

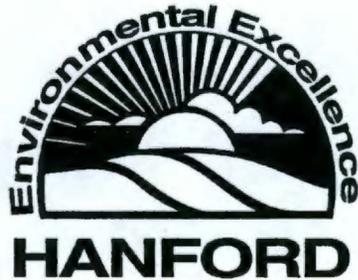
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Integrated Test Plan: In Situ Bioremediation Demonstration



Prepared for the U.S. Department of Energy
Office of Technology Development

Bechtel Hanford, Inc.
Richland, Washington



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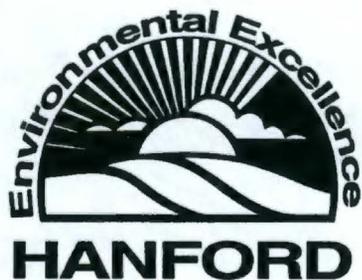
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Integrated Test Plan: In Situ Bioremediation Demonstration

Author
C.D. Kramer

Date Published
January 1995



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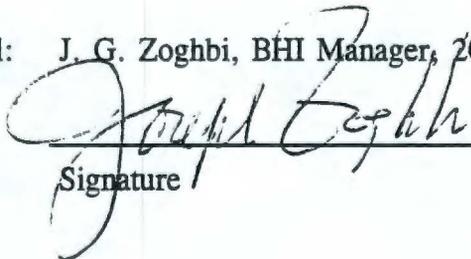
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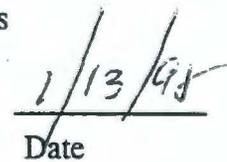
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EXECUTIVE SUMMARY

This document describes the objectives and activities for conducting an in situ bioremediation demonstration at the 200 West Area of the U.S. Department of Energy's (DOE) Hanford Site as part of the Volatile Organic Compound-Arid Integrated Demonstration. The purpose of the in situ bioremediation demonstration is to provide the necessary design, operating, and cost information for use in examining bioremediation as a treatment option for the carbon tetrachloride (CCl₄) and nitrate groundwater plumes at the Hanford Site. Information relative to microbial responses to injected nutrients, control of biofouling, and kinetics of cometabolic solvent destruction reactions gained from this application of in situ bioremediation will also be of use at other DOE, U.S. Department of Defense, and private sites.

Because of the complex technical issues and rigorous performance evaluation goals associated with the objectives of the demonstration, a design tool was developed for in situ bioremediation applications. This tool incorporates site characterization information, microbial kinetics, and transport modeling into a process simulator for use in examining potential field designs. This simulator was calibrated using laboratory kinetic and soil column experiments and will be further refined using field data. The resulting design tool is a primary product of the demonstration for use in full-scale design for in situ bioremediation.

The demonstration will consist of an abiotic recirculation control phase (Phase 1) and active bioremediation operations. During active bioremediation operations, the initial operating strategy will be implemented for at least two months (unless significant problems occur) to fully assess system performance (Phase 2). A thorough data review will be conducted after initial operations and a decision will be made either to continue the initial operating strategy or to implement a revised operating strategy (Phase 3). Primary measures of performance include (1) the in situ contaminant destruction rate for one pass of groundwater through the treatment zone and (2) field operation without a decrease in aquifer permeability that significantly hinders remediation (i.e., control of biofouling).

A well-to-well recirculation configuration will be used to mix the groundwater. Four monitoring wells are located on a line between the injection and extraction wells. This is not necessarily the configuration that would be used in a full-scale design but will provide the information needed to design a full-scale system. A biologically active zone will be created in the aquifer surrounding the injection well. Implementing, controlling, and assessing the performance of this zone are the primary tasks for the field demonstration.

The following process operations are planned for the field demonstration. Groundwater will be recirculated by extracting and injecting groundwater at a rate of 30 gallons per minute. This operation will establish a mixing zone in the aquifer. Before injecting nutrients, the concentration of contaminants can be measured over time to determine the effect of groundwater mixing on these concentrations. The nutrients, acetate and nitrate, will be injected into the aquifer to begin active bioremediation. These will be metered from separate bulk storage tanks to produce the desired in situ concentrations. Acetate and nitrate will be injected separately into the aquifer in pulses. Dispersion in the recirculation pattern around the injection well will mix the acetate and nitrate, resulting in the desired microbial activity. The exact strategy of nutrient pulsing is being determined in simulations and will be finalized for the demonstration after additional site characterization information is obtained from the wells installed in early fiscal year 1995 and from tracer test data. Injected nutrient

concentrations will be low initially when the sediment microbial population is low and will increase as the in situ utilization rate increases because of increased biomass. By introducing the appropriate nutrient flux to the aquifer, the contaminants CCl_4 and nitrate will be destroyed within the zone of microbial activity that is created.

The types of data to be collected during the demonstration include (1) routine monitoring to establish changes in constituent concentrations as the demonstration progresses and to meet sampling requirements for statistical demonstration of performance goals, (2) intense sampling to monitor the response of the treatment zone to nutrient pulses and the change in this response as the demonstration progresses, and (3) tracer tests to monitor changes in hydraulic properties of the treatment zone as a result of treatment activity. Pre- and post-demonstration monitoring will be implemented to establish the effects of the technology on overall water quality at the site.

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ACRONYMS

ARAR	applicable or relevant and appropriate requirements
BHI	Bechtel Hanford, Inc.
CAA	<i>Clean Air Act</i>
CCl ₄	carbon tetrachloride
CERCLA	<i>Comprehensive Environmental Response, Compensation, and Liability Act</i>
CH ₂ CL ₂	dichloromethane (methylene chloride)
CHCl ₃	chloroform
CO ₂	carbon dioxide
CRP	Community Relations Plan
CWA	<i>Clean Water Act</i>
DI	deionized
DOE	U.S. Department of Energy
DQO	data quality objectives
ECD	electron capture detector
Ecology	Washington State Department of Ecology
EII	Environmental Investigation Instruction
EPA	U.S. Environmental Protection Agency
ERC	Environmental Restoration Contractor
FTL	Field Team Leader
FY	fiscal year
GC	gas chromatograph
HPT	Health Physics Technician
ICP	Inductively Coupled Plasma
ID	Integrated Demonstration
IDW	Investigation-Derived Waste
ITP	integrated test plan
I/O	input/output
MPN	most probable number
NCP	National Contingency Plan
NEPA	<i>National Environmental Policy Act</i>
p/b	parts per billion
p/m	parts per million
PC	personal computer
PCE	tetrachloroethylene
PI	Principal Investigator
PNL	Pacific Northwest Laboratory
QA	quality assurance
RCRA	<i>Resource Conservation and Recovery Act</i>
RCW	<i>Revised Code of Washington</i>
RD&D	Research, Development, and Demonstration
RL	U.S. Department of Energy, Richland Operations Office
RQ	reportable quantities
SDWA	<i>Safe Drinking Water Act</i>
SOP	Safe Operating Procedure
SSO	Site Safety Officer
TCE	trichloroethylene

ACRONYMS (Continued)

TD	Technology Demonstrations
VOC	Volatile Organic Compound
WAC	<i>Washington Administrative Code</i>

1.0 INTRODUCTION

1.1 PURPOSE AND SCOPE

This document serves as an integrated test plan (ITP) to accomplish the in situ bioremediation demonstration at Hanford's 200-ZP-1 Operable Unit. It has been preceded by work yielding an understanding of site-specific characteristics and well network installation. This plan was produced by the Environmental Restoration Contractor (ERC) with help from the Principal Investigator (PI) of the technology, Pacific Northwest Laboratory (PNL). It incorporates the PI's conceptual test plan to create an site-specific operations document for the demonstration. The plan follows the template in the *VOC-Arid Integrated Demonstration Guide to Preparation of Demonstration Documents* (Jensen et al. 1994).

The Volatile Organic Compound (VOC)-Arid Integrated Demonstration (ID) is focused on developing and comparing technologies for characterization, removal, destruction, and monitoring of volatile organic wastes and associated contaminants at arid sites. The ID addresses vadose zone and groundwater contamination from VOCs, including carbon tetrachloride (CCl₄) and associated contaminants at the Hanford Site.

Cost-effective methods for in situ treatment of contaminated aquifers will be required at U.S. Department of Energy (DOE) sites, for the VOC-Arid ID, and for complete environmental restoration of the 200 West Area CCl₄ plume. In situ groundwater treatment technologies are a high priority for DOE's Environmental Restoration program because they offer a high likelihood for success in terms of cost-effective methods for remediation. However, key technical issues have slowed the development and implementation of in situ destruction. These include (1) methods for effective mixing and delivery of reagents to the subsurface, (2) adequate design methods and tools, and (3) monitoring and evaluating process effectiveness. Addressing these issues and determining appropriate engineered solutions relevant to implementation of full-scale in situ bioremediation are some of the targets for this project.

The purpose of the in situ bioremediation demonstration is to provide the necessary design, operating, and cost information for use in examining bioremediation as a treatment for the CCl₄ and nitrate groundwater plumes at the Hanford Site. Some additional scale-up and site-specific characterization would be necessary to implement the bioremediation technology for remediation of contaminant plumes or portions of contaminant plumes at the Hanford Site. However, the technology will have undergone field testing and will be developed to the point where the primary issues related to its deployment are related to macro-scale issues and site properties. The information obtained relative to microbial responses to injected nutrients, control of biofouling, and kinetics of cometabolic solvent destruction reactions gained from this application of in situ bioremediation will also be of use at other DOE, U.S. Department of Defense, and private sites.

The design tool developed for this demonstration can be used for other in situ bioremediation applications as well. The kinetic studies, treatability tests, process modeling, and deployment design are all generically applicable to many microbial remediation schemes. Methods for performing laboratory tests to obtain contaminant mass balance with VOCs were developed as part of this design process. In addition, the means to develop and optimize nutrient-feeding strategies were applied to the remediation design and are applicable to many other scenarios.

In summary, this demonstration is to provide detailed information to assess enhanced, in situ, microbial detoxification of CCl_4 -contaminated groundwater. The information will contribute to technology development and future decisions for cost-effective environmental cleanup. The scope will provide technology and design process information that can be readily transferred to expansion of the target application or to other related applications.

1.2 BACKGROUND

The current understanding of microbial degradation of CCl_4 is limited. However, CCl_4 biodegradation has been demonstrated with a number of different bacteria. The conditions that favor biodegradation of CCl_4 are anaerobic. For example, Bouwer and McCarty (1983a) observed that cultures of sewage treatment bacteria biodegraded CCl_4 to carbon dioxide (CO_2) and other metabolites under methanogenic (Bouwer and McCarty 1983b) and denitrification conditions. Sulfate-reducing microorganisms have also demonstrated the ability to destroy CCl_4 (Cobb and Bouwer 1991 and Egli et al. 1988). In addition, Semprini et al. (1991) speculated that sulfate-reducing bacteria were responsible for the CCl_4 degradation they observed during a field test of in situ bioremediation. Biodegradation of CCl_4 under denitrification conditions is of particular interest at Hanford because of the occurrence of both CCl_4 and nitrates in the unconfined aquifer. Hansen (1990), Criddle et al. (1990), Lewis and Crawford (1993), Bae and Rittmann (1990), and Bouwer and Wright (1988) demonstrated CCl_4 biodegradation with acetate as the electron donor and nitrate as the terminal electron acceptor. In addition, Bae and Rittmann (1990) speculated that CCl_4 competes as an electron acceptor with nitrate. This information, coupled with preliminary results for CCl_4 destruction by other subsurface microbes obtained from the Hanford Site (Brouns et al. 1990 and Koegler et al. 1989) led to the speculation that it may be possible to introduce the appropriate nutrients to the subsurface and induce the native bacteria to biodegrade both the nitrate and CCl_4 contamination in situ. Extensive kinetic studies were performed using a microbial consortium indigenous to the treatment demonstration site. These data were used to construct a predictive model of the reactions to determine rates and important parameters (Petersen et al. 1994 and Hooker et al. 1994). These reaction kinetics were also used in transport codes developed as a design tool and to simulate the in situ process.

The demonstration described in this ITP will implement in situ bioremediation of CCl_4 and nitrate. In this process, acetate will be injected into the aquifer where it will be used as an energy and carbon source by indigenous bacteria that can use nitrate as a terminal electron acceptor. During this process of dissimilatory denitrification, nitrate is reduced to nitrogen gas. Existing groundwater nitrate will be consumed and additional nitrate added as needed to support dechlorination reactions. In addition, while the primary cellular metabolism is directed for acetate utilization through denitrification, CCl_4 is dechlorinated by active cellular macromolecules that are functioning in the electron transport system of the bacteria. This dechlorination results primarily in the formation of CO_2 and chloride ion in addition to increased biomass, water, and nitrogen gas produced from denitrification and acetate utilization. Under some conditions of cellular activity, chloroform (CHCl_3) is produced as a byproduct. The production of CHCl_3 under initial field operating conditions will be quantified so that techniques to limit the production or degrade the CHCl_3 can be implemented in later phases of the field demonstration.

Thus, the goal of this in situ treatment process is to stimulate the native microorganisms to accelerate natural degradation of nitrate and CCl_4 . This will be achieved by introducing nutrient solutions to the contaminated aquifer to create a treatment zone. The treatment zone will be hydraulically established

with mixing wells to distribute nutrients and control groundwater flow within the treatment zone. Pump-and-treat is the primary alternative to in situ biological treatment. Multiple technologies including biological treatment, ion exchange, precipitation/filtration, or air stripping would likely be required to remove and treat the various contaminants from the groundwater. Performing aquifer remediation in situ is desirable because contaminant extraction is typically a rate-limiting step for restoration. Because of contaminant retardation and inefficient mass transfer, large volumes of water must be extracted and processed for ex situ treatments. In situ methods attempt to treat the contamination where it is found rather than where it can be moved. They offer an option to reduce costs associated with handling and treating massive quantities of dilute contamination. A cost-effective alternative, especially for contaminants that are tightly sorbed to the sediments or only slightly soluble in groundwater, is to stimulate the native microflora to destroy the contaminants, specifically nitrates and organics, through in situ bioremediation (Skeen et al., 1993).

1.3 SITE SETTING

1.3.1 Site Description

The demonstration site is located within the 200-ZP-1 Operable Unit. As shown in Figure 1-1, the demonstration site is located approximately 250 ft north of the sanitary tile field and 750 ft west of the 221-T plant. This location was chosen because it is hydraulically downgradient from the cribs where CCl_4 was disposed, it has a relatively uniform and well-determined hydraulic gradient, and it is not contaminated with radioactive constituents above levels that require special procedures. Groundwater samples collected from wells at the site indicate a fairly uniform CCl_4 level near 2 mg/L. Nitrate is present at about 300 mg/L as a co-contaminant, and other co-contaminants are minimal.

1.3.2 Hydrogeochemical and Hydrogeological Characterization

A detailed description of the site characterization data collected at the demonstration site is planned. The following is a summary of the characterization information. Figures 1-2 and 1-3 illustrate the overall properties of the site and the primary data from which these properties were generated. These data include sediment core analyses, hydrologic pumping tests, geophysical logs, and tracer tests. Site characterization information is based on three wells installed in fiscal years (FY) 1992 through 1994. These penetrate the uppermost part of the unconfined aquifer and are completed with stainless steel casing and screen. Two of the wells (299-W11-29 and 299-W11-30) have 4-in. inside diameter casings and are screened between 243 and 279 ft. These wells were altered in FY 1994 as described in Koegler (1994) by filling the internal well volume with bentonite from 263 to 279 ft so that the remaining open-screen interval is between 243 and 263 ft. The third well (299-W11-32) has an 8-in. inside diameter casing and was installed with three separate screened intervals at depth intervals of 243 to 258 ft, 273 to 278 ft, and 293 to 298 ft.

1.3.2.1 Stratigraphy. Figure 1-2 depicts the stratigraphy of the site from compiling the data collected during the drilling of wells 299-W11-29, 299-W11-30, and 299-W11-32. The stratigraphy of the saturated zone consists of alluvial sediments, primarily sandy gravels and muddy sandy gravels of the middle Ringold Formation. The particle size distribution of the clay, silt, and sand size fractions below the water table are relatively uniform with depth. However, the sediments contain

varying degrees of cementation and weathering. The clay size fraction makes up only 5% to 10% of the sediments, with half this fraction actually containing clay minerals. A 9-ft-thick caliche zone lies just above the water table between 238 and 247 ft. A lithology column (see Figure 1-2) summarizes the lithology between 240 and 310 ft. More data has been obtained as part of drilling activities (installation of wells 299-W11-33, 299-W11-34, and 299-W11-35) being completed in early FY 1995.

1.3.2.2 Hydrology. The characterization data summarized in Figures 1-2 and 1-3 indicate two distinct permeable units separated by a low-permeability unit. The high-permeability units lie at depths of approximately 245 to 255 ft and 286 to 300 ft, with an intervening low-permeability unit at a depth of 255 to 286 ft. The variation in hydraulic conductivity with depth is attributed primarily to variations in the degree of cementation of sediment clasts.

A series of constant-rate pumping tests, slug tests, laboratory hydraulic conductivity tests, and tracer tests was performed at the site to estimate hydraulic properties of the formation. The constant-rate pumping tests and slug tests indicated a range of approximately 10^{-2} to 10^{-4} cm/sec for hydraulic conductivity. The laboratory hydraulic conductivity values, measured with a falling head permeameter, range between 10^{-3} and 10^{-7} cm/sec. The point dilution tracer tests, performed in the fully screened wells (299-W11-29 and 299-W11-30), indicate higher flow velocity in the upper 13 to 18 ft of the test interval versus the lower part of the test interval. These tracer test profiles and the equivalent hydraulic conductivity estimated for each test interval are summarized in Figure 1-3. The hydraulic conductivity ranges shown adjacent to the lithologic log in Figure 1-2 are estimates for each equivalent lithologic unit. These ranges were estimated primarily from the field and laboratory hydraulic tests and from supporting characterization data, including lithology encountered during drilling and geophysical logging. The specific capacity for the aquifer units corresponding to the upper and lower screen intervals of the multiscreened well are also shown in Figure 1-2.

The neutron porosity log, shown in Figure 1-3, provides an indication of the relative porosity profile with depth. The log shows that the highest porosity is in the upper zone between a depth of about 247 and 258 ft, and in the lower zone, between 287 and 306 ft. The highest porosity values correspond to high-permeable zones indicated from the hydrologic tests.

1.3.2.3 Groundwater Chemistry. The groundwater chemistry at the demonstration site is typical for the unconfined aquifer conditions at the Hanford Site. Redox potential ranges between 235 and 357 mV, indicating oxidizing conditions. The dissolved oxygen content is depressed to approximately half of saturation. Total dissolved solids averaged about 440 mg/L, and pH ranges between 7.1 and 7.8. Sulfate and chloride range between 50 and 67 p/m and between 20 to 26 p/m, respectively. Metal ion concentrations detected in the groundwater are dominated by calcium, sodium, potassium, and magnesium. Figures 1-4, 1-5, 1-6, and 1-7 summarize water chemistry data collected from wells 299-W11-29, 299-W11-30, and 299-W11-32.

1.3.2.4 Contaminant Distribution. Sediment samples from all boreholes showed a relatively uniform CCl_4 concentration profile with depth and concentrations ranging between 10 and 300 $\mu\text{g}/\text{kg}$. Detectable CCl_4 concentrations in groundwater ranged from 586 to 2197 $\mu\text{g}/\text{L}$ in the upper and middle zones (247 to 278 ft) and between 1,900 and 3,789 $\mu\text{g}/\text{L}$ in the lower zone (293 to 309 ft). The lower concentrations detected in the sediment samples indicate that either volatile organics sorb little to the sediment particles or that some of the organic constituents were lost (volatilized) during sample collection. In all boreholes, CCl_4 was not detected in solid phase above the water table. Nitrates are present throughout the formation, with groundwater concentrations ranging from 190 to 310 mg/L. CHCl_3 was detected in both the solid and aqueous phases in the majority of the samples

taken below the water table. The concentration of up to 200 $\mu\text{g}/\text{kg}$ CHCl_3 in sediments is relatively constant with depth. The aqueous phase CHCl_3 concentrations ranged up to 540 $\mu\text{g}/\text{L}$. Figures 1-8, 1-9, 1-10, 1-11, 1-12, and 1-13 summarize the VOC data collected from wells 299-W11-29, 299-W11-30, and 299-W11-32. In addition to the above characterization efforts, background sampling for VOCs in groundwater was conducted from March 1994 until the start of the demonstration. Groundwater samples are being collected and analyzed from the upper and lower screened intervals of well 299-W11-32.

Figure 1-1. In Situ Bioremediation Demonstration Site Location.

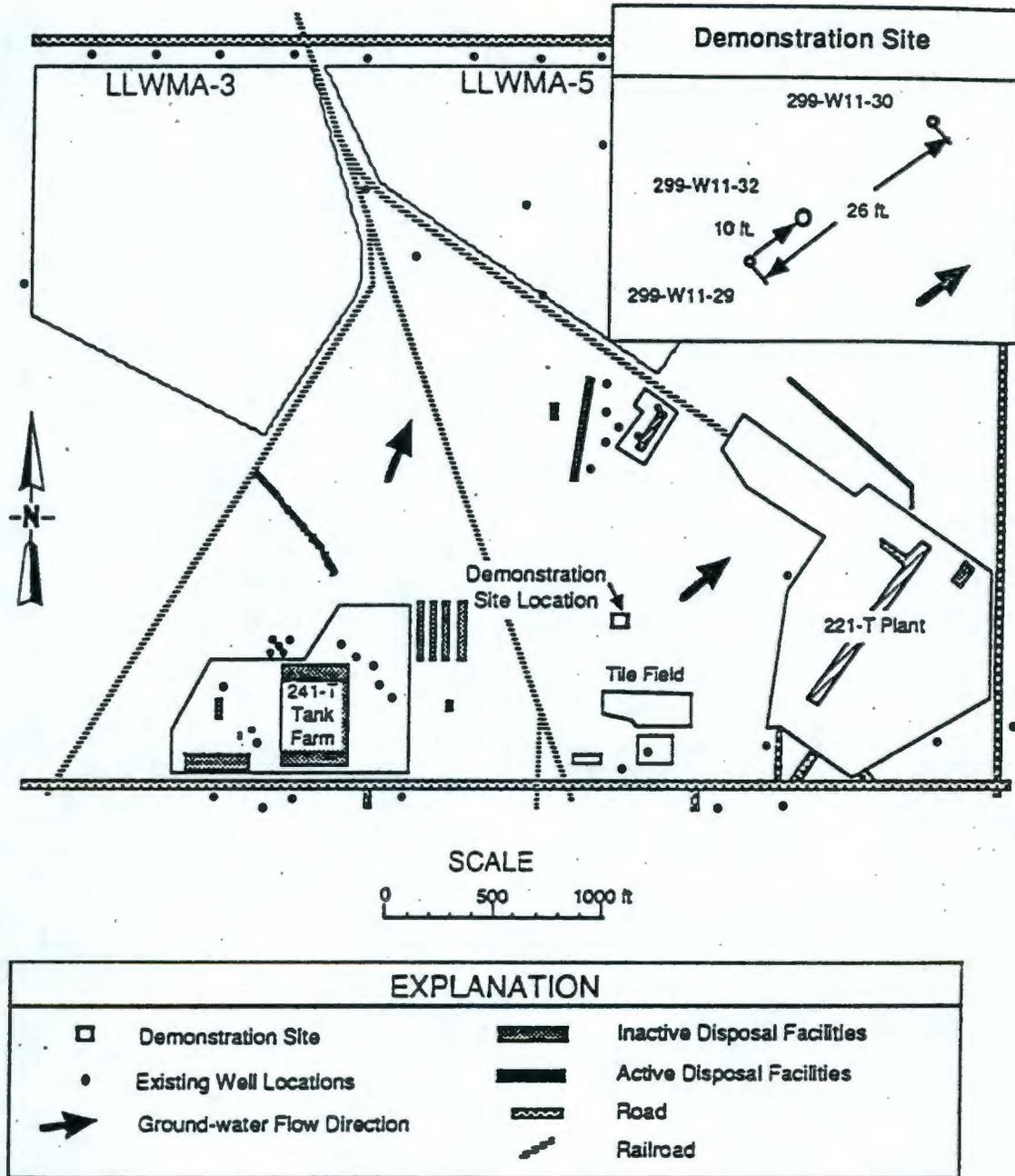


Figure 1-2. In Situ Bioremediation Demonstration Site Lithology Log.

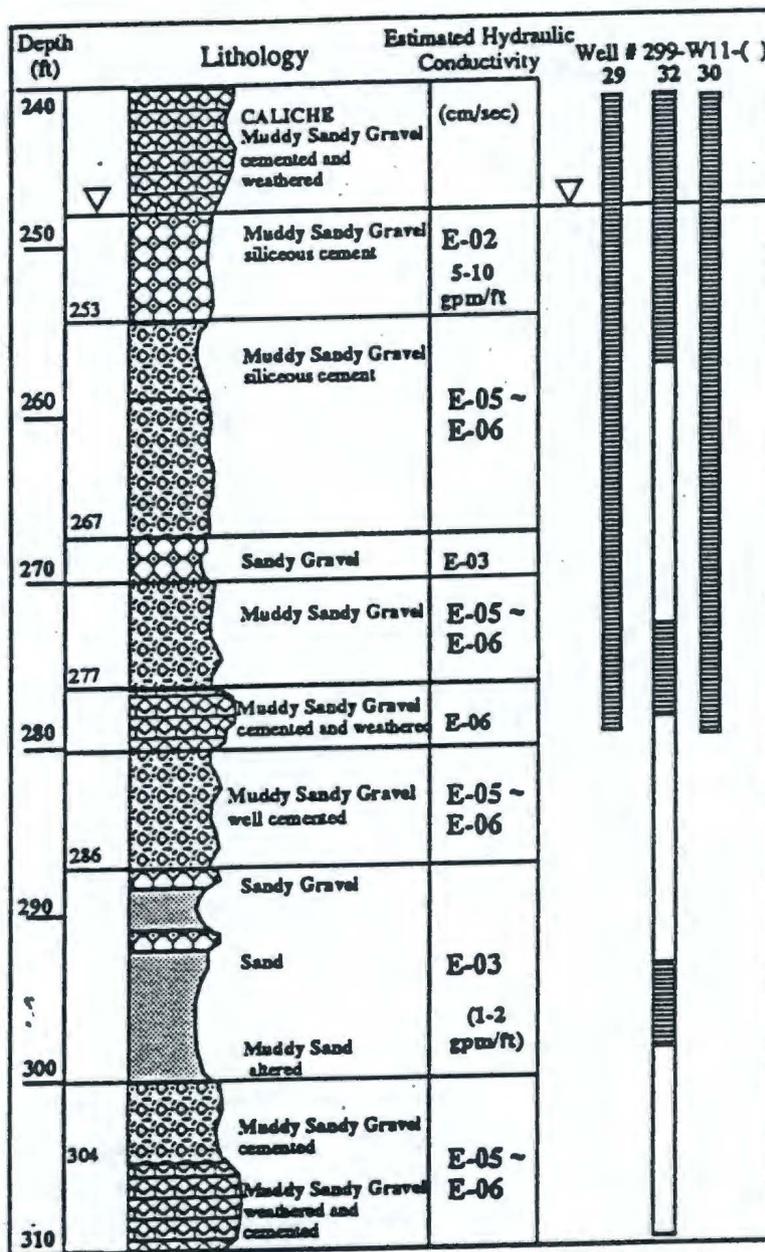


Figure 1-3. In Situ Bioremediation Demonstration Site Hydraulic Characterization.

