In Situ
Bioremediation of Perchlorate in Groundwater

SERDP and ESTCP Remediation Technology Monograph Series

Series Editor: C. Herb Ward, Rice University

In Situ Bioremediation of Perchlorate in Groundwater

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SERDP/ESTCP Remediation Technology Monograph Series

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SERDP and ESTCP have joined to sponsor 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 *in situ* bioremediation of perchlorate. Additional volumes planned for publication in the near future include:

- *In Situ* Remediation of Dissolved Chlorinated Solvents in Groundwater
- Delivery and Mixing in the Subsurface: Processes and Design Principles for *In Situ* Remediation
- Bioaugmentation for Groundwater Remediation
- Chlorinated Solvent Source Zone Remediation
- Monitored Natural Recovery at Contaminated Sediment Sites
- Remediation of Munition Constituents in Soil and Groundwater

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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 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 to 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 National Research Council 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 initiation of 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 with the mission "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 corporate 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 Department'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 Department'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 the DoD's 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 test 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 10 to 15 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, once recalcitrant environmental contamination problems for which little or no effective technology was 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 joined to sponsor 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 on *In Situ* Bioremediation of Perchlorate in Groundwater will be followed by others on such topics as the remediation of both soluble phase and DNAPL chlorinated solvents, bioaugmentation to enhance bioremediation processes, delivery and mixing strategies and technologies to enhance subsurface remediation, and remediation of contaminated sediments. Additional volumes will be written as new remediation technologies are developed and proven to be effective.

This volume provides a review of the past decade of intensive research, development, and demonstrations on the *in situ* bioremediation of perchlorate. The intended audiences include the decision makers and practicing engineers and hydrogeologists who will select, design, and operate these remedial systems, as well as researchers seeking to improve the current state-of-the-art. Our hope is that this volume will serve as a useful resource to assist remediation professionals in applying and developing the technology as effectively as possible.

A brief technology overview is provided in Chapter 1. Chapter 2 summarizes the development of *in situ* bioremediation of perchlorate to illustrate how we arrived at our state-of-understanding today. Chapter 3, on the principles of *in situ* bioremediation of perchlorate, presents the current state-of-the-science, covering the microbial processes, abiotic processes, and the engineering and implementation issues underlying the technologies described.

Chapter 4 deals with the important characterization issues relevant to perchlorate contamination, including a discussion on perchlorate sources (i.e., both the anthropogenic and natural sources of perchlorate) and the methods available to distinguish between differing sources (particularly the use of compound specific isotopic analysis).

Chapter 5 initiates a more detailed discussion of the different methods for implementing *in situ* bioremediation, beginning with a summary of the primary methods available, and the factors affecting the selection of *in situ* bioremediation at a specific site. Chapter 5 also discusses the specific remedial approaches available, with discussion on their design and monitoring, and the advantages and disadvantages of each approach under different site-specific conditions.

Chapters 6, 7, 8 and 9 detail the different options for implementing *in situ* bioremediation. These chapters describe the design and operation of the particular option, the current stage of development, and case histories that illustrate the issues involved and provide examples of the performance that is achievable. Chapter 6 discusses active bioremediation, in which substrates are continuously circulated through the target treatment zone. Chapter 7 discusses what is often described as semi-passive bioremediation, in which the substrate is added at intervals and mixing is intermittent. The final Chapters (8 and 9) discuss two different approaches to passive bioremediation, in which there is no active mixing. In the first case, edible oil is injected into the subsurface, and in the second, a biowall is created by installing a trench across the contaminant plume filled with a degradable material such as mulch.

Chapter 10 provides cost information for each technology, using analyses of several template sites to aid the reader in estimating the economics of applying these technologies at other sites. Cost information includes capital costs, as well as costs for laboratory testing, pilot-scale demonstration, design, system operation, monitoring and maintenance during operations, and demolition and restoration after remediation. In addition, analogous cost data are presented for pump-and-treat systems for each template site to illustrate the potential cost savings associated with the use of alternative approaches.

The final chapter on emerging technology (Chapter 11), describes three innovative bioremediation technologies still in the developmental stages. These technologies (monitored natural attenuation, phytoremediation, and vadose zone bioremediation) are described, and field demonstrations are used to illustrate the current stage of maturity and the potential applicability of these approaches for specific situations.

