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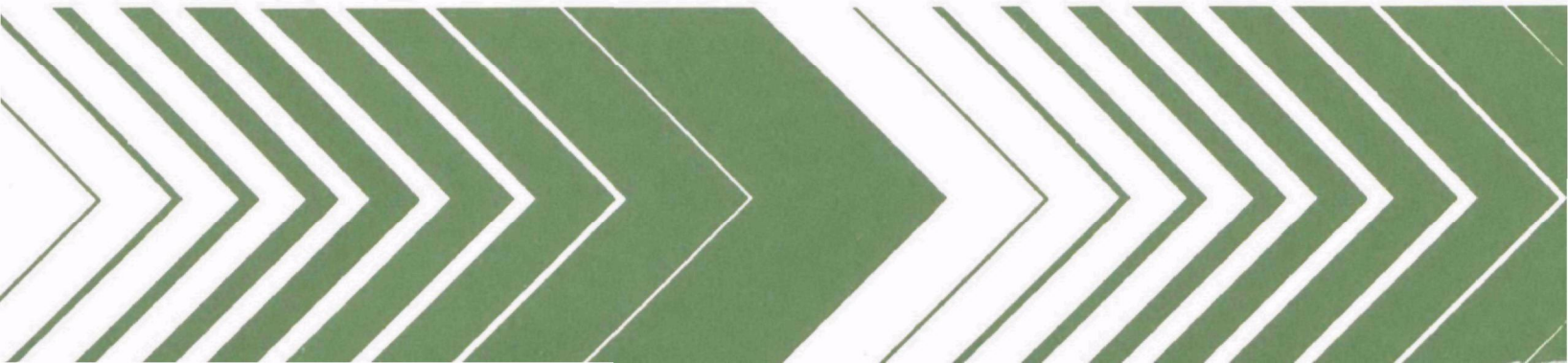
In-Situ Biotransformation of Carbon Tetrachloride under Anoxic Conditions



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IN-SITU BIOTRANSFORMATION OF CARBON TETRACHLORIDE UNDER ANOXIC CONDITIONS

by

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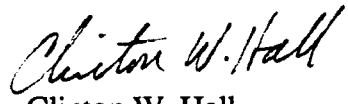
FOREWORD

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 describes research conducted to develop, evaluate, and demonstrate the efficacy of enhanced biotransformation of chlorinated organic contaminants for in-situ aquifer remediation. The research assesses biostimulation under denitrifying conditions as a means of transforming carbon tetrachloride, which is widely encountered as ground water pollutants.



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ABSTRACT

The results of this project showed convincingly that carbon tetrachloride (CT) was transformed to a significant extent and at a rapid rate under subsurface conditions in the absence of dissolved oxygen, when a natural bacterial population was biostimulated by the addition of acetate in the absence of dissolved oxygen and the presence of nitrate. The transformation of CT, which was introduced in a known quantity as the targeted contaminant, was demonstrated in both controlled experiments conducted in the laboratory and in injection-response experiments carried out in a shallow, confined aquifer at our Moffett test site. The CT did not degrade completely to harmless end products, however; chloroform (CF) appeared in significant quantity as an intermediate transformation product. The CF intermediate also transformed observably under the anoxic conditions created by biostimulation, but at a much slower rate than CT. Laboratory studies suggest that the other major product of CT transformation by an alternate pathway is carbon dioxide, although this could not be confirmed directly in the field experiment. Other halogenated organic compounds present as background contaminants in the Moffett aquifer also were transformed to a significant extent; these included 1,1,1-trichloroethane (TCA) and two chlorofluorocarbons (Freon-11 and Freon-113). With all of the organic compounds observed, the disappearance commenced some time after the beginning of active denitrification and the rate appeared to accelerate after the nitrate was depleted, suggesting that the transformation may have been mediated by a microbial subpopulation other than the active denitrifiers. The results of the laboratory and field experiments were for the most part consistent with one another, as well as with relevant previously published reports. The laboratory experiments, conducted with radiolabeled CT in semi-batch columns filled with solids from Moffett cores, confirmed the rapid course of denitrification and the subsequent onset of CT transformation, as well as the appearance of CF as an intermediate product accounting for roughly half the CT transformed. In addition, sorption equilibrium experiments were undertaken to quantify the extent of CT partitioning onto the Moffett solids, which was found to be relatively weak, but nonetheless sufficiently strong to affect transport significantly. A mathematical model was developed that accounted for the growth of the biostimulated community, the transformation of the target compound, the formation and subsequent transformation of the intermediate product, and the rate-limited partitioning of sorbing solutes, in the context of one-dimensional advective-dispersive transport in the aquifer. The mathematical model, which was implemented using independently determined parameters to the fullest possible extent, successfully reproduced the observed transient phenomena, i.e., the model simulations agreed acceptably with the transient and long-term concentrations observed in the field experiment.

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ACKNOWLEDGMENTS

The authors thank the personnel of the U.S. Navy, especially the Public Works Department at the Moffett Naval Air Station for allowing the Field Site to be located on their base. They have cooperated fully in helping us solve the many logistical problems associated with performing a field study of this type. Public works officer, John Heckman, has been especially helpful in this regard. We would also thank the staff at the Oakland Office of the California Regional Water Quality Control Board for permitting us to perform these experiments.

Members of the Kerr Laboratory of EPA have also provided input to the experimental design and the characterization of the test zone. John Wilson and Wayne Downs provided helpful technical information.

Graduate students who have made significant contributions to the study include Craig Criddle, Tom Harmon, and Sharon Just.

SECTION 1

INTRODUCTION

Chlorinated aliphatic C1 and C2 compounds are widely used as solvents, degreasing agents, and intermediates in chemical synthesis. Their widespread use has resulted in contamination of groundwater supplies (Westrick et al., 1984). The extent of the contamination has alarmed the public, legislators, and regulators. There is an urgent need to understand the behavior of the contaminants in the subsurface, to develop methods for monitoring the distribution and movement of the chemicals, and to clean up contamination once its extent is delineated. In-situ bioremediation of contamination by halogenated aliphatics is a promising alternative for aquifer restoration, since the process can lead to complete mineralization to non-toxic end products.

Our group at Stanford has assessed under field conditions the capacity of native organisms, i.e., bacteria indigenous to the subsurface environment, to metabolize halogenated synthetic organics, when the proper conditions are provided to enhance microbial growth. In this study, reducing conditions were promoted in the field by simulating a consortium of denitrifying bacteria, and perhaps sulfate-reducing bacteria, through the addition of acetate as a primary substrate for growth to the aquifer that contained both nitrate and sulfate. Under biostimulated conditions, the transformation of target compounds, including carbon tetrachloride (CT), 1,1,1-trichloroethane (TCA), Freon-11, and Freon-113, was assessed by controlled addition, frequent sampling, quantitative analysis, and mass-balance comparisons.

The field demonstration study was conducted at Moffett Naval Air Station, Mountain View, CA, with the support of the Robert S. Kerr Environmental Research Laboratory, through the Biosystems Program of the U.S. Environmental Protection Agency, and with the cooperation of the U.S. Navy. To provide guidance for the field work, laboratory studies were also performed to obtain a more basic understanding of key microbial and physical processes. This report summarizes the results of both the field study and associated laboratory studies.

BACKGROUND

Studies have shown that chlorinated aliphatic compounds can be biologically transformed under a range of environmental conditions (Vogel et al., 1987). An important factor influencing the biological transformations is the electron acceptor available to the microorganisms for deriving energy from oxidation of the electron donor (Vogel et al., 1987; Bouwer and McCarty, 1985; Vogel and McCarty, 1985; Vogel, 1988). Some halogenated compounds have been shown to be transformed under anaerobic conditions but to persist under aerobic conditions, while the reverse is true for other compounds. Vogel et al. (1987), in reviewing chemical, biological, and enzymatic studies, reported some clear trends between the ease of aerobic or anaerobic transformation and the oxidation state and chemical structure of the chlorinated aliphatics. Compounds that are more highly substituted with halides (more oxidized) are more likely to undergo reductive dehalogenation under anaerobic conditions, forming less halogenated intermediates. These less halogenated intermediates often are less reactive to subsequent reduction under anaerobic conditions. For

instance, Vogel and McCarty (1985) and Vogel (1988) found tetrachloroethylene (PCE) was transformed to trichloroethylene (TCE), dichloroethylene (DCE), vinyl chloride (VC), and carbon dioxide (CO_2) under methanogenic conditions. Vinyl chloride, as an intermediate, was the most persistent of the compounds under anaerobic conditions. However, VC can be rapidly degraded under aerobic conditions (Hartsmans et al., 1985; Fogel et al., 1986).

Previous Field Demonstration of Methanotrophic Biotransformation

The development of methods for in-situ treatment of halogenated aliphatics depends on the ability to promote the proper environmental conditions to enhance microbial biodegradation. In our previous field evaluation, the enhanced aerobic in-situ biodegradation of chlorinated aliphatics was studied; the process tested relied on the ability of methanotrophic bacteria to initiate the oxidation of chlorinated aliphatics. A two-meter-long test zone in a shallow confined aquifer was biostimulated through the addition of groundwater containing methane as the electron donor and oxygen as the electron acceptor. The extent of transformation in that biostimulated test zone was as follows: vinyl chloride > 95%; trans-DCE > 90%; cis-DCE > 40%; and TCE > 20-30%. These experiments demonstrated that enhanced in-situ biotransformation of chlorinated aliphatic compounds can be promoted when the proper conditions are applied in the subsurface. Based on the results of these field and laboratory studies, it is apparent that more highly chlorinated organics, such as CT and tetrachloroethylene are not amenable to aerobic transformations, but are more likely to be transformed under anaerobic conditions. Hence this study was undertaken to evaluate CT transformation under anoxic conditions.

Biotransformation of Halogenated Aliphatics under Anoxic Conditions

The occurrence of reductive transformation of halogenated aliphatic compounds in groundwater was first demonstrated in 1981 (Bouwer et al., 1981). Since then, several investigations have elucidated this process, with respect to the environmental conditions required and the transformation products to be expected. In general, anaerobic transformations of halogenated alkanes and alkenes lead to the production of a wide variety of transformations to less halogenated products (Bouwer and McCarty, 1983a,b; Gossett, 1985; Parsons and Barrio-Lage, 1985; Vogel and McCarty, 1985, 1987; Barrio-Lage et al., 1986; Belay and Daniels, 1987). Rates of transformation are generally faster under the more reducing anaerobic conditions in which methane is formed. Methane-producing bacteria are implicated in many of these transformations (Belay and Daniels, 1987), but other anaerobic bacteria can participate as well.

Anaerobic transformations of halogenated solvents follow the pathways illustrated in Figure 1.1. Compounds such as PCE and TCE are sequentially reduced to form 1,2-dichloroethylene (both cis- and trans-isomers) and vinyl chloride. Vinyl chloride can be transformed anaerobically, but the rate is slow. Another common solvent, TCA, can be transformed abiotically into 1,1-dichloroethene and acetic acid (Vogel and McCarty, 1987). TCA can also be reduced biologically to 1,1-dichloroethane (1,1-DCA), and then into chloroethane. However, 1,1-DCA is relatively stable and the rate of transformation into chloroethane is slow. Carbon tetrachloride is shown to undergo successive reduction with chloroform ($CHCl_3$) formed as one transformation intermediate.

Anoxic Biotransformation of Carbon Tetrachloride

Of the highly substituted chlorinated compounds shown in Figure 1.1, CT has been observed to be transformed under reducing conditions ranging from denitrifying to methanogenic. Thus, CT was considered an ideal compound to perform the initial evaluations of enhanced in-situ biotransformation under a range of anoxic conditions.

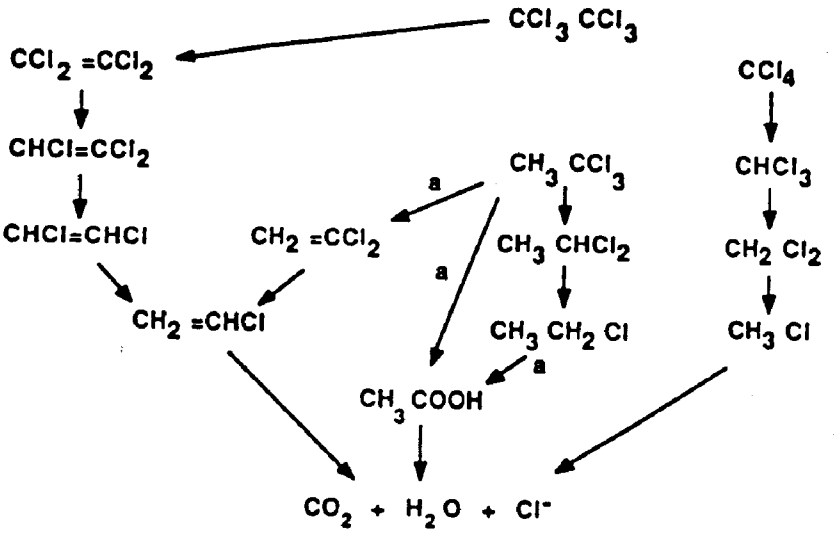

Figure 1.1. Anaerobic transformation pathways for selected chlorinated aliphatic compounds (after Vogel et al., 1987, and McCarty, 1988). Arrows with "a" indicate abiotic transformations; other arrows represent biotic transformations

Evaluation of in-situ biotransformation of CT under denitrifying conditions is of interest for several reasons. Denitrifying conditions represent an environment that is not strongly reducing. Promoting transformations under more highly reducing conditions of sulfate reduction or methanogenesis may be more objectionable in potential drinking-water aquifers due to the dissolution of iron and manganese and the production of sulfides and odorous compounds. Denitrifying conditions might be easier to promote, since denitrifiers grow more rapidly and are probably more ubiquitous in nature than sulfate-reducing or methanogenic bacteria.

