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A FIELD EVALUATION OF IN-SITU BIODEGRADATION FOR AQUIFER RESTORATION

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FOREWORD

EPA is charged by Congress to protect the Nation's land, air and water systems. Under a mandate of national environmental laws focused on air and water quality, solid waste management and the control of toxic substances, pesticides, noise and radiation, the Agency strives to formulate and implement actions which lead to a compatible balance between human activities and the ability of natural systems to support and nurture life.

The Robert S. Kerr Environmental Research Laboratory is the Agency's center of expertise for investigation of the soil and subsurface environment. Personnel at the Laboratory are responsible for management of research programs to: (a) determine the fate, transport and transformation rates of pollutants in the soil, the unsaturated and the saturated zones of the subsurface environment; (b) define the processes to be used in characterizing the soil and subsurface environment as a receptor of pollutants; (c) develop techniques for predicting the effect of pollutants on ground water, soil, and indigenous organisms; and (d) define and demonstrate the applicability and limitations of using natural processes, indigenous to the soil and subsurface environment, for the protection of this resource.

This report contributes to that knowledge essential to understanding the biological processes which control the transport and fate of contaminants in the subsurface and presents information on an emerging technology for the cost-effective remediation of contaminated aquifers.

Clinton W. Hall

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ABSTRACT

The in-situ remediation of aquifers contaminated with halogenated aliphatic compounds, commonly known in water supply as chlorinated solvents, is a promising alternative in efforts to protect groundwater quality. Biotransformation of the contaminants, by enhancing an indigenous microbial population capable of degrading the contaminants, has potential as an effective method for in-situ treatment.

This report presents the experimental methodology and the initial results of a field experiment evaluating the feasibility of in-situ biotransformation of TCE and related compounds. The method being tested relies on the experimentally proven ability of methane fed mixed cultures of bacteria to degrade these contaminants to stable, non-toxic, end products. Controlled experiments are performed in the subsurface in the presence and absence of biostimulation to evaluate the degree of biodegradation.

The field site is located at the Moffett Naval Air Station, Mountain View, Ca. The test zone is a shallow, confined aquifer composed of coarse grained alluvial sediments. The test zone has the following favorable characteristics: 1) high transmissivity, 2) an inorganic chemistry that will not inhibit aerobic microbial growth, 3) a background contamination with chlorinated solvents, and 4) the presence of methane-oxidizing bacteria. To create the test zone, an extraction well and injection wells were installed six meters apart, with three intermediate monitoring wells. A real time automated data acquisition and control system was developed which continuously monitors the concentrations of halogenated organic compounds, methane, oxygen, and bromide as a conservative tracer.

Bromide and TCE transport experiments were performed under induced flow conditions before the test zone was biostimulated. The bromide tracer tests indicated hydraulic residence times on the order of 0.5 to 2 days between the injection well and the observation and extraction wells. TCE was observed to be retarded compared to bromide, due to sorption onto the aquifer solids. Mass balances indicated that the injected TCE was recovered at the extraction well to the same extent as bromide, indicating little transformation of TCE before biostimulation.

Biostimulation of the test zone was achieved by injecting groundwater containing methane and oxygen in alternating pulses. Complete methane utilization was observed within a few weeks, confirming the presence of indigenous methanotrophic bacteria. By using pulse cycles of 8 to 12 hours, the biogrowth was distributed in the test zone, preventing biofouling of the area close to the injection well.

Under the influence of active biostimulation approximately 20 - 30% of the TCE was degraded within 2 meters of travel in the test zone, corresponding to the zone of methane utilization. The limited transformation most likely results from 1) a slow rate of degradation due to the high degree of chlorination of the TCE molecule, and 2) the limited enhancement of methane-utilizing population due to the limited quantity of methane and oxygen injected under saturated conditions. Laboratory experiments indicate compounds which are less chlorinated (i.e vinyl chloride and cis-and trans-DCE) are degraded more rapidly than TCE. In the second phase of field testing the biotransformation of several of these compounds, along with TCE, will be evaluated.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

C/Co	--	normalized concentration (measured/injected)
cm	--	centimeter
cm^3	--	cubic centimeter
conc	--	concentration
d	--	day
DAC	--	data acquisition and control
DCA	--	dichloroethane
DCE	--	dichloroethylene
d.l.	--	detection limit
DO	--	dissolved oxygen
f_{oc}	--	fraction of organic carbon
ft	--	feet
g	--	gram
gpd	--	gallons per day
hr	--	hour
kg	--	kilogram
m	--	meters
m^2	--	square meters
meq	--	milliequivalent
meq/l	--	milliequivalents per liter
mg	--	milligram
mg/l	--	milligrams per liter
min	--	minute
PCE	--	tetrachloroethylene
TCA	--	trichloroethane
TCE	--	trichloroethylene
ug	--	microgram
ug/l	--	micrograms per liter

SYMBOLS

D	--	dispersion coefficient (m^2/d)
K	--	hydraulic conductivity (m/d)
n	--	porosity (cm^3/cm^3)
K_d	--	distribution coefficient (cm^3/g)
p_b	--	bulk density (g/cm^3)
Pe	--	Peclet number (u x/D)
r	--	radius (m)
R	--	retardation factor (dimensionless)
r/L	--	leakage factor (dimensionless)
T	--	transmissivity (gpd/ft)
u	--	pore fluid velocity (m/d)
x	--	distance (m)

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SECTION 1

INTRODUCTION

The in-situ remediation of aquifers contaminated with halogenated aliphatic contaminants, commonly known in water supply as chlorinated solvents, is a promising alternative in efforts to protect groundwater quality. Chlorinated aliphatic compounds are frequently observed in groundwater. In a survey of 945 water supplies, Westrick et al. (1984) found trichloroethylene (TCE), tetrachloroethylene (PCE), cis- and/or trans-1,2-dichloroethylene (DCE), and 1,1-dichloroethylene to be the most frequently appearing compounds other than trihalomethanes. Approaches for the restoration of aquifers contaminated by these compounds based on extracting the contaminated groundwater by pumping and subsequently treating at the surface have been shown to be effective, but often entail great expense and also a risk of transferring the contaminants to another medium, i.e., the atmosphere. To circumvent these difficulties, in-situ treatment of the contaminants has come to be considered a potentially favorable alternative, with investigations centering on promoting biotransformation of the contaminants.

Our group at Stanford University is assessing under field conditions the capacity of native microorganisms, i.e., bacteria indigenous to the subsurface environment, to metabolize halogenated synthetic organic contaminants, when proper conditions are provided to enhance microbial growth. Specifically, the growth of a consortium of methane-utilizing bacteria is being stimulated in a field situation by providing ample supplies of dissolved methane and oxygen. Under biostimulation conditions, the transformation of representative halogenated organic contaminants, such as trichloroethylene (TCE), is assessed by means of controlled addition, frequent sampling, quantitative analysis, and mass balance comparisons.

The field demonstration study is being conducted at Moffett Naval Air Station, Mountain View, CA, with the support of the Kerr Environmental Research Laboratory of the U.S. Environmental Protection Agency, and with the cooperation of the U.S. Navy. To provide guidance for and confirmation of the field work, laboratory experiments and analyses are also being conducted, both at Stanford University's Water Quality Control Research Laboratory and at the Kerr Laboratory.

This report summarizes the experimental approach taken in the field study, the characterization of the test zone before the initiation of the evaluation experiments, and the results of the first phase of the field evaluation.

BACKGROUND

The in-situ restoration of aquifers contaminated with hydrocarbons is not a new idea. Raymond (1974) pioneered the development of the process for the in-situ reclamation of aquifers contaminated by liquid fuels. This work indicated that after promoting the proper conditions in the subsurface (i.e by the addition of oxygen and nutrients), a native population of microorganisms was stimulated that degraded the hydrocarbon contaminants. The microorganisms used the hydrocarbon contaminants as primary substrates for growth.

In-situ biorestoration of aquifers contaminated by halogenated aliphatic compounds requires a somewhat different approach, since in most cases the halogenated aliphatic compounds can not be utilized by native microorganisms as primary substrates for growth. However, they can be degraded as secondary substrates by microorganisms which utilize another primary substrate for growth. The in-situ degradation of these compounds is therefore promoted by the stimulation of a particular class of native microorganisms through the introduction of the appropriate primary substrate for growth (electron donor) and electron acceptor into the treatment zone.

The method being evaluated relies on the transformation of the chlorinated aliphatic compounds by methane-utilizing bacteria (methanotrophs). These bacteria grow on methane as a sole carbon source under aerobic conditions. The chlorinated aliphatic compounds are thought to be transformed by the methane monooxygenase enzyme, an enzyme with a broad range of specificity, that is produced by the methanotrophic bacteria.

The transformation has been demonstrated in laboratory studies using soil columns and mixed cultures. In experiments performed in an unsaturated soil column with an atmosphere of 0.6% natural gas and air, Wilson and Wilson (1985) found microorganisms were stimulated which degraded TCE fairly completely to carbon dioxide and cell material. Fogel et al. (1986) using mixed cultures of methane-oxidizing bacteria found TCE, vinyl chloride, vinylidene chloride, and cis- and trans-1,2-dichloroethylene to be rapidly degraded. Henson et al. (1987) found a range of both single and double carbon compounds to be degraded by mixed cultures. The rate of transformation was reported to be faster the less substituted the molecule with chlorine atoms and the more evenly distributed the chlorines on the molecule.

RESEARCH OBJECTIVES

The overall objective of this work is to assess the efficacy of a the proposed method for enhancing the in-situ degradation of the halogenated aliphatic compounds. The specific objectives of the field study are:

1) To demonstrate whether the proposed method of promoting the microbial decomposition of trichloroethylene and related compounds is effective under controlled experiments performed in-situ, in an aquifer representing conditions typical of groundwater environments;

2) To quantify the rate of decomposition and to identify intermediate transformation products, if any; and

3) To bracket the range of conditions under which the method is effective, and to establish criteria for dependable treatment of a real contamination incident.

SECTION 2

CONCLUSIONS

This reports summarizes the results of the first phase of the field experimentation evaluating in-situ methodologies for the restoration of aquifers contaminated with halogenated aliphatic compounds. The conclusions which can be drawn from these results are as follows:

1) In order to create a test zone in the subsurface in which controlled experiments can be conducted, detailed characterization of the zone must be performed, including:

   a) hydrogeology of the test zone based on coring, well logs, pump tests, piezometric measurements and published information on the local area;

   b) groundwater chemistry, including both major and minor inorganic compounds and trace organic compounds;

   c) aquifer solids analysis for microbial activity and sorption behavior of selected organic solutes; and

   d) hydraulics based on natural-gradient and induced-flow tracer tests.

2) The real time automated data acquisition and control system which was developed permits frequent sampling and reproducible analyses which are required for evaluation experiments of this type.

3) Tracer experiments provide valuable information on the test zone, including hydraulic residence times, dispersion, the degree of capture, and the retardation of TCE compared to bromide due to sorption on the aquifer solids. The tracer tests were found to be quite reproducible, which was required for the systematic, objective comparisons with the biostimulation results. Tracer tests before biostimulation indicated little transformation of TCE.

4) Indigenous methane-oxidizing bacteria were easily stimulated in the test zone within a few weeks by the pulsed addition of methane and oxygen. No nutrient addition was required to stimulate growth. Rapid growth kinetics were observed, with the microbial population increasing near the injection well such that all the methane was consumed within 1 meter of travel. Long pulse cycles of up to 12 hours were successfully used to distribute the bacterial growth, and to prevent biofouling of the aquifer.

5) Under active biostimulation conditions, 20 to 30 % of the TCE added to the test zone was degraded. Similar estimates of the degree of degradation were obtained using mass balances and comparisons with bromide as a conservative tracer. Degradation occurred within the test zone where methane was being utilized.

6) The limited degree of TCE transformation is attributed to the following factors:

   a) the high degree of chlorination of the TCE molecule, resulting in a slow rate of oxidation,

   b) the limited methane-oxidizing population, which can be stimulated with the amounts of methane and oxygen that can be delivered under saturated conditions, and

   c) possible competitive inhibition of TCE degradation by methane.

7) In the second phase of the field evaluation, other compounds which are less chlorinated, i.e. dichloroethylene isomers, will be tested along with TCE. Experiments will be performed to determine whether competitive inhibition is an important process and to assess the effect of the pulsed injection method on the rate of degradation.

SECTION 3

RECOMMENDATIONS

The limited degradation of TCE observed during the first phase of the field evaluation experiments indicates that more information is required before this process can be used on a larger scale for treatment of a real contamination incident. More basic laboratory research and pilot scale testing in the field are required. Laboratory investigations should address the factors which affect the rate of aerobic biotransformation of TCE and related compounds. The pilot scale field experimentation should determine whether the factors which enhance transformation in the laboratory can be successfully implemented in the field.

Important questions which must be addressed in laboratory experiments are:

1) How do the rates of transformation depend on the structure of the compound that is being degraded?

2) Do the conditions of biostimulation and maintenance of the microbial population affect the rate of transformation of different compounds?

3) Is there competitive inhibition between the methane and selected organic solutes which slows the rate of transformation?

4) Does biostimulation using different primary substrates or electron acceptors result in more effective degradation?

5) Is the addition of minor nutrients an important factor?

6) How does the sorption of the organics onto the aquifer solids affect the rate of biodegradation?

The pilot scale field tests should be continued, with new experiments being designed based on the results of the laboratory investigations. These pilot scale tests will help establish criteria for dependable treatment of real contamination incidents. Important criteria include; 1) the type of aquifers for which the process is best suited, 2) the range of environmental conditions in which the process may be applied, and 3) the most effective means of biostimulating the aquifer to achieve effective biotransformation.

Continuing both laboratory and pilot scale field studies at a well characterized site provides a basis for determining what information is needed for the design of in-situ restoration schemes at different sites. For instance, the comparison of field and laboratory results will evaluate how successfully parameters, which are generated in laboratory studies, predict biotransformation in the field. If parameters determined by laboratory studies, such as soil microcosm experiments, are of value, it would provide a low cost means of obtaining the necessary information to implement the treatment method at different sites.

Finally, pilot studies which evaluate this process in the unsaturated zone should be performed. Effective transformation, which requires the stimulation of a large microbial population, may be achieved by supplying ample quantities of methane and oxygen. This should be easier in the unsaturated zone compared to the saturated zone.

SECTION 4

FIELD EXPERIMENT METHODOLOGY

The experimental methodology developed to meet the goals of the field study is as follows:

1. Select a representative demonstration site based on available information regarding regional hydrology and geochemistry, and considering practical and institutional constraints;

2. Characterize the site by means of coring, pump tests, sampling and analysis of the native groundwater;

3. Construct a system of wells for injection, extraction, and monitoring of water at the site;

4. Design and install an automated system for sampling and analysis of the groundwater at the demonstration site;

5. Determine the velocity and direction of groundwater flow under natural gradient conditions, by means of bromide tracer tests;

6. Assess the mobility of trichloroethylene, relative to bromide tracer, at the demonstration site and quantify residence times in the system under injection/extraction conditions;

7. Stimulate the growth of native methane-oxidizing organisms by injecting dissolved methane and oxygen (biostimulation mode); and

8. Assess the transformation of trichloroethylene under biostimulation conditions.

This methodology provides a staged approach for evaluating the proposed technology. The initial stages of the study (1-5) focus on selecting the field site and characterizing its physical, chemical, microbiological and hydraulic properties. The latter stages of the experiment involve biostimulating methane-oxidizing bacteria in the test zone and evaluating the degree of transformation of a specific contaminant of interest.

The information obtained during the early stages of the experiments is critical to the success of subsequent evaluation experiments, which are dependent on the ability to run controlled experiments in the subsurface. The hydraulic information obtained in pump tests and tracer experiments is required in order to design a fluid injection and extraction system that creates an in-situ reaction zone. The chemical, physical and microbiological characteristics of the test zone also indicate whether favorable conditions exist for the biostimulation of a native population of methane-oxidizing bacteria. These data are necessary in determining whether a controlled evaluation of the proposed technology can be performed at the selected site.

The basic approach of the evaluation experiments is to create a test zone in the subsurface. The conceptual model for this approach is shown in Figure 1. A series of injection, extraction, and monitoring wells are installed within a confined aquifer. An induced flow field is created by the injection and extraction of fluid. The chemicals of interest for a specific experiment are metered into a stream comprising 10 to 15 percent of the extracted groundwater and then reinjected. The concentrations of the specific chemicals are monitored at several locations, including the injected fluid, the three monitoring wells, and the extracted fluid. The spatial and temporal responses of the chemicals in the test zone are determined by frequent monitoring, using an automated data acquisition and control system located at the site.

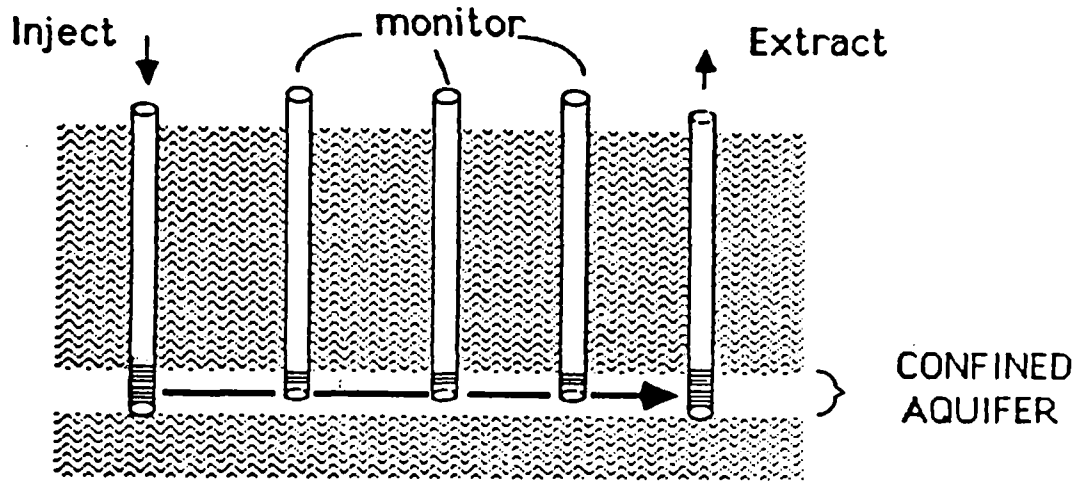


Figure 1. Conceptual model for the creation of the subsurface test zone.

