PNNL-11313

Accelerated In Situ Bioremediation of Groundwater

INNOVATIVE TECHNOLOGY SUMMARY REPORT

M.J. Truex
B.S. Hooker
D.B. Anderson

July 1996

Prepared for the U.S. Department of Energy
Office of Environmental Management
Office of Science and Technology

Pacific Northwest National Laboratory
Operated for the U.S. Department of Energy
by Battelle

DISCLAIMER

PACIFIC NORTHWEST NATIONAL LABORATORY
operated by
BATTELLE
for the
UNITED STATES DEPARTMENT OF ENERGY
under Contract DE-AC06-76RLO 1830

Printed in the United States of America

Available to DOE and DOE contractors from the Office of Scientific and Technical Information, P.O. Box 62, Oak Ridge, TN 37831; prices available from (615) 576-8401.

Available to the public from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Rd., Springfield, VA 22161

TABLE OF CONTENTS

SECTION 1: SUMMARY

Technology Description

In situ bioremediation, as applied in this project, is based on the principal of biostimulation: supplying nutrients to indigenous microbes to stimulate their metabolic activity and subsequent degradation of contaminants. Typically, a network of injection and extraction wells are used to recirculate groundwater into which amendments are added and distributed within the aquifer. The objective of the in situ process is to create in the aquifer a microbially active zone that maximizes contaminant destruction while controlling the distribution of microbial growth. It is important to control microbial growth to avoid plugging the aquifer near the injection well and to establish and sustain maximum treatment zones for each injection well. Figure 1 illustrates this concept for in situ bioremediation.

The technology described herein is innovative in its use of the computer-based Accelerated Bioremediation Design Tool (ABDT) to aid in selecting appropriate system designs and to determine optimal operating strategies. In addition, numerical simulations within the design dool proved to be valuable during remediation operations to determine appropriate changes in the operating strategy as the bioremediation process progressed. This is particularly important because in situ bioremediation is not a steady-state process, and corrective actions to operating parameters are typically needed to maintain both rapid destruction rates and hydraulic containment.

Technology Status

A field-scale demonstration of in situ carbon tetrachloride (CT) and nitrate bioremediation was conducted at the U.S. DOE Hanford site from January 1995 through March 1996. Two separate tests were performed in distinct, non-interacting aquifer layers at the same test site. Three phases of field operations were performed: 1) unamended groundwater recirculation, 2) upper zone biostimulation operations, and 3) lower zone biostimulation operations. The results of the demonstration indicate that in situ carbon tetrachloride bioremediation can be implemented with simultaneous denitrification and that the dechlorination byproducts can be controlled to avoid production of chloroform (CF), a hazardous and recalcitrant substance. This demonstration successfully validated the use of the ABDT for in situ bioremediation. Continued application and validation of the ABDT for in situ chlorinated solvent bioremediation is being initiated through several industrial partnerships.

Key Results

- The ABDT developed in this project was used effectively in design and operation of in situ bioremediation. The ABDT provided efficient process evaluation information for determining corrective actions during the field test. Field test validation suggests that the ABDT will be effective in future applications of in situ bioremediation.
- Carbon tetrachloride was destroyed under denitrification conditions with less than 2% conversion to chloroform in both upper and lower zone biostimulation operations.
- The rates of CT destruction measured in the field test (0.8 mg-CT/(g-biomass·day), upper zone; 0.9 mg-CT/(g-biomass·day), lower zone) were comparable to ABDT predictions based on the

laboratory-derived reaction kinetics.

- The growth of over 20 kg (dry weight) of bacteria in the upper zone test and 10 kg (dry weight) in the lower zone test was controlled to obtain good contaminant destruction without plugging of the groundwater reinjection well.
- Preliminary cost estimates indicate that in situ bioremediation is advantageous over pump-and-treat technology due to shorter treatment time duration to meet clean-up objectives as well as moderate capital and operating costs.

Contacts:

Technical

Daniel Anderson, Principal Investigator,
Pacific Northwest National Laboratory, (509) 376-9428

Management

Jim Wright, DOE, Office of Science and Technology, (803) 725-5608

Licensing Information

Richard Brouns, Pacific Northwest National Laboratory, (509) 372-6375

SECTION 2: TECHNOLOGY DESCRIPTION

Overall Process Schematic

A cross-section of the recirculation/monitoring well system used for the upper zone portion of the field demonstration is shown in Figure 2. Groundwater was extracted from well E1, passed through a 25-micron sediment filter and flow control valve, and reinjected into well I1 to create a recirculation pattern over a 12-m interval of the aquifer. Two monitoring wells, M1 and M2, were located on the centerline between the recirculation wells at distances of 3 m and 6 m from the injection well. This well configuration is not necessarily representative of other remediation applications;instead, it was selected to allow collection of the data needed to meet the field test objectives. For the lower zone test, the groundwater flow was in the reverse direction: extracted from well I1 and reinjected into well E1. Each well was screened in both the upper and lower zones. During individual zone tests, the screens were isolated with pneumatic packers.

Operations were conducted from a process-control trailer, where a personal-computer (PC) based process control system automated nutrient injection, sampling operations, and data collection. Concentrated nutrient stock solutions (15% to 20% by weight) of acetate and nitrate were stored in a separate section of the trailer.

Results of laboratory tests were used to define field conditions that would promote optimal CT dechlorination rates and minimal CF production. Acetate pulses were added to the aquifer to initiate biological activity and reduce the nitrate concentration. As required, nitrate was added in pulses to maintain a specified concentration range. Nitrate and acetate pulses were temporally skewed to facilitate biomass formation away from the injection well. The pulse injection strategy was used to limit well-bore biofouling and was optimized using the ABDT.

SECTION 3: PERFORMANCE

Demonstration Plan

The objectives of the field demonstration were:

- Demonstrate in situ biological destruction of CT and nitrate in the Hanford groundwater while minimizing unwanted byproducts.
- Demonstrate nutrient addition strategies that provide effective aqueous nutrient injection to remediate the contamination while minimizing biofouling near the injection well.
- Demonstrate a design method for deploying, controlling, and monitoring in situ bioremediation to restore contaminated aquifers.

Three phases of field operations were performed and are summarized in Table 1.

Table 1. Field Operations Summary

Phase	Duration	Objectives
unamended control and recirculation	January through March 1995	Quantify: 1) loss of contaminants during recirculation without biostimulation 2) baseline concentrations of contaminant species and the variability in these measurements 3) steady-state groundwater flow patterns under recirculation conditions
upper zone biostimulation	May through August 1995	1) demonstrate in situ bioremediation of CT 2) minimize production of CF 3) quantify the rate of contaminant destruction 4) effectively control the in situ bioremediation process
lower zone biostimulation	October 1995 through January 1996	1) confirm performance of the process obtained in the upper zone 2) demonstrate nutrient injection strategies that maximize the zone of influence around the injection well

Treatment Performance

Operational Summary

- Acetate pulses were added to the aquifer to stimulate the activity of indigenous denitrifying bacteria that can co-metabolically dechlorinate CT. Laboratory experiments had shown that the concentration of nitrate affects both the CT dechlorination rate and the rate of chloroform production by the indigenous bacteria.
    - A large plume of nitrate was already present in the aquifer. During initial operations, acetate pulses were added to deplete the nitrate that was present and then maintain nitrate concentrations conducive to rapid CT dechlorination while minimizing chloroform production. Because only partial hydraulic control was possible, nitrate was continuously replenished into the test area from the surrounding plume within the aquifer.
- To maintain active CT-degrading conditions while inducing microbial growth farther from the injection well, nitrate was added in pulses skewed in time from the acetate pulses.
- The ABDT was used to determine the concentration, pulse duration, pulse period, and skew time between acetate and nitrate pulses needed to maintain the desired conditions within the treatment zone of the aquifer.

Performance Summary

- Biostimulation was responsible for the simultaneous destruction of CT and nitrate with less than 2% conversion to chloroform in both upper and lower zone operations.
    - laboratory tests have demonstrated CT dechlorination by indigenous microorganisms under denitrification conditions.
    - during 2 months of unamended groundwater recirculation, CT and nitrate concentrations remained stable.
    - decreases in CT and nitrate concentrations coincided with addition and subsequent depletion of acetate.
    - increases in the numbers of denitrifying microorganisms coincided with addition of acetate, depletion of nitrate, and destruction of CT.
    - when acetate addition was suspended and groundwater recirculation continued, CT and nitrate concentrations increased as predicted based on the hydraulic control and the concentration of contaminants present outside the test site.
- The rates of CT destruction measured in the field test (0.8 mg-CT/(g-biomass·day), upper zone; 0.9 mg-CT/(g-biomass·day), lower zone) were comparable to ABDT predictions based on laboratory-derived reaction kinetics.
- The growth of over 20 kg of bacteria in the upper zone test and 10 kg in the lower zone test was controlled to obtain good contaminant destruction without plugging of the groundwater reinjection well.
- Inexpensive and quickly analyzed bromide tracer and groundwater anion measurements were effectively used in conjunction with the ABDT to control the CT destruction rate and limit the percent conversion to chloroform.
- Nitrate was destroyed at specific rate of 100 mg-nitrate/(g-biomass·day) during initial denitrification of the test site. The rate of denitrification was then controlled as needed to maintain appropriate conditions for efficient CT dechlorination.

◦ In both upper and lower zone tests, the numbers of microorganisms in specific areas of the aquifer were increased by 1 to 2 orders of magnitude, the target selected to reduce the potential of plugging the aquifer. Greater overall rates of CT destruction were achieved by maximizing the biostimulated volume of aquifer.
◦ The pressure required to reinject groundwater increased from 15 to 60 psi during the portion of lower zone operations with the highest input of nutrients into the aquifer. This was the maximum increase observed during testing and was well within operational limits.
◦ During initial biostimulation in both upper and lower zone tests, while the nitrate concentration was high, significant nitrite production occurred. In both tests, a transient operating strategy was used to deplete the accumulated nitrite and create conditions that limited nitrite production for the remainder of biostimulation operations. Transient nitrite production appears to be an inherent problem with denitrification of high nitrate concentrations. However, effective means to overcome nitrite accumulation were developed and successfully demonstrated.

SECTION 4: TECHNOLOGY APPLICABILITY AND ALTERNATIVE TECHNOLOGIES

Technology Applicability

◦ In situ bioremediation is effective for remediation of groundwater and aquifer sediments contaminated with VOCs and nitrate. While in situ CT bioremediation was demonstrated, the technology and, in particular, the ABDT are directly applicable to remediation of other VOCs.
• In laboratory tests, microbial destruction of CT resulted in a residual CT concentration below the drinking water standard.
• Because in situ bioremediation requires recirculation of groundwater to distribute nutrients within the treatment zone, low permeability zones may not be treated or may require additional technology advancements for in situ bioremediation to be effective.
◦ Bacteria catalyze the contaminant destruction reactions used for in situ bioremediation. Thus, aquifer conditions must be suitable for microbial growth or easily manipulated to become suitable. Conditions that may limit effectiveness include toxicity due to high concentrations of contaminant or other toxic substances such as metals, aquifer pH, and aquifer temperature. Treatability tests are typically required to assess the degradation potential present within a specific aquifer.
• In situ bioremediation is more cost effective than extraction technologies for remediation of sorbed contaminants.

Competing Technologies

Cost estimates (see section 5) indicate that in situ bioremediation can remediate CT faster and cheaper than conventional pump-and-treat methods based on a mass removal/destruction basis. Its advantage would be even greater at sites with a greater portion of sorbed contaminant than the one used for the cost estimate. At sites where contaminants are held up in adsorptive sediments, bioremediation can destroy the VOCs in place and reduce the mass transport limitations associated with VOC adsorption/desorption to sediments and dissolution into the groundwater that limits pump-and-treat technologies.

Other in situ contaminant removal technologies such as in-well vapor stripping are being developed. Bioremediation has an advantage over these technologies in that it destroys the contaminant in place rather than transferring the contaminant to another medium.

Technology Maturity

- Numerous published reports of laboratory and bench-scale experiments demonstrate the potential for field application of bioremediation
- A small field test of in situ carbon tetrachloride bioremediation at Moffett Field in California demonstrated and quantified successful biotransformation; however, it did not address in detail scale-up issues or mechanisms to control chloroform production.
- The ABDT is currently being applied at other sites for anaerobic in situ bioremediation in conjunction with industrial partners.