The transformation of several chlorinated aliphatics has been observed under denitrifying conditions. Bouwer and McCarty (1983b) observed the biotransformation of CT and brominated trihalomethanes in batch-fed mixed denitrifying cultures. Carbon tetrachloride was assimilated into cell mass and mineralized to CO_2. Chloroform was detected as a transformation intermediate, indicating that reductive dehalogenation, at least to some extent, was occurring under these conditions. Bouwer and Wright (1988) studied the transformation of CT and several other chlorinated aliphatics under conditions of denitrification, sulfate reduction, and methanogenesis, in anoxic biofilms. All compounds studied were transformed under methanogenic conditions. Bromoform, bromodichloromethane, CT, and hexachloroethane were transformed under the less reducing conditions of denitrification. Over 99% of the CT was biotransformed by the biofilm being supported on 30 mg/l acetate, and 83 mg/l $NaNO_3$. $^{14}CCl_4$ studies showed under conditions of denitrification that 41% of the labeled CT was mineralized to CO_2, 14% was transformed to $CHCl_3$, and 45% was converted to an unknown non-volatile product. The proportion of reaction products differed greatly for the different electron acceptors used. More CT was mineralized to $^{14}CO_2$ and less was converted to chloroform ($CHCl_3$) under denitrifying conditions compared to sulfate-reducing or methanogenic conditions. Thus, denitrifying conditions may be more beneficial for in-situ restoration of CT contamination since more complete mineralization is expected.

In a recent continuous-flow column study, Rittmann et al. (1988) investigated the biotransformation of CT and several other halogenated organics under denitrifying conditions. Several biologically active zones were created through the addition of nitrate at several locations along the column. Carbon tetrachloride was nearly completely transformed, while bromoform,

dibromomethane, trichloroethylene, and tetrachloroethene were transformed to lesser degrees. The observed steady-state removals of acetate, nitrate, and the chlorinated aliphatics, as secondary substrates, were found to agree closely with simulations using a computer model that couples the processes of one-dimensional solute transport and steady-state biofilm kinetics.

Little is known about the bacteria or other microorganisms that degrade CT in anaerobic environments. Several pure culture studies have been reported. Egli et al. (1987; 1988) found pure cultures transformed CCl_4 to $CHCl_3$ and CH_2Cl_2 quantitatively, under both sulfate-reducing and methane-producing conditions. They also found an acetogen that converted 92% of the $^{14}CCl_4$ to nonhalogenated products. One of the denitrifying cultures tested did not degrade CT. Organisms that possess acetyl coenzyme A (acetyl-CoA pathway) were able to transform CT, while those lacking the enzyme could not carry out the transformation.

Galli and McCarty (1989) identified a *Clostridium* sp. isolated from an anaerobic methanogenic reactor that was able to biotransform 1,1,1-TCA, chloroform, and CT. The transformation of CT led to the production of $CHCl_3$ as an intermediate, with further transformation to dichloromethane and unidentified products. Their studies indicate that the *Clostridium*, which can live on protein of decaying microorganisms, can bring about the transformation of CT. They indicated that *Clostridium* sp., which are ubiquitous in the environment due to their ability to produce endospores, may play an important role in dehalogenation of chlorinated compounds. It is possible for microorganisms, such as *Clostridium*, that can live on the decay products of stimulated denitrifiers, to grow and participate in the transformation of CT.

Criddle (1989) reported the isolation of a pure denitrifying culture, *Pseudomonas* sp. strain KC, that was capable of degrading CT. The microorganism was isolated from aquifer material taken from Orange County Water District Well #6. The pure culture transformed approximately 50% of the CT to carbon dioxide and about 40% to non-volatile compounds. A minimal amount of chloroform product was observed.

Parallel pathways for the transformation of CT and 1,1,1-TCA appear to exist for the reductive dehalogenation of compounds with three or more halogens substituted per carbon atom (Criddle, 1989). Parallel pathways for the degradation of CT, CF, and TCA under anaerobic conditions are presented in the simplified overview shown in Figure 1.2. The percentages indicate the range of conversions for each of the different pathways (Criddle, 1989). Figure 1.2 indicates that the extent of complete mineralization to carbon dioxide is high, ranging from 10 to 99% of the CT transformed. An important component of this work was to determine the extent to which chloroform was produced as a transformation product.

Biotransformation of Halogenated Organics

Recent research on the transformation of halogenated aliphatics has substantially advanced our understanding of the transformation processes and factors affecting transformation, including redox conditions, substrate concentration, microbial mass, temperature, and degradation by-products. McCarty (1988) reviewed these findings and discussed their significance in relation to in-situ treatment.

There is no evidence that demonstrates that microbes can live on highly chlorinated aliphatics as primary substrates for growth, but then again, there is no definitive evidence that excludes this possibility. In this work, the transformation process will be treated as a biological process under which CT can be transformed to various products via parallel transformation pathways, based on the pure culture studies that have been performed.

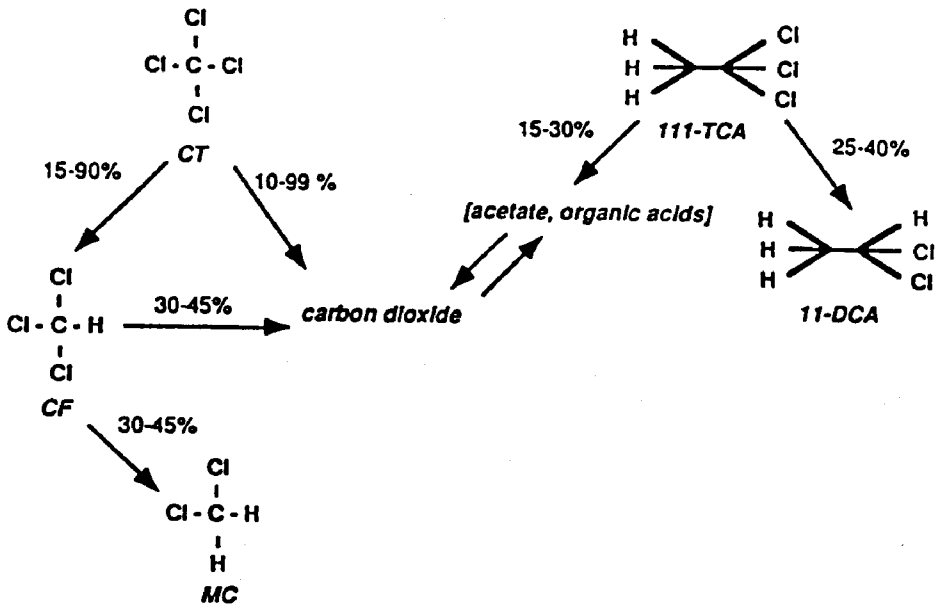

Figure 1.2. Parallel pathways for CT, CF, and TCA transformation (from Criddle, 1989).

Models have been developed to describe the transformation of the halogenated aliphatics by microbial processes. One of the simplest expressions used when the contaminant concentration is low is given by equation (1-1) (McCarty, 1984):

$$-\frac{dS}{dt} = \left(\frac{k}{K_S}\right)XS \qquad (1\text{-}1)$$

where S is the contaminant concentration, X is the microorganism concentration, and k/K_S is a ratio that is equivalent to a second-order rate constant. The k value ($time^{-1}$) represents the maximum specific substrate utilization rate per unit mass of microorganisms per unit time, and K_S is the half-velocity constant (mass/volume) which represents the organism's affinity for the substrate. At low substrate concentrations, the rate of reaction is dependent on both the concentration of microorganisms present and the concentration of the contaminant.

The rate of transformation is shown to be directly related to the microbial mass present. Thus, by increasing the microbial mass present, we can increase the rate of transformation. In-situ biotransformation of halogenated organics as secondary substrates relies on the enhancement of a specific microbial population (biostimulation) in the subsurface in order to increase transformation rates. Biostimulation is accomplished by supplying the appropriate electron donor (primary substrate for growth) and electron acceptor to the in-situ treatment zone to increase the specific microbial concentration (X), and thus increase the rate of biodegradation of the target compound(s).

As shown in equation (1-1), the rate of biotransformation also depends on the ratio of k/K_S. Values of k/K_S for the contaminant can be compared to those for the primary substrate. Favorable rates of transformation are achieved when k/K_S values are in the range of that of the primary substrate. Values of k/K_S were reported by Bouwer and McCarty (1985) for several halogenated organics transformed under methanogenic conditions, where acetate was used as the primary substrate for microbial growth. Rates were compound-dependent, with CT having a k/K_S ratio a

factor of three greater than the primary substrate, while the k/K_S value for tetrachloroethylene was a factor of 8 lower.

Limited data are available on the rates of transformation of CT under denitrifying conditions. In denitrifying column experiments, Bouwer and Wright (1988) found k/K_S values for CT of 0.36 L mg^{-1} d^{-1} compared to 1.4 L mg^{-1} d^{-1} for acetate, the primary substrate. This represents a fairly high rate of transformation of CT.

ABIOTIC PROCESSES

Abiotic Reactions

Carbon tetrachloride can also be transformed abiotically. Criddle (1989) reduced CT to CF in an electrolysis cell. As the cell conditions were made more reducing, CF was transformed to dichloromethane. In these experiments carbon monoxide and formate were also observed as transformation products, demonstrating parallel pathways for CT transformation by hydrolytic reduction and hydrogenolysis.

Recent CT abiotic transformation studies have been performed by Reinhard et al. (1990), Kriegman and Reinhard (in press), and Curtis (1990) for mineral systems representing groundwater environments. In studies of iron sulfide systems Kriegman and Reinhard (in press) found the rate of CT transformation at 50°C was two orders of magnitude faster in a heterogeneous system containing pyrite or marcasite than in a homogeneous solution containing ferrous iron or sulfide. They indicate that the reaction between haloaliphatics and environmental reductants is enhanced at mineral surfaces, and that rates of CT transformation could be significant on the scale of groundwater transport rates.

Curtis (1990) found that CT was transformed faster by solutions of Fe^{2+} or HS^- in the presence of humic acids, compared with solutions where humic acids were absent. Chloroform was detected as a product of CT transformation, but not equal to the amount of CT that disappeared. CT was also observed to be transformed by solutions of Fe^{2+} and HS^- in the presence of hematin, which served as a model compound for iron prophyrins.

These recent abiotic studies have indicated the difficulty in complex natural systems of distinguishing between biological and nonbiological reactions. Microbes can create the environmental conditions that produce reduced iron and/or HS^-, which might interact with humics to produce an agent capable of reducing CT (Curtis, 1990). This agent might act together with direct microbial processes in the transformation of CT. Thus, the microbial process studied here might act directly via biological processes or indirectly through abiotic processes in enhancing the transformation of CT.

Sorption and Its Effect on In-Situ Biotransformation

Carbon tetrachloride is a moderately hydrophobic compound, having a log octanol water partition coefficient (log K_{ow}) of 2.7 (Leo et al., 1971). Carbon tetrachloride is therefore likely to sorb appreciably onto the aquifer solids. In a natural gradient experiment conducted at Borden, Canada, CT transport was found to be retarded due to sorption onto the aquifer solids. Retardation factors ranged from 1.8 to 2.5 and were observed to increase with time, indicating that the sorption-desorption process may be kinetically controlled (Roberts et al., 1986). Significant sorption was observed at the Borden test site even though the solids organic carbon content was very low, 0.02% (Curtis et al., 1986). In contaminated aquifers with solids of higher organic

carbon content, CT is expected to sorb more strongly. This may create problems with the commonly used pump-and-treat method for aquifer restoration. Many pore volumes of water may be required to remove the CT sorbed onto the aquifer solids. The volumes required become even greater if CT is slowly desorbed from the aquifer solids. Model simulations by Valocchi (1986), using analytical solutions for a rate-limited desorption process, show that if desorption becomes rate limiting, the time required to reduce chemical concentrations is significantly increased with the pump-and-treat method.

In-situ biotransformation of CT may significantly reduce the time required for clean-up by increasing the driving force for removal of the sorbed CT from the aquifer solids. This would be accomplished by reducing the aqueous-phase concentrations over the treatment zone. As described by Rittmann et al. (1988), in-situ treatment might also permit successive reaction zones of a biostimulated population to be created in a contaminated aquifer, where groundwater is forced to flow through the zones, and CT is degraded. This could also reduce the time for aquifer restoration and greatly reduce the amount of water that must be treated at the surface.

OBJECTIVES

The overall objective of this work is to assess the feasibility of enhanced in-situ biodegradation of carbon tetrachloride (CT) under anoxic conditions. The specific objectives are to:

1) demonstrate in a controlled field experiment the ability to biostimulate an indigenous population of denitrifying bacteria under conditions representative of groundwater environments.

2) quantify the extent of enhanced biodegradation of CT, 1,1,1-TCA, Freon-11, and Freon-113, in the biostimulated zone, and identify intermediate products.

3) determine how to modify biostimulation conditions to achieve more complete mineralization of the halogenated aliphatics.

4) evaluate the treatment process in appropriate laboratory studies and compare these results with those obtained in the field test.

5) use mathematical models that incorporate key microbial and transport processes for interpreting the results of laboratory and field experiments.

In order to meet these objectives a combined field, laboratory, and modeling study was performed over a one-year period. The field study focuses on objectives 1,2 and 3, while the laboratory study focuses on objective 4. The modeling effort of objective 5 facilitated synthesis of the field and laboratory results.

REPORT ORGANIZATION

In keeping with EPA's required format, overviews of the report's contents and findings are provided in the Executive Summary (preceding the Table of Contents), and in the Summary and Conclusions (Section 2) and the Recommendations chapter (Section 3).

Section 4 presents the results of the laboratory microcosm studies using aquifer solids from the Moffett test zone. Studies presented here permit a direct comparison with results from the field experiments.

Section 5 presents the experimental methodology of the field experiments that was developed to provide a convincing and objective demonstration of the reductive transformation. The performance of the Automated Data Acquisition and Control System used to continuously monitor the field experiments, along with the injection system used to add controlled amounts of the compounds of interest, is reported here. The geologic, hydrogeologic, chemical, and microbiological characteristics of the field site are also summarized. Results of the field evaluation, comprised of the initial tracer tests and the biostimulation and biotransformation experiments,are presented in Section 6, which presents the principal findings of our field evaluations.

The mathematical simulation of the transport, biostimulation, and biotransformation observed in the field demonstration are presented in Section 7, along with the development of the mathematical models employed. Rate coefficients for CT transformation estimated based on the simulations are compared with those reported in the literature.

SECTION 2

SUMMARY AND CONCLUSIONS

The results of this project show convincingly that carbon tetrachloride (CT) was transformed to a significant extent and at a rapid rate under subsurface conditions in the absence of dissolved oxygen, when a natural bacterial population was biostimulated by the addition of acetate in the absence of dissolved oxygen and the presence of nitrate. The transformation of CT, which was introduced in known quantity as the targeted contaminant, was demonstrated in both controlled experiments conducted in the laboratory and in injection-response experiments carried out in a shallow, confined aquifer at our Moffett test site.