Each chapter in this volume has been thoroughly reviewed for technical content by one 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 perchlorate, remediation practitioners, and for those involved in development of advanced technology for the *in situ* remediation of perchlorate.

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 perchlorate.

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He has been a faculty member at Rice University for 42 years during which he has served as Chair of the Department of Environmental Science and Engineering and the Department of Civil and Environmental Engineering. He has also served as Director of the USEPA-sponsored National Center for Ground Water Research and the 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*.

The editors of this volume gratefully acknowledge the excellent organizational and editorial assistance of Catherine M. Vogel, PE, Noblis, Atlanta, Georgia.

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Dr. Borden received his BS and ME degrees in Civil and Environmental Engineering from the University of Virginia and a PhD in Environmental Engineering from Rice University.

He has been a faculty member at North Carolina State University since 1986, teaching and conducting research in surface water hydrology, groundwater hydrology, subsurface contaminant transport, and *in situ* remediation. His research includes laboratory studies, fieldwork and mathematical model development. Recently, much of his research has focused on remediation of chlorinated solvents, perchlorate, chromium and acid mine drainage using emulsified oils. At Solutions-IES, Dr. Borden supports many of the firm's projects including traditional remediation approaches, *in situ* bioremediation, *in situ* chemical oxidation, monitored natural attenuation, and expert witness testimony.

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Dr. Coates joined the faculty of the Plant and Microbial Biology Department at the University of California, Berkeley in 2002 where he is currently a Professor of microbiology. In addition, he holds a joint appointment in the Geological Scientist Faculty of the Earth Sciences Division, Ernest Orlando Lawrence Berkeley National Laboratory. He obtained an Honors BSc in Biotechnology in 1986 from Dublin City University, Ireland and his PhD in 1991 from University College in Galway, Ireland. His major area of interest is geomicrobiology applied to environmental problems. Specific interests include diverse forms of anaerobic microbial metabolism such as microbial perchlorate reduction, microbial iron oxidation and reduction, and microbial humic substances redox cycles. Other interests include bioremediation of toxic metals, radionuclides, and organics. He was the recipient of the 1998 *Oak Ridge Ralph E. Powe Young Faculty Enhancement Award*, joint recipient of the 2001 *DoD SERDP Project of the Year Award*, and the 2002 *Southern Illinois University College of Science Researcher of the Year Award*. He has given more than 90 invited presentations at national and international meetings and has authored and co-authored more than 80 peer-reviewed publications and book chapters. He has eight patent submissions based on technologies developed in his laboratory. He sits on the editorial boards of the journals *Applied and Environmental Microbiology* and *Applied Microbiology and Biotechnology*. He also sits on the microbiology editorial board of Faculty of 1000 databases.

Evan E. Cox

Mr. Cox is a Principal and Senior Remediation Microbiologist with 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, and metal-catalyzed reduction of chlorinated and recalcitrant chemicals in subsurface environments. Mr. Cox received his BSc and MSc degrees in Microbiology from the University of Waterloo. Over the past 18 years, Mr. Cox has pioneered the use of MNA and EISB technologies, including bioaugmentation for remediation of chlorinated solvents in porous media and fractured bedrock, co-authoring several guidance documents and presenting educational courses on these subjects. Mr. Cox has pioneered the use of EISB to treat rocket fuel components such as perchlorate in soil and groundwater. He has served as the program manager or technical lead for more than 50 perchlorate projects at more than 20 perchlorate-impacted sites nationwide. Mr. Cox has published more than 30 articles regarding the degradation of hazardous contaminants in subsurface environments.

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He has 20 years of experience in process engineering, chemistry, and microbiology. This experience has included research and development, bench-scale and pilot-scale evaluation of innovative technologies, design and construction of treatment facilities, and troubleshooting and optimization of operating processes. He has invented and holds patents for analytical methods and treatment processes.

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Dr. Hatzinger is a Senior Research Scientist in the Biotechnology Development and Applications Group of Shaw Environmental, Inc. in Lawrenceville, New Jersey. He holds a BS in Biology and Environmental Science from St. Lawrence University, and both a MS and PhD in Environmental Toxicology from Cornell University.

Dr. Hatzinger was previously employed as a staff scientist at Envirogen, Inc. and has more than 15 years of experience in biodegradation, bioremediation, groundwater geochemistry and microbiology.