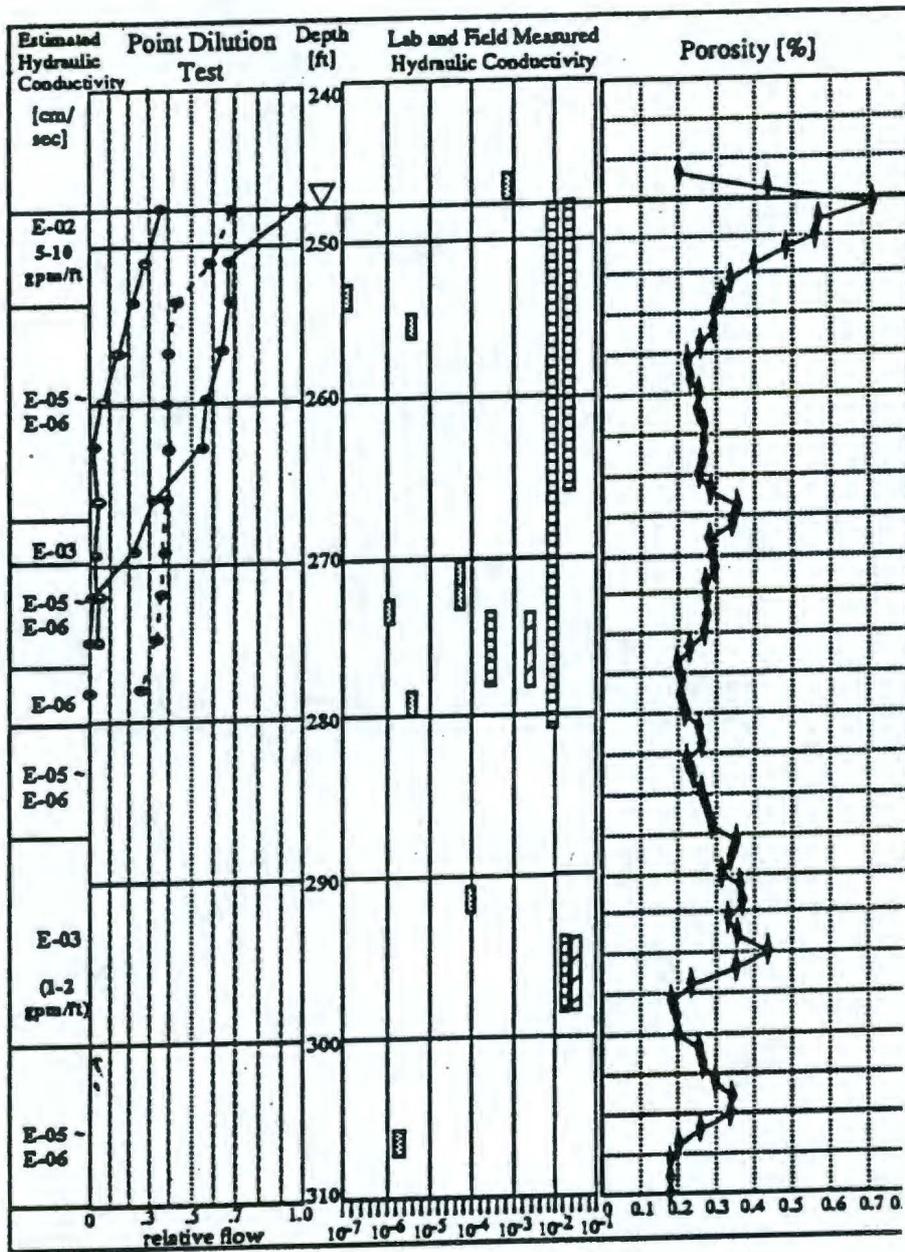


Figure 1-4. Anion Concentrations in Groundwater for Three Samples Collected from Well 299-W11-30.

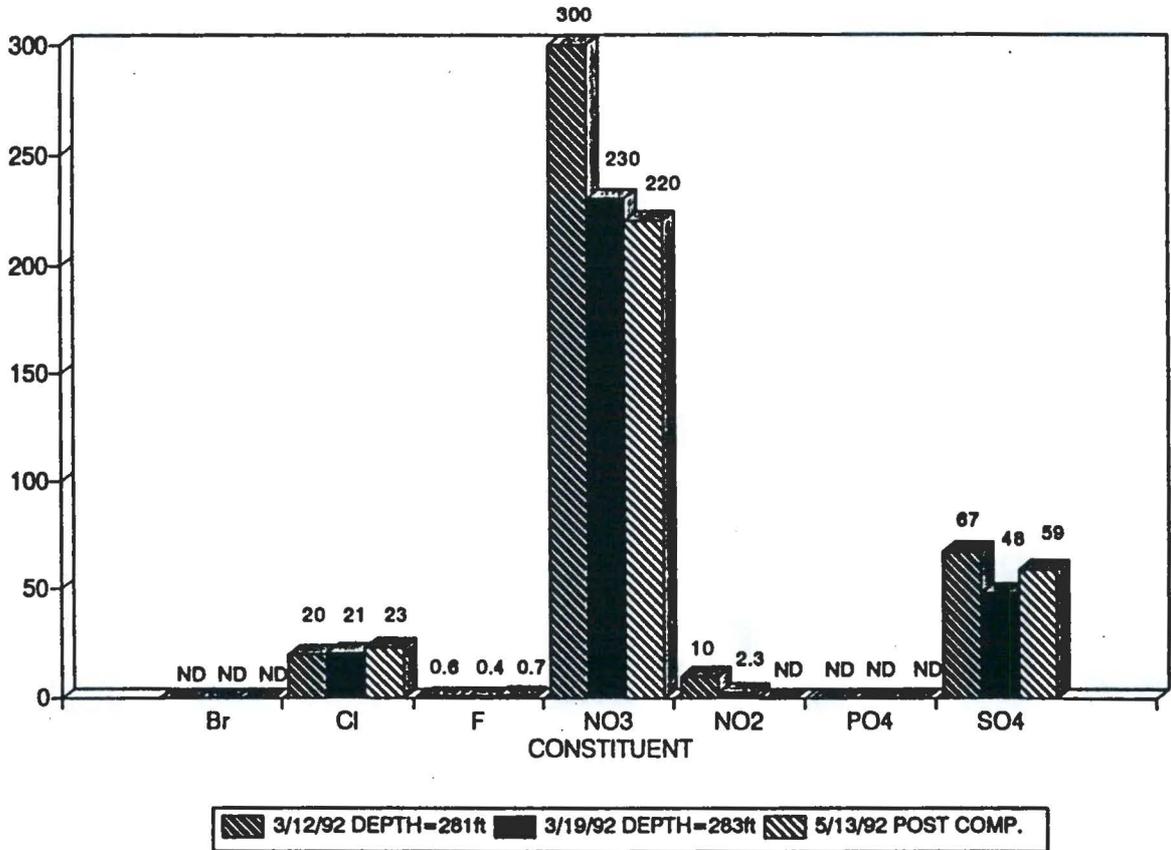
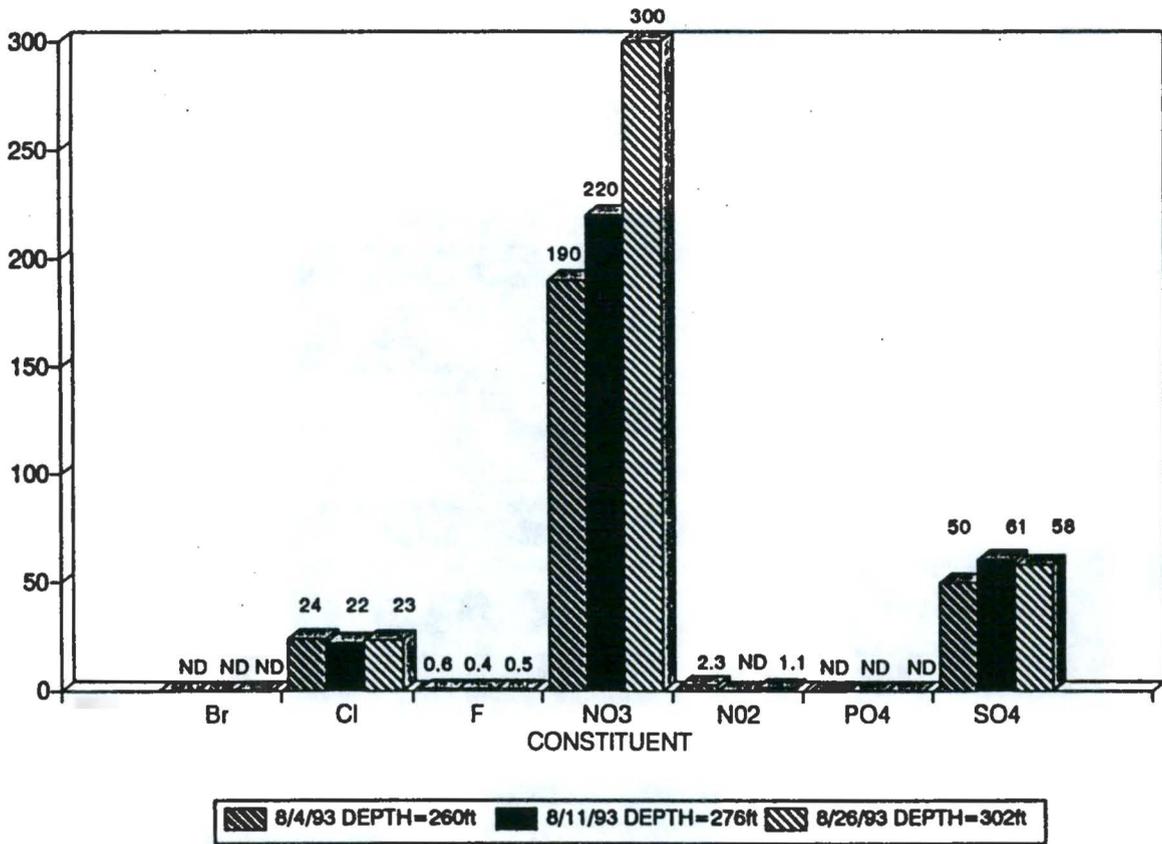


Figure 1-5. Anion Concentrations in Groundwater for Three Samples Collected from Well 299-W11-32.



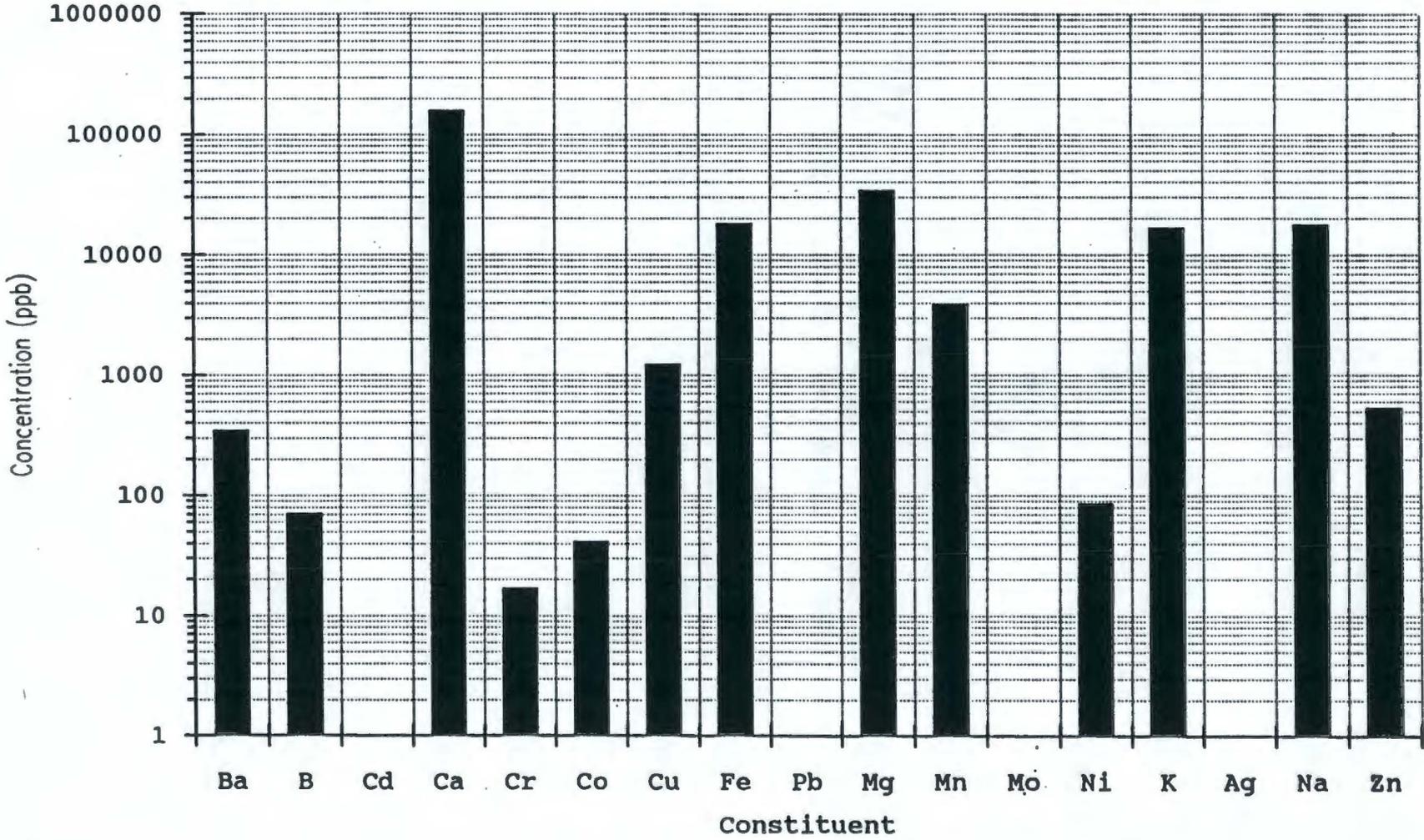


Figure 1-6. Results of Inductively Coupled Plasma Metals Analyses for Groundwater from Well 299-W11-29.

Figure 1-7. Results of Inductively Coupled Plasma Metals Analyses for Two Groundwater Samples from Well 299-W11-30.

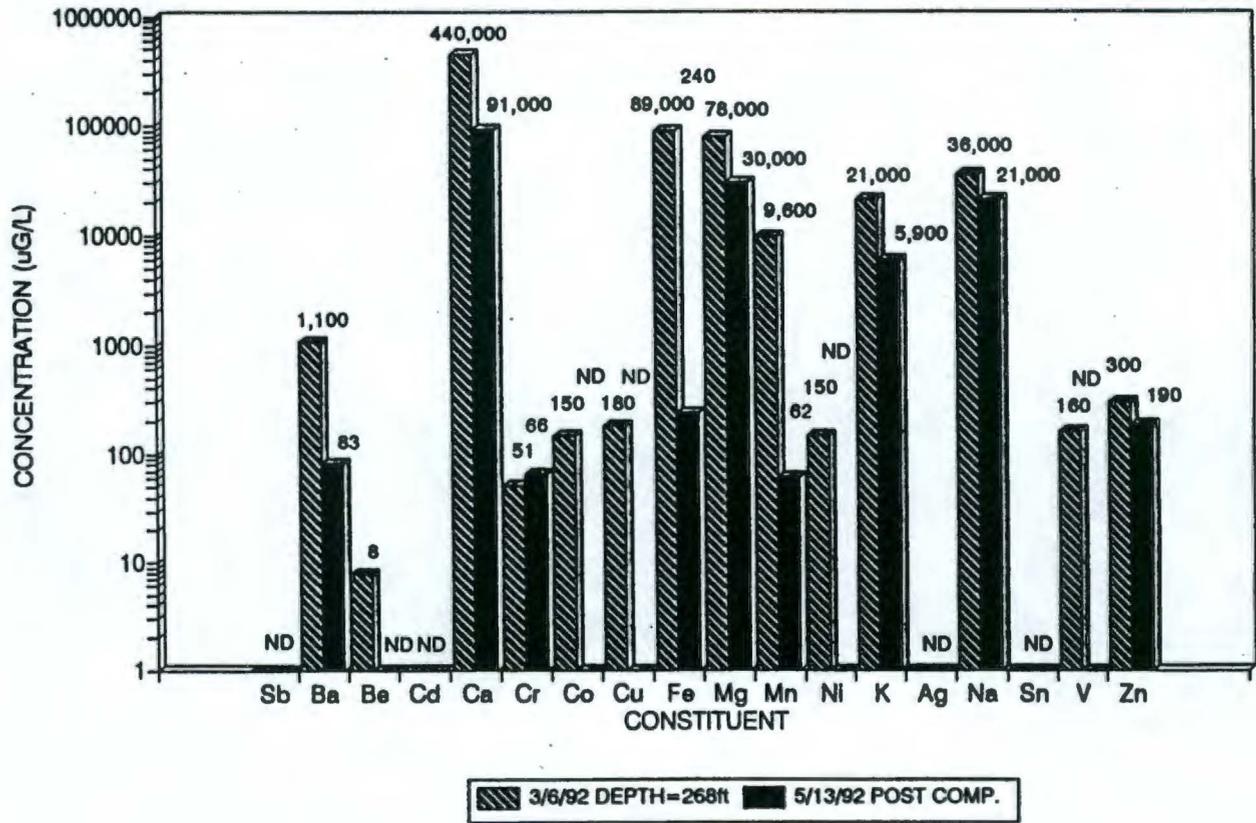
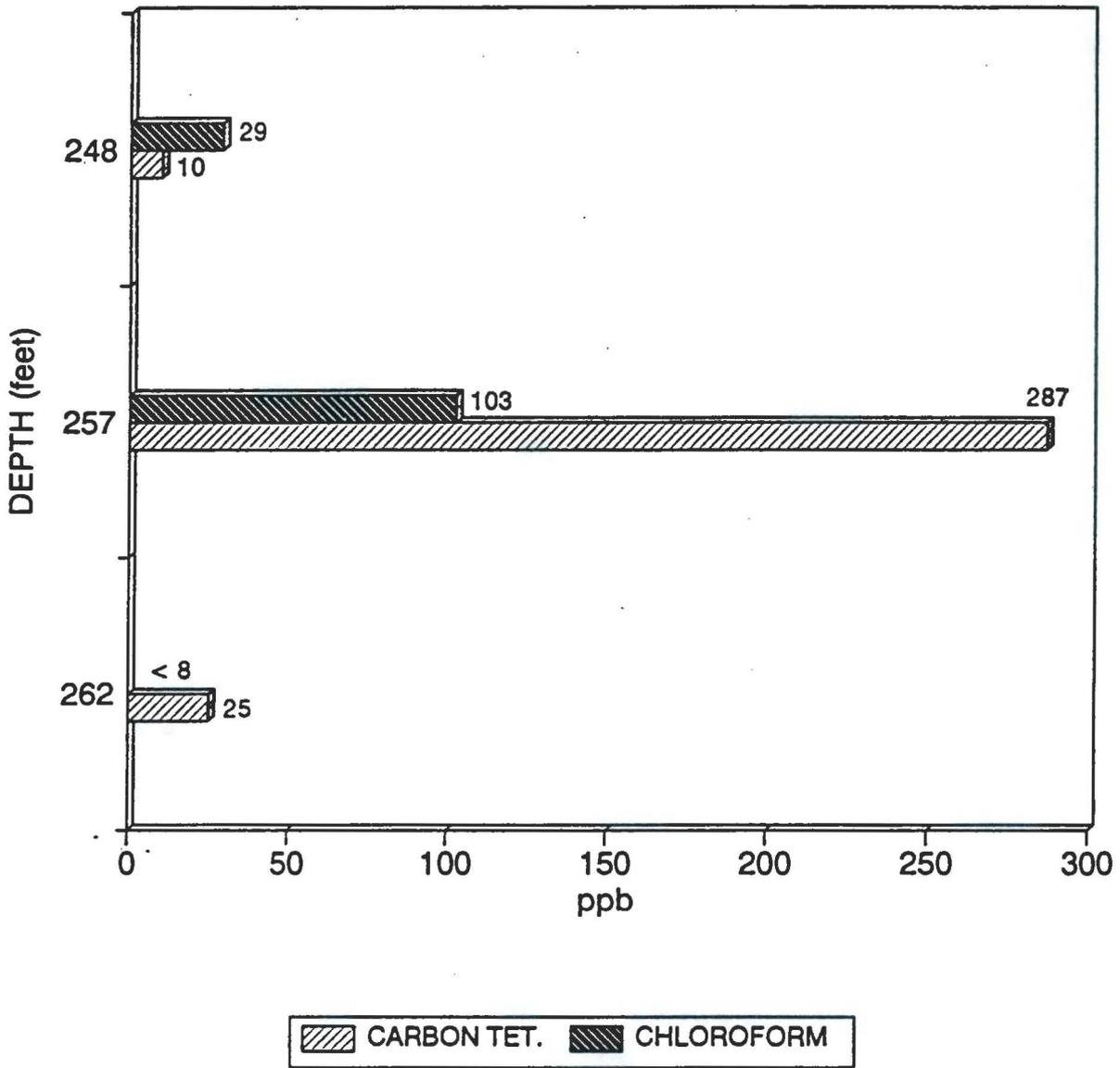


Figure 1-8. Volatile Organic Content of Sediments in Well 299-W11-29.



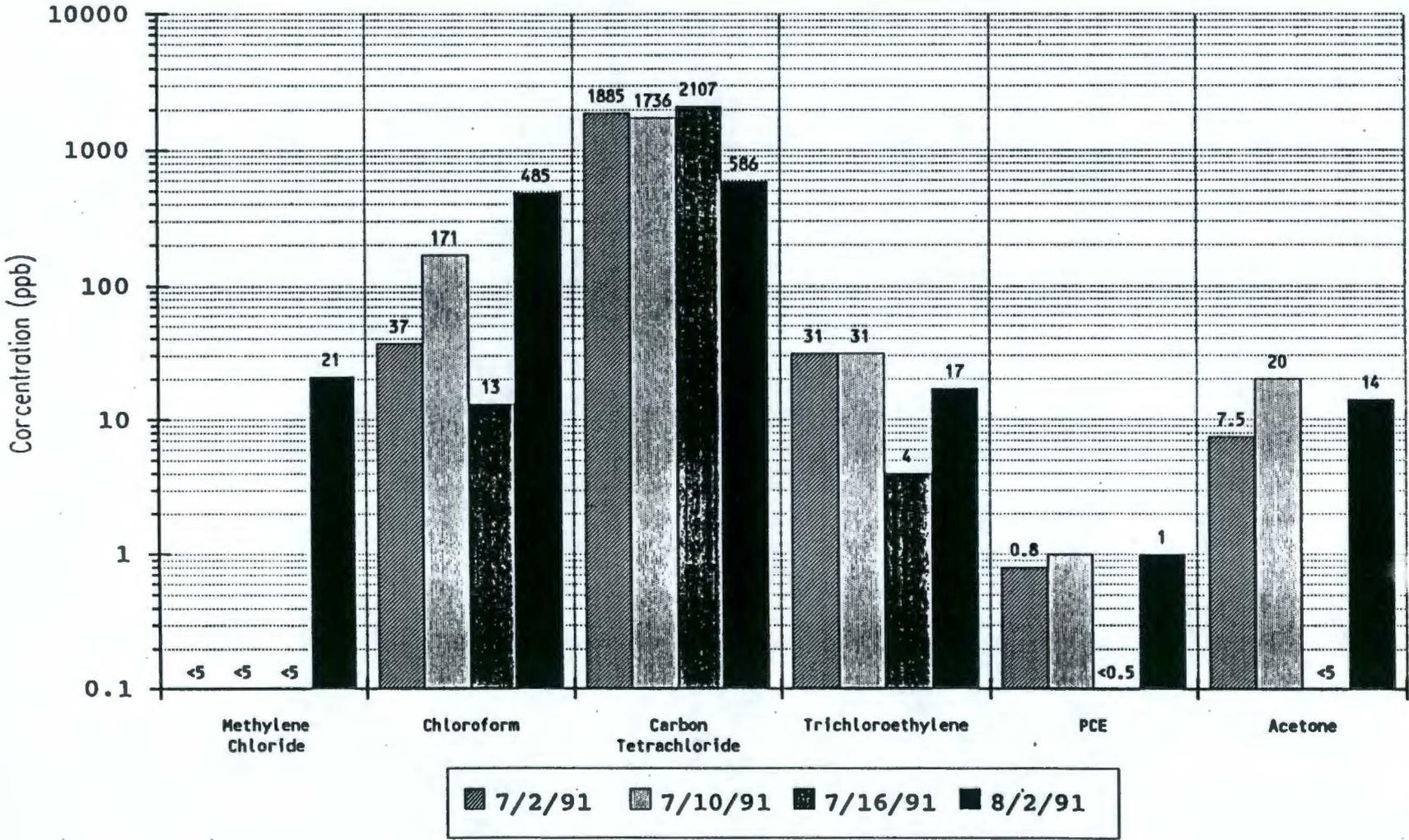
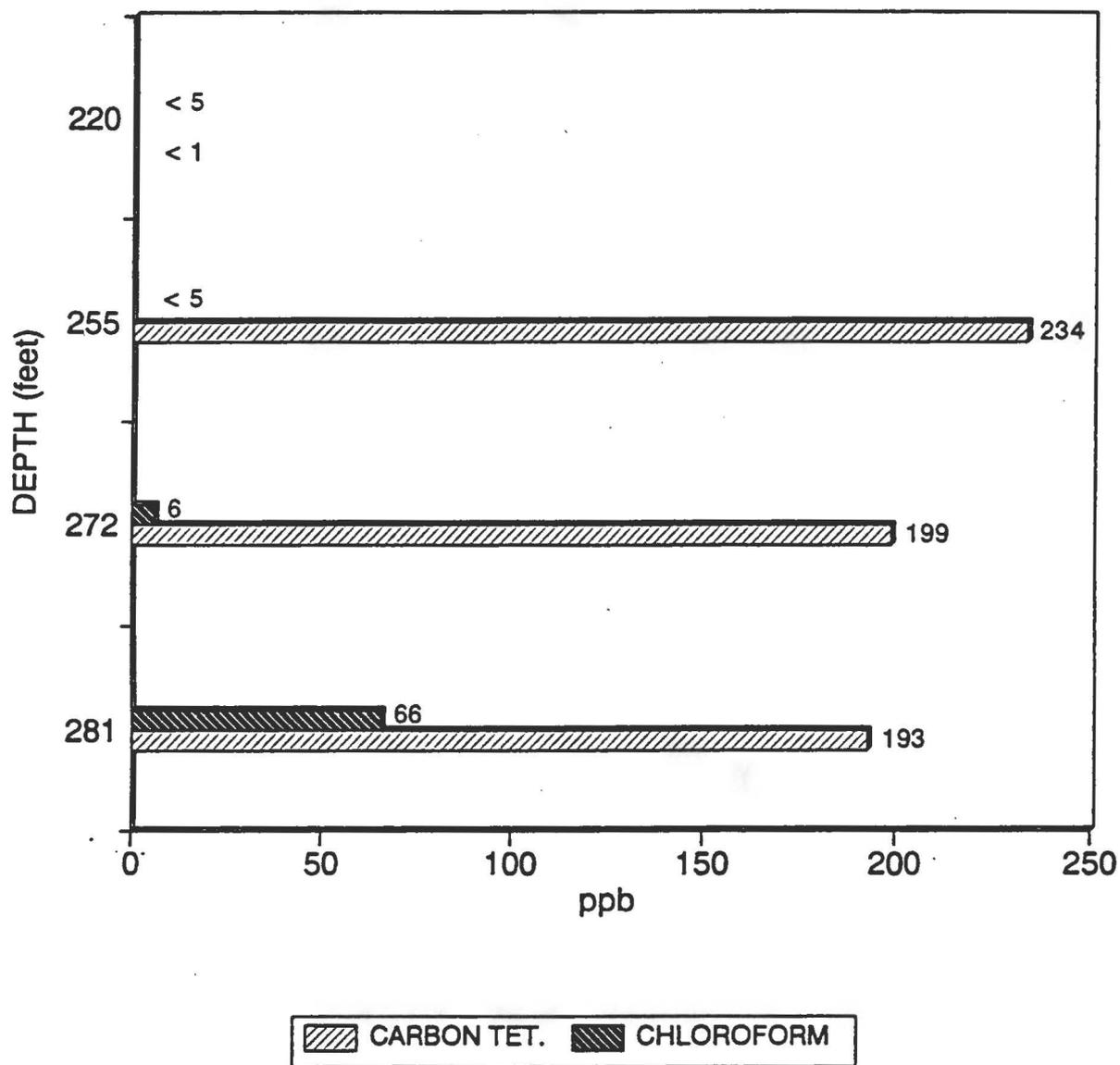


Figure 1-9. Results of Volatile Organic Analyses of Groundwater in Well 299-W11-29.