The principal laboratory experiments were conducted with ^{14}C-labeled CT in semi-batch columns filled with Moffett core material. The semi-batch protocol entailed exchanging the pore water on a regular basis, at a daily-to-weekly frequency. Denitrification commenced rapidly following biostimulation with either acetate, glucose, or ethanol. Both the nitrate and the organic substrate were utilized fully with exchange periods as short as one day. In the preliminary column studies, CT transformation began after approximately 30 days in the columns receiving acetate and ethanol, and more slowly in the column fed with glucose. Acetate was chosen for the subsequent experiments in the laboratory and the field, based on it being a less toxic compound to add to groundwater aquifers compared to ethanol.

Upon restimulation with acetate and nitrate following a hiatus of one year, biological activity commenced in the columns almost immediately, again showing full utilization of nitrate and acetate after the first exchange. CT transformation began immediately, ultimately reaching 40-60% conversion to CO_2. Chloroform (CF) appeared as a transformation product, to the extent of 30-40% of the CT fed; the data suggest that the CF transforms under anoxic conditions, but much more slowly than CT. No other halogenated products have been confirmed. With the information presently available, we cannot determine with certainty whether the CT transformation was accomplished by actively denitrifying bacteria or by other groups of microorganisms. The literature and experimental results here strongly suggest that transformation resulted from secondary organisms in the process and not denitrifiers *per se*. Further studies now underway aim to shed more light on that issue, but the question is complicated severely by the sequential progression of redox conditions during and following denitrification. This limitation notwithstanding, the semi-batch column methodology has again proven useful as a means of assessing the effect of biostimulation on facilitating transformation of targeted chlorinated organic compounds under controlled laboratory conditions, especially by permitting the use of radiolabeled compounds to assess the extent of complete degradation to CO_2.

In the field demonstration, the methodology proven to be effective in our earlier evaluation of aquifer restoration by biostimulation of a methanotrophic community was employed with minor modifications at the same site, located at Moffett Naval Air Station. Only minor adjustments were necessary in the analytical scheme to permit the determination of the primary substrate, acetate, and to allow for the acetate feed. Ample nitrate (25 mg/l, as NO_3^-) was present in the native groundwater, so that none needed to be added. In the final stages of the biostimulation/biotransformation

experiments, a bioreactor was installed in the feed loop at the surface to deplete the nitrate in the recycled, extracted groundwater, to assess performance in the complete absence of nitrate as an electron acceptor in the test zone. The ability to utilize the same basic configuration for the injection, extraction, sampling, and analysis as in our previous EPA-sponsored project made it possible to complete an ambitious field experimental program within the allotted one-year project period.

The field experimental program was conducted in stages, beginning with transport experiments to characterize the mobility and recovery of bromide, nitrate, and CT. The transport experiments showed that the planned experiments could be conducted along the south leg of injection/monitoring wells at extraction and injection rates of 10 and 1.5 liters/min, respectively. Under those conditions, the fractional permeation of the injected fluid at the relevant monitoring wells exceeded 97%, and the tracer residence times were on the order of 8 to 24 hrs at the nearest monitoring wells, S1 (1.0 m) and S2 (2.2 m). CT retardation proved to be relatively small, corresponding to a retardation factor of 1.5 to 2.0 estimated from transport studies. Approximately 3 to 4% of the CT added was converted to CF in the tracer experiment. When the test zone had been saturated with CT (in equilibrium with an input concentration of ca. 0.040 mg/l), the biostimulation experiment commenced.

In the biostimulation experiment, acetate was first introduced at a time-averaged concentration of 25-46 mg/l, albeit in the form of short pulses (1 hr of a 13-hr cycle) of higher concentration (330-600 mg/l) to avoid well clogging. Acetate was added in an amount that assured a slight stoichiometric excess over the primary electron acceptor, nitrate. Nitrate utilization commenced immediately after the introduction of acetate, and was complete within 100 hrs, while acetate utilization also commenced immediately but was expressed somewhat more slowly and less completely because of the stoichiometric excess. The onset of CT transformation was observed after approximately 350 hrs, after which the CT concentrations at the monitoring wells gradually declined, more rapidly at the more distant (S2) well than at the nearer S1 well. With time, the CT concentration decline slowed down, reaching approximately 30% (S1) and 80% (S2), respectively, in the period between 1160 and 1260 hrs, and the conversion was virtually complete over the longer path to the extraction well. Chloroform (CF) appeared as an intermediate product of the CT transformation at all of the sampling points, in an amount corresponding to approximately one-half to two-thirds of the CT that disappeared. As time proceeded, the ratio of CF observed to CT transformed decreased, indicating that CF also was transformed, but much more slowly than CT.

The pattern of CT concentrations suggested that the CT transformation proceeded more rapidly in the more distant portions of the biostimulated zone, beyond the point of nitrate depletion. To test the hypothesis that the absence of nitrate would enhance the CT transformation, nitrate was removed from the recycled water prior to injection, beginning at 1260 hrs; the acetate input was lowered by slightly more than half, giving a time-averaged injection concentration of 12 mg/l. Following the cessation of the nitrate feed, the CT concentration declined abruptly over the period 1300-1580 hrs at both monitoring wells (S1 and S2). During this period without nitrate feed, the fractional yield of the CF by-product declined to about one-third, based on CT transformed. Substantial acetate utilization persisted in the absence of nitrate feed, suggesting that sulfate (present at 700 mg/l in the native groundwater) may have served as an electron acceptor; however, no sulfide was detected in groundwater samples. Methane was not detected in groundwater samples, indicating the absence of methanogenic conditions. No attempt was made to monitor for Fe or Mn as intermediate electron acceptors.

Background contaminants, including 1,1,1-TCA and two chlorofluorocarbons (Freon-11 and Freon-113) were also transformed under the influence of anoxic biostimulation, whereas this had not been noticed previously under aerobic conditions. Although steady-state conditions were not reached by the end of the biostimulation experiment, the following average transformations for

well S2 give an impression of the minimum degrees of conversion for the several compounds over the 2.2 m distance: CT, 95%; Freon-11, 68%; Freon-113, 20%, and TCA, 15%. An analysis using a simple first-order rate model for the transformation indicated that these compounds were being transformed by the same process, but at different rates.

A non-steady state model was developed for simulating the results of the field experiments, to evaluate our knowledge of the processes governing CT transformation and to identify processes that may still be poorly understood. The model accounts for the basic phenomena of microbial growth, electron donor and electron acceptor utilization, the biotransformation of the chlorinated aliphatic compounds, and the formation of intermediate by-products. The major processes affecting transport were taken into account: advection, dispersion, and rate-limited sorption. The model simulated the growth and metabolism of two microbial populations: a denitrifying population and a second assumed population that utilizes the respiration products of the denitrifiers. The transformation of CT and other halogenated organics was assumed to be governed by Monod kinetics. Model simulations indicated that the main population of denitrifying bacteria was not responsible for the transformation of CT. In order to match the field observations, the model simulations required that the secondary population, whose growth was inhibited by the presence of nitrate, be the main population that transformed CT and the other halogenated aliphatics. This approach adequately simulated the CT transformation and CF formation data, although some parameter adjustments were necessary. Rate constants were determined for CT, CF, TCA, Freon-11, and Freon-113 by fitting the model to the data using coefficients within ranges reported in the literature. The resulting values for the apparent specific first-order rate constants (in units of liters•(mg cells)$^{-1}$•day^{-1})were as follows: CT, 0.4; Freon-11, 0.16; CF, 0.08; Freon-113, 0.04; and TCA, 0.01. As in previous projects, the modeling again proved its value as a useful tool in improving and synthesizing understanding of the salient processes that proceed concurrently in the complex field setting. The modeling also serves to determine important directions for further laboratory research and to sharpen questions that must be addressed in future field and laboratory studies.

SECTION 3

RECOMMENDATIONS

Regarding the objective of remediating aquifers contaminated with CT by biostimulating under denitrifying conditions, the present investigation has revealed that the interplay of processes is more complex than previously supposed. The finding that the CT transformation partially proceeds through a slowly transformed intermediate, CF, that is objectionable from a water quality standpoint poses a significant obstacle to the immediate deployment of the approach investigated in this project. To circumvent this obstacle, further laboratory studies are needed to improve understanding of the relevant transformation process, and thus to provide a basis for model refinement. These studies should aim to elucidate 1) whether nitrate inhibits the growth of the particular population that mediates the CT transformation, and if so what is the appropriate model for the inhibition; 2) how the presence of nitrate inhibits the rate of CT transformation, and whether a pertinent inhibition model can be formulated; 3) whether there is a set of conditions more favorable to transformation preferentially through the pathway leading directly to CT mineralization to CO_2 or otherwise expediting transformation of CF and subsequent by-products all the way to stable, harmless end products; 4) the role of redox conditions, and whether biotransformation models need to be coupled with geochemical models; and 5) whether complex biofilm models need to be considered, including microbial speciation within the biofilm. With respect to transport-related issues, further research should be directed toward better understanding the influence of heterogeneity (geochemical as well as physical) on the distribution and transport of electron donor and electron acceptor species, and hence on the spatial distribution of microbial populations, redox conditions, and the resulting effects on transformation rates.

SECTION 4

BATCH-EXCHANGE SOIL-COLUMN STUDIES OF CT BIOTRANSFORMATION UNDER ANOXIC CONDITIONS

INTRODUCTION

Laboratory-scale columns containing aquifer material have been effectively used to study the potential of aquifer and soil organisms to biotransform halogenated organics (Wilson and Wilson, 1985; and Siegrist and McCarty, 1987). The objective of this study was to evaluate the use of laboratory-scale soil columns for estimating the important factors in the development of in-situ treatment processes. The significant questions to be answered when considering in-situ bioremediation include: 1) are native bacteria present in the aquifer that are capable of growing on the added primary substrate, 2) what is the period of time required to increase the population of bacteria to adequate levels, and 3) are the indigenous bacteria capable of transforming the contaminants of concern after growth has been stimulated by the addition of primary substrate? The target contaminant in this study was carbon tetrachloride, CT. The primary substrate (electron donor) investigated was acetate. The primary electron acceptor was nitrate. Sulfate might also serve as an electron acceptor following complete nitrate consumption in the columns. Assuming that contaminant transformation was achieved, it was the goal of this investigation to explore the effects of changes in the amount of primary substrate and electron acceptor available.

MATERIALS AND METHODS

Column Preparation

The aquifer solids were obtained in July 1986 from the Moffett Naval Air Station, Santa Clara Valley, California, as described in Roberts et al. (1989). In June 1988, five laboratory columns containing Moffett aquifer solids were prepared for this study as described by Siegrist and McCarty (1987). The column design is shown in Figure 4.1. The columns were initially used in a 3 month batch column experiment from July to September 1988 to determine whether denitrifying bacteria were present in the Moffett aquifer and whether these bacteria could degrade CT. The current batch column experiments began in August 1989. Between the current and previous experiments, the columns had been saturated with Moffett groundwater and stored for 11 months at room temperature in the dark.

Chemicals and Stock Solutions

The unlabeled CT stock used in the initial stage of the experiment was prepared by saturating Milli-Q water with excess CT (Aldrich Chemical Co., Milwaukee, WI). The stock was stored in a 20-ml vial with an open-top screw cap sealed with a Teflon-lined silica septum. Prior to removing CT stock solution for use in the feed, the vial was stirred with a magnetic stir bar for two hours,

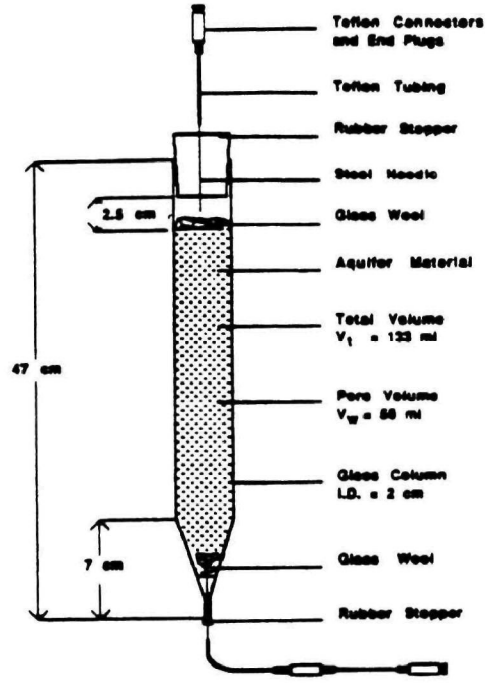

Figure 4.1. Column design.

then allowed to settle for 30 minutes after which an aliquot of stock solution was removed with a syringe and injected directly into the feed syringe.

The labeled CT (Sigma Chemical Co., St. Louis, MO) was extracted into Milli-Q water and stored in sealed ampules of approximately 15 ml. The activity of the labeled stock solution was approximately 400,000 dpm/ml. As necessary, the ampules were cracked and the contents were transferred to a 20 ml gas-tight syringe. The CT stock solution in the 20-ml syringe was injected directly into the feed syringe before each exchange to obtain the proper feed concentration.

Acetate stock solution was prepared using sodium acetate (J. T. Baker, Phillipsburg, NJ) in Milli-Q water. The stock solution concentration was 2.5 mg/ml, so that 1 ml injected into the 100-ml feed syringe created a feed acetate concentration of 25 mg/l. The acetate stock solution was stored at 4°C when not in use.

Feed Solution Preparation

Feed solutions were prepared using Moffett groundwater. The water was collected in 5-gallon quantities and stored in the dark at room temperature until needed. Prior to use, the water was stripped with nitrogen gas for 30 min, filter-sterilized using 0.2-μm sterile cellulose nitrate filters (Micro Filtration Systems, Dublin, CA) and placed in autoclaved glass bottles. All filtration apparatus and glassware contacting the water was autoclaved.

Feed for each column was prepared by filling the feed syringe with the filtered Moffett groundwater and injecting CT and, if applicable for the column, acetate. Sterile acetate and CT stock solutions were injected from the collection syringes directly into the feed syringe to achieve

the desired feed concentrations. The feed syringe contained a mixing device to assure that the feed concentration was uniform.

Analytical Methods

Acetate --

Acetate concentrations were determined by ion chromatography on a Dionex Ion Chromatograph. Column feed and effluent samples were diluted 25-fold with Milli-Q water prior to analysis. Borate buffer was used as the eluant.

