

Bioaugmentation for Groundwater Remediation

SERDP and ESTCP Remediation Technology Monograph Series
Series Editor: C. Herb Ward, Rice University

Bioaugmentation for Groundwater Remediation

Edited by

Hans F. Stroo

HydroGeoLogic, Inc., Ashland, OR

Andrea Leeson

SERDP & ESTCP, Alexandria, VA

C. Herb Ward

Rice University, Houston, TX

Authors

Wayne R. Amber
Carol E. Aziz
Cristin L. Bruce
Evan Cox
Craig S. Criddle
Michael J. Dybas
Elizabeth A. Edwards
Cloelle G.S. Giddings
James M. Gossett
Mark Harkness
Laura A. Hug
Laura K. Jennings
Paul C. Johnson

Stephen S. Koenigsberg
Thomas A. Krug
Thomas A. Lewis
Frank E. Löffler
Delina Y. Lyon
David W. Major
Erik A. Petrovskis
Kirsti M. Ritalahti
A. P. Robertson
Joseph P. Salanitro
Lewis Semprini
Jim C. Spain
Gerard E. Spinnler

Robert J. Steffan
Hans F. Stroo
Gregory M. Tatara
Simon Vainberg
Georgina Vidal-Gavilan
Timothy M. Vogel
Helen Vrionis
Christopher B. Walker
C. Herb Ward
Lance B. Warnick
Ryan A. Wymore
Stephen H. Zinder



Springer

Editors

Hans F. Stroo
HydroGeoLogic, Inc.
300 Skycrest Dr.
Ashland, OR 97520
USA
hstroo@hgl.com

Andrea Leeson
SERDP & ESTCP
4800 Mark Center Drive, Suite 17D08
Alexandria, VA 22350-3600
USA
andrea.leeson@osd.mil

C. Herb Ward
Rice University
Civil and Environmental Engineering
6100 Main Street, Mail Stop 316
Houston, TX 77005
USA
wardch@rice.edu

ISSN 1869-6864 ISSN 1869-6856 (electronic)
ISBN 978-1-4614-4114-4 ISBN 978-1-4614-4115-1 (eBook)
DOI 10.1007/978-1-4614-4115-1
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2012943697

© Springer Science+Business Media New York 2013

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Cover illustration: Electron micrograph of *Dehalococcoides mccartyi*, provided courtesy of Frank E. Löffler, University of Tennessee and Oak Ridge National Laboratory. Reprinted with permission from John Wiley and Sons, Inc. Copyright 2006.

Cover design: Cover layout designed by Kenneth C. Arevalo, Noblis Inc., Falls Church, VA.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

**SERDP and ESTCP Remediation Technology
Monograph Series
Series Editor: C. Herb Ward, Rice University**

SERDP and ESTCP have joined to facilitate the development of a series of monographs on remediation technology written by leading experts in each subject area. This volume provides a review of the state-of-the-art on bioaugmentation for groundwater remediation. Previously published volumes include:

- *In Situ* Bioremediation of Perchlorate in Groundwater
- *In Situ* Remediation of Chlorinated Solvent Plumes
- *In Situ* Chemical Oxidation for Groundwater Remediation
- Delivery and Mixing in the Subsurface: Processes and Design Principles for *In Situ* Remediation

Additional volumes planned for publication in the near future include:

- Chlorinated Solvent Source Zone Remediation
- Processes, Assessment and Remediation of Contaminated Sediment



U.S. Department of Defense Strategic
Environmental Research &
Development Program (SERDP)
4800 Mark Center Drive, Suite 17D08
Alexandria, VA 22350-3600



U.S. Department of Defense Environmental
Security Technology Certification
Program (ESTCP)
4800 Mark Center Drive, Suite 17D08
Alexandria, VA 22350-3600

Preface

In the late 1970s and early 1980s, our nation began to grapple with the legacy of past disposal practices for toxic chemicals. With the passage in 1980 of the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), commonly known as Superfund, it became the law of the land to remediate these sites. The U.S. Department of Defense (DoD), the nation's largest industrial organization, also recognized that it too had a legacy of contaminated sites. Historic operations at Army, Navy, Air Force, and Marine Corps facilities, ranges, manufacturing sites, shipyards, and depots had resulted in widespread contamination of soil, groundwater, and sediment. While Superfund began in 1980 to focus on remediation of heavily contaminated sites largely abandoned or neglected by the private sector, the DoD had already initiated its Installation Restoration Program in the mid-1970s. In 1984, the DoD began the Defense Environmental Restoration Program (DERP) for contaminated site assessment and remediation. Two years later, the U.S. Congress codified the DERP and directed the Secretary of Defense to carry out a concurrent program of research, development, and demonstration of innovative remediation technologies.

As chronicled in the 1994 National Research Council (NRC) report, "Ranking Hazardous-Waste Sites for Remedial Action," our early estimates on the cost and suitability of existing technologies for cleaning up contaminated sites were wildly optimistic. Original estimates, in 1980, projected an average Superfund cleanup cost of a mere \$3.6 million per site and assumed only around 400 sites would require remediation. The DoD's early estimates of the cost to clean up its contaminated sites were also optimistic. In 1985, the DoD estimated the cleanup of its contaminated sites would cost from \$5 billion to \$10 billion, assuming 400–800 potential sites. A decade later, after an investment of over \$12 billion on environmental restoration, the cost-to-complete estimates had grown to over \$20 billion, and the number of sites had increased to over 20,000. By 2007, after spending over \$20 billion in the previous decade, the estimated cost to complete the DoD's known liability for traditional cleanup (not including the Munitions Response Program for unexploded ordnance) was still over \$13 billion. Why did we underestimate the costs of cleaning up contaminated sites? All of these estimates were made with the tacit assumption that existing, off-the-shelf remedial technology was adequate to accomplish the task, that we had the scientific and engineering knowledge and tools to remediate these sites, and that we knew the full scope of chemicals of concern.

However, it was soon and painfully realized that the technology needed to address the more recalcitrant environmental contamination problems, such as fuels and chlorinated solvents in groundwater and dense nonaqueous phase liquids (DNAPLs) in the subsurface, was seriously lacking. In 1994, in the "Alternatives for Ground Water Cleanup" document, the NRC clearly showed that as a nation we had been conducting a failed 15-year experiment to clean up our nation's groundwater and that the default technology, pump-and-treat, was often ineffective at remediating contaminated aquifers. The answer for the DoD was clear. The DoD needed better technologies to clean up its contaminated sites, and better technologies could only arise through a better scientific and engineering understanding of the subsurface and the associated chemical, physical, and biological processes. Two DoD organizations were given responsibility for initiating new research, development, and demonstrations to obtain the technologies needed for cost-effective remediation of facilities across the DoD: the Strategic Environmental Research and Development Program (SERDP) and the Environmental Security Technology Certification Program (ESTCP).

SERDP was established by the Defense Authorization Act of 1991, as a partnership of the DoD, the U.S. Department of Energy, and the U.S. Environmental Protection Agency; its mission is “to address environmental matters of concern to the Department of Defense and the Department of Energy through support of basic and applied research and development of technologies that can enhance the capabilities of the departments to meet their environmental obligations.” SERDP was created with a vision of bringing the capabilities and assets of the nation to bear on the environmental challenges faced by the DoD. As such, SERDP is the DoD’s environmental research and development program. To address the highest-priority issues confronting the Army, Navy, Air Force, and Marine Corps, SERDP focuses on cross-service requirements and pursues high-risk and high-payoff solutions to the DoD’s most intractable environmental problems. SERDP’s charter permits investment across the broad spectrum of research and development, from basic research through applied research and exploratory development. SERDP invests with a philosophy that all research, whether basic or applied, when focused on the critical technical issues, can impact environmental operations in the near term.

