research Category: Groundwater, bioremediation, aquifer clogging

Goal: The objectives of this research are: (1) To advance methods for the observation and direct measurement of pore-scale processes that include flow, mass transport, and biomass growth. (2) To evaluate the effects of pore-scale reactive transport processes on Darcy-scale behavior. Thus, to determine the equations that numerical models should solve in engineering practice. (3) To examine the effect of biofouling on the efficiency of in-situ bioremediation.

Rationale: Many of the contaminants found in groundwater can be destroyed or immobilized through in-situ biological processes. The difficulties associated with bioclogging in aquifers are well known, yet basic studies to address this important issue are rare. For the successful implementation of in-situ bioremediation, one must be able to predict field-scale reaction rates and to devise practical methods for the addition and mixing of required nutrients and the control of biological clogging.

Approach: We perform experiments of biological growth in porous media conjunctively with mathematical modeling of the physical, chemical, and biological processes involved. The experimental studies utilize a unique experimental facility to evaluate the effect of reduction in porosity and hydraulic conductivity due to biological growth on the bio-enhanced PCE-DNAPL pool dissolution. The mathematical studies apply numerical and analytical methods to evaluate how groundwater flow and bioreactions are affected.

Status: *Experimental part:* We have performed micromodel experiments to observe the growth of *D. Multivorans* under anaerobic conditions. The pore-network micromodel devised by Hubert Dupin has been modified and tested to be able to keep the micromodel anaerobic. The results show that this particular strain, utilizing PCE and formate for growth, can form aggregates in the micro-channels. The hydraulic conductivity of the micromodel was reduced by a factor of 20 in 60 days. *Modeling part:* We have developed one-dimensional and two-dimensional finite-volume models to examine the growth of biomass in biologically enhanced PCE DNAPL dissolution. *Some conclusions:* (a) Experimental studies show that *D. Multivorans* can clog the pores. (b) The simulations indicate that biomass very close (about 1cm) to PCE source zones can, under the right conditions, enhance the dissolution of PCE DNAPL. (c) Biomass accumulation may divert groundwater flow away from a PCE DNAPL pool, decreasing the efficiency of bio-enhanced dissolution. (d) When the electron donor supply is unlimited, dissolution is bio-enhanced. The bio-reaction rate follows the analytical “half-order” solution in biofilm kinetics. (e) When the electron donor is consumed by populations competing with the dechlorinating bacteria, dissolution is not bio-enhanced. (f) To successfully enhance dissolution

of PCE DNAPL pools or residues, a clever method of substrate delivery is necessary to avoid competition and assure good mixing with PCE.

Project Publications:

Chu, M., Kitanidis, P. K. and McCarty, P. L., “Effects of Biomass Accumulation on Biologically Enhanced Dissolution of a PCE Pool: A Numerical Simulation.” *Journal of Contaminant Hydrology*, 65, 75-100 (2003).

**Western Region Hazardous Substance Research Center Project
SU99-11. Gene probes for detecting anaerobic alkylbenzene-degrading bacteria: Alfred M. Spormann, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1999-2001

Project Amount: \$143,770

Research Category: Groundwater, BTEX, anaerobic biodegradation

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

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

The specific objectives of this research proposal are:

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

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

benzoate. Using reverse genetics, the corresponding genes will be cloned, and probes derived from these genes will be printed on a DNA microarray together with the benzylsuccinate synthase genes.

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

Project Publications:

- Johnson H. A., D. A. Pelletier, and A. M. Spormann, "Isolation and Purification of Anaerobic Ethylbenzene Dehydrogenase, a Novel Mo-Fe-S Enzyme." *J. Bacteriol.*, **183**, 4536-4542 (2001).
- Achong, G. R., A. Rodriguez, A. M. Spormann, "Role of benzylsuccinate synthase in anaerobic toluene and *m*-xylene mineralization in *Azoarcus* sp. strain T: Transcriptional organization of the structural genes and analysis of a *bssA* mutant." *J. Bacteriol.* **183** (23), 6763-6770 (2001).

**Western Region Hazardous Substance Research Center Project
SU99-13. Investigation of Palladium Catalyzed Hydrodehalogenation for the Removal of
Chlorinated Groundwater Contaminants: Surface Chemistry of Catalyst Deactivation and
Regeneration: Martin Reinhard, Stanford University**

EPA Project Officer: Dale Manty

Project Period: 1999-2001

Project Amount: \$143,770

Research Category: Groundwater, chlorinated solvents, treatment, palladium catalyst

