CONF-910981--25

PNL-SA--19646

DE92 000799

BIOREMEDIATION OF HANFORD GROUNDWATER

T. M. Brouns
J. K. Fredrickson
R. S. Skeen
D. B. Anderson
S. P. Lettrell
D. J. Workman

September 1991

Presented at the
Conference on Environmental
Remediation 1991
September 8-11, 1991
Pasco, Washington

Work supported by
the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
Richland, Washington 99352

BIOREMEDIATION OF HANFORD GROUNDWATER

T. M. Brouns
D. B. Anderson
J. K. Fredrickson
S. P. Luttrell
R. S. Skeen
D. J. Workman

Pacific Northwest Laboratory[a]
P. O. Box 999, Richland, Washington 99352

ABSTRACT

Liquid wastes containing radioactive, hazardous, and regulated chemicals have been generated throughout the 40 years of operations at the U.S. Department of Energy's (DOE) Hanford Site. Some of these wastes were discharged to the soil column and many of the waste components, including nitrate (NO_3^-), carbon tetrachloride (CCl_4), and several radionuclides, have been detected in the Hanford groundwater. Current DOE policy prohibits the disposal of contaminated liquids directly to the environment, and remediation of existing contaminated groundwaters may be required. A research and development program is underway to develop bioremediation technologies for both ex situ and in situ groundwater treatment.

Although ex situ pump-and-treat remediation schemes have been criticized recently for their limited effectiveness in cleaning up contaminated aquifers to stringent regulatory standards, the use of extraction wells and above-ground treatment can be effective in removing bulk quantities of contaminants from the subsurface. Extraction processes can also be used on an interim basis to control the migration of contaminants. An ex situ bioremediation technology is being developed to provide cost effective methods for treating extracted groundwater. An in situ treatment process is also being developed. The goal of this process is to stimulate the native microorganisms and accelerate the natural degradation of NO_3^- and CCl_4. In situ treatment offers the additional benefits of lower operating costs, better removal of sorbed contaminants, and lower potential for exposure of workers to hazardous chemicals. A demonstration site at Hanford for in situ biological treatment was selected in 1990, and extensive hydrological, chemical, and biological characterization of the site is underway. Current research and development activities are focusing on developing methods for supplying nutrients to the subsurface, evaluating the effect of in situ bioremediation on the long-term mobility of metal and radionuclide co-contaminants, and modeling the bioremediation process using three-dimensional visualization tools to help design the field-scale demonstration site and predict performance. The in situ bioremediation process will be developed and tested at the laboratory- and intermediate-scale prior to field demonstration as part of the DOE's Integrated Demonstration for Cleanup of Volatile Organic Compounds at Arid Sites (VOC-Arid Integrated Demonstration).

(a) Pacific Northwest Laboratory is operated by Battelle Memorial Institute for the U.S. Department of Energy under Contract DE-AC06-76RLO 1830.

INTRODUCTION

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, electrical generation, 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 (Figure 1), 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. However, groundwater monitoring activities have been able to account for only less than 1% of the CCl_4 discharged to the subsurface (Last and Rohay 1991).

Place Figure 1 here

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 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.

BACKGROUND

The current understanding of the microbial degradation of carbon tetrachloride (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. In this study there were considerable differences in the capacity of samples from different depths to degrade CCl_4, even samples that were identical in appearance. This observation demonstrates that, in addition to physical and chemical heterogeneities in the subsurface, microbial populations and their associated activities can also be heterogeneous. Therefore, precautions need to be taken when interpreting microbial characterization and biotransformation feasibility results from one or a few samples, as a small number of samples may not be representative of the complete zone because of natural heterogeneities in the subsurface. Multiple samples need to be taken to fully assess the capacity of the in situ microflora for CCl_4 degradation.