A DoD partner organization, ESTCP, was established in 1995 as the DoD’s environmental technology demonstration and validation program. ESTCP’s goal is to identify, demonstrate, and transfer technologies that address the DoD’s highest priority environmental requirements. The program promotes innovative, cost-effective environmental technologies through demonstrations at DoD facilities and sites. These technologies provide a large return on investment through improved efficiency, reduced liability, and direct cost savings. The current cost and impact on DoD operations of environmental compliance is significant. Innovative technologies are reducing both the cost of environmental remediation and compliance and the impact of DoD operations on the environment, while enhancing military readiness. ESTCP’s strategy is to select laboratory-proven technologies with potential broad DoD application and use DoD facilities as test beds. By supporting rigorous testing and evaluation of innovative environmental technologies, ESTCP provides validated cost and performance information. Through these tests, new technologies gain end-user and regulatory acceptance.

In the 17–21 years since SERDP and ESTCP were formed, much progress has been made in the development of innovative and more cost-effective environmental remediation technology. Since then, recalcitrant environmental contamination problems for which little or no effective technology had been available are now tractable. However, we understand that newly developed technologies will not be broadly used in government or industry unless the consulting engineering community has the knowledge and experience needed to design, cost, market, and apply them.

To help accomplish the needed technology transfer, SERDP and ESTCP have facilitated the development of a series of monographs on remediation technology written by leading experts in each subject area. Each volume will be designed to provide the background in process design and engineering needed by professionals who have advanced training and five or more years of experience. The first volume in this series, *In Situ Bioremediation of Perchlorate in Groundwater*, met a critical need for state-of-the-technology guidance on perchlorate remediation. The second volume, *In Situ Remediation of Chlorinated Solvent Plumes*, addressed the diverse physical, chemical, and biological technologies currently in use to treat what has become one of the most recalcitrant contamination problems in the developed world. The third volume, *In Situ Chemical Oxidation for Groundwater Remediation*, provided comprehensive, up-to-date descriptions of the principles and practices of *in situ* chemical oxidation for groundwater remediation based on a decade of intensive research, development, and demonstration. The fourth volume, *Delivery and Mixing in the Subsurface: Processes and Design Principles for In Situ Remediation*, described the principles of chemical delivery and mixing systems, and their design and implementation for effective *in situ* remediation. Other volumes will follow on such topics as the remediation of DNAPL-chlorinated solvent source zones and remediation of

contaminated sediments. Additional volumes will be written as new remediation technologies are developed and proven to be effective.

This volume, *Bioaugmentation for Groundwater Remediation*, provides a review of the past 10–15 years of intensive research, development, and demonstrations that have been at the forefront of developing bioaugmentation into a viable remedial technology. In addition, both a primer on the basic microbial processes involved in bioaugmentation as well as a thorough summary of the methodology for implementing the technology is provided within this volume. It is our intention that this will serve as a valuable resource for environmental remediation professionals who seek to understand, evaluate, and implement bioaugmentation. Topics addressed in this volume include:

- A brief history and overview of bioaugmentation (Chap. 1).
- A detailed review of the discovery of *Dehalococcoides* and the development of reductive dechlorination of chlorinated solvents as a remedial technology (Chap. 2).
- The state-of-the-science for the production and handling of *Dehalococcoides* bioaugmentation cultures (Chap. 3).
- An overview of a decision process for determining whether to implement bioaugmentation with *Dehalococcoides* (Chap. 4).
- Design considerations for implementing bioaugmentation (Chap. 5).
- A summary of microbial monitoring options during bioaugmentation with *Dehalococcoides* (Chap. 6).
- A thorough review of the use of bioaugmentation for treatment of chemicals other than the more common chlorinated solvents (TCE and PCE), including DCE (Chap. 7), aerobic cometabolism of chlorinated solvents (Chap. 8), carbon tetrachloride (Chap. 9), and MTBE (Chap. 10).
- An analysis of cost considerations needed to evaluate whether bioaugmentation should be considered for the treatment of chlorinated aliphatic compounds in groundwater (Chap. 11).
- An assessment of important unknowns and uncertainties that impact the state-of-the-science that underpins bioaugmentation development and implementation. This chapter, written in consultation with a broad range of experts in the remediation field, should help guide the research agenda on bioaugmentation (Chap. 12).

Each chapter in this volume has been thoroughly reviewed for technical content by two or more experts in each subject area covered. The editors and chapter authors have produced a well-written and up-to-date treatise that we hope will prove to be a useful reference for those making decisions on remediation of contaminated sites, for remediation practitioners, and for those involved in development of advanced technology for the *in situ* remediation of contaminated groundwater.

SERDP and ESTCP are committed to the development of new and innovative technologies to reduce the cost of remediation of soil, groundwater, and sediment contamination as a result of past operational and industrial practices. We are also firmly committed to the widest dissemination of these technologies to ensure that our investments continue to yield savings for not only the DoD, but also the nation. In sponsoring this monograph series, we hope to provide the broader remediation community with the most current knowledge and tools available in order to bring these technologies to bear on the remediation of contaminated sites.

Jeffrey A. Marqusee, PhD, Executive Director, SERDP and ESTCP

Andrea Leeson, PhD, Environmental Restoration Program Manager, SERDP and ESTCP

About the Editors

Hans F. Stroo

Dr. Stroo is a Principal Technical Advisor with HydroGeoLogic, Inc. He provides technical support on large remediation projects for private- and public-sector clients and has served as a technical advisor to the Strategic Environmental Research and Development Program (SERDP) and the Environmental Security Technology Certification Program (ESTCP) for over 10 years.

Dr. Stroo received his BS degrees in Biology and Soil Science from Oregon State University, his MS in Soil Science from West Virginia University, and his PhD in Soil Science (Soil Microbiology) from Cornell University.

He was formerly a Principal with Remediation Technologies, Inc. (RETEC). He has over 20 years of experience in the assessment and remediation of contaminated soil and groundwater, particularly in the development and use of *in situ* bioremediation.

Dr. Stroo has served on several expert review panels for SERDP, other government agencies, and private companies. Recently, he served as co-chair of the SERDP workshops on Remediation of Chlorinated Solvents in Groundwater and Remediation of Dense Nonaqueous Phase Liquid (DNAPL) Source Zones. He is coeditor of the SERDP-facilitated monographs, *In Situ Bioremediation of Perchlorate in Groundwater* and *In Situ Remediation of Chlorinated Solvent Plumes*.

Andrea Leeson

Dr. Leeson is the Environmental Restoration Program Manager for SERDP and ESTCP. She received her BS degree in Biology from Eastern Kentucky University and her PhD in Environmental Engineering from The Johns Hopkins University.

Dr. Leeson has been the Environmental Restoration Program Manager at SERDP and ESTCP since 2001. She was formerly a Research Leader at Battelle Memorial Institute and worked on the design and implementation of innovative physical/chemical and biological treatment technologies for site remediation and treatment of industrial/hazardous wastewater for 10 years prior to working at SERDP and ESTCP.

Dr. Leeson served as chairperson of the Fourth, Fifth, and Sixth International *In Situ* and On-Site Bioremediation Symposium and was the founding Managing Editor for *Bioremediation Journal* from 1996 to 1999 and the Editor-in-Chief from 1999 to 2001. She has developed and implemented several expert panel workshops on topics ranging from contaminant bioavailability to chlorinated solvent-contaminated groundwaters.

C. Herb Ward

Dr. Ward holds the Foyt Family Chair of Engineering in the George R. Brown School of Engineering at Rice University. He is also Professor of Civil and Environmental Engineering and Ecology and Evolutionary Biology.

Dr. Ward has undergraduate (BS) and graduate (MS, PhD, MPH) degrees from New Mexico State University, Cornell University, and the University of Texas School of Public Health, respectively. He is a registered professional engineer in Texas and a Board Certified Environmental Engineer by the American Academy of Environmental Engineers.

