Introduction to In Situ Bioremediation of Groundwater

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1.0 INTRODUCTION

Introduction to In Situ Bioremediation of Groundwater was prepared by the Office of Superfund Remediation and Technology Innovation (OSRTI) as an introduction to in situ bioremediation (ISB) of groundwater. This information is intended for U.S. Environmental Protection Agency (EPA) and state agency site managers and may serve as a reference to designers and practitioners. Others may find the EPA's Citizen's Guide to Bioremediation (EPA 2012a) to be a more fundamental and concise reference.

Bioremediation is an engineered technology that modifies environmental conditions (physical, chemical, biochemical, or microbiological) to encourage microorganisms to detoxify organic and inorganic contaminants in the environment. The process can be applied above ground in land farms, stirred tanks, bio-piles, or other units (referred to as ex situ) or below ground in the soil or groundwater, referred to as in situ (“in place”) treatment.

This course focuses specifically on in situ groundwater bioremediation. In the context of this course, groundwater remediation is defined as remediation of contaminants that exist below the water table. As a result of phase equilibrium in the subsurface, groundwater remediation must address contaminants dissolved in groundwater as well as those sorbed to the aquifer matrix to be effective. In some cases, even treatment of non-aqueous phase liquid (NAPL) may be needed. Consideration must also be given to the capillary fringe and the smear zone, which can serve as an ongoing source of contaminants to groundwater. This report does not discuss phytoremediation (use of plants to treat groundwater and soil) or monitored natural attenuation (a technology based on monitoring the progress of natural, non-engineered processes that often include biodegradation). Those readers interested in more information on ex situ bioremediation, bioremediation of soil, monitored natural attenuation or phytoremediation may find useful information on EPA's CLU-IN website (www.cluin.org).

The course provides technical information on evaluating and implementing in situ groundwater bioremediation at contaminated sites. Superfund program guidance for selecting and implementing groundwater remedies at Superfund sites can be found in numerous program guidance documents, such as:

- Office of Solid Waste and Emergency Response (OSWER) 9283.1-33, *Summary of Key Existing Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Policies for Groundwater Restoration*, June 2009; and
- OSWER 9283.1-34, *Groundwater Road Map: Recommended Process for Restoring Contaminated Groundwater at Superfund Sites*, July 2011.

For other groundwater guidance, refer to the Superfund groundwater website (www.epa.gov/superfund/health/conmedia/gwdocs).

This course provides (1) a brief technical background on ISB, (2) a summary of the use of ISB for various contaminants, including information on its use for Superfund sites, (3) considerations for implementation of ISB, (4) brief summaries of some important emerging trends affecting ISB, and (5) links to additional sources of information.

1.1 Superfund Project Information

The list of in situ groundwater bioremediation projects accompanying this report in the appendix was derived primarily from the lists in *Treatment Technologies for Site Cleanup: Annual Status Report, Twelfth Edition*, and its successor *Superfund Remedy Report, Thirteenth Edition*, and represents a subset of in situ groundwater bioremediation projects at National Priorities List (NPL) sites. Projects on the list include remedial actions for in situ groundwater bioremediation selected in Superfund Records of Decision (RODs), ROD amendments, and Explanations of Significant Differences (ESDs) for fiscal years 1989 through 2008. These documents are referred as “decision documents.” Although decision documents select a general technology such as ISB, the final selection of a specific design is typically deferred to the remedial design phase. Detailed information regarding the remedial design and contaminants treated was compiled for each project on the list based on documents available either on line (for instance, http://cumulis.epa.gov/supercpad/cursites/srchsites.cfm) or in site files. These sources included 5-year reviews, Superfund site summary fact sheets, remedial action reports, and other pertinent documents. Remedial project managers (RPMs) and contractors were contacted for additional clarification as needed. Status information for most projects was last updated in November 2011. More information on project implementation status, design, and performance may be available on the websites related to each site found at the link given above.

1.2 History and Background

Bioremediation is not a new concept. Biological treatment of domestic wastewater has been in use since the mid-1800s, and land treatment has been used for several decades to treat oil and other petroleum wastes by aerobic biodegradation (Loehr 1979). The basic principles and experience from these technologies were adapted to ISB of petroleum (and other contaminants) in the 1980s (Thomas and Ward 1989). ISB has been further developed to treat a wide variety of other contaminants, particularly since the early 1990s, when the potential for enhanced anaerobic treatment became clear (NRC 1993; Alexander 1994).