The sequence of field experiments using this approach is outlined in Table 1. The initial experiments study the transport of bromide ion as a conservative tracer. The experiments determine fluid residence times in the system, the degree of dispersion, and the recovery of the injected fluid at the extraction well. In later experiments, bromide, dissolved oxygen and the chlorinated aliphatic compounds of interest are injected simultaneously. The retardation factors of the different chemicals with respect to bromide, owing to sorption, are determined. The transformation of the chlorinated aliphatic compounds in these experiments is evaluated based on comparisons with the bromide tracer. Two criteria are used: 1) the degree of steady-state fractional breakthrough achieved at the monitoring wells, and 2) mass balances on the amounts injected and extracted. These tracer experiments therefore, serve as pseudo controls, permitting a comparison of the observed responses before and after the test zone is biostimulated.

TABLE 1. SEQUENCE OF EXPERIMENTS AND PROCESSES STUDIED DURING THE FIRST PHASE OF THE FIELD EVALUATION.

Injected Chemicals	Process Studied
1) Br^-	Advection/Dispersion
2) $Br^- + O_2$	Retardation/Dispersion (TCA - Elution)
3) $Br^- + TCE + O_2$	Retardation (Transformation)
4) $CH_4 + O_2 +$ (nutrients)	Biostimulation
5) $CH_4 + O_2 +$ (nutrients) + TCE	Biotransformation

The biostimulation experiments (Stage 4) involve the addition of methane, oxygen, and nutrients (if required), to stimulate the growth of methane-consuming bacteria in the test zone. The transient response of the different chemical components is monitored, as previously discussed. This experiment determines: 1) how easily the methane-oxidizing bacteria are stimulated and whether nutrients are required, 2) stoichiometric requirements of oxygen to methane, 3) information on the kinetics and the rate of growth, and 4) the areal extent over which biostimulation is achieved.

The degree of biotransformation of the chlorinated aliphatic compound (TCE) is evaluated in the final stage (Stage 5) of the experiment. Known quantities of TCE are introduced into the biostimulated zone along with methane, oxygen and bromide. The extent of transformation of TCE is determined based on both mass balances and steady-state breakthrough concentrations at monitoring points, compared to those of bromide as a conservative tracer. The results are also compared with those obtained during the earlier pseudo-control experiments (Stage3) before the test zone was biostimulated.

SECTION 5

SELECTION AND OF THE CHARACTERIZATION FIELD SITE

FIELD SITE DESCRIPTION

After a reconnaissance study of several sites, a location at the Moffett Naval Air Station, Mountain View, Ca., was chosen (Figure 2). The site, designated SU-39, located on the lower part of the Stevens Creek alluvial fan is approximately 3 km south of the southwest extremity of San Francisco Bay. The surface elevation at the site is 8.5 m above mean sea level.

The experimental site is located in a region where the groundwater is contaminated with several organic solutes for which this biorestoration method might be applied. The area of groundwater contamination shown in Figure 2 represents the 1 mg/l TCE contour of the "A" Aquifer delineated in January, 1983 (Canonie Engineers, 1983). The plume contains concentrations of 1,1,1-trichloroethane (TCA) and trichloroethylene (TCE) of up to 100 mg/l, measured at points 700 and 1000 meters from the SU-39 site. Thus, if effective, the treatment method may have direct use in the area where it was evaluated.

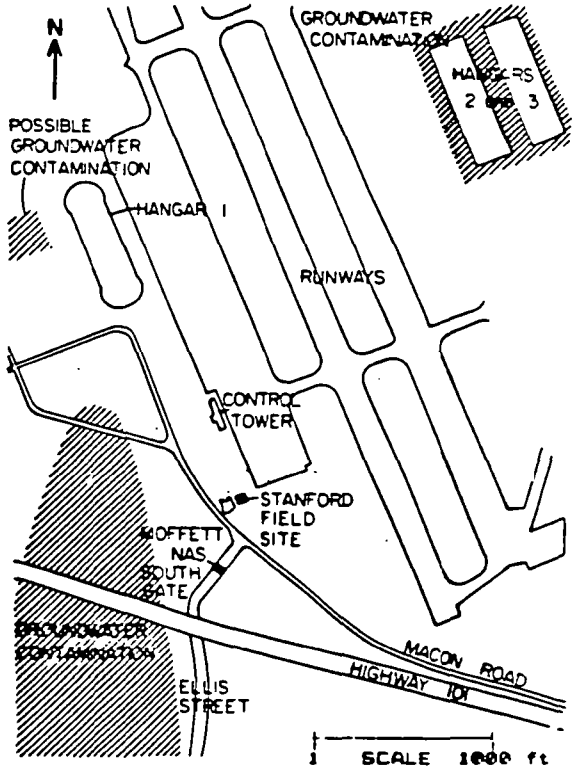


Figure 2. Location of the field site, SU-39, at the Moffett Naval Air Station, Mountain View, California.

GEOLOGIC CHARACTERISTICS

The geologic characteristics of the test zone have been examined using cores samples and well cuttings. Figure 3 shows the location of the wells installed at the test site to date. A series of exploratory wells (1,SI,3,4,5,6) were installed in July 1985, using the hollow stem auger drilling method. Cores were obtained using 2 inch pitcher barrels that were pushed ahead of the drill bit. The 6 test drillings identified a shallow, confined aquifer which is known as the "A" Aquifer, the shallowest of several in the region. Well logs indicate the aquifer is approximately 1.2 meters thick with a top 4.4 to 4.6 meters below the ground surface; the bottom ranges from 5.3 to 5.7 meters below the surface. The aquifer is confined between silt clay layers.

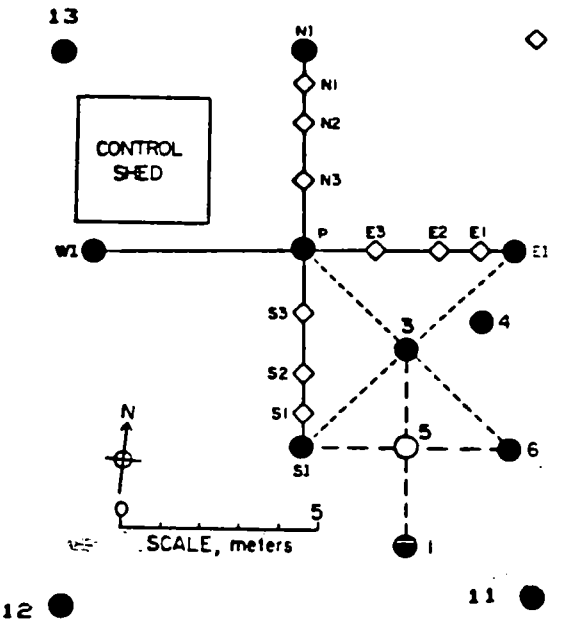


Figure 3. Map of the well field installed at the field site.

Figure 4 was constructed from the core well logs of the fully penetrating wells SI, P, NI. Well SI is the injection well and P is the extraction well used in the experiments. The three well logs have similar lithologic profiles. The uppermost 2 ft consists of silty sand with pebbles up to 8 cm in diameter. This surface layer is underlain by approximately 12 ft of silt and clay of a brownish-black to olive gray color, indicating that the sediment contains organic material. The bottom of this sequence is marked by a clayey sand that commonly separates the silt and clay overburden from the underlying aquifer.

The aquifer consists of fine- to coarse-grained sand and appears well bedded in most cores. Gravel lenses with pebbles up to 2.5 cm in diameter occur in some cores within the sand layers. Due to the presence of gravel, intact cores were difficult to obtain. Cores were often lost over the depth interval from 15.5 to 17 ft below the surface. This zone is considered to have the highest gravel fraction.

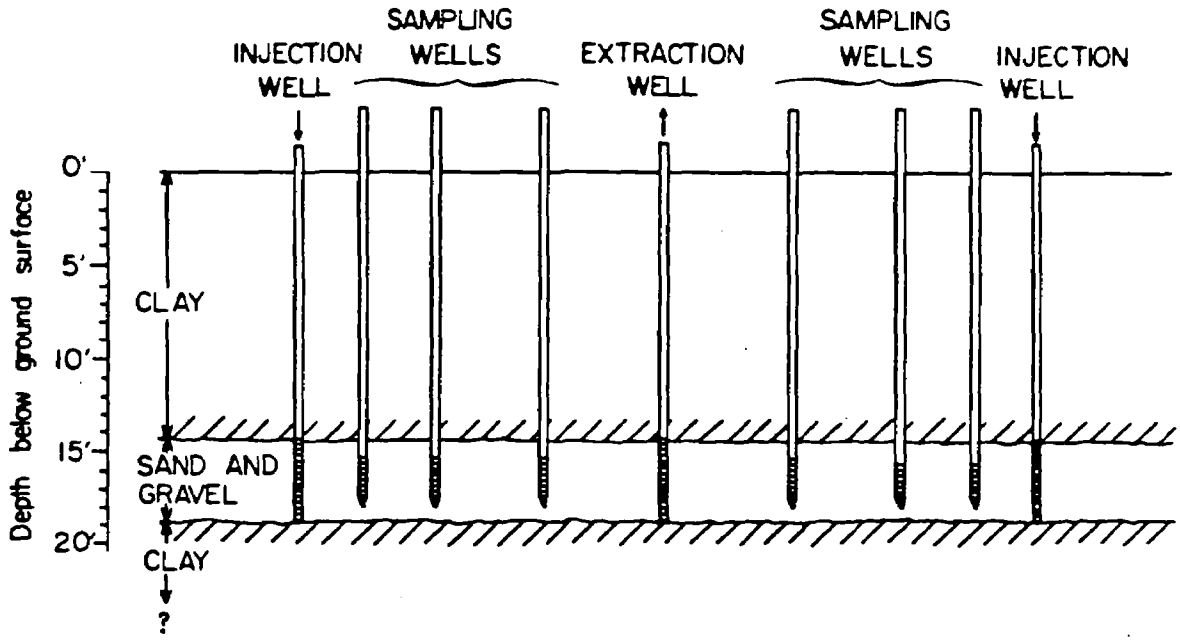


Figure 4. Vertical section of the test zone.

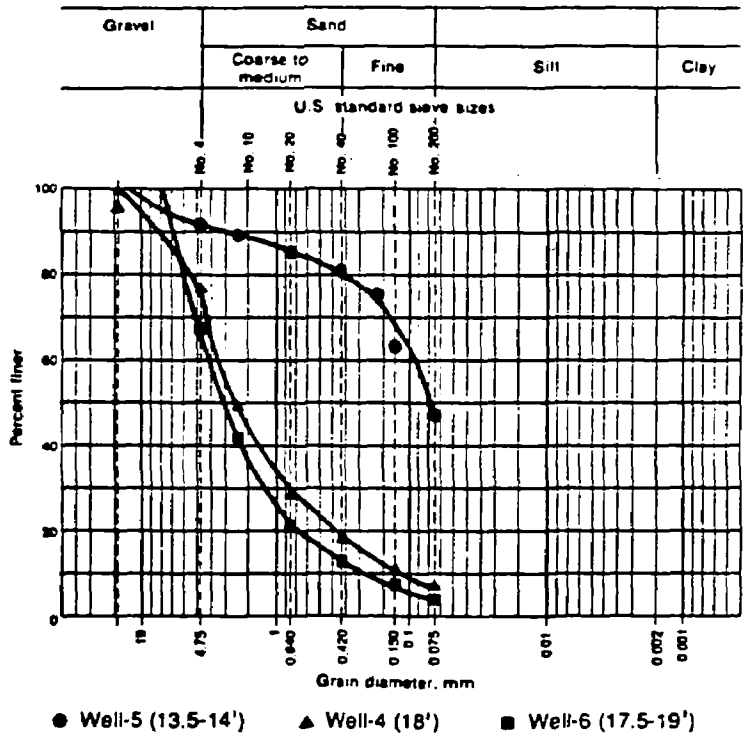


Figure 5. Particle size distribution of the aquifer core samples based on standard sieve analysis.

A layer of dark greenish-gray silty clay underlies the aquifer (top at 19 ft below the surface). While no well was drilled through this clay/silt layer at the project site, other studies in the vicinity have shown that this layer is approximately 20 ft thick and is underlain by another aquifer (Canonie, 1983).

The particle size distributions of aquifer cores are shown in Figure 5. Core samples taken from wells 4 and 6 at a depth of 18 ft and 17.5 - 18 ft respectively, have similar distributions of particle sizes, with a large fraction of the solids being coarse to medium sands and gravel. The core sample from well 5 at a depth 13.5 - 14 ft has a greater fraction of fine sand and silt, which is consistent with well log observations. Petrographic analysis shows the aquifer solids consist of rock fragments of the parent rock of the Santa Cruz Mountains. These include include graywackes, cherts, and volcanics of eugeosynclinal (slope) origin (Franciscan Series).

The observations at the test site are consistent with geologic studies in the region. The interlayering of coarse and fine sediments in the Santa Clara Valley results from changes in sea level caused by world-wide climate fluctuations (Atwater, et al, 1977). During times of high sea level (warm periods), fine grained estuarine sediments were deposited in the valley, resulting in the clay and silt aquitards. During times of low sea level (glaciation in northern latitudes), these sediments were covered by coarser grained alluvial deposits that form the aquifers. At the study site, the aquifer consists of alluvial sediments deposited during the last 5000 years. The test zone appears to have the structure of a buried stream channel containing sand and gravel. This structure is common in alluvial aquifers that are characterized by deposition from multiple channels with constantly shifting loci of deposition, resulting in discontinuous lenses of sand and gravel (Press and Siever, 1974).

HYDRAULIC CHARACTERISTICS

Hydraulic Gradient

Maps of the regional piezometric surface of the "A" Aquifer have been reported by Canonie Engineers (1983). The hydraulic gradient is northward at about 4.5 meters per kilometer. Piezometric measurements made with the original wells of the test zone, indicated that the aquifer was confined with a piezometric surface 2.5 meters above the top confining layer (21 ft above mean sea level). The magnitude and direction of the gradient in the test zone was in the range of regional values. The original gradient estimates had a large level of uncertainty due to the short spatial resolution. Wells 11, 12, 13, installed in August 1986, provide a more accurate estimate of the local gradient due to the greater distances between wells. A gradient of 0.0032 in a northerly direction was estimated using these wells.

Pump Tests

Numerous pump tests were performed to determine hydraulic properties of the aquifer. The tests determined the transmissivity, which permitted estimates of the hydraulic conductivity and the natural gradient groundwater velocity. The possible influence of leakiness, barriers and abnormalities was also examined. Finally, the steady-state drawdown was investigated in a long term pump test.

The drawdown pump tests were performed using a SE 200 A well test device, obtained on loan from the Robert S. Kerr Environmental Research Laboratory, USEPA; Joseph Keely of the Kerr Laboratory provided guidance in the use of the instrument. The equipment consisted of a central mini-computer and downhole pressure transducers. During the tests, six transducers were placed in the wells (SI, P, NI, P1, EI, 6). In all but one test, water was pumped from well P at a steady rate, while drawdown versus time was measured in other wells. To explore for directional variability, one test was run by extracting from well EI.

The pump tests were analyzed by standard methods as described in Freeze and Cherry (1979). A direct method for calculating transmissivity and the storage coefficient is based on semi-log method based on the equation of Cooper and Jacob (1946). The second method used is based on the method of log-log type curve matching.

The log-log plots of drawdown versus time showed that the responses matched type curves for a leaky aquifer (Walton, 1960). Freeze and Cherry (1979) indicate that when production wells are screened only in a single aquifer (as is the case of the test zone well) it is quite usual for the aquifer to receive flow from the adjacent beds. Thus leakage through the confining layers is likely occurring in region of the test zone. Matches were therefore made to the leaky aquifer type curves.

The results show good agreement between the semi-log method and the more rigorous type curve method. A semi-log method yielded an average transmissivity of 12600 gpd/ft. An average value of 11100 gpd/ft was obtained based on the type curve method. The match method gave an average value of the storage coefficient of 0.0013 and an average r/L value of approximately 0.05, indicating the aquitards are not very leaky. This result may explain the good agreement between the match method and the semi-log method, which assumes the system is confined system.

A summary of the results of the pump tests is presented in Table 2. The transmissivity values were fairly reproducible from test to test. The transmissivity values show no significant differences based on the location of the observations wells or the pumping well. Anisotropies in transmissivity in the horizontal plane were not indicated by the tests. However, a more detailed analysis of the data is currently being performed using a computer code which uses a non-linear least squares routine to estimate best fit parameters, for a given solution. These analyses will more accurately determine if anisotropies in transmissivity exist.

The high transmissivity results in an estimated hydraulic conductivity of 100 m/d (based on an aquifer thickness of 1.4 meters). The hydraulic conductivity is in the range of values given by Bouwer (1978) for coarse sand (20-100 m/d), gravel (100-1000 m/d) and sand-gravel mixes (20-100 m/d), which is consistent with the aquifer cores as indicated by the particle size distributions shown in Figure 5.

TABLE 2. SUMMARY OF THE PUMP TEST RESULTS

Pump Test	Duration (min)	Rate (gpm)	T(avg)[1] (gpd/ft)	K(avg)[2] (m/d)
5	90	5	13654	122
6	456	5	11272	101
7	495	5	9440	84
8	600	5	9625	86
9	3471	4	11505	102
		Average	11100	100
		std	1500	15

[1] match to leaky aquifer type curves
[2] based on an average aquifer thickness of 1.4 m

The long-term pump tests show that steady-state drawdowns were achieved, and that the aquifer was capable of supplying water at rates required for the experiments, with less than a 1 meter drawdown at the extraction well. The long-term pump tests did not detect any abrupt barriers to flow.

The pump tests indicated that the site had several favorable hydraulic features: 1) high transmissivity should permit the required pumping and injection of fluids into the test zone; 2) loss of permeability by clogging due to biological growth or chemical precipitation, would be limited, due to the original high permeability; 3) the aquifer is semi-confined, thus the test zone is fairly well bounded in vertical direction; and 4) the aquifer was capable of supplying groundwater at rates required for the experiments with less than one meter of drawdown at the extraction well.

One potential problem with the high hydraulic conductivity is that the velocity of the groundwater under natural gradient conditions is high. A velocity of 1 m/d was estimated based on the hydraulic conductivity of 100 m/d, the measured hydraulic gradient across the field of 0.0032, and an estimated porosity of 0.33. This high groundwater velocity limits the control of fluid residence times, since the induced flow field must be operated in such a manner as to overcome the natural flow in order to assure capture of the injected solutes.

CHEMICAL CHARACTERISTICS

Samples of the groundwater from the A-aquifer were obtained during the pump test program to determine the the background concentrations of inorganic and organic components. The analyses provided information on the quality of the groundwater in the area of the test zone and determined whether the aquifer was contaminated with chlorinated aliphatics of interest.