He has been a faculty member at Rice University for 47 years, where he has served as Chair of the Department of Environmental Science and Engineering and the Department of Civil and Environmental Engineering and as the Founding Director of the University's Energy and Environmental Systems Institute. He has also served as Director of the U.S. Environmental

Protection Agency (USEPA)-sponsored National Center for Ground Water Research and the U.S. Department of Defense (DoD)-sponsored Advanced Applied (Environmental) Technology Development Facility (AATDF).

Dr. Ward has been a member of the USEPA Science Advisory Board and served as Chair of the SERDP Scientific Advisory Board. He is the founding and current Editor-in-Chief of the international scientific journal *Environmental Toxicology and Chemistry*.

Dr. Ward received the Frederick George Pohland Medal for Outstanding Contributions to Bridging Environmental Research, Education, and Practice and the Brown and Caldwell Lifetime Achievement Award for Remediation in 2006, the Water Environment Federation Jack Edward McKee Medal for Achievement in Groundwater Restoration in 2007, and the Society for Industrial Microbiology and Biotechnology Charles Thom Award for bioremediation research in 2011.

ACKNOWLEDGMENTS

The editors gratefully acknowledge the outstanding assistance of Catherine Vogel, Christina Gannett, Gail Badenhop, and Dianna Gimon of Noblis, who have been instrumental in ensuring the quality of this monograph.

About the Authors

Wayne R. Amber

Dr. Amber is an Environmental Engineer with Geosyntec Consultants, Inc. in Ann Arbor, Michigan. He received his BEng (Civil and Environmental Engineering) and PhD (Geoenvironmental Engineering) degrees from Cardiff University, United Kingdom. Dr. Amber manages and executes remediation projects for private sector clients. His key professional experience has included work under the Resource Conservation and Recovery Act (RCRA) and Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) programs, bioremediation system designs and field implementations, and environmental due diligence support. Additionally, Dr. Amber assisted in the preparation of persistent organic pollutant (POP) guidance documentation for the United Nations Industrial Development Organization (UNIDO).

Carol E. Aziz

Dr. Aziz is a Senior Manager with ENVIRON in Mississauga, Ontario, Canada. She received her PhD in Environmental Engineering from the University of Texas at Austin, and her MSc and BSc degrees in Chemical Engineering from the University of Toronto.

Dr. Aziz specializes in assessment and remediation of industrial and military sites impacted by a broad range of contaminants, including chlorinated solvents, polycyclic aromatic hydrocarbons (PAHs), benzene, toluene, ethylbenzene, and xylenes (BTEX) and perchlorate. She has led the development, design, and implementation of several bioremediation/bioaugmentation technologies for public and private sector clients and has developed environmental software (BIOCHLOR) distributed through the U.S. Environmental Protection Agency (USEPA).

Cristin L. Bruce

Dr. Bruce is a Senior Consultant in the Soil and Groundwater Management Group at Shell Global Solutions US Inc., Houston, Texas. She received her PhD in Environmental Engineering from Arizona State University and BS and MS degrees in the environmental sciences from Rice University. Dr. Bruce is a remediation expert and technical assurance manager for Shell's US West Region.

Evan Cox

Mr. Cox is a Principal Remediation Scientist with Geosyntec Consultants in Guelph, Ontario, Canada. He received his BSc in Biology and his MSc in Microbiology from the University of Waterloo (UW) in Waterloo, Ontario, Canada. Mr. Cox has over 20 years of demonstrated experience in the development, feasibility evaluation, and application of innovative *in situ* remediation technologies, including monitored natural attenuation (MNA), enhanced *in situ* bioremediation (EISB), *in situ* chemical oxidation (ISCO), and metal-catalyzed reduction of chlorinated and recalcitrant chemicals in subsurface environments.

Mr. Cox has helped to pioneer the development of MNA and EISB technologies, including bioaugmentation, for remediation of chlorinated solvents, propulsion energetics (perchlorate, *n*-nitrosodimethylamine [NDMA]), and explosives (RDX, TNT) in porous media and fractured bedrock. He works with private sector interests and government research programs to develop innovative *in situ* treatment technologies and to demonstrate and validate their use at field-scale for widespread commercial use. As part of his *in situ* remediation research, development, and implementation work, he has authored over 30 professional publications and articles regarding

the degradation of hazardous contaminants in subsurface environments and has coauthored multiple guidance documents and educational courses on these subjects.

Craig S. Criddle

Dr. Criddle is a Professor of Civil and Environmental Engineering at Stanford University and Senior Fellow in the Woods Institute for the Environment. He received his BS in Civil and Environmental Engineering and his BA in Spanish from Utah State University in 1982, followed by an MS in Environmental Engineering in 1984. In 1990, he completed his PhD at Stanford University in Civil Engineering (Environmental Engineering and Science). His research focus is environmental biotechnology.

Dr. Criddle began his academic career in 1989 as a faculty member at Michigan State University (MSU). While at MSU, he served as Project Director for the Schoolcraft project, a field-scale test of bioaugmentation that involved faculty from diverse disciplines and institutions, staff scientists, students, consultants and outreach education to members of the public and officials in Michigan. The National Ground Water Association (NGWA) named it one of two Outstanding Remediation Projects for 2002. Since 1998, he has been a member of the Stanford faculty, serving as Associate Chair in 2003 and as Director of a bioremediation field project at the U.S. Department of Energy (DOE) Field Research Center in Oak Ridge, Tennessee (2000–2006). This project entailed a multi-year series of experiments focused on *in situ* sequestration of uranium in groundwater and involved faculty from several universities, DOE scientists, and many students. Dr. Criddle has over 100 peer-refereed publications and is coauthor with award-winning artist Larry Gonick of *The Cartoon Guide to Chemistry* (2006), a widely used supplementary text for high school and freshman chemistry.

Michael J. Dybas

Dr. Dybas is the President and Principal Consultant at M. Dybas and Associates, LLC, and is also a State of Michigan, Department of Environmental Quality (DEQ) Level of Effort contractor. He received his BS in Biology from Marquette University in 1985 and his MS from the University of Illinois at Urbana-Champaign in Microbiology in 1988. In 1992, he completed his PhD at the University of Illinois at Urbana-Champaign in Microbiology, focusing on anaerobic and methanogenic processes. His research and professional focus has been applied biotechnology and microbial physiology.

Dr. Dybas began his academic career as a postdoctoral researcher with the Center for Microbial Ecology at MSU in 1992, where he became a faculty member in 1994 and remained until 2007. At MSU, Dr. Dybas served as Project Director of the Schoolcraft project from 1998 to 2007. This project involved a multidisciplinary academic research effort and a set of large-scale field projects funded by both state and federal agencies. The project was a recipient of the NGWA award as an Outstanding Project in Groundwater Remediation (2002), and Dr. Dybas was recognized as the Groundwater Management Professional of the Year (2004) by the Michigan Water Environment Association. Since 2007, Dr. Dybas has led an environmental consulting firm providing microbiological, chemical, and phytoremediation design and implementation services. He has also coauthored over 30 academic publications and has received six U.S. patents for applied biotechnology.

Elizabeth A. Edwards

Dr. Edwards, PEng, is a Professor in the Department of Chemical Engineering and Applied Chemistry at the University of Toronto. She received her BS and MS degrees in Chemical Engineering from McGill University, Montreal, and her PhD degree (1993) in Civil and Environmental Engineering from Stanford University.