SECTION 5: COST

A cost comparison was performed using the framework established by a previous study (1) which evaluated in situ bioremediation and ex situ air stripping/activated carbon (AS/GAC) in terms of the costs to remove/destroy CT from a groundwater plume of defined extent. The size of the groundwater plume selected for the comparison is based on a conservative estimate of the volume of aquifer that can be treated by in situ bioremediation using only two wells. Costs for each technology were developed based on removing/destroying the initial aqueous (1 mg/L) and sorbed contamination (calculated based on a soil partitioning coefficient of 0.2 mL/g) to reach a final aqueous concentration of 0.005 mg/L.

- Volume of Aquifer Treated - 30,000 m^3 (2000 m^2 by 15 m depth)
- Initial Aqueous Contaminant Concentration - 1 mg/L
- Initial Mass of Contaminant - Aqueous Mass = 9 kg, Sorbed Mass = 9.6 kg
- Estimated Cost/Performance
    - In Situ Bioremediation
        - Overall Treatment Cost - \$5.8/m^3
        - Capital Cost - \$88K
        - Operating Cost - \$46K/yr
        - Overall Treatment Time - 1.9 yr
    - AS/GAC
        - Treatment Cost - \$13.3/m^3
        - Capital Cost - \$172K
        - Operating Cost - \$50K/yr
        - Overall Treatment Time - 4.5 yr

Comparison

Within the framework of this study, in situ bioremediation requires 42% the treatment time and costs 44% of AS/GAC. Calculated treatment costs do not include inflation or other economic

factors such as economic life, depreciation, taxes, and salvage value. Thus, comparisons are not based on life-cycle present value costs. Depending on the values of the economic factors, the relative costs of in situ bioremediation and AS/GAC may change when a life-cycle analysis is performed.

Costs for AS/GAC in this study were significantly affected by the estimated time required to extract contaminant. However, those estimates were based on two conservative assumptions: 1) effective porosity is equal to actual porosity and 2) the aquifer is homogeneous. Some differences in the relative costs of in situ bioremediation and AS/GAC are also dependent on the selected treatment volume. A single extraction well may extract contamination from a larger volume of aquifer than that used in the cost study. While this factor may lower the capital cost per volume of aquifer treated for the AS/GAC system, an increase in the treatment time due to an increase in the purge volume may outweigh these savings.

Costs for in situ bioremediation in this study were developed using process knowledge from the Hanford field test to determine the amount of active biomass and the volume of aquifer affected by the treatment. Use of process simulations is necessary to provide better estimates of these variables. The relative cost of in situ bioremediation and AS/GAC is affected by the well costs because in situ bioremediation requires twice the number of wells. For this comparison, a 25-m well depth with a 15-m screened interval was selected. Well installation costs were assumed to equal $100/ft.

SECTION 6: REGULATORY/POLICY ISSUES

Regulatory Considerations

- No specific permits were required for operation of the field test at Hanford: a categorical exclusion was granted based on NEPA information.
- Application of the technology might require permits for injection of nutrients and groundwater.

Safety, Risks, Benefits, and Community Reaction

Worker Safety

- There are no unusual health and safety issues related to operation of in situ bioremediation.
- Reagents used in the process (acetate and nitrate) are relatively innocuous and can be handled using standard chemical handling practices.
- Level D personnel protective clothing was used during installation and operation of the system.

Community Safety

- In situ bioremediation does not routinely release and has a low potential for release of any reagent or contaminant harmful to the public.
- There are no unusual hazards associated with transport of materials required for in situ bioremediation.
- No pathogen indicator organisms were detected in aquifer samples after biostimulation operations.

Environmental Impacts

- Overall, in situ bioremediation has a positive environmental impact.
  - Surface disturbance at the site can be minimal.
  - The primary environmental issue not fully addressed in this project is the fate of the biomass after active biostimulation ceases.

Socioeconomic Impacts and Community Perception

- In situ bioremediation was perceived as an acceptable and preferred technology based on stakeholder input obtained during the project at Hanford.

SECTION 7: LESSONS LEARNED

Design Issues

- Sufficiently sophisticated process simulators such as those incorporated into the ABDT are critical for selecting effective system designs and for process control during operations.
- An advantage of using simulations to aid in process control is the ability to predict the destruction rate from inexpensive, rapid measurements of anions and conservative tracers. This mechanism allows optimization of operating strategies using measurements of the directly controlled variables (flow rate and nutrient pulses) and measurements of groundwater flow within the treatment zone (conservative tracers).

- In situ bioremediation is an inherently non-steady-state process because it occurs in an open system. Stimulating microbial growth directly affects the permeability of the aquifer. Even moderate changes in the permeability of a region in the aquifer may change the flow patterns induced by groundwater recirculation and therefore change the characteristics of the overall system. Use of the ABDT to evaluate changes in anion and tracer responses and their ramifications on contaminant destruction rate allows quick corrective action to maintain rapid contaminant destruction during these changes.

Implementation Considerations

- A significant portion of operations for in situ bioremediation can be automated. During the field test, a PC-based system controlled the nutrient pulses, collected electronic data for pressure, flow rate, pH, redox, and T, controlled a fraction collector and groundwater pumps to collect samples at a designated frequency, and signaled project personnel if a measured parameter was outside established control limits. Site personnel were required to change operating parameters within the control system, perform sampling for VOCs and microbiology, retrieve samples from the fraction collector, mix nutrient solutions, and perform general maintenance. Operations were monitored based on analysis of anion and conservative tracer data with the aid of the ABDT. Project engineers used these data and ABDT process optimization to select appropriate corrective actions when necessary.
- Periodic sampling of groundwater constituents is required to control the in situ bioremediation process.

Technology Limitations/Needs for Future Development

- The key issue in sustained operation of in situ bioremediation is managing the permeability changes caused by microbial growth. These inherent permeability changes affect the flow patterns, and therefore nutrient distribution, within the treatment zone and may affect hydraulic control of the contaminant plume. The ABDT developed in this project provides an excellent platform for managing the effects of permeability changes, but more field experience is needed to fine tune the approach.
- Mechanisms for effective distribution of nutrients into low permeability zones of an aquifer are needed to expand the range of applicability for in situ bioremediation.
- In situ treatability tests to determine the presence of degradative potential and to determine key reaction and transport parameters are needed to facilitate more cost effective implementation of in situ bioremediation. These tests would circumvent expensive laboratory testing and provide key design information under true in situ conditions. The ABDT developed in this project provides a means to design and analyze in situ treatability tests.

Technology Selection Considerations

- In situ bioremediation compares favorably to extraction technologies with respect to cost and treatment time because it destroys contamination and minimizes mass transfer limitations for destruction of sorbed contaminants.
- In situ bioremediation may be effectively used in combination with plume management and natural attenuation to provide low cost treatment of contamination.
- In situ bioremediation may be effective for treating or mitigating the plume caused by dissolution of non-aqueous phase liquids (NAPLs).
- Site specific conditions affect the performance of in situ bioremediation; therefore, treatability tests must be part of the design process.

APPENDIX A: DEMONSTRATION SITE CHARACTERISTICS

Site History/Background

Hanford, a U.S. Department of Energy site, began defense materials production in 1944. From 1955 to 1973, as part of plutonium recovery processes, carbon tetrachloride, nitrate, water, and other materials were discharged to subsurface liquid waste disposal facilities. In this manner, as much as 600,000 kilograms of CT may have entered the soil column and a portion of this has contaminated the underlying aquifer.

Contaminants of Concern

Carbon tetrachloride (2 mg/L) and nitrate (250 mg/L) are the only contaminants of concern at the field test site.

Nature and Extent of Contamination

An estimated 20,000 to 70,000 kg of CT now contaminates a 10 km^2 plume at Hanford (Figure 3). The highest groundwater concentration of CT is approximately 7 mg/L. Additional information on the CT plume and relevant hydrogeology of the Hanford Site are described in the report entitled "1994 Conceptual Model of the Carbon Tetrachloride Contamination in the 200 West Area at the Hanford Site" (2).

Contaminant Locations and Hydrogeologic Profiles

The test site is in a portion of the groundwater contaminant plume that contains approximately 2 mg/L CT and 250 mg/L nitrate. Contaminant concentrations are relatively constant over a large area of the plume surrounding the test site. The unsaturated zone is 75-m thick and is uncontaminated. Distribution of permeability at the test site is highly stratified with depth due to cementation by carbonate deposits. Hydrologic measurements revealed two highly permeable zones (~10^{-3} cm/s) in the groundwater at depths of 75-to-78 m (245-to-255 ft) and 87-to-92 m (286-to-300 ft) and an intervening low permeability unit (~10^{-6} cm/s). The two permeable zones do not interact significantly even under pumping stresses. Separate biostimulation operations were conducted in both permeable zones. Site hydraulic and chemical characteristics for the upper zone are listed in Table 2. The properties of the lower zone were similar except as noted in the text.

Table 2. Pre-Test Site Characterization Data, Upper Zone

Constituent	**Value**	**Constituent**	**Value**
Hydraulic Properties (average)		*Groundwater Chemistry* (average)	
horizontal hydraulic conductivity	5×10^{-3} cm/s	carbon tetrachloride	1918 µg/L
longitudinal dispersivity	1.0 m	chloroform	12 µg/L
effective porosity	0.15	nitrate	240 mg/L
regional gradient	0.001 m/m	nitrite	nd
Sediment Chemistry		sulfate	55 mg/L
carbon tetrachloride	nd - 0.287 µg/g	oxygen	5 mg/L
chloroform	nd - 0.103 µg/g	pH	7.4
organic carbon	< 0.1 %	temperature	19 C
carbonate	1 - 4 %		
leachable phosphorus	0.027 - 0.053 %		

nd - not detected

APPENDIX B: TECHNOLOGY DESCRIPTION DETAIL

System Configuration

The field test equipment consisted of an injection/extraction well pair, monitoring wells, a nutrient injection system, and a groundwater sampling system. These overall systems are shown in Figure 4. The well configuration shown on the figure is not necessarily representative of other remediation applications; it was selected to allow collection of the data necessary to meet the field test objectives.

The injection/extraction well system used in the field test consisted of a 5-HP submersible centrifugal pump, pneumatic packers to isolate well screen intervals, a 25-µm sediment filter, a manual flow control valve, a turbine flow meter, and pressure transducers in addition to standard plumbing. The flow meter and pressure transducers were monitored with a PC-based control system running AIMAX (TA Engineering Co., Inc., Moraga, CA) process control software.

The nutrient injection system consisted of externally-controllable gear pumps, flow meters, solenoid valves, and pressure transducers connected to the PC-based system to control nutrient injections. Stock solutions were stored in 250-gallon storage tanks. Injection lines released nutrients within the screened intervals of the wells. Check valves provided back pressure for the deep well injection.

The groundwater sampling system consisted of Redi-Flo 2 (Grundfos, Clovis, CA) submersible centrifugal pumps connected to a PC-based control system, solenoid and manual valves to select specific wells for sampling, a fraction collector, and an air compressor. The fraction collector and Redi-Flo pumps were controlled to automatically collect samples for anion analysis. The sample pump system could also be operated manually to retrieve samples for VOCs and microbiology. An in-well Hydrolab (Hydrolab, Austin, TX) probe was connected to the PC-based system to monitor groundwater pH, redox potential, and temperature. Compressed air was used to clear groundwater from sample lines between sampling events to inhibit the growth of biofilms within the sampling system.

Operational Requirements

The use of process automation minimized requirements for operation of the field system. The site labor required is approximately 0.25 full-time equivalent. See also Section 7, "Lessons Learned: Implementation Considerations".

Monitoring Systems

For the field test, only two monitoring wells between the injection/extraction well pair were available due to the cost constraints associated with installation of 320-foot-deep wells. Use of the ABDT allowed extrapolation of measurements from these wells to predictions of conditions in the aquifer within the treatment zone. Because the ABDT provided accurate predictions, process control was maintained with a minimal number of monitoring locations.

APPENDIX C: PERFORMANCE DETAIL

Performance Analysis

Introduction

Laboratory studies have demonstrated microbial dechlorination of CT by denitrifying (3, 4, 5), fermentative (6, 7), iron-reducing (8), sulfate-reducing (9), and methanogenic (9, 10) bacteria. Dechlorination products include primarily chloroform (CF), CO_2, and non-volatile material; the distribution of these products is a function of the microbial system and the properties of the medium. Chloroform is an undesirable product because it is recalcitrant to further dechlorination under most anaerobic conditions and is a regulated groundwater contaminant. Although pathways for specific microbial systems have not been fully elucidated, Criddle and McCarty (11) outlined potential abiotic and biotic transformations of CT. Biotic conversion of CT to intermediate radicals may occur through either one or two electron transfers. Under anoxic conditions, CF is the dominant product of one-electron transfer; two-electron transfer yields formate or carbon monoxide, which are subsequently converted to CO_2. In contrast to these dechlorination mechanisms, *Pseudomonas* sp. strain KC may initiate CT transformation by a one- electron transfer and produce CO_2 and nonvolatile materials without producing CF (12).