The first use of ISB was in 1972, when aerobic treatment was used to clean up a Sun Oil pipeline spill in Ambler, Pennsylvania. Treatment consisted of withdrawing groundwater, adding oxygen and nutrients, and recirculating it through the subsurface (Raymond 1977). Aerobic biological treatment or oxidation of petroleum releases gained acceptance throughout the 1970s and 1980s and has been used in several large-scale applications, including the effort to clean up numerous Superfund sites (see for example EPA 1989; Ross 1988).

Anaerobic bioremediation gained popularity when it was recognized as an effective method to remediate chlorinated solvents in groundwater. In 1997 scientists isolated a bacterium originally referred to as *Dehalococcoides ethenogenes* strain 195, the first organism known to completely dechlorinate the common groundwater contaminant perchloroethene (PCE, also known as tetrachloroethene) (Maymo-Gatell 1997). Further studies showed that several related bacteria, all now referred to as strains of *Dehalococcoides mccartyi* (Löffler and others 2012), had the ability to partially or completely dechlorinate PCE and the related chloroethenes. To date, these are the only known organisms with the ability to completely degrade these compounds, which are particularly prevalent groundwater contaminants at Superfund sites. As a result, several demonstration-scale applications of anaerobic bioremediation was completed in the late 1990s and early 2000s. Several of the demonstration projects went full scale, and today reductive dechlorination, as it is now known, is a widely accepted method for treating halogenated ethenes, ethanes, and methanes (Stroo 2010).

Figure 1 shows the surge in popularity of anaerobic bioremediation for use at NPL sites after the method was successfully demonstrated in the early 2000s, while the use of aerobic bioremediation has remained relatively steady. As indicated in Figures 1 and 2, the selection of anaerobic bioremediation to remediate groundwater at Superfund sites increased dramatically over recent years, and this method is now used at the majority of Superfund sites where ISB technologies have been selected.

As shown in Figure 3, the most common groundwater contaminants addressed by ISB at NPL sites were halogenated volatile organic compounds (VOCs); followed by nonhalogenated VOCs; nonhalogenated semi-volatile organic compounds (SVOCs); and BTEX compounds (benzene, toluene, ethyl benzene and xylenes).

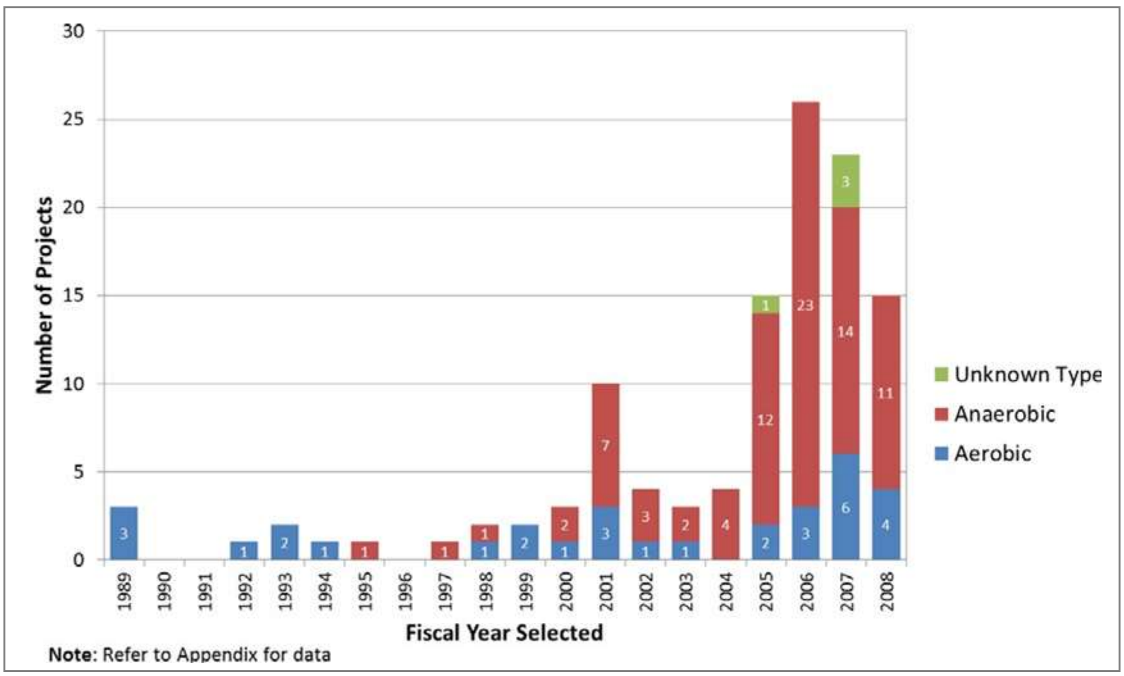

Figure 1. Use of In Situ Groundwater Bioremediation Technologies at Superfund Sites.