Dr. Edwards is internationally known for her work on anaerobic bioremediation, the application of molecular biology and metagenomics to uncover novel microbial processes, and the transition of laboratory research into commercial practice to develop bioremediation and bioaugmentation strategies for groundwater pollutants. Dr. Edwards and Geosyntec Consultants were recognized with the 2009 National Science and Engineering Research Council of Canada Synergy Award for their long-standing partnership that led to the development of KB-1[®], a successful commercial microbial bioaugmentation culture. She has been inducted into the Canadian Academy of Engineering and is a Fellow of the American Association for the Advancement of Science.

Cloelle G.S. Giddings

Dr. Giddings is a lecturer in the Picker Engineering Program at Smith College in Northampton, Massachusetts. She received her BS degree in Engineering Science from Smith College and her MS and PhD degrees (Civil and Environmental Engineering) from Cornell University. In 2004, Dr. Giddings proudly joined the first class to receive a degree from an engineering program at an all-women's college in the United States. While she has taught a variety of courses, she most enjoys coaching the senior design project teams.

James M. Gossett

Dr. Gossett is Professor of Civil and Environmental Engineering at Cornell University, Ithaca, New York. He received his BS in Chemical Engineering and his MS and PhD in Civil and Environmental Engineering, all from Stanford University. He has been both a faculty research fellow and a visiting professor with the U.S. Air Force Research Laboratory (AFRL), a North Atlantic Treaty Organization (NATO) fellow, and a Pacific Northwest National Laboratory (PNNL) affiliate staff scientist. At Cornell, he served as Director of the School of Civil and Environmental Engineering from 2003 to 2008.

Dr. Gossett's research interests are in the general area of applied microbiology – factors influencing biodegradability, microbial kinetics, and understanding the complex interactions occurring in microbial communities. Since 1984, he has principally worked on topics related to bioremediation of chlorinated solvent-contaminated groundwaters.

Mark Harkness

Mr. Harkness is a remediation engineer at GE Global Research in Niskayuna, New York, where he is part of a multidisciplinary team providing consulting support to GE project managers who wish to apply innovative remedial solutions to soil and groundwater issues. Mr. Harkness received his BS and MS degrees in Chemical Engineering from Rensselaer Polytechnic Institute.

In 23 years at GE, his work has focused on the development of novel remedial solutions for polychlorinated biphenyls (PCBs), petroleum hydrocarbons, and chlorinated solvents. His current specialty is the design of passive bioremediation systems for chlorinated solvents in groundwater. He has served as the GE representative on the steering committee of the Remediation Technologies Development Forum (RTDF) Bioremediation Consortium, and more recently Project SABRE, and is a frequent contributor to journal articles and book chapters in the field of bioremediation.

Laura A. Hug

Laura A. Hug, MSc, is currently pursuing her PhD in Dr. Elizabeth Edwards' laboratory at the University of Toronto, Ontario. She received her BSc degree in Molecular Biology and Genetics from the University of Guelph, Ontario, and her MSc degree (2007) from Dalhousie University

in Nova Scotia in the field of molecular evolution. Her research focus is the bacterial interrelationships within reductively dechlorinating microbial consortia.

Laura K. Jennings

Dr. Jennings is currently a Senior Fellow in the Microbiology Department at the University of Washington, Seattle. She received her BS in Chemical and Biological Engineering from Montana State University in 2003 and her MS and PhD degrees in Civil and Environmental Engineering from Cornell University in 2009. As a postdoctoral research associate at Montana State University, she aided in the establishment of a metabolomics facility. Her research interests center around environmental and biomedical applications of microbial metabolism, including the investigation of (1) biodegradation pathways in a bacterium that uses the suspected carcinogen *cis*-1,2-dichloroethene for growth and (2) biofilm metabolic signatures in *Staphylococcus aureus*.

Paul C. Johnson

Dr. Johnson is a Professor in the School of Sustainable Engineering and the Built Environment and is also the Dean of the Fulton Schools of Engineering at Arizona State University (ASU). He received his BS degree from the University of California, Davis, and his MA and PhD degrees from Princeton University, all in Chemical Engineering.

Prior to joining the faculty at ASU in 1994, he was a Senior Research Engineer at Shell Development in Houston, Texas. His research, teaching, and professional activities are focused on the development, evaluation, and optimization of *in situ* remediation technologies and on modeling and monitoring related to risk assessment. From 1993 to 2012, he was the editor of *Ground Water Monitoring and Remediation*.

Stephen S. Koenigsberg

Dr. Koenigsberg is Vice President and Director of Advanced Remediation Technologies at Brown and Caldwell in Irvine, CA. He has worked as a team member on numerous projects involving *in situ* and on-site treatment protocols and has published over 150 technical articles focusing on bioremediation and environmental biotechnology. He received his BA from the City College of New York (CCNY) and his MS and PhD degrees from Cornell University.

Dr. Koenigsberg was a Founder of Regenesis, where he coinvented or managed the development of the company's major products including ORC[®], HRC[®], MRC[®] and Regenox[®]. Also during his tenure, Regenesis produced one of the first commercial *Dehalococcoides* products (BDI[®]) and associated deoxyribonucleic acid (DNA) probes (Censussm) through the work of Dr. Frank Löffler at the Georgia Institute of Technology. Upon retiring from Regenesis, Dr. Koenigsberg was a Partner at WSP and a Principal at ENVIRON. In 2010, he received a Lifetime Achievement Award from the Association for Environmental Health & Sciences (AEHS) Foundation. He is a member of several editorial and advisory boards and is an Adjunct Professor at California State University at Fullerton where he also serves on the Dean's Advisory Council.

Thomas A. Krug

Mr. Krug, PEng, is an Associate and Senior Environmental Engineer with Geosyntec Consultants in Guelph, Ontario, Canada. He received his BSc and MSc degrees in Chemical Engineering from Queen's University in Kingston, Ontario, Canada, and has over 25 years of professional experience developing technical solutions to solve challenging environmental problems.

Mr. Krug has extensive experience in the development and evaluation of innovative technologies for remediation of soil, groundwater, and sediment at contaminated properties

for Fortune 500 companies, branches of the U.S. Department of Defense (DoD), and the National Aeronautics and Space Administration (NASA). The focus of his professional practice has been in taking new technologies for remediation of environmental contamination from the early development stage to successful field-scale application and adapting new and conventional technologies to solve client's real-world problems. He has been a pioneer in the development, demonstration, and application of zero-valent metal and biological processes for the treatment of chlorinated organic compounds. In 2007, he was inducted into the Space Foundation Technology Hall of Fame for his contributions to the development of emulsified nanoscale zero-valent iron technology for treatment of chlorinated solvent dense nonaqueous phase liquid (DNAPL) source zones.

Thomas A. Lewis

Dr. Lewis is an Associate Professor in the Department of Biological and Physical Sciences at Montana State University, where he teaches courses in microbiology and biochemistry and conducts research. He received his BS degree (Microbiology) from Northern Arizona University, his PhD (Microbiology) from Oregon State University, and was a Project Leader at the University of Idaho Environmental Biotechnology Institute prior to his academic appointments. His research interests lie in the area of microbial physiology and genetics with a focus on pollutant transformations contributing to bioremediation.

Frank E. Löffler

Dr. Löffler is a Governor's Chair at the University of Tennessee and Oak Ridge National Laboratory with appointments in the Department of Microbiology, the Department of Civil and Environmental Engineering, and the Biosciences Division. He received his BS degree in Biology/Agricultural Sciences in 1986 and his MSc (Microbiology) in 1990, both from the University of Hohenheim in Germany, and his PhD from the Department of Technical Biochemistry at the Technical University Hamburg-Harburg, Germany, in 1994.