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

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

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

Status: Column tests were run for 40-60 days using groundwater, de-ionized (DI) water, or DI water amended with sulfate or carbonate (at 3 pH levels: 4.4, 8.6 and 10.9). Spectroscopic analysis indicated biogrowth or accumulation of organics on the catalyst for all tests, regardless of the water source. This accumulation decreased as pH increased; however, correlation with catalyst activity was poor, which implies limited or no fouling/sorption. Sulfate and/or silicon

were found on catalysts exposed to sulfate-amended DI water and groundwater, but were removed by water-washing; again, this implies no chemical binding. Calcium was also detected on the groundwater-exposed catalyst but was not removable; it has not been determined whether this is bound to the Pd or alumina. No other species were observed. In all tests, Pd was found to oxidize from Pd(0) to Pd(II); this correlated roughly with activity. In order to get more accurate measurements of the Pd(II): Pd(0) ratio, detect any trace species bound to the Pd, and separate out effects of the Pd and support, a model catalyst and corresponding new reactor and numerical model were developed. In preparation for determining the reaction and deactivation rates, the reactor flow was characterized and TCE sorption to the Pd was fitted with a Freundlich isotherm (desorption was tested but not observed); this reactor is near ready for use in continuous flow experiments.

Project Publications:

- Munakata, N., G. Lowry, W. Sriwatanapongse, and M. Reinhard, "Pd-Catalyzed Hydrodehalogenation of Chlorinated Compounds: Spectroscopic Analysis," in Battelle Conference, Monterey April (2000).
- Munakata, N., and M. Reinhard. "Palladium Catalysis for the Treatment of Waters Contaminated with halogenated Hydrocarbons, Nonhalogenated Aromatics, Oxidized Carbon, and Oxidized Nitrogen Species," *Environmental Science and Technology*, in press (2001).
- Munakata, N. and M. Reinhard, "Palladium Catalysis for the Treatment of Waters Contaminated with Halogenated Hydrocarbons, Nonhalogenated Aromatics, Oxidized Carbon and Oxidized Nitrogen Species," in *Physicochemical Groundwater Remediation*, J. A. Smith and S. E. Burns, Eds. Kluwer Academic/Plenum Publisher, Dordrecht, The Netherlands, pp. 45-71 (2001).
- Munakata, N., J.A. Cunningham, R. Ruiz, C. Lebron, and M. Reinhard. "Palladium Catalysis in Horizontal-Flow Treatment Wells: Field-Scale Design and Laboratory Study," *Remediation of Chlorinated and Recalcitrant Compounds: Advances in ex situ Treatment of Groundwater*, eds. A. Gavaskar and A. Chen, Batelle Press, 2002.

TRAINING AND TECHNOLOGY TRANSFER

The training and technology transfer activities of the Western Region Hazardous Substance Research Center included field demonstration projects, hazardous waste training workshops, sponsorship of conferences, Technical Outreach Services for Communities (TOSC), and Technical Assistance to Brownfields Communities (TAB). The demonstration projects conducted under Training and Technology Transfer are all listed under Research and Demonstration Projects. The TOSC program is only about six years old and the TAB program about four years old. Their addition to the WRHSRC represented a major change in the Training and Technology Transfer activities of the WRHSRC and represent the major focus of its most recent efforts. In the following, Hazardous Waste Training Activities and Conference Sponsorships are listed first, following by summaries of TOSC and TAB activities.

Hazardous Waste Training

Principal Investigators: Peter O. Nelson, Ann Kimlering, and Kenneth Williamson, Oregon State University

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

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

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

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

Continuing Education

Principal Investigator: Kenneth J. Williamson, Oregon State University

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

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

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

Conference Sponsorship

Principal Investigators: Kenneth J. Williamson, Oregon State University and Perry L. McCarty, Stanford University

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

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

Status: The WRHSRC sponsored or organized sessions for the following conferences:

International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, CA, July 23 to 26, 1989, organized by the WRHSRC.

1990 Idaho Regional Conference on Hazardous Materials and Wastes in Pocatello, Idaho.

1990 Responsible Hazardous Materials Management Conference in Portland.

International Symposium on In-Situ and On-Site Bioreclamation, March 19-21, 1991, San Diego, California.

1991 Regional Conference on Hazardous Materials and Wastes, April 23-24, 1991, Pocatello, Idaho.

Environmental Programs: Challenges for the 1990s, National Conference of the National University Continuing Education Association, May 13-14, San Diego, California.

New Development in Hazardous Waste Management, May 16, 1991, Oregon State University, Corvallis, Oregon.

Five-Centers' On-Site Bioremediation Conference, May 19-22, 1991, Gull Lake, Michigan.

Sixth Conference on Environmental Engineering Education, August 18-20, Oregon State University, Corvallis, Oregon, organized by the WRHSRC.

Hazardous Materials Management Conference and Trade Show, September 16 and 17, 1991, Portland, Oregon.

Five-Centers' Conference on Technology Transfer, November 12-14, Fallen Leaf Lake, California, organized by the WRHSRC.

