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BIOREMEDIATION OF NITRATES AND CARBON TETRACHLORIDE IN GROUNDWATER

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A development and demonstration program is presently underway to test an in situ bioremediation technology for treating nitrates and carbon tetrachloride in contaminated Hanford groundwaters. Several key technical issues have prevented widespread use of bioremediation for cleanup of organic and inorganic subsurface contaminants, including the development of adequate nutrient delivery systems; effective mixing technologies for contacting microorganisms, nutrients, and contaminants; methods to control biofouling or excessive microbial growth; and adequate tools for designing, predicting, and monitoring the performance of in situ technologies in heterogeneous subsurface environments. The goal of this program is to address these technical issues in an integrated laboratory-, bench-, and field-scale demonstration by stimulating native microorganisms and accelerating the natural degradation of NO_3^-, CCl_4 and chloroform. A demonstration site at Hanford for in situ bioremediation testing was selected in 1990, and extensive hydrological, chemical, and biological characterization of the site has been conducted. Microorganisms from the site are being tested in batch reactors and soil columns to determine the most appropriate reaction rate expressions and confirm nutrient injection strategies. Three-dimensional simulation and visualization tools are being used to facilitate design of this complex field test by incorporating flow, transport, and biological reaction of the nutrients, contaminants, and microorganisms (Chiang, Dawson, and Wheeler 1991). Current activities are focused on detailed design of the field well network, predicting the performance of the remediation system, designing the nutrient delivery system and protocols, and procuring the monitoring and nutrient delivery equipment in preparation for field testing in 1994.

BACKGROUND

Soil and groundwater contamination from volatile organic compounds (VOCs) is a serious problem at federal, state, and commercial facilities. In a recent survey of 91 waste sites at 18 of the U.S. DOE's facilities across the United States (U.S. DOE, 1992), the most common soil and groundwater contaminants were identified as fuel hydrocarbons and chlorinated solvents, respectively. More than sixty percent of the DOE facilities had soil or groundwater contamination from the presence of highly volatile chlorinated solvents. In addition, at least fifty percent of the facilities had anions, radionuclides, or metal contaminants co-existing with the chlorinated solvents. Remediation of VOCs, especially chlorinated solvents, is complicated by the tendency of these contaminants to be transported rapidly through the subsurface in the vapor phase, to sorb strongly to fine silts and sediments, to

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be relatively insoluble in water, and to be resistant to natural biotic or abiotic degradation. Cleanup of VOCs in soils has focused on the use of soil vapor extraction (SVE) to volatilize and extract the contaminants from soils, followed by above-ground off-gas treatment to minimize release of the contaminants to the environment. Cleanup of VOCs in groundwater has traditionally been done using groundwater extraction technology followed by air stripping and off-gas treatment (pump-and-treat). More recently, techniques for air sparging and/or in-well vaporization have been deployed to reduce the cost associated with groundwater pumping, and will work in conjunction with SVE to remove bulk contaminants from the subsurface (Schroeder et al., 1992). SVE and complementary techniques for volatilization of groundwater VOCs are generally quite effective in permeable soils; however, highly adsorptive soils and less permeable silts, sediments, and clays are sinks for most VOCs and cannot be effectively vented or flushed with air or water to remove contaminants. In situ bioremediation provides a potential benefit with these types of soils, especially in groundwater, by destroying the VOCs in place and reducing the mass transport limitations associated with VOC adsorption and desorption to sediments, and dissolution into the groundwater. The time and cost of cleanup could be substantially reduced if bioremediation could be effectively employed alone, or in conjunction with other bulk-contaminant removal technologies.

The majority of the DOE sites with VOC contamination have mixtures of chlorinated solvents (e.g, trichloroethylene, tetrachloroethylene, chloroform, carbon tetrachloride) and mixtures of other metal or inorganic ions with chlorinated solvents. Bioremediation of these contaminants is accomplished through both aerobic and anaerobic processes. For many chlorinated solvents, aerobic degradation is possible by methanotrophic or toluene-degrading microorganisms. A primary substrate is supplied to the subsurface to provide an energy source for the microorganisms while the VOC is degraded cometabolically. A demonstration of aerobic, methanotrophic bioremediation of trichloroethylene is currently underway at the DOE's Savannah River Site (Hazen, 1992). However, other chlorinated solvents such as perchloroethylene and carbon tetrachloride are degraded only under anaerobic conditions by denitrifying, iron-reducing, sulfate-reducing, or methanogenic microorganisms. Therefore, at a majority of the sites containing mixtures of chlorinated solvents, or mixtures of anions and chlorinated solvents, a combination of aerobic and anaerobic bioremediation may need to be employed to adequately remediate the site. Aerobic bioremediation has been the primary focus of recent research, development, and field testing. Techniques for supplying oxygen to the subsurface using a variety of techniques such as air venting, air injection, and hydrogen peroxide have been extensively tested. Unfortunately, anaerobic processes benefit very little from these oxygen addition techniques. The technical issues related to nutrient delivery systems and effective mixing technologies become even more significant when working with an anaerobic system.