Dr. Löffler was awarded a Feodor-Lynen Fellowship from the Alexander von Humboldt Foundation and joined the Center for Microbial Ecology at MSU in 1994. Before moving to Tennessee, he was on the faculty of the Schools of Civil and Environmental Engineering and of Biology at the Georgia Institute of Technology. Discoveries in the Löffler laboratory have contributed to the advancement of bioremediation, and he has contributed more than 80 peer-reviewed publications to this field. He currently serves on the Interstate Technology and Regulatory Council (ITRC) Environmental Molecular Diagnostics (EMD) team.

Delina Y. Lyon

Dr. Lyon is currently a research associate at Howard University in Washington, D.C. She received her BA in Biology from St. Mary's College of Maryland, her MS in Microbiology from the University of Georgia, and her PhD in Environmental Engineering at Rice University. She spent 2 years as a postdoctoral researcher at the Ecole Centrale de Lyon, France. Her research interests are primarily in applied microbiology and biotechnology.

David W. Major

Dr. Major is a Principal of Geosyntec Consultants Inc., Associate Editor of *Ground Water Monitoring and Remediation*, and an Adjunct Professor in the Department of Chemical Engineering and Applied Chemistry, University of Toronto, and in the Department of Earth Sciences, University of Waterloo (UW). He received his BSc (1981), MSc (1984), and PhD (1987) in Biology from UW. Dr. Major has over 23 years of experience working with clients, researchers, and regulators to develop practical biological and chemical solutions to remediate

contaminated sites. He was inducted into the Space Hall of Fame® and received a UW Science's Alumni of Honor Award. Dr. Major has served on various national scientific and regulatory advisory boards.

Erik A. Petrovskis

Dr. Petrovskis is an Associate/Engineer with Geosyntec Consultants, Inc. in Ann Arbor, Michigan. He received his BS (Honors) in Biochemistry from The University of Wisconsin and his MSE and PhD degrees in Environmental Engineering from The University of Michigan. Dr. Petrovskis began his career as a molecular biologist. His 17 years of environmental consulting experience have focused on development and implementation of innovative technologies for remediation of chlorinated solvents, including bioaugmentation and surfactant-enhanced aquifer remediation. He has served as Principal Investigator (PI) or Co-PI on four Small Business Innovative Research or SERDP/ESTCP projects related to chlorinated solvent bioremediation. He is a member of the ITRC EMD team. He is also a Lecturer in the Department of Civil and Environmental Engineering at The University of Michigan.

Kirsti M. Ritalahti

Dr. Ritalahti is a Research Assistant Professor in the Department of Microbiology at the University of Tennessee, Knoxville. She received her BS degree in Microbiology in 1992 from Oregon State University and her PhD from the Center for Microbial Ecology and Department of Microbiology at MSU in 2000. Her primary research focus is to design and employ molecular tools to detect and quantify relevant target genes from microbes pertinent to bioremediation applications. She is also attempting to harness the horizontal gene transfer mechanisms by which organohalide respiring organisms acquire new capabilities.

A. P. (“Sandy”) Robertson

Dr. Robertson is a Senior Research Engineer and Lecturer working in the areas of physical chemical processes for water and wastewater treatment as well as aquatic geochemistry. He received his bachelor's degree from Harvard College in Engineering and Applied Physics. He did his MS and PhD work in Environmental Engineering and Science at Stanford University. Dr. Robertson spent 2 years in Thailand as a Peace Corps volunteer working on village water projects. He has also worked as a consulting engineer in Sacramento, California. At Stanford, his work has included studies related to copper partitioning in soils and groundwaters, photocatalytic oxidation of contaminants, membrane processes and children's exposure to pesticides. He has helped develop and run the Clean Water (research) and Singapore Stanford Partnership (graduate education) programs – joint efforts involving the Stanford Environmental and Water Studies group and Singapore's Nanyang Technological University.

Joseph P. Salanitro

Dr. Salanitro is an Adjunct Research Professor in the Department of Civil and Environmental Engineering at the University of Houston, Texas, where he teaches environmental chemistry, environmental engineering, water quality engineering, and environmental microbiology. He received his PhD in Microbiology from Indiana University in 1968.

Dr. Salanitro was previously employed by Shell for 31 years, where he was involved in the chemical and oil sectors of environmental research and development, including the biodegradability assessment of surfactants, solvents, pesticides, and petrochemical waste effluents; the role of microbes in sour gas formation in oilfield waterfloods; biocorrosion potential; oilfield biocide evaluations; and defining the potential and limits of natural attenuation, biostimulation, and bioaugmentation processes for crude oils, fuels, and fuel oxygenates in soils and

groundwater. During the last several years, he has consulted for companies on the biodegradation evaluation of drilling fluids in the seafloor environment and the degradation of petroleum products and chlorinated solvents in bioreactors using immobilized microbial cultures.

Lewis Semprini

Dr. Semprini is a Distinguished Professor of Environmental Engineering at Oregon State University. He received his BS degree in Chemical Engineering from the University of California, Berkeley, and MSE and PhD degrees in Civil and Environmental Engineering from Stanford University. His research involves laboratory, field, and modeling investigations on the aerobic and anaerobic transformations of chlorinated solvents. He has served as a PI or Co-PI on several SERDP/ESTCP projects, including the SERDP project “Development of Effective Aerobic Cometary Systems for *In Situ* Transformation of Problematic Chlorinated Solvent Mixtures.” He has also authored over 80 peer-reviewed scientific papers and book chapters.

Jim C. Spain

Dr. Spain has been a Professor in Civil and Environmental Engineering at the Georgia Institute of Technology since 2005. He received his PhD in Microbiology from The University of Texas at Austin. Dr. Spain has 30 years of experience studying the mechanisms of biodegradation of organic compounds and the ecology of the bacteria that catalyze the processes. He worked on fate and transport of marine pollutants including pesticides and petroleum for 5 years at the USEPA Marine Environmental Research Laboratory before joining the Air Force Research Laboratory (AFRL) in 1985. As head of Biotechnology Research at AFRL, he directed a program to discover and develop strategies for biodegradation of industrial and military chemicals, including fuels, solvents, and explosives. Research at the Air Force also included the exploration of biocatalysts for transformation and synthesis of materials. He is a former editor of *Applied and Environmental Microbiology* and has published extensively on biodegradation and biotransformation of synthetic organic compounds.

Gerard E. Spinnler

Dr. Spinnler is a Senior Consultant in the Soil and Groundwater Management Group at Shell Global Solutions US Inc., Houston, Texas. He received his PhD in Geology from Arizona State University and is a Professional Geoscientist in Texas. Dr. Spinnler is a remediation expert for Shell’s East Region of the United States. He has been active in research on fuel oxygenate remediation, bioaugmentation, biostimulation, oxygen distribution, chemical oxidation, and most recently on the application of isotopic and molecular biological tools.

Robert J. Steffan

Dr. Steffan is Director of Biotechnology Development and Applications at Shaw Environmental, Inc. He received his PhD in Biology from the University of Louisville in 1988, and then received an Alexander von Humboldt Fellowship to perform research at the National Institute for Biotechnology (GBF) in Braunschweig, Germany, from 1988 to 1990. He joined Envirogen, Inc. as a research scientist in 1990, and from 2001 to 2003 served as Envirogen’s Vice President of Technology Development. He has served in his current position since the acquisition of Envirogen by Shaw Environmental and Infrastructure, Inc. in March 2003. He has worked for more than 20 years on the development and application of biotechnologies for treating some of the nation’s most challenging pollutants, including chlorinated solvents, methyl *tert*-butyl ether (MTBE), and 1,4-dioxane. He has published more than 70 research papers, manuscripts, and book chapters, has earned ten U.S. and international patents, and has served on a wide array of national and international committees and scientific review panels. In addition, he earned a

Juris Doctorate degree from the Temple University School of Law in 1997 and is licensed to practice law in the states of Pennsylvania and New Jersey.