Nitrate, Nitrite, Bromide, and Sulfate --

Nitrate, nitrite, bromide, and sulfate were also analyzed by ion chromatography using a Dionex Ion Chromatograph. Samples required dilutions of 25 or 50 to 1 prior to analysis. Carbonate buffer was used as the eluant.

CCl_4 and CCl_3H --

CCl_4 and CCl_3H were analyzed using liquid/liquid extraction into pentane. Samples of column influent and effluent were collected in syringes to prevent losses by volatilization. The collected volumes were transferred to 5 ml vials with open-top screw caps sealed with Teflon-lined silica septum. The extraction procedure consisted of simultaneously introducing 1 ml of pentane while extracting 1 ml of sample. The vials were shaken on a shaker table for 30 minutes and analyzed by gas chromatography.

Gas chromatographic analyses were done on a Tracor GC equipped with a squalene packed column and an ECD detector. The column temperature was 60°C. Argon/methane was the carrier gas at 7 ml/min and the detector gas makeup gas was at 70 ml/min. Calibration was achieved by injecting 3 standards bracketing the expected concentrations of CT and CF and comparing relative areas to the column samples; the calibration standards were subjected to the full sample treatment. Internal standards were not used.

Radioactivity Analyses --

Carbon-14 activity was determined using a Tricarb Model 4530 scintillation spectrometer (Packard Instrument Co., Downers Grove, IL). For each sample, three separate aliquots were counted. A 1.0-ml sample was injected into a glass counting vial containing 6 drops 1N HCl, another 1.0-ml sample into a vial containing 6 drops 1N NaOH, and a third 1.0 ml into a vial containing 10 ml liquid scintillation cocktail (Universol, ICN Biomedicals Inc.). The first two vials were stripped with nitrogen gas for 15 min and then 10 ml of scintillation cocktail was added. This procedure allowed for estimation of the production of $^{14}CO_2$, which is stripped at low pH but not at high pH. The volatile ^{14}C-activity which was air stripped at any pH was assumed to represent non-transformed CT and CF.

Column Operation

The column fluids were exchanged with 100 ml of new feed solution. The column exchange interval was one to three days in the initial set of experiments, and three to seven days in the main set of experiments. A syringe pump (Sage Instruments; Division of Orion Research, Inc., Cambridge, MA) with one 100 ml gas-tight syringe (Spectrum, Houston, TX) was used to exchange the liquid in an upflow direction at a flow rate of 5 ml/min. Breakthrough curves of bromide as a conservative tracer indicated that the first 18-20 ml of liquid removed during an exchange was not contaminated by the influent feed. All effluent samples were taken from the first 15 ml of liquid removed from the columns.

During each exchange, samples of influent feed and the column effluent were collected for analysis. The influent sample was obtained directly from the feed syringe prior to contact with the columns. The effluent sample consisted of the first 15 ml removed from the column. Effluent samples were collected in a syringe to avoid losses from volatilization and distributed from the syringe according to the volume needs of the analyses being performed. The effluent sample was assumed to represent the composition of the pore fluid within the column at the time of the exchange. Column effluent was compared to the influent of the previous exchange to determine acetate and nitrate consumption, CT degradation, and CF formation.

Results of the Initial Batch Column Experiment

The columns were initially used in a short-term study to characterize the indigenous bacteria and determine their ability to degrade CT under denitrifying conditions. A variety of primary carbon sources including, acetate, glucose, ethanol,and methanol were evaluated. Table 4.1 summarizes the treatment of each column during this initial study. Column fluids were exchanged every two days, but on a few occasions the exchange period was three days.

TABLE 4.1. OVERVIEW OF COLUMN OPERATION DURING THE INITIAL EXPERIMENTS

Day[a]	Column 1	Column 2	Column 3	Column 4	Column 5
0	no carbon substrate	no carbon substrate	70 mg/l acetate	50 mg/l methanol	50 mg/l ethanol
27	no carbon substrate	inoculated with strain KC 140 mg/l acetate	140 mg/l acetate	50 mg/l methanol	100 mg/l ethanol
60	no carbon substrate	140 mg/l acetate	140 mg/l acetate	100 mg/l glucose	100 mg/l ethanol
62	no carbon substrate	140 mg/l acetate	43 mg/l acetate	100 mg/l glucose	100 mg/l ethanol
64	no carbon substrate	140 mg/l acetate	43 mg/l acetate	100 mg/l glucose	poisoned with 0.02% NaN_3

[a] All columns began receiving CT on day 0. All changes in operation listed above held for the whole period after the timepoint indicated. Column fluids were replaced every two days, but in a few cases after three days.

Nitrate analyses performed 10 days after the initial column exchange showed that nitrate was completely removed in all columns receiving an organic substrate. Feed nitrate concentration during this initial study was 42 mg/l and effluent concentrations were < 0.1-0.02, 0.1-0.9, and < 0.1 mg/l for columns 3, 4, and 5, respectively (0.1 mg/l is the detection limit). Nitrate concentrations were not determined for columns 1 and 2, which did not receive organic substrate. Acetate uptake was measured on several occasions. Column 3, receiving 140 mg/l of acetate, showed an acetate concentration in the effluent of 74 mg/l on day 17. On the same day, nitrate was

reduced from 42 mg/l to 0.5 mg/l. Stoichiometrically, 0.5 mg of acetate would be degraded per milligram of nitrate, which corresponds to an expected value of 25 mg/l acetate removed, not 66 mg/l. Apparently, part of the acetate removal was not caused by denitrification.

Figure 4.2 shows the percent breakthrough of CT for each of the columns. For the first 27 days, it was found that little removal of CT took place in any of the columns. Concentrations in the effluents of the columns varied between 58 and 72% of the influent concentration. None of the columns receiving an organic substrate showed significantly higher removal than the control columns.

After approximately 27 days, the removal of CT was still low for columns 1-4, which showed about 40% removal of CT. In columns 3 and 4, the amount of CT removal again was not higher than in the sterile controls receiving no organic substrate, namely columns 1 and 2. Some degradation had occurred in column 5 which was receiving ethanol as the oxidizable substrate.

No CT degradation was occurring in the acetate-fed denitrifying column, 3. Therefore, it was attempted to start CT degradation in column 2 using acetate as the primary substrate and by inoculating column 2 with a pure bacterial culture that has the ability to degrade CT during denitrification. This bacterial culture, called KC and tentatively identified as a strain of *Pseudomonas* (Criddle, 1989), was isolated from aquifer material collected at Orange County, CA. The inoculation did not result in a significant increase in CT removal. Just like column 3, which was started with acetate at day zero, significant removal of CT was not observed in the inoculated column 2 during the first 20 days of operation.

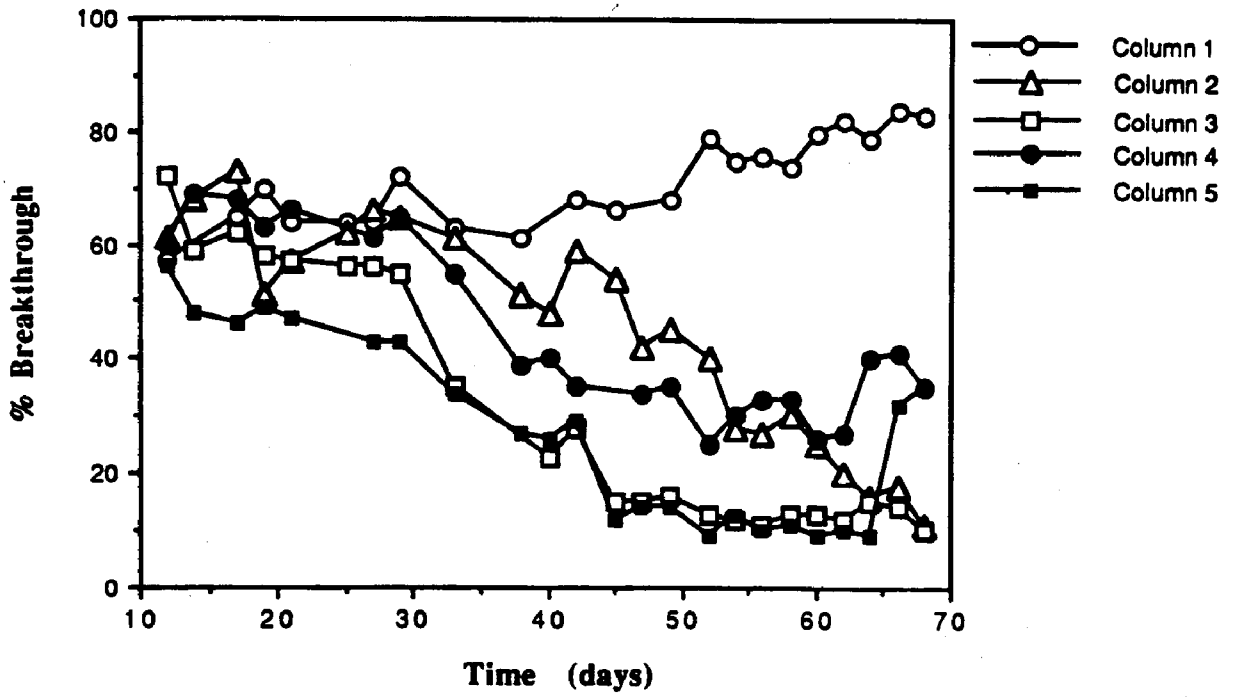

Figure 4.2. Percent breakthrough of CT for all five columns in the initial experiment.

Six days after inoculation, the effluent of column 2 was analyzed for the presence of CT degrading bacteria. On MMY plates supplemented with acetate and on Moffett groundwater plates supplemented with acetate, colonies were detected that appeared very similar to colonies of strain KC with respect to cell morphology, colony texture, and pigmentation. Four of these colonies were purified and checked for the degradation of CT in liquid MMY-acetate medium. It was found that these organisms were not able to degrade CT under conditions that allowed degradation of CT by strain KC, used for inoculation. Microscopically, strain KC and the isolates could not be distinguished. Upon close observation of colony morphology, slight differences were observed between the organisms present in the effluent and strain KC. Furthermore, similar organisms were detected in column 3, which was not inoculated. Apparently, bacteria with properties very similar to strain KC used for inoculation, but unable to degrade CT, are present in the Moffett aquifer. These indigenous organisms dominate strain KC when it was used for inoculation. Colonization of the column by introduced strain KC did not appear to occur.

Throughout the first month of the column experiments, the highest percentage of CT removal was found in the column, 5, receiving ethanol. After five weeks of operation, 55-65% CT was removed, compared to 35% for the columns receiving no substrate. Removal in the ethanol column increased to 85% after 8 weeks. The column that was fed acetate also showed a slowly increasing rate of CT removal. After a delay of approximately 4 weeks, CT degradation in the acetate columns, 2 and 3, had reached the levels observed in the ethanol column. The column that was fed glucose, column 4, showed a slow increase in CT removal up to about 65%. The extent of removal in the glucose-fed column remained significantly lower than the columns receiving either ethanol or acetate.

In order to determine whether the increase in CT removal observed in the acetate column was due to biological conversion or to increased physical absorption by accumulating biomass, Moffett water amended with sodium azide (0.02%) was fed to column 5 on day 64. This treatment caused a rapid decrease of the extent of CT removal to 60%. The sodium azide experiment showed that at least part of the CT degradation was due to biological activity.

The CT removal observed in the columns receiving organic carbon compounds could be due to several processes including 1) degradation by denitrifying, fermentative or sulfate-reducing bacteria or 2) absorption into increasing biomass. Since nitrate was removed within a week after starting the addition of acetate or ethanol to the columns whereas removal of CT required a much longer adaptation period, it cannot be concluded from these data that denitrifying microorganisms in Moffett aquifer material degrade CT during denitrification.

Theoretically, denitrifying organisms that can degrade CT could have colonized the soil column only after prolonged adaptation. It was attempted to isolate denitrifying bacterial cultures from the effluents of columns 3 and 5 after CT degradation had started. Twenty pure cultures were isolated anaerobically on MW plates and MMY plates with acetate or ethanol as carbon source. All these cultures were able to grow anaerobically with nitrate as electron acceptor, but the organisms did not degrade CT in liquid cultures. Strain KC, used as a control, completely degraded CT under the same conditions within 2 days, but the isolates obtained from the columns showed no significant CT degradation within 6 days. Thus, there is no evidence for any denitrification-coupled CT degradation in the soil columns or for the presence of microorganisms that have this ability.

Criddle (1989) performed batch experiments in sterile and unsterile Moffett groundwater to determine the ability of strain KC to degrade CT under the conditions present in the Moffett aquifer. The batch experiments examined four different additions: 1) acetate and KC; 2) acetate,

KC, and phosphorus; 3) acetate, KC, phosphorus, and trace metals; and 4) acetate only. Results indicated that in the unsterile Moffett water, the highest removal of CT occurred in the batch with acetate, KC, and phosphorus. However, the batch with just acetate performed better than the batch with acetate and KC inoculation. The addition of trace metals appeared to inhibit the degradation of CT by KC. These findings suggest that 1) Moffett water actually inhibits the transformation of CT by strain KC, 2) this inhibition can be partially alleviated by addition of phosphorus, and 3) inhibition is aggravated by addition of trace metals.

Results of Current Batch Column Experiments

The five columns described above were left undisturbed for 11 months, with no addition of groundwater amended with growth substrates or CT. The columns were then reactivated using the batch feed method. The purpose of this set of column experiments was to mimic the field experiments and to identify important processes and process sensitivities. The exchange periods used in this study varied from 3 to 7 days, compared to 1 to 3 days for the previous study. The final three months of this study also employed ^{14}C-labeled CT in order to quantify the fraction of influent CT that was completely mineralized to CO_2.

Table 4.2 summarizes the column operation for the current batch column experiments. As in the previous study, column 1 was operated as a control, receiving only Moffett groundwater with CT at approximately 50 µg/l. Columns 2, 3, and 5 were operated identically and received Moffett groundwater amended with acetate, approximately 25 mg/l, and CT, 50 µg/l. Column 4 was initially intended to be operated with concentrations of acetate sufficiently low to guarantee excess nitrate. However, even at 4 mg/l acetate, no excess nitrate was observed. After the first two exchanges, both column 1 and 4 were operated without acetate feed, receiving only Moffett groundwater with approximately 50 µg/l CT.