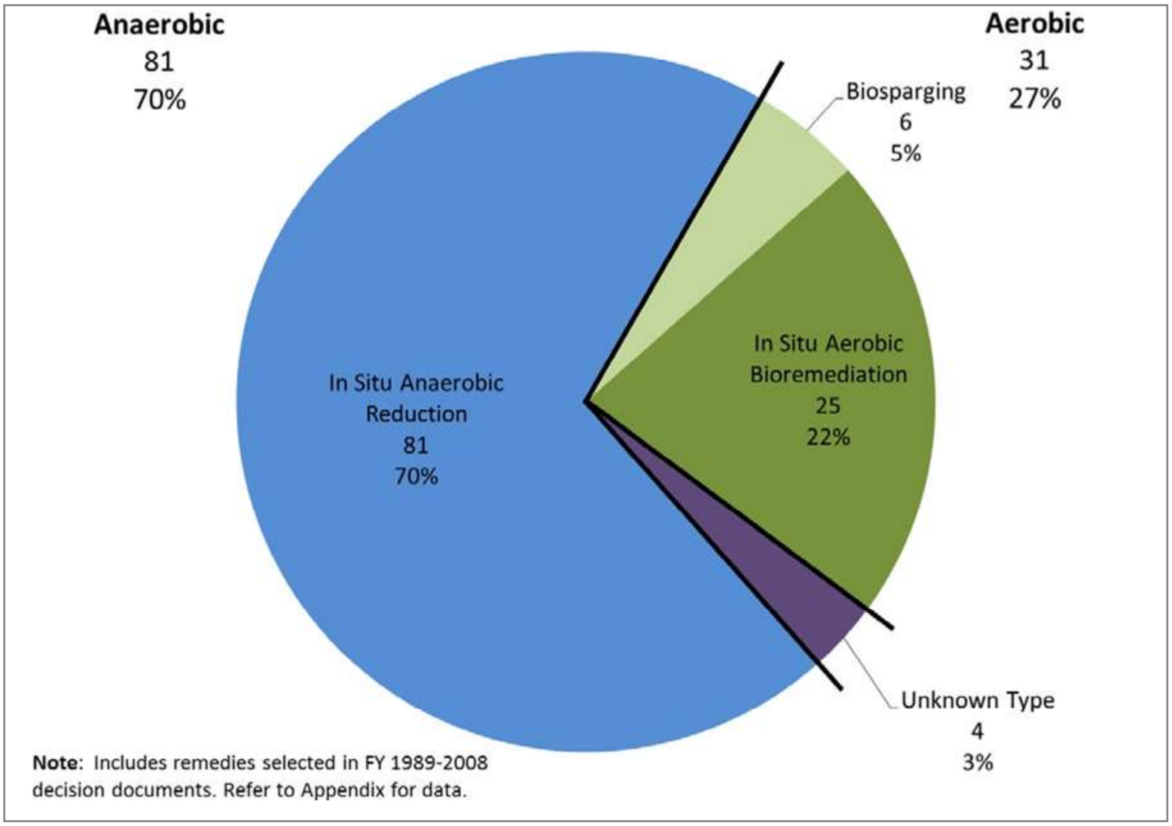

Figure 2. Aerobic and Anaerobic Bioremediation Projects at NPL Sites.