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. However, strain KC isolated by Criddle et al. (1990a) degraded CCl_4 more rapidly and completely than did the *P. stutzeri* strains studied by Hansen (1990), but was sensitive to the presence of metals, particularly Fe(II) and Co(II). This sensitivity could have implications on the application of this organisms for bioremediation of CCl_4-contaminated groundwater if there are metal co-contaminants or if the pH of the groundwater is acidic.

In addition to direct bacterial degradation, abiotic reductive dechlorination of CCl_4 has been shown to be catalyzed by the corrinoid cobalamin (vitamin B12) (Krone et al. 1989) and other metal-containing co-enzymes, including F430 and hematin (Gantzer and Wackett 1991). Interestingly, proteins such as ferredoxin and azurin that carry out electron transfer reactions were unable to reductively dechlorinate polychlorinated ethylenes, including CCl_4, or benzenes. The reductive dechlorination of CCl_4 by these compounds is rapid, although the rate decreases with a decreasing number of Cl substituents. The rapid reductive dechlorination of CCl_4 by co-enzymes suggests that this is the mechanism of biodegradation of CCl_4 by bacteria, although this mechanism has yet to be demonstrated. If co-enzymes are involved in the bacterial degradation of CCl_4, then this mechanism provides a strategy for isolating or developing strains that have an enhanced capacity for CCl_4 degradation and for designing ex situ and in situ approaches for bioremediation of CCl_4 in groundwater.

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.

To apply the degradative capability of these microorganisms, an ex situ process that uses the native microorganisms to destroy both NO_3^- and CCl_4 was developed based on laboratory- and bench-scale studies. A pilot-scale treatment process was demonstrated with a simulated groundwater feed in 1989 (Brouns et al. 1990). In these demonstration tests, greater than 99% of the NO_3^- and 93% of the CCl_4 was destroyed at influent concentrations of 400 ppm and 200 ppb, respectively. Analysis of all product streams indicated that the concentrations of NO_3^- and CCl_4 were below the drinking water standards of 44 ppm and 5 ppb, respectively. This process is continuing to be developed and tested to support remediation of contaminated groundwater. However, the primary focus of the bioremediation program is to evaluate the potential for in situ remediation. A location within the 200 West Area was selected in FY 1990 for development of a bioremediation test site. Characterization and preparation of the test site was initiated in FY 1991 as part of DOE's VOC-Arid Integrated Demonstration.

SITE CHARACTERIZATION AND SEDIMENT ANALYSIS

The primary objectives of the drilling and site characterization activities at the test site are to 1) confirm that the desired contaminant types and concentrations are present for bioremediation testing; 2) determine the microbial populations present in groundwater and the feasibility of CCl_4 degradation by the indigenous population; 3) determine the aquifer's hydraulic, transport, and chemical characteristics for remediation system design; and 4) provide a well network to be used for testing and demonstrating groundwater bioremediation.

Sediment Sampling

During FY 1991, a borehole for site characterization was drilled using cable tool-percussion drilling to a total depth of approximately 85 m (279 ft). The depth to groundwater at the site is approximately 74 m (244 ft). Samples were collected and analyzed for physical, chemical, and microbiological characterization according to requirements provided in a written work plan. Deionized, sterilized water was used for drilling from approximately 6 m (20 ft) above the water table to the bottom of the borehole to prevent contamination from nonindigenous microorganisms. Tools used for sampling were cleaned at the site with a high-pressure hot-water cleaner prior to sample collection.

Sediment samples were collected; described for textural, general mineralogy, and color characteristics; and archived for the entire drill depth. Three sediment samples were collected below the water table. Subsamples were taken from these split-barrel sediment samples, collected in amber glass vials containing methanol, and kept cool until analysis for volatile organics. The depth of the samples and the respective results are given in Table I.