Figure 1-10. Volatile Organic Content of Sediments in Well 299-W11-30.



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Figure 1-11. Results of Volatile Organic Analyses of Groundwater in Well 299-W11-30.

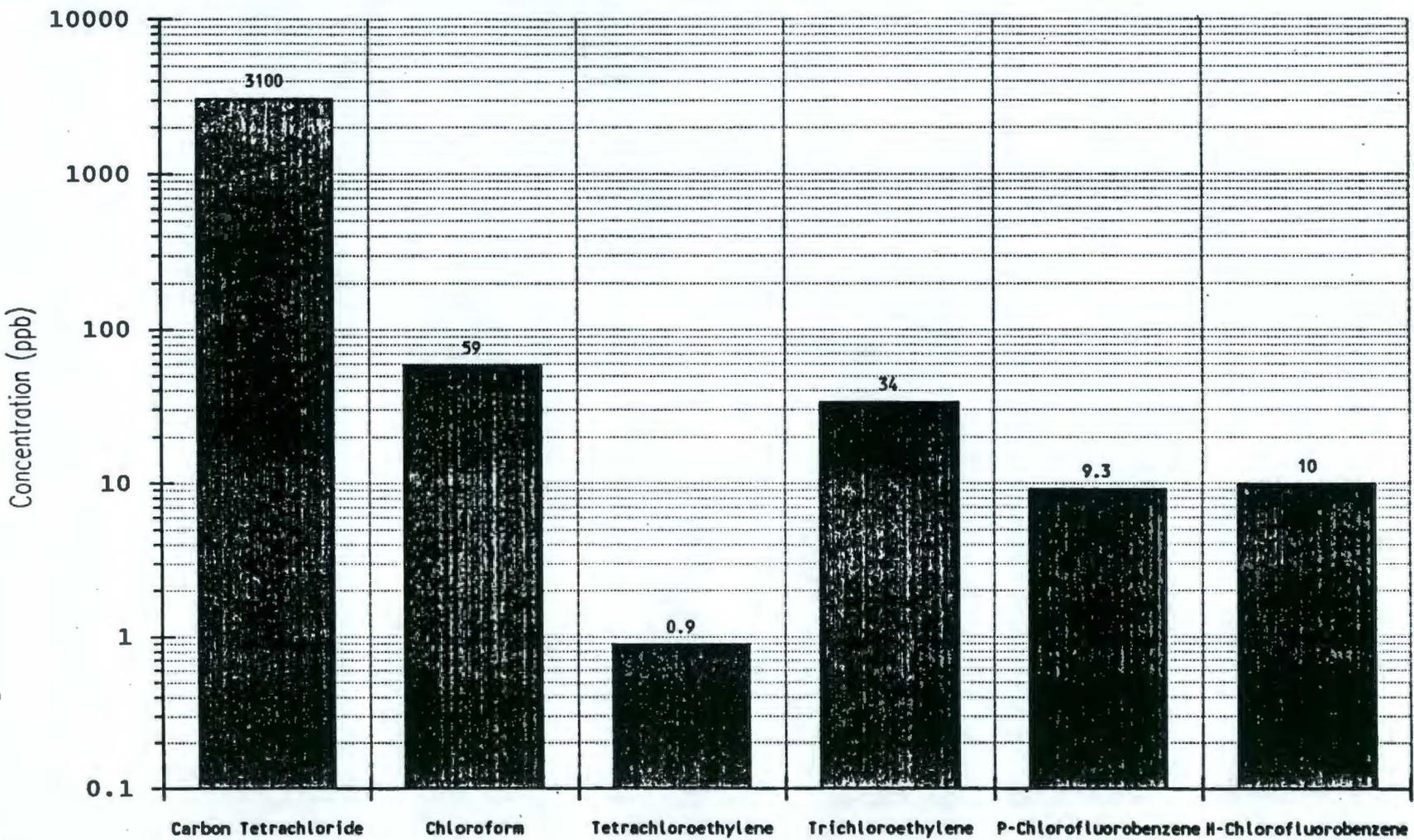


Figure 1-12. Volatile Organic Content of Sediments in Well 299-W11-32.

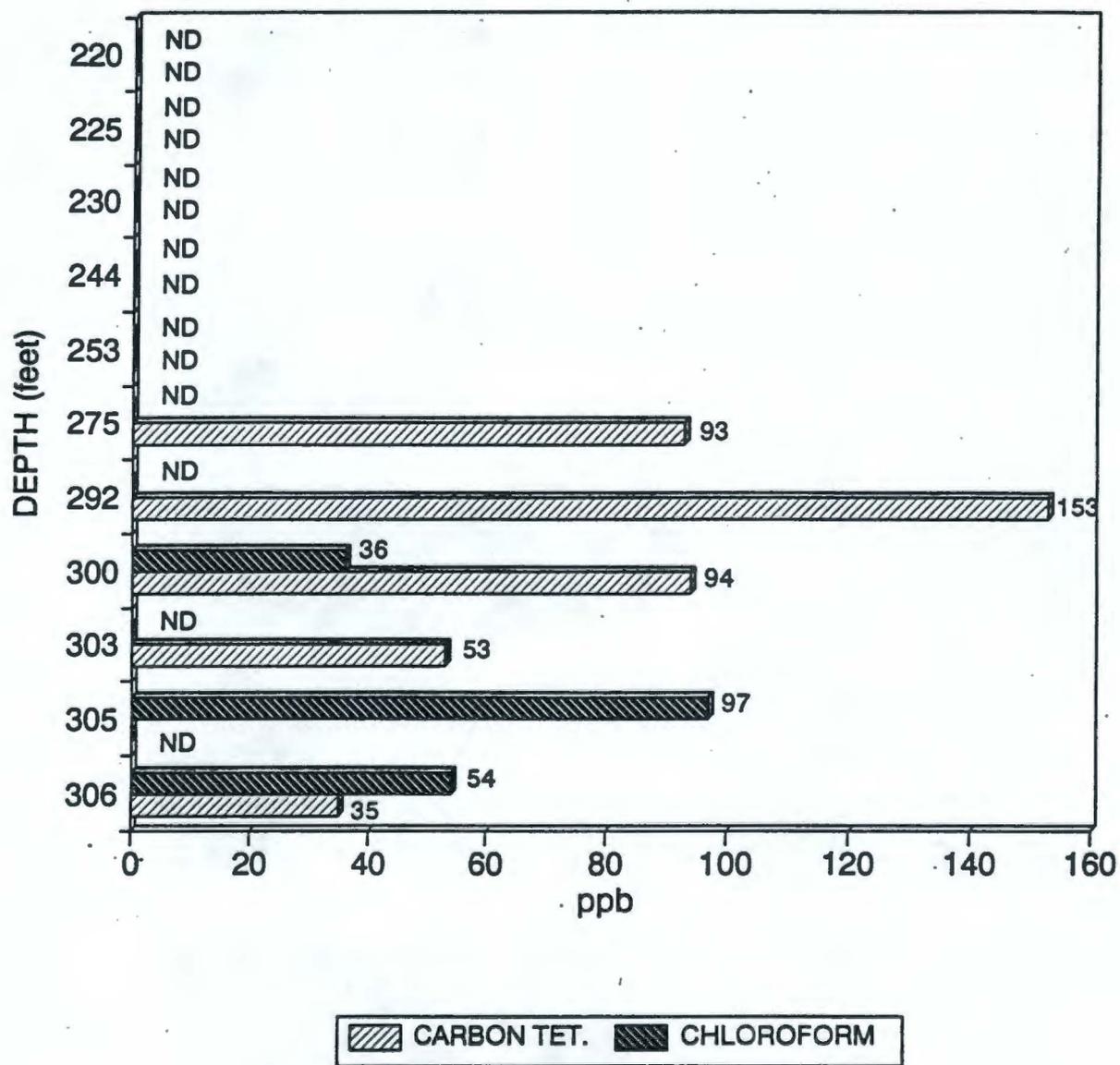
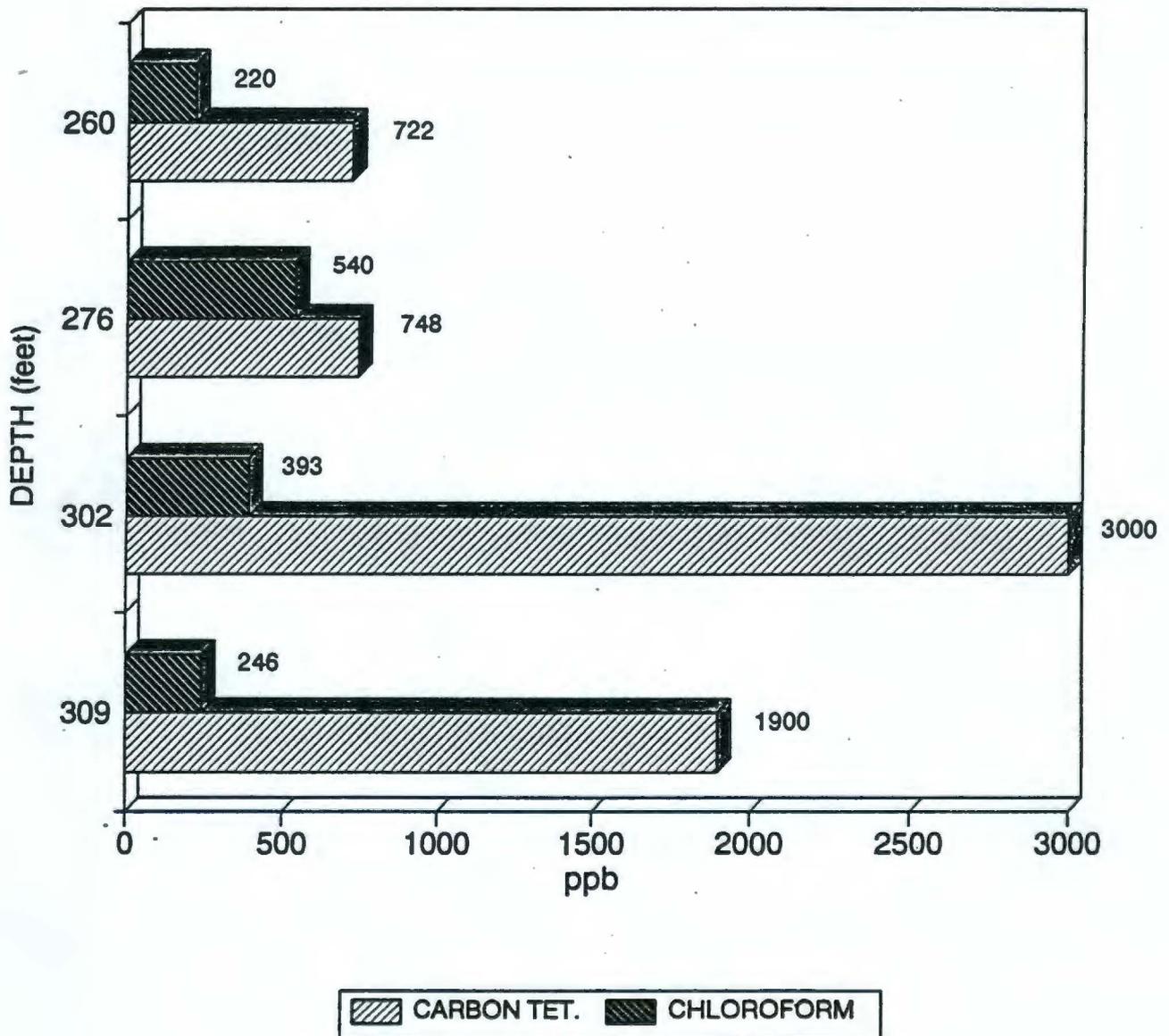


Figure 1-13. Results of Volatile Organic Analyses of Groundwater in Well 299-W11-32.



2.0 TECHNOLOGY DESCRIPTION

2.1 FUNDAMENTALS

Biochemical degradation is one of the primary mechanisms by which environmental contaminants are destroyed in nature. Decomposition is mediated by microbes. It is dependent on the presence of a sufficient concentration of other components for microbial growth. Bioremediation is based on the principle of supplying appropriate nutrients to stimulate microbial metabolic activity and subsequent degradation of contaminants by either metabolic or cometabolic processes. Microbes are found in habitats as diverse as boiling hot springs to soils of the Antarctic wilderness.

The demonstration described in this ITP will implement enhanced in situ bioremediation of CCl_4 and nitrate in an aquifer. This will be achieved by metering nutrient solutions into the contaminated aquifer to promote development of a biological treatment zone. The treatment zone will be hydraulically established with mixing wells to distribute nutrients and control groundwater flow.

In this process, acetate will be utilized as an energy and carbon source by indigenous bacteria that utilize nitrate as a terminal electron acceptor. While primary cellular metabolism is directed for acetate utilization through denitrification, CCl_4 is dechlorinated by active cellular macromolecules that are functioning in the electron transport system of the bacteria. (Microbial degradation of CCl_4 has been discussed in Section 1.2.) Denitrification results in the reduction of nitrate to harmless nitrogen gas, while the residuals of dechlorination are CO_2 and chloride ion. One of the compounds produced by incomplete dechlorination is CHCl_3 . Methods for limiting CHCl_3 production and/or degrading CHCl_3 are being examined in the laboratory so that techniques to control this unwanted byproduct can be assessed after the CHCl_3 production rate is observed during initial field operations.

Implementing, controlling, and assessing the performance of a subsurface detoxifying zone are the primary tasks for the field demonstration. These tasks are supported by laboratory and hydrologic testing, process monitoring technologies, computational simulations, and bench-scale experiments.

The technological basis for enhancing degradation is dependent on parameters associated with the design of the groundwater recirculation system and the nutrient injection strategy.

2.2 DESIGN OF A WELL NETWORK

In evaluating potential designs for the In Situ Bioremediation Demonstration, simulations to assess the effect of hydraulic control (i.e., the percentage of groundwater that remains in the recirculation pattern and is not lost and replaced by far-field groundwater) on CCl_4 destruction and biomass distribution were used to select the design.

2.2.1 Nonreactive Tracer Simulations

A nonreactive tracer test was conducted under groundwater recirculation conditions to determine the hydraulic control during well-to-well interaction using the first three wells installed at the site. In this test, recirculation was established between two wells and the third well, located in a line between the recirculation wells, was used as the monitoring well. After steady-state recirculation was established,

a bromide pulse was introduced at the injection well. Progress of the pulse was monitored using bromide-specific probes and groundwater samples.

The hydraulic control and dispersivity within the zone of influence for the recirculation wells was determined in the tracer test. Other recirculation scenarios were assessed based on these data to select the most appropriate design for the demonstration.

2.2.2 Feeding Strategies Simulations

Supplemental acetate and nitrate solutions are planned to develop and maintain an active subsurface biological treatment zone. Simulations were completed to predict the effect of feeding strategy on (1) the extent of biofouling and (2) the single-pass destruction efficiency of CCl_4 . Reaction kinetics routines used parameter values for k_d , a parameter related to biofilm development, at 0.005 min^{-1} and K_{i,CCl_4} , a parameter related to the inhibition of CCl_4 destruction by nitrate, at 10^{-4} gmol/L as suggested by batch and soil column experimental data. Simulation predictions show minimum biofouling and maximum CCl_4 destruction obtained when using sequential 1-hour pulses of acetate (1,000 mg/L) and nitrate (1,400 mg/L) followed by a 10-hour interval of no nutrient addition. Pumping was continuous at a rate of 5 gal/min per meter well screen. This prediction is being refined further using an optimization scheme that minimizes maximum field biomass concentration and effluent CCl_4 concentration based on nutrient pulse concentration, duration, frequency, and interval between acetate and nitrate injections. The results of feeding strategies simulations were used in additional studies that directly tested different well configurations being considered for the demonstration.

2.2.3 Integrated Assessment

These simulations directly tested the effect of hydraulic control on biofouling and CCl_4 destruction efficiency when using a sequential pulsing nutrient feeding strategy. Because the test site possesses background nitrate concentrations of approximately 300 mg/L, any level of hydraulic control below 100% causes a continuous breakthrough of nitrate at the injection well(s). Thus the pulsed nature of electron donor and electron acceptor feeding is diminished with decreasing level of hydraulic control. To simulate this phenomenon, breakthrough concentrations of nitrate corresponding to the selected level of hydraulic control were maintained at the injection well(s) throughout the duration of the simulation. Levels of hydraulic control investigated directly corresponded to those values tested in non-reactive tracer simulations. Pulses of nitrate, stoichiometric to 1000 mg/L acetate pulses, were superimposed on background nitrate levels over a 1-hour duration every 12 hours.

Figure 2-1 shows steady-state CCl_4 destruction based on variable hydraulic control between 60 and 100%. This figure demonstrates that system performance decreases rapidly as hydraulic control drops below 65%. The hydraulic control in the field can drop to as low as 70% with only a small decrease in CCl_4 destruction performance compared to the performance at 95% hydraulic control.

Biomass distribution was satisfactory throughout the 30-day simulated test duration for all tested levels of hydraulic control using a skewed-pulse nutrient injection strategy. This is demonstrated in Figure 2-2, which shows spatial changes in porosity caused by biofilm development for each test case after 30 days of simulated operation.

2.2.4 Conclusions

The data from the above simulations were combined with other characteristics of different well configurations to select the best design for use in the demonstration. Two primary issues were key to determining the best design: the ability to obtain measurable responses in necessary monitoring parameters and the versatility of the design in allowing the demonstration to test bioremediation strategies relevant to full-scale applications. Based on these and other site-related criteria, a well-to-well recirculation configuration was selected. This design provides adequate hydraulic control so that reaction kinetics and biomass distribution can be controlled to within an acceptable range. It also allows multiple monitoring points at varying radial distances from the injection well. Figure 2.3 illustrates a cross section of the site with the proposed well configuration.

The system will be operated using well 299-W11-33 for injection of groundwater and nutrients. The bioactive zone will be created in the subsurface within the upper permeable layer as shown in Figure 2-3. Groundwater will be extracted from well 299-W11-30. The extracted groundwater will be directly plumbed to the injection well through an in-line 25 μ m filter, flow meter, and pressure gauge. A schematic of the system is shown in Figure 2-4.

2.3 PROCESS EQUIPMENT

A demonstration site layout is shown in Figure 2-5. The primary components of the above-ground system are as follows. The process trailer will contain all the nutrient injection equipment, automated sampling equipment, process control, and data management systems. The well network equipment schematic is illustrated in Figure 2-6. An office trailer will be used for operations management. Primary storage of materials and personal protective equipment will be in a conex box. Bulk chemicals will be stored in drums on a containment pan directly behind the process trailer so that they do not need to be moved for chemical mixing operations. Clean make-up water will be contained in four 200-gal tanks. Additional storage capacity may be added. Ample room for general storage and operations will be available on the graded gravel pad. The demonstration site has been fenced as shown in Figure 2-6. This provides for site access control; however, the site also lies within the 200 West Area perimeter fence line.

Equipment and monitoring for nutrient injection, sampling, and process control are described in Sections 2.3.1, 2.3.2, and 2.3.3. Process monitoring ranges and methods are summarized in Table 2-1.

Table 2-1. Operational Monitoring Parameters.

Measurement Parameter	Range Capability	Method
Temperature (down-well)	-5 to 50 °C	Hydrolab* multiprobe
Temperature (aboveground)	-50 to 200 °C	Type T thermocouples
pH	0 to 14 pH	Hydrolab multiprobe
Redox potential	-999 to 999 mV	Hydrolab multiprobe
Pressure (down-well)	0 to 30 lb/in ² abs	Pressure transducer

Table 2-1. Operational Monitoring Parameters.

Pressure (nutrient feedline)	0 to 100 lb/in ² gauge	In-line pressure transmitter
Liquid flow rate (feedlines)	0.05 to 0.5 gal/min	Magnetic, in-line turbine flowmeter
Liquid flow rate	4 to 60 gal/min	On-line turbine flowmeter
Liquid level	ok or low	Liquid level switch
Time	N/A	Process control software, stopwatch

*Hydrolab Corporation

2.3.1 Nutrient Injection Equipment

The nutrient solutions are contained in 250-gal bulk storage tanks that are located within a secondary containment tub to prevent any spills to the environment. Nutrient feed stock concentrations are greater than the toxicity limit for bacteria so that no growth occurs in the tanks. The process control computer controls nutrient injection intervals, duration, and rate into the well by in-line solenoid valves and by a variable speed gear pump. Input to the computer includes line pressure and flowrate. Because the injection point is 250 ft below the trailer; a check valve is placed in-line at the bottom of each injection line to provide backpressure against the static water head. A gear pump was selected for the design to provide the positive pressure necessary to open this backpressure valve. Manual shutoff valves for each nutrient line are provided inside the process trailer, outside the process trailer, and at the wellhead. In addition, a pressure relief valve on the downstream side of the pump will vent excess pressure back to the bulk storage tank if normal operating pressures are exceeded. Level switches will provide a low-level alarm for each bulk storage tank as an indication of either a leak into the containment pan of the trailer or siphoning of the nutrient solution into the well. The alarm system is further described in Section 2.3.3.

2.3.2 Sampling Equipment for Reaction Parameters

Sampling equipment was designed to obtain representative samples of groundwater for analysis of VOCs, anions, microbe numbers, temperature, pH, and redox potential. In situ probes will be used to measure the temperature, pH, and redox potential in water at the monitoring and extraction wells. Dedicated in-well submersible centrifugal pumps will be used to pump water to the surface for obtaining samples from each monitoring well in the well network. Groundwater will be also sampled from ports in the surface piping that connects the extraction and injection wells (recirculation piping). The submersible centrifugal sampling pumps in the monitoring wells will be directly connected to an autosampler to collect samples every 30 minutes, as required, for specific tests during the demonstration. These intense sampling periods will be used to monitor the progression of nutrient or tracer pulses within the recirculation area as part of the testing strategy. The autosampler will consist of a fraction collector, solenoid valves for the sample lines, and metering valves. The metering valves will be used to control the flowrate to the fraction collector because there is no flow control built into the fraction collector. The groundwater directed to the fraction collector will be first purged through a three-way valve on the fraction collector, then the sample will be collected in a test tube. The process will repeat for each sample line at the desired times as controlled by the process control computer.

Manual samples for VOCs, anions, cations, and microbes will be collected from each sampling location using a syringe and specially designed sampling port on the effluent line of the in-well pump or the recirculation piping. The sampling port will consist of a tee fitting where a gas-tight ball valve is connect to the effluent line. A septa will be installed on the outlet of the valve such that the valve may be opened and a syringe needle inserted into the flow stream of the effluent line to extract a sample without exposing the sample to surface air. Specific sampling protocols for each constituent are described in Chapter 3.0 and will be written for onsite personnel as Safe Operating Procedures (SOP). In addition to primary sampling, groundwater samples will be withdrawn periodically from the lower piezometer of well 299-W11-35 using a bladder pump. These samples will be used to determine whether groundwater constituents in the nontreated zone of the aquifer are changing.

To prevent biomass from growing in the in-well submersible centrifugal sampling pumps and discharge tubing, the discharge line will be drained and blown out with air after each sampling event. A 5% household bleach solution will be used periodically to clean the pumps and effluent tubing at the surface.

Gas samples will be collected using a gas-sample pump (borehole sampler). The pump is part of a device normally used for collecting samples during drilling operations. For this demonstration, the above-ground sample retrieval pump and equipment will be connected to in-place soil gas probes to obtain samples of the vadose zone. The soil gas probes will be placed to monitor the unsaturated zone just above the water table at two of the wells (299-W11-33 and 299-W11-34). Details of this installation are contained in the FY 1994 drilling and characterization work plan (Koegler 1994).

All waste lines from in-well pumps will be routed to the onsite, 20,000-gal waste tank. Sample pumps will be connected by tubing to the waste tank. Waste water will be periodically transported to the Hanford purgewater facility and disposed of in the same manner as for well purgewater generated as part of standard hydrological tests and sampling from other wells at the Hanford Site.

2.3.3 Process Control and Data Acquisition

Operation of the in situ bioremediation demonstration requires process control for nutrient injection and sample collection, as well as data management for process monitoring equipment. An IBM (a registered trademark of International Business Machines, Inc.) compatible personal computer (PC) with appropriate input/output (I/O) equipment will be used for this control system. TA Engineering Company offers a man-machine-interface software called AIMAX/Plus-WIN (a registered trademark of TA Engineering Company, Inc.). This software is capable of multiple control and data acquisition functions and can use a number of input devices which are connected to the PC via a RS-232 connection. The I/O Plexer PCx (a registered trademark of duTec) was chosen for I/O signal processing.

The AIMAX software will be set up to monitor all the signals collected by the I/O Plexer and store this information in data files on the PC. The AIMAX software will also send out control signals to the I/O Plexer, which will convert the signals to the proper type by using removable I/O modules, thus controlling valves and pumps as necessary. The wiring for the I/O signals will be separated into high and low voltage and will be run in metal conduit with the intention of minimizing signal interference.

As another piece of the PC control system, an automatic telephone dialer will be connected to the I/O Plexer. If an alarm is activated and no one is at the demonstration site, the telephone dialer will be activated to notify project personnel of the alarm.