Semprini et al. (13) demonstrated in situ bioremediation of CT in a field test at Moffett Field Naval Air Station. In this test, pulses of acetate were introduced into a thin, confined aquifer to stimulate microbial activity. Chloroform was observed as a significant dechlorination product. Although operations under nitrate-limited conditions produced the least CF, the amount of CF produced corresponded to more than 30% of the CT transformed (13). The Moffett Field bioremediation test demonstrated and quantified successful in situ CT biotransformation; however, it did not address scale-up issues in detail nor mechanisms to control chloroform production.

In situ bioremediation, as applied in the Hanford field test, is based on the principal of biostimulation: supplying substrate and nutrients to indigenous microbes to stimulate metabolic activity and consequent degradation of contaminants. The field test conducted at Hanford was focused on 1) determining effective methods to control the in situ bioremediation process, 2) evaluating system performance, 3) formulating a useful scale-up technique for in situ bioremediation, and 4) minimizing in situ CF production by controlling the concentration of nitrate as demonstrated in the laboratory by Sherwood et al. (14). The field test was preceded by laboratory studies of reaction kinetics and biofilm processes in porous media that defined the appropriate conditions for the desired dechlorination reactions. These data and field hydrological information were incorporated into reactive flow and transport computer models for use in design and process control of the field test.

Field Test Method Summary

Microbial Characterization

Facultative denitrifiers were the dominant culturable bacteria (approximately 10^4 denitrifiers/g soil) in aquifer sediments at the test site. The denitrifying microbial consortium was further

characterized in laboratory treatability tests using batch reactors (5, 14) and soil columns (15, 16). Batch tests showed that nitrate concentration and duration of nitrate deprivation affected both the rate of CT dechlorination and end product distribution (14). In the presence of less than 20 mg/L nitrate, CT was rapidly transformed without the production of CF, while higher levels of nitrate significantly inhibited dechlorination. After active nitrate reduction was induced, absence of nitrate for greater than 12 hours while substrate (acetate) was present caused CF production as a significant dechlorination product, accounting for up to 30% of the CT destroyed.

Field Equipment

A cross-section of the recirculation/monitoring well system used for the upper zone portion of the field demonstration is shown in Figure 2. Groundwater was extracted from well E1, passed through a 25-micron sediment filter and flow control valve, and reinjected into well I1 to create a recirculation pattern over a 12-m interval of the aquifer. Two monitoring wells, M1 and M2, were located on the centerline between the recirculation wells at distances of 3 m and 6 m from the injection well. For the lower zone test, the groundwater flow was in the reverse direction: extracted from well I1 and reinjected into well E1. Operations were conducted from a process-control trailer, where a computer system automated nutrient injection, sampling operations, and data collection. Concentrated nutrient stock solutions (15% to 20% by weight) were stored in a separate section of the trailer.

Field Test Operation

Three phases of Hanford field operations were performed: 1) unamended control and recirculation, 2) upper zone biostimulation operations, and 3) lower zone biostimulation operations. The unamended control phase was completed between January and March 1995. During this phase, groundwater was recirculated without addition of nutrients to quantify 1) any loss of contaminants during recirculation without biostimulation, 2) baseline concentrations of contaminant species and the variability in these measurements, and 3) steady-state groundwater flow patterns under recirculation conditions.

The upper zone biostimulation phase was initiated in May 1995 and continued for four months. The objectives of this phase of biological operations were to 1) demonstrate in situ bioremediation of CT, 2) minimize production of CF, 3) quantify the rate of contaminant destruction, and 4) effectively control the in situ bioremediation process. Biostimulation activity can be divided into Start-Up, Continuous Operations, and Chloroform Test operational stages as shown on Figure 5. This figure details the recirculation flow rate and amount of nutrients injected during each stage. Start-Up and Continuous Operations are stages of primary biostimulation operations. The Chloroform Test was a separate test performed to assess factors that control the accumulation of CF in the field.

The lower zone biostimulation phase was initiated in October 1995 and completed in January 1996. The objectives of this phase were to 1) confirm the performance of the process and 2) demonstrate nutrient injection strategies that maximize the zone of influence around the injection well. Figure 6 illustrates the recirculation flow rate and amount of nutrients injected during this

phase. This phase can be divided into two operational periods, CT Rate Confirmation and Biomass Distribution Test, as outlined on the figure. The period of no flow between day 28 and day 51 was due to a failure of some in-well equipment.

Results of laboratory tests were used to define field conditions that would promote optimal CT dechlorination rates and minimal CF production. Acetate pulses were added to the aquifer to initiate biological activity and reduce the nitrate concentration. As required, nitrate was added in pulses to maintain the nitrate concentration within a specified range. Nitrate and acetate pulses were temporally skewed to distribute biomass away from the injection well. A pulse injection strategy was used to limit well-bore biofouling as recommended by Roberts et al. (17).

Results

Unamended Groundwater Recirculation

Carbon tetrachloride and CF concentrations remained constant during unamended groundwater recirculation at 1.92 (0.022) mg/L and 0.011 (0.002) mg/L, respectively. The numbers in parentheses indicate the 95% confidence intervals. The nitrate concentration was also stable at 240 (16) mg/L. Stable measured CT and nitrate concentrations indicate that contamination was relatively uniform throughout the volume of aquifer affected by recirculation.

Upper Zone Biostimulation Operations

Dechlorination results for upper zone biostimulation operations are plotted in Figure 7. The solid line on the graph represents the predicted CT concentrations at the extraction well. Predictions were based on numerical simulations using the actual acetate and nitrate feeding schedule and the measured recirculation flow rate over the course of the field test. The flow field was calibrated to field tracer tests at three times during upper zone biostimulation operations. Because the reaction kinetic parameters within the simulators were not modified to fit field data, the agreement between measured and predicted concentrations was not quantified. Reported results, however, demonstrate an overall agreement between measured and predicted CT concentrations and suggest that *a priori* simulations can be successful in selecting designs and operating strategies for in situ bioremediation.

Start Up

During start up, an operating strategy to rapidly denitrify the test site was selected to demonstrate in situ denitrification and establish conditions that promote rapid CT destruction. During the first 8 days of operation, high concentration pulses of acetate were injected to stimulate microbial growth and nitrate reduction (Figure 5), and CT concentration fell significantly (from 1920 to 1630 µg/L) (Figure 7). After 5 days of operation, the concentration of nitrite, an intermediate of microbial nitrate reduction, increased to an unacceptable concentration of approximately 100 mg/L. Earlier kinetic studies (15) show some toxicity to denitrifiers at nitrite concentrations as low as 25 mg/L. At the same time, growth of biomass in the proximity of the extraction well was sufficient to decrease permeability. Because the extraction well is screened to a depth of only 3 m below the water table, the small change in aquifer permeability (causing an observed 1-2 psi pressure drop in the extraction well) resulted in dewatering of the well. Subsequently, the

recirculation flow rate had to be reduced. Between days 8 and 26, nitrite accumulation and reduced pumping capacity problems were addressed.

To reduce nitrite concentration in the field, a large pulse of acetate (10.5 kg)was introduced into the aquifer, centered around well M1, and recirculation was stopped. By providing more acetate than required to convert nitrate to nitrite, sufficient acetate remained to stimulate microbial nitrite reduction. A second pulse of acetate (7.8 kg) was also introduced and centered around well M2 with the same effect. After this stimulation of nitrite reducing ability, nitrite concentration remained within acceptable operating limits for the remainder of the field test.

Groundwater recirculation was reinitiated at day 20, and pumping continued without addition of acetate for 7 days. The permeability of the aquifer increased during this time as indicated by the pressure response in the site wells (data not shown). It is likely that this permeability change resulted from a decrease in biomass through endogenous respiration. Carbon tetrachloride concentrations increased during this time due to the reintroduction of contaminant from far-field regions (Figure 7). On day 27, the recirculation flow rate was stabilized at 45 L/min (12 gpm) to provide a residence time in the system that was conducive to favorable distribution of biomass, yet limited its accumulation near the extraction well. A revised biostimulation strategy was developed to induce good CT destruction under these operating conditions. The required operational constraints were not associated with injection well fouling; but with biomass buildup 12 m away from the injection well.

Continuous Operations

Continuous Operations began on day 27. The initial recirculation flow rate was 45 L/min (12 gpm) produced a hydraulic control of about 72% and a mean residence time in the aquifer of approximately 24 hours as determined from tracer test data. Further changes in aquifer permeability made it necessary to reduce the recirculation flow rate between days 40 and 44 and to implement tighter constraints on the operating conditions to prevent dewatering of the extraction well. The recirculation flow rate was decreased and remained relatively constant at near 32 L/min (8.5 gpm). The resulting hydraulic control and mean residence time in the aquifer were about 62% and 48 hours, respectively, as confirmed by tracer test data. Carbon tetrachloride destruction activity was maintained while operating under these constraints, and the Continuous Operations stage ended at day 97.

During the Continuous Operations stage, CT concentration was reduced from 1875 µg/L to approximately 1300 µg/L and CF formation was insignificant (Figure 7). Throughout this operational period, active denitrification occurred without significant nitrite accumulation. The evaluation of in situ bioremediation performance was primarily based on estimated rate parameters (Table 3). The same time intervals were used for calculated and predicted estimates.

Table 3. Estimated CT Destruction Rate. Rates are reported from simulator predictions (P) and as calculated using a reactor-based analysis (C). Numbers in parentheses indicate the 95% confidence interval on the calculated mean value using a Monte Carlo simulation. The average nitrate concentration (N) within the biologically active areas of the aquifer as estimated in process simulations is shown for each stage of operation.

Stage of Operation	Overall CT Destruction Rate (g-CT/d)		Overall Specific CT Destruction Rate (mg-CT/g-biomass·d)		First Order Rate Constant (m^3/mol-biomass·d)	
	C	P	C	P	C	P
Start Up (N = 194 mg/L)	6.9 (1.2)	1.3	0.6 (0.12)	0.13	0.057 (0.012)	0.0085
Day 30-45 (N = 76 mg/L)	5.4 (1.7)	3.5	0.61 (0.23)	0.46	0.057 (0.021)	0.051
Day 80-95 (N = 31 mg/L)	15.6 (1.9)	13.2	0.79 (0.095)	0.71	0.094 (0.018)	0.119

Rates of CT destruction increased consistently during the field test. As the field test progressed, low nitrate and nitrite concentrations, conditions that favor CT destruction, were obtained and then stabilized during the last month of field testing. Changes in the overall CT destruction rate at the time intervals listed in Table 3 illustrate the effect of nitrate on the CT dechlorination rate. The average nitrate concentration within the microbially active zone of the aquifer during these times are also shown in the table. Operating at lower concentrations of nitrate in the field may provide higher contaminant destruction rates, but this strategy may also increase the potential for chloroform production.

Chloroform Test

For more than 90 days of field operations, CT was destroyed without significantly increasing the CF concentration. To demonstrate that this success was due to proper control of in situ conditions, a test was performed to determine whether CF production could be induced in the field under conditions favorable to CF formation in the laboratory. In this portion of the field test, a 10.5-kg pulse of acetate was introduced into the aquifer and recirculation was discontinued when the pulse was centered around well M1. Seven days later another large pulse of acetate (19.5 kg) was again positioned around well M1 in the same manner. The result was excess acetate and a complete depletion of nitrate and nitrite in the aquifer around the well. Laboratory studies had shown that these conditions, i.e. the prolonged absence of electron acceptor, favors reductive dechlorination of CT with accumulation of CF (14). Once produced, CF is recalcitrant to further metabolism by the Hanford denitrifying consortium. During the Chloroform Test, CF concentrations increased from 15 to 183 µg/L (a change of about 0.17 mg/L) (Figure 7) and CT concentrations fell by 0.9 mg/L. These changes in the CT concentration were larger than those observed during Continuous Operations because there was no inflow to reintroduce additional

CT. Furthermore, the low nitrate concentrations induced around the monitoring well were conducive to rapid CT dechlorination. During the Chloroform Test, 35% of the CT destroyed was converted to CF. In contrast, during Continuous Operations, the percent conversion of CT to CF was 1.6%.