Water samples were collected at four depths in the saturated zone and were analyzed for volatile organics, metals, anions, and gross beta concentrations. One sample was analyzed for alkalinity, total organic carbon, and gross alpha in addition to the analytes previously identified. The sampling interval was opened by driving the casing to the bottom or near the bottom of the borehole then drilling an open hole below the bottom of the casing. The borehole was open from as little as 0.15 m (0.5 ft) to as much as 0.76 m (2.5 ft) when the sample was collected. The samples were collected from the well by bailer and transferred into containers. Samples were kept cool until they were analyzed. The open intervals from which samples were collected, and the respective results from their analyses, are given in Table II for volatile organics and anions.

Samples were collected from eight depth intervals, one above and seven within the saturated zone, for studies to determine indigenous microbial populations and contamination degradation by indigenous microorganisms. For the sampling intervals during which sediments were collected for microbiological analyses, cores were collected using a split spoon sampling tool containing a Lexan liner (Figure 2). Numerous precautions were taken to ensure the microbiological integrity of the core samples. The precautions included steam cleaning and ethanol flame disinfection of the split spoon, shoe, and core catcher prior to each sampling interval. In addition, the Lexan liners were autoclaved and aseptically placed into the disinfected split-spoon tool immediately before the trip down the borehole. Following coring and transport to the surface, the shoe was removed and the split spoon disassembled. The Lexan-encased core was immediately capped and placed into a polyethylene bag that was then flushed repeatedly with Ar, sealed, and transported to the laboratory. In contrast to in situ bioremediation of petroleum hydrocarbons in groundwater, the approach described herein requires the development and maintenance of anaerobic conditions. Therefore, sampling procedures for site characterization were designed to minimize the introduction of air into subsurface samples. In the laboratory, the cores in the polyethylene bags were placed inside an anaerobic (N_2) glovebox. Upon removal from the polyethylene bag, the Lexan core liners were wiped clean and cut longitudinally with a cordless power saw. Depending on the nature of the core material, the core was either split and the interior portions sampled for use in microbiological analyses, or one-half of the Lexan liner was removed and the outer core material pared to avoid sampling sediment that may have come in direct contact with surfaces of the drilling tools. Table III lists the intervals from which samples were collected.

Figure 2 here

Samples were collected from two depth intervals in the saturated zone for hydraulic characterization analyses. The analyses, for which the results are not yet available, include water retention characteristics, bulk and particle density, and hydraulic conductivity. These samples were collected in Lexan liners in a manner similar to those collected for microorganism studies. The intervals from which samples were collected are 78.8 - 79.3 m (258.4 - 260.3 ft) and 81.7 - 82.0 m (268 - 269.1 ft).

Microbiological Analyses

Microbiological analyses included sediment microcosm studies to

determine the potential for denitrification and biodegradation of CCl_4. Nitrate disappearance from the solution phase of anaerobic microcosms upon addition of acetate was determined by colorimetric analysis for nitrate and nitrite. Carbon tetrachloride biodegradation is degraded primarily under anaerobic conditions, although recently its degradation by *Escherichia coli* was reported under aerobic conditions, albeit at low (1%) O_2 tensions (Criddle et al. 1990b). Ideally, the in situ degradation of CCl_4 under denitrifying conditions is preferable as, in theory, the main requirement to drive both denitrification and CCl_4 degradation would be the addition of an electron donor since NO_3^- would serve as the electron acceptor. Experiments with core sediment microcosms were initiated using several different electron donors and alternative electron acceptors to identify the conditions that will promote the degradation of CCl_4 by the indigenous subsurface microflora in the Hanford unconfined aquifer. The electron donors being evaluated include acetate, glucose/fructose mix, and glycerol. In addition to nitrate, fumarate is also being evaluated as a potential electron acceptor for the degradation of CCl_4. In addition to biological degradation, an experiment was performed to determine if CCl_4 could be reductively dechlorinated in sediments by the addition of exogenous corrinoids. To this end cobalamin (vitamin B12) was added to sediment samples with dithiothreitol as a reductant. All microcosm experiments were conducted in 150-ml serum bottles containing 20-30 g of sediment, groundwater or mineral salts solution (Shelton and Tiedje 1984), and the various electron donors and acceptors. The serum bottles were sealed with Mini-Nert valves to permit repeated sampling and were incubated at 15°C. All treatments were replicated three times and included one autoclaved (sterile) control. Carbon tetrachloride solution concentrations were determined by gas chromatography (Hewlett-Packard).