TABLE 4.2. SUMMARY OF COLUMN OPERATION DURING THE CURRENT STUDY

Day	Column 1	Column 2	Column 3	Column 4	Column 5
0	no acetate	25 mg/l acetate	25 mg/l acetate	8 mg/l acetate	25 mg/l acetate
14	no acetate	25 mg/l acetate	25 mg/l acetate	acetate addition ends	25 mg/l acetate
125	begin ^{14}C-labeled CT additions for all columns				

Note: Influent nitrate concentration was approximately 25 mg/l in all columns.

Electron Donor and Acceptor Utilization --

Influent nitrate concentrations were the ambient concentration present in the Moffett groundwater at the time of collection, 24.92 mg/l (st. dev. = 0.78 mg/l), compared to the previous sampling season concentration of 42 mg/l. The columns receiving acetate, 2, 3, and 5, consumed all of the available nitrate for even the shortest exchange period, 3 days. The control column, 1, consumed approximately 15% of the influent nitrate, i.e. 3.69 mg/l (st. dev. = 0.42 mg/l). Column 4 consumed all available nitrate for two exchanges without acetate additions. Column 4 continued to receive only Moffett water with CT and no acetate and excess nitrate began appearing

at the third exchange. After six exchanges without acetate addition, the level of nitrate consumed by column 4 appeared to level off at about 50%.

Sulfate is present at high concentration (> 700 mg/l) in the Moffett groundwater. Sulfate could be acting as an electron acceptor in the columns that completely consumed nitrate. Sulfate concentrations were measured initially but the high concentration prevented using differences in the influent and effluent sulfate concentrations as an indication of sulfate reduction. No evidence of hydrogen sulfide production was detected.

Columns 2, 3, and 5 received acetate at concentrations of 25.08 ± 1.32, 24.35 ± 1.80, and 25.16 ± 1.68 mg/l respectively. Column 5 showed no signs of inhibited microbial activity due to the sodium azide poisoning in the initial experiment, the available nitrate and acetate was completely consumed. Excess acetate has never been observed in any column effluent for even the shortest exchange interval.

CCl_4 Degradation and CCl_3H Formation--

The columns degrading CT in the original study appear to have retained the ability to degrade CT after a long dormant period. Figure 4.3 shows the influent and effluent CT and effluent CF concentrations for column 5. It appears that column 5 had retained its ability to degrade the CT after the long dormant period, as the lag period observed in the initial study was not observed. Similar responses were observed for columns, 2 and 3, also receiving acetate.

Figure 4.4 shows the influent and effluent CT and effluent CF concentrations for the control, column 1. Carbon tetrachloride effluent concentrations for column 1 showed an initial increase, suggesting possible adsorption of CT. As in the previous study, column 1 showed less CT removal than the columns receiving organic substrate. Approximately 70% of the influent CT was present in the effluent. Chloroform production was observed in all columns.

Figures 4.5, 4.6, and 4.7 show the effluent CT and CF concentrations normalized to the influent CT concentration for columns 1, 4, and 5 respectively. The large fluctuation observed around day 130 was caused by the switch to the ^{14}C-labeled CT feed, which was at a lower concentration (approx. 40 µg/l) than the unlabeled CT feed (approx. 60 µg/l). Effluent CT in the control, column 1, was on the order of 70% of the influent CT, while chloroform varied from 5 to 20% of the influent CT. Column 5 effluent CT was much lower than the control, approximately 15% of the influent CT. Chloroform from column 5 varied between 1 and 12%. Column 4 results were intermediate between columns 1 and 5, with effluent CT about 35 - 40% of influent CT and effluent CF about 18% of influent CT.

After 125 days of operation CT addition was switched from unlabeled to ^{14}C-labeled CT in an attempt to identify the extent of mineralization of CT to CO_2. Figure 4.8 shows the typical labeled effluent fractions for each of the columns. The columns receiving acetate had much greater levels of ^{14}C-labeled CO_2, ranging from about 35% for columns 2 and 3 to 52% for column 5. The control column produced very little CO_2, 1-2%. Column 4 showed behavior intermediate between the control and the columns receiving acetate, with around 20% conversion of ^{14}C-labeled CT to CO_2. Column 4 had been biostimulated in the initial work and was initially operated at low acetate levels, but acetate additions were discontinued after day 14 (exchange 2). The column appears to have retained some ability to degrade CT without the addition of acetate. Recovery of ^{14}C-labeled material ranged from 65% in column 5 to around 40% in column 3.

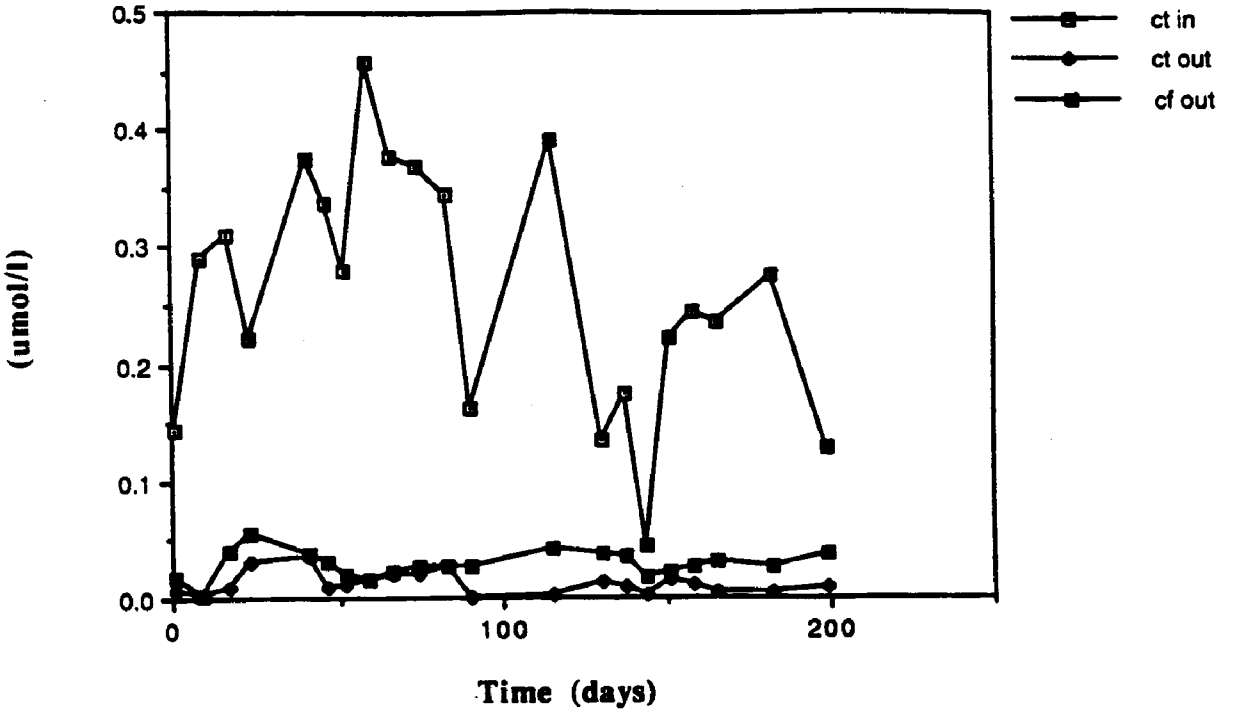

Figure 4.3. Influent and effluent CT and effluent CF concentrations for column 5.

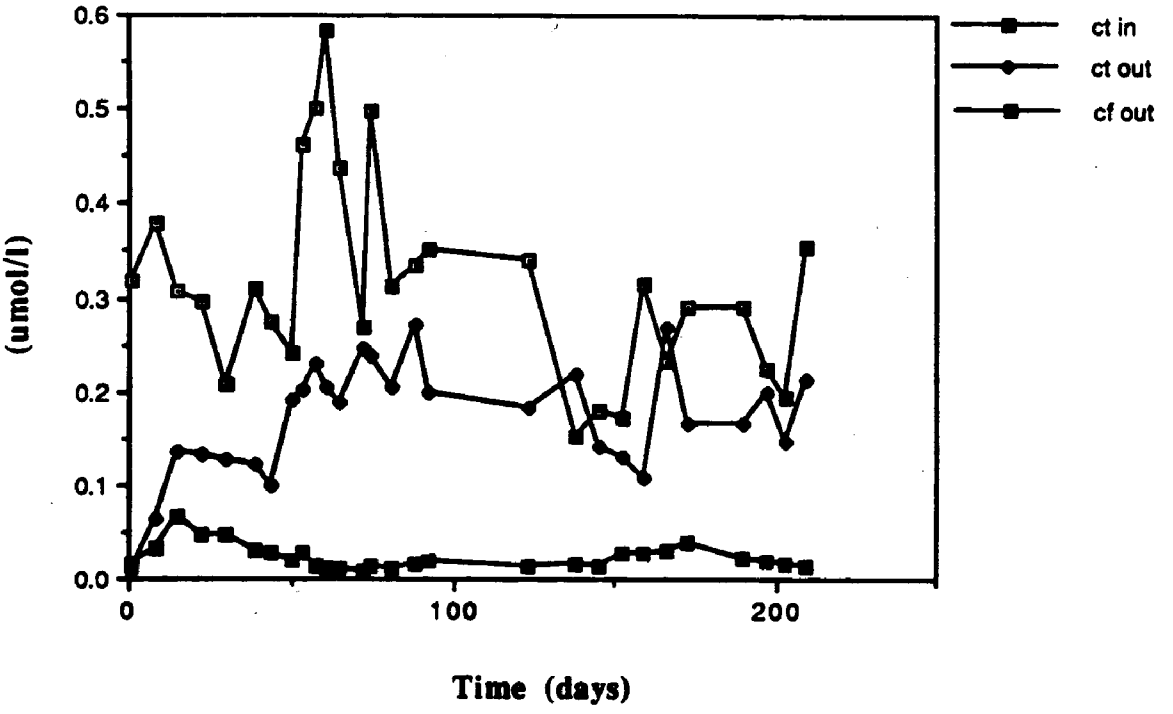

Figure 4.4. Influent and effluent CT and effluent CF concentrations for column 1.

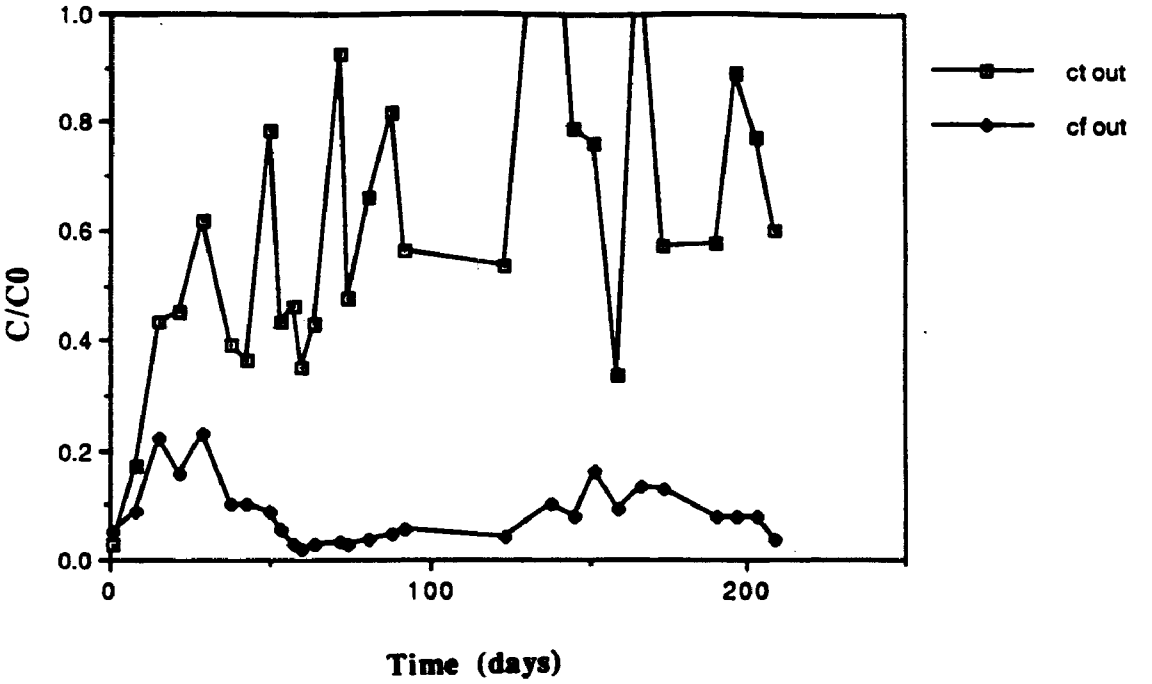

Figure 4.5. Effluent CT and CF concentrations normalized to the influent CT concentration for column 1.

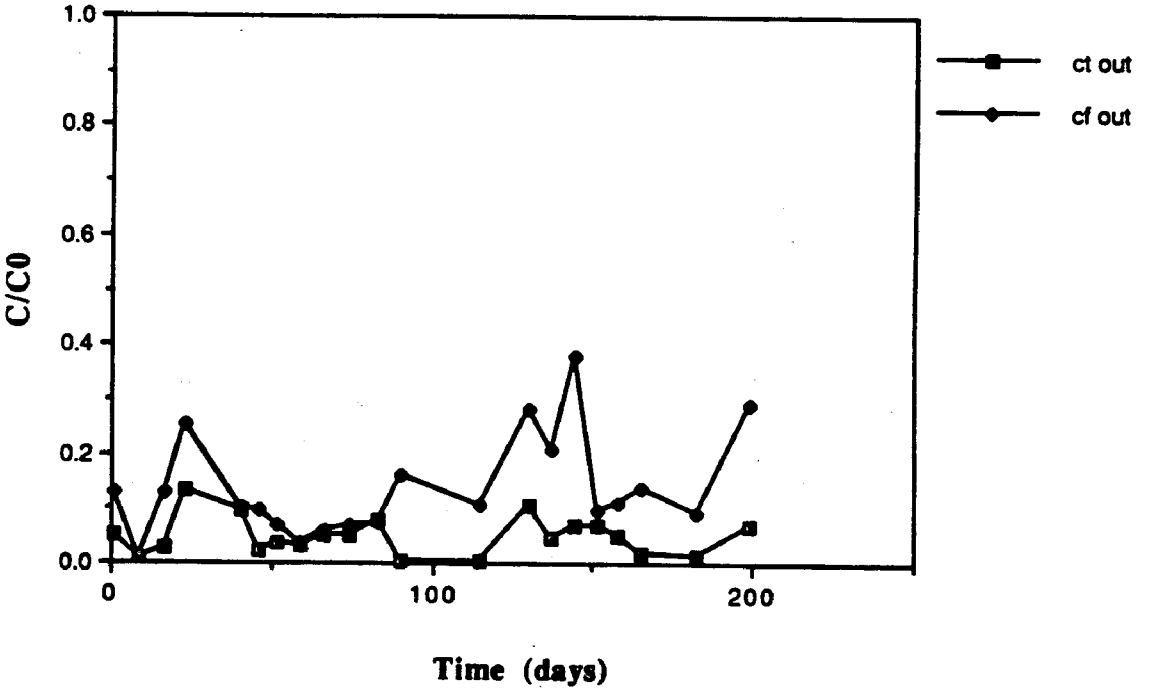

Figure 4.6. Effluent CT and CF concentrations normalized to the influent CT concentration for column 5.