Dr. Hatzinger's research is focused on the development of *in situ* and *ex situ* bioremediation technologies for emerging contaminants. He has been studying perchlorate biodegradation since 1999, and has served as the Principal Investigator on several field projects evaluating *in situ* remedial approaches for the oxidant. In addition, Dr. Hatzinger has worked closely with the engineering group at Shaw that has designed and constructed five full-scale bioreactor systems for treatment of perchlorate in groundwater.

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Mr. Henry provides project management and technical direction for the *in situ* remediation of fuel hydrocarbons and chlorinated solvents in groundwater. In particular, he has worked with the U.S. Air Force to develop technical protocols for enhanced *in situ* biroemediation of chlorinated solvents and is the primary author of the Air Force *Technical Protocol for Enhanced Anaerobic Bioremediation Using Permeable Mulch Biowalls and Bioreactors.* Mr. Henry also provides technical support on remediation projects for several private- and public-sector clients.

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He has been a faculty member at Texas Tech University for 10 years. He is very active in the general area of contaminant fate (perchlorate, RDX, chlorinated solvents) in natural environments and the use of engineered wetlands as passive sustainable treatment systems. His work includes the design of biological reactors for source separated waste streams and their consequences on water quality in closed loop recycled systems.

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The focus of his professional practice has been in taking new technologies for groundwater remediation 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 permeable reactive barriers for the treatment of chlorinated solvents. In 2007, he was inducted into the Space Foundation Technology Hall of Fame for his contributions to the development of emulsified nano-scale zero-valent iron technology for treatment of chlorinated solvent dense nonaqueous phase liquid source zones.

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Mr. Wolfe has undergraduate degrees in Industrial Engineering and International Relations from Lehigh University and an MS Degree in Environmental Engineering from Virginia Tech. He is a registered professional engineer in Delaware.

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ACRONYMS AND ABBREVIATIONS

°C	degrees Celsius
µg/kg	micrograms per kilogram
µg/L	micrograms per liter
µm	micrometer
µmoles/L	micromoles per liter
µS/cm	microsiemens per centimeter
1,1,1-TCA	1,1,1-trichloroethane
1,1-DCA	1,1-dichlororethane
16S rRNA	ribosomal RNA
AWMA	Air & Waste Management Association
AFB	Air Force Base
AFCEE	Air Force Center for Environmental Excellence (renamed the Air Force Center for Engineering and the Environment)
AIChE	American Institute of Chemical Engineers
amsl	above mean sea level
AN	ammonium nitrate
ANFO	ammonium nitrate-fuel oil
APA	American Pyrotechnics Association
bgs	below ground surface
BOD	biochemical oxygen demand
CA	chloroethane
CCL	Contaminant Candidate List
CD	chlorite dismutase
CDC	Center for Disease Control
CDHS	California Department of Health Services
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
***cis*-1,2-DCE**	*cis*-1,2-dichloroethene
cm	centimeter
cm/sec	centimeters per second
COC	Contaminant of Concern
COD	chemical oxygen demand
CSM	Conceptual Site Model
DAP	di-ammonium phosphate
DCE	dichloroethene
DDT	dichlorodiphenyltrichloro ethane
DERP	Defense Environmental Restoration Program
DHG	dissolved hydrocarbon gas
DNAPL	dense nonaqueous phase liquid
DO	dissolved oxygen
DOC	dissolved organic carbon
DoD	U.S. Department of Defense
DPRB	dissimilatory perchlorate-reducing bacteria
EDQW	Environmental Data Quality Workgroup
EISB	enhanced *in situ* bioremediation
ELISA	enzyme-linked immunosorbent assay
EOFA	edible oil fatty acids
EOS®	Edible Oil Substrate
ER	extraction and reinjection
ESI	electrospray ionization
ESTCP	Environmental Security Technology Certification Program
EVO	emulsified vegetable oil
EWG	Environmental Working Group
FBR	fluidized bed reactor
ft	feet
g	gram
g/L	grams per liter
GAC	granular activated carbon
gal	gallon
GCIRMS	gas chromatography-isotope ratio mass spectrometry
GEDIT	Gaseous Electron Donor Injection Technology
gpm	gallons per minute
HFTW	horizontal flow treatment well
HPLC	high performance liquid chromatography
hr	hour