Process Control

Accurate prediction of transient electron donor and acceptor concentrations at the monitoring wells is an important indicator of process control. Transient concentrations of acetate and nitrate were tracked periodically at the monitoring wells. Measured responses were compared to simulator predictions to confirm that the numerical simulations accurately predicted microbial growth and substrate consumption within the flow field. This confirmation is important because the simulations were used to estimate the biomass concentration in the aquifer for rate calculations. No direct measurement of attached biomass for in situ bioremediation was practical for the Hanford field test. Agreement between measured and simulated responses is also important because the success of a particular operating strategy depends on determining how to manipulate the system to produce the desired in situ conditions. Optimizing CT dechlorination without CF accumulation, required controlling the activity of the indigenous bacteria by altering the flux of acetate and nitrate. Specifically, the acetate flux controlled the total biomass, and the nitrate flux/concentration controlled the formation of CF and the CT destruction rate. Figure 8 shows the measured and predicted acetate and nitrate concentrations at well M1 for one monitoring event during the Continuous Operations stage of the field test. Throughout the field test similar agreement between predicted and measured results was observed. These data and the close agreement of measured and predicted CT concentrations suggest that the approach used for this field test was successful in controlling in situ conditions to obtain CT destruction rates comparable to those achieved in the laboratory while avoiding production of CF.

Microbial Response

During upper zone operations, the microbial concentration was increased by 1 to 2 orders of magnitude within specific portions of the aquifer. Maintaining this moderate increase in microbial concentration was desired to limit the chance of aquifer plugging. Biomass was increased by one order of magnitude up to 5.6 m away from the injection well.

Lower Zone Biostimulation Operations

Preceeding biostimulation operations in the lower zone, the groundwater was recirculated for 3 weeks without addition of nutrients to establish baseline conditions. During these unamended operations, the contaminant concentrations were stable. The average and 95% confidence intervals for the contaminant concentrations were; CT 2.725 (0.039) mg/L, chloroform 0.030 (0.0017) mg/L, and nitrate 290 (5) mg/L.

CT Rate Confirmation Test

The specific rate of CT destruction obtained during the CT Rate Confirmation Test portion of lower zone operations was 0.9 mg-CT/(g-biomass·day). In contrast to the long start up period required for upper zone operations, this rate of CT destruction was obtained within only 15 days

of initiating biostimulation in the lower zone. We were able to more quickly approach optimal operating conditions by better understanding the dynamics of the initial microbial activity. While we still encountered a buildup of nitrite after 15 days of operation, we were able to smoothly transition to operations that depleted nitrite yet retained appropriate conditions for good CT destruction. Chloroform production was controlled and represented less than 2% of the CT destroyed during the CT Rate Confirmation Test.

During the CT Rate Confirmation portion of lower zone operations, the microbial concentration was increased by 1 to 2 orders of magnitude within specific portions of the aquifer. Biomass was increased by one order of magnitude up to 8 m away from the injection well.

Biomass Distribution Test

During the Biomass Distribution Test, we doubled and then tripled the initial flux of acetate into the injection well. Nitrate was added as required to maintain appropriate nitrate concentrations in the aquifer using pulses that were offset in time from the pulses of acetate. This technique of skewed pulsing for acetate and nitrate was used to help spread microbial activity over a large volume of the aquifer and minimize biomass buildup in close proximity to the injection well. The aquifer microorganisms have significant activity only when acetate and nitrate are present simultaneously.

During the Biomass Distribution Test, 368 kg of acetate and 176 kg of nitrate were injected. This amount of nutrients would produce approximately 63 kg (dry weight) of biomass based on a denitrification yield of 0.17 g-biomass/g-acetate estimated from the kinetics reported by Hooker et al. (18). The biomass produced during the Biomass Distribution Test is in addition to the 28 kg of biomass already present in the lower zone of the aquifer due to the acetate injected during the CT Rate Confirmation Test. The injection well pressure increased from 21 to 57 psi in response to these increases in aquifer biomass (well within operational limits) and the recirculation flow rate remained relatively constant between 13.5 and 14.5 gpm.

To further confirm that the nutrient strategy used during the Biomass Distribution Test was successful in minimizing bioimass buildup in close proximity to the injection well, a constant-discharge pumping test was performed 1 week after the end of biostimulation operations. In this test, water was extracted at a constant rate of 3 gpm from well M2 and the pressure response in well M2 and the well used as the injection well during biostimulation operations were monitored. Pressure responses in both wells were also monitored after pumping was halted until pressures returned to pre-test static conditions. Based on analysis of these data, the hydraulic conductivity of the aquifer between the injection well and monitoring well M2 was estimated as 8.5×10^{-3} cm/s. This value of hydraulic conductivity represents only a small decrease from the pre-test hydraulic conductivity of 9.4×10^{-3} cm/s determined during hydraulic characterization of the lower zone.

While it is apparent that excessive near-well biomass buildup was avoided, production of high biomass concentrations within the aquifer did significantly alter the flow pattern of groundwater

between injection and extraction wells. Prior to injection of nutrients into the lower zone, the flow pattern between the wells was well characterized by a recirculation tracer test in which a pulse of sodium bromide was introduced into the injection well and tracked at monitoring well M2 and the extraction well. Results of this test indicate an shortest-path residence time of approximately 25 hr at a recirculation flow rate of 14.2 gpm and produced a defined pulse at well M2 (Figure 9). In contrast, bromide was not detected at well M2 or the extraction well even after 72 hrs in a tracer test performed near the end of the Biomass Distribution Test at a recirculation flow rate of 14.9 gpm (Figure 10). Thus, the flow pattern had been significantly altered such that the injection and extraction wells had effectively lost hydraulic communication. These type of changes in the hydraulic flow patterns due to growth of biomass in the aquifer were also observed to a much smaller extent in the upper zone tests. Because of these large changes in flow patterns during the Biomass Distribution Test and the resulting loss of hydraulic control, evaluation of the CT destruction rate was not possible. The nutrient addition strategy used for the Biomass Distribution Test provided a large biomass distribution while minimizing biomass buildup near the injection well, but caused difficulty in monitoring of the process and hydraulic control within the relatively small well network of the field test system.

Water Quality

A limited number of groundwater samples for coliform bacteria and cations were collected before and after biostimulation operations. Coliform bacteria were not detected in any samples. RNA molecular probe techniques and other microbial enumeration methods provided additional information about the types of microorganisms present during biostimulation operations. These data indicate that denitrifiers were the dominant organisms during biostimulation operations. Methanogen and sulfate-reducing bacteria numbers did not increase during the field test. However, the number of eukaryotes, presumably protozoa, did increase significantly in response to the increased numbers of bacteria in the aquifer. Sodium was the only cation concentration that changed significantly during biostimulation. The sodium concentration increased by approximately 30 mg/L due to injection of nutrient solutions which were the sodium salts of acetate and nitrate.

Sampling, Monitoring, Analysis, and QA/QC Issues

Sampling, Monitoring, and Analysis

Pulses of acetate and nitrate were tracked periodically at monitoring wells and the extraction well to determine the extent of microbial activity throughout the flow field. These data were then compared to the concentration of a conservative tracer to determine the extent of degradation, production, and/or retardation of a particular species. Anion data were generally collected weekly, although a more intensive sampling schedule was used during start-up of biostimulation operations. Carbon tetrachloride, CF, and dichloromethane concentrations were measured and planktonic bacteria were enumerated every two weeks. Groundwater pH, temperature, and oxidation/reduction potential were monitored continuously and logged on a computer.

A field sampling system allowed both manual and automated sampling of groundwater. Purge

times for the sampling system were determined using a bromide tracer to verify that the concentrations measured in groundwater samples were representative. During manual sampling, use of a needle and syringe to withdraw aliquots from a flow-through line sealed with a butyl rubber septum prevented exposure of groundwater samples with air. For manual anion sampling, a 10-mL sample of groundwater was withdrawn with a 10-mL syringe, sterilized by filtration through a 0.2-μm filter into a sterile 15-mL snap-top test tube, and frozen. For VOC sampling, a 25-mL gas-tight syringe containing 10-mL of hexane (an extractant for CT and other chlorinated organics) was used to withdraw 10-mL of groundwater. The hexane and water were mixed in the syringe, dispensed to a 25-mL glass screw-top centrifuge tube, and frozen. Samples were transported from the field to the laboratory and analyzed within 24 hours. After sampling, pressurized air was used to clear the sample line of groundwater and thereby inhibit biomass buildup, which could affect sample quality.

In automated sampling for anion analyses, a fraction collector was used to dispense 10-mL of groundwater to a 15-mL snap-top test tube containing 200 μL of 0.5 M sodium carbonate (to keep the pH above pH 10 and thereby inhibit microbial growth). Within 24 hours of sampling, samples were removed from the fraction collector and frozen. The sample lines were drained and cleared as described for the manual samples.

Anion concentrations were measured using a Dionex 500 ion chromatograph equipped with a PAX 100 anion exchange column, conductivity detector and ultraviolet detector (Dionex, Sunnyvale, CA). Thawed VOC samples were prepared for analysis by mixing the hexane and groundwater for 2 min on a vortex mixer and then separating the hexane phase by centrifugation at 2700 g for 5 min. The VOCs in the hexane were measured using a HP5890 Series II gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with an electron capture detector and a 30-m by 0.52-mm(ID) DB-624 column (J&W Scientific, Fulsom, CA).

QA/QC

Data Quality Objectives (DQO) were established for variability, accuracy, precision, completeness, representativeness, and comparability. In addition, procedures for calibration and standards preparation were developed for analysis of samples. Quality assurance and control procedures were developed based on EPA guidance documents for conducting CERCLA treatability studies.

Performance Validation

- During operation of the field test, none of the DQO parameters were outside the limits established in the Integrated Test Plan.
- Results of onsite sample analysis for all constituents were compared to offsite analysis by a separate contract laboratory using EPA methods. In all cases onsite methods produced comparable or superior results.

APPENDIX D: COMMERCIALIZATION/INTELLECTUAL PROPERTY

The intellectual property (IP) developed in this project is centered around the ABDT, process control, and monitoring methods for in situ bioremediation. Commercialization and technology transfer activities include: 1) co-development and transfer to industry, and 2) transfer and deployment to the EM-40 programs. Memoranda of Understanding have been signed with three full service environmental engineering/remediation firms, OHM Remediation Services Corporation, Montgomery Watson, and Parsons Engineering Science, as the first step in developing partnerships to validate and transfer this technology to industry. Several joint projects are planned to begin in FY97 with these industrial partners.

In addition, this technology and related IP are being discussed for use by EM-40 programs in addressing environmental issues at Hanford and several other DOE sites.

APPENDIX E: REFERENCES

1) Skeen, R. S., S. P. Luttrell, T. M. Brouns, B. S. Hooker, M. Shouche, and J. N. Petersen. 1993. In Situ Bioremediation of Hanford Groundwater. *Remediation* 3:353-367.

2) Rohay, V. J. 1994. 1994 Conceptual Model of the Carbon Tetrachloride contamination in the 200 West Area at the Hanford Site. WHC-SD-EN-TI-248, Westinghouse Hanford Company, Richland, Washington.

3) Criddle, C.S., J.T. DeWitt, D. Grbic-Galic, and P.L. McCarty. 1990. Transformation of Carbon Tetrachloride by *Pseudomonas* sp. Strain KC under Denitrification Conditions. *Appl. Environ. Microbiol.* 56:3240-3246.

4) Bouwer, E.J. and P.L. McCarty. 1983. Transformation of halogenated organic compounds under denitrification conditions. *Appl. Environ. Microbiol.* 45:1295-1299.

5) Petersen, J. N., R. S. Skeen, K. M. Amos, and B. S. Hooker. 1994. Biological Destruction of CCl_4: Part 1. Experimental Design and Data. *Biotechnol. Bioeng.* 43:521-528.

6) Galli, R. and P.L. McCarty. 1989. Biotransformation of 1,1,1-Trichloroethane, Trichloromethane, and Tetrachloromethane by *Clostridium* sp. *Appl. Environ. Microbiol.* 55:837-844.

7) Criddle, C.S., J.T. DeWitt, and P.L. McCarty. 1990. Reductive Dehalogenation of Carbon Tetrachloride by *Escherichia coli* K-12. *Appl. Environ. Microbiol.* 56:3247-3254.

8) Picardal, F.W., R.G. Arnold, H. Couch, A.M. Little, and M.E. Smith. 1993. Involvement of Cytochromes in the Anaerobic Biotransformation of Tetrachloromethane by *Shewanella putrefaciens* 200. *Appl. Environ. Microbiol.* 59:3763-3770.

9) Egli, C., T. Tschan, R. Scholtz, A.M. Cook, and T. Leisinger. 1988. Transformation of Tetrachloromethane to Dichloromethane and Carbon Dioxide by *Acetobacterium woodii*. *Appl. Environ. Microbiol.* 54:2819-2824.