VOC contamination at Hanford is typical of the VOC problem across the DOE complex. The Hanford Site, located in southeastern Washington State, is an area of approximately 600 square miles that was selected in 1943 for producing nuclear materials, primarily plutonium, in support of the United States' effort in World War II. Hanford's operations over the last 40+ years have been dedicated to nuclear materials production, generation of electricity, diverse types of research, and waste management. Some of these operations have produced aqueous and organic wastes that were subsequently discharged to the soil column. In the 200 West Area of the Hanford Site, plutonium recovery processes at Z-Plant discharged CCl_4-bearing solutions to three liquid waste disposal facilities: a trench, tile field, and crib. A minimum of 637 t of CCl_4 was disposed to the subsurface, primarily between 1955 and 1973, along with co-contaminants and/or degradation products such as tributyl phosphate; lard oil; cadmium; nitrates; hydroxides; fluorides; sulfates; chloroform; and various radionuclides, including plutonium (Last and Rohay 1991). The subsurface in the vicinity of Z-Plant consists of approximately 58 to 65 m of unsaturated sediments and approximately 70 m of saturated soil overlying low permeability silts and clays and basalt bedrock. Carbon tetrachloride vapors have been encountered in the vadose zone during well-drilling operations, and groundwater contamination from CCl_4 is extensive in the 200 West Area, covering more than 5 km^2. The highest concentrations of CCl_4 in the groundwater plume were measured approximately 450-m downgradient of the three disposal facilities at levels exceeding 1000 times the U.S. Environmental Protection Agency's (EPA) drinking water standard of 5 ppb. The DOE and its operating contractor at Hanford, Westinghouse Hanford Company, are currently pursuing an Expedited Response Action (ERA) to remove volatile CCl_4 from the vadose zone using soil vapor extraction (vacuum extraction) technology (Hagood and Rohay 1991). In collaboration with the ERA, DOE's Office of Technology Development has selected the 200 West Area CCl_4 site as the host site for the Integrated Demonstration for Cleanup of Volatile Organic Compounds at Arid Sites (VOC-Arid Integrated Demonstration). The objective of the integrated demonstration is to develop, demonstrate, evaluate, and transfer for deployment new technologies for all phases of cleanup of VOCs and associated contaminants in the subsurface. The goal is to bring new technology forward to provide more effective, cheaper, and safer methods for cleanup. Bioremediation is one technology being developed as part of the VOC-Arid Integrated Demonstration to meet the need for cost effective technologies to clean groundwater contaminated with CCl_4 and other organic and inorganic contaminants.

STATUS OF DEVELOPMENT

The current understanding of the microbial degradation of CCl_4 is limited, particularly in comparison to other chlorinated aliphatic compounds such as trichloroethylene (TCE) that are common groundwater contaminants. However, progress has been made in recent years in identifying microorganisms and understanding mechanisms of CCl_4 biodegradation. Bouwer and McCarty (1983a) made some of the initial observations on the microbial transformation of CCl_4 in groundwater in batch microcosms under denitrifying conditions. Degradation of CCl_4 to CO_2 and chloroform in the microcosms was observed after 3 weeks of incubation. Bouwer and McCarty (1983b) also observed that CCl_4 was completely degraded to CO_2 under methanogenic conditions. In another microcosm study, Wilson et al. (1987) observed the biodegradation of CCl_4 in unamended aquifer sediments. Carbon tetrachloride biodegradation has been demonstrated with a number of different bacteria. The conditions that favor biodegradation of CCl_4 are predominantly anaerobic. Carbon tetrachloride biodegradation to CO_2 and other metabolites has been demonstrated in pure cultures and consortium of denitrifying *Pseudomonas* sp. (Criddle et al. 1990a; Hansen 1990), the acetogen *Acetobacterium woodii* (Egli et al. 1988), *Clostridium* sp. (Gälli and McCarty, 1990), and under anaerobic and microaerophilic conditions by *E. coli* (Criddle et al. 1990b). Biodegradation of CCl_4 under denitrifying conditions is of particular interest because of the occurrence of both nitrates and CCl_4 in the unconfined aquifer on the Hanford site. Both Hansen (1990) and Criddle et al. (1990a) identified *Pseudomonas* species capable of degrading CCl_4, with acetate as the electron donor and nitrate as the terminal electron acceptor.