HRC®	Hydrogen Release Compound
HRT	hydraulic retention time
IC	ion chromatography
IHDIV	Indian Head Division
IME	Institute of Makers of Explosives
in	inch
IRMS	isotope ratio mass spectrometry
ISE	ion specific electrode
ISEE	International Society of Explosives Engineers
ITRC	Interstate Technology & Regulatory Council
JPL	Jet Propulsion Laboratory
K	conductivity
K_d	partition coefficient
kg	kilogram
km	kilometer
kPa	kilopascal
L	liter
L/min	liters per minute
lb	pound
LC	liquid chromatography
LCFA	long-chain fatty acid
LHAAP	Longhorn Army Ammunition Plant
LLC	Limited Liability Company
LLNL	Lawrence Livermore National Laboratory
LPG	liquefied petroleum gas
LUFT	Leaking Underground Fuel Tank
LVW	Las Vegas Wash
m	meter
MADEP	Massachusetts Department of Environmental Protection
MCL	maximum contaminant level
MCT	matrix conductivity threshold
MDL	method detection limit
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
mi	mile
min	minute
mL	milliliter

mM	millimolar
mmol	millimole
MNA	monitored natural attenuation
MPN	most probable number count
MRL	method reporting limit
MS	mass spectrometry
mS/cm	millisiemens per centimeter
MSDS	Material Safety Data Sheet
MTBE	methyl tertiary butyl ether
mV	millivolt
NAVAIR	Naval Air Systems Command
NFESC	Naval Facilities Engineering Service Center
NPV	Net Present Value
NRC	National Research Council
NWIRP	Naval Weapons Industrial Reserve Plant
O&M	operation and maintenance
OB/OD	open burn, open detonation
OM&M	operation, maintenance and monitoring
OMB	Office of Management and Budget
OMRI	Organic Materials Review Institute
ORP	oxidation reduction potential
OSW	Office of Solid Waste
PAH	polycyclic aromatic hydrocarbon
p-CBS	p-chlorobenzenesulfonate
PCE	perchloroethene (also termed tetrachloroethene)
PCL	protective concentration limit
PCR	polymerase chain reaction
ppb	part per billion
PPE	personal protective equipment
ppm	part per million
PQL	practical quantitation limit
PRB	permeable reactive barrier
PRG	preliminary remediation goal
PTA	pilot test area
PV	pore volume

QA/QC	quality assurance/quality control
RAO	Remedial Action Objective
RCRA	Resource Conservation and Recovery Act
rDNA	Recombinant Deoxyribonucleic Acid
RDX	cyclotrimethylenetrinitramine or **R**oyal **D**emolition e**X**plosive
RfD	reference dose
RFI	RCRA Facility Investigation
RO	reverse osmosis
ROD	Record of Decision
ROI	radius of influence
SAMNAS	Surface Application and Mobilization of Nutrient Amendments
SERDP	Strategic Environmental Research and Development Program
SMOC	standard mean ocean chloride
SU	standard unit
TCE	trichloroethene
TCEQ	Texas Commission on Environmental Quality
TDS	total dissolved solids
TEAP	terminal electron accepting process
THPS	tetrakis(hydroxymethyl)phosphonium sulfate or Tolcide
TIC	total inorganic carbon
TNT	2,4,5-trinitrotoluene
TOC	total organic carbon
UCMR	Unregulated Contaminant Monitoring Rule
UMD	University of Massachusetts at Dartmouth
USACE	U.S. Army Corps of Engineers
USDOC	U.S. Department of Commerce
USEPA	U.S. Environmental Protection Agency
USFDA	U.S. Food and Drug Administration
USGS	U.S. Geological Survey
UTC	United Technologies Corporation
UV	ultraviolet
VC	vinyl chloride
VFA	volatile fatty acid
VOC	volatile organic compounds
vol/vol	volume per volume
WMP	Waste Management Plan
wt/vol	weight per volume
ZVI	zero valent iron