Specific microbial populations were also quantified to gain information on the size of bacterial populations that could potentially be manipulated for bioremediation. Aerobic and anaerobic heterotrophic bacteria were measured using direct agar plate contact methods. Populations of denitrifying bacteria were estimated using a most probable number method with nitrate-tryptic soy broth and in Hanford groundwater amended with acetate and nitrate.

Bacteria capable of reducing nitrate were present in all sediment samples as determined by microbial growth in TSA + nitrate enrichments coupled with complete loss of nitrate after 20 days. In all core samples there was greater than 10^4 MPN denitrifiers/g sediment. In sediment samples amended with groundwater, nitrate, and acetate, nitrate and nitrite were present after 20 days, indicating only a partial removal of nitrate. It is likely that microbial growth in the groundwater treatments was much less than in the tryptic soy treatments, accounting for the slower rate of nitrate removal. These results indicate that in situ biodenitrification is feasible in the unconfined aquifer on the Hanford site, as the bacteria capable of reducing nitrate are present and can be stimulated by the addition of an electron donor such as acetate.

Experiments to determine CCl_4 biodegradation are currently incomplete, but preliminary results indicate there was considerable growth in sediment enrichments with mineral medium (a defined salts solution) containing nitrate + acetate or glycerol + fumarate, and there was some growth in enrichments receiving the sugar mix (glucose + fructose). These results indicate that bacteria are present in the Hanford unconfined aquifer that are able to

metabolize and grow on electron acceptors and donors that have been demonstrated to promote the degradation of CCl_4. After 20 days of incubation there was no significant biodegradation of CCl_4 in any of the microbiological enrichments. However, the presence of $CHCl_3$ has been detected in several of the sediment samples amended with glucose + fructose mix, fumarate/glycerol, and acetate/nitrate, but not in the sterile controls. This indicates that there has been some transformation of CCl_4 by indigenous microbial populations and, with additional time, the degradation of CCl_4 may increase as the microbial populations grow and adapt. Previous research indicates a strong kinetic relationship between electron donor (acetate) concentration and CCl_4 metabolism (Brouns et al. 1990, Hansen 1990). Therefore, additional laboratory studies will be conducted to determine whether depletion of the electron donor may be affecting the biodegradation of CCl_4.

To date, the most dramatic removal of CCl_4 from core aquifer sediment samples has occurred with B12 and dithiothreitol amendments. In all core samples, including the autoclaved control, CCl_4 could not be detected after 4 days, although trace amounts of $CHCl_3$ were detected. These later results indicate that CCl_4 can be reductively dechlorinated in the presence of aquifer sediments. Although it would not be practical to add corrinoids to the subsurface for purposes of remediation, some bacteria are capable of biosynthesizing B12 and may be candidates for use for in situ and ex situ reductive dechlorination of CCl_4.

ON-GOING RESEARCH

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. Several experiments are planned to develop an accurate foundation for designing the in situ remediation system. First, bench-scale batch reactors will be used to rigorously 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 will be included in this study. Concurrently, field studies will be conducted to characterize the test site for spatial differences in hydrologic, chemical, and microbiological parameters. The results of these bench- and field-scale studies will be incorporated into an existing three-dimensional model for subsurface transport (Chiang, Dawson, and Wheeler 1991) to provide a complete description of the in situ remediation process. Next, one-dimensional bench-scale as well as three-dimensional intermediate-scale flow cell experiments will be conducted 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.