2.3.3.1 Process Control. There are three main equipment systems where process control is important: (1) the nutrient injection system, (2) the autosampler, and (3) the sampling pump system.

In the process trailer, the feed pumps, the feed line pressure, the feed line flowrate, and the feed tank liquid level will all be controlled or monitored. The feed pumps will be turned on or off and their flowrate adjusted as appropriate to deliver nutrients to the well. The feed line pressure and flowrates will be monitored to determine that (1) nutrients are delivered to the well in the proper amounts, (2) there is no plugging of the feed lines, and (3) there are no leaks in the feed lines. If an adverse condition is encountered, an alarm will be activated on the PC and the feed pump will be shut down (if appropriate). The feed tank liquid level will be monitored for a low liquid level that would indicate siphoning or a leak in the feed tank. A low liquid level condition will activate an alarm on the PC.

The autosampler system will be connected to the control system. The fraction collector will be manually programmed but will be turned on at the start of an intense sampling period by the PC. The three-way solenoid valves that control which line is being sampled will be cycled by the control system to allow proper purging and sampling.

The submersible centrifugal sampling pumps and compressed air system used to blow down the discharge tubing of the in-well pumps will be automatically controlled when operating in conjunction with the autosampler. For manual samples, the pumps will be manually activated using keyboard controls on the process control computer. The blow-down system will be activated during manual sampling by operating a manual valve in the air supply manifold.

The large recirculation pump will be manually activated and will operate continuously during the demonstration except that it can be automatically shut down if flow and pressure alarms indicate a catastrophic failure. The submersible centrifugal pump for monitoring the lower portion of the test site will be manually activated as needed during the routine sampling events.

2.3.3.2 Data Acquisition. Data to monitor and analyze the performance of this demonstration will be collected both manually and automatically. Sample analysis and data collection for VOCs, anions, and microorganisms will be done manually, not within the control system. Data collected by the control system will include down-well measurement of pressure, flowrate, temperature, pH, and redox potential. In addition, pressure, flowrate, and storage-tank liquid level will be measured for the nutrient injection system.

Pressure transducers will be used to obtain pressure readings from specific depths in all wells. Most of these pressure transducers will be the Keller PSI 210 S series, which transmit a 4-20 mA signal directly to the I/O Plexer. The flowrate of the recirculation pump will be measured using a turbine flowmeter located in the recirculation plumbing aboveground; the flowmeter generates a 4-20 mA signal. Series 169 pressure transducers (Keller PSI) and a Campbell Scientific, Inc. data logger will be used for some of the pressure measurements. The data logger will convert the Series 169 transducer signals to 4-20 mA signals which will then be read by the I/O Plexer.

The temperature, pH, and redox potential will all be measured using the H2O-G multiprobe (a registered trademark of Hydrolab Corporation). This is a collection of probes and circuitry assembled into one probe. Signals from the multiprobe are converted to 4-20 mA signals by an analog converter. Signals will then be read by the I/O Plexer.

2.4 FIELD APPLICATION

A well-to-well recirculation configuration will be used to mix the groundwater. Four monitoring wells are located on a line between the injection and extraction wells. This is not necessarily the configuration that would be used in a full-scale design but will provide the information needed to design a full-scale system. (Figure 2-3 illustrates the well network design including the groundwater recirculation wells and the monitoring wells for the demonstration.) Details of well construction for each of the site wells are given in the *FY 93 Site Characterization Work Plan for the VOC-Arid ID and 200 West Area Carbon Tetrachloride ERA* (Rohay et al. 1993) and the *In Situ Bioremediation Drilling and Characterization Work Plan* (Koepler 1994). A generalized cross section of the well completions is shown in Figure 2-7. The demonstration will be performed using the screens in the upper portion of the aquifer. Screens were installed in the lower part of the aquifer for monitoring and for potential future operations.

The following operations are planned for the field demonstration to promote a biologically active zone in the aquifer surrounding the injection well. Groundwater will be recirculated by extraction and injection at a rate of 30 gal/min. This operation will establish a mixing zone in the aquifer. Before injecting nutrients, the concentration of contaminants can be measured over time to determine the effect of groundwater mixing on these concentrations.

The nutrients, acetate and nitrate, will be injected into the aquifer to begin active bioremediation. These will be metered from separate bulk storage tanks to produce the desired in situ concentrations. One bulk storage tank will contain 20% by weight acetate (sodium acetate), and the other will contain 20% by weight nitrate (sodium nitrate and nitric acid). Nitric acid will be used as a partial source of nitrate (up to 5% by weight in the storage tank) because protons are consumed during denitrification and the acid will supply protons to help buffer pH changes. Acetate and nitrate will be injected separately into the aquifer in pulses of 0.5- to 2-hour duration at a frequency of 5 to 24 hours. The acetate and nitrate pulses will be skewed by between 0 and 5 hours. Dispersion in the recirculation pattern around the injection well will mix the acetate and nitrate, resulting in the desired microbial activity. The concentration of acetate in pulses will be between 100 and 10,000 mg/L. Nitrate pulses will be between 0 and 10,000 mg/L. The maximum concentration of nitric acid in the pulse after injection will be 200 mg/L as nitrate (pH 2.5).

The exact strategy of nutrient pulsing is being determined in simulations and will be finalized for the demonstration after additional site characterization information is obtained from the wells installed in early FY 1995 and from tracer test data. In any case, the nutrient injection will be designed so that injected nutrients are completely consumed within the aquifer and none are extracted into the recirculation system where they may cause fouling. Thus, injected nutrient concentrations will be low initially when the sediment microbial population is low and increase as the in situ utilization rate increases because of increased biomass.

By introducing the appropriate nutrient flux to the aquifer, the contaminants CCl_4 and nitrate will continue to be destroyed within the zone of microbial activity that is created.

Figure 2-1. Simulated Carbon Tetrachloride Destruction as a Function of Hydraulic Conductivity.

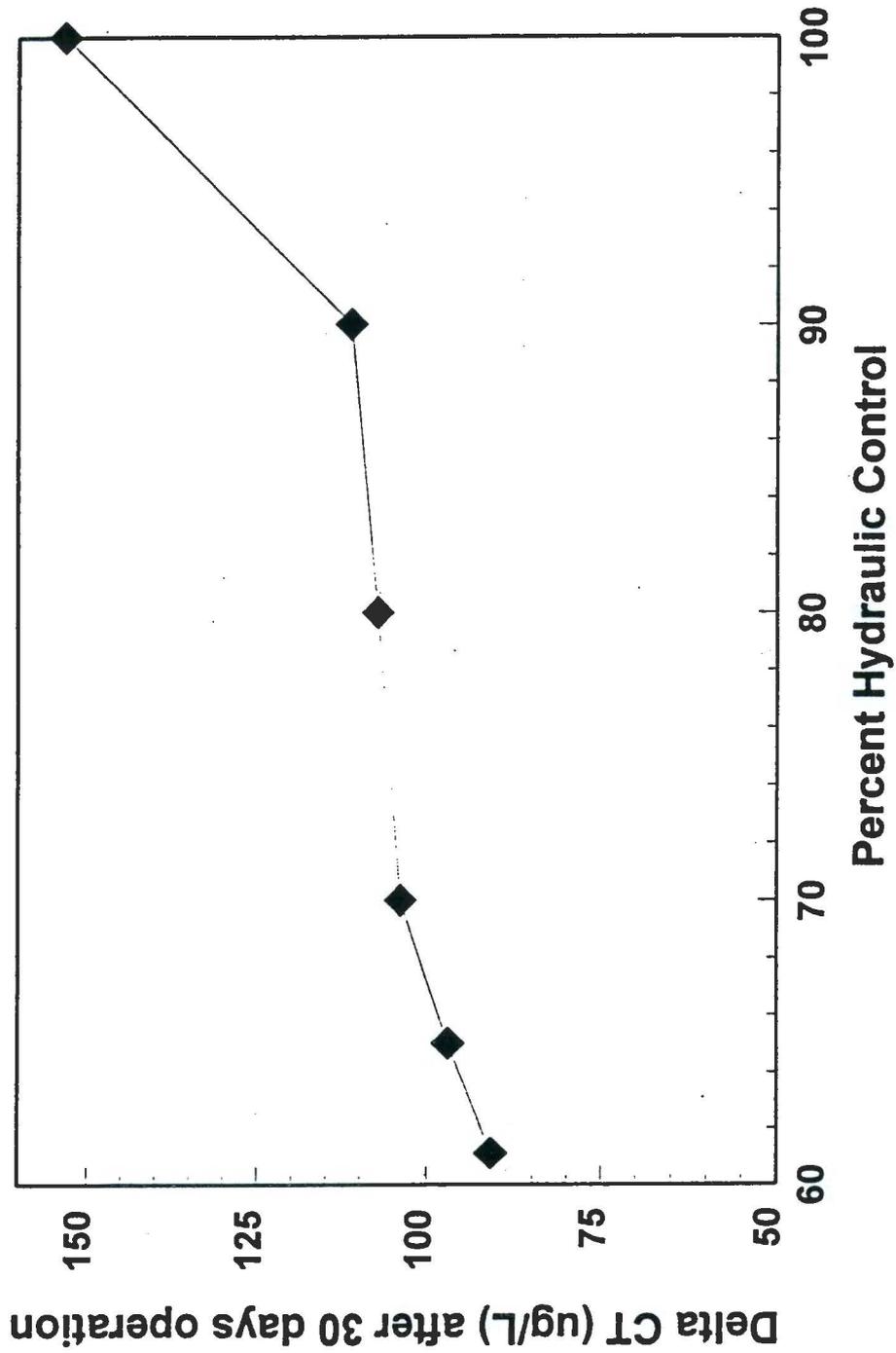
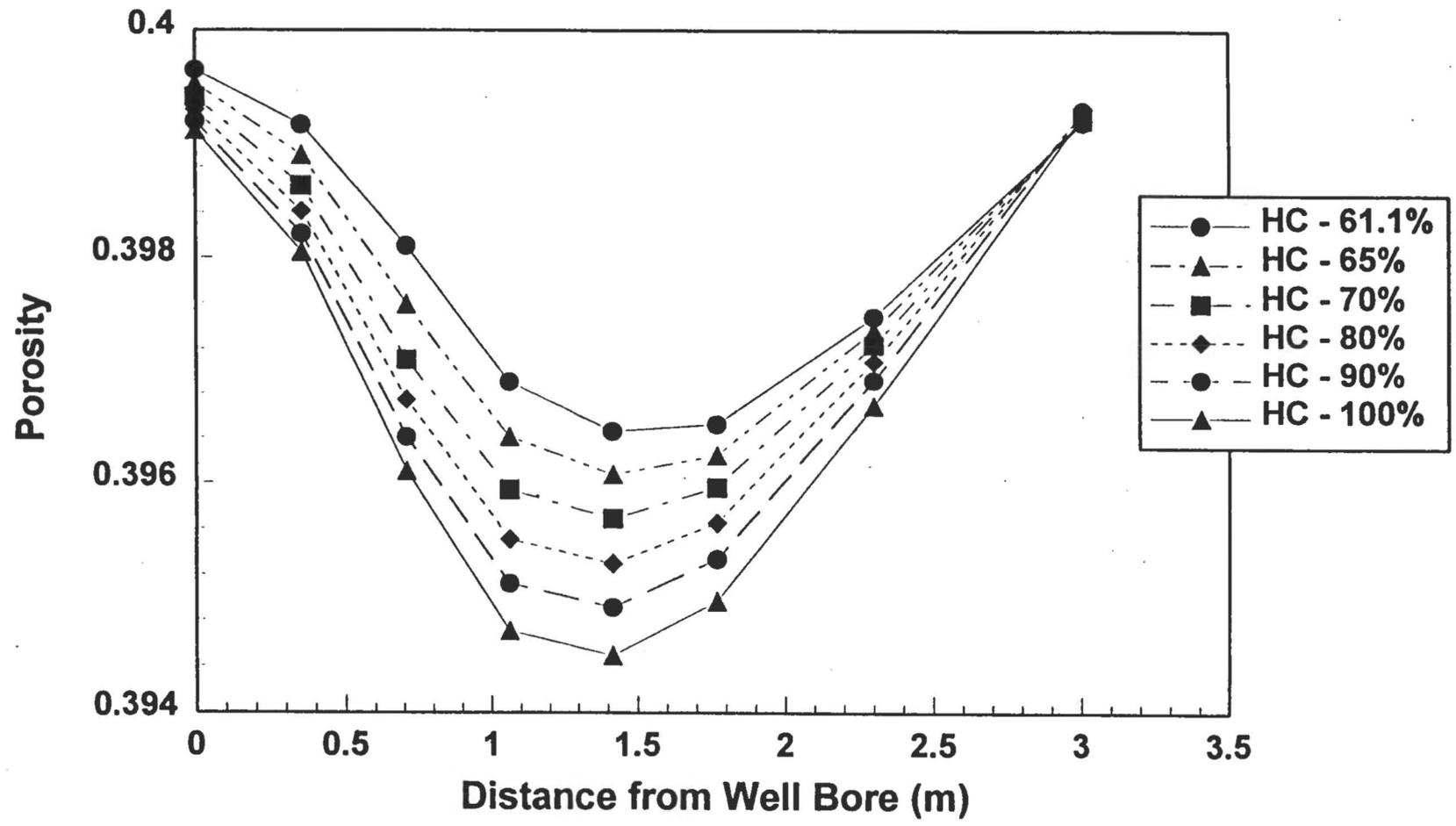


Figure 2-2. Simulated Biomass Distribution as a Function of Hydraulic Conductivity.



Surface recirculation with sample ports and in-line filters for entrained sediment

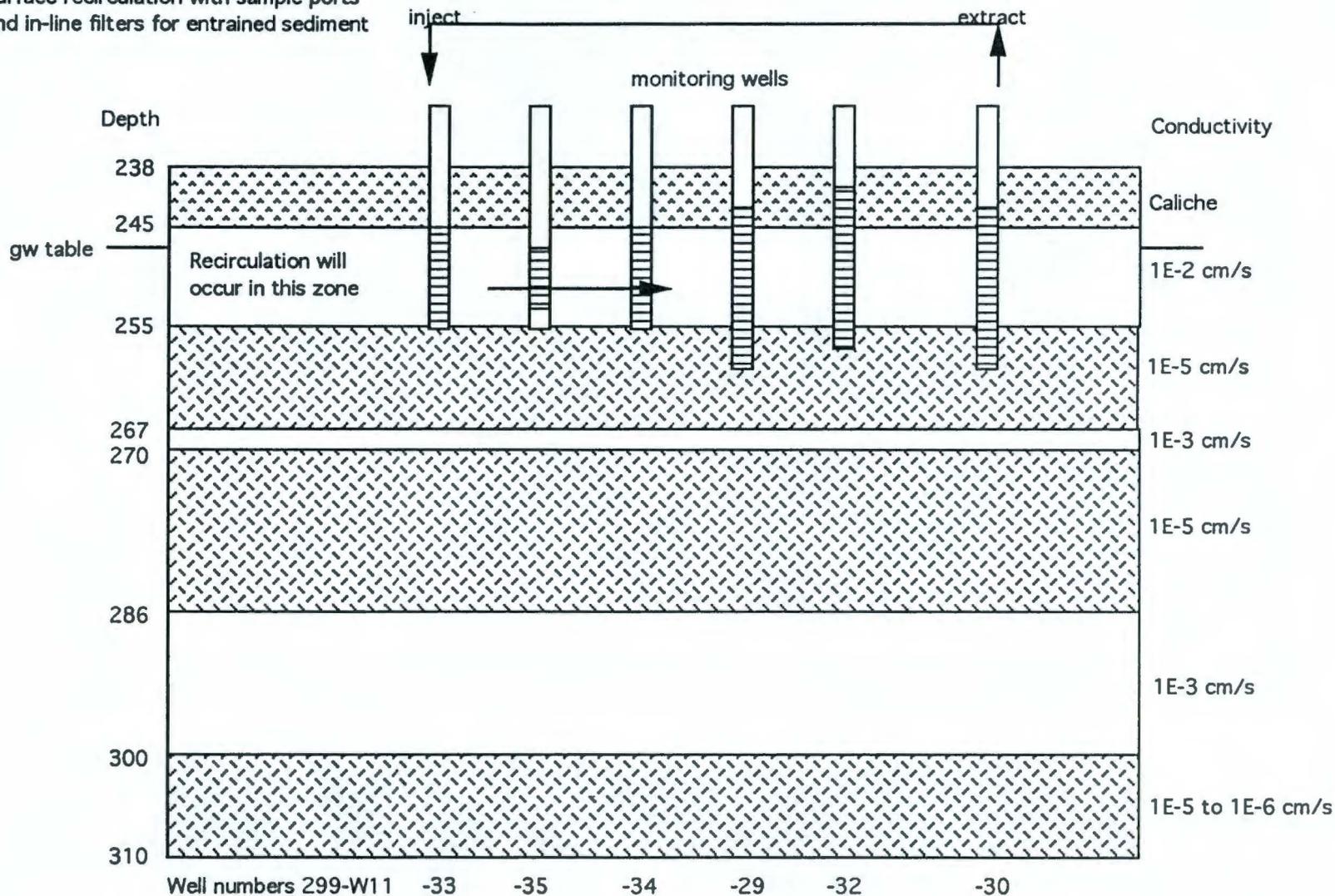
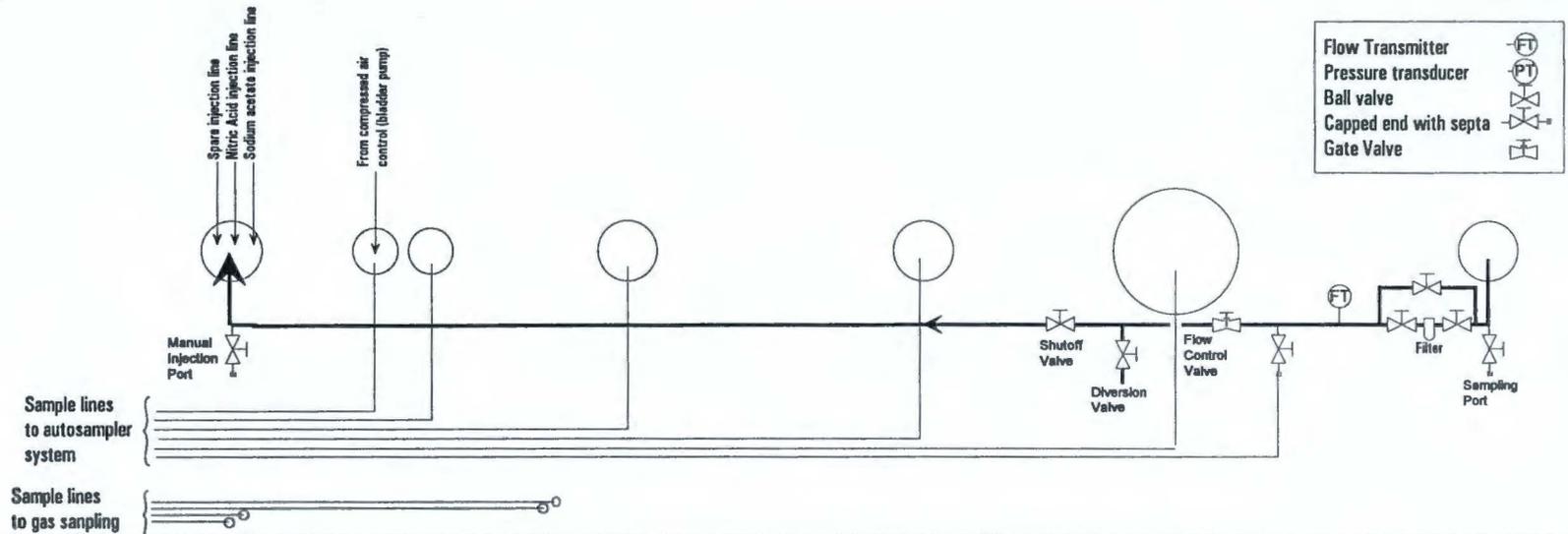


Figure 2-3. Groundwater Recirculation Pattern for Bioremediation Demonstration.

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Top view of well heads and piping going into and out of the wells.



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Vertical section through wells at the level of the aquifer.

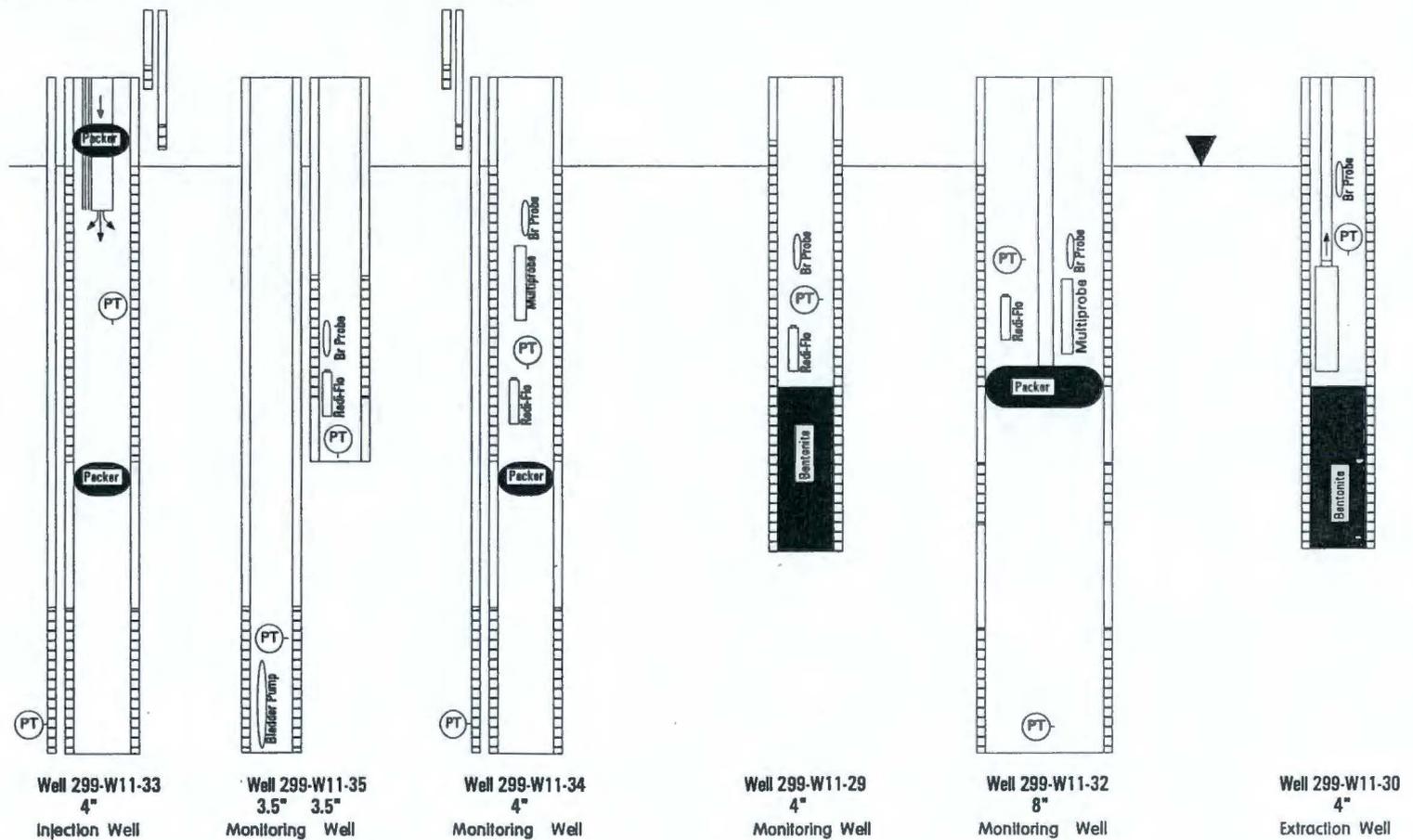


Figure 2-4. Equipment Schematic of Groundwater Recirculation System.

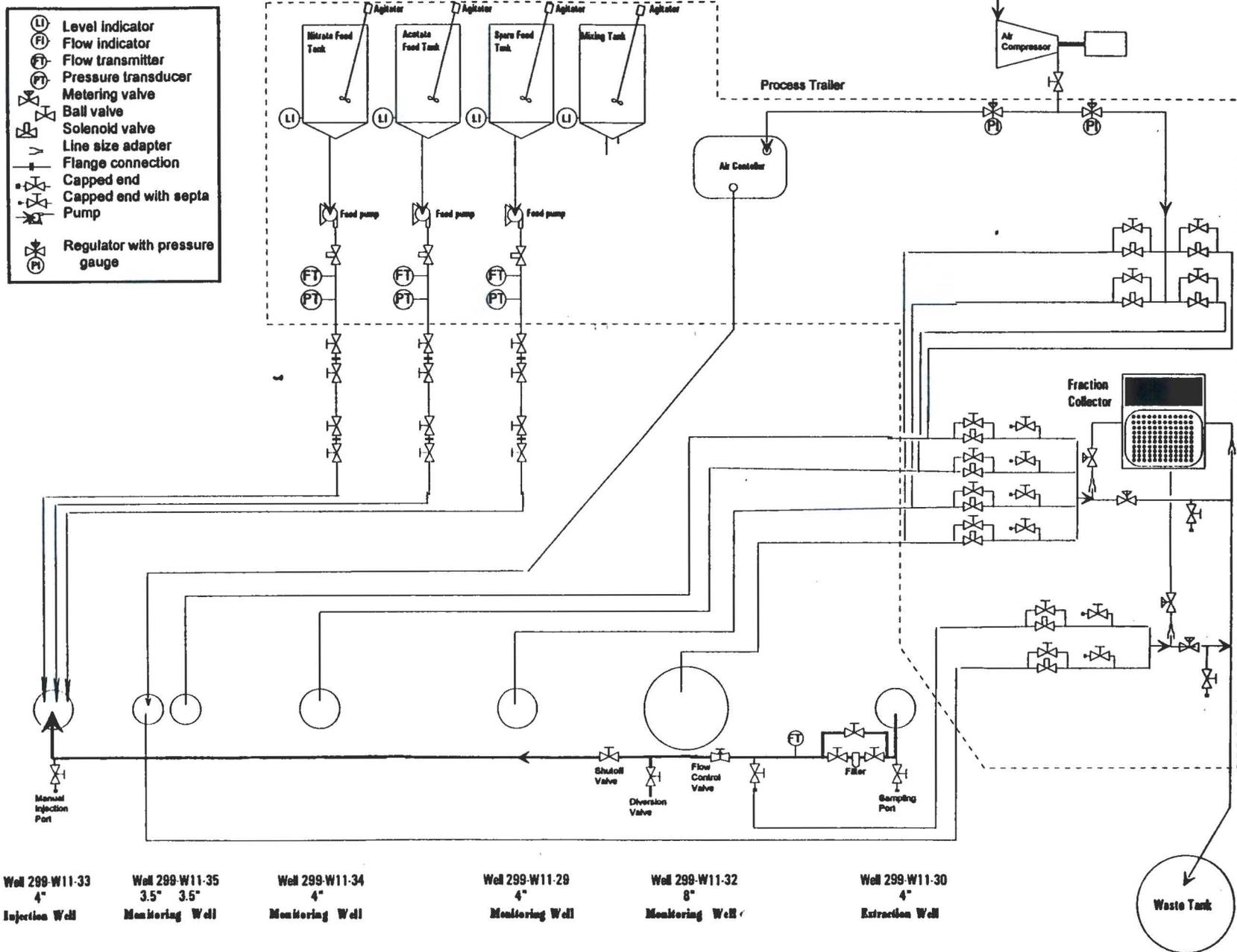


Figure 2-6. In Situ Bioremediation Demonstration Site Equipment Schematic.