10) Bouwer, E.J. and P.L. McCarty. 1983. Transformation of 1- and 2- carbon halogenated aliphatic organic compounds under methanogenic conditions. *Appl. Environ. Microbiol.* 45:1286-1294.

11) Criddle, C.S. and P.L. McCarty. 1991. Electrolytic Model System for Reductive Dehalogenation in Aqueous Environments. *Environ. Sci. Technol.* 25:973-978.

12) Lewis, T.A. and R.L. Crawford. 1995. Transformation of Carbon Tetrachloride via Sulfur and Oxygen Substitution by *Pseudomonas* sp Strain KC. *J. Bacteriol.* 177:2204-2208.

13) Semprini, L., G.D. Hopkins, P.L. McCarty, and P.V. Roberts. 1992. In Situ Transformation of Carbon Tetrachloride and Other Halogenated Compounds Resulting From Biostimulation Under Anoxic Conditions. *Environ. Sci. Technol.* 26:2454-2461.

14) Sherwood, J.L., J.N. Petersen, and R.S. Skeen. 1996. Effects of Nitrate and Acetate Availability on Chloroform Production During Carbon Tetrachloride Destruction. *Biotechnol. Bioeng.*, in press.

15) Peyton, B.M. 1996. Improved Biomass Distribution in Porous Media Using Pulsed Nutrient Delivery. *Water Res.* 30(3):756-758.

16) Jennings, D. J., J. N. Petersen, R. S. Skeen, B. S. Hooker, B. M. Peyton, and D. J. Johnstone. 1995. Effects of Slight Variations in Nutrient Loadings on Pore Plugging in Soil Columns. *Appl. Biochem. Biotechnol.* 51/52:727-734.

17) Roberts, P.V., G.D. Hopkins, L. Semprini, P.L. McCarty, and D.M. MacKay. 1991. Pulsing of Electron Donor and Electron Acceptor for Enhanced Biotransformation of Chemicals. U.S. Patent No. 5,006,250.

18) Hooker, B.S., R.S. Skeen, and J.N. Petersen. 1994. Biological Destruction of CCl_4, Part II: Kinetic Modeling. *Biotechnol. Bioeng.* 44:211-218.

Bibliography of Publications to Date Resulting from the In Situ Bioremediation Project

In Situ Bioremediation Design

Clement, T.P., B.S. Hooker, and R.S. Skeen. 1996. Numerical modeling of biologically reactive transport from a nutrient injection well. *ASCE Journal of Environmental Engineering Division*, in press.

Clement, T.P., B.S. Hooker, and R.S. Skeen. 1995. Modeling biologically reactive transport in porous media. In: Proceedings of the International Conference on Mathematics and Computations, Reactor Physics, and Environmental Analyses, Portland, Oregon, April-May 1995, Vol. 1., p. 192-201.

Clement, T.P., B.S. Hooker, and R.S. Skeen. 1995. Modeling of In-Situ Biological Processes in Contaminated Aquifers. In: Proceedings of the Annual meeting of Air & Waste Management Association, San Antonio, Texas, in press.

Clement, T. P., B. S. Hooker, and R. S. Skeen. 1995. Modeling Biologically Reactive Transport in Porous Media. In: Proceedings of the International Conference on Mathematics and Computations, Reactor Physics, and Environmental Analyses, Portland, Oregon. in press.

Peyton, B.M., M.J. Truex, R.S. Skeen, and B.S. Hooker. 1995. The Use of Bench and Field-scale Data for the Design of an *In Situ* Carbon Tetrachloride Bioremediation System. In: Proceedings of the Third International Symposium on *In Situ* and On-Site Bioreclamation, San Diego, CA, April 24-27, Battelle Press, Columbus, OH.

Cunningham, A.B. and B.M. Peyton. 1995. Bench-Scale/Field-Scale Interpretation of *In Situ* Bioremediation. In Proceedings of the Third International Symposium on *In Situ* and On-Site Bioreclamation, San Diego, CA, April 24-27, Battelle Press, Columbus, OH.

Kramer, C.D. 1995. Integrated Test Plan: In Situ Bioremediation Demonstration. BHI-00154, Bechtel Hanford, Inc., Richland, WA.

Hooker, B. S., R. S. Skeen, and J. N. Petersen. 1994. Application of a Structured Kinetic Model to the Bioremediation of Hanford Groundwater. pp. 387-392. In: Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds, R.E. Hinchee *et al.*, eds. Lewis, Boca Raton, FL.

Shouche, M. J., J. N. Petersen, R. S. Skeen, and B. S. Hooker. 1994. Alternating Extraction/Injection Well Interactions for In Situ Bioremediation. *Appl. Biochem. Biotechnol.*, 45, 775-785.

Hooker, B.S., R.S. Skeen, M.J. Truex, and B.M. Peyton. 1994. A Demonstration of *In Situ* Bioremediation of CCl_4 at the Hanford Site. pp. 281-292 In: In Situ Remediation: Scientific Basis for Current and Future Technologies, Part 1, G. W. Gee and N. R. Wing, eds., Battelle Press, Columbus.

Truex, M. J., C. D. Johnson, D. R. Newcomer, L. A. Doremus, B. S. Hooker, B. M. Peyton, R. S. Skeen, and A. Chilikapati. 1994. Deploying *In Situ* Bioremediation at the Hanford Site. pp. 209-231 In: In Situ Remediation: Scientific Basis for Current and Future Technologies, Part 1, G. W. Gee and N. R. Wing, eds., Battelle Press, Columbus.

Truex, M.J., D.R. Brown, and D.C. Elliott. 1993. Cost/Benefit Analysis Comparing Ex Situ Treatment Technologies for Removing Carbon Tetrachloride from Hanford Ground Water. PNL-8334, Pacific Northwest Laboratory, Richland, Washington.

Skeen, R. S., S. P. Luttrell, T. M. Brouns, B. S. Hooker, M. Shouche, and J. N. Petersen. 1993. In Situ Bioremediation of Hanford Groundwater. *Remediation* 3:353-367.

Shouche, M. J., J. N. Petersen, and R. S. Skeen. 1993. Use of a Mathematical Model for Prediction of Optimum Feeding Strategies for *In situ* Bioremediation. *Appl. Biochem. Biotechnol.* 39:763-779.

Skeen, R. S., S. M. Cote, M. J. Truex, and J. N. Petersen. Kinetics of *In Situ* Bioremediation of Hanford Groundwater. 1992. In: Proc. Spectrum '92 Conference, August 12-27, Boise, Idaho, 1209-1213.

Skeen, R. S., K. R. Roberson, D. J. Workman, J. N. Petersen, and M. Shouche. 1992. *In Situ* Bioremediation of Hanford Groundwater. In: Proc. Federal Environmental Restoration '92 Conference, April 15-17, Vienna, Virginia, 113-117.

Brouns, T. M., D. B. Anderson, J. K. Fredrickson, S. P. Luttrell, R. S. Skeen, and D. J. Workman. 1991. Bioremediation of Hanford Groundwater. In: Proc. Environmental Remediation *'91*, 101-106.

Microbial Kinetics

Sherwood, J.L., J.N. Petersen, and R.S. Skeen. 1996. Effects of Nitrate and Acetate Availability on Chloroform Production During Carbon Tetrachloride Destruction. *Biotechnol. Bioeng.*, in press.

Skeen, R. S., N. B. Valentine, B. S. Hooker, and J. N. Petersen. 1995. Kinetics of Nitrate Inhibition of Biological Transformation of Carbon Tetrachloride. *Biotechnol. Bioeng.* 45:279-284.

Skeen, R. S., K. M. Amos, and J. N. Petersen. 1994. Influence of Nitrate Concentration on Carbon Tetrachloride Transformation by a Denitrifying Microbial Consortium. *Water Research* 28, 2433-2438.

Hooker, B. S., R. S. Skeen and J. N. Petersen. 1994. Biological Destruction of CCl_4, Part II: Kinetic Modeling. *Biotechnol. Bioeng.* 44:211-218.

Petersen, J. N., R. S. Skeen, K. M. Amos, and B. S. Hooker. 1994. Biological Destruction of CCl_4: Part 1. Experimental Design and Data. *Biotechnol. Bioeng.* 43:521-528.

Skeen, R. S., M. J. Truex, J. N. Petersen, and J. S. Hill. 1994. A Batch Reactor for Monitoring Process Dynamics During Biodegradation of Volatile Organics. *Environmental Progress* 13:174-177.

Truex, M. J., R. S. Skeen, S. M. Caley, and D. J. Workman. 1994. Comparative Efficiency of Microbial Systems for Destroying Carbon Tetrachloride Contamination in Hanford Ground Water. In: Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds, R. E. Hinchee, L. Semprini, and S. K. Ong (eds.), Lewis Publishers, Ann Arbor, MI, 80-85.

Biofilms and Microbial Transport

Clement, T.P., B.S. Hooker, and R.S. Skeen. 1995. Macroscopic models for predicting changes in saturated porous media properties cause by microbial growth. *Ground Water.* in press.

Jennings, D. J., J. N. Petersen, R. S. Skeen, B. S. Hooker, B. M. Peyton, and D. J. Johnstone. 1995. Effects of Slight Variations in Nutrient Loadings on Pore Plugging in Soil Columns. *Appl. Biochem. Biotechnol.* 51/52:727-734.

Peyton, B.M. 1996. Improved Biomass Distribution in Porous Media Using Pulsed Nutrient Delivery. *Water Res.* 30(3):756-758.

Peyton, B.M., R.S. Skeen, B.S. Hooker, R.W. Lundman, and A.B. Cunningham. 1995. Evaluation of Bacterial Detachment Rates in Porous Media. *Appl. Biochem. Biotechnol.*, 51/52:785-797.

Jennings, D.A., J.N. Petersen, R.S. Skeen, B.M. Peyton, B.S. Hooker, T.P. Clement, D.L. Johnstone, and D.R. Yonge. 1995. An Experimental Study of Microbial Transport in Porous Media. In: Proceedings of the Third International Symposium on *In Situ* and On-Site Bioreclamation, San Diego, CA, April 24-27, Battelle Press, Columbus, OH.

Hanneman, T.F., D.L. Johnstone, D.R. Yonge, J.N. Petersen, B.M. Peyton, and R.S. Skeen. 1995. Control of Bacterial Exopolysaccharide Production and Plugging in the Subsurface. In: Proceedings of the Third International Symposium on *In Situ* and On-Site Bioreclamation, San Diego, CA, April 24-27, Battelle Press, Columbus, OH.

Site Characterization

Newcomer, D.R., S.H. Hall, and V.R. Vermeul. 1996. Use of Improved Hydrologic Testing and Borehole Geophysical Logging Methods for Aquifer Characterization. *Ground Water Monitoring and Remediation*, Winter.

Newcomer, D.R., L.A. Doremus, S.H. Hall, M.J. Truex, V.R. Vermeul, and R.E. Engelman. 1995. Geology, Hydrology, Chemistry, and Microbiology of the In Situ Bioremediation Demonstration Site. PNL-10422, Pacific Northwest Laboratory, Richland, Washington.

Field Test Results

Truex, M.J. 1995. In Situ Bioremediation Destroys Carbon Tetrachloride in a Contaminated Aquifer. *Environmental Solutions.* 8(11):30-33.