Inorganic Composition

Table 3 presents the major anions and cations, along with other parameters. The charge balance, as well as the measured and calculated TDS, indicate that all of the major ions have been identified. The major cations in decreasing milliequilavent concentrations are as follows: calcium > magnesium > sodium > potassium. The major anions are, in declining order: sulfate > bicarbonate > chloride > nitrate. The groundwater hardness is 920 mg/l, based on the calcium and magnesium concentrations, and would be classified as very hard water. Bicarbonate is the major form of alkalinity at the measured groundwater pH of 6.5. The dissolved oxygen content of the groundwater is below 0.2 mg/l.

The analysis of the major chemical components indicates that the test zone is suitable for the experiments. The chemical composition, including the pH, is suitable for the microbial growth. However, because the concentration of dissolved oxygen in the groundwater is very low, all of the oxygen required for microbial growth must be added to the test zone. The presence of high nitrate and low ammonia concentrations indicate that the aquifer is not anaerobic. Thus, major problems associated with the change in the oxidation state by the addition of oxygen are not anticipated, at least from the microbiological point of view. The high calcium concentrations may present problems, e.g., the precipitation of sulfates and carbonates with changes in fluid chemistry. The chemical composition of the groundwater indicates that the fluid phase concentrations are close to the solubility limits of gypsum ($CaSO_4$) and calcite ($CaCO_3$). Owing to the high sulfate concentration, the groundwater is not considered of drinking water quality, which facilitated obtaining regulatory approval to perform the experiments.

Trace Chemical Analysis

Analysis for trace element composition was performed by Inductively-Coupled Argon Plasma Spectrometry at the Robert S. Kerr Environmental Research Laboratory (Bledsoe, 1985, unpublished data). Table 4 shows concentrations of all inorganic elements were below 1000 ug/l, and in most cases below the detection limit of the analysis. Concentrations were below levels that would be considered toxic to microorganisms, and indicate that the addition of trace nutrients may be required to promote effective microbial growth.

TABLE 3. GROUNDWATER CHEMISTRY: MAJOR IONS AND OTHER PARAMETERS

MAJOR IONS	Concentrations (mg/l)	Concentrations (mg/l)	Milliequivalents Calculated from Lab 1 results (meq/l)
CATIONS	Lab 1*	Lab 2*	
Na^+	53.	44.	2.3
K^+	2.6	1.5	<0.1
Ca^{++}	200.	216.	10.0
Mg^{++}	100.	93.	8.2
NH_4^+	<0.1	nd	<0.1
TOTAL	356.	355.	20.5
ANIONS	Lab 1*	Lab 3*	
Cl^-	42.	39.	1.2
Br^-	0.6	<0.2	<0.1
HCO_3^-	270.	227.	4.4
NO_3^-	6.9	14.9	<0.1
PO_4^{3-}	0.1	nd	<0.1
SO_4^{2-}	750.	699.	15.6
TOTAL	1070.	980.	21.3

CHARGE BALANCE ERROR = 2%

OTHER PARAMETERS

Total Dissolved Solids (TDS, mg/l)
Measured = 1456 ± 15 (by gravimetric analysis)
Calculated = 1426 (from major ion analyses)
Estimated = 1000-1400 (from specific conductance)

pH = 6.5 (measured in the field)

DO < 0.2 mg/l
Temperature = 18°C (measured in the field)

*Major ion analyses conducted by different laboratories. Lab 1, Lab 2, and Lab 3 refer to Sequoia Analytical Laboratory, Kerr Environmental Research Laboratory, and Stanford University Civil Engineering Laboratory, respectively.

TABLE 4. TRACE CHEMICAL COMPOSITION OF THE GROUNDWATER FROM THE SU-39 SITE.

TRACE INORGANIC CONSTITUENTS*

Element	DISSOLVED (ug/l)	TOTAL (ug/l)
Fe	nd	540
Mn	300	310
B	150	200
Zn	10	30
Sr	67	76
Ba	20	20
Al	<100	<100
As	<30	<30
Be	<3	<3
Ag	<10	<10
Cd	<3	<3
Co	<10	<10
Cr	<10	<10
Cu	<10	<10
Hg	<30	<30
Li	<10	<10
Mo	<10	<10
Ni	<10	<10
Pb	<20	<20
Ti	<100	<100
Se	<30	<30
Tl	<20	<20
V	<10	<10

TRACE ORGANIC CONSTITUENTS**

Chemical	Concentration (ug/l)
1,1-dichloroethylene (1,1 DCE)	14
1,1-dichloroethane (1,1 DCA)	0.5
1,1,2-trichloro-1,2,2-trifluroethane (Freon113)	9.4
1,1,1-trichloroethane (TCA)	97.4 ± 30

* determined by Inductively-Coupled Argon Plasma Spectrometry; when results were below detection limit (d.l.), the results are listed as less than (<) the d.l. for the method

** determined by gas chromatography or gas chromatography/mass spectrometry. Values listed are averages of duplicate determinations, except for TCA. TCA analyses were conducted on seven samples taken during the period 7/9/85-11/10/85; the TCA concentrations in the samples ranged in concentration from 56 to 131 ug/l.

Analyses were conducted to determine the type and concentrations of trace organic compounds at the field site. Four volatile organic compounds were detected, as shown in Table 4. The highest concentrations in the native ground water were found for 1,1,1-trichloroethane (TCA), which is present at a concentration on the order of 100 ug/l, varying over a range of 56-131 ug/l for analyses conducted over several months. Trace amounts of other halogenated compounds are present, as shown in Table 4. Trichloroethylene (TCE) was not detected in these samples.

Analyses were as performed for purgeable aromatics. No such compounds (e.g., benzene, xylene, toluene, chlorinated aromatics), were detected. Total (non-purgeable) organic carbon was determined to be approximately 2 mg/l, within the range of 0.1 - 10 mg/l reported for groundwaters due the presence of natural humic and fulvic acids (Freeze and Cherry, 1979).

These analyses showed that the native groundwater in the test zone had the following important characteristics with respect to chlorinated compounds:

1) It was contaminated with halogenated organics at low concentrations. This was considered an important criterion for obtaining regulatory approval to conduct the experiments. The concentrations, however, were low and would not be toxic to the native bacteria.

2) The TCE concentration was below the detection limit. Thus, controlled experiments can be performed by adding small but measureable quantities of TCE to the test zone.

The results of the initial inorganic and organic analyses indicated that the groundwater was of a suitable chemical composition for performing the experiments. The chemical composition would not inhibit the stimulation of the methanotrophic bacteria, and it appears feasible to inject and transport dissolved oxygen in the test zone without undue consumptive losses.

AQUIFER SOLIDS ANALYSIS

Core samples of the aquifer material were obtained in order to characterize the aquifer material's physical, chemical, and microbiological properties. Some of the core material was to be used for microbiological studies in the laboratory. Aseptic procedures as outlined by Wilson et al. (1983) were used for obtaining the cores samples and transferring the materials to storage containers.

Microbial Enumeration

The acridine orange-epifluorescence procedure of Ghiorse and Balkwill (1983) was used to enumerate the active bacteria attached to solid samples from the test zone. The analysis indicated that the microorganisms were typically attached to particles of organic matter. The bacterial numbers per gram of dry solids varied from 2 - 39 x 10^6 within the range of values of 1 - 50 x 10^6 obtained in subsurface investigations of Ghiorse and Balkwill (1983), Wilson et al (1983), and Webster et al. (1985). No apparent trend with depth was indicated. The highest value, however, was observed in the sand and gravel zone, 17 -

17.5 ft below the surface. The bacteria counts may be associated with the high permeability of this zone and a corresponding greater flux of nutrients.

The presence of methanotrophic bacteria was not established using this enumeration procedure, since the method is not type specific. The presence of methane-consuming bacteria on aquifer solids was, however, demonstrated in columns studies discussed by Wilson et al. (1987). In these studies, columns were packed with core solids obtained from well SI. After exchanging the pore water with water containing methane and oxygen, oxygen and methane consumption was observed. This study and the bacteria enumeration study indicated that the test zone had an indigenous microbial population that could be successfully biostimulated.

Organic Carbon Content

The organic carbon content of the Moffett aquifer material was determined by measurement on a Dohrmann DC-80 organic carbon analyzer following pretreatment consisting of acidification with H_3PO_4 and heating under vacuum to remove carbonate, addition of $K_2S_2O_8$, and autoclaving at 121°C for 4 hours in sealed ampules to oxidize the organic matter to CO_2. The ampules were then broken into the oxygen stream of the DC-80 analyzer, and the CO_2 production was quantified by a Horiba nondispersive IR spectrometer. Coarse-grained samples were preground for 10 seconds in a tungsten carbide mill to facilitate complete removal of inorganic carbon and complete recovery of the organic carbon.

Results are summarized in Table 5. For the bulk material, the average value was 0.11 percent carbon, with no significant influence of pregrinding. The organic matter appeared to be concentrated in the clay fraction, with organic carbon contents six times that of the bulk material, whereas the coarse-grained fractions have organic carbon contents as much as 40 percent less than the bulk average.

TABLE 5. ORGANIC CARBON CONTENT OF MOFFETT AQUIFER SOLIDS

Size Fraction	Organic Carbon Content Percent, mean + std. dev. ground	not ground
Bulk	0.112 ± 0.020#	0.110 ± 0.014
Clay-top		0.649 ± 0.039
Clay-bottom		0.638 ± 0.090
U.S. Std. Mesh		
<200		0.161 ± 0.014##
-100+200	0.113 ± 0.009	
-60+100	0.087 ± 0.005	
-40-60	0.100 ± 0.008	
-20+40	0.062 ± 0.005	
-8+20	0.095 ± 0.009	
-4+8	0.082 ± 0.007	

* 4 replicates, unless otherwise noted.
\# 3 replicates
\## 6 replicates

Based on these measurements, it appeared likely that the Moffett aquifer material would exhibit substantial sorption capacity, significantly greater than observed at the Borden site in our previous field experiment (Roberts et al., 1986; Curtis et al, 1986), where the organic carbon content was measured as 0.02 percent.

Sorption onto Aquifer Solids

The degree of sorption of several chlorinated aliphatic compounds onto aquifer solid samples was determined in batch sorption experiments. The procedure used in the batch sorption experiments is that described by Curtis et al. (1986). The size fraction studied was that which passed through a #10 U.S Standard sieve, i.e particle diameters less than 2 mm. This fraction represents approximately 33% of the particle mass present in cores as shown in Figure 5.

The results of 3-day batch experiments for PCE, TCE and 1,1,1-TCA are plotted in Figure 6, with the corresponding fit to a Freundlich isotherm. It is evident that TCA sorbs less that TCE, while PCE sorbs most strongly. The isotherms are fairly linear, as indicated by the exponent term being greater than 0.90. Linear fits resulted in K_d values of 0.42, 1.4 and 4.0 cm^3/g for TCA, TCE, and PCE respectively.

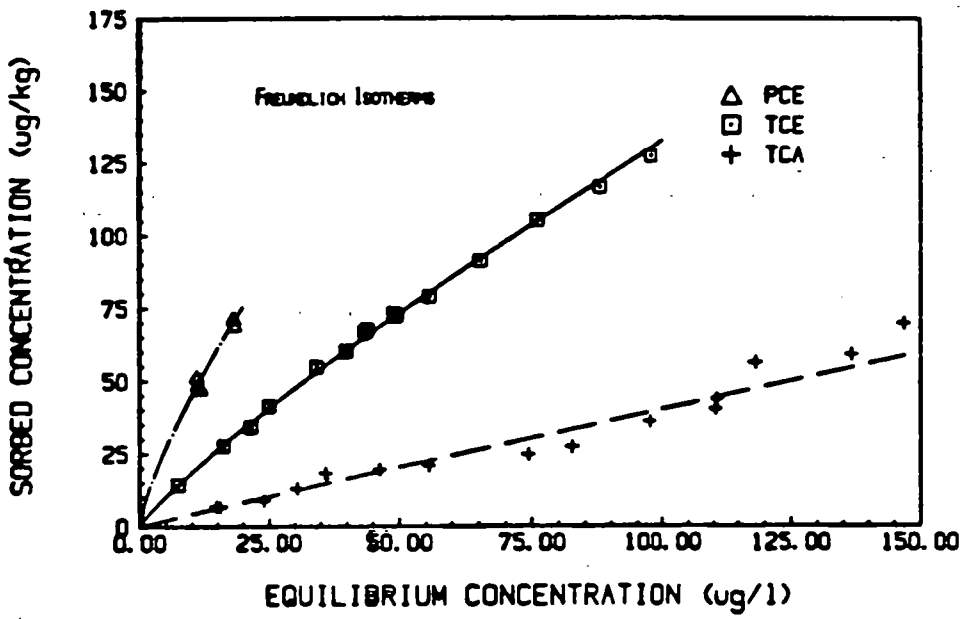


Figure 6. Freundlich isotherms for PCE, TCE and 1,1,1-TCA based on 3 day batch sorption experiments onto aquifer solids.

Estimates of the K_d values were made based on the empirical relationship of Karickhoff et al. (1979), where K_d is dependent on the fraction organic carbon of the solid and the sorbing solute's solubility in water. Solubility data tabulated in Horvath (1982) was

used in the estimates. The organic carbon content of the bulk solids used in the sorption studies was 0.001. The estimated K_d values were 0.266, 0.318, and 1.06 cm^3/g for TCA, TCE and PCE, respectively. The measured values show a trend similar to that predicted from partitioning theory, with higher K_d values for the less soluble compounds. However, the estimated K_d values are consistently lower, by factors 1.6, 4.4, and 3.8 for TCA, TCE and PCE, respectively. Similar results were obtained for low carbon content materials by Curtis et al. (1986b) and Schwarzenbach and Westall (1981), who explained the larger measured partition coefficients in terms of sorption to mineral surfaces.

TABLE 6. MEASURED AND PREDICTED K_d VALUES FOR PCE, TCE, AND 1,1,1-TCA, AND ESTIMATED RETARDATION FACTORS.

Compound	Measured Sorption Coefficient K_d (cm^3/g)	Predicted Sorption[2] Coefficient K_d (cm^3/g)	Retardation[3] Factor R
TCA	0.42	0.27	2.5-3
TCE	1.4	0.32	6.5-8.5
PCE	4.0	1.06	17-22

1) based on measured linear sorption isotherm
2) based on the empirical correlation with water solubility of Karickhoff et. al. (1979) and the measured f_{oc} = 0.001
3) based on Eq 1. with p_b= (1.6-1.9 g/cm^3), and n = (0.3-0.4)

Estimates of the degree of retardation of the sorbing solutes relative to a nonsorbing solutes were made based on the retardation factor as described by Freeze and Cherry (1979), given by

$$R = 1 + p_b K_d / n \qquad (1)$$

where p_b is the bulk density of the aquifer material (g/cm^3); n is the porosity (cm^3/cm^3); and K_d is the equilibrium distribution coefficient. The estimated retardation factors are presented in Table 6. Based on these estimates, the movement of TCE through the test zone would be expected to be retarded by a factor of 6.5 to 8.5. This has important implications for the time required to establish steady-state concentrations during the tests, and the effect the sorption process may have on the biotransformation of the TCE.

SECTION 6

SITE INSTRUMENTATION

THE WELL FIELD

Figure 4 presents a vertical section of the test zone and the well field used in the experiments. The well field was designed to permit simultaneous experiments by creating two test zones through the injection of fluids at both the south (SI) and north (NI) injection wells, and extraction at the central extraction well (P). The operation of the extraction well is intended to dominate the regional flow field in the study area in an approximation of radial flow.

The injection wells are located 6 meters from the extraction well. The monitoring wells are located 1.0, 2,2 and 4.0 meters from the injection wells. This spacing should result in roughly equivalent fluid residence times between monitoring wells if radial flow conditions exist. The extraction and injection wells are constructed of 2" PVC wellstock which is slotted over a 5 ft screened section. The screened section is positioned 14 ft to 19 ft below the surface in order to fully penetrate the aquifer. After installation with a hollow stem auger, the borehole around the screened section was back filled with sand (Monterey #8). The monitoring wells are 1.75" O.D. stainless steel well casing with a 2 ft screen drive point (Johnson Wirewound #35 slot). The wells were installed with minimal disturbance of the aquifer by augering to within one foot of the aquifer top and hand-driving the wellpoint into the middle of the aquifer. The 2 ft screen section was placed to intercept what was considered to be the most permeable zone consisting of sands and gravels.

In order to prevent losses by volatilization and sorption, the fluid injection and sampling lines are 1/4 inch O.D. stainless steel tubing. The tubing runs from the well bottom to inside the control shed, with a maximum length of approximately 16 meters. The tubing has a series of orifices along the well's slotted interval, in order to collect a representative fluid sample from the formation.

THE AUTOMATED DATA ACQUISITION AND CONTROL SYSTEM

An automated data acquisition system has been devised at the site to implement the field experiments. The system permits the continuous measurement of the experiment's principal parameters: the concentrations of the bromide ion tracer, methane, halogenated aliphatic compounds of interest, and dissolved oxygen, as well as pH. The methods of analysis and the practical detection limit for each parameter under field conditions are summarized in Table 7.

A schematic of the system is shown in Figure 7. The system is driven by a microcomputer. A data acquisition and control program (DAC) has been designed and programmed that can be operated in either manual or automated mode. Manual mode permits selection of samples, creation of a sample sequence for automated operation, calibration of various instruments, and graphing the results as the sampling proceeds. During automated operation, the DAC selects the sample to be analyzed, opens the proper solenoid, and activates a peristaltic sampling pump, located in the control building. After withdrawing approximately 1.2 liters of

TABLE 7. METHODS OF ANALYSIS AND PRACTICAL DETECTION LIMITS FOR EACH PARAMETER UNDER FIELD CONDITIONS.