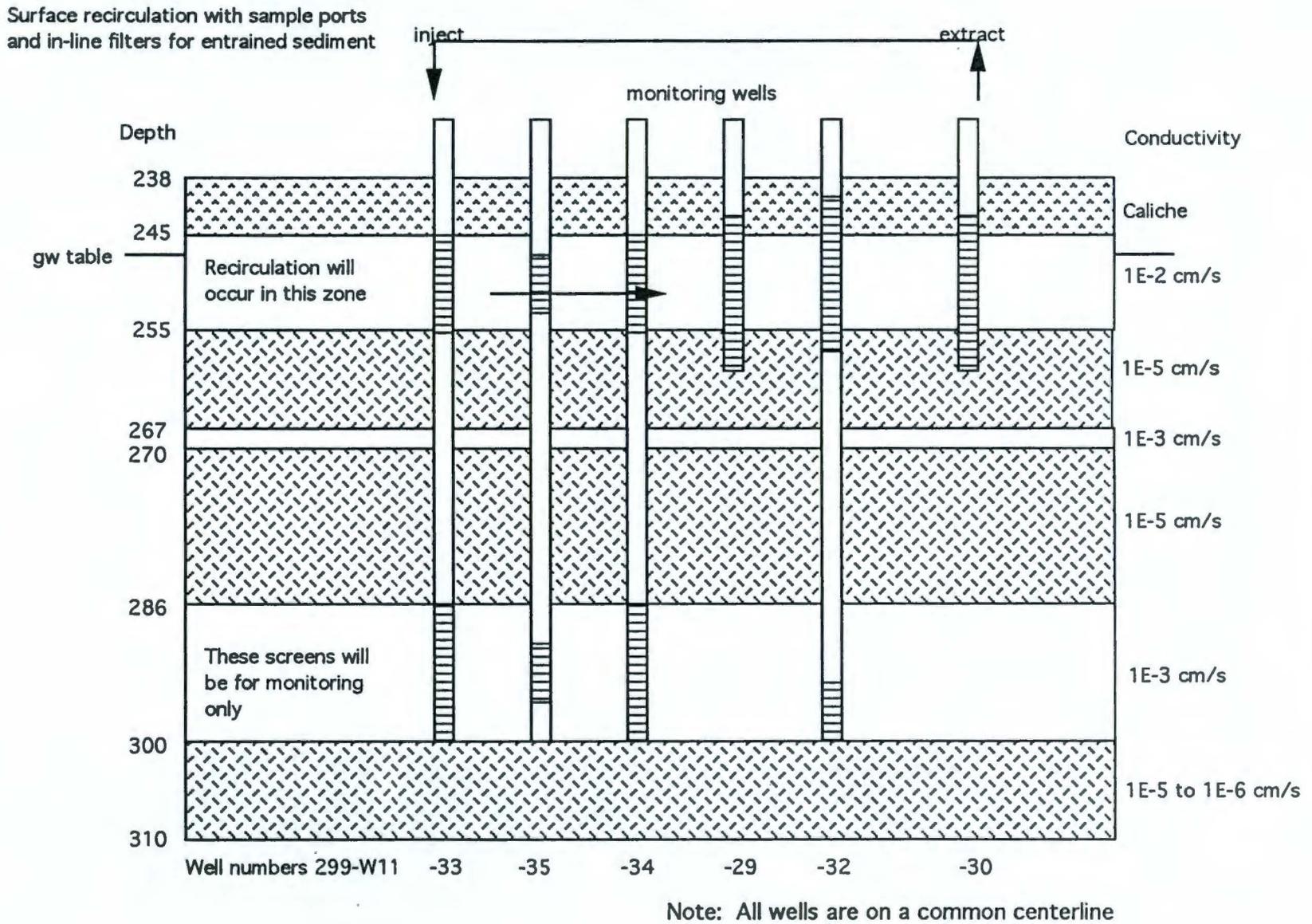


Figure 2-7. Well Configuration for the Bioremediation Demonstration.

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3.0 DEMONSTRATION OBJECTIVES AND PARAMETERS

3.1 DEMONSTRATION OBJECTIVES

The goal of this demonstration is not only to successfully degrade CCl_4 in the saturated zone, but also to collect the data with which to compare the bioremediation technology to other groundwater remediation methods and to develop and validate the knowledge and tools to apply this technology successfully to other saturated subsurface environments. This ITP is a guide to meet objectives. Specifically, primary objectives of the in situ bioremediation demonstration are to:

- Collect data for assessing the technology performance related to the baseline technology, pump-and-treat, stakeholder consideration, and the National Contingency Plan (NCP) Criteria for Evaluating Technology Alternatives (40 CFR 300.430).
- Demonstrate in situ biological destruction of CCl_4 and nitrate in the Hanford groundwater while minimizing unwanted byproducts. The performance goal is 15 g of CCl_4 destroyed per day based on process simulations.
- Demonstrate nutrient addition strategies that provide effective aqueous nutrient injection to remediate the contamination while minimizing the effects of biofouling around the injection well.
- Demonstrate a design methodology for deploying, controlling, monitoring, and determining the rate of in situ bioremediation to restore contaminated aquifers.

Detailed project objectives and data quality objectives (DQO) were developed based on the objectives of the demonstration. The objectives were used to develop the DQOs and in particular the sampling protocol and statistical analyses required to quantitatively show with field data that goals have been achieved. The following list of detailed project objectives was developed to guide data collection and analysis.

- Determine the area in which biodestruction of CCl_4 occurs
- Maximize and quantify the rate of CCl_4 destruction
- Quantify the mass of CCl_4 destroyed
- Quantify the rate of nitrate destruction
- Minimize and quantify the production of CHCl_3 and nitrite
- Minimize changes in aquifer hydraulic conductivity and well screen plugging.

Each detailed objective comprises specific tasks and data collection to (1) determine specific information for each parameter and (2) integrate information from specific data into analysis of the overall success of the demonstration. Measurement of demonstration parameters is further discussed in Section 3.2. The specific tasks and data required for the above detailed objectives are discussed in conjunction with the project DQOs and quality assurance (QA) procedures in Appendix A.

3.2 DEMONSTRATION PARAMETERS

3.2.1 Summary of Measured Parameters

Technical success will be evaluated by contaminant, water chemistry, microbiological, and process control measurements. Table 3-1 summarizes the primary parameters that will be measured during the demonstration and the devices used for these measurements. Further detail, including analytical methods, is contained in Section 3.3.

Table 3-1. Process Measurements.

Measurement	Device
VOCs	Gas chromatography (GC)
Anions	Ion chromatography
Cations	Inductively Coupled Plasma
Pressure	Pressure transducer
Flow	Turbine flowmeter
Temperature	Thermocouple on in situ multiprobe
pH	In situ multiprobe
Redox potential	In situ multiprobe
Aerobic heterotrophs	Spread plate enumeration
Denitrifiers	Most probable number (MPN) enumeration
Sulfate reducers	MPN enumeration
Iron reducers	MPN enumeration (sediment samples only)
Microbial identification	Biomolecular probes (detection independent of ability to culture)
Coliforms	Membrane filter enumeration
Microbial samples	Biocoupons

3.2.1.1 Contaminants. VOC and oxides of nitrogen compounds will be analyzed using groundwater samples extracted with submersible centrifugal sampling pumps or through ports in the recirculation plumbing at the surface.

3.2.1.2 Water Chemistry. Anions and cations will be analyzed using groundwater samples extracted with submersible centrifugal pumps or through ports in the recirculation plumbing at the surface. Other chemistry parameters such as pH, temperature, and redox potential will be monitored using an in situ probe located in two of the monitoring wells.

3.2.1.3 Microbiology. Samples for microbial enumeration and characterization will be collected in two ways. Enumerations and biomolecular probe analysis will be performed using groundwater samples as a baseline microbial monitoring technique. The biocoupons will be used to collect samples of biofilm for use in biomolecular probe analysis, enumerations, and biofilm characterization. The biocoupon analyses will be compared to results from groundwater samples to assess the effectiveness of the biocoupon monitoring technique. Sediment samples may also be collected from a sampling borehole installed during the demonstration. The technical details of the biomolecular probes and biocoupons are described in Appendix B.

3.2.1.4 Process Control. Process control measurements have been described in previous sections (see Section 2.3.3).

3.2.2 Performance Evaluation for Demonstration Objectives

Each of the four primary demonstration objectives listed in Section 3.1 is individually addressed in this section.

3.2.2.1 Collect Data for Technology Evaluation. Data will be collected using the plan outlined in Chapter 7.0 to provide enough information to assess the technology in terms of (1) overall protection of human health and the environment; (2) compliance with applicable or relevant and appropriate requirements (ARAR); (3) long-term effectiveness and permanence; (4) reduction of toxicity, mobility, or volume; (5) short-term effectiveness; (6) implementability; (7) cost; and (8) stakeholder acceptance. Using these parameters, in situ bioremediation can be compared to pump-and-treat technology based on the NCP criteria for technology evaluation and specific stakeholder criteria. The process simulator that has been calibrated with field and laboratory data will function as a tool allowing appraisal of full-scale applications.

This type of comparison is necessary because the design used for the demonstration is not the same as would be used for many full-scale applications. Because of the overall plume size and the need to obtain measurable responses for determining process performance, the field demonstration was designed to meet specific demonstration objectives not necessarily associated with full-scale design. In addition, before performing the demonstration, data was insufficient to determine a sound full-scale design.

3.2.2.2 Demonstrate In Situ Bioremediation of Nitrate and Carbon Tetrachloride. This objective will be successfully met if the experimental data indicate that biological activity was responsible for removing nitrate and CCl_4 from the groundwater. The experimental evidence required to support this conclusion is a simultaneous reduction in nutrient concentrations, increase in biomass levels, disappearance of contaminants, and appearance of metabolic intermediates or products (Madsen 1991). The performance goal is 15 g of CCl_4 destroyed per day based on process simulations. These simulations use kinetic information determined in laboratory studies as the basis for the reaction component of the simulator. The ramifications of this performance goal are discussed below. In addition, the production rate of nitrite and CHCl_3 will be quantified during initial active bioremediation operations (Phase 2). In the latter phase of field operation (Phase 3), techniques will be implemented to demonstrate control or degradation of these byproducts if the initial operating strategy is not effective in limiting CHCl_3 or nitrite production. These techniques will be based on laboratory experiments and field experience during initial operations.

Performance goals were influenced by the selected field design. The performance is a function of the reaction kinetics and the hydraulic control obtained. The reaction kinetics and hydraulic control are linked because the hydraulic control determines the amount of nitrate and CCl_4 that enter the reaction zone through the injection well. The kinetics of the CCl_4 destruction are first order in CCl_4 concentration and inhibited by the presence of nitrate above about 20 mg/L. The desired process operation requires dispersion-induced mixing of acetate and nitrate pulses at a distance away from the well bore so that (1) nitrate concentration is kept below 20 mg/L when in the presence of acetate pulses, and (2) biomass production does not occur adjacent to the well bore, leading to biofouling. Results of process simulations performed at varying percentages of hydraulic control are illustrated in Figure 2-1. For 100% hydraulic control, no additional chemical species would enter the reaction zone, and the reaction can be controlled completely via injection of desired amounts of acetate and nitrate. For low percentages of hydraulic control, the system is essentially "open," and the amount of nitrate entering the system renders process control difficult. Influent nitrate allows biomass to grow adjacent to the well when an acetate pulse is introduced. The performance of the system in terms of steady-state CCl_4 destruction is essentially constant from about 70% to 95% hydraulic control. Performance is greatly enhanced very near 100% and rapidly declines below 65% hydraulic control. Since 100% hydraulic control is not possible in the field, the design for the demonstration was selected to achieve a "simulated" hydraulic control of about 85%. Control in the field may not be as good as predicted in the simulation but can drop as low as 70% and the predicted performance will be the same. Thus, a performance goal was selected based on the process simulation. At the performance goal for CCl_4 destruction rate, the steady-state CCl_4 concentration at the monitoring well would be about 1.3 mg/L for 85% hydraulic control and 1.7 mg/L for 70% hydraulic control, assuming that the far-field water entering the reaction zone has a constant CCl_4 concentration of 2 mg/L.

The number of samples necessary to demonstrate that there is a difference in CCl_4 concentration between the injection well and a monitoring well was determined based on the anticipated CCl_4 destruction rate. A statistical analysis was performed using a Student's t test and assuming a standard deviation of 25% of the mean for the field samples and an initial CCl_4 concentration of 2 mg/L. Over the course of the demonstration, the performance goal of destroying 15 g of CCl_4 per day would result in a 100 p/b difference in concentration between the injection and monitoring wells. To demonstrate statistically that the concentration at the monitoring well is 100 p/b lower than the concentration at the injection well with 80% confidence, 40 samples at each location are required. For a confidence level of 90%, 100 samples are required. After reaching steady-state, the CCl_4 concentration for the performance goal results in a monitoring well concentration of about 1.6 mg/L compared to the initial concentration of 2 mg/L. To demonstrate that steady-state concentration is lower than the initial concentration with a confidence of 95%, 20 samples are required. However, it will be important to demonstrate statistically that the steady-state concentration is lower by a specific amount in order to use the data to back-calculate the actual CCl_4 destruction rate. For an observed difference of 0.4 mg/L with a standard deviation of 25% of the mean, 100 samples are needed to statistically demonstrate that the difference in the initial and steady-state concentrations is 0.3 mg/L with a confidence of 90%. Thus, to quantify the CCl_4 destruction rate, a sampling strategy based on the above calculations will be implemented (see Chapter 7.0). Using an example CCl_4 destruction rate, Figure 3-1 shows example field data for the selected sampling plan.

Loss of CCl_4 due to biotic reactions will be determined by comparing the losses measured during operation of the treatment zone in a control mode to losses measured during active bioremediation. The first two months of the demonstration will be used to establish abiotic losses of contaminants because of the groundwater mixing employed at the site. Groundwater will be mixed with no addition

of nutrients so that these losses can be quantified. In the subsequent phases of the demonstration, nutrient injection will be employed to stimulate bioremediation of the target contaminants. Therefore, by comparison of the control and treatment losses, the effectiveness of the bioremediation treatment can be quantified.

Reduction in nutrient concentration will be demonstrated by comparing tracer pulse concentration profiles to the concentration profiles of nutrient species at specific monitoring locations. Any reduction in the concentration profiles of the nutrient species beyond that exhibited by the tracer can be attributed to chemical or biological reactions because laboratory experiments with sediment from the test site indicate that there will be almost no sorption of the injected species. These conclusions will be validated in several ways.

To demonstrate that acetate and nitrate are biologically destroyed, the following activities will be performed.

- The standard injection strategy, in which acetate and nitrate are allowed to mix within the treatment zone, will result in the degradation of both acetate and nitrate. Pulses of nitrate only will result in much lower degradation of the injected species because microbial degradation of nitrate is linked to acetate utilization through the energy-yielding metabolic pathways. Some loss of nitrate may occur as a result of endogenous respiration or bioaccumulation related to survival strategies of the microbes. This loss is estimated to be small in comparison with the degradation of nitrate using the primary metabolic pathways. Thus, responses of the nutrient species at the monitoring points for the standard injection strategy can be compared to the response when only one of the nutrient species is injected. For instance, increased concentrations of nitrate at a monitoring point compared to concentrations observed when both acetate and nitrate are injected together will demonstrate that the loss of nitrate in the treatment zone is linked to the availability of acetate. This implies that biological processes are responsible for the loss of acetate and nitrate.
- Less frequently, the responses of nutrient species will be compared to the response of a conservative tracer to demonstrate that the nutrients are not conservative within the treatment zone. These tests will be performed in conjunction with hydraulic tracer studies that will be conducted each month. The frequency of these tests is limited by the buildup of the conservative tracer within the treatment zone.
- The microbial reactions will also produce unique products that are measurable. The reaction intermediates most readily measured are nitrite and CHCl_3 . Thus, aqueous samples will also be analyzed for the appearance of nitrite and CHCl_3 . In addition, the pH of the groundwater along with the CO_2 levels just above the water table will be monitored since the denitrification will cause an increase in these parameters. To monitor for increased assimilatory biotic activity, the biomass concentration in the groundwater at specific monitoring locations will be measured. In addition, a soil sampling well may be installed in FY 1996 to collect sediment samples for biomass measurement.
- Standard enumerations, DNA probes, and biocoupon analyses will be used to demonstrate that microbial growth and/or activity has been stimulated within the subsurface. Section 3.2.1 and Appendix B describe the specific role of biocoupons and biomolecular probes in providing key data for this purpose.

3.2.2.3 Demonstrate Engineering Strategies to Minimize the Effects of Biofouling. This objective will be successfully met if the design tool/process simulator is effective for process control during Phase 2 operations and in determining an appropriate operating strategy for Phase 3 operations. The experimental evidence required to support this conclusion is the variation of pressure and flow rate profiles for the injection well during the remediation period. In addition, laboratory flow cell experiments using porous media to demonstrate the ability to control biofouling will provide confirmation of the field results.

The primary measure used to demonstrate that biofouling did not limit bioremediation operations is the pressure and flow data at the injection screen. The groundwater pump will be designed such that increases in pressure of up to 100% of the initial pressure can be tolerated without a reduction in flow rate. Ideally, the pressure at the injection screen will increase to no more than 200% of the initial pressure during the course of remediation. Greater increases may be acceptable if the remediation process can still proceed at these higher pressures and potentially reduced flow rate. Tracer tests will be used as a means to measure whether hydraulic control of the treatment zone is being maintained during the demonstration and, in particular, during changes in the pressure at the well. Significant increases or decreases in conservative tracer travel times to monitoring points will be a qualitative indication that the hydraulic recirculation zone has changed. Unfortunately, models will not be useful for prediction of the precise changes in the recirculation pattern because of these changes in tracer travel times. Thus qualitative decisions will be made with respect to this issue. The primary quantitative measure of biofouling will be the injection pressure coupled with the continued operation of the remediation process.

3.2.2.4 Demonstrate a Design Methodology for In Situ Bioremediation. This objective will be successfully met if the effectiveness of the design tool/process simulator (process model developed based on site characterization and laboratory transport and kinetic experiments) can be assessed. Operating strategies and the predicted in situ process response generated using the design tool will be compared to field measurements. This will verify the ability of the design tool to provide useful and accurate information that can be used for in situ bioremediation design and operation. The utility of the design tool will be directly tested in determining the operating strategy for Phase 3 operations with respect to controlling CHCl_3 concentrations and increasing rates of contaminant destruction. In choosing the operating strategy for Phase 3, a timely integrated analysis of field data and laboratory data is required so that changes in the operating strategy can be implemented during the field demonstration.

3.3 SAMPLING AND ANALYTICAL PROCEDURES

The following summarizes the sampling and analytical procedures that will serve to measure demonstration parameters. A discussion of DQOs and precision, accuracy, representativeness, completeness, comparability parameters is included as Appendix A.

A brief summary of each method is provided. Trained personnel will conduct all the procedures. Details of project-specific procedures performed at the Hanford Site will be written as SOPs and maintain as part of the project documentation. (Summaries of some of the primary operations associated with the demonstration are found in Chapter 7.0.)

3.3.1 Volatile Organic Compound Sampling and Analysis

3.3.1.1 Analytical Method Summary. Water effluent is sampled with a syringe containing hexane from the sample pump effluent line. The sample is extracted and added to a graduated sample tube for storage and further analysis by EPA Method 8010/8020.

The extracted sample is injected into a GC with a electron capture detector (ECD) and separated with a DB-624 capillary column. The compounds are identified on the basis of retention time by comparison to standards. Concentrations of the samples are quantified by area response using a standard curve to convert area to concentration.

3.3.1.2 Applicability. The demonstration will employ this procedure for the analysis of halogenated volatile organics in groundwater. CCl_4 , CHCl_3 , dichloromethane (methylene chloride) (CH_2CL_2), tetrachloroethylene (PCE), and trichloroethylene (TCE) will be reported with an expected detection limit of 5- $\mu\text{g/L}$ each. Additional high volatile halogenated organics may be analyzed by this procedure.

Depending upon the concentration of the samples, sensitivity of the ECD, and the low-level detection requirements, the halogenated volatile organic compounds may need further analysis by GC/mass spectrometer for a lower level sensitivity.

3.3.1.3 Summary of Sampling Method. The sampling system will be purged for 10 minutes before sampling at each sample location. Purgewater will be collected in the onsite purgewater tank for subsequent disposal. Samples will be obtained using a flow-through cell equipped with a septa for withdrawing samples that have not been exposed to the atmosphere. A 1-mL sample will be withdrawn from the cell into a syringe containing 1-mL of hexane. The syringe contents will then be dispensed directly into a screw-cap graduated cylinder containing 4-mL of hexane with the cylinder in a beaker with ice. The hexane and sample will be thoroughly mixed to extract the VOCs and then allowed to settle so that the hexane and water phases separate. The volumes of sample and hexane will be recorded and the hexane portion dispensed into GC vials so that no head space remains. Vials will be immediately crimp sealed and placed in the onsite freezer with the septa on the bottom. Three samples from each sampling location will be collected. A hexane blank will be prepared for each sampling event. Samples will be transported to the 324 building for analysis by GC.

3.3.1.4 Gas Samples for Volatile Organic Compounds. The concentration of VOCs in gas samples will be determined with the EPA Method 524 using purge-and-trap GC. VOCs targeted for measurement include CCl_4 , CHCl_3 , CH_2CL_2 , PCE, and TCE. Full details of the method will also be documented in an SOP per Chapter 7.0 of this ITP.

Sampling will be performed using the borehole sampler as described in Section 2.3.2.

3.3.2 Anion Sampling and Analysis

3.3.2.1 Analytical Method Summary. Samples diluted with a carbonate solution are analyzed with an ion chromatograph per American Society for Testing and Materials Method D4327-88. The samples are injected into a stream consisting of carbonate eluent and passed through an ion suppressor, guard column, and separator column. The separated ions are then measured by conductivity or a conductivity/absorbance combination. The ions are identified on the basis of

retention time by comparison to standards. Concentration of the samples is quantified by area response using a standard curve to convert area to concentration.

3.3.2.2 Applicability. The demonstration will employ this procedure for the analysis of the following anions in groundwater: acetate, bromide, chloride, fluoride, nitrate, nitrite, phosphate, and sulfate. Each will be reported with an expected minimum detection limit of 1 mg/L. Sample dilutions can be varied according to the concentrations of the stock samples and to resolve interference difficulties.

Varying methods of sample preparation will allow different analyses to be conducted. Bioreactive samples may be analyzed after filtration to remove particulate matter. Water samples may also be analyzed after extraction, filtration, and dilution.

3.3.2.3 Manual Sampling for Anions. Manually obtained samples will be collected using the same sample port as described for VOC sampling (Section 3.3.1). The sampling system will be purged for 10 minutes before sampling at each sample location. Purgewater will be collected in the onsite purgewater tank for subsequent disposal. Sampling will consist of 5-mL aliquots dispensed through 0.2 μm sterile syringe filters into sterile 15-mL snap-capped sample tubes. Three samples from each sampling location will be collected and placed in the onsite freezer. Samples will be placed in a cooler on ice and transported to the 324 building for analysis by ion chromatography.

3.3.2.4 Automated Sampling for Anions. During the intense sampling period, samples will be collected for analysis of anion concentrations (nitrate, acetate, etc.). This automatic sample collection will take place using the autosampler system, which basically consists of down-well pumps and a fraction collector (see Section 2.3.2 for a description of the autosampler system equipment). The procedure for collecting samples will involve controlling the autosampling rate via the PC, collecting the samples with the autosampler system, and storing the samples for transport and later laboratory analysis.

The control of automatic sampling of groundwater is described in Section 2.3.3. A PC will be running the AIMAX/Plus-WIN software and will use an I/O Plexer network to collect and disburse I/O signals. The fraction collector, sample pumps, and solenoid valves will be controlled by this control system.

The PC control system will activate the submersible centrifugal pump controller and select the appropriate pump for sampling. The controller will allow the pump to operate for a predetermined time to purge the sampling lines and then activate the autosampler system. The PC control system will then coordinate the actions of the fraction collector, the pump controller and selector, and a series of three-way solenoid valves to collect samples automatically from the appropriate locations.

The samples will be collected in open test tubes held in the fraction collector's test tube rack. The test tubes will contain 0.5 mL of 300 mM carbonate solution so that after 4.5 mL of groundwater sample is added (1) the sample will contain the appropriate carbonate concentrations for use on the ion chromatograph and (2) the sample will be above pH 10 to inhibit microbial activity. When the fraction collector is within 10 to 20 test tubes of finishing all 120 test tubes, one of the site personnel will cap the test tubes with samples and move the capped test tubes to a test tube rack. The empty spots in the fraction collector will be filled with new test tubes (with the 0.5 mL of 300 mM carbonate solution). The collected samples will be stored in a freezer until needed for analysis. Test

tubes filled with samples will be removed and replaced as needed to obtain all of the samples. After dispensing 120 samples, the fraction collector starts over with the test tube in the initial spot.

3.3.3 Microbiological Sampling and Analysis

The sampling system will be purged for 10 minutes before sampling at each sample location. Purgewater will be collected in the onsite purgewater tank for subsequent disposal. Samples will be obtained using a flow-through cell equipped with a septa for withdrawing samples that have not been exposed to the atmosphere. A 15-mL sample will be withdrawn from the cell with a sterile prepurged gas-tight syringe and dispensed into sterile prepurged test tubes sealed with a butyl rubber septa. Six samples from each sampling location will be collected. Microbial measurements are expected to have a 100 colony forming unit/mL detection capability. Samples will be placed in a cooler on ice and transported to the 324 building. Samples will be handled in an anaerobic glove box Model 1025M (a registered trademark of Forma Scientific, Inc.). Three samples will be used for analysis of denitrifiers and sulfate reducers using the specific modifications of the standard MPN technique outlined in a project SOP. All the dilutions will be made with 0.1% sodium pyrophosphate. Three samples will be packaged for overnight shipment to an offsite laboratory for analysis of total coliforms using the membrane filter technique (APHA 1989). Aerobic heterotrophic bacteria will also be measured at the 324 building by spread plate method (APHA 1989).

In addition to the above analyses, biocoupons and biomolecular probes will be used for microbial characterization. Details of these procedures are described in Appendix B.

3.3.4 Cation Sampling and Analysis

The sampling system will be purged for 10 minutes before sampling at each sample location. Purgewater will be collected in the onsite purgewater tank for subsequent disposal. Sampling will consist of 5-mL aliquots dispensed through 0.2 μ m sterile syringe filters into sterile 15-mL snap-capped sample tubes. Three samples from each sampling location will be collected and placed in the onsite freezer. Samples will be preserved using nitric acid to reduce the pH of the sample below pH2 and shipped to a contract laboratory for analysis.