Efforts to evaluate the potential for bioremediation of Hanford's CCl_4-contaminated groundwater were initiated in 1987. Evidence of CCl_4 degradation by microorganisms indigenous to the Hanford Site was first obtained with a denitrifying consortium from groundwater samples (Koegler et al. 1989). Additional studies conducted at laboratory-, bench-, and pilot-scales confirmed that NO_3^- and CCl_4 were degraded by the bacterial consortium (Brouns et al. 1990). The CCl_4-degrading *P. stutzeri* strain identified by Hansen (1990) was isolated from the Hanford groundwater consortium in 1989. Based on the results of early laboratory studies, and limited pilot testing, a program was established to evaluate the feasibility of in situ bioremediation through a field demonstration. The approach to the field demonstration was to rigorously evaluate the feasibility of the technology with parallel laboratory-, intermediate-, and field-scale activities. The primary activities included 1) test site hydrological, geochemical, and microbiological characterization, 2) remediation design through laboratory, flow-cell, and 3-D modelling studies, 3) installation and verification of the well network and process and monitoring equipment, 4) demonstration testing and data analysis, and 5) post-demonstration site characterization. A location within the 200 West Area at Hanford was selected in FY 1990 for development of the bioremediation test site. Characterization and preparation of the test site was initiated in FY 1991 as part of DOE's VOC-Arid Integrated Demonstration (Brouns et al. 1991). Two of the five primary test wells have been installed, and the third test well is being designed for installation this fiscal year. As part of the effort to address inadequate mixing, innovative mixing well designs are being considered for the remaining three wells. Collaborative efforts have been established with EPA's Western Region Hazardous Substance Research Center to design and test innovative single well recirculation systems at the Hanford test site (Semprini et al., 1992).

Engineering research activities are developing methods for supplying nutrients to the subsurface and modeling the bioremediation process using three-dimensional visualization tools to help design the field-scale demonstration site. A series of rigorous kinetic studies have been conducted to develop an accurate foundation for designing the in situ remediation system (Skeen et al. 1992). Bench-scale batch reactors were used to study the kinetics of contaminant degradation and the growth of the microorganisms. The effects of important environmental conditions such as pH, temperature, redox potential, and the concentrations of substrate, electron acceptor, and contaminants were included in this study. The results of these studies have been incorporated into an existing three-dimensional model for subsurface flow, transport, and reaction (Chiang et al. 1991) to provide a complete description of the in situ remediation process. This simulation tool is currently being used to design the field test site and operational procedures. Concurrently, field studies are continuing to characterize the test site for spatial differences in hydrologic, chemical, and microbiological parameters. One-dimensional bench-scale flow cell experiments have been initiated to provide remediation and transport data under well-defined conditions. These experiments will be used to calibrate the bioremediation model and help minimize the error associated with applying a simulation based on laboratory-scale data to field remediation. The column studies are particularly important for evaluating microbial growth and fouling, and determining the effectiveness of methods to minimize fouling.

Through laboratory study, intermediate-scale flow-cell experiments, and three-dimensional computer simulations, a field test site is under development, and will be completed in early FY 1994. Field testing will focus on demonstrating 1) destruction of CCl_4 and nitrate, 2) effective nutrient addition strategies that minimize biofouling, 3) novel well designs and mixing strategies to enhance subsurface mixing, and 4) a design methodology for deploying bioremediation that includes laboratory and column studies, and 1-, 2-, and 3-D design simulations. The in situ bioremediation field testing in FY 1994 and FY 1995 will be integrated with other characterization, remediation, and monitoring demonstrations that comprise the VOC-Arid Integrated Demonstration, and with ongoing environmental restoration efforts within Hanford's 200 West Area.

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