UNIT CONVERSION TABLE

MULTIPLY	BY	TO OBTAIN
acres	0.405	hectares
acres	1.56 E-3	square miles (statute)
centimeters	0.394	inches
cubic feet	0.028	cubic meters
cubic feet	7.48	gallons (U.S. liquid)
cubic feet	28.3	liters
cubic meters	35.3	cubic feet
cubic yards	0.76	cubic meters
feet	0.305	meters
feet per year	9.66 E-7	centimeters per second
gallons (U.S. liquid)	3.79	liters
hectares	2.47	acres
inches	2.54	centimeters
kilograms	2.20	pounds (avoir)
kilograms	35.3	ounces (avoir)
kilometers	0.62	miles (statute)
liters	0.035	cubic feet
liters	0.26	gallons (U.S. liquid)
meters	3.28	feet
miles (statute)	1.61	kilometers
ounces (avoir)	0.028	kilograms
ounces (fluid)	29.6	mililiters
pounds (avoir)	0.45	kilograms
square feet	0.093	square meters
square miles	640	acres

GLOSSARY[1]

Abiotic—Occurring without the direct involvement of organisms.

Active treatment—*In situ* bioremediation approach in which water-soluble amendments are added to the subsurface intermittently, frequently, or even continuously, by pumping liquid solutions into injection wells. Extraction may also be used to recover water prior to amendment addition and/or to recirculate amendments through the target treatment zone.

Advection—Transport of molecules dissolved in water along the groundwater flow path at an average expected velocity.

Aerobic—Environmental conditions where oxygen is present.

Aerobic respiration—Process whereby microorganisms use oxygen as an electron acceptor to generate energy.

Analytical model—A mathematical model that has a closed form solution (i.e., the solution to the equations used to describe changes in a system can be expressed as a mathematical analytic function). Analytical solutions can be more exact and aesthetically pleasing than numerical models, but analytical solutions to equations describing complex systems can often become very difficult.

Anaerobic—Means “without air”. It generally refers to occurring or living without oxygen present. Thus, in an anaerobic groundwater system, the chemistry is characterized by reductive conditions. Sometimes (e.g., in wastewater treatment) anaerobic is used to indicate a lack of any electron acceptors (i.e., including nitrate and sulfate). In groundwater, a dissolved oxygen concentration below 1.0 mg/L is generally considered anaerobic.

Anaerobic bioventing—Delivery of gases such as hydrogen to the subsurface to stimulate the activity of anaerobic microorganisms.

Anaerobic respiration—Process whereby microorganisms use an electron donor such as hydrogen and a chemical other than oxygen as an electron acceptor. Common “substitutes” for oxygen are nitrate, sulfate, iron, carbon dioxide, and other organic compounds (fermentation).

Anoxic—Literally means “without oxygen.” For example, anoxic groundwater is groundwater that contains no dissolved oxygen.

Aquifer—An underground geological formation that stores groundwater. A confined aquifer lies beneath a confining unit of lower hydraulic conductivity. An unconfined aquifer does not have a confining unit and is defined by the water table.

Aquitard—An underground geological formation of low permeability that does not readily transmit groundwater.

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[1] This glossary is a compilation of definitions of terms synthesized by the volume editors and chapter authors. Select definitions are reprinted from *In Situ Bioremediation: When does it work?* (National Research Council, 1993) with permission from the National Academies Press, Copyright 1993, National Academy of Sciences.

Attenuation—Reduction of contaminant concentrations over space or time. It includes both destructive (e.g., biodegradation, hydrolysis) and non-destructive (e.g., volatilization, sorption) removal processes.

Attenuation Rate—The rate of contaminant concentration reduction over time. Typical units are milligrams per liter per year (mg/L/yr).

Bacterium—A single-celled organism of microscopic size (generally 0.3 to 2.0 micrometers in diameter). As opposed to fungi and higher plants and animals ("eukaryotes"), bacteria are "prokaryotes" (i.e., they are characterized by the absence of a distinct, membrane-bound nucleus or membrane-bound organelles, and by DNA that is not organized into chromosomes).

Bedrock—The solid or fractured rock underlying surface solids and other unconsolidated material or overburden.

Bioaugmentation—The addition of microbes to the subsurface to improve the biodegradation of target contaminants. Microbes may be "seeded" from populations already present at a site, or from specially-cultivated strains of bacteria.

Biobarrier—The concept of intercepting and treating a contaminant plume as it passes through a permeable subsurface barrier. Biobarriers are created by installing wells or trenches across the width of a plume to deliver substrate to the microorganisms in the groundwater as it flows through the barrier.

Biochemical—Produced by, or involving, chemical reactions of living organisms.