Samples will be analyzed using EPA Methods 3005A and 6010A. The former is an acid digestion procedure used to prepare water samples for analysis. The latter is a commonly employed multianalyte analytical procedure using inductively coupled argon plasma spectroscopy. The demonstration will measure each of the following elements at an expected minimum instrumental detection limit in μ g/L:

aluminum (45)	calcium (10)	magnesium (30)	silver (7)
antimony (32)	chromium (7)	manganese (2)	sodium (29)
arsenic (53)	cobalt (7)	molybdenum (8)	thallium (40)
barium (2)	copper (6)	nickel (15)	vanadium (8)
beryllium (0.3)	iron (7)	potassium (*)	zinc (2)
cadmium (4)	lead (42)	selenium (75)	

(*) Highly dependent upon specific operating conditions.

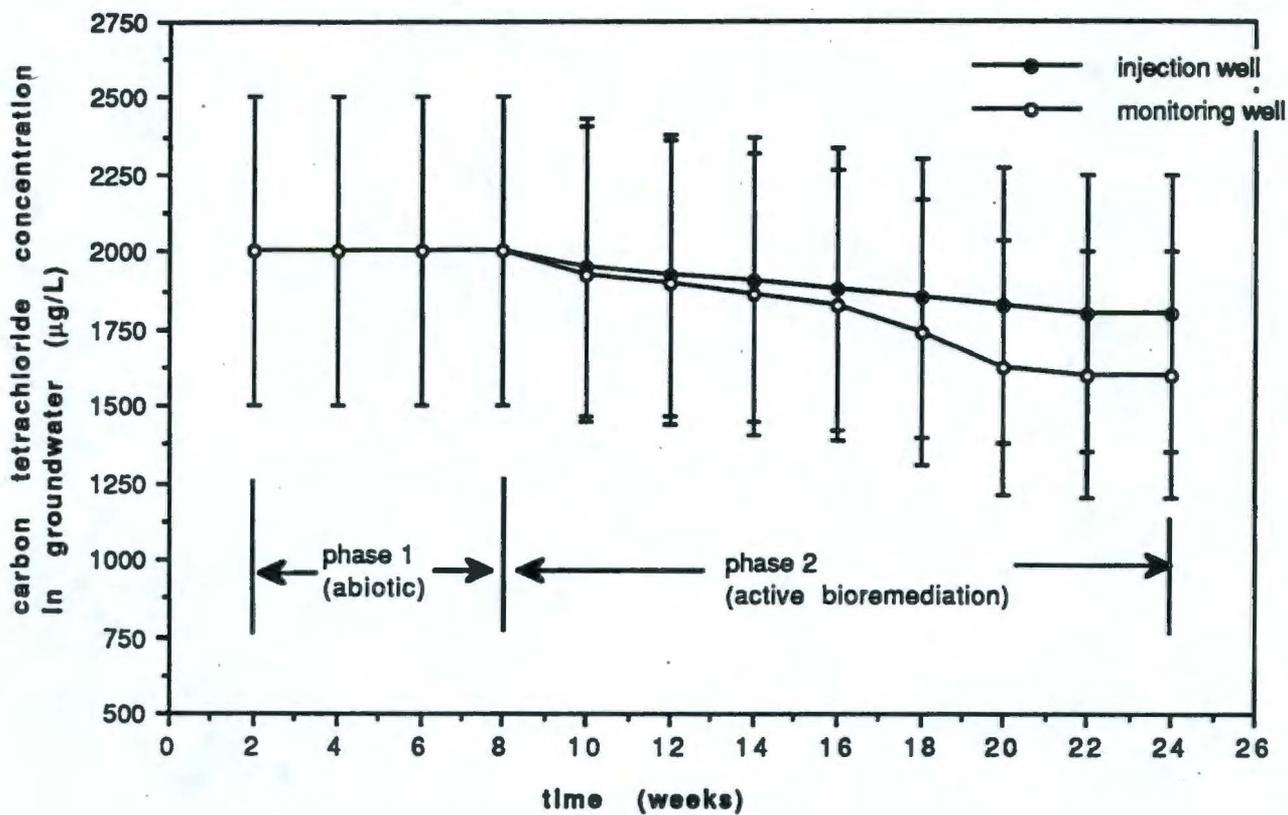
Instrumental detection limits are from Winge, Peterson, and Fassel (1979). These concentrations are identical to those appearing in EPA Method 6010A (EPA 1992). The actual method detection limits are sample dependent and may vary as the sample matrix varies (EPA 1992).

3.3.5 Gas Sampling and Analysis for Carbon Dioxide and Methane

Samples for CO₂ will be analyzed in the field using a field instrument and in the laboratory with a respirometer. Methane will be analyzed in the laboratory using a GC. A project-specific SOP will be prepared detailing the method per Chapter 7.0 of this ITP.

Samples will be collected with the borehole sampler as described in Section 2.3.2.

Figure 3-1. Example Results Based on Bioremediation Demonstration Sampling Schedule.



4.0 REGULATORY COMPLIANCE

This section discusses the regulatory compliance requirements for the in situ bioremediation field demonstration project. The major federal and state environmental laws that must be considered in the technology demonstration include the *National Environmental Policy Act* (NEPA); the *Comprehensive Environmental Recovery, Compensation, and Liability Act of 1980* (CERCLA); the *Resource Conservation and Recovery Act* (RCRA); the *Safe Drinking Water Act* (SDWA); the *Clean Water Act* (CWA); the *Clean Air Act* (CAA); the *Emergency Planning and Community Right-to-Know Act* (EPCRA); and the *Model Toxics Control Act*. Because of the limited nature of the test, no requirements under the CWA or the CAA are expected to exist.

4.1 NATIONAL ENVIRONMENTAL POLICY ACT

The in situ bioremediation NEPA is the basic federal charter for protecting the nation's environment. NEPA's focus is to ensure that federal agencies such as DOE give appropriate consideration to environmental impacts in their decision-making processes. DOE is required to examine all actions that affect the environment to determine whether they are major Federal actions that may significantly affect quality of the human environment. Actions may require preparation or supplement of an environmental impact statement, an environmental assessment, or may be categorically excluded from further study under existing documentation.

On November 23, 1994, DOE determined the proposed demonstration is among the class of activities subject to categorical exclusion under B6.2 found in 10 CFR 1021, Subpart D. Such actions, individually or cumulatively, do not have a significant effect on the human environment or are otherwise precluded by regulation from additional NEPA review.

A Cultural Resource Review (HCRL #93-200-158) of the site was performed by the Hanford Cultural Resources Laboratory. The review found no known cultural resources or historic properties located at the demonstration site. Survey results were considered in the NEPA compliance process.

Ecological surveys performed in the spring and summer of 1993 have determined no plant or wildlife species of concern will be impacted by work activities associated with the bioremediation wells, (Westinghouse Hanford Company survey number 93-200-57). Results were confirmed by PNL survey (#94-PNL-023) and considered in NEPA compliance process.

4.2 COMPREHENSIVE ENVIRONMENTAL RESPONSE, COMPENSATION, AND LIABILITY ACT

CERCLA is the governing statute for response actions being conducted in the 200 West Area to remediate CCl_4 releases. CERCLA was designed to manage the unplanned, uncontrolled releases of hazardous substances as well as provide a roadmap for the cleanup of abandoned sites. CERCLA is one of several federal laws that require reporting of "hazardous substance releases to the environment" above certain threshold amounts—reportable quantities (RQ). These limits can be found in 40 CFR 302, and apply to a 24-hour release period. Qualifying releases must be reported to the National Response Center in Washington, DC. (The final RQ for CCl_4 is currently 10 lb, which is

equivalent to over 500,000 gal of water contaminated at 2 p/m. The final RQ for nitric acid is 1,000 lb [454 Kg]. Less than 10 kg/24 hour of nitric acid is planned for maintaining bioremediation.)

The CERCLA cleanup framework governs CCl₄ remedial efforts at the 200 West Area. Treatability studies benefit from relaxed requirements under the CERCLA process found at 40 CFR 300.430 as part of the remedial investigation/feasibility study process. The EPA and DOE's Environmental Restoration program have directed that the demonstration of innovative treatment technologies be included as part of the remedial action program. The in situ bioremediation demonstration will serve as a treatability test in support of the site remediation goals. Under CERCLA Section 121, no federal or state permits will be required for the field demonstration, although substantive ARARs must be met in the final remedy action.

The EPA and the Washington State Department of Ecology (Ecology) have been briefed on the plans for the field demonstration and have indicated their support to proceed subject to the review and approval of this ITP. Concurrence with this ITP on a National Priority List Change Form will be the avenue by which EPA and Ecology formally affirm that no permits are required and any necessary requirements have been addressed.

A Community Relations Plan (CRP) has been developed for the Hanford Site Environmental Restoration Program and is applicable to remedial actions at the demonstration site. The CRP discusses Hanford Site background information, history of community involvement at the Hanford Site, and community relations programs that the DOE, Richland Operations Office (RL), the EPA Region X Office, and Ecology will cooperatively implement throughout the cleanup of all the operable units at the Hanford Site. Community relations activities associated with the 200-ZP-1 Operable Unit will be conducted under this overall Hanford Site CRP. Additionally, the DOE technology development program has provided stakeholder participation in the development phases of this bioremediation feasibility study. Participation will be continued with a stakeholder acceptance analysis upon conclusion of the final performance evaluation report.

4.3 RESOURCE CONSERVATION AND RECOVERY ACT

Subtitle C of RCRA establishes a comprehensive program to regulate the generation and management of hazardous waste. Administered by Ecology and EPA, RCRA Subtitle C requirements are contained in *Washington Administrative Code* (WAC) Chapter 173-303 and in 40 CFR 260-272, and apply to the generation, accumulation, treatment, storage, and disposal of hazardous/dangerous waste. Releases of nonradioactive effluent containing dangerous waste are regulated by WAC 173-303.

Groundwater at the proposed bioremediation site has been found to be contaminated with an F-listed spent solvent waste, CCl₄. The in situ bioremediation technology does not require interfaces with other remediation technologies because the two primary contaminants present in the groundwater, CCl₄ and nitrate, can be remediated using this technology. Furthermore, there are no remedial process waste streams containing contaminants that require additional treatment other than the small quantity of sample waste and purgewater. Additionally, as a general rule, any waste generated during this test would ostensibly fall under the Research, Development, and Demonstration (RD&D) permit requirements of RCRA and be considered Investigation-Derived Waste (IDW). However, since this action is being performed under the auspices of CERCLA, no RCRA permit is required for the generation, treatment, storage, or disposal of this waste if it is managed onsite. It should be noted

that all substantive requirements that are established as ARARs will be met in the final remedial action if not previously waived.

Field operations will conduct all regulated solid waste disposal, regardless of whether a regulated hazardous or dangerous waste, in accordance with established procedures. Control of CERCLA and other past practice IDW is performed according to Environmental Investigation Instruction (EII) 4.3 (BHI 1994). Plans issued under this procedure are approved by RL and the Lead Regulatory Agency (EPA), before implementation. Groundwater waste that is generated from groundwater sampling, well development, aquifer tests, etc., will be handled according to established Hanford purgewater requirements. Management procedures are described in EII 10.3 (BHI 1994). Purgewater containing constituents in excess of collection criteria will be collected and stored in Modutanks (a registered trademark of Modutanks, Inc.) immediately east of the 200 East area. This water will be treated in accordance with all applicable regulations before discharge to the soil column or surface waters on the Hanford Site.

It should be noted that the proposed addition of bionutrients to the recirculating groundwater does not constitute waste disposal. The nutrient supplements are essential elements to control the in situ remedial processes and are not waste by the definition associated with hazardous/dangerous waste regulations.

4.4 SAFE DRINKING WATER ACT

It is anticipated that the in situ bioremediation will reach clean-up levels better than other innovative or baseline technologies. EPA SDWA standards are the final target values for full-scale application of the technology. These maximum contaminant levels are 5 p/b for CCl_4 and 10 p/m (as N) for nitrate. Because the demonstration involves only a very limited volume within a much larger contamination plume, there is no expectation that these levels can be achieved or persist upon conclusion of field demonstration.

Under state regulations, WAC 173-218 sets forth the procedures and practices applicable to the injection of fluids through wells. Provisions of the chapter are designed to accomplish the following:

- Satisfy the intent and requirements of Part C of the federal SDWA 42 U.S.C. 300h et seq. as authorized by *Revised Code of Washington (RCW) 43.21A.445* and of the *Water Pollution Control Act, Chapter 90.48 RCW*
- Preserve and protect groundwater, including underground sources of drinking water, for existing and future beneficial uses.

The above goals are consistent with the objectives and procedures proposed by this ITP. The recirculation of groundwater within the contaminated plume and the proposed controlled addition of nutrients do not constitute discarded, abandoned, unwanted, or unrecovered waste fluid(s). Permits normally required for injection under the SDWA or under an RD&D effort for RCRA are not necessary because this test is being conducted as part of a CERCLA remedial action and all wastes derived therefrom are expected to remain onsite.

4.5 EMERGENCY PLANNING AND COMMUNITY- RIGHT-TO-KNOW ACT

The in situ bioremediation nitrate source is considered a hazardous substance under CERCLA and will be released to the environment as a part of the treatability study. As such, the material will be included in the overall sitewide inventory requirements under EPCRA.

5.0 HANFORD SITE COMPLIANCE

This chapter identifies Hanford Site compliance areas for this field demonstration and describes compliance. Operations activities shall recognize that environmental protection, safety, and productivity are compatible goals.

5.1 CONDUCT OF OPERATIONS

Conduct of Operations is a set of standards that establishes an overall philosophy for achieving excellence in the operation of DOE facilities. These standards shall be considered by organizations that conduct or support operations in their efforts to improve overall organizational performance. The elements of the Conduct of Operations Requirements for DOE Facilities (DOE 1990) are tools to do our work. These elements shall provide a framework for well-operated facilities committed to excellence and not just compliance. The goal is to promote greater ownership and accountability by each individual worker and supervisor. Evidence of success will include accountability and a technical inquisitiveness by employees at all levels. The fundamental purpose is to provide goods and services to the DOE in a safe, high-quality, timely, cost-efficient manner. Striving for excellence will be a team effort in this demonstration.

5.2 SAFETY

Hanford activities under this plan will be governed by a site-specific safety document meeting DOE and contractor requirements. Plans will be periodically reviewed and updated and will be supported by a risk or safety assessment, as appropriate.

Field activities will be governed by the health and safety procedures found in the *Environmental Investigations Procedures* (BHI 1994). Bechtel Hanford, Inc. (BHI) will have oversight regarding safety at the field test site. Practices on the job will comply with current site-specific health and safety plans meeting all applicable requirements of 29 CFR 1910.120. Each staff member will be responsible for his/her actions regarding safety at the field site. No person shall be required to perform work that s/he feels would jeopardize the safe operation of equipment, other personnel, or the general public. Only authorized staff will be allowed to enter any exclusion zone at the test site.

Characterization data has demonstrated the site is not significantly contaminated with radionuclides or chemical contamination. Although groundwater contamination exceeds drinking water standards, contaminant concentrations are such that incidental contact poses no acute exposure risk to onsite personnel.

Work conducted at onsite laboratories, such as the 324 facility, shall be governed by health and safety plans of the associated DOE contractor.

This demonstration shall not seed genetically engineered or non-native, cultured bacteria to affect remediation of the contaminated aquifer.

5.3 QUALITY ASSURANCE

All work at the Hanford Site is subject to the requirements of DOE orders that establish broadly applicable QA program requirements. QA records shall be managed according to the respective procedures of the originating DOE-contractor organization. All field SOPs will be approved and maintained by BHI in a project file.

The objective of the demonstration plan is to ensure that the data obtained and the conclusions drawn are sufficiently accurate and reliable to support decisions associated with the evaluation of the demonstration. A QA plan has been prepared and incorporated into Appendix A to ensure project success. This plan incorporates QA sampling to support measurement objectives.

5.4 TRAINING

Standard training, including Occupational Safety and Health Administration training for personnel working at hazardous waste sites plus training relevant to assigned duties, will be required for any personnel entering the exclusion zone.

Safety training requirements shall be listed in the site-specific health and safety plan. Sign-off acknowledging familiarization with the field health and safety plan may be required for entry into particular areas of the site.

5.5 SECURITY

The demonstration site is located within the Hanford Site's 200 West Area. Personnel require Hanford Site security access. Visitor access to the demonstration site shall follow established Hanford Site procedures and shall be approved jointly by the ERC Project Engineer and PNL PI before all site visits.

The site has been fenced to allow access control. Field equipment will be secured during off-normal hours and may need to be stored in a locked area. The well heads shall be covered when the site is not occupied.

Emergency access phone numbers shall be listed in the field health and safety plan and at the perimeter of the demonstration site.

6.0 ORGANIZATION AND RESPONSIBILITIES

The field demonstration will be performed by Technology Demonstrations (TD) working with the PI (see Figure 6-1). There is only one team at the demonstration site, and all participants are members. Field aspects of the demonstration will be supported by the infrastructure of the assigned ERC Project. This organization will provide the PI necessary support to perform the demonstration field work. It will include such functions as site facilities support, regulatory oversight, and operable unit integration. The PI will provide for the technical accomplishment of all project objectives, including nonfield work vital to the objectives of the overall project. The following sections contain more detailed descriptions of roles and responsibilities.

6.1 ENVIRONMENTAL RESTORATION CONTRACTOR TEAM PROJECT MANAGER

The ERC Team Project Manager is responsible to provide operational support to do the field demonstration. Management shall ensure that a high-level of performance in facility operations is achieved through cost-effective implementation and control of operations activities. The ERC will provide the following specific resources to host the PI.

- TD Engineer/Field Team Leader (FTL)
- Health and Safety support
- Health Physics Technician (HPT) support
- Analyses for HPT release
- Facilities support including
 - Deionized (DI) water
 - Fuel
 - Portable toilets
 - Cellular phones
 - Drinking/wash water
 - Trash collection
 - Hazardous waste management
 - Power
 - Yard lights
- Interface with Operable Unit Coordinator and Regulators
- Provide regulatory compliance support related to field activities
- Provide support for installing in-well/retrieving downhole equipment.

Support will be requested by the TD Project Engineer working in conjunction with the PI.

The ERC Team Project Manager will designate reviewers of all field plans to ensure safety, regulatory and DOE/Hanford compliance for field demonstrations. Management will authorize field implementation. The team is also encouraged to provide feedback to the PI to enhance the value of future technology development activities.

6.2 PROJECT ENGINEER

The TD Project Engineer is responsible for coordinating with the PI, the FTL, and the ERC Project team to do the field demonstration. This includes ensuring that the above support equipment and materials are requested to meet project needs. The Project Engineer will coordinate preparation and approvals of necessary ERC project documentation for the field work and submit records to the ERC project file. The Project Engineer will be essential in providing information for development and maintenance of ERC budget and schedules. Duties also include periodic reporting of project status to the ERC and authorship of this ITP. After the field demonstration is complete, the Project Engineer will prepare an informal "Lessons Learned" letter report with the PI to identify successes and failures pertinent to the conduct of future site demonstrations.

6.3 FIELD TEAM LEADER

The FTL will coordinate with the Project Engineer to ensure field delivery of ERC Team resources. The FTL will be the primary ERC field contact to support the demonstration. Demonstration site safety requirements will be established and enforced per team effort of the FTL and Site Safety Officer (SSO). The FTL will stay in regular contact with the PI field personnel to stay knowledgeable of demonstration progress and to resolve needs or problems.

6.4 SITE SAFETY OFFICER

The ERC SSO is responsible for the generation of the site-specific health and safety plan meeting all applicable requirements of 29 CFR 1910.120. The SSO efforts shall be commensurate with the field activity's potential safety and/or health impacts. Field activities will be governed by the health and safety procedures found in the *Environmental Investigations Procedures* (BHI 1994).

Each organization has some part in the safe and efficient operation of the demonstration. All personnel are obligated to conduct activities in a safe and professional manner in compliance with the site-specific health and safety plan. The SSO will perform site visits as necessary to determine compliance with safety and health requirements. Any deficiencies will be communicated to management for correction.

6.5 PRINCIPAL INVESTIGATOR

The PI shall:

- Ensure the technical objectives of the demonstration are met
- Conduct the field demonstration through coordination with the TD Project Engineer including the preparation of SOPs
- Provide all monitoring and process equipment to be demonstrated
- Provide personnel to set up the equipment, perform the demonstration, and analyze the results

- Prepare a performance evaluation report that reviews the results of the demonstration related to each objective.

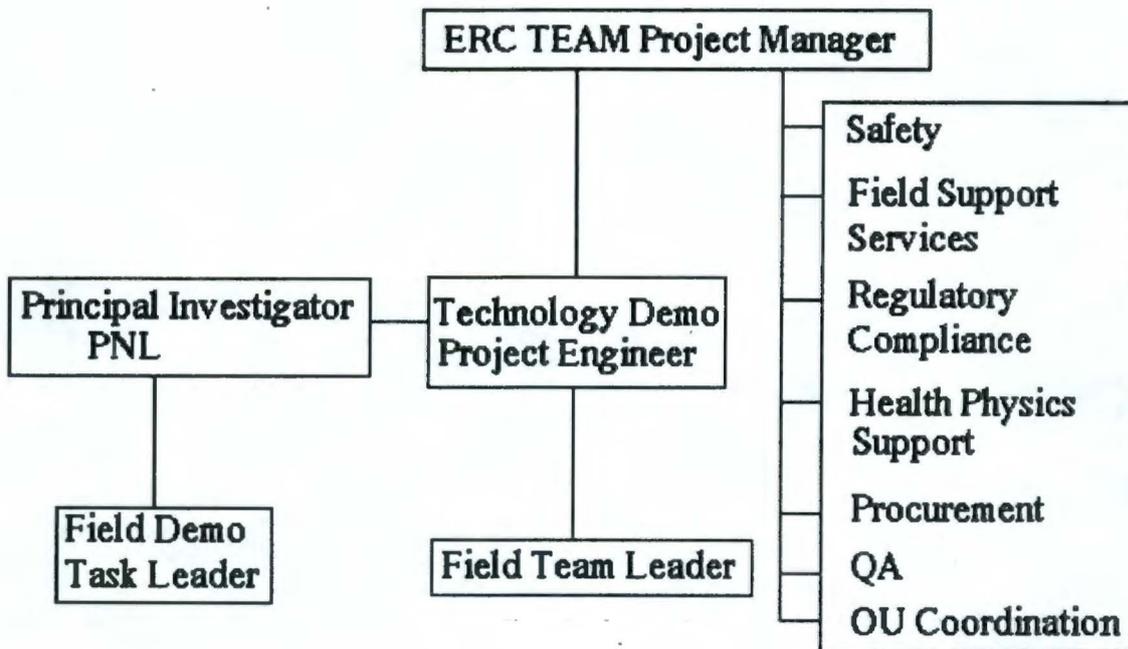
The PI will integrate field activities with remediation design via laboratory, flow-cell, and 3-D modeling studies, laboratory work, data analysis, technology transfer, and reporting activities. QA and peer review for the technical aspects of the demonstration shall be the responsibility of the PI's organization.

The PI has contributed to the creation of this ITP. After the demonstration, the PI will summarize the demonstration results and prepare a final evaluation report. (The *VOC-Arid Integrated Demonstration Guide to Preparation of Demonstration Documents* [Jensen et al. 1994] or subsequent revisions describe the documentation process.)

6.6 FIELD DEMONSTRATION TASK LEADER

The Field Demonstration Task Leader is delegated technical responsibility for performance of field aspects of the demonstration. This individual reports to the PI Demonstration Manager and is one of several technical specialists on the PI team. Field personnel from the PI's organization will report to the Task Leader.

Figure 6-1. Organization Chart.



7.0 DESCRIPTION OF TASKS AND PROCEDURES

Field testing of in situ bioremediation will involve the following major tasks:

- Predemonstration site hydrological, geochemical, and microbiological characterization and well construction
- Predemonstration remediation modeling
- Installation and verification of the process and monitoring equipment
- Evaluation of in situ biotreatment and data analysis during test operation
- Shutdown of the injection system and continued operation of the monitoring equipment
- Shutdown of the remaining equipment
- Post-demonstration site characterization
- Final analysis of test data and reporting.

Basic site operations and required project-specific procedures to accomplish the above tasks are defined through this ITP. Table 7-1 lists each planned procedure. The depth of procedure detail shall be commensurate with the intended use. Two types of written, project-specific procedures are planned for the in situ bioremediation demonstration: field and laboratory. All field procedures will be approved by ERC Project management per Chapter 6.0 of this ITP. Special laboratory procedures will be approved by the PI.

7.1 PREDEMONSTRATION SITE HYDROLOGICAL, GEOCHEMICAL, AND MICROBIOLOGICAL CHARACTERIZATION AND WELL CONSTRUCTION

Characterization information associated with installation of the first three wells at the site is discussed in Section 1.3. Additional characterization efforts are described in detail in the *In Situ Bioremediation Drilling and Characterization Work Plan* (Kogler 1994). These activities include sampling of both sediment and groundwater media during construction of the final three site wells. This has been conducted simultaneously with preparation of this document. A characterization report is planned. Numerous hydrological, geochemical, and microbiological parameters have been measured to support implementation of the bioremediation field demonstration.

Table 7-1. Project-Specific Procedures.

Procedure	Title	Type
SOP 18687-1	Analysis of Denitrifiers, Sulfate Reducers, Iron Reducers, and Enumeration of Aerobic Heterotrophs	Lab
SOP 18687-2	Manual Groundwater Sampling Procedures	Field
SOP 18687-3	Determination of Anions by Ion Chromatography	Lab
SOP 18687-4	Analysis of Selected Halogenated Organic Compounds Methane and Carbon Dioxide by Gas Chromatography	Lab
SOP 18687-5	Routine Operation of the Process Control Computer	Field
SOP 18687-6	Data Transfer Operations using the Process Control Computer	Field
SOP 18687-7	Restarting the Process Control Computer	Field
SOP 18687-8	Sample Handling, Logging, and Storage at the Test Site	Field
SOP 18687-9	Sampling for Methane and Carbon Dioxide in Gas Samples	Field
SOP 18687-10	Sampling for VOCs in Gas Samples	Field
SOP 18687-11	Chemical Mixing Procedures for Nitric Acid Stock Solution	Field
SOP 18687-12	Chemical Mixing Procedures for Acetate Stock Solution	Field
SOP 18687-13	Chemical Mixing Procedures for 5% Bleach Solution	Field
SOP 18687-14	Transfer Operations for Stock Solutions in the Process Trailer	Field
SOP 18687-15	Plumbing Maintenance Operations for Nutrient Injection System	Field
SOP 18687-16	Process Water System Operation	Field
SOP 18687-17	Groundwater Autosampler Operation	Field
SOP 18687-18	In Situ Bioremediation Test Site Daily Checklist	Field
SOP 18687-19	In-Well Sample Pump Operation	Field
SOP 18687-20	Procedure for Conducting a Two-Well Groundwater Recirculation Tracer Test	Field
SOP 18687-21	Procedure for Conducting Extended Two-Well Groundwater Recirculation	Field
SOP 18687-22	In-Line Filter Operation	Field
SOP 18687-23	Procedure for Conducting a Two-Well Groundwater Recirculation Tracer Test using Nitrate as the Tracer	Field
SOP 18687-24	Emergency Shutdown Procedures	Field
SOP 18687-25	Draining the Secondary Containment Pan	Field
SOP 18687-26	Storage Tank Fan Operation and Maintenance	Field
SOP 18687-27	Valve Setup for Normal Nutrient Injection Operations	Field
SOP 18687-28	Sampling of Nutrient Stock Solutions	Field
SOP 18687-29	Change Procedure for SOPs	Field

7.1.1 Baseline Water Chemistry and Microbiology Sampling

A series of measurements is being performed by PNL to determine the baseline concentrations of important constituents in the groundwater at the bioremediation field site. Sampling is being conducted from March 1994 until the end of November 1994. Samples will be withdrawn from the upper and lower screened intervals of well 299-W11-32.