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

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

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.

The 1993 Idaho Regional Conference on Hazardous Materials, Pocatello, Idaho, April, 1993.

The 1993 Responsible Hazardous Materials Management Conference, Portland, Oregon, September, 1993.

In-situ and Onsite Bioreclamation Conference, San Diego, California, April, 1993.

The 1994 Idaho Regional Conference on Hazardous Materials, Pocatello, Idaho, April, 1994.

The 1994 Responsible Hazardous Materials Management Conference, Portland, Oregon, September, 1994.

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

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

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

The 1995 Five Centers' Research Conference, "From Flask to Field", Gleneden Beach, Oregon, July, 1995, organized by the WRHSRC.

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

The 1996 Idaho Regional Conference on Hazardous Materials, April 1996.

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

The 1997 Idaho Regional Conference on Hazardous Materials, April, 1997.

Five Centers Technology Transfer Conference, October 1997.

Fourth International In Situ and On-Site Bioremediation Symposium, Battelle, April 1997.

The 1997 Idaho Regional Conference on Hazardous Materials, Pocatello, ID, April, 1998.

Remediation of Chlorinated and Recalcitrant Compounds, Battelle, San Diego, CA, May 18-21, 1998. WRHSRC participated in organizing the conference, and WRHSRC researchers presented 11 papers including one of the keynote addresses.

1998 Joint Conference on the Environment, Albuquerque, NM, March 31-April 1, 1998.
WRHSRC cosponsored the conference and its researchers presented 6 papers.

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

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

Remediation of Chlorinated and Recalcitrant Compounds, The Second International Conference, May 22-25, Monterey, California, 2000.

HSRC Research Symposium, July 9-12, 2000, Asilomar, Pacific Grove, CA, organized by the WRHSRC.

Remediation of Chlorinated and Recalcitrant Compounds, The Third International Conference, , Monterey, California, 2002.

Technical Outreach Services for Communities

The Technical Outreach Services for Communities (TOSC) Program of the Western Region hazardous Substance Research Center has been a community advocacy project designed to aid communities confronted with environmental contamination by hazardous waste sites. The TOSC program is conducted with a staff of faculty, consultants, and research assistants including:

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

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

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

A listing of communities served by TOSC is given below. A description of the services provided to each community follows.

Communities Served by TOSC

Alameda Naval Air Station, San Francisco, California
Bay Area Drum, San Francisco, California
Bayview-Hunters Point, California
Blair Community, Eugene, OR

Brewster, WA
Bunker Hill NPL Site, Kellogg, Idaho
California Trade and Commerce Agency
Casmalia, California
Concerned Blair Area Neighbors, Eugene, OR
Eastern Michaud Flats NPL Site, Pocatello, Idaho
Garden Valley, CA
Makua Military Reservation, Oahu Island, Hawaii
Mare Island Naval Shipyard, Vallejo, California
Marine Corps Air Facility, Tustin, California
Naval Air Station North Island, Coronado, California
Oakland, California
Oregon State Penitentiary, Salem, Oregon
Presidio of San Francisco, California
Quincy, WA
Rayonier Mill Site, Port Angeles, Washington
Santa Fe Springs, California
Sitka, Alaska
South Phoenix, Arizona
Tempe, Arizona
Union Hills Subdivision, Phoenix, Arizona
Vallejo, California
Waste Disposal, Inc. NPL Site, Santa Fe Springs, California
Rayonier Mill Site, Port Angeles, Washington
Oakland, California

Summary of Communities Served by TOSC and Services Provided

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

Chemicals of Concern: VOCs, PAHs, Pesticides, PCBs, and Metals identified in soils and groundwater.

Services Included: Review and comment on the Remedial Investigation, Human Health Risk Assessment, and the Ecological Risk Assessment associated with soil and groundwater contamination at OU-1

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

Chemicals of Concern: PCE, PCBs, Pesticides, Arsenic, and Lead in soils and groundwater.

Services Included: Review and evaluation of soil and groundwater sampling results,

Conduct a community educational workshop focused on potential health concerns associated with contaminants found in local residential soils,
Review and comment on Remedial Investigation and Feasibility Study (RI/FS) documents.

TOSC will review the next Draft RI/FS.

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

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

Blair Community, Eugene, OR – Provided assistance to the Blair Community related to health concerns from presently unknown sources. Services included:

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

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

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

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

Work included:

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

California Trade and Commerce Agency: CTCA received an EPA pilot grant to conduct site assessments at select group of former lumber mill sites in California. Assisted TCA in presenting its workshop in June 1999. Workshop was targeted to small communities in California interested in redeveloping former mill sites as well as other former commercial/industrial facilities. Agreed to assist pilot mill community in their assessment, cleanup, and redevelopment efforts on an ongoing basis. Communities assisted and assistance provided include the following:

- North Fork – review of data summary report
- Del Norte County – redevelopment charrette
- Foresthill – redevelopment charrette

Casmalia, California: Worked with Citizen's Advisory Committee (CAC) associated with the Casmalia Resources Hazardous Waste facility.