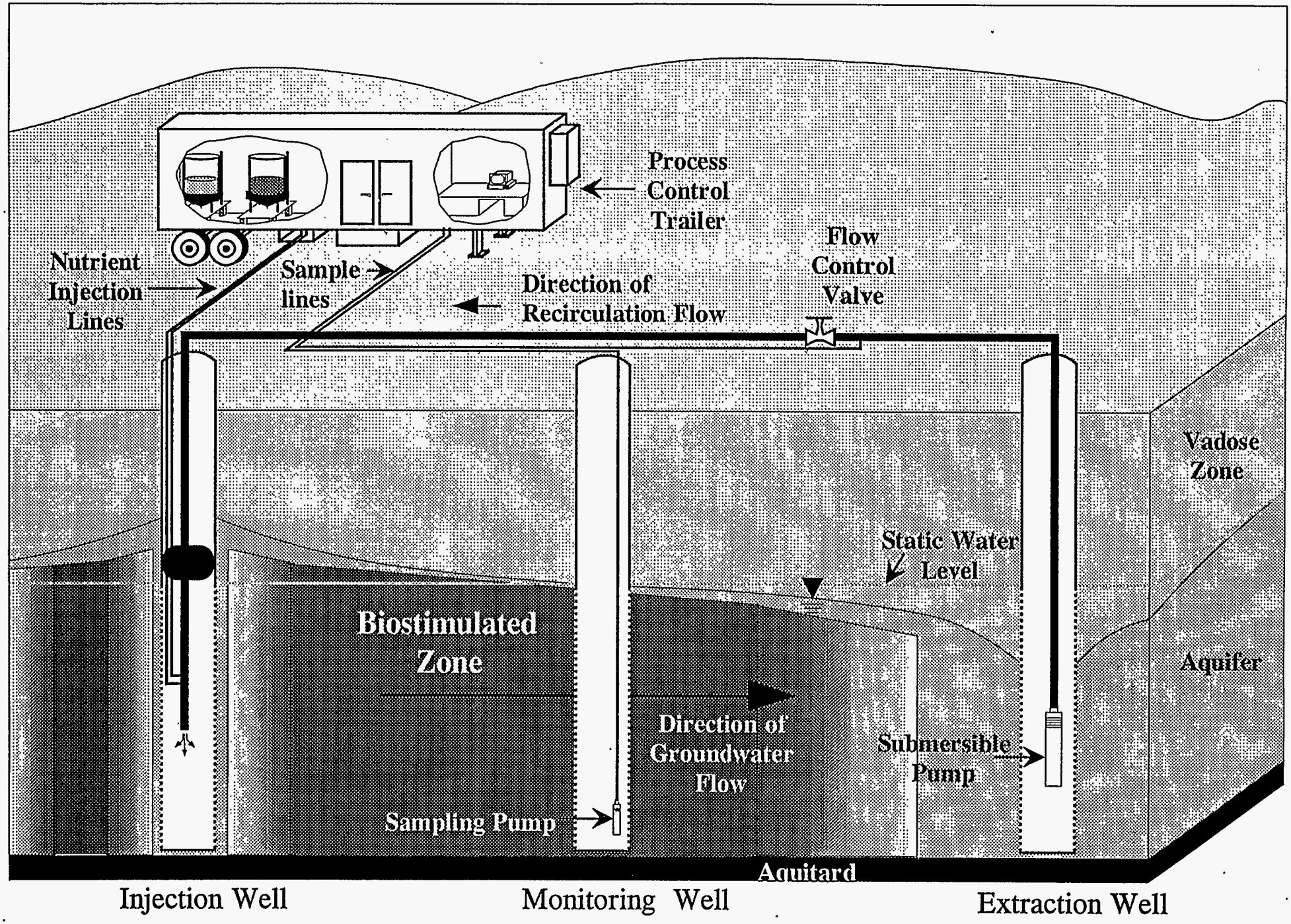

Figure 1. In Situ Bioremediation Concept

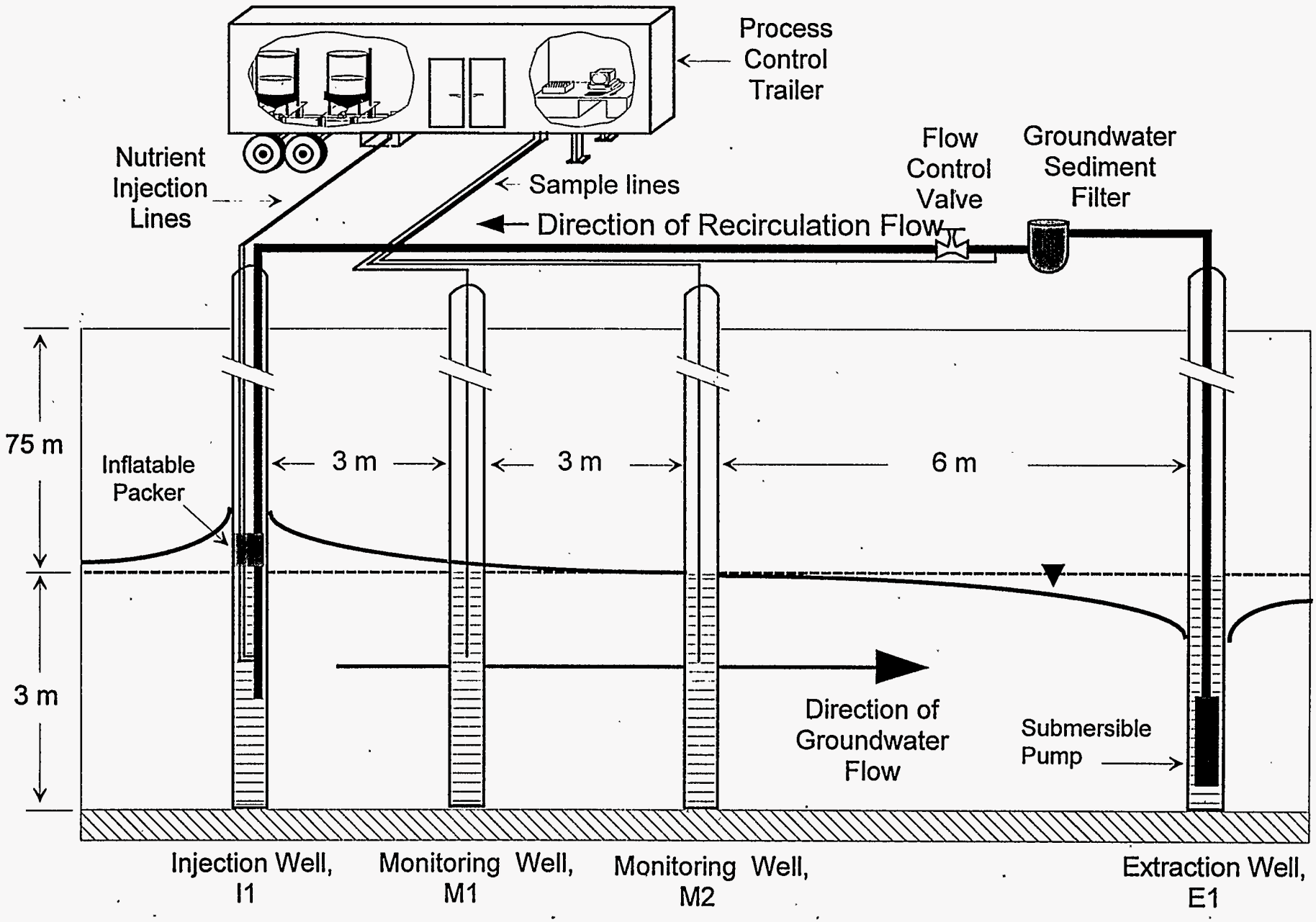

Figure 2. Recirculation/Monitoring Well System Used for Biostimulation Operations