7.1.2 Sampling Constituents and Frequencies

The following lists the baseline sampling constituents and sampling frequency.

Constituent	Frequency	Number/Event
VOCs ^a	biweekly	4/interval
anions ^b	monthly	4/interval
microbiological denitrifiers	monthly	3/interval
sulfate reducers	bimonthly	3/interval
coliforms	bimonthly	3/interval

^aVOCs include CCl₄, CHCl₃, CH₂Cl₂, PCE, and TCE.

^bAnions include nitrate, nitrite, sulfate, phosphate, chloride, and fluoride.

Sampling Protocols for the baseline sampling are described in detail in Appendix C.

7.2 PREDEMONSTRATION REMEDIATION MODELING

Predemonstration modeling will be continued using several parallel paths. The 3-D model will be used as a process simulation tool to examine transport and reaction for the probable operating strategies for the demonstration. To develop the candidate strategies, a combination of several types of modeling will be used. Stanford University will focus on transport modeling to examine hydraulic control of the treatment zone and to develop monitoring strategies. These simulations will be performed using a 2-D radial flow code developed at Stanford. PNL will focus on developing two design tools in addition to the 3-D simulator. A 2-D transport and reaction model will be developed for use in testing nutrient addition strategies to maximize the rate of CCl₄ destruction. This code is needed to formulate and test design cases for use in process development in a quick and efficient manner. A 1-D radial transport and reaction model will be used to assess near-well effects of the nutrient addition strategy with respect to biofouling. The primary tools used during the demonstration for data analysis will be the 2-D and 3-D transport and reaction models.

7.3 INSTALLATION AND VERIFICATION OF THE WELL NETWORK AND PROCESS AND MONITORING EQUIPMENT

Specifications for the wells installed before FY 1994 are contained in the *FY 93 Site Characterization Work Plan for the VOC-Arid ID and 200 West Area Carbon Tetrachloride ERA* (Rohay et al. 1993). Specifications for process equipment are summarized in Chapter 2.0. The wells installed in FY 1994 and FY 1995 are described in the *In Situ Bioremediation Drilling and Characterization Work Plan* (Koegler 1994).

7.4 EVALUATION OF IN SITU BIOTREATMENT AND DATA ANALYSIS DURING TEST OPERATION

7.4.1 Test Outline

Monitoring and data collection will be performed continuously throughout operation of the demonstration. The demonstration will consist of an abiotic recirculation control phase (Phase 1) and active bioremediation operations. During active bioremediation operations, the initial operating strategy will be implemented for at least three months (unless significant problems occur) to fully assess system performance (Phase 2). A thorough data review will be conducted after three months of operation and a decision will be made to either continue the initial operating strategy or to implement a revised operating strategy (Phase 3).

7.4.1.1 Phase 1 - Abiotic Recirculation. Phase 1 will consist of control operations in which the groundwater will be mixed and hydraulic control of the region demonstrated with no addition of nutrients. For this phase, routine sampling and tracer sampling as described below will be utilized to monitor concentrations of contaminants and to determine the characteristics of the mixing zone. Data from this phase will be used to establish any abiotic removal of contaminant because of groundwater mixing and to calibrate the transport portion of the process model. At the completion of this phase, a technical review meeting will be conducted to analyze data from the test and determine any necessary changes to the planned operating parameters.

7.4.1.2 Phase 2 - Active Bioremediation. Nutrient injection will be initiated in this phase. An operation strategy based on process simulations and data from Phase 1 will be implemented and adhered to for at least three months in order to collect sufficient information to assess the success of the remediation strategy. Sampling schedules described below will be used for collection of data during this phase of the demonstration. The operation strategy will be changed before the end of the three months of operation only if major problems occur. Data will be reviewed after this initial operation to determine if any changes in the operation strategy should be implemented. Examples of data that may lead to changes in the operation strategy include the production of noninert persistent byproducts (such as CHCl_3 and nitrite), changes in the hydraulic mixing pattern that are not desired, insufficient or excessive biomass accumulation, and insufficient contaminant destruction. If it is determined in the technical data review that the operating strategy will be changed for Phase 3 operations, a constant-rate discharge test will be conducted to determine changes in hydraulic conductivity during Phase 2. Groundwater will then be recirculated as needed to reestablish stable field parameters (e.g., CCl_4 and nitrate concentrations) before initiating nutrient injection in Phase 3.

7.4.1.3 Phase 3 - Active Bioremediation, Refined Operation. A revised bioremediation operating strategy may be implemented as a means to optimize the performance of the process based on field experience from initial operation. Key issues that may need to be addressed in Phase 3 are CHCl_3 production and CCl_4 destruction rate. It is anticipated that demonstration goals will be reached within this phase of operation if not achieved during initial operations. Operations may require changes in the types of nutrients and/or their injection rate into the aquifer to meet demonstration goals. Potential changes related to CHCl_3 production will be determined based on initial field operations and laboratory experiments being conducted to determine CHCl_3 production kinetics and means to reduce production or degrade CHCl_3 . At the end of this phase, a constant-rate discharge test will be conducted to determine changes in hydraulic conductivity compared to earlier tests.

7.4.2 Data Collection

During the demonstration, data will be collected according to the schedules listed below. Also, data from in situ probes and nutrient injection monitoring will be collected by the process control computer. The strategy of data analysis during demonstration operations will be to collect data to establish the rate and extent of remediation, determine how the treatment zone is changing (microbial growth, chemical species, flow patterns) because of the injection of nutrients, maintain hydraulic control of the treatment zone, and maintain proper system operation. These data will be compiled and analyzed to determine whether the objectives of the test are met. Data analysis will be continuous throughout the demonstration but will be focused by a data review scheduled after each phase of operation.

Data, including in-well pressures and flow rates, contaminant loss, nutrient utilization, and background chemical species, will be plotted to establish trends during the demonstration. These trends will be compared to simulations of the process as a means to estimate the in situ reactions that are occurring. Data from intense sampling will be analyzed using simulations to estimate changes in biomass and other reaction parameters that are causing any changes in response observed at the monitoring locations. Equipment monitoring data will be used in conjunction with software-generated control charts to maintain proper system operation.

Sampling will be performed to collect several different types of data. Because each data set has different requirements, the sampling schedule for each will be different. The types of data to be collected during the demonstration include (1) routine monitoring to establish changes in constituent concentrations as the demonstration progresses and to meet sampling requirements for statistical demonstration of performance goals, (2) intense sampling to monitor the response of the treatment zone to nutrient pulses and the change in this response as the demonstration progresses, and (3) tracer tests to monitor changes in hydraulic properties of the treatment zone as a result of treatment activity. Post-demonstration monitoring will be implemented to establish the effects of the technology on overall water quality at the site.

7.4.2.1 Sampling Constituents and Frequencies for Routine Sampling Events. Tables 7-2 and 7-3 list constituent, frequency of sampling, and locations for routine monitoring of the treatment zone during the demonstration. These data will be used to establish trends in important process parameters and provide samples for statistically determining whether performance goals have been achieved. Production of noninert byproducts of the treatment process will also be monitored with this data.

Table 7-2. Routine Sampling Schedule.

Chemical/Microbiological Parameter	Frequency	Sample Locations
VOCs ^a	See Table 7-3	MW, EW
Anions ^b	daily	MW, EW (first 8 weeks of injection)
Anions ^b	twice per week	MW, EW (when at steady-state)
Cations ^c	monthly	MW
Gas Samples ^d	biweekly	MW
aerobic heterotrophs	biweekly	MW
denitrifiers	biweekly	MW
sulfate reducers	monthly	MW
coliforms	monthly	MW

NOTE: Biocoupons and biomolecular probes will be used according to the description in Appendix B.

EW = extraction well

MW = monitoring well

^aVOCs include CCl₄, CHCl₃, CH₂CL₂, PCE, and TCE.

^bAnions include acetate, nitrate, nitrite, sulfate, phosphate, chloride, and fluoride.

^cCations include aluminum, antimony, arsenic, barium, beryllium, calcium, cadmium, chromium, cobalt, copper, iron, lead, magnesium, manganese, molybdenum, nickel, potassium, selenium, silicon, silver, sodium, thallium, tin, titanium, vanadium, and zinc.

^dGas samples include the VOCs listed above and CO₂.

Table 7-3. Volatile Organic Compound Sampling Schedule.

Period of Operation ^a	Number of Samples ^b
Phase 1 operations	180 (15/week)
First 2 months of nutrient injection - every 2 weeks	40 samples taken over a 24-hour period
Last month of nutrient injection (steady-state) - every week	50 samples taken over a 24-hour period
After any discontinuation of nutrient injection	100 samples taken over a 48-hour period after determining that concentration is stable

^aPeriods refer to general modes of operation. Time for each period was estimated based on process simulations.

^bNumber of samples is based on the confidence levels discussed in Section 3.2.

7.4.2.2 Sampling Constituents and Frequencies for Intense Sampling Events. Table 7-4 lists the constituent, frequency of sampling, and locations for intense sampling events that will be used to monitor the response of the treatment zone to nutrient pulses during the demonstration. These intense pulse-monitoring events will occur biweekly during demonstration operations. The duration of these intense sampling events will be determined based on bromide tracer test results. These data will be used to determine changes in nutrient uptake that correlate to biomass increases and changes in system parameters such as biomass distribution or changes in pulse concentration and duration. These tests will be effective in determining the movement of nutrients in the subsurface until biomass increases sufficiently that the nutrients are consumed before they reach a specific monitoring point. Data on the concentration profile of the nutrient while it still appears at a specific monitoring point can be used for comparison to model predictions to calibrate for biomass concentrations and distribution. Once the nutrients no longer reach a monitoring point, sampling at that point will be discontinued. The time required until nutrients no longer appear at a specific location will also be a calibration point for the model of the process.

Table 7-4. Intense Sampling Schedule.

Constituent	Frequency	Sample Locations
Anions ^a	Every half hour	MW, EW
Anions ^a in pulse	During pulse	IW
Bromide	Continuous	MW, EW

EW = extraction well

IW = injection well

MW = monitoring well

^aAnions include acetate, nitrate, nitrite, sulfate, phosphate, chloride, and fluoride.

7.4.2.3 Sampling Constituents and Frequencies for Tracer Sampling Events. Table 7-5 lists the constituent, frequency of sampling, and locations for tracer sampling events that will be used to monitor changes in hydraulic properties of the treatment zone during the demonstration. Tracer tests will be performed monthly during Phases 2 and 3 using nitrate and acetate in separate events as the tracer. Conservative tracer tests using bromide as the tracer will also be performed once during abiotic recirculation (Phase 1) and in the middle and at the end of Phases 2 and 3. These data will be analyzed to assess changes in tracer breakthrough curves that correlate to biomass increases or changes in biomass distribution. The effectiveness of the design in controlling biofouling will also be determined using these data in conjunction with pressure and flow rate data. As another means to assess hydraulic changes induced during bioremediation, constant-rate discharge tests will be conducted before nutrient injection and after Phases 2 and 3.

Table 7-5. Tracer Test Sampling Schedule.

Constituent	Frequency	Sample Locations
Anions ^a	Every half hour	MW, EW
Bromide	Continuous	MW, EW

EW = extraction well
MW = monitoring well

^aAnions will be nitrate, nitrite, acetate, or bromide as required in the tracer test protocol.

7.5 SHUTDOWN OF THE INJECTION SYSTEM AND CONTINUED OPERATION OF THE SAMPLING AND MONITORING EQUIPMENT

At the conclusion of the demonstration, the nutrient injection system will be shut down and the field network will continue to be operated in a circulation mode. This operation will allow post-treatment chemical and microbiological data to determine the long-range effect of the bioremediation treatment on the aquifer. Background sampling as described in Section 7.1.2 will be used during this monitoring period except that analyses for fermentation products will be added. The fermentation products will be analyzed to assess the effect of biomass autodigestion on water quality. To assess changes in hydraulic conductivity during the decay of biomass in the subsurface, constant-rate discharge tests will be conducted at the end of post-test monitoring.

7.6 SHUTDOWN OF THE REMAINING EQUIPMENT

At the completion of post-treatment monitoring, the recirculation equipment will be shut down, processing equipment will be emptied and decontaminated, secondary wastes will be dispositioned, and the process equipment will be placed in a storage condition. Nonessential equipment will be removed from the demonstration site. All remaining sampling and data analysis operations will be completed.

7.7 POST-DEMONSTRATION SITE CHARACTERIZATION

Following process shutdown, post-demonstration characterization efforts may be initiated to provide sediment core samples and groundwater monitoring. Sediment samples will be analyzed for comparison to predemonstration characterization. On-going groundwater monitoring will be continued and will be incorporated into the routine Hanford site-wide groundwater monitoring program. These data will provide the longer term measure of the impacts of the bioremediation demonstration.

7.8 FINAL ANALYSIS OF TEST DATA AND REPORTING

Final data analysis and the demonstration performance evaluation report will be prepared following the conclusion of the test. A draft report will be issued before completion of activities described in Section 7.5 to provide the opportunity for technical input to the post demonstration site characterization and longer-term monitoring requirements. The final project report will be completed after Section 7.7 activities have concluded.

7.9 SITE OPERATIONS

The following are summaries of some of the primary operations associated with the demonstration. Detailed SOPs will be completed for use at the site during operations.

7.9.1 Nutrient Stock Solutions

Nutrient stock solutions will be mixed to the appropriate concentration and stored in the bulk nutrient storage tanks located in the process trailer. These tanks will serve as the feed tanks for the nutrient injection system. One bulk storage tank will contain 20% by weight acetate (sodium acetate), and the other will contain 20% by weight nitrate (sodium nitrate and nitric acid). Nitric acid will be used as a partial source of nitrate (up to 5% by weight in the storage tank) because protons are consumed during denitrification and the acid will supply protons to help buffer pH changes. Up to a one-month supply of bulk chemicals will be stored on site. The bulk chemicals will be 40% (wt) nitric acid, sodium nitrate, and sodium acetate. Empty drums of nitric acid will be picked up each month when the next month's drums are delivered. The drum storage will be located directly behind the process trailer on a containment pad. This location will allow filling of the bulk storage tanks in the process trailer without moving the drums. Chemical mixing procedures are described in detail in site-specific SOPs.

7.9.2 Process Operation and Control

Processes will be controlled from the front section of the process trailer. The process control computer will provide all primary process control and will be the interface for all systems. Disconnects and circuit breakers will be used as required for manual operations and to shut out sections of power as needed for maintenance. Coordination of manual sampling with automatic operations will be performed using the process computer interface. Manual sampling will be

performed for some locations by actuating manual valves to control air driven pumps and actuators or manual operation of groundwater sampling pumps.

7.9.3 Waste Management

All sampling waste will be dispensed to the onsite purgewater tank. Periodically, this tank will be emptied and its contents transported to the Hanford purgewater facility. All nutrient solutions will be used as fully as practical. Any excess will be stored in the extra bulk nutrient tanks in the process trailer and disposed. Every effort will be made to minimize excess nutrient solutions. Some system flush water will be generated as part of maintenance procedures. This waste will be disposed. Empty drums of bulk chemicals will be returned to the vendor. There will be no lab waste on site. Filter cartridges will be disposed in compliance with hazardous waste regulations. All wastes and materials associated with laboratory procedures will be handled in the 324 building according to PNL procedures.

7.9.4 Equipment Cleaning and Maintenance

For any maintenance or cleaning activity, the plumbing will be flushed with DI water to clear residual solutions before disconnecting any fittings. Groundwater sampling pumps will be cleaned periodically at the surface using a 5% household bleach solution. All waste associated with this operation will be disposed.

8.0 SITE SERVICES REQUIREMENTS

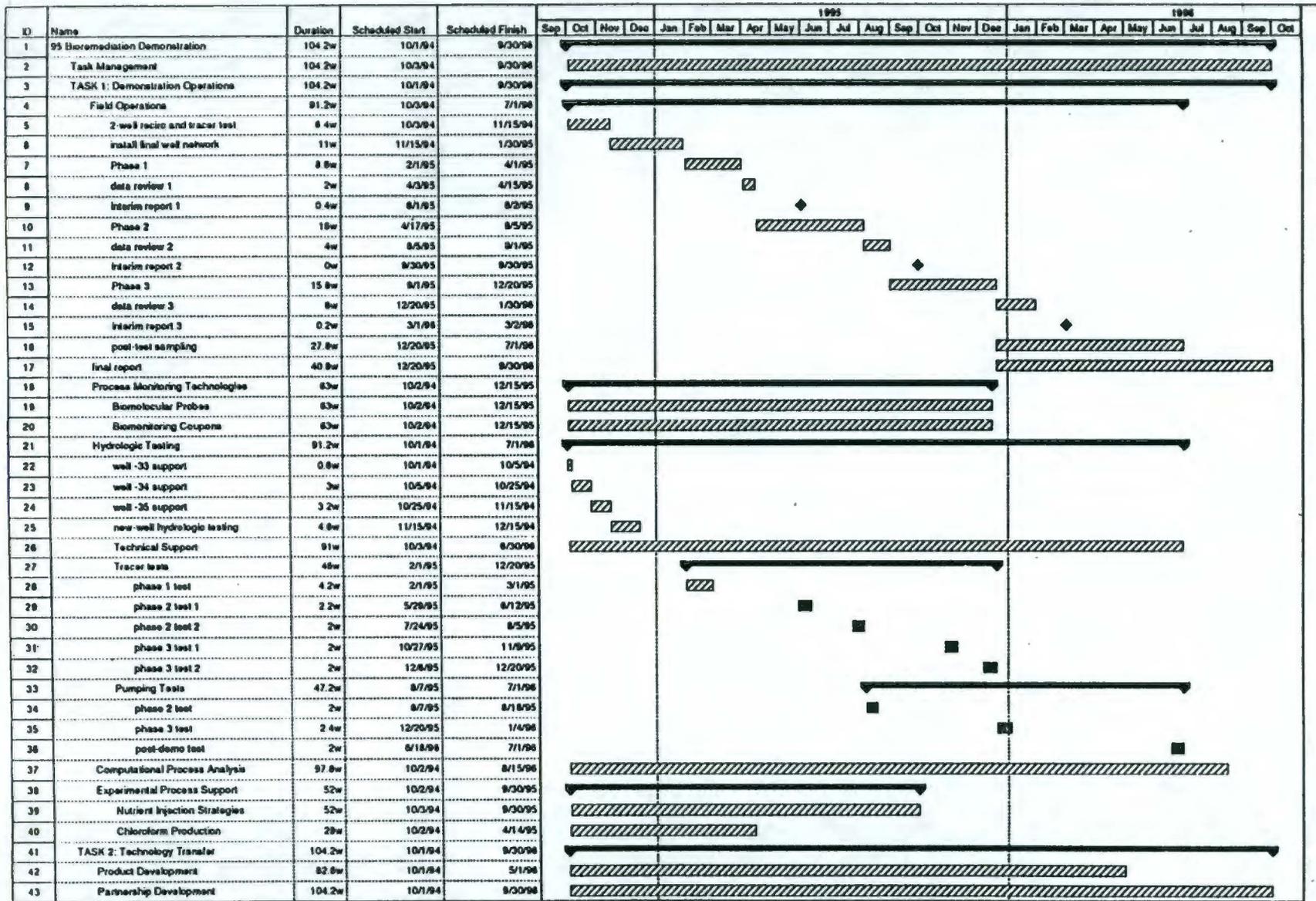
Operations at the site will require 200 amps of 208V ac, 3-phase power. In addition, DI water must be supplied by filling transportable 200-gal water tanks or acquiring a suitable onsite processing capability. For waste disposal, purgewater tank contents must be periodically emptied and transported to the Hanford purgewater facility.

9.0 DEMONSTRATION SCHEDULE

Table 9-1 provides the schedule for the primary activities of the demonstration. Figure 9-1 is a chart showing more details of the planned activities.

Table 9-1. Activity Schedule.

Dates	Task
02/01/95	Start demonstration
02/01/95 to 04/01/95	Phase 1 of test, abiotic control operation
04/01/95 to 04/15/95	Data review
04/15/95 to 08/01/95	Phase 2 of test, initial nutrient injection strategy
08/01/95 to 09/01/95	Data review
09/01/95 to 12/15/95	Phase 3 of test, revised nutrient injection strategy (as required)
12/95 to 06/96	Post-test monitoring
03/96	Draft evaluation report complete
09/96	Evaluation report complete



Project Date 8/25/94
 Critical [Hatched] Noncritical [Solid] Progress [Line] Milestone [Diamond] Summary [Arrow] Rolled Up [Diamond]

Figure 9-1. Bioremediation Demonstration Tasks and Schedule Details.

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APPENDIX A

**DETAILED PROJECT OBJECTIVES, DATA QUALITY OBJECTIVES,
AND QUALITY ASSURANCE PLAN**

APPENDIX A**DETAILED PROJECT OBJECTIVES, DATA QUALITY OBJECTIVES,
AND QUALITY ASSURANCE PLAN****1.0 DETAILED PROJECT OBJECTIVES**

Meeting the specific project objectives as described in Chapter 3.0 of this integrated test plan will require collection and analysis of data described below. The collected data will be used to operate the demonstration and evaluate the success of the demonstration as defined in the four overall demonstration objectives. The following detailed objectives are described below as part of the demonstration plan:

- Determine the area in which biodestruction of carbon tetrachloride (CCl_4) occurs
- Maximize and quantify the rate of CCl_4 destruction
- Quantify the mass of CCl_4 destroyed
- Quantify the rate at which the nitrate concentration can be decreased
- Minimize and quantify the production of chloroform (CHCl_3) and nitrite
- Minimize changes in aquifer hydraulic conductivity and well screen plugging.

Accomplishment of each detailed objective is discussed in the following sections.

**1.1 QUANTIFY THE AREAL EXTENT OF BIOSTIMULATION BECAUSE
OF RECIRCULATION OF NUTRIENTS**

Approach: Measure in situ parameters, compare with parameters predicted using with numerical simulations.

Parameters to be measured during demonstration:

- width (thickness) of saturated zone biostimulation
 - screened interval thickness
 - hydraulic conductivity vertical distribution
- horizontal extent of saturated zone biostimulation
 - hydraulic conductivity horizontal distribution
 - measurement of nutrient and tracer concentrations during recirculation.

**1.2 MAXIMIZE AND QUANTIFY THE RATE OF
CARBON TETRACHLORIDE DESTRUCTION**

Approach: The performance goal is 15 g of CCl_4 destroyed per day based on process simulations. These simulations use kinetic information determined in laboratory studies as the basis for the reaction components of the process. Measured field concentrations of contaminant and byproducts during

demonstration in accordance with the sampling schedule described in Chapter 7.0 will be used in conjunction with the process simulator to determine destruction rates.

Parameter measurements necessary for calculation:

- CCl_4 concentration over time in the aquifer measured at monitoring wells and in the recirculation stream
- Nutrient and tracer concentrations over time in the aquifer measured at monitoring wells and recirculation stream
- Byproducts (nitrite, carbon dioxide (CO_2), and CHCl_3) concentrations over time in the aquifer measured at monitoring wells and in the recirculation stream.

Ancillary information required for estimation:

- Aquifer geochemical parameters, measured in situ (pH, redox potential, dissolved oxygen, temperature, pressure).

1.3 QUANTIFY THE MASS OF CARBON TETRACHLORIDE DESTROYED

Approach: Using the CCl_4 destruction rates determined from field data, integrate over time using the process simulator to determine the mass destroyed.

1.4 QUANTIFY THE RATE OF NITRATE DESTRUCTION

Approach: Process simulations incorporate nitrate destruction kinetics from laboratory experiments. Because nitrate will be amended to the subsurface in defined quantities, measurements of in situ reductions in nitrate concentrations can be used to determine the destruction rate.

1.5 MINIMIZE AND QUANTIFY THE PRODUCTION OF CHLOROFORM AND NITRITE

Approach: Laboratory experiments were used to develop nutrient amendment strategies that minimize the formation of unwanted byproducts. When the nutrient strategy is implemented in the field, byproduct concentrations will be measured at monitoring wells (and in the vadose zone for CHCl_3).

1.6 MINIMIZE CHANGES IN AQUIFER HYDRAULIC CONDUCTIVITY AND WELL SCREEN PLUGGING

Approach: Laboratory experiments were used to determine the relationship of nutrient utilization to biomass production. Based on the nutrient flux added to the subsurface, the total biomass within the reaction zone can be estimated. Using the predicted biomass concentrations and measurements of pressure and flow, biofouling will be monitored during nutrient injection. At the end of Phase 2,

a pump test will be performed to determine changes in hydraulic conductivity relative to a pump test performed before nutrient injection.

2.0 DATA QUALITY OBJECTIVES

2.1 DATA QUALITY OBJECTIVE DEFINITIONS

2.1.1 Variability

A standard deviation will be used as a measure of the variability in the mean concentration of a species. This variation can be due to (1) variability within the sampled system, (2) sampling variability, and (3) analytical variability. This data quality objective (DQO) is primarily for use in analysis of Volatile Organic Compound (VOC) concentrations. However, analysis of other constituents will also use this DQO. The standard deviation calculation primarily applies to sample clusters used to determine the change in a constituent concentration over time.

The standard deviation will be calculated as:

$$\text{standard deviation} = \sqrt{\left(\frac{1}{n-1}\right)\left(A - \frac{B}{n}\right)}$$

where, A = the sum of the square of the sample concentrations
B = the square of the sum of the sample concentrations
n = number of samples (i.e., observations).

The standard deviation will be used in Student's t test analyses to compare the mean sample concentrations of sample clusters taken at different times during the demonstration. A discussion of this statistical analysis is contained in Chapter 3.0.

2.1.2 Accuracy

Accuracy is a measure of the bias of a system or measurement. It is the closeness of agreement between an observed value and an accepted value.

For this project, accuracy of chemical analysis will be determined through the analysis of method blanks, transfer blanks, and standard reference material (SRM). SRMs are materials that have been certified by a recognized authority (e.g., National Institute of Standards and Technology) and that are treated and analyzed as an actual sample. Method blanks will be used to measure contamination associated with laboratory processing and analyses. Transfer blanks will be used to measure contamination associated with sampling procedures and reagents.

For analysis of SRMs, the following calculation will be used to determine the percent difference.

$$ePD = 100 \left(\frac{C_1 - C_2}{C_2} \right)$$

where, PD = percent difference
C₁ = measured value
C₂ = certified value.

Blank sample analysis will be performed as outlined in the individual analytical procedures for VOCs, anions, and cations.