Biodegradation—Biologically mediated conversion of one compound to another.

Biofouling—Impairment of the functioning of wells or other equipment as a result of the growth or activity of microorganisms.

Biomass—Total mass of microorganisms present in a given amount of water or soil.

Bioremediation—Use of microorganisms to control and destroy contaminants.

Biotransformation—Biologically catalyzed transformation of a chemical to some other product.

Biowall—A form of passive *in situ* bioremediation, in which the contaminant plume is intercepted and treated as it passes through an emplaced porous barrier (e.g., trenches filled with sand-mulch mixtures). Microorganisms growing on the wall materials remove contaminants through biodegradation processes as groundwater passes through the barrier.

Catalyst—A substance which promotes a chemical reaction, but does not itself enter into the reaction.

Chlorinated solvent—A hydrocarbon in which chlorine atoms substitute for one or more hydrogen atoms in the compound's structure. Chlorinated solvents commonly are used for grease removal in manufacturing, dry cleaning, and other operations. Examples include trichloroethene, perchloroethene and trichloroethane.

Chlorite dismutase—An enzyme that catalyzes the disproportionation (i.e., a chemical reaction in which a single reactant breaks up to produce two different products) of chlorite to chloride and oxygen. Chlorite dismutase is present in bacteria capable of cell respiration using perchlorate or chlorate.

Conceptual site model—A hypothesis about how contaminant releases occurred at a site, the current state of the contaminant source, an idealized geochemical site type, and the current plume characteristics (plume stability).

Dechlorination—A type of dehalogenation reaction involving replacement of one or more chlorine atoms with hydrogen.

Degradation—The transformation of a compound through biological or abiotic reactions.

Dehalogenation—Replacement of one or more halogens (e.g., chlorine, fluorine, or bromine) with hydrogen atoms.

Dense nonaqueous phase liquid (DNAPL)—A liquid that is denser than water and does not dissolve or mix easily in water (it is immiscible). In the presence of water it forms a separate phase from the water. Many chlorinated solvents, such as trichloroethene, are DNAPLs.

Desorption—Opposite of sorption; the release of chemicals from solid surfaces.

Diffusion—Dispersive process resulting from the movement of molecules along a concentration gradient. Molecules move from areas of high concentration to areas of low concentration.

Dilution—The combined processes of advection and dispersion resulting in a net dilution of the molecules in the groundwater.

Dispersion—The spreading of molecules along and away from the expected groundwater flow path during advection as a result of mixing of groundwater in individual pores and channels.

Dissimilatory—A biochemical process in which an inorganic compound is used for an energy source but is not assimilated into the organism (i.e., as occurs in perchlorate reduction to chloride, the metabolites are all inorganic compounds).

Electron—A negatively charged subatomic particle that may be transferred between chemical species in chemical reactions. Every chemical molecule contains electrons and protons (positively charged particles).

Electron acceptor—Compound that receives electrons (and therefore is reduced) in the oxidation-reduction reactions that are essential for the growth of microorganisms and for bioremediation. Common electron acceptors in the subsurface are oxygen, nitrate, sulfate, iron and carbon dioxide. Chlorinated solvents (e.g., trichloroethene) can serve as electron acceptors under anaerobic conditions.

Electron donor—Compound that donates electrons (and therefore is oxidized) in the oxidation-reduction reactions that are essential for the growth of microorganisms and bioremediation. Organic compounds (e.g., lactate) generally serve as an electron donor during anaerobic bioremediation. Less chlorinated solvents (e.g., VC) can also serve as electron donors. Hydrogen generated in fermentation reactions also can serve as an electron donor.

Emulsified edible oil—A formulation in which an edible oil (such as soybean oil) is dispersed into water (e.g., through stirring or use of homogenizers) to form a mixture of oil droplets in water. Emulsifying the oil greatly improves the distribution of the oil in the subsurface.

Enzyme—A protein created by living organisms to use in transforming a specific compound. The protein serves as a catalyst in the compound's biochemical transformation.

Ex situ—Latin term referring to the removal of a substance from its natural or original position, e.g., treatment of contaminated groundwater aboveground.

Fermentation—Process whereby microorganisms use an organic compound as both electron donor and electron acceptor, converting the compound to fermentation products such as organic acids, alcohols, hydrogen and carbon dioxide.