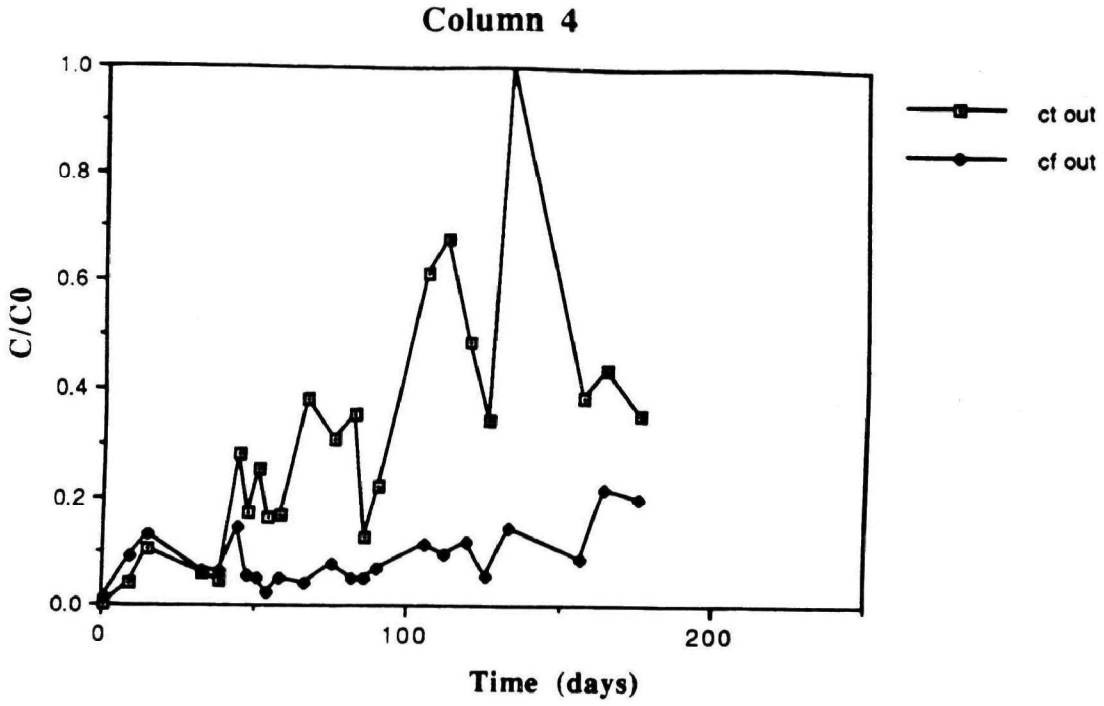

Figure 4.7. Effluent CT and CF concentrations normalized to the influent CT concentration for column 4.

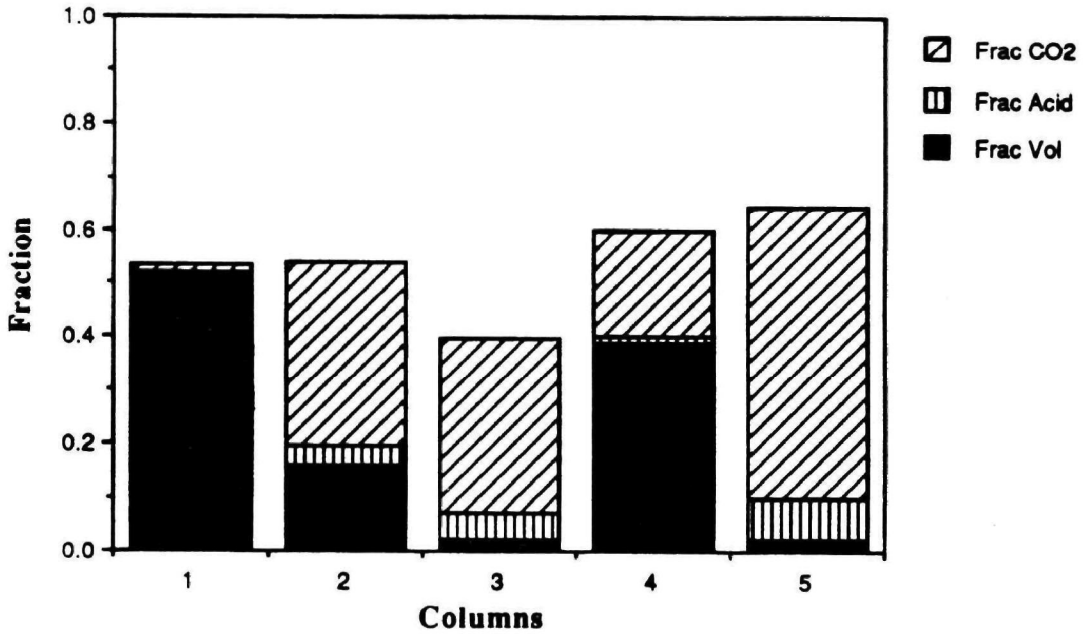

Figure 4.8. Carbon-14-labeled effluent fractions from all columns.

Figure 4.9 shows the fraction of the influent CT that was detected as CT or CF in the column effluent (data shown is from the same exchange as shown in Figure 4.8). The columns receiving acetate, columns 2, 3, and 5, show the highest transformation of CT and also the highest production of CF. Removal of CT for these three columns ranged from 95 to 82% and CF production ranged from 30 to 40% for a seven day exchange period. The control column had about 89% of influent CT accounted for as effluent CT and about 7% transformed to CF. Column 4 showed approximately the same fraction of CT and CF combined as columns 2, 3, and 5 but much more of this effluent fraction was as CT than CF indicating that less degradation of CT had occurred in column 4.

The differences between the labeled fraction of volatiles (Figure 4.8) and the labeled + unlabeled sum of CT and CF (as measured by GC) (Figure 4.9) may result from steady-state conditions not being achieved for the labeled compound. Thus, as indicated by the control results, the labeled + unlabeled fractions are closer to unity than those of the labeled. Isotopic exchange between the C-labeled aqueous CT and nonlabeled sorbed CT fraction is probably occurring in the columns. This may partly explain the sum of all the labeled fractions being less than unity.

Evidence of biomass accumulated was also observed as pore clogging by biomass made it increasingly difficult to perform the column exchanges in the columns receiving acetate. Column 5 can no longer be exchanged and the time necessary to perform a column exchange for all other columns at the standard pump speed employed throughout the experiment has increased.

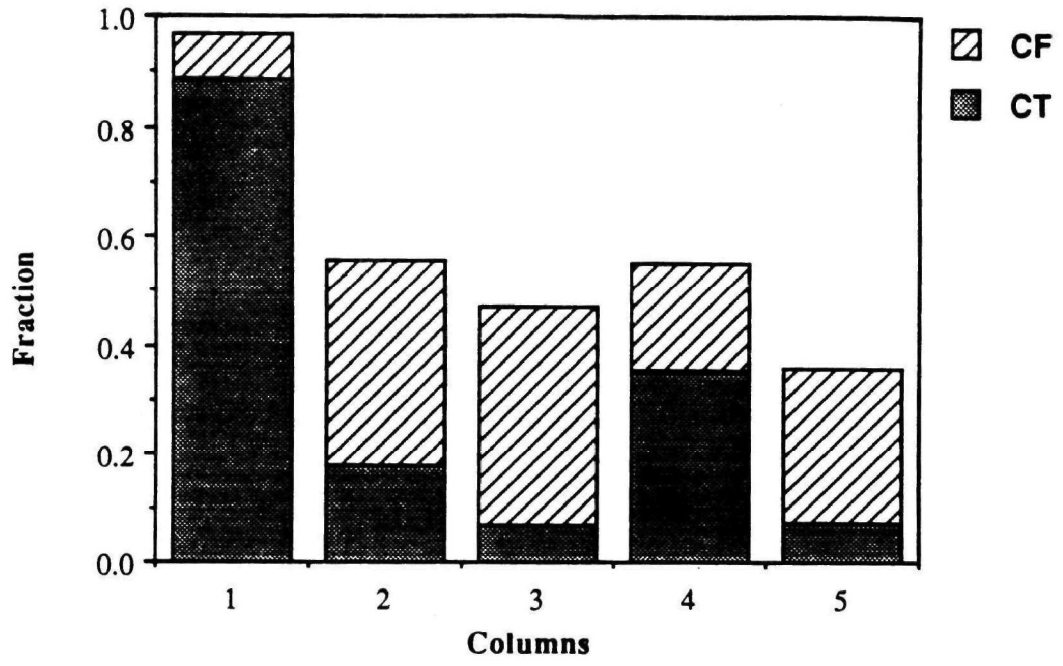

Figure 4.9. Unlabeled and labeled fractions of effluent CT and CF for all columns, as determined by GC analysis.

SUMMARY

In semi-batch column experiments with pore fluid exchanges performed daily to weekly, denitrification activity commenced rapidly in columns containing Moffett solids following biostimulation with various organic substrates. The nitrate as well as the organic substrate was fully utilized with exchange periods as short as one day. Upon restimulation with acetate and nitrate, following a hiatus of one year, biological activity commenced immediately, again showing full utilization of nitrate and acetate after the first exchange.

In the initial study, CT transformation began after approximately 30 days in the columns receiving acetate and ethanol, and more slowly in the column receiving glucose. Acetate was chosen for the subsequent set of experiments in the field and laboratory. Upon restimulation with acetate, CT transformation began immediately and ultimately reached 40 to 60% conversion to CO_2. Chloroform appeared as a transformation product, to the extent of 30 to 40% of the CT fed. No other halogenated byproducts have been confirmed.

With the information presently available, we have not yet determined whether the transformation was mediated by actively denitrifying bacteria or by a secondary population of microorganisms. Further studies now underway aim to shed light on that issue. The question is complicated in systems containing aquifer solids because of the interacting biotic and abiotic transformations and the progression of redox conditions during and following denitrification. This limitation notwithstanding, the semi-batch column methodology has proven useful as a means of assessing the effect of biostimulation on facilitating transformation of targeted chlorinated compounds under controlled laboratory conditions.

SECTION 5

FIELD EXPERIMENT METHODOLOGY AND SITE CHARACTERIZATION

This section will discuss the experimental methodology used in the field evaluation. Characteristics of the field site as they pertain to the experiments will be presented. The facilities instrumentation used in the field experiments will be described, followed by an analysis of its performance.

EXPERIMENTAL METHODOLOGY

The experimental approach taken was based on that which was successfully used in our previous evaluations of in-situ bioremediation at this field site (Roberts et al., 1989). The methodology developed to meet the goals of the study was as follows:

A) Select an appropriate zone in the subsurface to conduct the evaluation.

B) Modify the Automated Data Acquisition and Control System at the field site in order to conduct the evaluation.

C) Assess the mobility of CT, relative to the bromide tracer, to quantify residence times in the system under injection and extraction conditions and to determine if transformation occurred before biostimulating in the test zone.

D) Evaluate how easily an enhanced population of denitrifiers was biostimulated when the appropriate growth conditions are supplied, to quantify the resulting transformation of CT and the intermediate products formed, and to determine if background contaminants such as TCA, Freon-11, and Freon-113 are also transformed.

E) Investigate under what biostimulation conditions the biotransformation was most effective and the formation of chlorinated intermediate products best minimized.

The methodology described above required the creation of a test zone in the subsurface that permitted controlled chemical addition and fluid extraction. The concentrations of the chemicals of interest in the groundwater of the test zone are determined with and without biostimulation. Both the temporal and spatial changes in concentration are used in the evaluation. The degree of transformation is assessed using mass-balance comparisons before and after the test zone is biostimulated, in conjunction with direct comparisons with mass balances for bromide, used as a conservative nonreacting tracer during the biotransformation experiments.

The field evaluation consists of a series of stimulus-response experiments. The stimulus in these experiments was the injection of known quantities of the chemicals of interest in a controlled

manner into the test zone. The response is measured in terms of the chemical concentrations of fluid samples taken at observation wells and at the extraction well.

Figure 5.1 illustrates the well system that was developed to perform these experiments. The test zone includes a series of injection, extraction, and monitoring wells installed in a shallow, semi-confined aquifer. The monitoring wells are located 1, 2.2, and 3.8 m from the injection well in a direct line with the extraction well located 6 m from the injection well. The test zone was created by the controlled injection and extraction of groundwater. The chemicals of interest for a given stimulus-response experiment are added as soluble components to the injected groundwater. Groundwater was injected in well SI at a rate of 1.5 l/min and extracted at well P at rate of 10.0 l/min. At these rates the region surrounding the observation wells was completely dominated by the injected groundwater and the injected fluid was effectively captured by the extraction well (i.e., 90% recovery or greater). The response of the system is studied by continually monitoring the concentrations of the chemicals of interest in the injected and extracted fluids, and in groundwater samples obtained from the three monitoring wells.