Additional measures of accuracy will be employed as appropriate through the use of standards and calibration procedures as defined for the individual analytical procedures.

2.1.3 Precision

Precision is a measure of mutual agreement among individual measurements of the same property, usually under prescribed similar conditions.

For this project, measures of analytical precision will be determined by the analysis of laboratory replicates. Laboratory replicates will be prepared by splitting a sample in the laboratory and carrying the subsamples through the entire analytical process. Precision is expressed in terms of the relative percent difference.

$$RPD = \left(\frac{C_1 - C_2}{\frac{1}{2}(C_1 + C_2)} \right) 100$$

where, RPD = relative percent difference
C₁ = larger of the two observed values
C₂ = smaller of the two observed values.

2.1.4 Completeness

Completeness is a measure of the amount of valid data obtained from a measurement system compared to the amount that was expected to be obtained under normal conditions.

Percent completeness is defined as follows for all measurements:

$$\%C = 100 \left(\frac{V}{n} \right)$$

where, %C = percent completeness
V = number of measurements judged valid
n = total number of measurements necessary to achieve a specified statistical level of confidence in decision making.

2.1.5 Representativeness

Representativeness expresses the degree to which data accurately and precisely represent a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition.

Representativeness will be addressed primarily in the sample design, through the selection of sampling sites and procedures. Representativeness also will be ensured by the proper handling and storage of samples and analyses within the specified holding times so that the material analyzed reflects the material collected as accurately as possible. Representativeness of data will be discussed, when appropriate, in technical memoranda.

2.1.6 Comparability

Comparability expresses the confidence with which one data set can be compared to another. Data obtained during this investigation should be either directly comparable or comparable within defined limits to literature, existing data, or any applicable criteria.

Comparability of the data will be maintained by using U.S. Environmental Protection Agency (EPA)-defined procedures in both the sampling activities and the analytical methods used. Sampling procedures are discussed in Section 3.3. Analytical methods and detection limits are summarized in Table 2-1.

2.2 DATA QUALITY OBJECTIVE VALUES

DQOs for this project will be applicable to VOC, anion, and cation analyses. Data quality for other measurements or procedures will be controlled by adherence to specified methods and equipment calibration procedures. The DQOs are as follows:

- **Variability:** Standard deviation of sample clusters used to determine the change in a constituent concentration over time must be less than or equal to 25% of the mean.
- **Accuracy:** Percent difference must be in the range of $\pm 25\%$, inclusive. Additional accuracy determinations for individual analytical methods are defined within the method procedure.

- **Precision:** Relative percent difference must be in the range of $\pm 20\%$, inclusive.
- **Completeness:** Percent completeness (%C) must be greater than 90%.

2.3 CORRECTIVE ACTION FOR RESULTS OUTSIDE ESTABLISHED DATA QUALITY OBJECTIVES

Results outside the established criteria in Chapter 2.0 shall be brought to the attention of the Field Operations Task Manager who shall determine and document the appropriate corrective action on a Request For Data Review Form. Documents describing these activities will be submitted to the Pacific Northwest Laboratory (PNL) Project Manager for review. Corrective actions may include, but are not limited to, review of data and calculations, flagging of suspect data, or reanalyses of individual or entire batches of samples. The following describes guidelines to be followed when established criteria are not met.

- **Replicates:** All samples associated with replicates that are outside the established control limits will be noted in the narrative and flagged in the final data report. In addition, the number of results that exceed the range per batch shall be noted to determine if the problem affects the sample data for that batch and to determine any other appropriate corrective action.
- **Standard Reference Materials:** SRM values exceeding the percent difference range from the certified values shall be noted in the narrative and flagged in the final data report. In addition, the number of results that exceed the range per batch shall be noted to determine if the problem affects the sample data for that batch and to determine any other appropriate corrective action.
- **Method Blanks:** Any blank values detected above the established criteria should be noted in the narrative and the corresponding data should be flagged as blank contaminated. In addition, the number of results that exceed the range per batch shall be noted to determine if the problem affects the sample data for that batch and to determine any other appropriate corrective action.

3.0 QUALITY ASSURANCE PLAN

3.1 QUALITY CONTROL SAMPLES

Table A-1 contains the applicable quality control (QC) measurements and the minimum frequency with which the measurements need to be performed during sampling and analysis. Table A-1 applies only to VOC, anion, and cation analyses.

Table A-1. Quality Control Measurements for Volatile Organic Compound, Anion, and Cation Analyses.

Type of Sample	Collection Frequency
Field Duplicate	1 per 10 samples (10%)
Transfer Blank	1 per 20 samples (5%)
Laboratory Duplicate	1 per 20 samples (5%)
Method Blank	1 per 20 samples (5%)

Chapter 3.0 lists the analyses, applicable methods, and detection limits for use in the demonstration.

The following holding times will be used for aqueous samples: VOCs (30 days); anions (30 days); cations (6 months); microbial enumeration samples (5 days). Holding times begin on the day of sampling at the time of sampling. The analytical labs shall be notified of these holding times.

3.2 OPERATIONAL DOCUMENTATION

3.2.1 Sample Chain-of-Custody

The chain-of-custody of samples from the field to the analytical lab will be established for aqueous and soil samples. This chain-of-custody must be the equivalent of the PNL or EPA chain-of custody.

3.2.2 Field Record Forms

As a minimum, forms must be used for documentation of the following activities:

- sample collection
- instrument calibration (see below and Chapter 9.0)
- chain-of-custody
- deficiencies.

These forms shall contain all pertinent information for traceability and re-creation of the details of the activity.

The instrument calibration shall have, as a minimum, the name and signature of the person doing the calibration, the date of the calibration, the frequency with which calibrations need to be performed, the procedure for calibration (or a traceable reference to the procedure), information about what standards were used and where they were obtained (include lot numbers, if appropriate), the results of the calibration, whether the results were acceptable or not, and the action taken to correct unacceptable results (if any).

3.2.3 Field Log Book

The following instructions shall be adhered to for use of the field log book (FLB):

- Record each new activity in the FLB table of contents with the starting page.
- Record observations/data chronologically. Use sketches or narratives to describe experimental apparatus, equipment, procedures, data sheets, etc. that are used.
- Make entries legibly in black, permanent ink.
- Do not erase or obliterate entries. Mark out errors or corrections with single lines. Initial and date all changes other than editorial corrections. If the change is substantive, record the reason for the change.
- Attach loose pages by (1) making an entry in the FLB to introduce or describe the loose sheet, (2) drawing a line through the FLB page where the loose sheet will be attached, (3) attaching the loose sheet with tape or glue, (4) signing and dating the loose sheet, and (5) writing the FLB number and page number on the loose sheet in case it is detached.
- The author will sign each page at least once. List person(s) who performed the work. A supervisor will sign the FLB at least weekly.

3.2.4 Corrections to Documentation

If an error is made on any field documentation, an individual may correct the error by drawing a single line through the error and entering the correct information. The error shall not be obliterated. All noneditorial corrections shall be initialed and dated.

3.3 CALIBRATION PROCEDURES AND FREQUENCY

3.3.1 pH Meter Calibration

The pH meters will be calibrated by the user according to the manufacturer's instructions. Calibrations will be documented in the FLB. Calibrations will include checks of two reference standards expected to bracket the area of measurements. Calibrations will occur at the end of each phase of the demonstration or at intermediate times if the pump string is pulled out of the well.

3.3.2 Thermocouple Calibration

Temperature measurement devices must be calibrated before installation by using a two-point calibration (100 °C with boiling water and 0 °C with melting ice). Calibration checks will be documented in the FLB.

3.3.3 Analytical Chemistry Calibration

Calibration methods for all chemical analytical processes shall be addressed in each specific procedure. As a minimum, calibrations should include:

- standards that are traceable to nationally recognized standard organization(s)
- standards that are within their expiration date
- concentrations of standards that bracket the expected concentration of the sample(s)
- documentation of the calibration in the FLB or with the analysis data packet.

3.3.4 Down-Well Multiprobe Calibration

The temperature, pH, and redox potential probes on the Hydrolab (a registered trademark of Hydrolab Corporation) down-well probe will be calibrated by the user according to the manufacturer's instructions. Calibration will occur before installation. The multiprobe calibration will be checked at the completion of each phase of operation.

3.3.5 Fraction Collector

The fraction collector of the autosampler system will be calibrated by the user according to the manufacturer's instructions. Calibrations will be performed before each intense sampling event. Calibrations will be used to determine the relationship between the number of drops dispensed and the collected volume.

3.3.6 Flowmeter

The flowmeter will be calibrated according to the manufacturer's instructions. Differential pressure will be used to calculate the flow rate. Calibration will occur at the time of installation and will be checked at the end of the bioremediation demonstration.

3.3.7 Down-Well Pressure Transducers

The down-well pressure transducers will be calibrated by the manufacturer. A calibration check will be performed by the user before installation and at the end of each phase during the bioremediation test.

3.3.8 Feed-Line Flowmeter

The feed-line flowmeter will be calibrated by the user according to the manufacturer's instructions. Calibration will occur monthly.

3.3.9 Feed-Line Pressure Transmitter

The feed-line pressure transmitters will be calibrated by the user according to the manufacturer's instructions. Calibration will occur monthly.

3.4 DATA MANAGEMENT

3.4.1 Data Reduction and Reporting

Chemistry data will be stored in the project files and will include:

- Results of sample analyses will be reported in the units presented in Section 7.3. Analytes that were not detected will be reported as less than the established detection limit.
- Results of procedural blank analyses and other QC measurements.
- Results of replicate analyses reported as relative percent difference.

3.4.2 Process for Handling Suspect or Unacceptable Data

When the initial data review identifies suspect data, those data must be investigated to establish whether they reflect true conditions or an error. The investigation shall be documented on a Request For Data Review form. If the data value is determined to be in error, the source of the error must be investigated, the correct value established if possible, and the erroneous value replaced with the correct value. If the investigation concludes that the data are suspect (possibly in error) but a correct value cannot be determined, the data must be flagged to indicate the suspect status.

3.4.3 Standard Units

The standard units used to report data are:

Chemistry Parameters:	
VOCs ^a	µg/L (aqueous)
anions ^b	µg/L (aqueous)
cations ^c	µg/L (aqueous)
gas samples ^d	p/m

Field Parameters:	
air flow rate	ft ³ /min
current	mA
length	ft, in.
liquid flow rate	gal/min
liquid volume	gal
pH	pH units
pressure	ft H ₂ O, lb/in ² abs, lb/in ² gauge
redox potential	mV
temperature	°C
time	hr, min
volume	mL, gal
Microbiological:	
aerobic heterotrophs	colony forming units (CFU)/mL
coliforms	CFU/mL
denitrifiers	CFU/mL
sulfate reducers	CFU/mL

^aVOCs include CCl₄, CHCl₃, dichloromethane (CH₂CL₂), tetrachloroethylene (PCE), and trichloroethylene (TCE).

^bAnions include acetate, nitrate, nitrite, sulfate, phosphate, chloride, and fluoride.

^cCations include aluminum, antimony, arsenic, barium, beryllium, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, molybdenum, nickel, potassium, selenium, silver, sodium, thallium, vanadium, and zinc.

^dGas samples include the VOCs listed above and CO₂.

3.4.4 Data Validation

A review by PNL technical personnel will be implemented to ensure that the data generated for this project meets the DQOs. These reviews will be kept in the project files and will include the following:

- Data will be reviewed by the field personnel at the end of each working day to ensure that sample collection activities are completely and adequately documented. A signature and date by the PNL site supervisor will document the review.

- Reviews of analytical results and supporting documentation will be the responsibility of the Field Operations Task Manager. This will include review of sample holding times, sample preservation, equipment calibration, and sample integrity. The results of QC measurements will be compared to pre-established criteria (DQOs) as a measure of data acceptability.
- Periodic copies of data and entries in the FLB will be made and stored separately from the FLB by the PNL site supervisor to ensure that key data are not lost.

Validation will utilize the DQOs in Chapters 1.0 and 2.0 in accordance with the applicable and appropriate parts of the EPA guidance in *Laboratory Data Validation Functional Guidelines for Evaluating Inorganic Analyses* (EPA 1988) and *USEPA Contract Laboratory Program National Functional Guidelines for Organic Data Review, Multi-Media, Multi-Concentration (OLM01.1) and Low Concentration (OLC01.1)* (EPA 1994).

3.4.5 Reports

The final technical memoranda to the client shall contain citations of the methodology(s) used during the technical activities of this project. The technical memoranda shall undergo internal technical review (by PNL personnel) and a quality assurance (QA) review. The PNL Project Manager shall select technical reviewers who will be able to ensure that the report is technically adequate, complete, and correct. Selection shall be based on:

- Technologies and disciplines represented in the report.
- Qualifications of the reviewer(s). Those selected shall have proven competence in the subject matter of the report and shall have been given an adequate understanding of the requirements for, and objectives of, the technical report.
- Reviewer independence. Those selected shall be independent of the original work performed.

3.5 SPECIFIC ROUTINE PROCEDURES USED TO ASSESS DATA PRECISION, ACCURACY, COMPARABILITY AND COMPLETENESS

Because of the nature of environmental measurements, it is frequently difficult or impossible to know the "true" value of the measured parameter. The accuracy of the measured value must instead be inferred through the use of QC samples of known composition. This project uses this method to verify that the established DQOs have been met. Precision, accuracy, comparability, and completeness will be calculated following equations presented in Chapter 2.0. The results will be reported in data tables in the final technical memoranda. These results will be compared against the established DQOs; this comparison will also be reported in the final technical memoranda.

3.6 CORRECTIVE ACTION

The need for corrective action may be identified by the technical staff during the course of their work or through QA surveillances or audits. Each individual performing field or data processing activities

will be responsible for notifying the appropriate supervisory personnel of any circumstance that could affect the quality or integrity of the data.

Deviations typically result from unforeseen circumstances. Deviations apply when the quality of reportable data is indeterminate, (i.e., no objective evidence is available to substantiate data quality or to indicate that established procedures/requirements were met). All unplanned deviations from approved Safe Operating Procedures (SOP) or the project work plan must be documented on a Deficiency Report. A list of Deficiency Reports and their corresponding numbers will be kept in the site FLB. Deviations that are planned and approved in advance by the Field Operations Task Manager and the PNL Project Manager do not require documentation on a Deficiency Report. However, planned deviations will be documented in a memorandum issued by PNL. The following are guidelines to resolving deficiencies:

- Technical problems relating to the field program (e.g., schedule delays, inability to sample certain locations, frequency of sampling, loss/breakage of sampling equipment) will be resolved by the Field Operations Task Manager and the PNL Project Manager.
- The need for corrective action at the laboratory level, such as broken samples, improper instrument calibration, etc. will be addressed by the Field Operations Task Manager or by means specified in the statement of work to a contracted laboratory.
- Corrective actions for results outside established DQOs are addressed in Section 2.3.

4.0 REFERENCES

EPA, 1988, *Laboratory Data Validation Functional Guidelines for Evaluating Inorganic Analyses*, EPA-540/2-88/503, Hazardous Waste Evaluation Division, U.S. Environmental Protection Agency, Washington, D.C.

EPA, 1994, *USEPA Contract Laboratory Program National Functional Guidelines for Organic Data Review, Multi-Media, Multi-Concentration (OLM01.1) and Low Concentration (OLC01.1)*, EPA/540/R-94/013, U.S. Environmental Protection Agency, Washington, D.C.

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APPENDIX B

BIOCOUPON AND BIOMOLECULAR PROBE APPLICATION

APPENDIX B

BIOCOUPON AND BIOMOLECULAR PROBE APPLICATION

1.0 BIOCOUPON APPLICATION

Current methods of microbial characterization rely on expensive sediment samples or are applied to groundwater samples where only planktonic populations are assayed. Biocoupon monitoring as a technique to obtain and analyze samples representative of subsurface biofilm growth will be developed and tested. This technique employs a unique combination of three biological coupons that allow quantification of biofilm formation in monitoring and nutrient injection wells used for in situ bioremediation. The combination of three coupons that will be used in this work element to characterize subsurface microbial activity are the following:

- **Bio-Potential Coupon:** This coupon is a vial with an internal supply of nutrients held in place by a micro-porous ceramic membrane. Its design is based on a characterization tool developed at the Idaho National Engineering Laboratory. The ceramic membrane allows the slow diffusion of nutrients from the vial to the groundwater-membrane interface where microorganisms will attach and grow. After a specific time period, the coupons will be retrieved from the well and the biomass can be quantified and characterized by selective media techniques and biomolecular probes. Primary use for this coupon is for site prescreening and characterization. Thus, this coupon will be deployed during Phase 1 of field operations, where groundwater is recirculated with no added nutrients.
- **Accumulation Coupon:** This coupon measures the overall accumulation rate of the in situ biofilm formation during the active bioremediation process and will provide high concentrations of biomass required by the biomolecular probes. The coupon itself is a flat uniform surface that has been pre-engraved with an analytical grid for quantification of the areal biomass concentration. In addition to biomass growth, this coupon will reflect the effects of in situ biomass transport processes such as cellular attachment and detachment. This coupon will be deployed in conjunction with the growth coupon during Phase 1 to collect baseline data and in Phases 2 and 3 where nutrients are added to the subsurface to monitor the bioremediation process.
- **Growth Coupon:** This coupon is a modification of a coupon developed at Montana State University. It employs cells from a pure culture that have been artificially immobilized in a counting chamber by an agar matrix. The agar matrix ensures that native bacteria from the groundwater do not enter the counting chamber, but allows for diffusion of groundwater constituents in to the immobilized bacteria. Thus, the cells in the chamber will grow in numbers in response to conditions in the groundwater. This growth rate can then be quantified without the effects of cellular attachment and detachment. This coupon will be deployed in conjunction with the attachment coupon during Phase 1 to collect baseline data and in Phases 2 and 3 where nutrients are added to the subsurface to monitor the bioremediation process.

The above coupons will be employed as a system for monitoring microbial activity throughout the bioremediation demonstration. During Phase 1, the bio-potential coupon will be deployed containing the nutrients to be injected in Phases 2 and 3 so that the resulting biofilm can be characterized. Important data from this application include characterization of the biofilm microbial community induced by the nutrients using standard enumeration and biomolecular probe techniques, biofilm density, and baseline data to compare to attachment coupons deployed during the nutrient injection phases of the demonstration. The accumulation and growth coupons will also be deployed in Phase 1 to establish baseline data for comparison to data from when they are deployed in the process monitoring wells during Phases 2 and 3. During Phases 2 and 3, while there is not sufficient biomass to completely delete injected nutrients between the injection and monitoring wells, growth and/or attachment on coupons deployed in the monitoring wells will mimic the biofilm growth in the subsurface. To ensure a flux of groundwater to the coupons, periodic small pump pulses can be used to draw groundwater into the monitoring well. Coupons can be retrieved at specific intervals for use in characterizing the biofilm community and for comparing to simulator predictions. As biomass in the subsurface increases, nutrient concentrations in the monitoring wells will decrease so that direct measures of concentrations will be difficult. Because nutrient pulses will be used at the injection well, it may be difficult using groundwater samples to accurately determine the amount of nutrients reaching the monitoring well. The coupons, however, will provide an integrated measure of the nutrient flux past the monitoring point for comparison to simulator predictions.

The biomonitoring system is relevant to most applications of in situ bioremediation and provides process information that is critical to the technology, but not yet available with current monitoring techniques. One of the components used to determine the success of in situ bioremediation is to show increases in microbial biomass and activity as a result of the bioremediation process (Madsen 1991). The biomonitoring coupons will provide key information that can be used for process control and to demonstrate to regulators, technology users, and the public that the bioremediation process is working.

2.0 BIOMOLECULAR PROBE APPLICATION

In addition to certain denitrifying bacteria, it is known that other anaerobes (acetogens, iron[III] reducers, methanogens, and presumptive sulfate reducers) can degrade carbon tetrachloride (CCl_4). It is known that reductive dehalogenation of CCl_4 will be energetically more favorable as the redox decreases below that of denitrification. For this reason, it may be desirable to further decrease the redox to enhance CCl_4 destruction after nitrate is removed from the demonstration site. The demonstration will use rRNA analysis to assist in determining what types of obligate anaerobic microorganisms are present, stimulated to grow, or displaced in situ during the bionitrification phase of the demonstration. This information may be very useful in decisions regarding how redox status should be manipulated at the site for optimal in situ CCl_4 destruction.

The functional objective of the nucleic acid probe task is to extract and purify nucleic acids from groundwater and sediments from the Volatile Organic Compound-Arid Integrated Demonstration, for the purpose of providing information on the presence and density of obligate anaerobes capable of CCl_4 destruction before, during, and after bioremediation activities.

There are two major advantages of rRNA analysis of nucleic acids extracted from the environment. First, it is independent of the ability to culture the microorganisms in the laboratory. This is very important because 90 to 99.9% of the viable microbial population can not be cultured in the laboratory. Second, it provides information on level of metabolic activity, rather than simply the presence of the microorganisms.

The majority of analyses will be conducted on biocoupons because they afford more rapid, cost-effective, and sensitive analysis. However, analyses will also be conducted on groundwater and sediment to investigate the relationship between colonization of the biocoupons and microorganisms present in groundwater and sediment.

To preserve the in situ metabolic status of microorganisms in samples, sediments and biocoupons are frozen at -70 °C immediately upon arrival from the subsurface and groundwater is immediately filtered and the filters frozen at -70 °C. Before extraction from groundwater, large volumes (hundreds of liters) are rapidly and sequentially filtered through a large, hollow-fiber filtration unit (300,000 molecular weight cutoff), a smaller hollow-fiber filtration unit, and finally onto a 142 mm-diameter Millipore Durapore (a registered trademark of Millipore Corporation) filter (0.2 µm pore size).

After extraction and purification of rRNA, it will be blotted and fixed to charged nylon membranes, and hybridized to various oligonucleotide probes. After hybridization, excess single-stranded probe molecules are removed by washing the membrane under specific conditions. Probe molecules hybridized to target molecules are visualized by various methods to estimate the number of rRNA molecules corresponding to the probe that were originally present in the sample. Oligonucleotide probes will include the following:

- Delta-Proteobacteria probe (a registered trademark of Delta X Corporation) identifies sulfate reducing bacteria plus some nonsulfate reducing bacteria including some iron[III] reducing bacteria
- Five genera/genus-specific probes for sulfate reducing bacteria
- Archaea probe identifies methanogenic bacteria plus some nonmethanogenic bacteria
- Seven genera/genus-specific probes for methanogenic bacteria,

With the addition of acetate to the groundwater, it is expected that obligate anaerobe populations will increase to above the level of detection required for hybridizations. However, obligate anaerobe populations will probably be below the level of detection (approximately 10⁴ cells) before acetate addition, and it is possible that they will be below the level of detection during portions of the acetate injection. For this reason, technical staff will assess the utility of detecting and measuring rRNA corresponding to obligate anaerobes by amplification methods such as polymerase chain reaction of copy DNA produced by reverse transcriptase. The approach will begin with primers targeting the kingdom and group levels (i.e., archae and delta-proteobacteria), and if successful, progress to more specific primers targeting specific genera.

3.0 BIOCOUPON AND BIOMOLECULAR PROBE SAMPLING

Project phases and approximate number and type of these samples are shown in the following sections. Baseline sampling for the demonstration has taken place as part of the predemonstration site characterization activities. Sampling is summarized by media: groundwater (GW), sediment (SD), and biocoupons (BC).

3.1 BASELINE CHARACTERIZATION

- September 1993: Obtained SD samples from borehole 299-W11-32
3 depths X triplicate samples = 9 SD samples
- Spring 1994: Obtained GW samples from recirculation well
4 samples X duplicate samples = 8 GW samples
- Fall 1994: SD samples from two additional boreholes
2 boreholes X 3 depths X triplicate samples = 18 SD samples.

3.2 DEMONSTRATION

Additional sampling will be conducted during Phase 1 (Abiotic Recirculation), Phase 2 (Active Bioremediation), and Phase 3 (Active Bioremediation, Refined Operation) of the in situ bioremediation demonstration. Each month of active GW recirculation, six samples will be taken for biological characterizations described in this appendix. One GW sample will come from the recirculation well and from each of two monitoring wells, and one BC from each of the two monitoring wells and a background well. Planned samples total:

- 3 demo phases X 9 GW samples/demo phase = 27 GW samples
- 3 demo phases X 9 BC samples/demo phase = 27 BC samples.

3.3 POST-DEMONSTRATION SITE CHARACTERIZATION

The following samples are planned for post-demonstration biocoupon and biomolecular probe application:

- SD samples from one borehole (3 depths X triplicate samples = 9 SD samples)
- GW samples from monitoring wells (3 wells X 3 sampling points = 6 GW samples)
- BC from monitoring wells (3 wells X 2 depths = 6 BC samples).

4.0 REFERENCE

Madsen, E.L. ., 1991, *Determining In Situ Biodegradation*, Environmental Sciences Technology, 25:1663-73.

APPENDIX C

BASELINE SAMPLING PROTOCOLS

APPENDIX C**BASELINE SAMPLING PROTOCOLS****1.0 VOLATILE ORGANIC COMPOUND SAMPLING PROTOCOL**

The sampling system will be purged for 10 minutes before sampling at each sample location. Purgewater will be collected in the onsite purgewater tank for subsequent disposal. Samples will be obtained using a flow-through cell equipped with a septa for withdrawing samples that have not been exposed to the atmosphere. A 1-mL sample will be withdrawn from the cell into a syringe containing 1 mL of hexane. The syringe contents will then be dispensed directly into a screw-cap graduated cylinder containing 4 mL of hexane with the cylinder in a beaker with ice. The hexane and sample will be thoroughly mixed to extract the Volatile Organic Compounds and then allowed to settle so that the hexane and water phases separate. The volumes of sample and hexane will be recorded and the hexane portion dispensed into gas chromatography (GC) vials so that no head space remains. Vials will be immediately crimp sealed and placed on ice with the septa on the bottom. Three samples from each sampling location will be collected. A hexane blank will be prepared for each sampling event. Samples will be transported to the 324 building for analysis by GC.

2.0 ANION SAMPLING PROTOCOL

The sampling system will be purged for 10 minutes before sampling at each sample location. Purgewater will be collected in the onsite purgewater tank for subsequent disposal. Sampling will consist of 5-mL aliquots dispensed through 0.2 μm sterile syringe filters into sterile 15-mL snap-capped sample tubes. Three samples from each sampling location will be collected. Samples will be placed in a cooler on ice and transported to the 324 building for analysis by ion chromatography.

3.0 MICROBIOLOGICAL SAMPLING PROTOCOL

The sampling system will be purged for 10 minutes before sampling at each sample location. Purgewater will be collected in the onsite purgewater tank for subsequent disposal. Samples will be obtained using a flow-through cell equipped with a septa for withdrawing samples that have not been exposed to the atmosphere. A 15-mL sample will be withdrawn from the cell and dispensed into sterile prepurged test tubes sealed with hungate septa. Six samples from each sampling location will be collected. Samples will be placed in a cooler on ice and transported to the 324 building. Three samples will be used for analysis of denitrifiers, sulfate reducers, and iron reducers using the most probable number technique. Three samples will be packaged for overnight shipment to an offsite laboratory for analysis of coliforms using the membrane filter technique.

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