A conceptual design of the in situ bioremediation system that may be used in the field test has been developed. Both surface treatment of extracted water and subsurface recycle will be evaluated. Methods for bioremediation of extracted groundwater have previously been developed at PNL (Brouns et al. 1990), and other chemical and physical technologies for treating contaminated groundwater will be available for testing through the VOC-Arid Integrated Demonstration. Subsurface recycle techniques will be

evaluated to determine the advantages and disadvantages of enhanced hydraulic gradients within the test zone. In the conceptual design, nutrient solutions introduced at the injection well will inevitably displace some percentage of the contaminants. Subsurface recycle may enhance contact between the injected solution and contaminants by establishing a mixing region between the injection and extraction wells. Reduced well clogging from excessive bacteria growth near the injection well is another potential advantage of subsurface recycle. This can be realized by injecting nutrients at concentrations that will inhibit growth, and relying on mixing of the injected solution with the subsurface fluid to dilute the nutrients to a level that will enhance degradation. Thus, near the injection well cell growth will be inhibited, but in the bulk of the soil conditions suitable for degradation will prevail. In addition to evaluating the technical feasibility of microbial stimulation and biodegradation techniques, efforts will focus on evaluating the regulatory feasibility of the remediation strategy. Techniques that minimize the need for nutrient, microorganism, or recycled water injection will certainly have a higher likelihood of regulatory acceptability.

Through laboratory study, intermediate-scale flow-cell experiments, and three-dimensional computer simulations, a field test site will be designed and installed in FY 1992 and FY 1993. 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.

REFERENCES

BOUWER, E. J., and P. L. McCARTY, "Transformations of Halogenated Organic Compounds under Denitrification Conditions," Appl. Environ. Microbiol. 45:1295-1299 (1983a).

BOUWER, E. J., and P. L. McCARTY, "Transformations of 1- and 2-carbon Halogenated Aliphatic Organic Compounds under Methanogenic Conditions," Appl. Environ. Microbiol. 45:1286-1294 (1983b).

BROUNS, T. M., S. S. KOEGLER, W. O. HEATH, J. K. FREDRICKSON, H. D. STENSEL, D. L. JOHNSTONE, and T. L. DONALDSON, "Development of a Biological Treatment System for Hanford Groundwater Remediation: FY 1989 Status Report," PNL-7290, Pacific Northwest Laboratory, Richland, Washington (1990).

CHIANG, C. Y., C. N. DAWSON, and M. F. WHEELER, "Modeling of In Situ Biorestoration of Organic Compounds in Groundwater," Transport in Porous Media (in press).

CRIDDLE, C.S., J. T. DeWITT, D. GRBIC-GALIC, and P. L. McCARTY, "Transformation of Carbon Tetrachloride by *Pseudomonas sp.* Strain KC under Denitrification Conditions," Appl. Environ. Microbiol. 56:3240-3246 (1990a).

CRIDDLE, C. S., J. T. DeWITT, and P. L. McCARTY, "Reductive Dehalogenation of Carbon Tetrachloride by *Escherichia coli* K-12," Appl. Environ. Microbiol. 56:3247-3254 (1990b).

EGLI, C., T. TSCHAN, R. SCHOLTZ, A. M. COOK, and T. LEISINGER, "Transformation

of Tetrachloromethane to Dichloromethane and Carbon Dioxide by *Acetobacterium woodii*. Appl. Environ. Microbiol. 54:2819-2824 (1988).

GÄLLI, R., and P. L. McCARTY, "Biotransformation of 1,1,1-trichloroethane, trichloromethane, and tetrachloromethane by a *Clostridium sp.*," Appl. Environ. Microbiol. 56:3240-3246 (1990).

GANTZER, C. J. and L. P. WACKETT, "Reductive Dechlorination Catalyzed by Bacterial Transition-Metal Coenzymes," Environ. Sci. Technol. 25:715-722 (1991).

HAGOOD, M. C., and V. J. ROHAY, "200 West Area Carbon Tetrachloride Expedited Response Action Project Plan," WHC-SD-EN-AP-046, Westinghouse Hanford Company, Richland, Washington (1991).