Parameter	Method	Detection Limit
Dissolved Oxygen	Probe	0.1 mg/l
pH	Probe	NA
Anions (Cl^-, Br, NO_3, SO_4)	Ion Chromatography	0.5 mg/l
Halogenated Organics (Freon 113, TCA, TCE)	Gas Chromatography-ECD	1.0 ug/l
Methane	Gas Chromatography-FID	0.2 mg/l

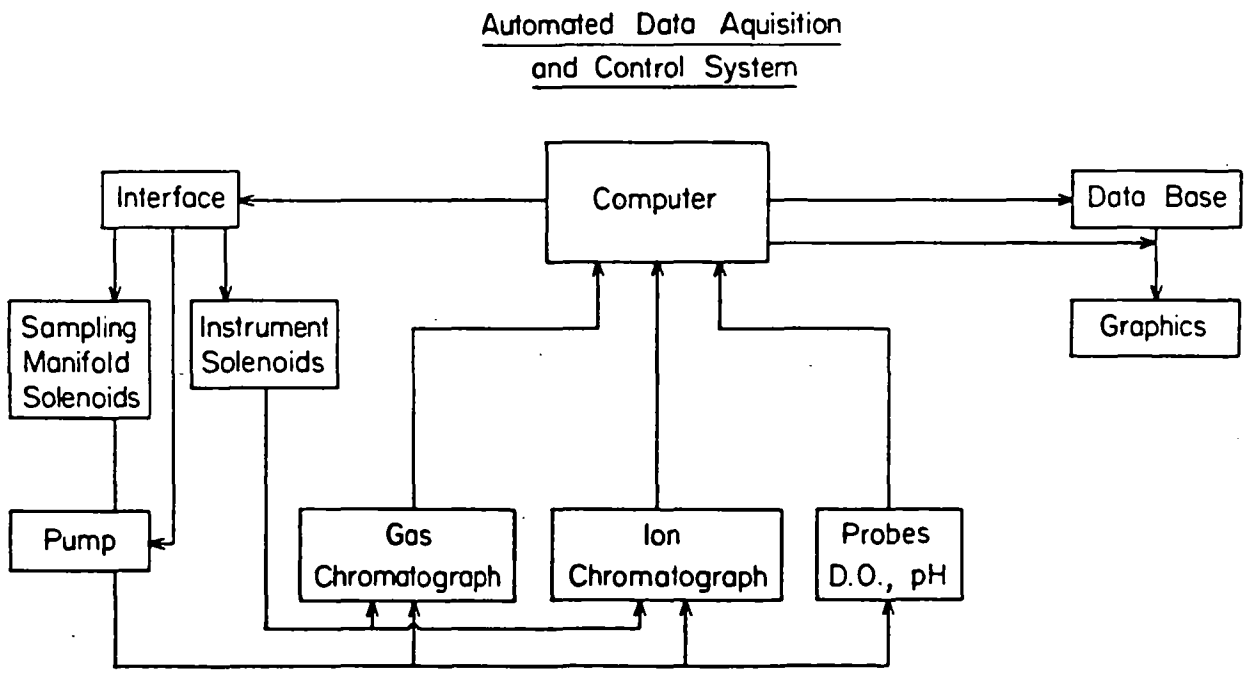


Figure 7 Schematic of the automated data acquisition and control system.

sample, pumping is stopped and samples are analyzed using the methods given in Table 7. After completion of the data acquisition cycle, the DAC integrates the chromatogram (in the case of ion and gas chromatography), calculates and stores the results, and proceeds with the next sample.

In order to realize real-time control and interpretation, measurements are made continually throughout a period of several weeks or months at a frequency corresponding to approximately two per hour. The sampling points are typically six in number, the injected fluid, extracted fluid, three intermediate monitoring points, and the effluent from the air stripper (for monitoring the groundwater discharged to a storm sewer). In order to obtain precise and reproducible measurements during an experiment, the instruments are calibrated daily.

A series of experiments were performed using the DAC system to study the transport characteristics of the test zone under a variety of flow conditions. Natural gradient tests were performed in order to estimate the groundwater velocity and direction at the site. Induced flow tests were performed, in which groundwater was injected and extracted, to study transport under conditions similar to those used in the biostimulation and biodegradation stages of the experiment. The DAC system was found to work reliably and generated more than enough data to observed the transient responses at observation locations. The results of these tracer tests will be presented in the following section.

SECTION 7

RESULTS OF TRACER TESTS

NATURAL GRADIENT TRACER TESTS

Two natural gradient tracer tests were performed, Tracer2 and Tracer3. The tests were performed as follows: a slug of 460 liters of bromide tracer was injected over a period of 3 to 4 hrs into a well along the main line of wells SI through NI, and then allowed to drift under natural gradient conditions. Responses at monitoring wells encompassed both the the breakthrough and the elution of the bromide tracer. In the Tracer2 test, well P was used to inject the tracer and wells N3, N2, and N1 were used as monitoring wells. In the Tracer3 test, well SI was used to inject the tracer, and all the wells along south to north legs were monitored.

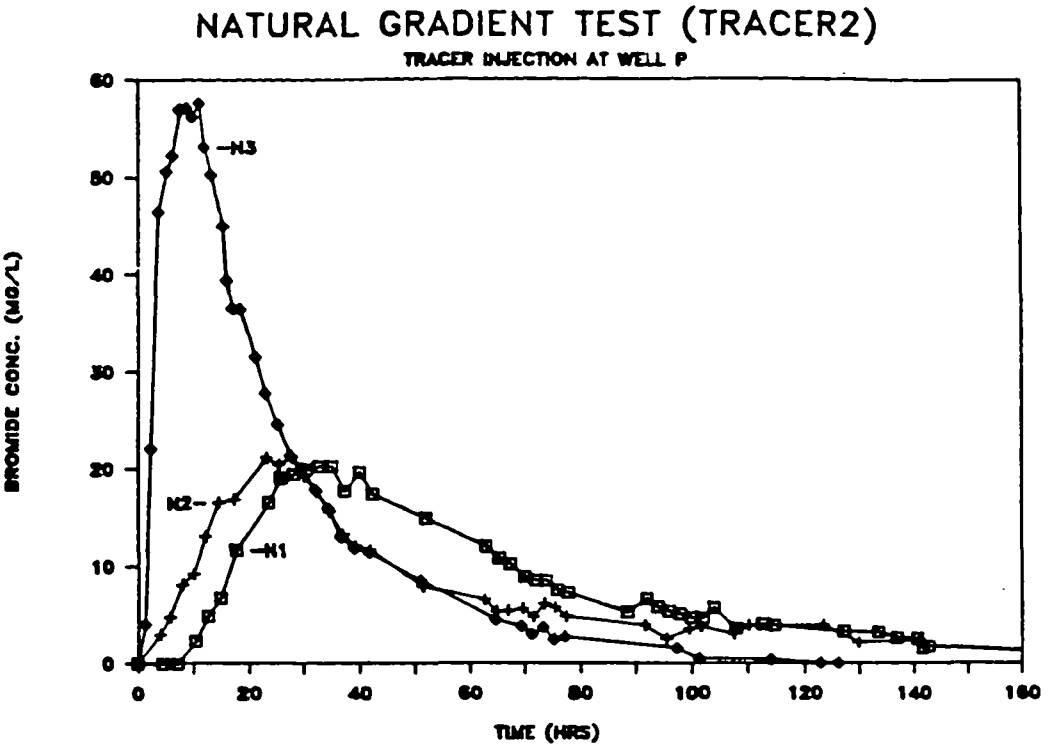
The experiments indicated that the groundwater flow was primarily in a northerly direction. Figure 8 shows responses at the monitoring wells for the Tracer2 and Tracer3 tests, respectively. The response curves are skewed in shape, with a sharp rise in concentration followed by a gradual decrease, or tailing, to background concentrations. The areas under the response curves are shown to be reduced as the distance from the injection well increases, especially for the Tracer3 test. The maximum concentrations are significantly lower than the injected concentrations. The decrease in area with distance and the low maximum concentrations suggest either a flow direction that deviates slightly from being parallel to the line of the observation wells and/or a large amount of lateral dispersion.

Table 8 summarizes the results from the natural gradient tracer tests. The skewed shape of the response curves are indicated by the greater time associated with the center of mass of the response curves

TABLE 8. ESTIMATES OF REGIONAL VELOCITIES BASED ON THE RESULTS OF THE NATURAL GRADIENT TRACER EXPERIMENTS.

	Well	Distance from the Inj. Well (m)	Time Max Conc. (hrs)	Time Center of Mass (hrs)	Velocity[1] (m/hr)	Area Under Response Curve (mg-hr/l)
Tracer2	N3	2.0	8.8	17.9	0.11	1555
	N2	3.8	27.8	38.6	0.10	1059
	N1	5.0	32.8	50.5	0.10	1250
Tracer3	S1	1.0	16.4	32.9	0.7	3658
	S2	2.2	32.5	44.3	1.2	2131
	S3	3.8	12.9	20.0	4.8	1019

[1] Velocity based on center of mass



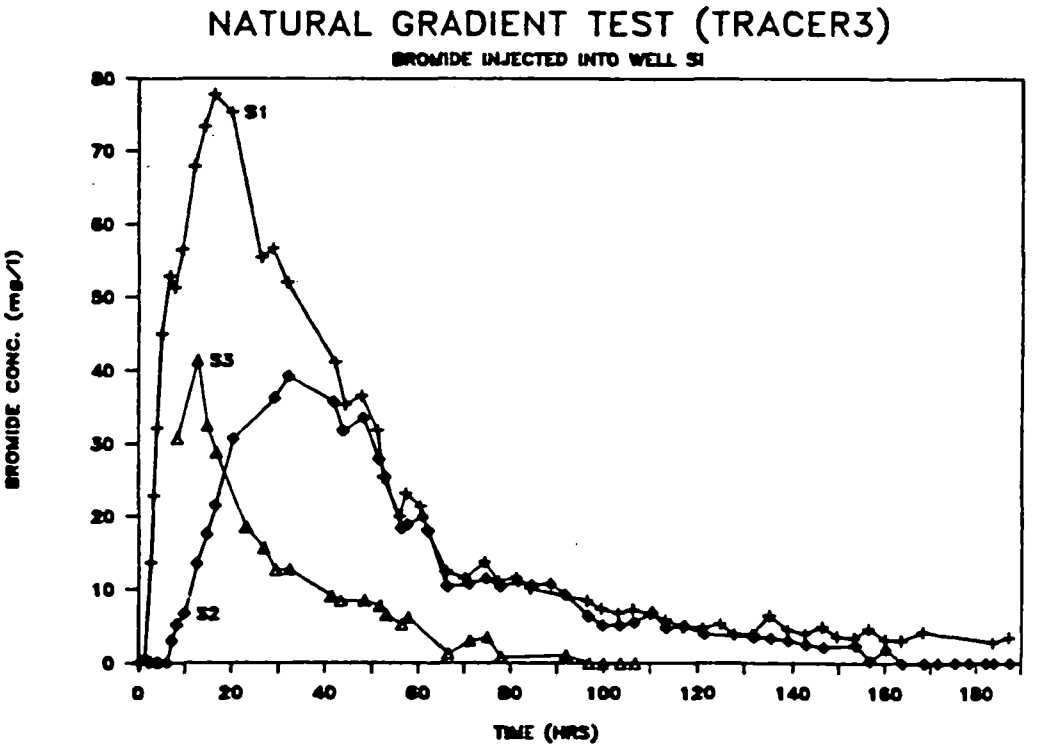


Figure 8. Results of the natural gradient tracer tests (Tracer2 and Tracer3)

compared to the time to the maximum observed concentration. The groundwater velocity estimates based on the time corresponding to the center of mass of the response curve are in good agreement for the Tracer2 test. An average value of 2.6 m/d was obtained. The results obtained from the Tracer3 test are more variable, with higher values obtained the farther the observation well is from the injection well. The higher velocities are seen to be associated with a decrease in area under the response curves.

The rapid transport in the test zone is typified by the initial response at the S3 monitoring well, which precedes that of the S2 well, even though the latter well is located closer to the injection well for this test. This earlier breakthrough is reproduced in all the tracer experiments performed to date. These data suggest that the aquifer is quite heterogeneous, with high permeability zones rapidly conveying the tracer to the distant wells, while the responses at observation wells closest to the injection well represent contributions from a range of permeability zones. The observation wells are not fully penetrating. Thus, if there is layering and vertical structure in the test zone, the monitoring wells may be sampling different zones, especially along the south experimental leg, where the variations in estimated velocity are great. The extensive tailing in the response curves would also suggest multi-permeability zones, as discussed by Moltz et al. (1986).

The results of the two natural gradient tests indicate a fairly high groundwater velocity at the site: approximately 2.4 m/d. The velocity is somewhat higher than the 1 m/d value obtained from the measured gradient and hydraulic conductivity estimated from pump tests, but nonetheless of the same order of magnitude. The hydraulic conductivity, however, is based on an aquifer thickness of 1.5 meter. If the thickness were less, higher estimates of groundwater velocity would result.

INDUCED FLOW TRACER TESTS

Two induced flow tracer experiments -- Tracer4 and Tracer5 -- were performed under the conditions used in the later evaluation experiments. The Tracer4 experiment studied the transport of bromide ion and dissolved oxygen through the test zone. The Tracer5 experiment studied the transport of bromide ion and TCE. The south experimental leg was chosen for the experiments, with fluid being injected into the SI well and extracted at well P. This configuration results in an induced gradient which is superimposed on the natural gradient, thus creating conditions for the effective capture of the injected fluid at the extraction well.

The induced-flow tracer tests were performed as follows: groundwater was extracted at a rate of 8 l/min and reinjected at a constant rate of 1 l/min and 0.6 l/min in the Tracer4 and Tracer5 experiments, respectively. The groundwater was air stripped prior to reinjection to remove background concentrations of organics and to oxygenate the groundwater to a DO concentration of 8 mg/l. Bromide (Tracer4) and bromide and TCE (Tracer5) were added to the air-stripped ground water to achieve the desired concentration and injected at a constant rate. The injection of tracers was performed as a broad pulse. Bromide was added at an average concentration of 120 mg/l for 107 hrs in the Tracer4 test and at a concentration of 230

mg/l for 250 hrs in the Tracer5 experiment. In the Tracer5 experiment TCE was injected concurrently with bromide at an average concentration of 160 ug/l for 350 hrs. The tracer breakthroughs as well as their elution from the test zone were continuously measured at the monitoring wells S1, S2 ,S3 and the extraction well.

Figure 9 shows the DO responses observed in the Tracer4 experiment. The data show a tightly spaced temporal response over four days, with approximately ten samples at each observation point per day. The results show a rapid breakthrough at the S1 observation well. The breakthrough at the S3 observation well, located 4 meters from the injection well, preceded that at well S2, 2.2 meters from the injection well, which indicates short circuiting of flow resulting from aquifer heterogeneities. Steady-state concentrations were achieved at S1 after a period of injection of approximately 50 hrs, while 80 hrs were required to achieved steady-state values at well S3.

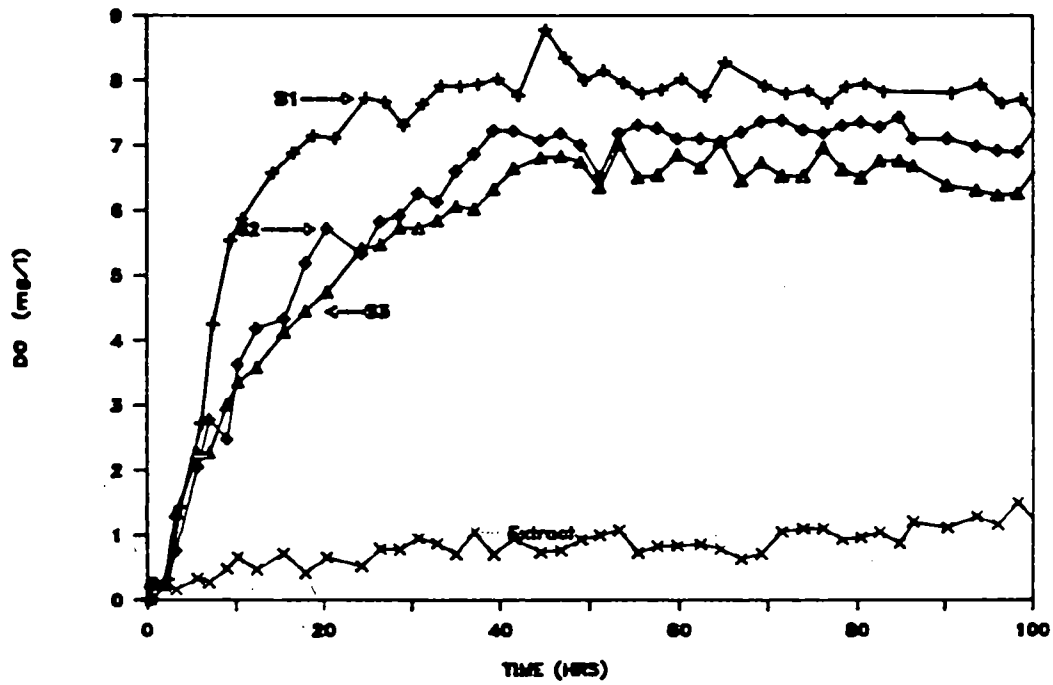


Figure 9. Response of DO at the observation locations in the induced flow tracer test (Tracer4).

The steady-state values show lower concentration values the greater the distance from the injection well. This probably results from one of the following factors: 1) a small degree of DO consumption along the flow path, or 2) some dilution of the injected water by native groundwater having a low DO concentration. The extraction well definitely shows the effect of mixing with the native groundwater, owing to the injection rate (1 l/min) being 1/8 of the extraction rate (8 l/min). The maximum extraction well concentration was approximately 12 percent of the injection concentration, consistent with dilution estimates. The breakthrough of DO at these concentrations at the extraction well indicated that little utilization of DO occurred during transport through the aquifer. Thus, the ability to transport DO through the

aquifer, which is required during the biostimulation phase of the experiment, was demonstrated.

Figure 10 shows the bromide responses for the same experiment, normalized to the injection concentration. Both the initial breakthrough and the elution from the test zone after ceasing bromide injection are shown. The bromide breakthroughs have the same characteristics as the DO breakthroughs, discussed above. The decrease in steady-state bromide concentrations with distance from the injection well indicates dilution by the native groundwater. Thus, with the injection-extraction conditions used, the test zone was not being completely dominated by the injected fluid.