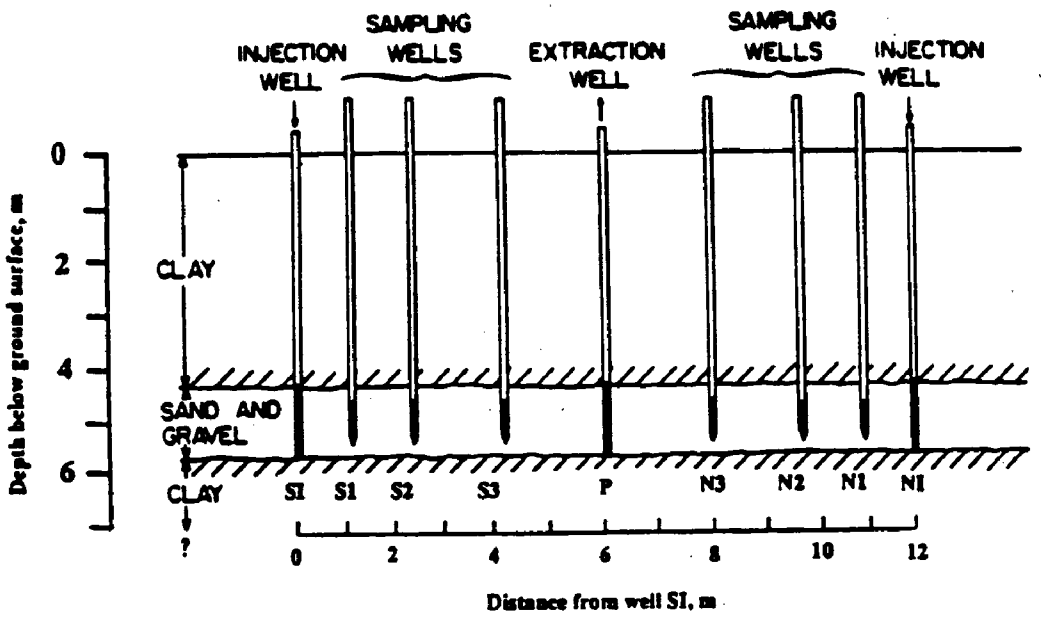

Figure 5.1. A vertical section of the test zone.

Series of Stimulus-Response Experiments

The series of stimulus-response experiments conducted and the processes evaluated, based on the methodology described above, are presented in Table 5.1. The field evaluation was to be conducted in four stages.

In Stage 1 the hydraulic characteristics of the test zone operating under induced gradient conditions of injection and extraction were evaluated through bromide tracer tests. Experiments along different experimental legs were performed to determine which leg had the best hydraulic characteristics for the controlled experiments. These experiments showed that it was most favorable to inject at SI and extract at P (Figure 5.1), using S1, S2, and S3 as the intermediate monitoring points. This strategy, identical to that used in the earlier investigation of methanotrophic treatment, assures the most complete capture of injected fluid by the extraction well.

TABLE 5.1. SEQUENCE OF EXPERIMENTS AND PROCESSES STUDIED IN THE FIELD EVALUATION

Stage	Injected Chemicals	Process Studied
1)	Br^-	Advection/Dispersion
2)	$Br^- + CT + NO_3$	Retardation/Dispersion (Transformation)
3)	$Br^- + CT + NO_3$ + Acetate	Biostimulation + Biotransformation
4)	$Br^- + CT$ + Acetate	Enhanced Biotransformation

In Stage 2 the transport of CT through the test zone was studied and the possible transformation of CT by abiotic or biotic processes, in the absence of biostimulation, was assessed. The experiment also served to saturate the test zone with CT prior to the biostimulation-biotransformation experiment. In this test, CT (the target compound) along with bromide (as a conservative tracer) were continuously injected into the test zone under induced gradient conditions. The concentration breakthroughs of CT and Br at the observation wells and at the extraction well were monitored, along with anticipated transformation products. The retardation of the CT with respect to the bromide tracer, due to sorption onto the aquifer solids, was quantified based on the breakthrough response. The concentration of background contaminants in the test zone was also monitored to establish their steady-state background concentrations. Thus, this phase of the experiment acts as a quasi-control experiment before biostimulation.

In Stage 3 the main results of the field evaluation were obtained. The growth of an indigenous population of denitrifying bacteria was stimulated through the addition of acetate as a substrate for energy and growth (electron donor) into the test zone that contained nitrate as an electron acceptor. Biostimulation was evaluated by observing the concentration decreases of the electron donor and acceptor, both spatially and temporally, in the test zone. Upon biostimulation the transformation of CT was evaluated based on its decrease in concentration. The concentration of the background contaminants and expected intermediate products was also monitored throughout the experiment.

The final stage of the experiments (Stage 4) involved a transient test to determine how changes in operating conditions affected transformations. In this test, nitrate was removed from the injected fluid using a bioreactor at the surface. The experiment evaluated whether enhanced transformation of CT resulted after nitrate was completely removed from the test zone. The results of Stages 1 through 4 will be discussed in Section 6.

The degree of biotransformation achieved in the experiments was determined based on comparisons with bromide as a conservative tracer. The percent biotransformed was based on the ratio of the normalized breakthrough of the CT to that of bromide at each observation location, after steady-state conditions were achieved. The percent biotransformed is given by equation (5-1):

$$\text{Percent biotransformed} = \left(1 - \frac{C_{fCT}}{C_{fBr}}\right) \times 100\% \qquad (5\text{-}1)$$

where, C_{fCT} is the mean fractional breakthrough of CT after biostimulation and C_{fBr} is the mean fractional breakthrough of bromide over the same time interval. This estimate gives the total degree

of transformation achieved during transport through the biostimulated zone. Biotransformation can also be estimated using the quasi-control data from the Stage 2 experiment. In this case the fractional breakthrough of CT achieved in that experiment was substituted from the Br fractional breakthrough in equation (5-1). This estimate represents the degree of enhanced transformation that results from the biostimulation of the test zone.

Mass balances can also be performed on the amounts of CT injected and extracted. These mass balances were used to confirm the estimates based on the CT/bromide breakthrough comparisons described above.

The above series of quantitative assessment experiments provided the information required for an effective evaluation of the proposed technology, within the limited funding period. The staged approach optimized the amount of information that could be achieved in one season of field testing. The data also provided a realistic basis for model calibration and verification, as discussed in Section 7.

The controlled evaluation was conducted at a site that had been instrumented and characterized in our previous experimental evaluation of "In-Situ Aquifer Restoration of Chlorinated Aliphatics by Methanotrophic Bacteria," (EPA/600/2-891033). The characteristics of this site have been presented in detail by Roberts et al. (1989, 1990), and will not be repeated in detail here. A brief summary of the characteristics as they pertain to these experiments are summarized below.

SITE CHARACTERISTICS

The field site designated SU-39 is located at the Moffett Naval Air Station, Mountain View, CA. The site is located on the lower part of the Stevens Creek alluvial fan, 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 halogenated aliphatic compounds. The major contaminant in the test zone is 1,1,1-TCA at a concentration of 50 µg/l. Freon-11 and Freon-113 are also present at concentrations of 3 µg/l and 6 µg/l, respectively.

GEOLOGIC CHARACTERISTICS

The geologic characteristics of the test zone were examined extensively in our previous work at the site. The test zone is located in an aquifer that is confined between silty clay layers and is approximately 1.2 m thick; the top border is located 4.4 to 4.6 m below the ground surface, and the bottom ranges from 5.3 to 5.7 m below the surface. The aquifer consists of fine- to coarse-grained sand and gravel and appears poorly sorted in most cores. The aquifer, as indicated by the slotted well screens in Figure 5.1, is located 4.3 to 5.8 m below the surface. Gravel lenses with pebbles up to 2.5 cm in diameter occur in some cores within the sand layers. Cores were often lost over the depth interval from 4.7 to 5.2 m below the surface. Hence, this zone was considered to have the highest gravel fraction.

Along the north-south series of wells (SI, P, NI) the aquifer is composed of a mix of sands and gravels, of fairly uniform thickness. Petrographic analysis shows that the aquifer solids consist of rock fragments of the parent rock of the Santa Cruz Mountains. These include graywackes, cherts, and volcanics of eugeosynclinal (slope) origin (Franciscan Series). At the study site, the aquifer consists of alluvial sediments deposited during the last 5000 years. The aquifer is spatially heterogeneous, with the composition varying appreciably over short distances. The test zone

appears to have the structure of a buried stream channel, containing sand and gravel in some areas and only sand in others. This structure is common in alluvial aquifers, which 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

The hydraulic characteristics of the test zone were evaluated in our previous studies using the series of monitoring wells shown in Figure 5.2. Piezometric measurements made during the test indicated that the aquifer was confined with a piezometric surface 1.5 m above the top confining layer (5.4 m above mean sea level). The gradient of 0.0032 in a northerly direction was estimated using piezometric measurements from the monitoring wells shown in Figure 5.2.

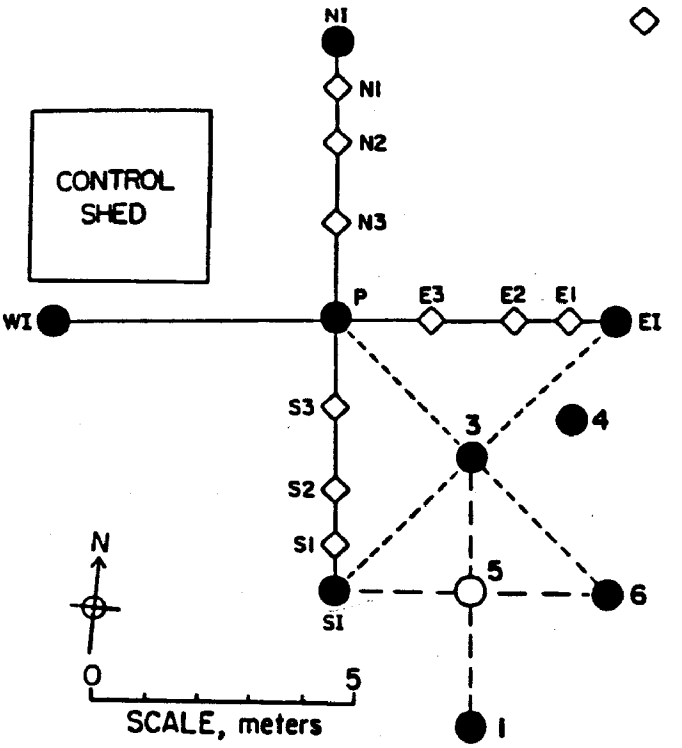

Figure 5.2. Map of well field installed at the test site.

The results of pump tests performed at the site are discussed in detail by Roberts et al. (1989) and Johns et al. (1990). Average transmissivity values in the western portion of the well field were very high, ranging from 130 m^2/d at well NI to 151 m^2/d at well SI. The pump tests match fairly well the response of a leaky aquifer with an average transmissivity of 150 m^2/d, an average storativity of 0.0023, and average leakage parameter of 0.19. The results of pump tests and model simulations indicate minimal leakage across the aquitard under the hydraulic conditions of the field tests. The high transmissivity results in an estimated hydraulic conductivity of 100 m/d (based on an aquifer thickness of 1.4 m), in the range of values for coarse sand (20-100 m/d), gravel (100-1000 m/d), and sand-gravel mixes (20-100 m/d) (Bouwer, 1978). The pump tests indicated that the site had several favorable hydraulic features: 1) high transmissivity would permit the required pumping and injection of fluids into the test zone; 2) loss of permeability by clogging, which might

result from biological growth or chemical precipitation, might be limited, owing to the original high permeability; 3) vertical leakage is insignificant, because the test zone is fairly well bounded above and below; and 4) the aquifer is capable of supplying groundwater at rates required for the experiments with less than one meter of drawdown at the extraction well.

Chemical Characteristics

The chemical characteristics of the site groundwater were reported by Roberts et al. (1989). The major cations' milliequivalent concentrations are as follows: calcium, 10.0; magnesium, 8.2; sodium,2.3; potassium,< 0.1. The major anions' milliequivalent concentrations are as follows: sulfate, 15.6; bicarbonate, 4.4; chloride, 1.2; nitrate, 0.2. The groundwater hardness is 920 mg/l, based on the calcium and magnesium concentrations, and the groundwater would be classified as very hard water. Bicarbonate is the major form of alkalinity at the measured groundwater pH of 6.7. 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. Nitrate and sulfate are both available as electron donors. The chemical composition, including the pH, is suitable for the microbial growth. The high calcium concentration was a potential problem, e.g., the precipitation of sulfates and carbonates with changes in fluid chemistry. The chemical composition of the groundwater indicates that the pore water 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; the poor quality of the formation water facilitated obtaining regulatory approval to perform the experiments.

The trace element composition of the groundwater was reported by Roberts et al. (1989). Concentrations were below levels that would be considered toxic to microorganisms. Iron and manganese were present at total concentrations of 0.5 mg/l and 0.3 mg/l, respectively.

The total concentration of background halogenated contaminants was less than 100 µg/l. 1,1,1-trichloroethylene was the major background contaminant, with an average concentration during the test of 50 µg/l. Freon-113 and Freon-11 were also present at average levels of 6 µg/l and 3.5 µg/l. Carbon tetrachloride, the target compound, was not detected in the groundwater.

Previous analyses showed no purgeable organics (e.g., benzene, xylene, toluene, chlorinated aromatics) present. 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 to the presence of natural humic and fulvic acids (Freeze and Cherry, 1979).

The fact that the groundwater at the test zone is contaminated with halogenated aliphatic compounds at low concentrations facilitated obtaining regulatory permission to inject CT, the target compound, at concentrations below 100 µg/l. Thus, controlled experiments could be performed by adding small but measurable quantities of CT to the test zone.

Organic Carbon Content

The organic carbon content of the Moffett aquifer material was reported by Roberts et al. (1989). The average carbon content of the bulk material is 0.11%. The organic matter appeared to be concentrated in the clay fraction, which had an organic carbon content six times that of the bulk material, whereas the coarse-grained fractions have organic carbon contents as much as 40% less than the bulk average. Based on these measurements, it appeared likely that the Moffett aquifer material would exhibit substantial sorption capacity, significantly greater than that 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%. In the Borden experiment CT had an estimated retardation factor of approximately 2.0.

SITE INSTRUMENTATION

The site instrumentation used in the field evaluation was that used in our previous studies. A complete description of the instrumentation is given by Roberts et al. (1989). Modifications to the system and a brief summary are presented here for completeness.

The Well Field

Figure 5.1 presents a vertical section of the test zone and the well field used in the experiments. The well field was originally 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 injection wells are located 6 m from the extraction well. The monitoring wells are located 1.0, 2,2, and 4.0 m from the injection wells.