Gregory M. Tatara

Dr. Tatara is the Utility Director for the Marion, Howell, Oceola, and Genoa (MHOG) Sewer and Water Authority located in Howell, Michigan. He received his BS in Biology from Gannon University in 1991. In 1996, he completed his PhD at MSU in Microbiology (Center for Microbial Ecology). His research focus was environmental biotechnology.

Dr. Tatara began his professional career in 1996 as a Project Manager with the Traverse Group in Ann Arbor, Michigan. In 2000, he became the Deputy Drain Commissioner for Livingston County, coordinating the operation and maintenance of the county's numerous sanitary sewer systems and overseeing the installation of remediation and methane gas mitigation systems for the Livingston County Landfill. In 2006, he became the Utility Director for the MHOG Sewer and Water Authority, which serves 20,000 customers in central Livingston County. As Director, Dr. Tatara has upgraded existing microbial wastewater treatment systems and initiated hydraulic modeling of water systems to improve quality and pressure. Dr. Tatara oversees a staff of 22 personnel.

Simon Vainberg

Dr. Vainberg is the Fermentation Manager in the Biotechnology Development and Application Group of Shaw Environmental, Inc. in Lawrenceville, New Jersey. He received his MS and PhD degrees in Chemical Engineering and Industrial Biotechnology from D. Mendeleev University of Chemical Technology of Russia (Moscow). He also received a Certificate of Completion from the Patent Training Academy of the U.S. Patent and Trademark Office (USPTO).

Dr. Vainberg has more than 30 years of experience in industrial microbiology and biotechnology and has been involved in the isolation of microorganisms for bioremediation, development of aerobic and anaerobic fermentation processes, and production of bacterial cultures for a wide variety of environmental projects. He has worked as a Patent Examiner for the USPTO, has coauthored more than 20 scientific articles, and has received four patents in the United States and the former Union of Soviet Socialist Republics (USSR) in the area of bioremediation and applied biotechnology.

Georgina Vidal-Gavilan

Ms. Vidal-Gavilan is Director of Research, Development and Innovation (R&D&I) and Remediation Department at D'ENGINY BIOREM, an engineering bioremediation firm in Barcelona, Spain. She received her BS in Environmental Sciences from the Autonomous University of Barcelona (1996) and MSc in Environmental Engineering from Michigan State University (MSU) (2000). She is an expert in soil and groundwater remediation. Currently, she is completing her PhD at the University of Barcelona, with a focus on the application of isotopic techniques for enhanced attenuation of nitrate-polluted groundwater.

Ms. Vidal-Gavilan began her remediation career at MSU, participating in the Schoolcraft project, in which she developed her Master's Thesis on the bioremediation of carbon tetrachloride in a three-dimensional laboratory-scale reactor. She then returned to Barcelona, where she worked in the private sector, developing characterization and remediation projects for industry and government. At BIOREM, she leads remediation activities with local and international projects that use new applications of bioremediation and natural attenuation technologies for groundwater recovery. She combines research, innovation, consultancy, and field-scale

remediation, networking between involved stakeholders with the goal of technology transfer and enhancement, and often participates in research seminars, lectures, and congresses.

Timothy M. Vogel

Dr. Vogel is professor of Microbiology and Environmental Engineering at the University of Lyon. He received his BS degrees in Geology and Oceanography from the University of Washington and his MSE and PhD degrees in Environmental Engineering from Stanford University. He has worked for 30 years on the biodegradation and bioremediation of environmental contaminants and has over 70 scientific publications and 8 patents. He works as a consultant for both environmental consulting firms and various industries, was on the faculties of Michigan State University and the University of Michigan, and was responsible for environmental biotechnology at Rhône-Poulenc for 8 years. He is a partner in the recent environmental biotechnology start-up, ENOVEO.

Helen Vrionis

Dr. Vrionis has expertise in environmental and anaerobic microbiology. She received her BSc degree in Biology from the University of Windsor, Ontario, her MSc in Microbiology and Immunology from Queen's University in Kingston, Ontario, and her PhD (2002) in the Department of Chemical Engineering, Queen's University, Ontario. She has postdoctoral experience from the laboratory of Dr. Derek Lovley in the Department of Microbiology at the University of Massachusetts Amherst, from the Department of Biology at the University of Calgary, and from the Department of Chemical Engineering and Applied Chemistry at the University of Toronto.

Christopher B. Walker

Dr. Walker is an Environmental Engineer with Geosyntec Consultants, Inc., in Seattle, Washington. He received his BS in Environmental Engineering from Northwestern University and his PhD in Environmental Engineering from the University of Washington. Dr. Walker provides operational and strategy consulting for commercial, industrial, and federal clients on remediation and water management issues. His experience includes the evaluation, design, and installation of *in situ* and *ex situ* remedial systems for chlorinated solvents, energetics, heavy metals, and organics, as well as liability valuations and lifecycle assessments of contaminated sites.

Lance B. Warnick

Mr. Warnick is a Principal Engineer with Aspen Engineers in Nampa, Idaho. He received his MS degree in Environmental Engineering from Michigan State University (MSU) and his BS degree in Environmental Engineering from Utah State University. While a student at MSU, he performed bench-scale experiments to help simulate the anticipated geochemical changes associated with the Schoolcraft Bioaugmentation Project in Michigan. As a consultant for the last 14 years, he has worked on many projects, managing and treating contaminated soils and groundwater at decommissioned oil refineries in Oklahoma and Wyoming, rail yards in Nebraska, and wood treatment facilities in Colorado. He has experience in developing and implementing pilot-scale evaluations of biologically enhanced remediation. Much of his current work is centered in Idaho, where he focuses on assisting private land owners and local government entities in developing plans and management systems to help protect land and soil from contamination and help them comply with the state Department of Environmental Quality and USEPA regulations.

Ryan A. Wymore

Mr. Wymore is a Principal Environmental Engineer with CDM Smith in Denver, Colorado, where he serves as a national resource for evaluation, selection, and implementation of remediation strategies. He received his BS in Biological Systems Engineering from the University of Nebraska-Lincoln and his MS in Civil/Environmental Engineering from the University of Idaho. Mr. Wymore has spent the last 14 years specializing in innovative groundwater remediation technologies, particularly bioremediation, natural attenuation, and chemical oxidation. He also serves as the administrator for CDM's Research and Development Program, where he coordinates all of the company's internally and externally funded research. He joined ITRC in 2002, has had membership on six technical teams, and currently serves on the ITRC's Board of Advisors as the industry representative. He is a registered professional engineer in Colorado and Idaho.

Steven H. Zinder

Dr. Zinder is Professor of Microbiology at Cornell University, Ithaca, New York, where he has been on the faculty for over 30 years. He received his BA in Chemistry from Kenyon College and his PhD in Bacteriology from the University of Wisconsin, where he was mentored by the microbial ecologist T. D. Brock and was a Postdoctoral Fellow at the University of California, Los Angeles (UCLA). His research has centered on anaerobic microbes that carry out environmentally important chemical transformations, and over the years he has studied metabolism of organic sulfur compounds, methanogenesis from acetate, nitrogen fixation, and reductive dehalogenation. In the latter arena, his laboratory isolated and characterized *Dehalococcoides ethenogenes* strain 195 and helped analyze its genome sequence. He participated in the writing of the *Treatability Test Protocol for the Reductive Anaerobic Biological In Situ Treatment Technology (RABITT)*.