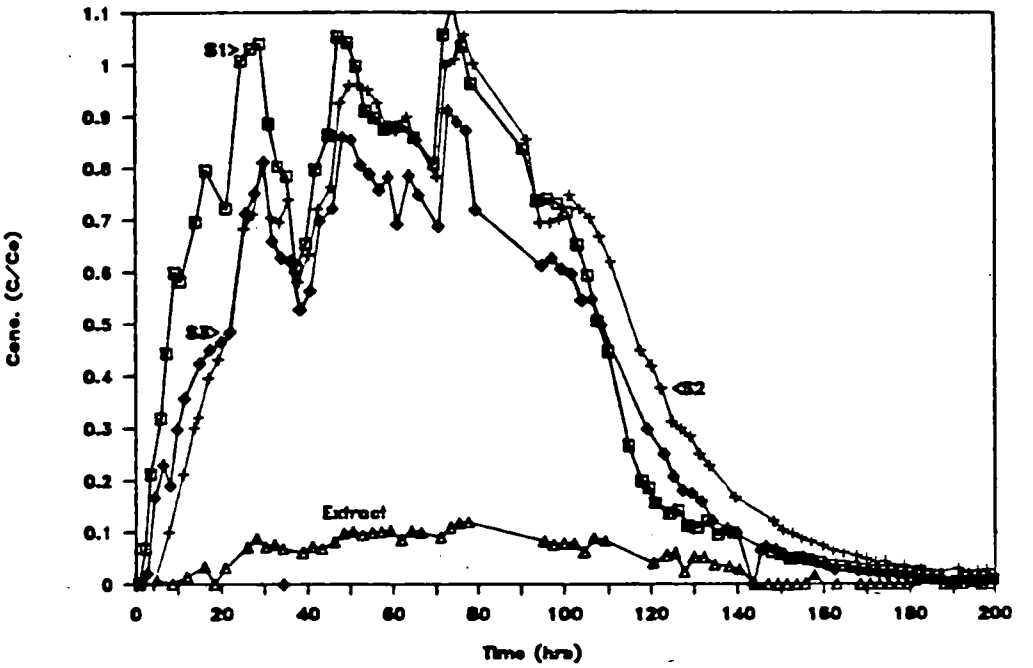


Figure 10. Normalized response of bromide in the Tracer4 test.

Figure 11 shows the response of both bromide and TCE at the S1 observation well, during the early stages of the Tracer5 experiment. The movement of TCE is shown to be retarded with respect to bromide, with a more gradual approach to the injected concentration. The shapes of the breakthrough curves do not conform to that predicted from transport theory for homogeneous media, assuming local equilibrium sorption. These observations suggest: 1) the influence of rate limitation effects on sorption, or 2) the effects of multi-conductivity zones in the aquifer. The observations during the elution phase of the experiment, after the TCE addition was stopped, show extended tailing as shown in Figure 12. The extended tailing is is another indication of the processes described above.

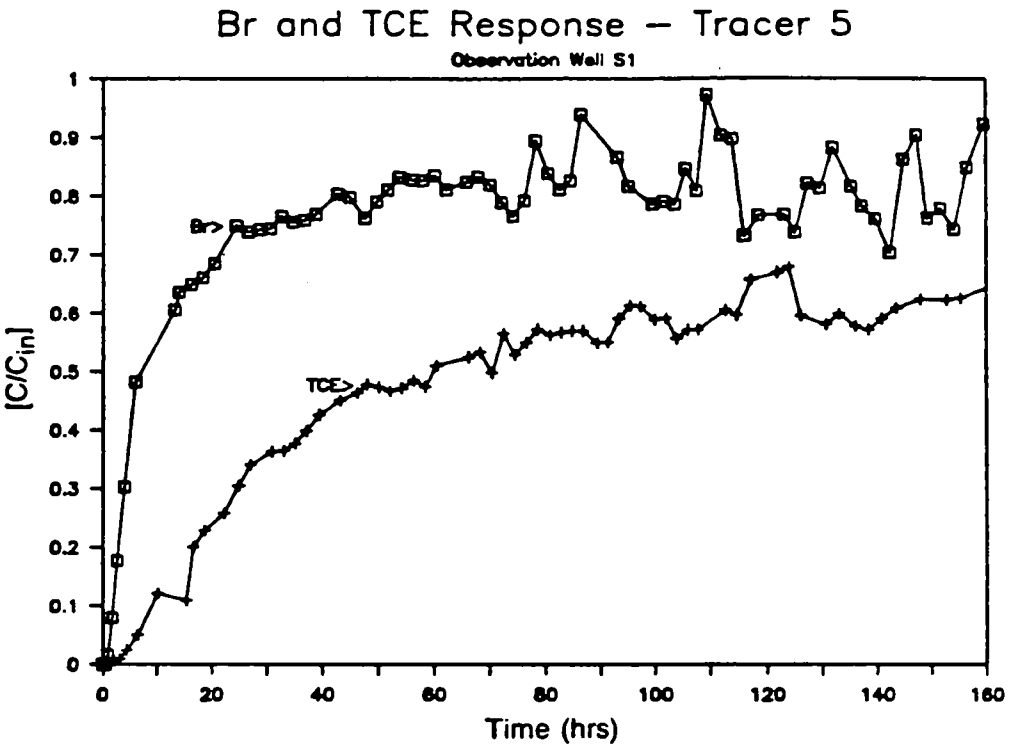


Figure 11. Normalized response of bromide and TCE at the S1 observation well in the Tracer5 experiment.

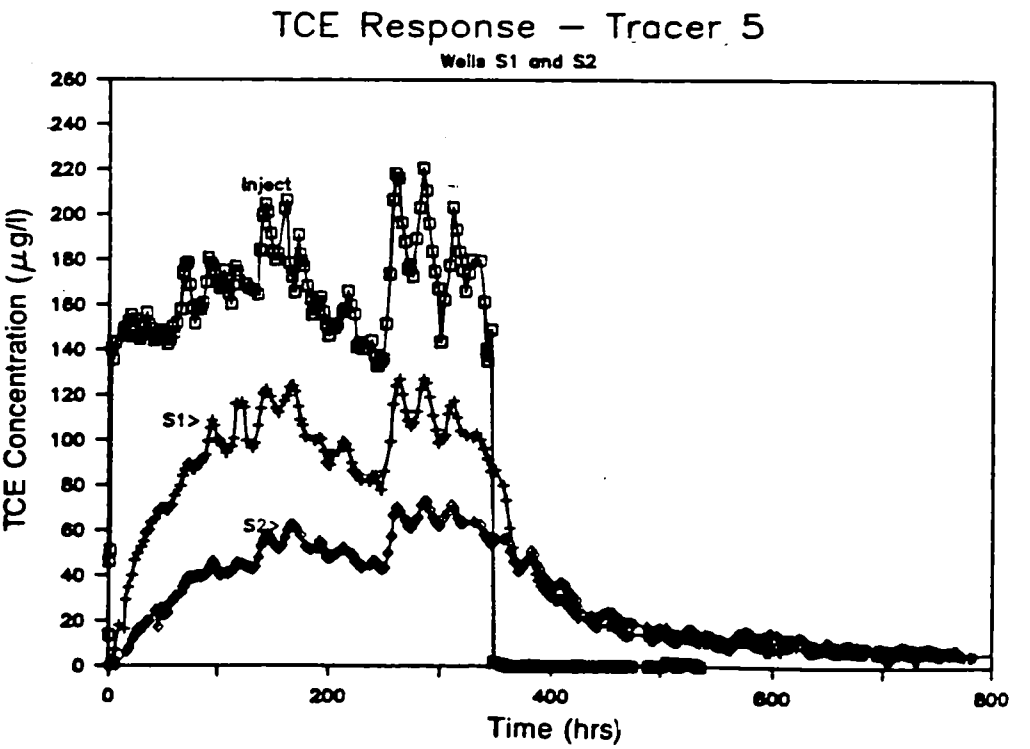


Figure 12. The TCE responses at observation locations in the Tracer5 experiment.

Mass balances based on the Tracer5 results indicated that 61 percent of the injected bromide was recovered by the extraction well, whereas 55 percent percent of the TCE was recovered, over the 800 hr period during which continuous observations were made. Due to the slow elution from the test zone, the total TCE recovered with continued pumping is probably equivalent to that of bromide. The mass balances indicate that the injected fluid is not completely recovered at the extraction well under the injection-extraction conditions used during the first year of the field testing. The recovery of the TCE is similar to that obtained for bromide, indicating that the loss of TCE results primarily from the flow conditions, and not degradation. Thus, the Tracer5 test serves as a pseudo control experiment to which the biotransformation studies can be compared.

Estimated Transport Times

The average fluid residence times from the injection to the observation wells and corresponding fluid velocities were estimated based on the results of the tracer experiments and the initial biostimulation experiment. The estimates are based on the time required to achieve 50% of the steady-state breakthrough concentrations. During the injection period of 350 hrs, the steady-state TCE concentrations, however, were not obtained, due possibly to the slow rate of sorption onto the aquifer solids. The long term steady-state fractional breakthroughs for TCE were therefore assumed equal to that achieved by the bromide tracer. Transport times were also estimated for TCA, based on its elution from the test zone during the Tracer4 experiment. The elution resulted since TCA is present as a background contaminant in the groundwater and air-stripped groundwater, such that the TCA concentration injected during the experiment was always lower than that of the native groundwater.

Table 9 presents the average residence times for the S1 and S2 observation wells. These two wells are presented since they will be discussed in most detail in the latter evaluation experiments, and the shape of their breakthrough response is relatively symmetrical such that the 50% value is fairly representative of the center of mass of the response curve. Several important transport characteristics are indicated by the results. The transport times and corresponding velocities are shown to be very reproducible from test to test. The average fluid residence times based on the bromide tracer are 7.3 hrs and 16.0 hrs from the injection well to the S1 and S2 observation wells, respectively. This corresponds to an average fluid velocity of 0.14 m/hr in both cases. Methane and DO analyses were found to yield similar residence time estimates as obtained using the bromide. This result indicates that these dissolved gases are easily transported through the test zone and are not retarded.

TABLE 9. RESIDENCE TIMES AND TRANSPORT VELOCITIES OF DIFFERENT COMPOUNDS IN INDUCED FLOW EXPERIMENTS.

Test	Compound	Obs. Well	Residence Time (hrs)	Velocity (m/hr)
Tracer4	Bromide	S1	7.6	0.130
Tracer5	Bromide	S1	7.7	0.132
Biostm1	Bromide	S1	6.7	0.149
Tracer4	Bromide	S2	17.9	0.122
Tracer5	Bromide	S2	14.8	0.148
Biostm1	Bromide	S2	15.4	0.143
Tracer4	DO	S1	7.2	0.138
Biostm1	Methane	S1	6.0	0.167
Tracer4	DO	S2	16.7	0.131
Biostm1	Methane	S2	16.1	0.136
Tracer4	TCA	S1	10.0	0.100
Tracer5	TCE	S1	42.5	0.024
Tracer4	TCA	S2	30.0	0.073
Tracer5	TCE	S2	156	0.014

The data for TCA and TCE indicate that these compounds are retarded. The residence times for transport from the injection well to the S1 observation well are 10 hrs and 42 hrs, for TCA and TCE, respectively, compared with 7.3 hrs for bromide. The resulting retardation factors are 1.4 for TCA and 5.75 for TCE. Estimates based on the S2 well data yield retardation values of 1.9 and 9.8 for TCA and TCE, respectively. The values are in good agreement with those predicted from the batch experiments performed in the laboratory (Table 6). The data show an increase in the retardation value, with a greater residence time in the test zone. This may result from a rate-limited sorption process as discussed by Roberts et al. (1986).

Summary of Tracer Test Results

The results of the tracer experiments indicate that reproducible transport experiments can be performed in the test zone. The fluid residence times in the test zone are fairly short, on the order of 8 hrs to the first observation well to 30 hrs at the extraction well. Owing to the high groundwater velocity under natural gradient conditions, longer transport times are not possible, since an extraction rate of at least 8 l/min is required to ensure effective recovery of the injected fluid at the extraction well. The tracer experiments indicated recovery of 60 to 75 of the bromide injected. TCE was recovered to the same degree as bromide, indicating negligible loss of TCE. There is some dilution of the injected groundwater by the native groundwater with the degree of dilution increasing with distance from the injection well. Evidence of aquifer heterogeneities were observed,

for example, with tracer being rapidly transported to well S3. Observation wells S1 and S2 yield similar transport velocities and, based on modeling discussed in detail later, conform to the behavior expected for the case of an induced flow field superimposed upon a natural potential field. TCE was found to be retarded due to sorption onto the aquifer solids. The degree of retardation was within the range of values predicted based on batch sorption experiments with aquifer solids performed in the laboratory.

Modeling the Tracer Test Results

Preliminary modeling of the results of the tracer experiments has been performed using 1-D and 2-D models. The semi-analytical model, RESSQ, developed by Lawrence Berkeley Laboratory and described by Javandel, Doughty, and Tsang (1984) was used to simulate 2-D advective transport under the injection, extraction and natural gradient conditions of the tracer experiments. 1-D analytical solutions were used to estimate dispersion coefficients and to determine if a 1-D modeling approach could be used in the development of a numerical model to simulate the biostimulation and biotransformation processes.

The RESSQ model was used to estimate: 1) the areal extent of the injected fluid front that develops around the injection well and observation wells, 2) the fluid residence times from the injection well to the observation wells, and 3) the degree of recovery of the injected fluid at the extraction well.

Simulations were performed to illustrate the original design of the well field to permit simultaneous experiments along three experimental legs. The model input parameters were: fluid injection at a rate of 0.5 l/min at three wells; an extraction rate of 8 l/min; no regional flow velocity; a porosity of 0.35; and an aquifer thickness of 1.2 meters. Figure 13 shows the results of the simulations. An injected fluid front of uniform size develops around each of the three experimental legs. The maximum width of the front is approximately 1.6 meters around the S1 and S2 observation wells.

Figure 14 shows the fronts that develop when a regional groundwater velocity of 300 m/yr in a northerly direction is imposed on the simulation discussed above. The front around the east injection well is shifted northward due to the groundwater flow. The regional flow also results in a thinning of the front along the southern leg and a broadening along the northern leg. These results indicated that the southern leg (SI,S1,S2,S3) should be used in the experiments for the following reasons: 1) the injected fluid supplying the nutrients becomes less dispersed, and hence a more dense microbial population can be stimulated, and 2) by injecting upstream of the natural groundwater flow, the injected tracers and chlorinated hydrocarbons can be most effectively recovered at the extraction well. The area dominated by the injected fluid does become smaller, however, which helps explain the dilution of the injected fluid by the native ground water that was observed in the tracer experiments.

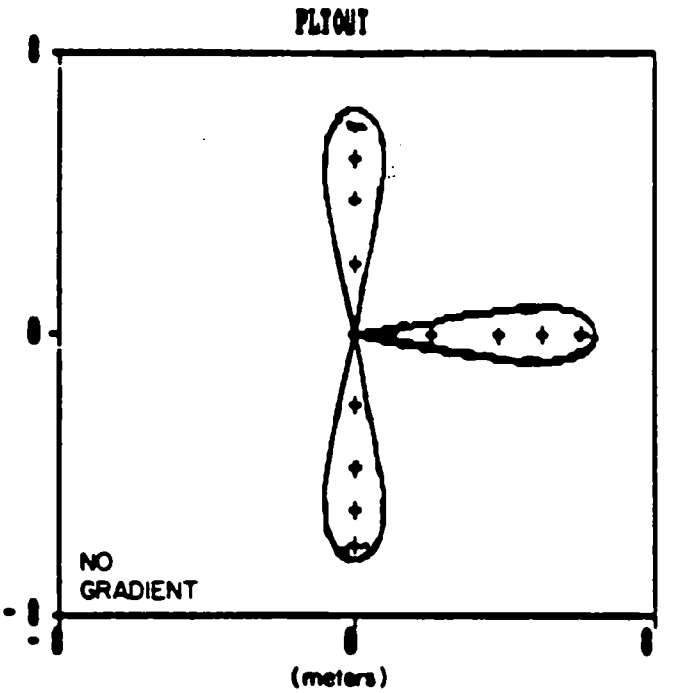


Figure 13. RESSQ simulations of the injected fluid fronts which develop under induced flow conditions of the tracer experiments with no regional flow.

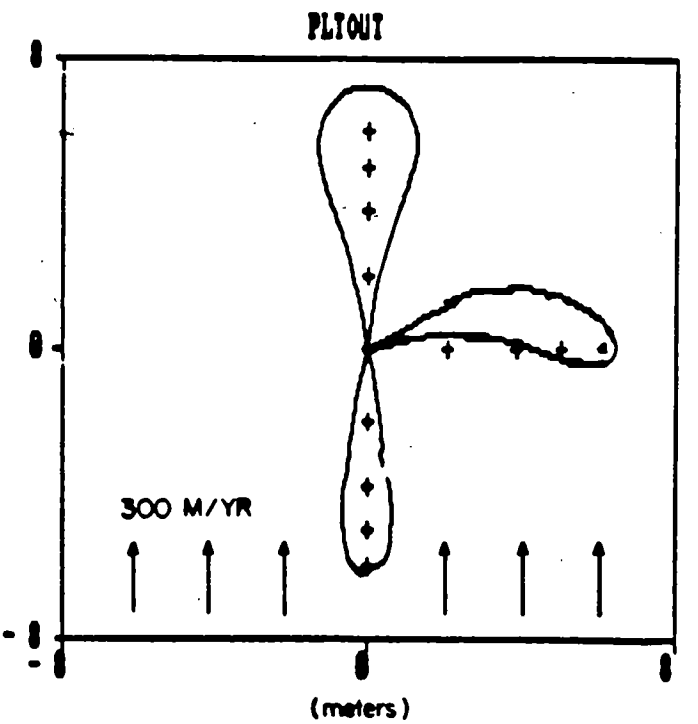


Figure 14. RESSQ simulations of the injected fluid fronts which develop under induced flow conditions of the tracer experiments with a regional flow of 300 m/yr.

Simulations were performed with the RESSQ model to determine whether the predicted fluid residence times are in the range of values estimated by the tracer tests. The model predicted fluid residence times of 8 hrs and 21 hrs for wells S1 and S2, respectively, in fairly good agreement with the Tracer4 test values of 7.6 and 18 hours. Aquifer properties used in the simulation were a regional fluid velocity of 300 m/yr, a porosity of 0.35, and an aquifer thickness of 1.25 m, which are in good agreement the measured and estimated values. The simulations indicate that the injected fluid should be totally captured by the extraction well under these conditions. The tracer tests, however, indicated that only 70 percent of the bromide was captured. The reason for this lower degree of capture is not known, but heterogeneities in aquifer properties are likely responsible.

The simulations indicate that the region near the injection well does not conform to uniform flow, but that the flow is fairly uniform at distances more than approximately 0.5 meters from the point of injection, and hence in the region of the observation wells. To determine the degree of dispersion required to fit the observed breakthrough response at the S1 and S2 wells, 1-D simulations were performed. The non-linear least squares fitting program described by van Genuchten (1981) was used in fitting the data to the solution to the 1-D convective-dispersive transport equation.