HANSEN, E. J., "The Transformation of Tetrachloromethane under Denitrifying Conditions by a Subsurface Bacterial Consortium and Its Isolates," M.S. Thesis, Washington State University (1990).

KOEGLER, S. S., T. M. BROUNS, W. O. HEATH, and R. J. HICKS, "Biodenitrification of Hanford Groundwater and Process Effluents: FY 1988 Status Report," PNL-6917, Pacific Northwest Laboratory, Richland, Washington (1989).

KRONE, U. E., R. K. THAUER, and H. P. C. HEGENKAMP, "Reductive Dehalogenation of Chlorinated C1-hydrocarbons Mediated by Corrinoids," Biochemistry 28:4908-4914 (1989).

LAST, G. V. and V. J. ROHAY, "Carbon Tetrachloride Contamination, 200 West Area, Hanford Site," PNL-SA-19564, Pacific Northwest Laboratory, Richland, Washington (1991).

SHELTON, D. R. and J. M. TIEDJE, "General Method for Determining Anaerobic Biodegradation Potential," Appl. Environ. Microbiol. 47:850-857 (1984).

WILSON, J. T., G. B. SMITH, J. W. COCHRAN, J. F. BARKER, and P. V. ROBERTS, "Field Evaluation of a Simple Microcosm Simulating the Behavior of Volatile Organic Compounds in Subsurface Materials," Water Resour. Res. 23:1547-1553 (1987).