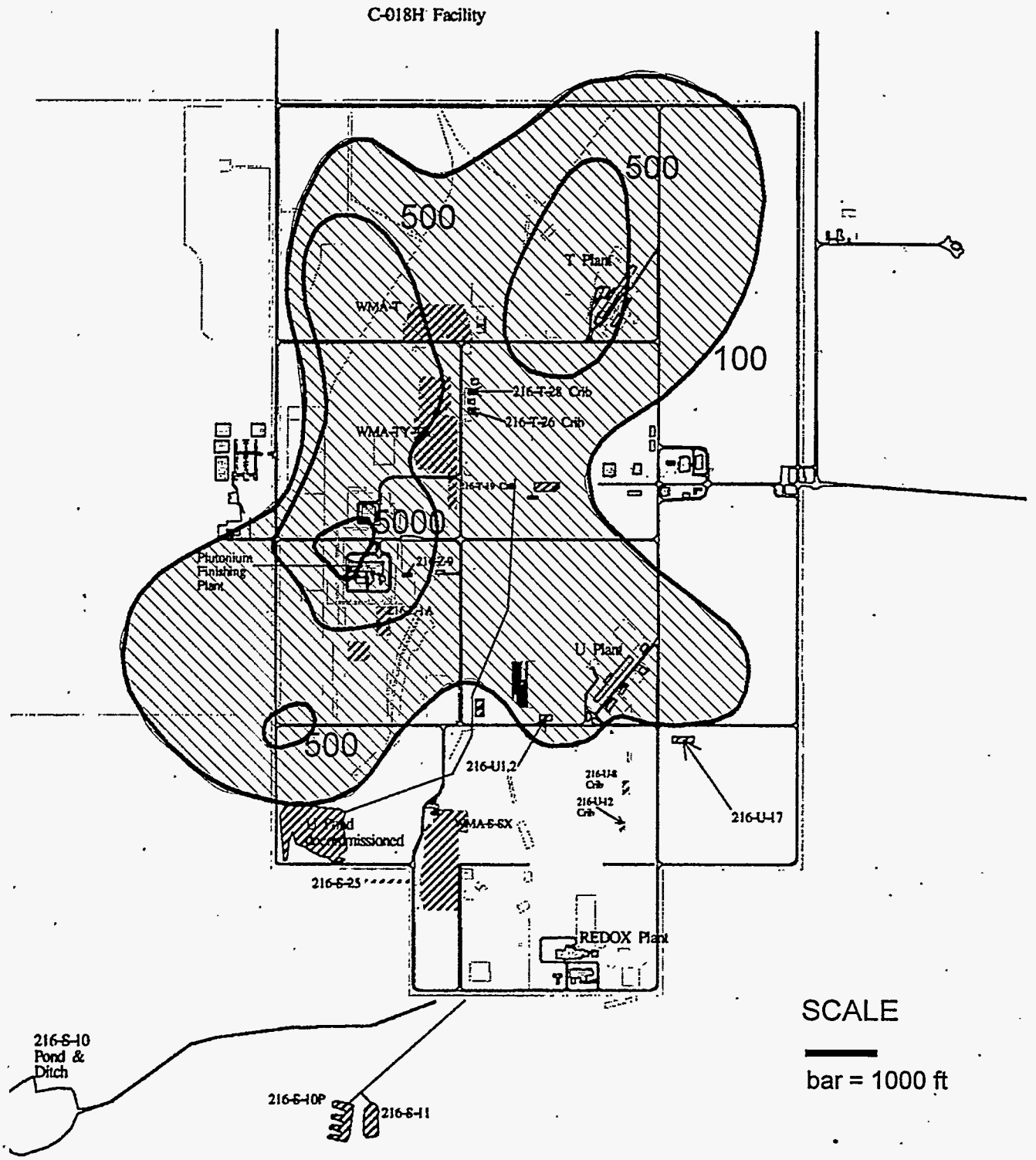

Figure 3. Carbon Tetrachloride Plume in the Groundwater at Hanford

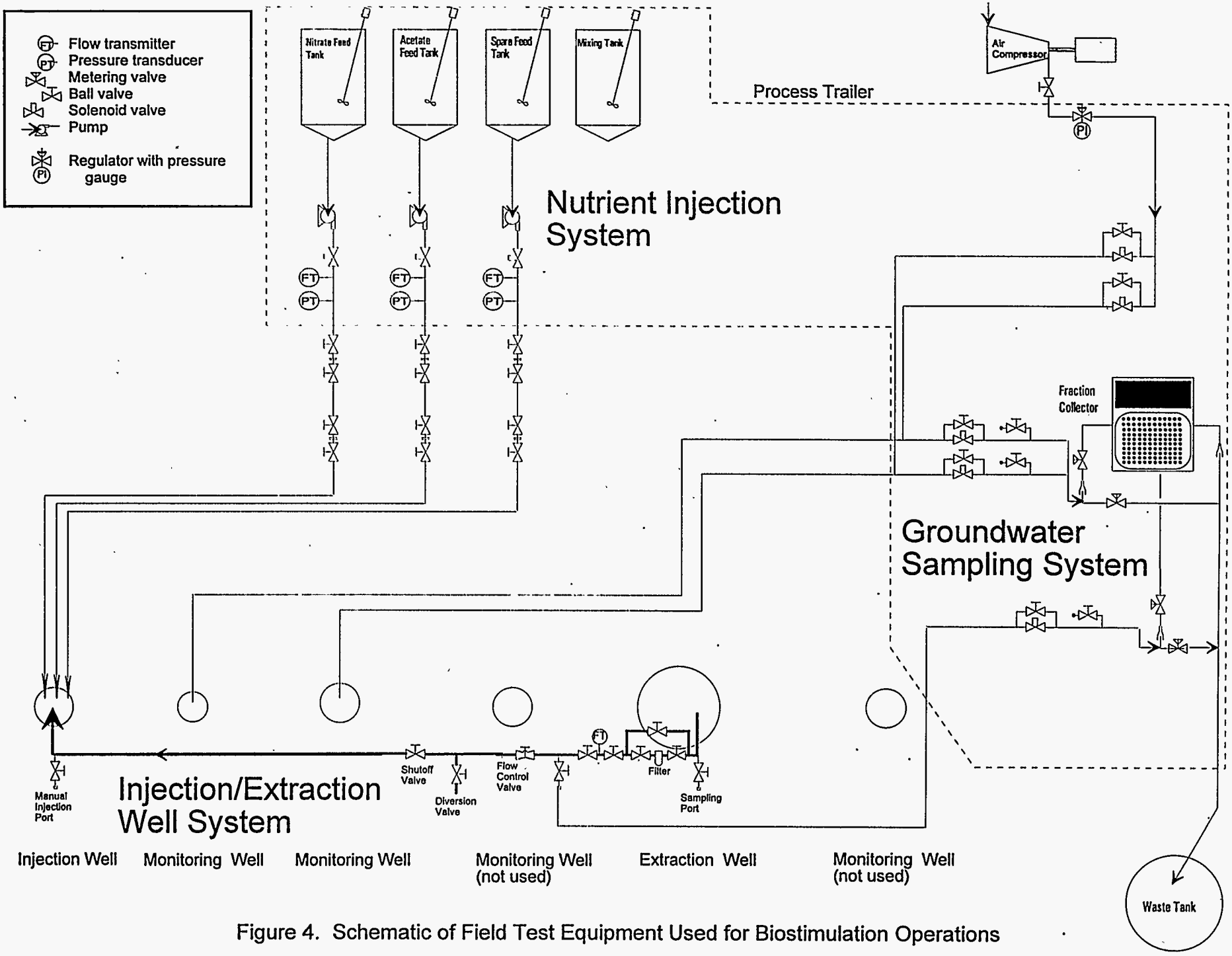

Figure 4. Schematic of Field Test Equipment Used for Biostimulation Operations

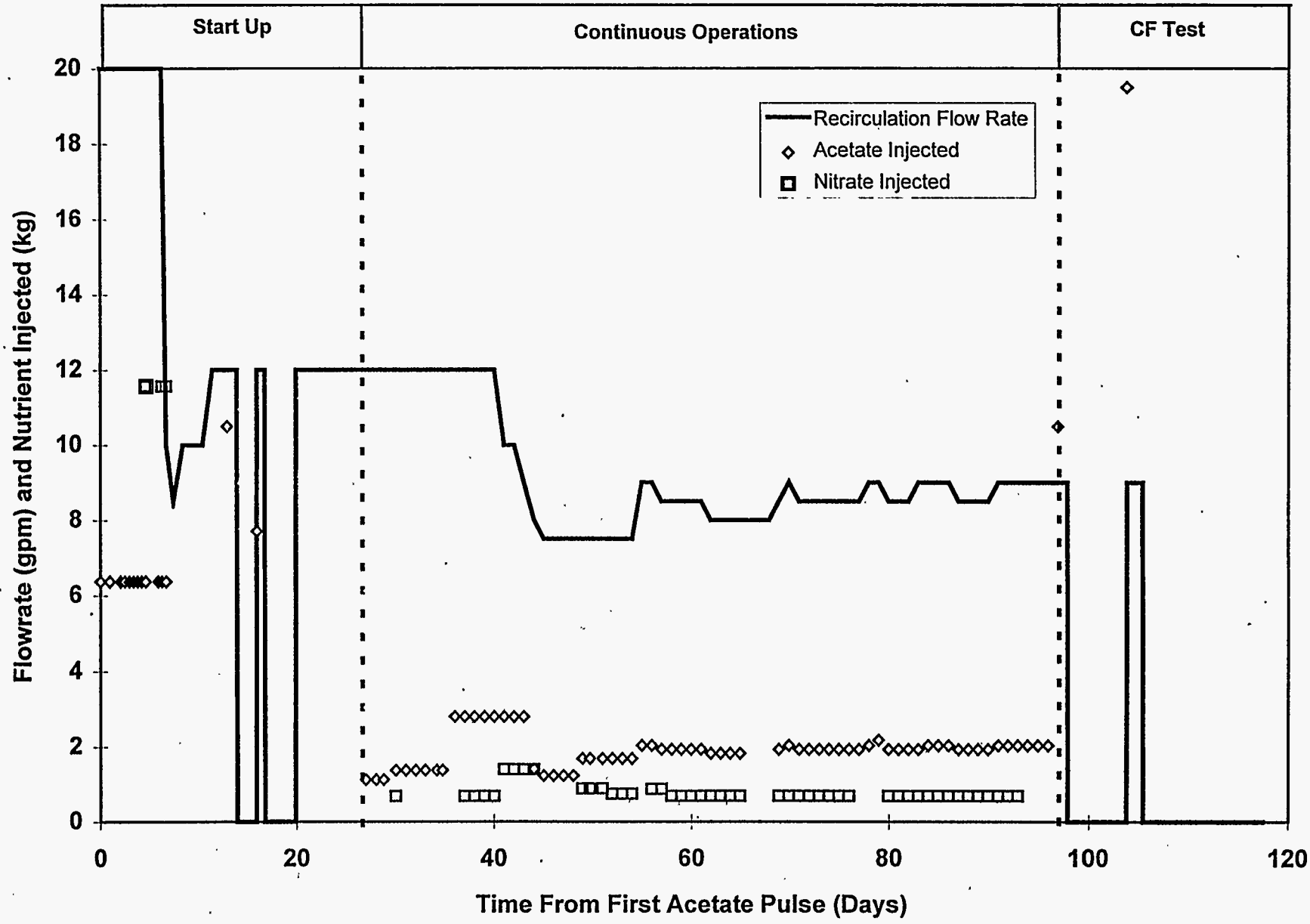

Figure 5. Operational Summary for Upper Zone Biostimulation

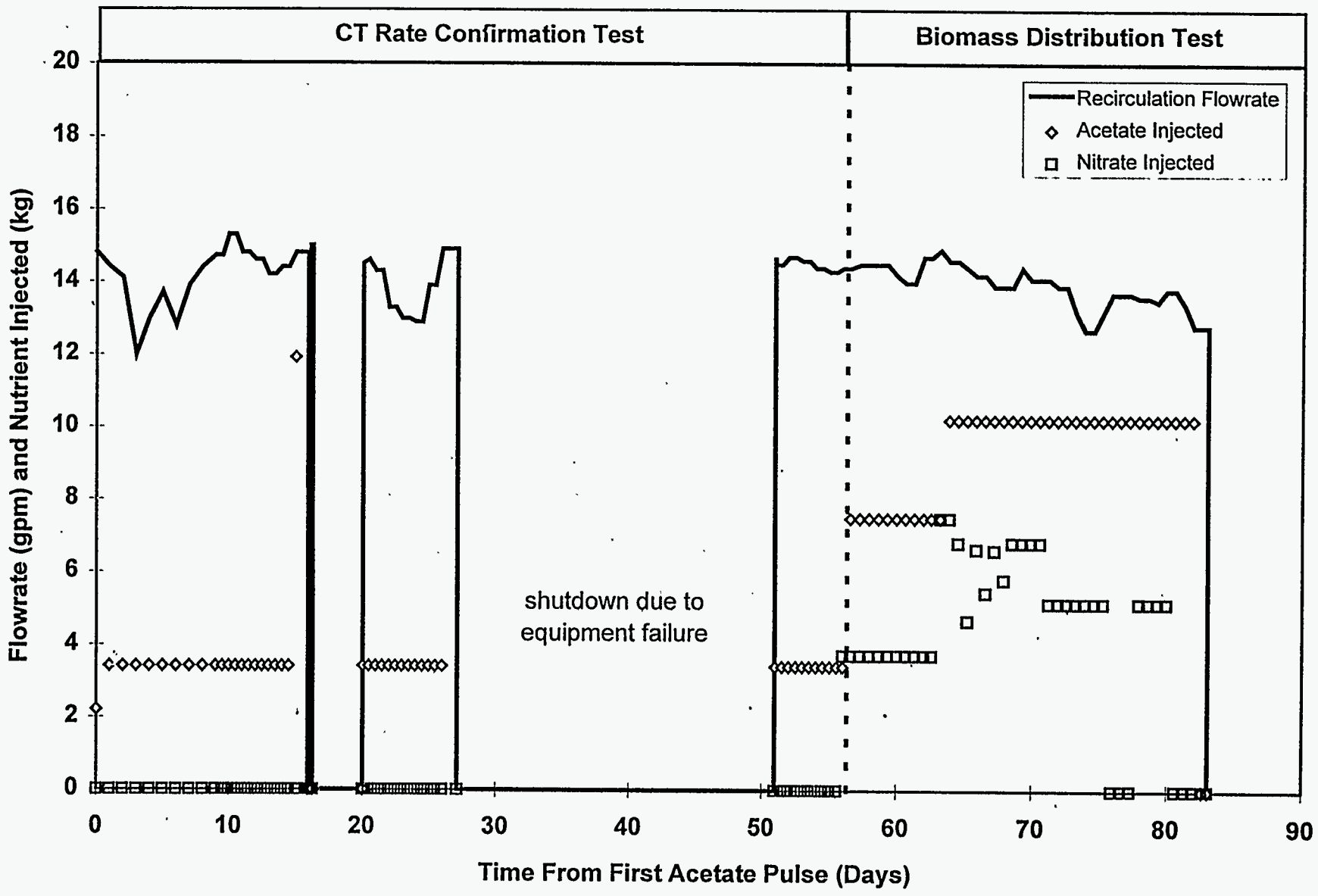

Figure 6. Operational Summary for Lower Zone Biostimulation

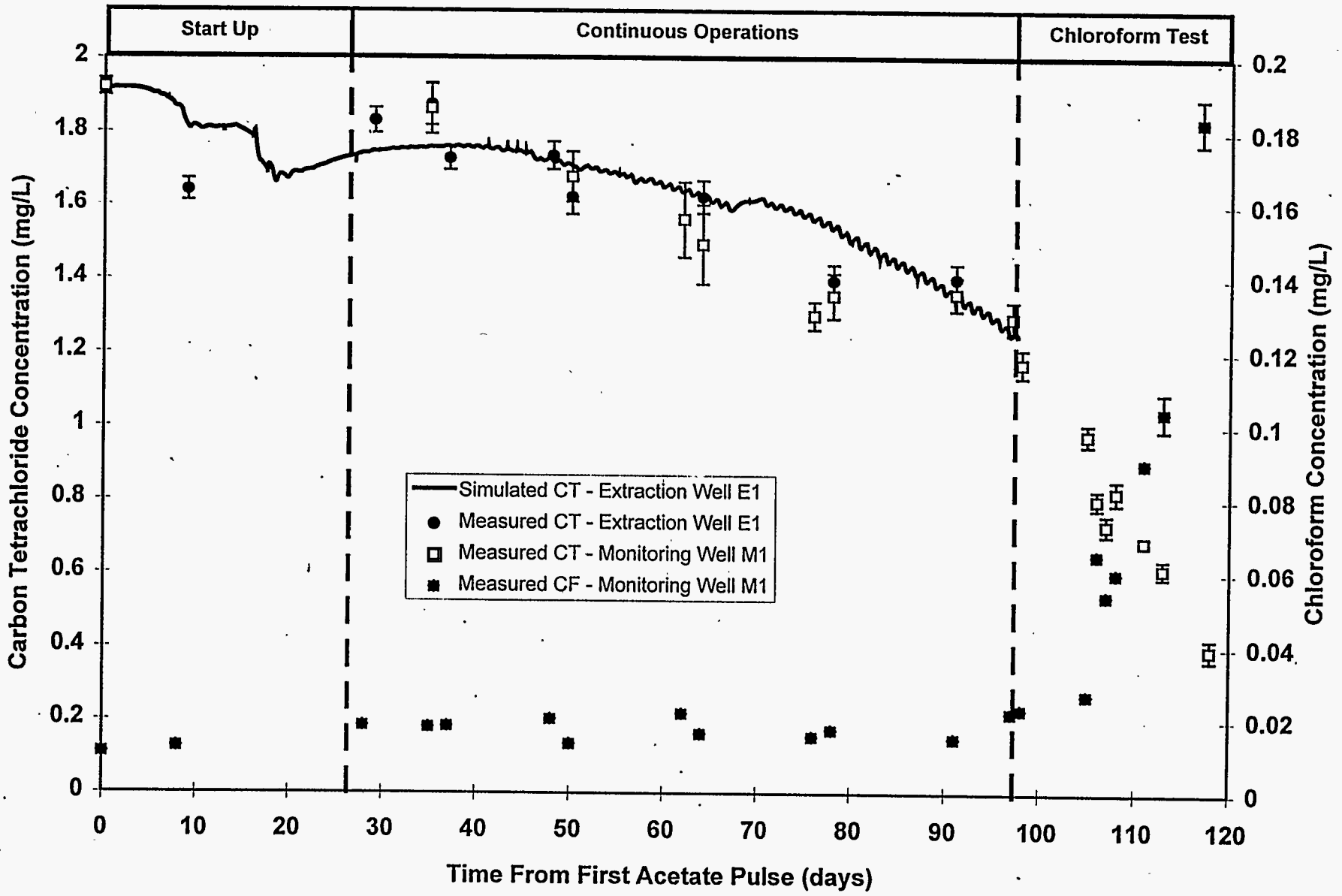

Figure 7. Dechlorination Results During Upper Zone Biostimulation

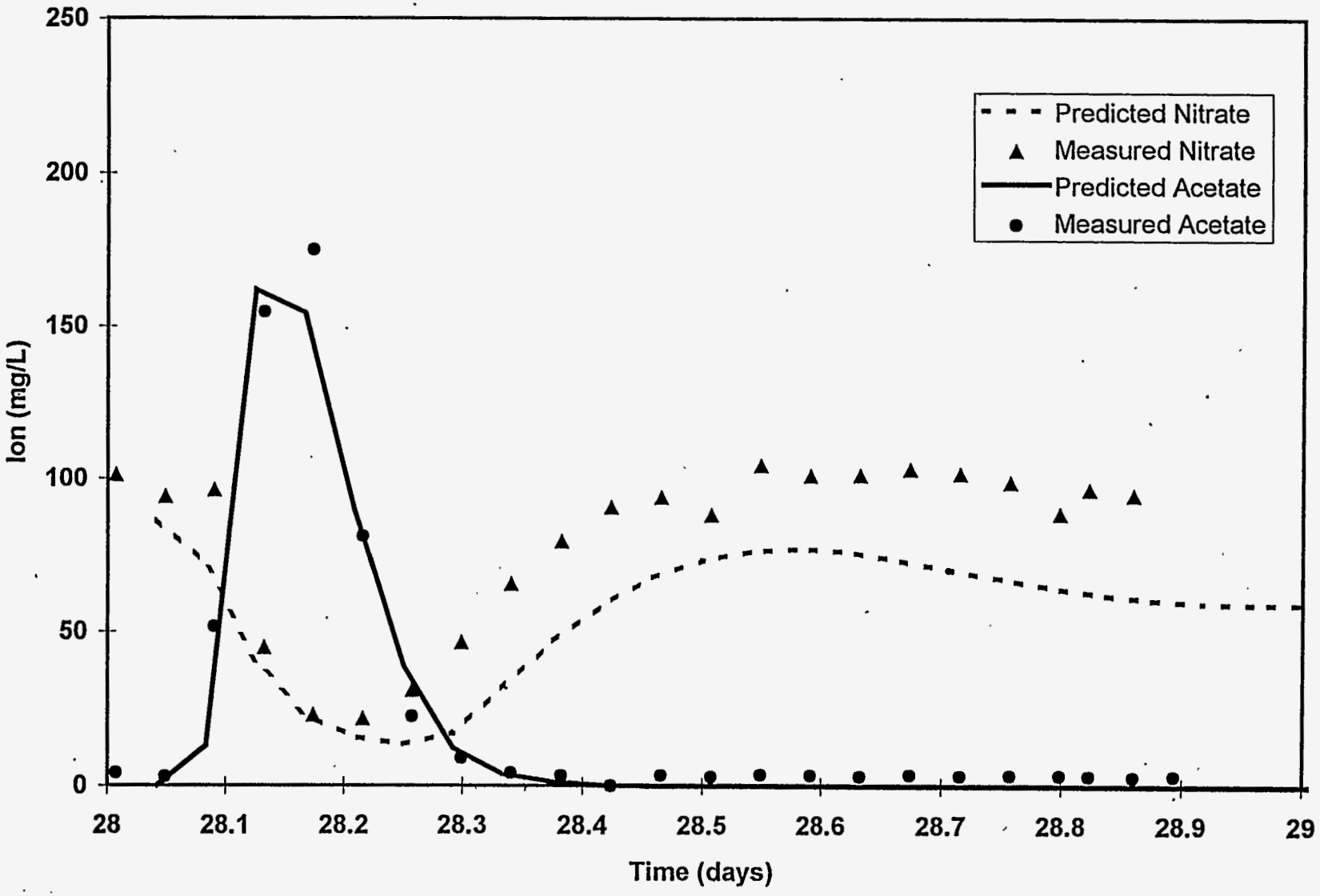