Figure 15 shows the fit to the DO breakthrough response at the S-2 observation well in the Tracer4 experiment. A fairly good fit is

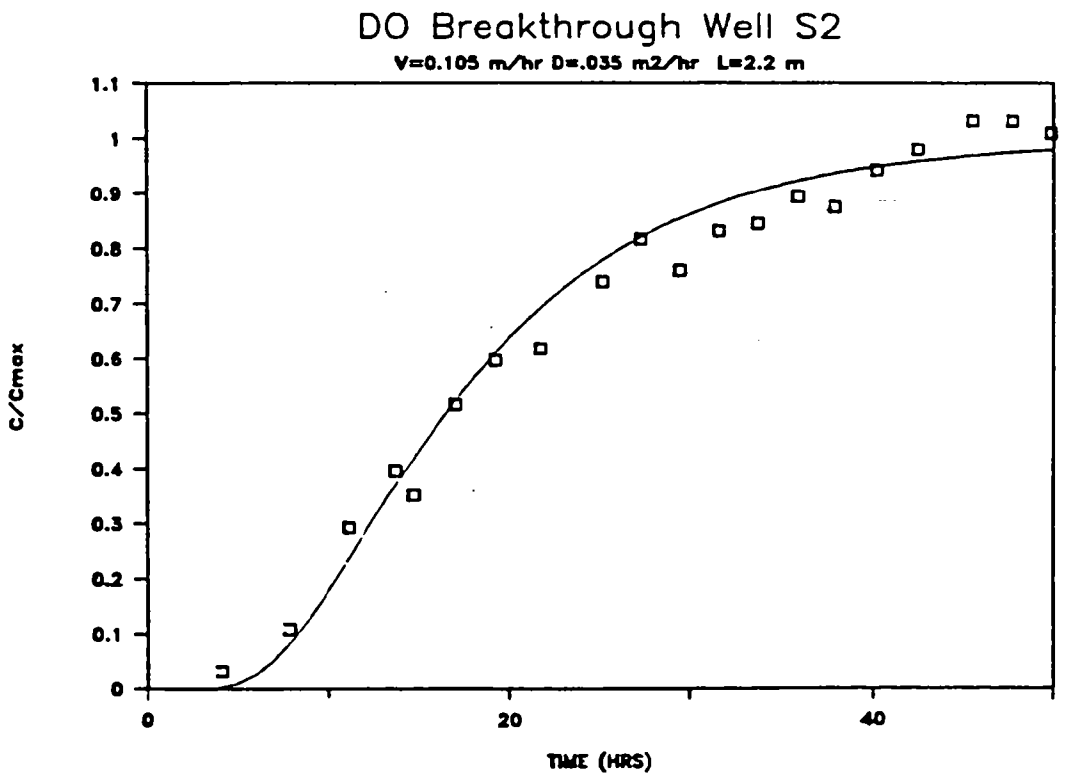


Figure 15. Fit of the 1-D advective-dispersion transport model to tl breakthrough of DO at the S2 observation well during the Tracer4 test.

obtained with the 1-D model, with a resulting Peclet number (Pe) of 6.6, which corresponds to an aquifer dispersivity of 0.33 m (Length/Pe). Model fits to the different experiments and for Br, DO, and methane were performed for the S1 and S2 wells. The best-fit Peclet number based on the S1 well ranged 2.7 to 4.0, with an average value of 3.1. The values based on the S2 well ranged from 3.4 to 6.6 with an average value of 4.4. The resulting average dispersivities were 0.32 and 0.50 meters based on the S1 and S2 wells, respectively. The 1-D analysis resulted in best fit dispersivity values that are in a similar range from the analysis S1 and S2 data. The results indicate that 1-D transport modeling is of value in the initial stages of experimental design and data interpretation, when complex biostimulation and biotransformation processes must be taken into consideration. A more detailed analysis of the tracer data is currently being performed with more complex 1-D and 2-D transport models.

PULSED INJECTION

To enhance the effectiveness of biostimulation, it was decided to introduce the methane (primary substrate) and oxygen (electron acceptor) as alternating, timed pulses. This decision was reached based upon consideration of two crucial requirements: 1) the need to avoid clogging of the injection well and borehole interface, and 2) the need to achieve as uniform a distribution of microbial growth as possible throughout a substantial portion of the aquifer. Failure to fulfill the first requirement would cause loss of hydraulic capacity and premature termination of our experiments, as the drastic chemical measures such as chlorination or strong acid treatment that are customarily employed to rejuvenate clogged wells would interfere with biostimulation. Failure to satisfy the second requirement would lead to conditions of extremely high microbial densities near the injection point and low bacterial populations elsewhere, which would not be conducive to secondary substrate utilization as needed to degrade halogenated aliphatic compounds by methanotrophs. It was thought that introducing the two essential additives, methane and oxygen, as alternating timed pulses would assure their separation in the injection well and borehole, thus discouraging biological growth in that critical region. The methane and oxygen would then mix gradually, owing to the action of hydrodynamic dispersion and associated mixing processes, during transport through the aquifer, stimulating the growth of methanotrophic bacteria over the zone in which mixing occurs. In designing the pulsed injection system, two important variables had to be selected: 1) the ratio of the individual pulses of methane and oxygen, and 2) the overall pulse length.

The ratio of the individual pulses of methane and oxygen can be estimated approximately from knowledge of the stoichiometry of methane oxidation. The oxygen requirement for complete oxidation of methane is 2 moles oxygen per mole methane, which corresponds to a mass ratio of 4 g O_2 per g methane. In choosing the pulse lengths, the concentrations achieved by the saturation columns for oxygen and methane also must be taken into account.

The overall pulse length was evaluated by employing a transport model that incorporates a periodic input (Valocchi and Roberts, 1983). The form of periodic input that corresponds most closely to the case of alternating inputs of methane and oxygen is the rectangular pulse, or saw-toothed wave. The model of Valocchi and Roberts (1983) takes into account the effects of advection, dispersion, and sorption on transport

and mixing of rectangular pulses under conditions of uniform flow. Although the situation at the Moffett field site certainly differs appreciably from the simple case of uniform flow in a homogeneous medium, the model computations based on the idealized case are instructive in exploring the effects of pulse length on mixing, and serve as a point of departure for experimental design.

In the absence of reaction, the normalized amplitude ratio is the most convenient measure of the degree to which the pulses remain separated during transport, or conversely the degree to which mixing has occurred. The amplitude ratio is the ratio of observed magnitude of concentration fluctuations measured at an observation a distance X removed from the injection point to the magnitude of the fluctuations measured at the injection point. The amplitude ratio varies from zero to unity: a value near unity means that concentration fluctuations are damped nearly completely, and signifies virtually complete mixing over the distance traversed, whereas a value near zero implies negligible mixing.

Model computations were conducted under conditions simulating those at the Moffett site. The important variables were the integral distance, x; the pore water velocity, u; and the Peclet Number for dispersion, Pe. The values for the simulation were chosen as x = 1 m, u = 0.12 m/h, and Pe = 5 (dimensionless), to correspond to the results at the nearest observation, S1, based on the results of the early tracer tests., i.e., the dissolved oxygen breakthrough in the initial stages of the Tracer4 set. The computation's results (Figure 16) indicated that substantial mixing over a transport distance of 1 m (the distance from SI to S1) would be attained using a pulse length on the order of several hours, and that pulse lengths on the order of several tens of hours would prevent adequate mixing prior to the first observation well.

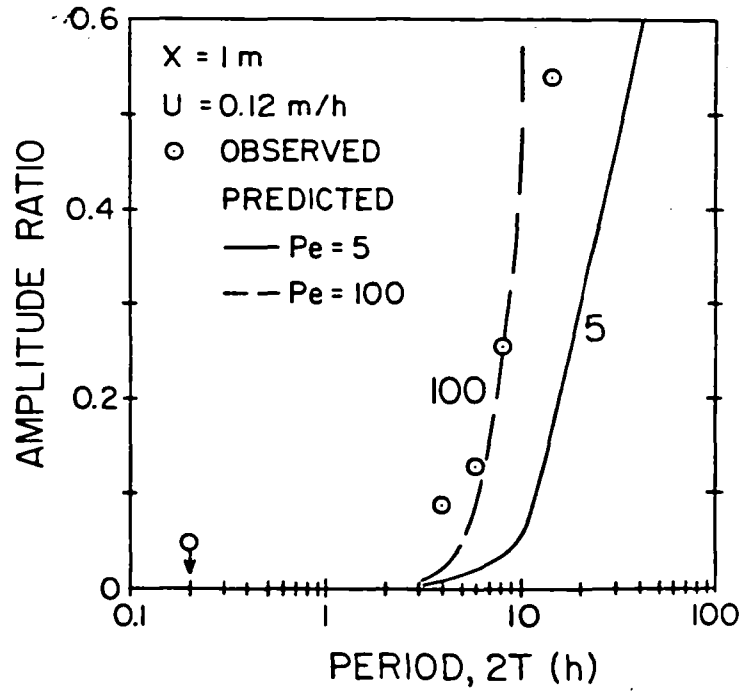


Figure 16. Comparison of predicted and observed effects of dissolved oxygen pulsing

To test the model, toward the end of the Tracer 5 experiment the dissolved oxygen injection was switched to an on/off mode, with pulse lengths chosen to span the range of potential choices for experimental operation, i.e., less than one hour to 12 hours. The observed values are shown in Figure 16 as open circles.

The observations show qualitatively the kind of trend predicted by the model: with short pulses (<1 hour), the mixing is complete within the first meter, but, as the pulse period is increased beyond a critical value of several hours, substantial concentration fluctuations begin to appear at the observation well, indicating that mixing is less than complete. The prediction does not agree quantitatively with the data, as the onset of substantial observed concentration fluctuations occurs at a lower critical value of the pulse period. Indeed, the value of the Peclet Number must be chosen as 100, rather than the observed value of 5, to simulate the pulsing data satisfactorily. These deviations may well be caused by deviations from the model assumptions of uniform flow in a homogeneous medium. Nonetheless, the qualitative agreement between predicted and measured values for the effect of pulsing was deemed adequate as a framework for experimental design of the biostimulation phase.

SECTION 8

BIOSTIMULATION AND BIOTRANSFORMATION EXPERIMENTS

The biostimulation and biotransformation experiments in the 1986 field season were conducted in two stages. First, the test zone was biostimulated by the pulse injection of methane and oxygen into the test zone. After the zone had been successfully stimulated, TCE was continuously injected as previously discussed.

Figure 17 shows the injection system used in the biostimulation and biodegradation experiments. The system uses two counter-current columns to sorb the methane and oxygen to approximately 80 percent saturation,

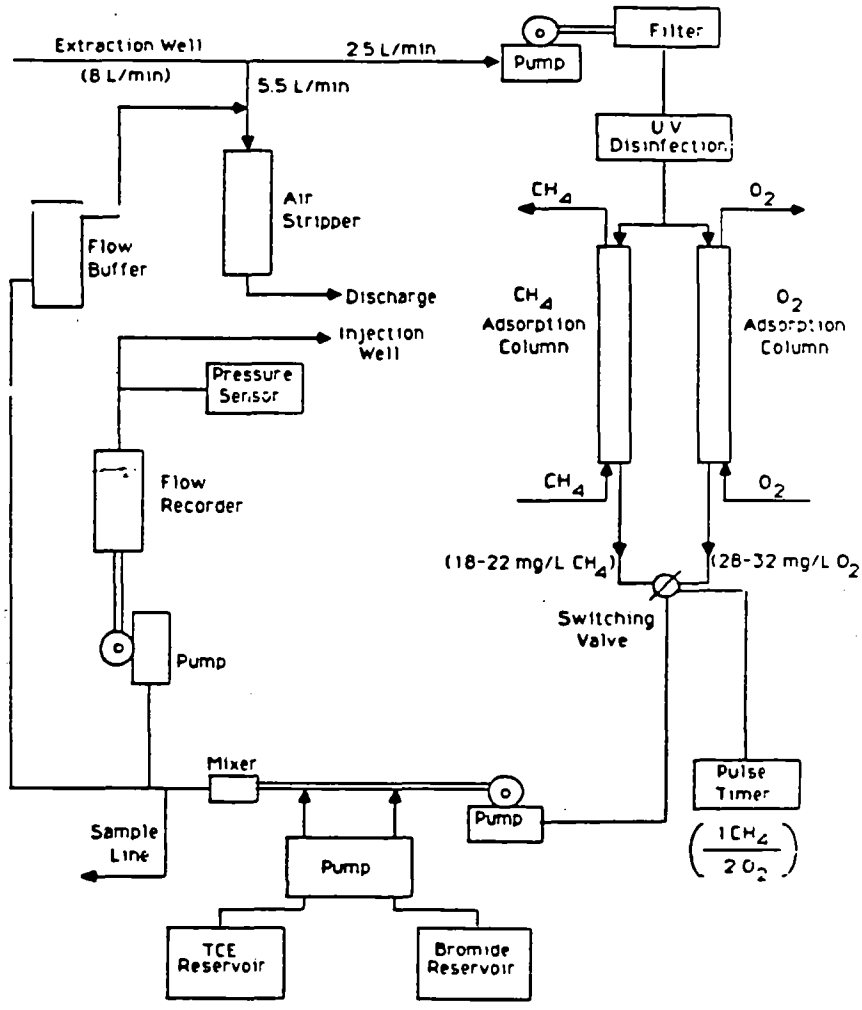


Figure 17. Schematic of the injection system used in the biostimulation and biodegradation experiments.

resulting in concentrations that are approximately 20 mg/l for CH_4 and 32 mg/l for DO. These solutions are alternately pulsed, with a pulse time ratio of about 2:1 (methane:oxygen), based on the stoichiometric requirements. A pulse timer permits the ratio and the length of the pulses to be varied. The other components of the injection system permit the accurate and continuous addition of the bromide tracer and TCE into the injection stream, the monitoring of the injection rates, and the sampling of the injection fluid, while maintaining a constant rate of injection.

BIOSTIMULATION EXPERIMENT

The biostimulation experiment was performed under same induced flow conditions as the earlier tracer tests. The pulse cycle for the injection of either methane or oxygen containing groundwater was varied during the course of the experiment, from less than 1 hr during start-up to ensure the pulsing would not interfere with growth, to a 12 hr period during the later stages to distribute growth in the test zone. No additional nutrients were added to the groundwater.

Figure 18 shows the dissolved oxygen concentration as a function of time at the three observation wells and the extraction well. The consumption of oxygen increases with time, indicating the stimulation of a microbial population. During the initial stages of the experiment (0-50 hrs) there is little evidence of oxygen consumption. The maximum DO concentrations of 19, 17, and 14 mg/l at wells S1, S2, and S3, respectively, result from the combined effects of the pulsed injection of DO water and dilution by the native groundwater. At this stage of the experiment, the microbial population in the test zone was sufficiently low so that the DO consumption was not observable.

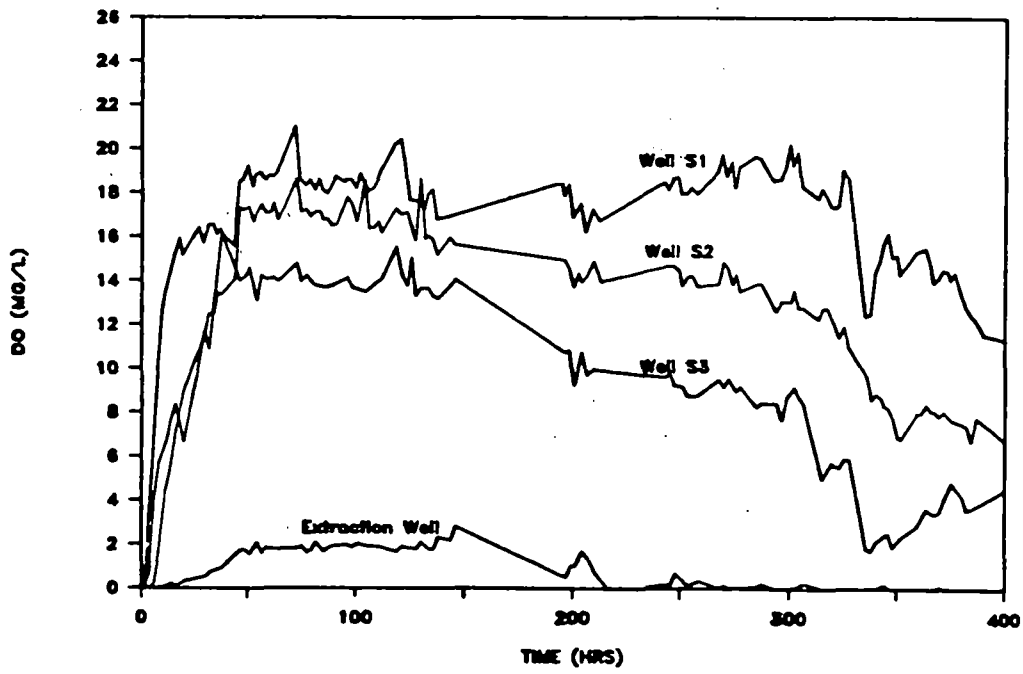


Figure 18. DO response during the biostimulation experiment.

The first signs of consumption were observed in the extraction well and the S3 observation well after approximately 200 hrs of injection. The concentration at the extraction well decreased below the detection limit after 300 hrs of injection. Owing to the continuous removal by microorganisms, the decrease in DO was greater the longer the travel paths through the aquifer. As time proceeds, the increase in the growth of microbial population throughout the test zone results in an increase in the DO consumption along the flow path.

The methane response was similar to that observed for the DO, which is expected, as methane is the electron donor and oxygen the electron acceptor for microbial growth. Figure 19 shows the response of the methane and DO at the S2 observation well. The fairly rapid decrease in the methane concentration over the period of 200 to 400 hrs indicates fairly rapid growth kinetics typical of aerobic microorganisms. A significant amount of methane substrate is also incorporated into cells. Based on the concentration values during the period of 350 - 375 hrs, the ratio of oxygen to methane consumed was 2.25 mg O_2/ mg CH_4, which is significantly lower than the ratio of 4 required for complete oxidation. The lower value suggests incorporation of the methane substrate into the cell mass, with a yield coefficient of approximately 0.5 mg cells per mg CH_4, in the range values for methane-utilizing bacteria reported by Anthony (1977).