External Reviewers

Lisa Alvarez-Cohen

Civil and Environmental Engineering
University of California, Berkeley
Berkeley, CA 94720 USA
Email: chair@ce.berkeley.edu

Alison M. Cupples

Civil and Environmental Engineering
Michigan State University
East Lansing, MI 48824 USA
Email: cupplesa@egr.msu.edu

Greg Davis

Microbial Insights, Inc.
Rockford, TN 37853 USA
Email: gdavis@microbe.com

Rula Deeb

ARCADIS U.S., Inc.
Emeryville, CA 94608 USA
Email: Rula.Deeb@arcadis-us.com

Donna E. Fennell

School of Environmental and Biological
Sciences, Rutgers, The State University
of New Jersey, New Brunswick
NJ 08901 USA
Email: fennell@envsci.rutgers.edu

Jim K. Fredrickson

Biological Sciences Division, Fundamental
Science Directorate, Pacific Northwest
National Laboratory
Richland, WA 99352 USA
Email: jim.fredrickson@pnl.gov

David L. Freedman

Environmental Engineering & Earth Sciences
Clemson University
Clemson, SC 29634 USA
Email: dfreedm@clemson.edu

James M. Gossett

School of Civil and Environmental
Engineering, Cornell University
Ithaca, NY 14853 USA
Email: jmg18@cornell.edu

Michael R. Hyman

Department of Microbiology
North Carolina State University
Raleigh, NC 27695 USA
Email: Michael_hyman@ncsu.edu

Laurie T. LaPat-Polasko

Ciris Energy, Inc.
Centennial, CO 80112 USA
Email: llapat@cirisenergy.com

Carmen A. Lebrón

Naval Facilities Engineering Command—
Engineering Service Center
Port Hueneme, CA 93043 USA
Email: carmen.lebron@navy.mil

Frank E. Löffler

Department of Microbiology
University of Tennessee
Knoxville, TN 37996 USA
Email: frank.loeffler@utk.edu

Jon Magnuson

Pacific Northwest National Laboratory
Richland, WA 99352 USA
Email: Jon.Magnuson@pnnl.gov

Perry L. McCarty

Department of Civil and Environmental
Engineering, Stanford University
Stanford, CA 94305 USA
Email: pmccarty@stanford.edu

Charles J. Newell

GSI Environmental, Inc.
Houston, TX 77098 USA
Email: cjnewell@gsi-net.com

Aaron D. Peacock

Haley & Aldrich
Oak Ridge, TN 37830 USA
Email: apeacock@HaleyAldrich.com

Robert A. Sanford

Department of Geology
University of Illinois at Urbana-Champaign
Urbana, IL 61801 USA
Email: rsanford@illinois.edu

Jim C. Spain

School of Civil & Environmental
Engineering, Georgia Institute
of Technology, Atlanta
GA 30332 USA
Email: jspain@ce.gatech.edu

Alfred Spormann

Chemical Engineering and Civil/
Environmental Engineering
Stanford University
Stanford, CA 94305 USA
Email: spormann@stanford.edu

Rob Steffan

Biotechnology Development and
Applications, Shaw Environmental, Inc.
Lawrenceville, NJ 08648 USA
Email: Rob.Steffan@shawgrp.com

Anna Willett

Interstate Technology & Regulatory Council
Washington, DC 20001 USA
Email: awillett@ecos.org

John T. Wilson

USEPA Office of Research
and Development, National Risk
Management Laboratory
Ada, OK 74820 USA
Email: wilson.johnt@epa.gov

Contents

CHAPTER 1	BIOAUGMENTATION FOR GROUNDWATER REMEDATION: AN OVERVIEW	1
1.1	Introduction	1
1.1.1	Background: The Pollution Problem	1
1.1.2	Definitions: General Bioremediation Terminology	2
1.1.3	Chapter Overview	3
1.2	Development of Bioaugmentation for Groundwater Bioremediation	3
1.2.1	Historical Development of Bioaugmentation	3
1.2.2	Recent Developments: Bioaugmentation with <i>Dehalococcoides</i> for Reductive Dehalogenation of Chlorinated Ethenes	4
1.3	Types of Bioaugmentation	6
1.3.1	Currently Practiced Methods	6
1.3.2	Potential Bioaugmentation Strategies	9
1.4	Making the Decision to Bioaugment	12
1.4.1	Technical Analysis/Site Evaluation	14
1.4.2	Select and Test Bioaugmentation Strategy	15
1.4.3	Implement the Treatment	16
1.4.4	Monitoring Effectiveness	17
1.4.5	Other Considerations: Economics and Degradation Kinetics	19
1.5	Bioaugmentation Issues	19
1.5.1	Development of Effective Bioaugmentation Cultures	20
1.5.2	Successful Inoculum Delivery and Dispersion	20
1.5.3	Inoculum Survival	20
1.5.4	Pollutant Bioavailability	21
1.5.5	Potential Undesirable Side-Effects	21
1.6	Bioaugmentation to Remediate Chlorinated Compounds	22
1.6.1	Chlorinated Aliphatic Hydrocarbons (CAHs): <i>Dehalococcoides</i> and the Chloroethenes	23
1.6.2	Applications for Other Chlorinated Compounds	24
1.7	Bioaugmentation to Remediate Other Contaminants	24
1.7.1	Petroleum and BTEX	25
1.7.2	Polycyclic Aromatic Hydrocarbons (PAHs)	25
1.7.3	Methyl Tert-Butyl Ether (MTBE)	26
1.7.4	Pesticides	26
1.7.5	Metals	27
1.7.6	Mixed Pollutants	27
1.8	Summary	28
	References	28

CHAPTER 2	DEHALOCOCCOIDES AND REDUCTIVE DECHLORINATION OF CHLORINATED SOLVENTS	39
2.1	Introduction	39
2.1.1	The Chlorinated Ethene Problem	39
2.1.2	Anaerobic Microbial Degradation of Chlorinated Ethenes	43
2.1.3	Discovery of <i>Dehalococcoides</i>	46
2.2	<i>Dehalococcoides</i> Isolation and Cultivation Strategies	49
2.2.1	General Considerations	49
2.2.2	Electron Acceptor	50
2.2.3	Electron Donor	50
2.2.4	Carbon Source	51
2.2.5	Reducing Agent (Reductant)	51
2.2.6	Incubation Conditions	52
2.2.7	Isolation	52
2.3	<i>Dhc</i> Pure Cultures	52
2.3.1	Isolation of <i>Dhc mccartyi</i> Strain 195	52
2.3.2	Isolation of <i>Dhc</i> sp. Strain CBDB1	53
2.3.3	Isolation of <i>Dhc</i> sp. Strain FL2	53
2.3.4	Isolation of <i>Dhc</i> Strains That Respire VC: Strains BAVI, GT and VS	53
2.3.5	Isolation of <i>Dhc</i> Strain MB	54
2.4	Maintenance of <i>Dehalococcoides</i> Pure Cultures	55
2.4.1	General Considerations	55
2.4.2	Growth Factors	55
2.5	<i>Dehalococcoides</i> Morphology and Physiology	56
2.6	Phylogeny of <i>Dehalococcoides</i> and Related Bacteria	58
2.7	<i>Dehalococcoides</i> Genetics	61
2.7.1	Insights from <i>Dehalococcoides</i> Genomes	61
2.7.2	<i>Dehalococcoides</i> Reductive Dehalogenases Gene Operons	63
2.8	<i>Dehalococcoides</i> Reductive Dehalogenases (RDASES)	64
2.9	Biochemistry of Reductive Dechlorination by <i>Dehalococcoides</i>	65
2.10	<i>Dehalococcoides</i> Biomarkers	67
2.11	<i>Dehalococcoides</i> Evolution and Dissemination of Reductive Dehalogenase Genes	72
2.12	<i>Dehalococcoides</i> Biogeography	73
2.13	<i>Dehalococcoides</i> Ecology	74
2.14	Outlook	75
2.15	Implications for Bioremediation Practice: Take Home Messages	76
	References	76