Geochemical—Produced by, or involving, non-biochemical reactions of the subsurface.

Growth substrate—An organic compound upon which a bacteria can grow, usually as a sole carbon and energy source.

Hydraulic conductivity—A measure of the rate at which water moves through a unit area of the subsurface under a unit hydraulic gradient.

Hydraulic gradient—Change in head (i.e., water pressure) per unit distance in a given direction, typically in the principal flow direction.

Hydrophobic compound—A "water-fearing" compound, such as oil, that has low solubility in water and tends to form a separate phase.

In situ—Latin term meaning "in place"—in the natural or original position, e.g., treatment of groundwater in the subsurface.

Inorganic compound—A chemical that is not based on covalent carbon bonds. Perchlorate is an inorganic compound, as are metals, nutrients such as nitrogen and phosphorus, minerals, and carbon dioxide.

Intrinsic bioremediation—A type of *in situ* bioremediation that uses the innate capabilities of naturally occurring microbes to degrade contaminants without requiring engineering steps to enhance the process.

Intrinsic remediation—*In situ* remediation that uses naturally occurring processes to degrade or remove contaminants without using engineering steps to enhance the process.

Isotope—Any of two or more species of an element in the periodic table with the same number of protons. Isotopes have nearly identical chemical properties but different atomic masses and physical properties. For example, the isotopes chlorine 37 (^{37}Cl) and chlorine 35 (^{35}Cl) both have 17 protons, but ^{37}C has two extra neutrons and thus a greater mass.

Isotope fractionation—Selective degradation of one isotopic form of a compound over another isotopic form. For example, microorganisms degrade the ^{35}Cl isotopes of perchlorate more rapidly than the ^{37}Cl isotopes.

Kinetics—Refers to the rate at which a reaction occurs.

Life cycle cost—The overall estimated cost for a particular remedial alternative over the time period corresponding to the life of the program including direct and indirect initial costs plus any periodic or continuing costs of operation and maintenance.

Mass balance—An accounting of the total inputs and outputs to a system. For dissolved plumes, it refers to a quantitative estimation of the mass loading to a dissolved plume and the mass attenuation capacity within the affected subsurface environment.

Mass flux—The rate of mass flow across a unit area (typically measured in grams per square meter per day [g/m^2/day]). Typically calculated by integrating measured groundwater contaminant concentrations across a transect. Often used interchangeably with mass discharge or mass loading (expressed in grams per day [g/day] to describe the mass emanating from a source zone or the mass passing a given transect across the plume.

Mass spectrometer—Instrument used to identify the chemical structure of a compound. Usually, the chemicals in the compound are separated beforehand by chromatography.

Mass transfer—The general term for the physical processes involving molecular and convective transport of atoms and molecules within physical systems. In this context, the term refers to the transport of solute mass from the nonaqueous phase (e.g., NAPL) into the aqueous phase. The rate of mass transfer is controlled by the differences in concentrations between the phases, as well as the interfacial tension at the NAPL:water interface.

Metabolic intermediate—A chemical produced by one step in a multistep biotransformation (e.g., chlorite produced during stepwise reduction of perchlorate to chloride).

Metabolism—The chemical reactions in living cells that convert food sources to energy and new cell mass.

Methanogen—A microorganism that exists in anaerobic environments and produces methane as the end product of its metabolism. Methanogens use carbon dioxide, or simple carbon compounds such as methanol, as an electron acceptor.

Methanogenesis—Process of producing methane gas during biological metabolism.

Microcosm—A laboratory vessel set up to resemble as closely as possible the conditions of a natural environment.

Microorganism—An organism of microscopic or submicroscopic size. Bacteria are microorganisms.

Mineralization—The complete degradation of an organic chemical to carbon dioxide, water, and possibly other inorganic compounds.

Monitored natural attenuation (MNA)—Refers to the reliance on natural attenuation processes (within the context of a carefully controlled and monitored site cleanup approach) to achieve site-specific remediation objectives within a time frame that is reasonable compared to that offered by other more active methods.

Natural attenuation—Reduction in the mass, toxicity, mobility, volume or concentration of contaminants in soil and/or groundwater caused by natural processes that act without human intervention. These *in-situ* processes include biodegradation, dispersion, dilution, sorption, volatilization, radioactive decay, and chemical or biological stabilization, transformation, or destruction of contaminants.