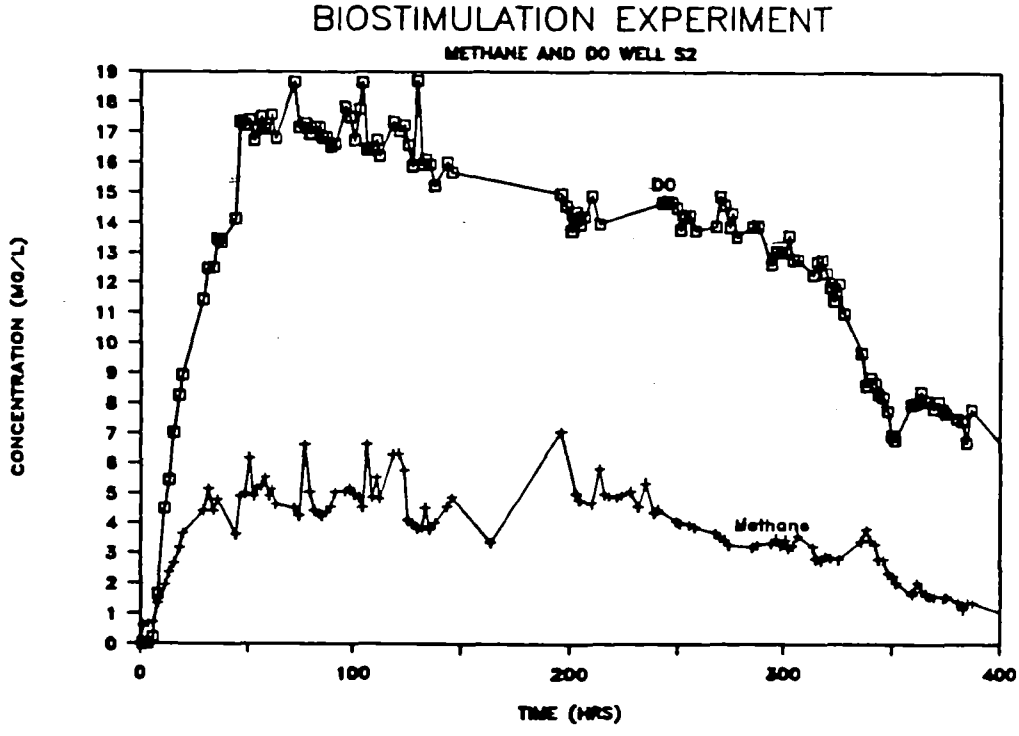


Figure 19. The response of methane and DO at the S2 observation well during the biostimulation of the test zone.

After 450 hrs of injection, the methane concentration at the S2 observation well decreased below the detection limit, indicating that the microbial mass was increasing near the injection well. The pulse cycles were therefore lengthened to 12 hrs in order to prevent

biofouling near the wellbore. Figure 20 shows the response of the system to the pulsing at the S2 observation well. Peak methane values are shown to increase from below detection to approximately 1mg/l, as a result of the longer pulse duration. Peak methane concentrations are noted to occur when minimum DO concentrations are observed, which is anticipated based on transport theory.

Long pulse cycles were continued throughout the biostimulation and biodegradation experiments, with durations ranging from 6 hrs to 12 hrs. Based on continued methane breakthrough at the observation wells, the pulsing is believed to have helped to distribute the microbial population in the test zone and prevented biofouling of the aquifer.

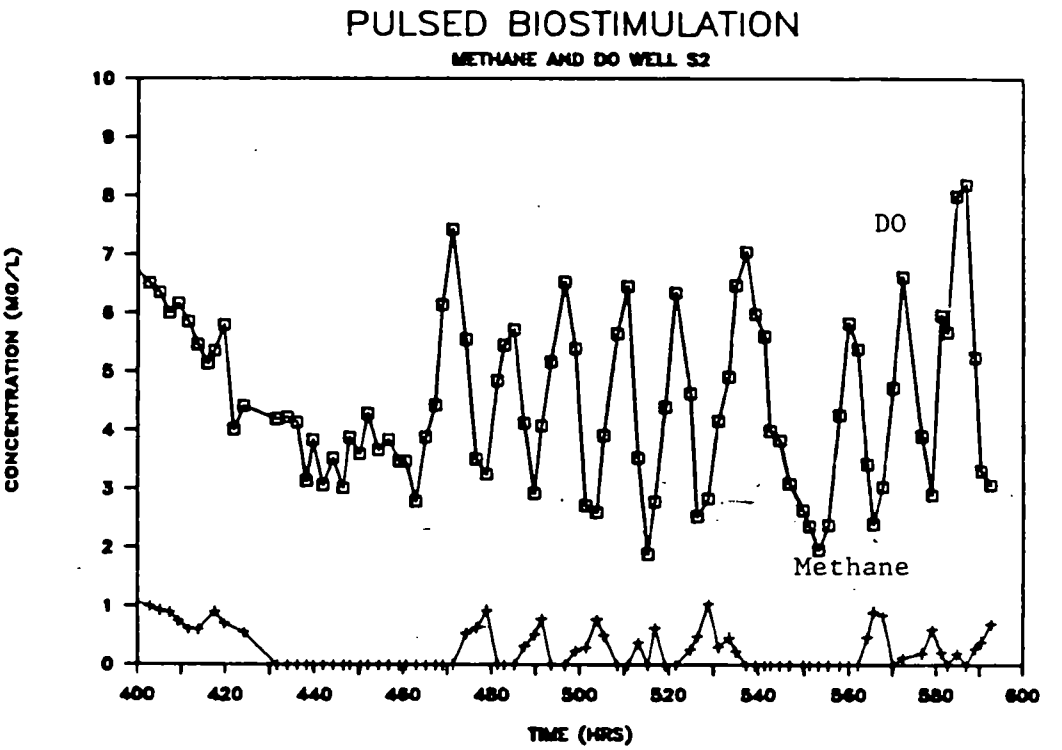


Figure 20. The effect of long term pulsing of DO and methane on the response at the S2 observation well.

The biostimulation experiment demonstrated that methane-oxidizing bacteria could be successfully established in the test zone. No additional nutrients were required to stimulate growth. The transient methane and DO responses indicated that a population was stimulated which grew closer to the injection well with time. The response indicates that microorganisms have fairly rapid growth kinetics, typical of aerobic organisms. Thus, pulsing was required to distribute the growth in the test zone and to prevent biofouling of the aquifer.

BIOTRANSFORMATION EXPERIMENTS

Biotransformation experiments were performed after the test zone was biostimulated. TCE was continuously injected over a three month period. During the initial stages TCE was injected at an average concentration of 100 ug/l. During the later stages, the concentration was lowered to 60 ug/l. Methane and oxygen (no nutrients) were continuously pulse-injected during this period to support the methane-oxidizing microorganisms which had been biostimulated.

During the initial phase of the experiment, the TCE concentrations slowly approached steady-state values. The normalized breakthroughs are presented in Figure 21. The response at the S1 well is very similar to that observed in the Tracer5 experiment, shown in Figure 11. Both experiments show fractional breakthroughs of approximately 60 percent of the injection concentration after 100-150 hrs of injection. The similar response indicates that little degradation of TCE was occurring in the biostimulated zone, within 1 meter from the injection well. This is further supported by the long term steady-state values of 80 percent of the injected concentration that are obtained after 400 hrs of injection.

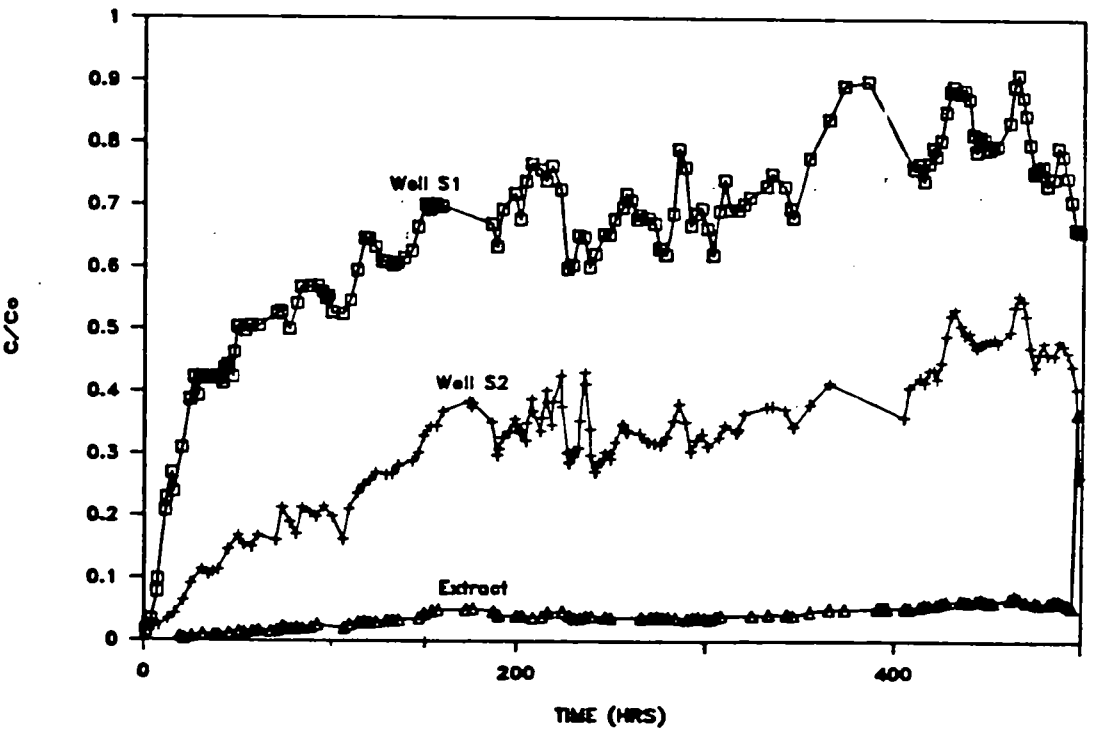


Figure 21. Normalized breakthrough of TCE at observation locations during the initial stage of the biotransformation experiment.

The breakthrough at the S2 observation well indicates that some degradation was occurring during the early stages of TCE addition. In the pseudo control experiment (Tracer5), the TCE reached

approximately 30 percent of the injected concentration at the S2 well after 100 hrs of addition, while in the biostimulation experiment the concentration reached only 20 percent. The early breakthrough results indicated that degradation may be as high as 30 percent.

Comparisons of mass balances of the amount injected and extracted in the two experiments also suggests some degree of degradation was occurring during the early stages of the biodegradation experiment. In the Tracer5 experiment, 2.24 g of TCE were injected over a 338 hrs period, of which 0.9 g were recovered over the same period, representing a recovery of 39 percent at the extraction well. In the biotransformation experiment, 2.09 g of TCE were injected over a 347 hr period, of which 0.53 g were recovered in the extracted water, representing a recovery of 25 percent. The 11 percent lower recovery, or 35 percent on a relative basis, indicates that partial degradation occurred.

Toward the end of the biotransformation experiment, the TCE injection concentration was lowered from 100 to 60 ug/l, to ensure that no sorptive losses of TCE onto the aquifer solids would occur and that maximum steady-state concentrations were being achieved. This permitted an estimation of the degree of degradation based on the steady-state concentrations of TCE compared to bromide, a non-reactive conservative tracer.

Figure 22 shows bromide tracer results during a period when TCE concentrations were at steady-state (Figure 23). The fractional breakthroughs of bromide are shown to be significantly higher than those obtained by TCE. This comparison indicates that the lower normalized concentration of TCE results from degradation.

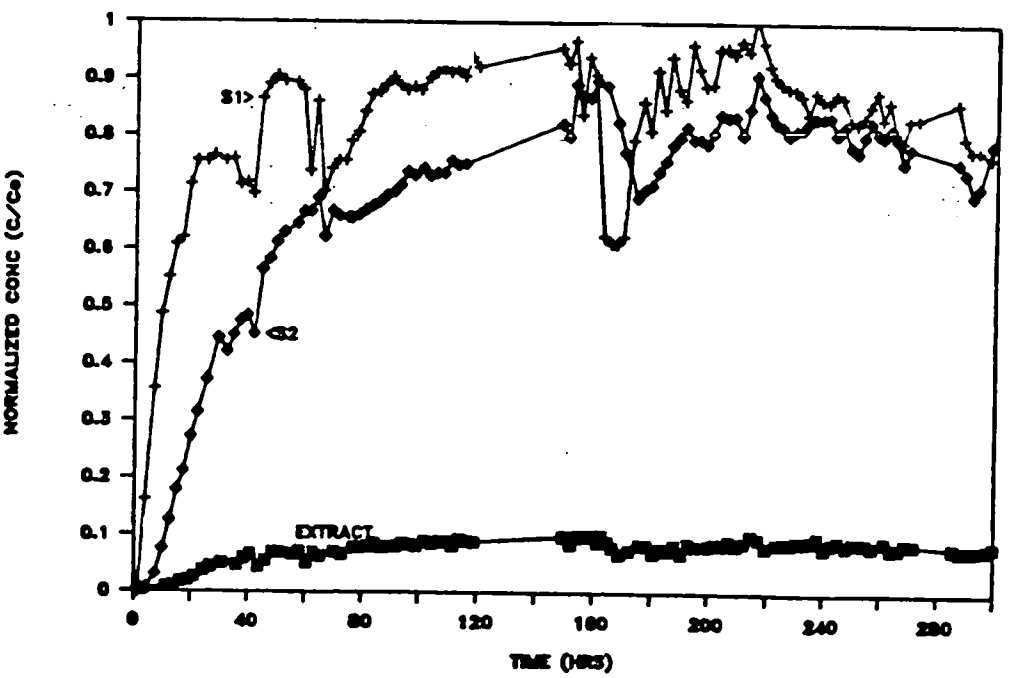


Figure 22. Normalized bromide tracer response during a steady-state period of the TCE biotransformation experiment.

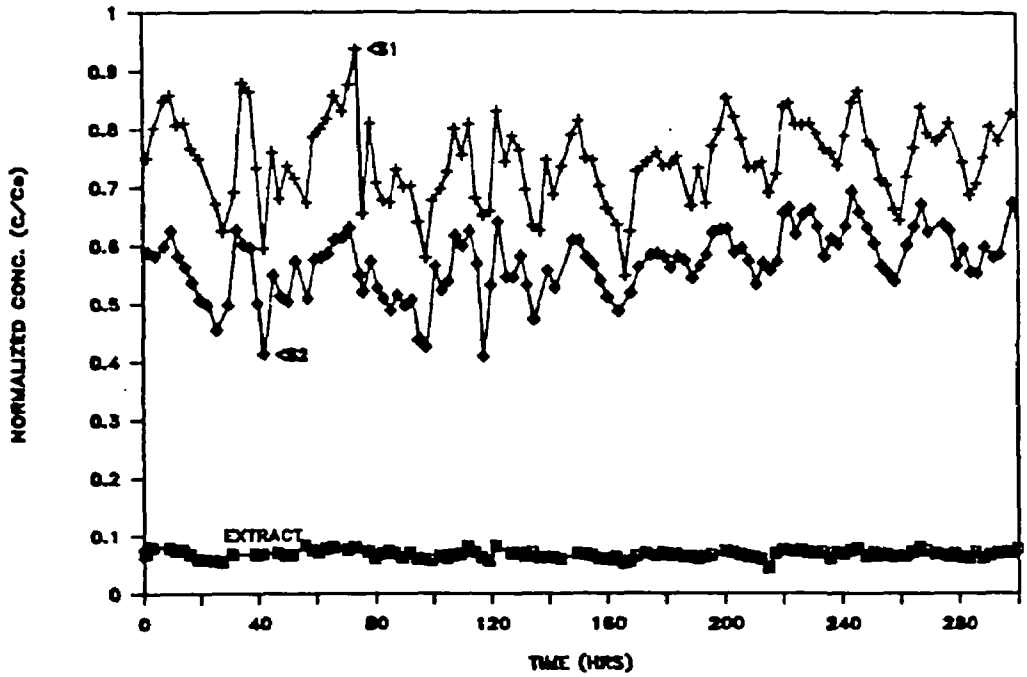


Figure 23. Steady-state TCE concentrations corresponding to the same time period as the bromide data in Figure 22.

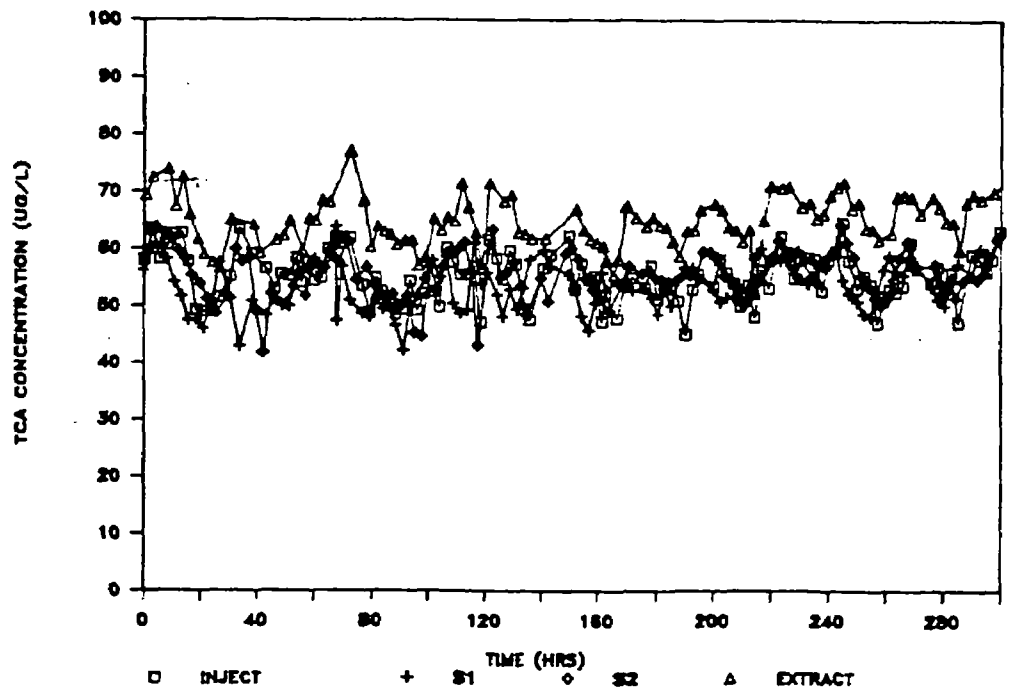


Figure 24. Steady state TCA concentrations corresponding to the same time period as the bromide and TCE data in Figures 22 and 23.