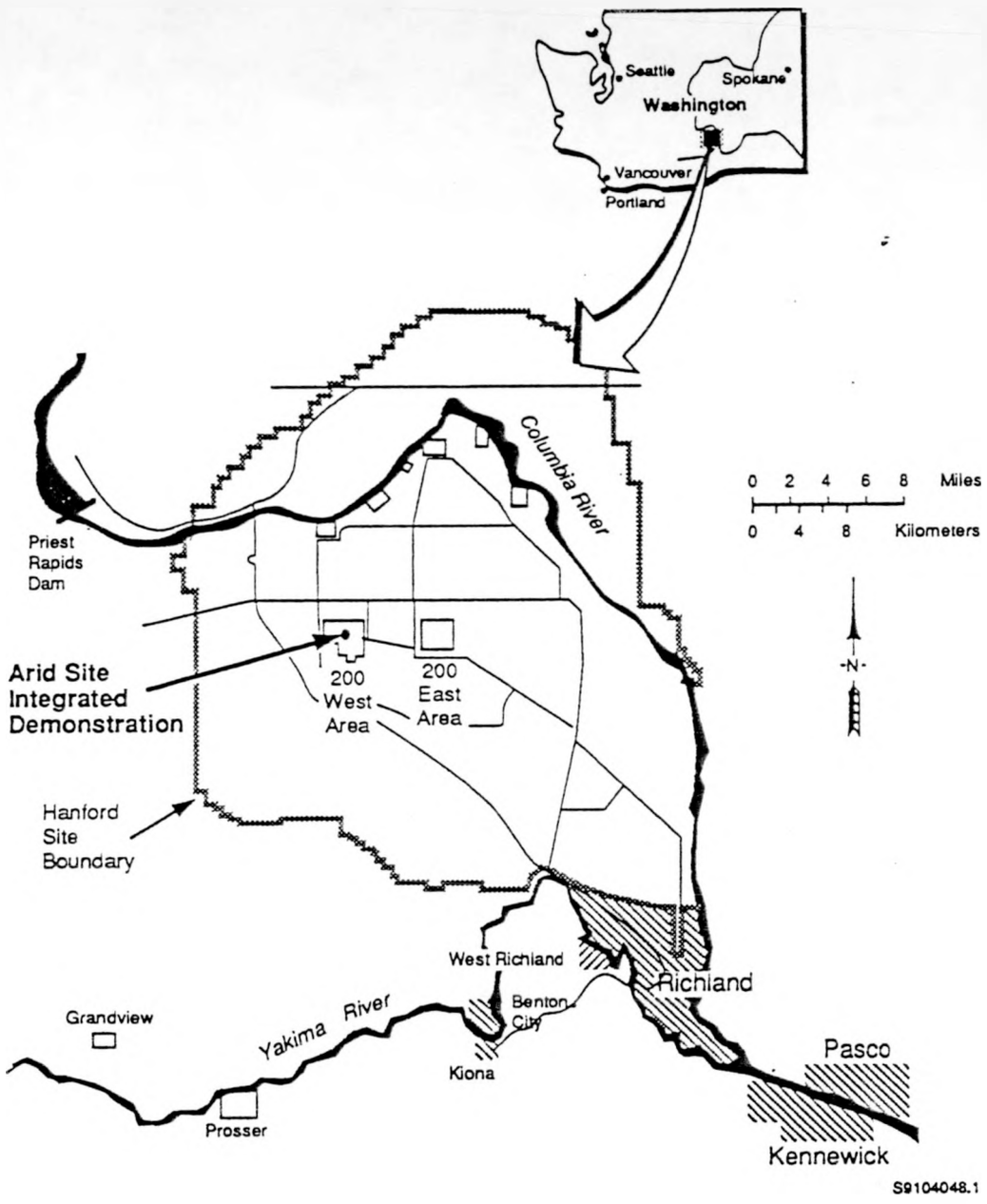

FIGURE 1. Location of 200 West Area on the Hanford Site in Washington State.

FIGURE 2. Sterile Sediment Sample Collected for Microbiological Analyses Using a Split Spoon Sampling Tool Containing a Lexan Liner.

Table 1. Sediment sample analysis results from ground-water biological treatment well.

Sample Depth, m (ft)	Date	Methylene Chloride, ng/gm	Chloroform ng/gm	Carbon Tetrachloride ng/gm	Trichloro-ethylene ng/gm
75.6 (247.9)	7/01/91	962	29	10	1
78.3 (257.0)	7/03/91	4505	103	287	7
79.9 (262.0)	7/09/91	<1677*	<8	25	<2

* The high "less than" value (<) for this sample is caused partly by methylene chloride impurity in the purge and trap grade methanol and the small amount of sediment in the sample vial.

Table 2. Water sample analysis results for volatile organics and anions from groundwater biological treatment well.

Sampling Date	7/2/91	7/10/91	7/16/91
Sample Depth Interval (m)	75.3-76.0	79.2-80.0	81.7-82.1
(ft)	247.0-249.4	260.0-262.5	268.0-269.5
Methylene Chloride (ppb)	<5	3.2**	<5
Chloroform (ppb)	37	171	13
Carbon Tetrachloride (ppb)	1885	1736	2107
Trichloroethylene (ppb)	31	31	4
Tetrachloroethylene (ppb)	0.8	1	<0.5
Acetone (ppb)	7.5*	20*	<5*
Fluoride (ppm)	0.6	0.93	0.61
Chloride (ppm)	19.2	20	21.4
Nitrate (ppm)	232	231	228
Phosphate (ppm)	<0.20	<0.20	<0.20
Sulfate (ppm)	58.4	53.1	56.8

* Non-calibrated estimates.
** Observed, but below linear detection limits.

Table 3. Sample depth intervals from which sterile samples were collected for microbiological characterization.

Core No.	Depth Interval, m	(Depth Interval), ft
1	71.9-72.3	(235.8 - 237.3)
2	75.6-76.0	(247.9 - 249.4)
3	78.3-78.6	(257.0 - 258.0)
4	79.3-79.9	(260.3 - 262.0)
5	80.2-80.7	(263.2 - 264.7)
6	81.3-81.7	(266.6 - 268.1)
7	82.7-83.3	(271.2 - 273.2)
8	83.3-83.5	(273.2 - 274.0)