Figure 8. Measured and Predicted Acetate and Nitrate Concentrations at Well M2 on Day 30 of Upper Zone Operations

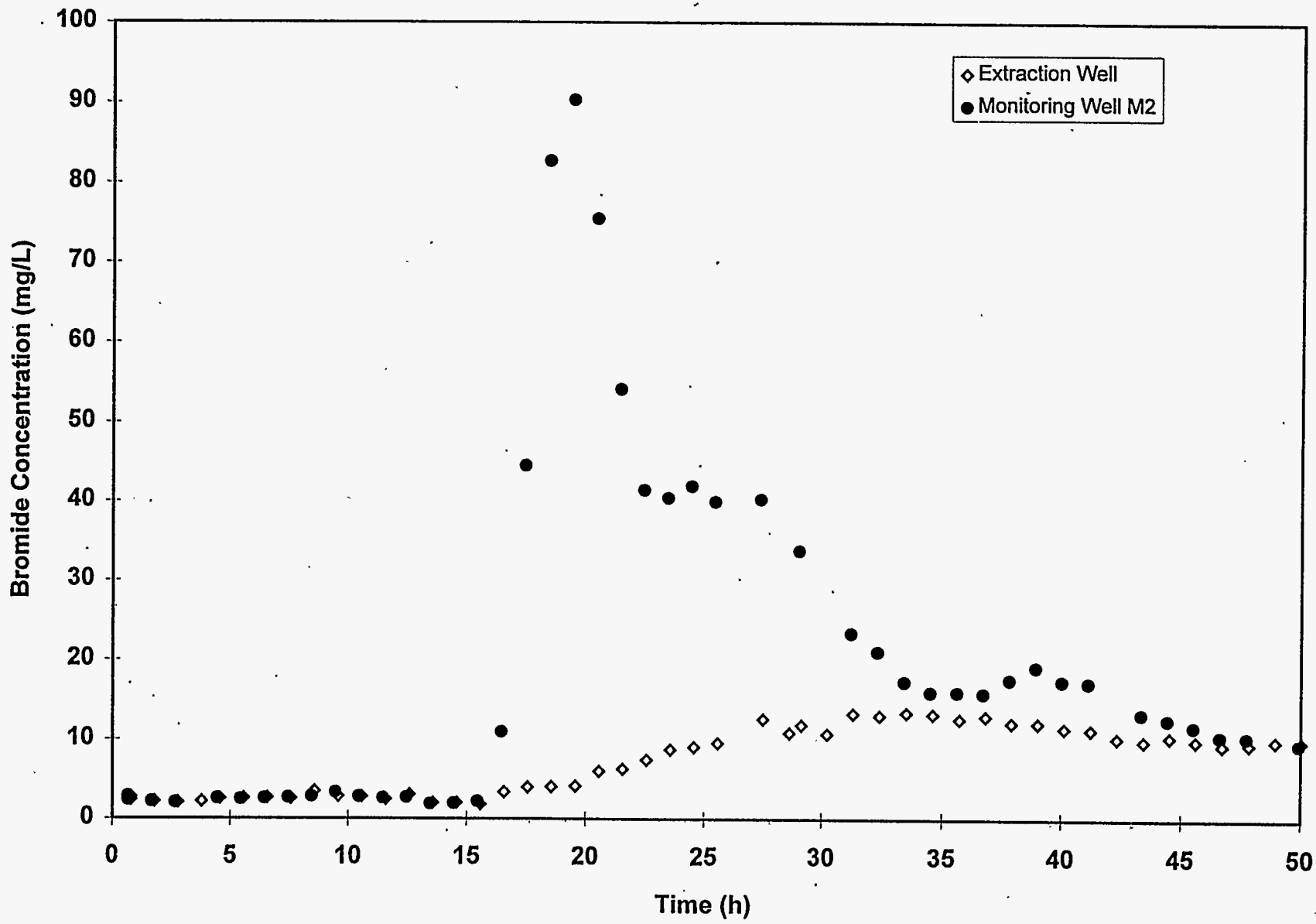

Figure 9. Recirculation Tracer Test Results for Lower Zone Unamended Operations

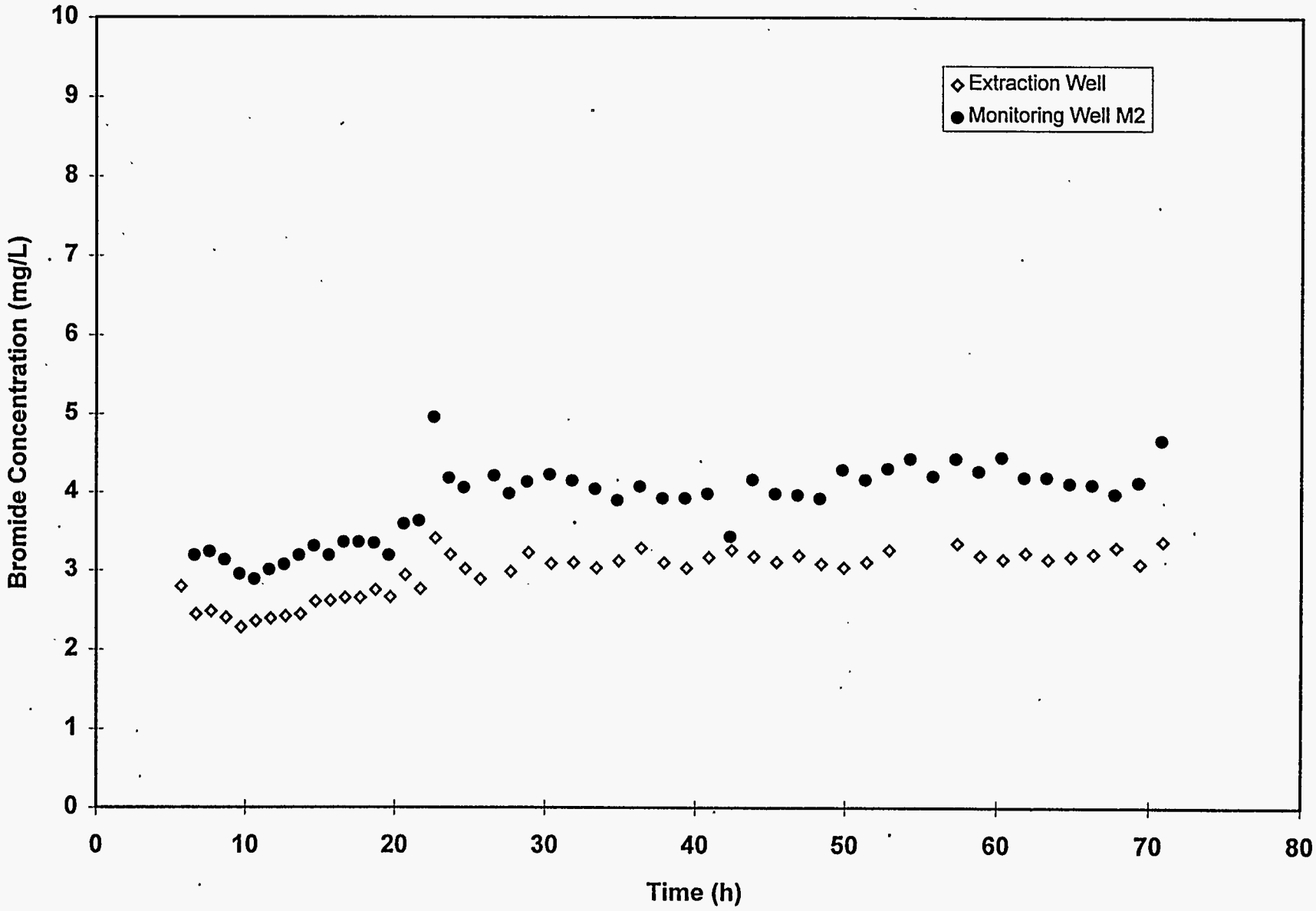

Figure 10. Recirculation Tracer Test Results at the End of Lower Zone Biostimulation Operations

DISTRIBUTION

No. of
Copies

ONSITE

18 Pacific Northwest National Laboratory

M. J. Truex (10)
B. S. Hooker
D. B. Anderson
Publishing Coordination
Technical Report Files (5)

3 Bechtel Hanford Company

K. J. Koegler
T. D. Anderson
C. D. Kramer