The concentrations of TCA during this steady-state phase of the experiment are presented in Figure 24, permitting comparison with the TCE results. TCA is present as a background contaminant, as indicated by the extraction well concentration which has an average concentration of 65 ug/l. The concentrations in the injected fluid and at the S1 and S2 monitoring wells are essentially equal with average values of 55.6, 53.3 and 55.3 ug/l, respectively. Little biotransformation of TCA occurred during transport through the test zone. After normalizing for the degree of mixing of the injected fluid with the native fluid, over 95% of the estimated TCA concentration was observed at the S1 and S2 monitoring locations.

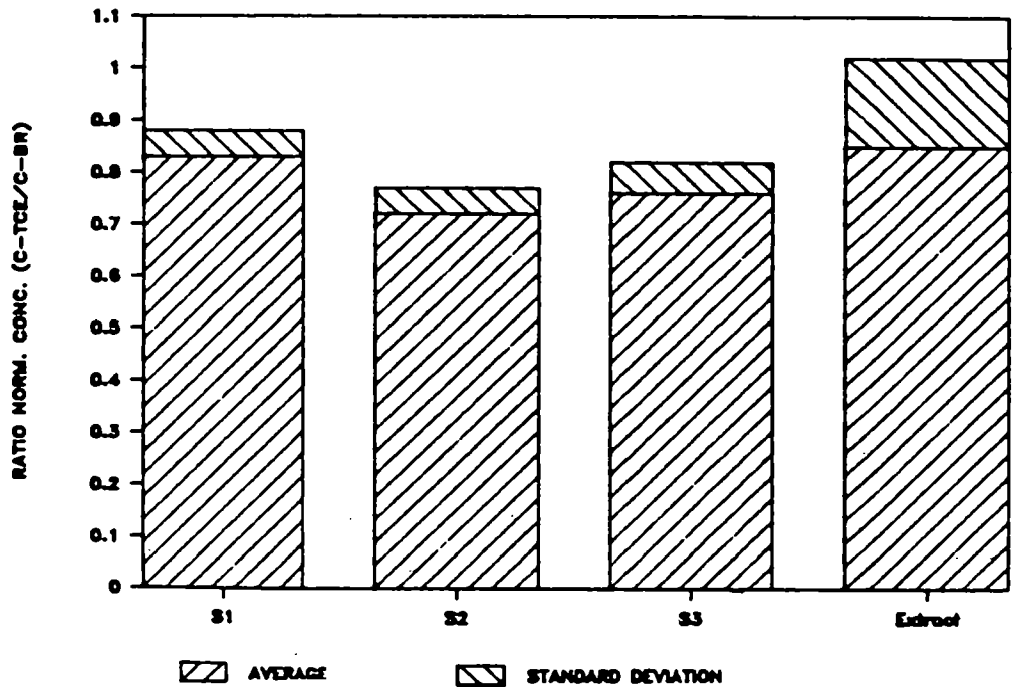


Figure 25. Estimated TCE biotransformation based on comparisons with bromide as a conservative tracer.

Figure 25 represents a summary of these experiments, where the fractional breakthroughs of TCE relative to bromide ion (TCE/Br) at the observation wells are compared. The ratios range from 70 percent to 80 percent, indicating a maximum degree of degradation of 30 percent. Degradation is noted to occur in the area of the test zone in which methane is present to support the methane-oxidizing bacteria. In the area between S2-S3 and the extraction well, no methane is present to support the bacteria, and accordingly no additional degradation of TCE is observed.

The degree of degradation indicated in this experiment is consistent with the earlier results, conducted approximately 100 days earlier. This suggests that the extent of degradation did not increase with time. Thus, acclimation to TCE, resulting in an enhancement in biodegradation, was not observed in the experimental period of 100 days.

The different methods of assessing the degree of degradation -- including mass balances, comparison of TCE breakthrough concentrations with the pseudo control experiment, and comparisons with bromide concentrations at steady-state within an experiment -- yield similar estimates of the degree of TCE degradation in the test zone. The degree of degradation is in the range of 20 to 30%. The results demonstrate that, if sufficient care is taken in obtaining the experimental data, quantitative evidence of degradation can be obtained in a real aquifer situation.

Discussion

The degree of TCE transformation observed in the test zone during the first field season is less than complete, and indeed barely within our ability to reach quantitative conclusions. Several factors may have contributed to the limited degree of transformation:

1) low solubility of methane and oxygen in water limits the TCE-degrading microbial population which can be stimulated under saturated conditions.

2) methane and TCE compete for the methane monooxygenase enzyme, such that the presence of methane can inhibit TCE degradation.

3) the high degree of chlorine substitution in the TCE molecule leads to a relatively low rate of aerobic degradation, relative to the rate of methane utilization.

4) extended acclimation to TCE is required before degradation begins to occur at a rapid rate.

5) minor nutrients are required for effective degradation.

6) the bacteria that were stimulated were not of the type that effectively degrades TCE.

7) the sorption of TCE onto the aquifer solids affects the rate of transformation.

The first three factors appear to be most important, based on the results of other studies. In the experiments of Wilson and Wilson (1985) discussed earlier, an unsaturated medium was exposed to a constant atmosphere of about 0.6% methane. A constant flux of methane was available to develop a high microbial population of methane-oxidizing bacteria. The population, however, developed under conditions of low methane concentrations in solution due to the low partial pressure of methane in the gas phase. Thus, the competition between methane and TCE for the methane monooxygenase enzyme was limited. In these experiments, the high microbial population and the lack of inhibition may have resulted in the high degree of degradation achieved.

Based on several experimental studies, Wilson and White (1986) developed an empirical correlation which relates the degree of TCE transformation to the amount of methane consumed. Based on their correlation, a reduction in TCE concentration of 35 percent is predicted when 6 mg/l of methane is consumed, the amount present in the biostimulation experiments. The estimate is in good agreement with the field results and indicates that the degradation is limited by the amount of methane and oxygen which can be delivered under saturated conditions.

TCE may have slow degradation kinetics, owing to its high degree of substitution by chlorine. Studies of Fogel et al. (1986) and Henson et al. (1987) indicate increasingly rapid disappearance of chlorinated aliphatics with decreasing degree of chlorine substitution. Recently Vogel et al. (1987), and McCarty (1987) indicated that the rate of degradation under aerobic or anaerobic conditions is related to the degree of oxidation of the compound. Vogel et al. (1987) indicate that, based on the available data, the rate of oxidation is higher for compounds containing fewer chlorine atoms per carbon atom, while for anaerobic conditions the reverse is true. Thus vinyl chloride and DCE would be expected to degrade faster than TCE or PCE. Also, the lack of degradation of TCA is consistent with the laboratory results of Henson et al. (1987), as well as with the arguments of Vogel et al. (1987).

Insufficient data are available to determine if acclimation to TCE is required to achieve effective degradation. The test zone was not previously contaminated with TCE. The test zone was contaminated with TCA, however, for which no degradation was observed. The results indicate that the rate of degradation is more highly dependent on structure than acclimation. TCE degradation has been observed in the laboratory cultures isolated from sediments not exposed to TCE. Whether a previous exposure to the chemical of interest has an effect on the rate of transformation is not clear at the present time.

Not enough is currently known about the final three possibilities listed above to determine their relative importance. Research work is currently being performed in our laboratory and elsewhere to gain a better understanding of how importantly these factors may effect the rate of degradation of TCE by methane-utilizing mixed cultures of bacteria.

The results indicate that the degradation resulted from the stimulation of methane-utilizing bacteria, which promoted TCE transformation. Another possible explanation is that the TCE was degraded anaerobically through the creation of anaerobic microzones resulting from the decrease of DO concentration with biostimulation. However, anaerobic degradation is considered unlikely owing to the presence of high nitrate concentrations (50 mg/l) throughout the test zone during the biotransformation experiments. Bouwer and McCarty (1983, 1985) found 1,1,1-TCA to be degraded under methanogenic conditions but not under denitrification conditions. Based on theoretical considerations presented by Vogel et al. (1987) TCE should behave similarly with little degradation under denitrification conditions. If methanogenic conditions existed, transformation of 1,1,1-TCA as well as TCE should be observed (Vogel et al. 1987). There, however, was no evidence of biotransformation of 1,1,1- TCA in the field experiment.

ELUTION OF TCE FROM THE TEST ZONE

After the first phase of the biostimulation experiment was completed, TCE injection was terminated, and the TCE remaining in the test zone was eluted by continuing to extract at the same flow rate as before (8 l/min). Methane and oxygen addition was also stopped and the injection well was used as a monitoring location. Figure 26 presents the monitoring data over the 2400 hour period during which this purging operation was continued; the gradual decrease in TCE concentrations at the observation wells and in the extracted water is easily seen. During the elution period, 50 to 100 pore volumes of water passed through the test zone, resulting in concentration decreases by a factor of 5 to 20.

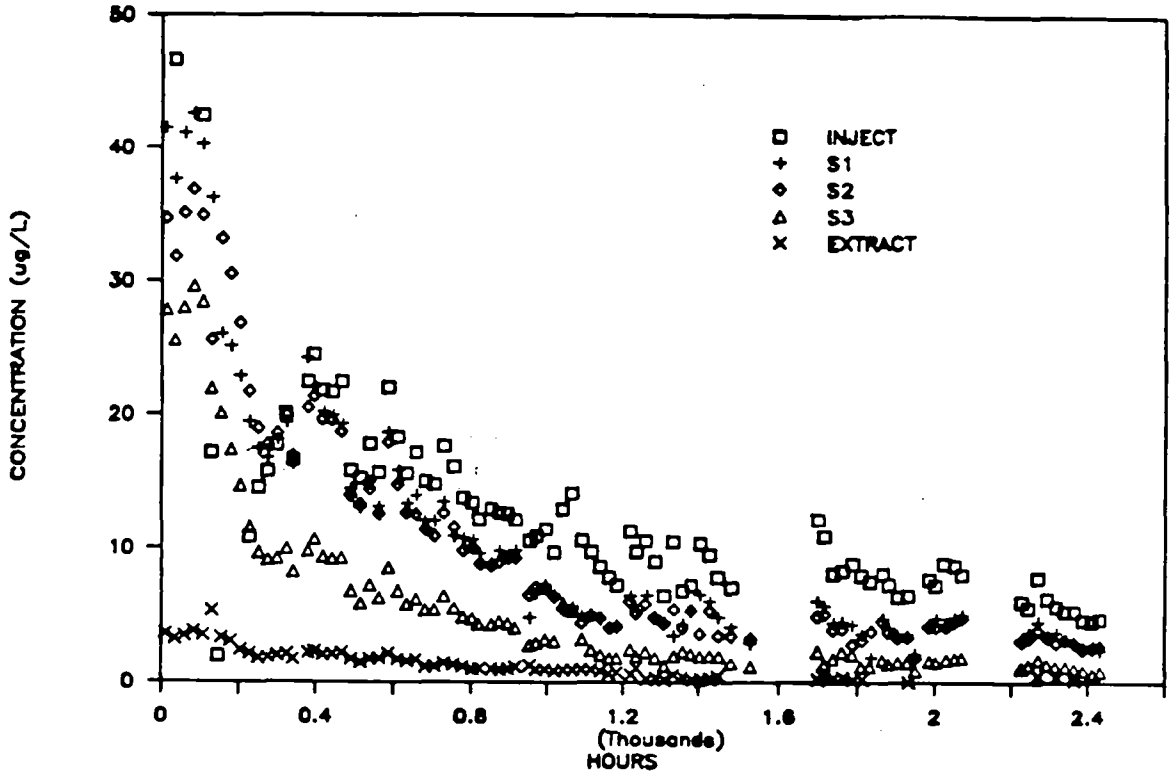


Figure 26. Elution of TCE from the test zone under induced flow conditions after stopping biostimulation and TCE addition.

A mass balance for TCE over the course of the TCE biostimulation experiment shows that of the total 10.1 g that were injected during the course of this experiment, 4.5 g were recovered in the water pumped from the extraction well, representing a recovery of 45 percent. During this same overall period, 65 percent of the bromide tracer was recovered. The lower recovery of TCE supports the conclusion that 25 to 30 percent of the injected TCE was degraded, consistent with our interpretation of the monitoring well data shown in Figure 25.

REFERENCES

1. Anthony, C., "The Prediction of Growth Yields in Methylotrophs", Journal of General Microbiology, 104:91-104, (1977).

2. Atwater, B.F, C.W. Hedel, and E.J. Helley, "Late Quaternary Depositional History, Holocene Sea-Level Changes, and Vertical Crustal Movement, Southern San Francisco Bay", California, U.S.G.S Prof. Pap. 1014, Washington D.C., (1977).

3. Bledsoe, B.E., (Personnel communication) Robert S. Kerr Environmental Research Laboratory, (1985).

4. Bouwer, E.J. and P.L. McCarty, "Transformations of Halogenated Organic Compounds Under Denitrification Conditions", Applied and Environmental Microbiology, 45:1295-1299 (1983).

5. Bouwer, E.J. and P.L. McCarty, "Utilization Rates of Trace Halogenated Organic Compounds in Acetate-Grown Biofilms", Biotechnology and Bioengineering, 27:1564-1571,(1985).

6. Bouwer, H. Groundwater Hydrology, McGraw-Hill Book Company, N.Y., (1978).

7. Canonie Environmental," Subsurface Hydrogeologic Investigation, Mountain View Facility", Project CES 82-023, June (1983).

8. Cooper, H.H. and C.E. Jacob, "A generalized graphical method for evaluating formation constants and summarizing well field history", Trans. Amer. Geophys. Union, 27:526-535, (1946).

9. Curtis, G.P., P.V. Roberts, and M. Reinhard, "A Natural Gradient Experiment on Solute Transport in a sand Aquifer: IV. Sorption of Organic Solutes and its influence on Mobility", Water Resour. Res., 22(13):2059-2067, (1986).

10. Curtis, G.P., M. Reinhard and P.V. Roberts, "Sorption of Hydrophobic Organic Compounds by Sediments", in: Geochemical Processes at Mineral Surfaces, J.A. Davis and K.F. Hayes (eds.) ACS Symposium Series 323:191-216, American Chemical Society, Washington, D.C. (1986).

11. Fogel, M. M., A. R. Taddeo, and S. Fogel, "Biodegradation of Chlorinated Ethenes by a Methane-Utilizing Mixed Culture", Applied and Environmental Microbiology, 51:720-724, (1986).

12. Freeze, R.A., and J.A. Cherry, Groundwater, Prentice-Hall, Englewood Cliffs, N.J., (1979).

13. Ghiorse, W. C. and D. L. Balkwill, "Enumeration and Morphological Characterization of Bacteria Indigenous to Subsurface Environments", Developments in Industrial Microbiology, 24:213-224, (1983).

14. Henson, J. M., M. V. Yates, and J. W. Cochran, "Metabolism of chlorinated aliphatic hydrocarbons by a mixed bacteria culture growing on methane", Abstr. Q-97, Annual Meeting Amer. Soc. Microbiol., Atlanta, GA. pp 298, (1987).

REFERENCES (continued)

15. Horvath, A.L. Halogenated Hydrocarbons Solubility-Miscibility with Water, Marcel Dekker Inc., N.Y., (1982).

16. Javandel, I., C. Doughty, and C. Tsang, Groundwater Transport: Handbook of Mathematical Models, Water Resources Monograph Series 10, Amer. Geophys. Union, Washington, D.C. (1984).

17. Karickhoff, S.W., D.S. Brown, and T.A. Scott, Sorption of hydrophobic pollutants on natural sediments, Water Res., 13:241-248, (1979).

18. McCarty, P.L., "Bioengineering issues related to in-situ remediation of contaminated soils and groundwater", in Proceedings: Reducing risks of environmental chemicals through biotechnology, University of Washington, Seattle, Washington, July 21, 1987.

19. Moltz, F.J., O. Güven, J.G. Melville, R.D. Crocker, and K.T. Matteson, "Performance, Analysis, and Simulation of a Two-Well Tracer Test at the Mobile Site", Water Resour. Res.,22(7):1031-1037, (1986).

20. Press, F. and R. Siever, Earth, Freeman & Co., San Francisco, (1974).

21. Raymond, R. L.,"Reclamation of Hydrocarbon Contaminated Groundwaters", United States Patent Office, Pat. No. 3,846,290 Nov. 5, 1974.

22. Roberts, P.V., M. N. Goltz, and D.M. Mackay, "A natural gradient experiment on solute transport in a sand aquifer: III. Retardation estimates and mass balances for organic solutes". Water Resour. Res., 22(13):2047-2058, (1986).

23. Schwarzenbach, R.P. and J. Westall, "Transport of non-polar organic compounds from surface water to groundwater", Environ. Sci. Technol.,15(11):1360-1367, (1981).

24. Walton, W.C., "Leaky artesian aquifer conditions in Illinois", Illinois State Water Surv. Rept. Invest. 39, (1960).

25. Webster, J.J, G.J. Hampton, J.T. Wilson, W.C. Ghiorse, and F. R. Leach, "Determination of Microbial cell numbers in subsurface samples", Groundwater 23(1):17-25, (1985).

26. Westrick, J. J., J. W. Mello, and R. F. Thomas, "The Groundwater Supply Survey," J. Amer. Water Works Assoc. 76(5):52-59, (1984).

27. Wilson. B.H., and M.V. White, (1986), "A fixed film bioreactor to treat trichloroethylene-laden water from interdiction wells", in: Proceedings of the Sixth National Symposium and Exposition on Aquifer Restoration and Groundwater Monitoring, Columbus, Ohio, National Water Well Association, May 19-22, 1986.

28. Wilson, J.T, S. Fogel, and P.V. Roberts, "Biological treatment of trichloroethylene in situ", In Proceedings: Symposium on groundwater contamination, ASCE National Convention, Atlantic City, N.J. April 27-30, 1987.

REFERENCES (continued)

29. Wilson, J.T., J.F. McNabb, D.L. Balkwill, and W.C. Ghiorse, "Enumeration and characterization of bacteria indigenous to a shallow water table aquifer", Groundwater, 21(2):134-141, (1983).

30. Wilson, J. T., and B. H. Wilson, "Biotransformation of Trichloroethylene in Soil," Applied and Environmental Microbiology, 29:242-243, (1985).

31. Valocchi, A.J. and P.V. Roberts, "Attenuation of groundwater contaminant pulses", Jour. Hydr. Eng. (ASCE), 109(12):1665-1682, (1983).

32. van Genuchten, M.Th., "Non-equilibrium transport parameters from miscible displacement experiments", Res. Rep. 119, U.S. Salinity Lab., Riverside, Calif. (1981).

33. Vogel, T. M., C. S. Criddle, and P. L. McCarty, "Transformations of halogenated aliphatic compounds", Env. Sci. & Tech., 21(8):722-736, (1987).