Work included:

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

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

Work included:

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

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

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

Garden Valley, CA: Worked with this El Dorado County community relative to airborne asbestos.

Work included:

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

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

Chemicals of Concern: Lead, Arsenic, 2,4-DNT, and 2,6-DNT in Soils,
Groundwater has not yet been detected beneath the investigation area.

Services Included: Review and comment on documents related to the potential closure of a former Open-Burn Open-Detonation (OBOD) site on this base,
Review and comment on Army's investigation of area groundwater and near-shore water contamination.

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

Chemicals of Concern: VOCs, Metals, Pesticides, PAHs, and PCBs in Soils,
Groundwater, and Marine Sediments.

Services Included: Review and evaluation of the Human Health Risk Assessment and the Ecological Risk Assessment

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

Chemicals of Concern: TCE and Other VOCs in Groundwater

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

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

Chemicals of Concern: Primarily TCE and Metals

Services Included: Review of Navy contractor reports and documents associated with soil and groundwater contamination at two operable units (sites 9 and 11).

Attend RAB meetings to comment on proposed investigation, treatability study, and remediation plans.

TOSC served on RAB Technical Workgroup to address Site 9 RI/FS issues.

Oakland, California: Worked with Citizen's Advisory Committee, Environmental Services, City of Oakland, Oakland, California. Work involved:

- Development of soil cleanup matrix based upon major chemical(s) of concern for use with Oakland Brownfield sites
- Conduct a series of meetings involving City personnel, the Citizen's Advisory Committee, and other involved citizens related to adoption of soil cleanup matrix and limited risk cleanup strategies.

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

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

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

Services Included: Research to determine whether proposed institutional control remedies at any DOD remediation sites have been successfully contested by RABs,
Research into non-hazardous landfill debris recycling

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

Rayonier Mill Site, Port Angeles, Washington: Worked cooperatively with Haskell Indian Nations University to provide services to the Lower Elwha Klallam Tribal community regarding the ongoing remedial investigation and proposed cleanup of the former Rayonier Paper Mill, a possible USEPA Superfund site. Services included:

- Conduct a needs assessment of the tribal community

- Review various technical reports associated with the investigation and remediation plans

Santa Fe Springs, California: Provided assistance to the Protect Our Neighborhood Committee (PONC) in their involvement with the Waste Disposal, Inc. Superfund Site, a former landfill. Services included:

- Review the Record of Decision
- Review past and current data associated with groundwater, soil, and air sampling
- Attempt to ascertain health related concerns of residents living adjacent to the site
- Provide an updated list of contaminants affecting groundwater, soil, and air both at the site and in the adjacent community

Sitka, Alaska: Worked with Citizen's Advisory Committee, Sitka, Alaska related to hazardous material releases from Alaska Pulp Corporation pulp mill.

Work included:

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

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

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

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

The chemicals of concern were benzene, hexavalent chromium, and other metals. Services included a review of the air emissions sampling plan and report:

Review of Final Draft Operational Air Permit, with presentation of findings to the community.

TOSC monitored and reviewed the emissions sampling to be conducted by the facility prior to the issuance of an operational permit.

Union Hills Subdivision, Phoenix, Arizona: Assisted neighborhood citizens group to investigate symptoms related to possible chemical exposures. These exposures are likely

a causative factor of some resident morbidity, however the source(s), are unknown following Arizona Dept. of Environmental Quality and U.S. EPA investigations. Work included:

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

Vallejo, California - Provided assistance to an established RAB in dealing with remediation activities at the Mare Island Naval Shipyard where the RI/FS is being completed. Work included:

- Review and evaluation of the Human Health Risk Assessment and the Ecological Risk Assessment

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

Chemicals of Concern: Metals, VOCs, PAHs, PCBs, and Pesticides in Soils, Methane, Benzene, Vinyl Chloride, TCE and Other VOCs in Soil Gas, VOCs in Groundwater.

Services Included: Review the Record of Decision, Review past and current data associated with groundwater, soil, and air sampling, Determine health related concerns of residents living adjacent to the site, Provide an updated list of contaminants affecting groundwater, soil, and air both at the site and in the adjacent community, Will review data from several new groundwater and soil gas sampling events conducted in Fall '98 due to the re-opening of the ROD. Submit comments to the State Dept. of Health Services (DHS) on their Draft Public Health Assessment, on behalf of the PONC.

Technical Assistance to Brownfields Communities (TAB) Program

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

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

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

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

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

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A. Refereed Journal Articles

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