CHAPTER 3	PRODUCTION AND HANDLING OF <i>DEHALOCOCCOIDES</i>	
	BIOAUGMENTATION CULTURES	89
3.1	Introduction	89
3.1.1	Microbial Cultures Used for Bioaugmentation.....	89
3.1.2	Why High Density Microbial Cultures Are Important.....	91
3.2	Growing Inocula	91
3.2.1	Microbial Growth Options: Batch Versus Continuous.....	91
3.2.2	Culture Growth Protocol.....	93
3.3	Full-Scale Production Results.....	95
3.3.1	Factors Affecting Culture Growth.....	99
3.4	Quality Assurance/Quality Control Considerations	104
3.4.1	Pathogen Analysis	104
3.4.2	<i>Dhc</i> Concentrations	104
3.4.3	Specific Activity.....	105
3.4.4	Other QA/QC Considerations	106
3.5	Concentrating and Storing Inocula.....	106
3.5.1	Concentrating Cultures.....	107
3.5.2	Culture Stability and Storage.....	109
3.6	Shipping Cultures	110
3.7	Onsite Handling	111
3.7.1	Direct Injection	111
3.7.2	Dilution	112
3.7.3	Mixing with Other Reagents Before Injection.....	112
3.8	Summary	113
	References	113
CHAPTER 4	BIOAUGMENTATION WITH <i>DEHALOCOCCOIDES</i>:	
	A DECISION GUIDE	117
4.1	Introduction	117
4.2	Need for Decision Guidance.....	118
4.3	Decision Guidance Overview	119
4.4	Is Complete Dechlorination Occurring?.....	119
4.5	Are the Site Conditions Inhibitory?	121
4.6	Is the Site Highly Aerobic?.....	122
4.7	Will Biostimulation Work?.....	123
4.7.1	Laboratory Diagnostic Tests	124
4.7.2	Field Testing.....	128

4.8	How Valuable is Time?	131
4.9	Is the Risk of Exposure to Toxic Intermediates Unacceptable?	132
4.10	Economic Assessments of Bioaugmentation	132
4.11	Summary and Recommendations	134
	References	135
CHAPTER 5	BIOAUGMENTATION CONSIDERATIONS.....	141
5.1	Introduction	141
5.2	Effect of Site Conditions on Effectiveness of Bioaugmentation	141
5.2.1	Exposure to Oxygen.....	141
5.2.2	Temperature and pH.....	142
5.2.3	Competition for Electron Donor/Geochemical Conditions.....	142
5.2.4	Volatile Organic Compound (VOC) Concentration.....	143
5.2.5	Inhibitory Constituents.....	143
5.2.6	Hydrogeology	144
5.3	Field Methods	144
5.3.1	Injection Infrastructure Considerations	144
5.3.2	Preconditioning Requirements.....	145
5.3.3	Culture Requirements.....	148
5.3.4	Injection Techniques	148
5.3.5	Distribution Techniques.....	152
5.4	Bioremediation Configurations Employing Bioaugmentation	153
5.4.1	Active Recirculation Approach.....	153
5.4.2	Semi-Passive Approach	159
5.4.3	Passive Approach.....	160
5.5	Conclusions.....	161
	References	162
	Appendix 5A Background on Inoculum Density and Dechlorination Rates.....	162
CHAPTER 6	MICROBIAL MONITORING DURING BIOAUGMENTATION WITH <i>DEHALOCOCCOIDES</i>.....	171
6.1	Introduction	171
6.2	MBTs for Chlorinated Ethene Biodegradation	173
6.3	Developing a Monitoring Strategy	174
6.3.1	Defining Monitoring Objectives	174
6.3.2	Temporal Considerations	174
6.3.3	Selection of Sampling Wells.....	174
6.4	MBT Sampling Methods	175
6.4.1	General Sampling Considerations	175
6.4.2	Groundwater Sampling Protocol	177

Contents	xxix
6.5 Quantitative-PCR	180
6.5.1 Description and General Methodology	180
6.5.2 Standards	182
6.5.3 Limitations	182
6.5.4 <i>Dhc</i> Analysis	184
6.5.5 Conclusion	185
6.6 Fluorescent <i>In Situ</i> Hybridization	185
6.6.1 Introduction	185
6.6.2 Description and General Methodology	186
6.6.3 Limitations	187
6.6.4 Conjunctive Technologies	188
6.6.5 Conclusion	188
6.7 Community Profiling	189
6.7.1 Gel Electrophoresis	189
6.7.2 Cloning and Sequencing	189
6.7.3 Terminal-Restriction Fragment Length Polymorphism	190
6.7.4 Denaturing Gel Gradient Electrophoresis	190
6.7.5 Temperature Gel Gradient Electrophoresis	190
6.7.6 Microarrays and High-Throughput Sequencing	191
6.7.7 Conclusion	192
6.8 Data Evaluation and Interpretation of MBTs	193
6.9 Future Research Needs	194
References	194
CHAPTER 7 BIOAUGMENTATION FOR AEROBIC DEGRADATION OF <i>CIS</i>-1,2-DICHLOROETHENE.....	199
7.1 Introduction	199
7.2 <i>Polaromonas</i> sp. Strain JS666.....	200
7.2.1 Isolation.....	201
7.2.2 Kinetics, Thresholds and Tolerances to <i>cis</i> -DCE and Oxygen	201
7.2.3 Insight About Metabolic Pathways from Genomics and Proteomics ...	202
7.2.4 Cometabolism of Other Chlorinated Solvents	204
7.2.5 Development of a Molecular Probe for Process Monitoring.....	205
7.2.6 Development of Strategy for Growth of Inocula.....	206
7.3 Microcosm Assessment of Site-Suitability.....	207
7.3.1 Microcosm Preparation	207
7.3.2 Previous Experiences with Microcosm Assessment	208
7.4 Field Demonstration	208
7.4.1 Test Site Selection	208
7.4.2 Preliminary Microcosm Study	209

7.4.3	Titration Studies with SJCA Groundwater	210
7.4.4	Field Test	210
7.5	Summary and Future Prospects	212
	References	213
CHAPTER 8 BIOAUGMENTATION FOR THE <i>IN SITU</i> AEROBIC COMETABOLISM OF CHLORINATED SOLVENTS.....		
8.1	Introduction	219
8.2	Aerobic Cometabolic Processes	219
8.3	Aerobic Cometabolism by Indigenous Microorganisms.....	222
8.3.1	Microcosm Studies with Indigenous Microorganisms.....	222
8.3.2	Field Studies with Indigenous Microorganisms.....	222
8.4	Bioaugmentation Approaches	228
8.4.1	Bioaugmentation Approach I	228
8.4.2	Bioaugmentation Approach II.....	237
8.4.3	Bioaugmentation Approach III.....	242
8.4.4	Bioaugmentation Approach IV.....	248
8.5	Summary	249
	References	251
CHAPTER 9 BIOAUGMENTATION WITH <i>PSEUDOMONAS STUTZERI</i> KC FOR CARBON TETRACHLORIDE REMEDIATION.....		
9.1	Introduction and Rationale	257
9.2	Physiological Function of PDTC Production.....	258
9.3	CT Transformation by <i>P. Stutzeri</i> KC as a Novel Dechlorination Reaction	259
9.3.1	Pathway of PDTC-Promoted CT Dechlorination.....	260
9.3.2	Transition Metal Chelation of PDTC.....	261
9.4	Genetic Requirements for PDTC Production	263
9.5	PDTC-Mediated CT Transformation.....	265
9.5.1	Trace Metals.....	266
9.5.2	Cell and CT Concentration	266
9.5.3	Cell Membrane Components	268
9.5.4	An Overall Model for CT Transformation by <i>Pseudomonas stutzeri</i> KC	269
9.6	Bioaugmentation with <i>P. Stutzeri</i> KC: Transport, Growth and Competition ...	269
9.6.1	Inoculation and Transport	270
9.6.2	Growth and Competition	271
9.7	pH Adjustment	273