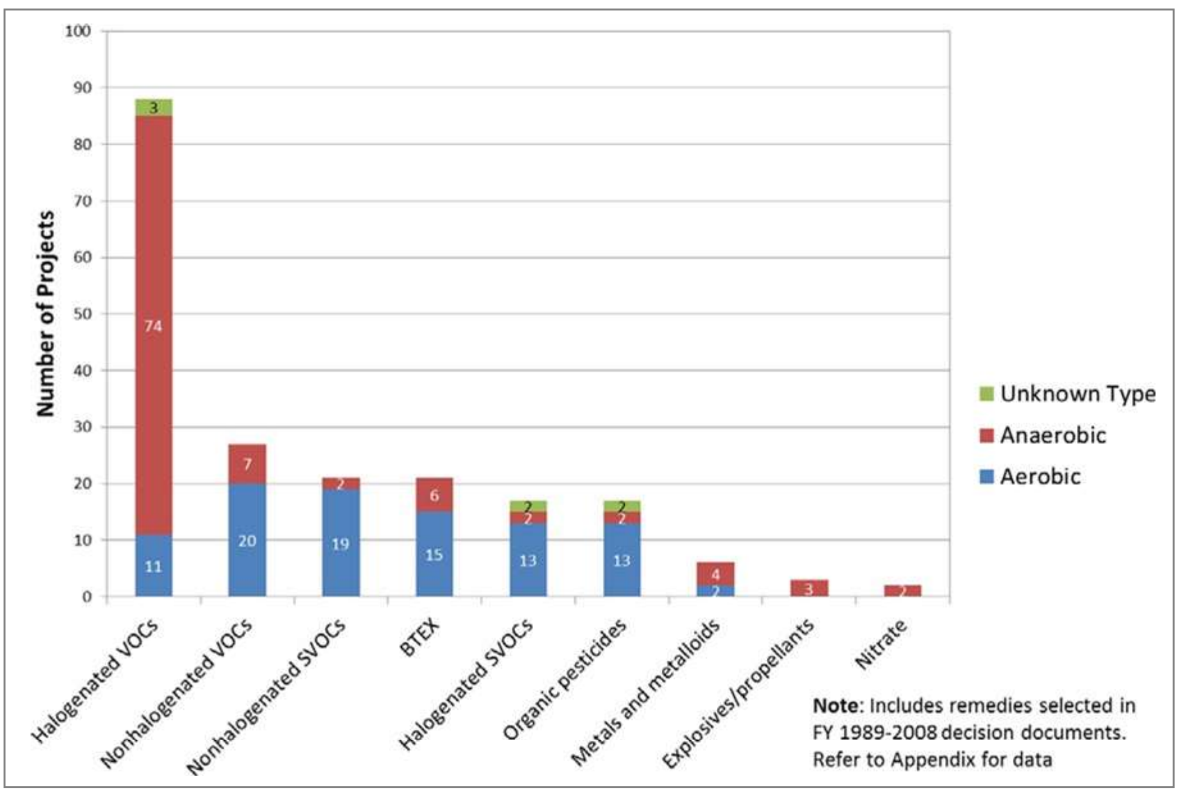

Figure 3. Contaminant Groups Addressed by Bioremediation Technologies at Superfund Sites.

1.3 Microbiology

One component of designing an effective ISB system is to understand the fundamental ecology and physiology of microbes. Microbes have been found everywhere on earth, including environments of extreme heat, cold, and pH, without oxygen, and in the presence of radiation. They are adaptive, resilient, and can thrive in environments impaired by most contaminants. Bioremediation most commonly uses bacteria for treatment, but also includes remediation performed by archaea, protists, and fungi. Microbes used for bioremediation are often referred to collectively as “bacteria,” or “bugs” in the bioremediation field.

All microbes have basic requirements for life and growth. The six elements considered essential for life are carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur. The needs of bacteria can be further simplified to three requirements: a carbon source that can be used to build its biomass, an electron donor (such as hydrogen) for the energy it needs to live and reproduce, and a terminal electron acceptor (for example, oxygen) to receive the electrons the bacteria use for energy. Often, the carbon source will serve as the electron donor. Nitrogen, phosphorus, and sulfur will sometimes fulfill the role of electron donor or acceptor, but are more often considered nutrients and are required in smaller proportions than are carbon, hydrogen, and oxygen.

The specific growth rate of bacteria depends on the concentration of a carbon source (substrate) or the nutrient that is most limiting. This growth kinetics are modeled by the Monod equation (Okpokwasili and Nweke 2005), which shows how, at low substrate concentrations, the specific growth rate increases directly with an increase in substrate concentration, while it levels out to approach a constant maximum growth rate when substrate is plentiful. The specific growth rate of bacteria is particularly relevant in bioremediation because bacterial growth rates (and proportionally, breakdown of the contaminant) will likely slow as cleanup progresses, if the contaminant is the substrate.

1.4 Reduction and Oxidation Chemistry and Microbial Metabolism

Bacteria generate the energy they need to live by catalyzing (increasing, initiating, or transforming) chemical reactions that transfer electrons from one molecule, known as the electron donor or reductant, to another molecule, called the electron acceptor or oxidant. When the right electron donor and acceptor are present, bacteria will consume them to grow and divide. The amount of energy generated and available for bacterial growth by each reduction and oxidation or redox pair varies, and each species of bacteria has enzymes to take advantage of only certain redox pairs. The contaminants of concern may act as reductants or oxidants for in situ groundwater remediation.

The various terminal electron acceptors that exist naturally in groundwater are preferentially used and exhausted in a specific order, according to their decreasing redox potential. In the environment, organic matter in the aquifer matrix and groundwater plays the role of electron donor. The vast majority of microbial metabolisms relevant to bioremediation use organic matter as an electron donor, and the bacteria able to generate the most energy from it tend to dominate the microbial population. The amount of energy released during electron transfer is controlled by the redox potential of the terminal electron acceptor. There are a few important groups of bacteria that use inorganic reduced compounds as a substrate. These microbes oxidize many of the same species reduced by anaerobic respiration and