Nonaqueous phase liquid (NAPL)—An organic liquid that is maintained as a separate phase from water.

Numerical model—A mathematical model that uses a numerical time-stepping procedure to estimate behavior of a system over time (as opposed to an analytical model). The mathematical solution is represented by a generated table and/or graph. Numerical models require greater computing power, but they can allow more realistic simulations of complex systems.

Oxidation—Transfer (loss) of electrons from a compound, such as an organic contaminant. The oxidation can supply energy that microorganisms use for growth and reproduction. Often (but not always), oxidation results in the addition of an oxygen atom and/or the loss of a hydrogen atom.

Oxygenase—An enzyme that introduces oxygen into an organic molecule.

Passive treatment—*In situ* bioremediation approach in which amendments are added to the subsurface on a one-time, or infrequent basis. Passive treatment relies on the use of slow-release electron donors, which can be injected into the subsurface or placed in trenches or wells.

Perchlorate—An anion consisting of one chlorine atom and four oxygen atoms, with the chlorine atom present at an oxidation state of +7. Perchlorate occurs naturally, and because it is a potent oxidizer, it has also been manufactured and used for solid rocket propellants and explosives.

Permeable reactive barrier—A permeable zone containing or creating a reactive treatment area oriented to intercept and remediate a contaminant plume.

Phytoaccumulation—Plant uptake and retention of a compound within plant tissues.

Phytodegradation—Degradation of a contaminant within a plant.

Phytoremediation—The use of plants and in some cases the associated rhizosphere (root zone) microorganisms for *in situ* remediation of contaminants.

Plume—A zone of dissolved contaminants. A plume usually originates from a contaminant source zone, and extends for some distance in the direction of groundwater flow.

Primary substrates—The electron donor and electron acceptor that are essential to ensure the growth of microorganisms. These compounds can be viewed as analogous to the food and oxygen that are required for human growth and reproduction.

Radius of influence—The radial distance from the center of an injection point or well to the point where there is no significant impact from the injected material.

Reduction—Transfer of electrons to a compound such as oxygen. It occurs when another compound is oxidized.

Reductive dechlorination—The removal of chlorine atoms from an organic compound and their replacement with hydrogen atoms (subset of reductive dehalogenation).

Reductive dehalogenation—The process by which a halogen atom (e.g., chlorine or bromine) is replaced on an organic compound with a hydrogen atom. The reactions result in the net addition of two electrons to the organic compound.

Rhizodegradation—Degradation of compounds by organisms living on or near plant roots.

Saturated zone—Part of the subsurface that is beneath the water table and in which the pores are filled with water.

Semi-passive treatment—*In situ* bioremediation approach in which amendments are added to the subsurface intermittently (i.e., at intervals of a few weeks to a few months). Generally, water-soluble compounds serve as the electron donor. The accumulation of biomass can also serve as a longer-term source of electron donors.

Sorption—Collection of a substance on the surface of a solid by physical or chemical attraction. Can refer to either absorption (in which one substance permeates another) or adsorption (surface retention of solid, liquid, or gas molecules, atoms, or ions).

Source zone—A subsurface zone that serves as a reservoir of contaminants that sustains a dissolved plume. The source includes the material that is or has been in contact with the separate phase (DNAPLs for chlorinated solvents), and the source zone mass includes the sorbed and aqueous phase contaminants as well as any residual NAPL.

Stakeholder—A person other than regulators, owners or technical personnel, who has a legitimate interest in a contaminated site.

Substrate—A compound that microorganisms can use in the chemical reactions catalyzed by their enzymes.

Sulfate reducer—A bacterium that converts sulfate to hydrogen sulfide. Because they can act without oxygen, sulfate-reducing bacteria can be important players in the oxygen limited subsurface.

Transcription—Transfer of information in DNA sequences to produce complementary messenger RNA sequences, which are then translated into functional polypeptides and proteins.

Translation—The decoding of messenger RNA to produce specific polypeptides. It occurs after transcription.

Vadose zone—Subsurface solids above the water table, where pores are partially or largely filled with air. Also called the unsaturated zone.

Volatilization—Transfer of a chemical from the liquid to the gas phase (as in evaporation).

Water table—The top of an unconfined aquifer. Indicates the level below which subsurface solids and rock are saturated with water.

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