The extraction and injection wells are constructed of 2" PVC wellstock that is slotted over a 1.5-m screened section. The screened section was positioned 4.3 to 5.8 m below the surface in order to fully penetrate the aquifer. The monitoring wells were 1.75"-OD stainless steel well casing with a 0.6-m screened drive point (Johnson Wirewound #35 slot). The 0.6-m screen section was placed to intercept what was considered to be the most permeable zone, consisting of sands and gravels 4.7 to 5.3 m (± 1 cm).

The Automated Data Acquisition and Control System as well as the injection system were housed in a control house adjacent to the well field. Samples from the test zone were pumped to the surface with a Cole-Parmer, multihead peristaltic pump, located in the control building. In order to prevent losses by volatilization and sorption, the fluid injection and sampling lines were fabricated with 1/4"-OD stainless steel tubing. The total maximum volume of the sampling lines and the tubing to the well screen was approximately 300 ml.

The Extraction System

The groundwater extraction system was designed to maintain a very constant rate of fluid withdrawal and permit changes in rates, if desired. The central extraction well was equipped with a shallow-well jet pump, essentially a combination of centrifugal and eduction systems. The inlet to the suction pipe was located at a depth of 5.1 m, the center of the screened section. The extracted water was delivered to the instrument/control house, where the flow rate was controlled using a pressure regulator and needle valve. Flow rate was measured by an electronic paddle-wheel sensor backed-up by a standard rotameter. Induced gradient conditions were created by extracting groundwater at a rate approximately 7 to 8 times greater than the injection rate. This was required in order to dominate the regional groundwater flow. Extraction rates were maintained at 10 l/min in the experiments.

In order to meet discharge requirements, the excess extracted water was air-stripped before it was discharged to a storm sewer. The air stripper removed more than 95% of the measurable chlorinated aliphatics, and was capable of achieving the discharge requirement of 5 µg/l for each compound (Roberts et al. 1989).

Injection System

In order to maintain a constant injection concentration, the injection system required spiking of a metered flow of constant concentrated solutions of bromide (the conservative tracer) and CT (the target compound). The pulsed addition of acetate, the primary substrate for biological growth, was also required over a short duration in a repeating cycle. The bromide and acetate spike solutions were made up in batches (500 g NaBr in 4 L and 500 g $NaC_2H_3O_2$ in 2 L). The CT spike solution was near its solubility limit in water at 21°C. The solubility of CT in water at 20°C is 805 mg/l (Horvath, 1982).

Figure 5.3 shows the system used for preparing of the CT spike solution. The system for delivering spike solutions of CT was designed to 1) maintain constant injection concentrations; 2) change the injected concentration, if desired; and 3) add an aqueous spike solution free of any co-solvent such as methanol. The system consisted of a refillable water reservoir, a solute saturation flask, and a multichannel peristaltic pump to inject the spike solution at the desired flow rate. Water was drawn from the refillable reservoir through the solute saturation flask via the peristaltic pump. The solute saturation flask, containing a sufficient quantity of pure CT to have an immiscible phase present, was mixed by a magnetic stirrer to form a saturated aqueous solution of the chlorinated organic. The flask was immersed in a water bath maintained at 21°C.

The injection system was that used in prior field experiments (Roberts et al., 1989) with minor modifications. The system is shown in Figure 5.4. The extraction water, which contained native nitrate, was used as the injection water supply. The extracted water was recycled before being air-stripped in order to reduce the buildup of carbonates in the system. A gear pump pumped the supply water through a nominal 5-μm polyester filter and a UV disinfection unit (rated at 4.5 log reduction of *E. coli* at 4 l/min). The supply water from the UV disinfection reactor is then passed through a gas-stripping column to remove excess N_2 gas, that would be formed in the denitrification process. The gas-stripping tower was 4" OD, of 40" long plexiglass, filled with 5/8" polypropylene flexrings (Koch). The column was operated in counter-current flow with helium used as a purge gas (< 100 ml/min). Excess water was allowed to overflow to the drain at the effluent end of the column.

Effluent water from the gas-stripping column was pumped via a gear pump through a rotameter to the mixing chamber. Spike solutions of bromide, CT, and acetate were pumped with peristaltic pumps to this mixer. The power to the acetate pump was switched on and off with a mechanical timer allowing control of both pulse width and duration of the cycle. In the experiments the typical duration of acetate additions was 1 hr followed by 12 hrs without acetate addition.

The effluent of the mixer is connected to the injection gear pump, an injection sample line, and a constant-head reservoir. The injected fluid was pumped through a rotameter and transferred to the injection well via stainless-steel tubing. A pressure switch connected to the rotameter was used to shut down the injection pumps when any component upstream fails. The constant head reservoir permits the sampling of the injection solution without disturbing the system. Thus, a constant injection rate was maintained, even during sampling.

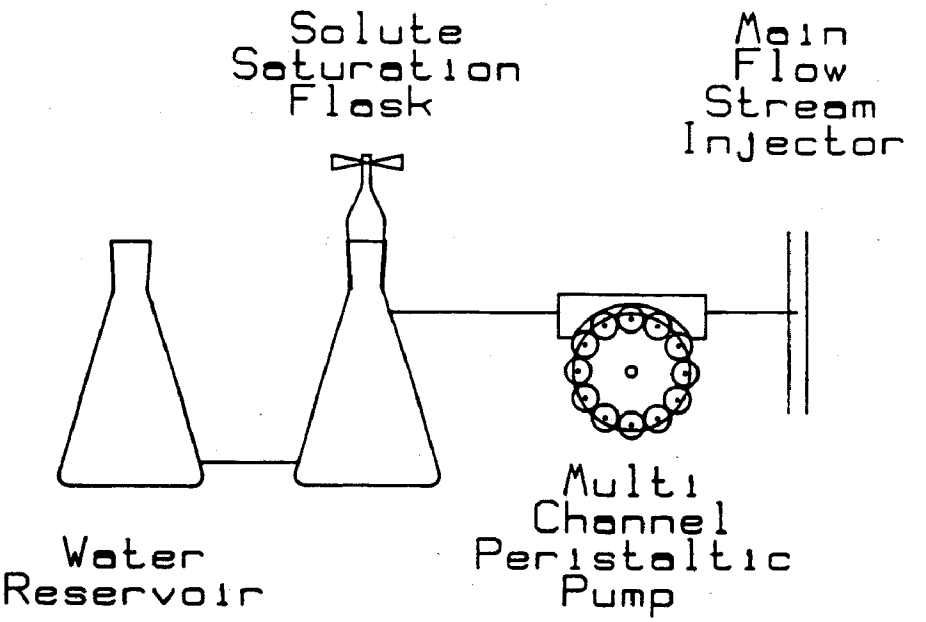

Figure 5.3. System used for preparation and delivery of spike solutions.

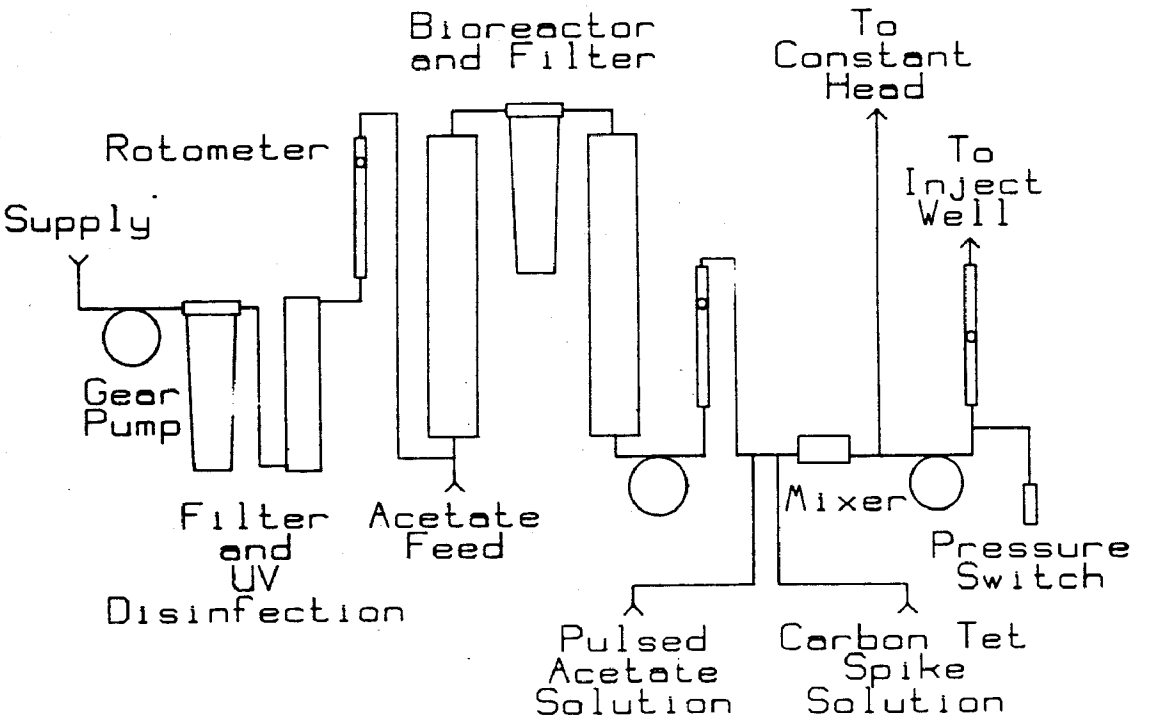

Figure 5.4. Schematic of the injection system.

In the latter portion of the biostimulation experiment, a surface bioreactor was installed to biologically remove nitrate in the recycled groundwater. The plexiglass bioreactor was 4" OD and 40" long and was filled with 5/8" polypropylene flexrings (Koch), which served as a support medium for attached biological growth. The reactor had a packed-bed contact time of less than 3 min. Acetate was spiked into the influent groundwater at an average concentration of 55 mg/l. After 10 days of acetate addition the reactor was found to be 80% efficient at nitrate removal. Three nominal 1-μm cotton filters were added behind the reactor to achieve complete nitrate removal. The first filter had to be removed daily and the others shifted in position, such that the first filter was always the oldest and the third filter the newest. In this arrangement nitrate was effectively removed and the effluent had a residual of 1 mg/l acetate. Analyses indicated no CT removal in the bioreactor. The CT, bromide, and acetate were then added to the injection solution that lacked nitrate, as previously discussed. The reactor was operated for 14 days before being brought into the injection system for the final transient test (Stage 4).

The Automated Data Acquisition and Control System

The Automated Data Acquisition and Control System (DAC) used for the evaluation was upgraded from that presented by Roberts et al. (1989). As in previous studies the system was constructed to permit real-time monitoring of the experimental parameters. The data were collected from three gas chromatographs (GC), two ion-liquid chromatographs (IC), and two probes. During the field evaluation, the DAC system was expanded to store and graphically display up to 34 measured parameters. The DAC system could be run in an automated mode, where samples were collected and analyzed in a predefined sequence, or in a manual mode which permits instrument calibration and sampling out of the predefined sequence.

A schematic of the DAC system is presented in Figure 5.5. Details of the system's construction are presented by Hopkins et al. (1988). The system is driven by a 6-Mhz microcomputer. The computer is equipped with a Techmar Lab Master A/D board for transforming from analog to digital response. The system also includes a Techmar MegaFunction board, CGA composite monitor, 20-Mb hard disk, and modem. The program controlling the DAC system was written and compiled with Microsoft's Quick Basic.

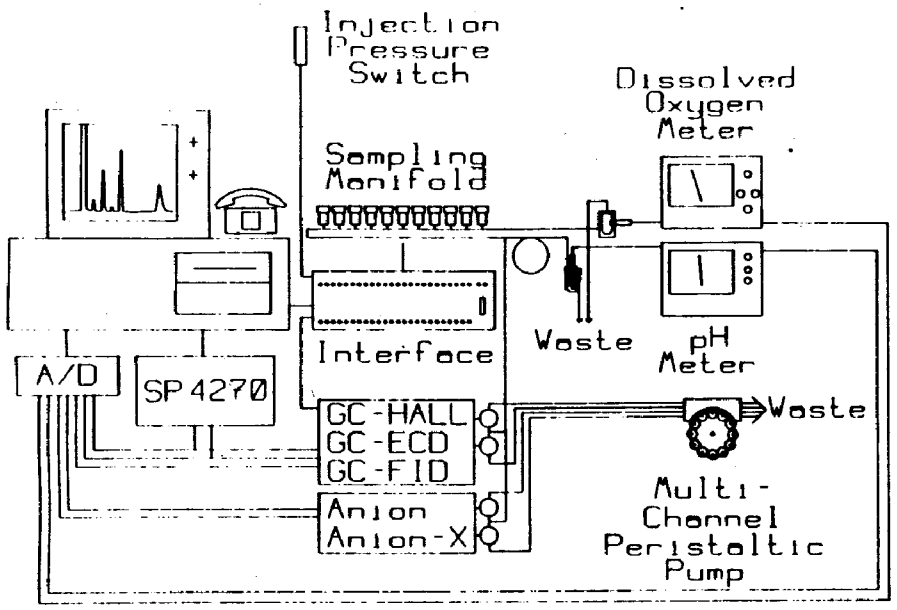

Figure 5.5. Schematic of the automated Data Acquisition and Control system.

There are three independent gas chromatographs in the DAC system, each with a different detector: an ECD, a Hall detector, and a FID. The ECD was used to measure halogenated solutes with three or more substitutions: Freon-11, Freon-113, TCA, CF, and CT. This was our primary detector and produces the most stable measurements. The Hall detector was used to measure mono- and di-substituted halogenated solutes, compounds insensitive on the ECD, which are potential CT transformation products: methylene chloride and chloromethane, and 1,1,1-TCA transformation products of chloroethane and 1,1-dichloroethane. The Hall detector also functioned as a backup to the ECD, since it is capable of measuring the same compounds as the ECD. The FID channel monitored for methane production, should methanogenic conditions be induced in the field.

The DAC system had two ion chromatographs (ICs) on line, one for the analysis of bromide, nitrate, and nitrite (standard IC column), and another for the analysis of acetate analysis (ion-exclusion column). Both ICs used conductivity detectors. The probes in the system were a dissolved oxygen probe and a pH probe. A thin piece of pure silver metal sheet was also installed in the dissolved oxygen probe's plexiglass flow-through cell to detect for sulfide production, if sulfate-reducing conditions were promoted.

Table 5.2 lists the major components measured, the method of measurement, and the detection limits. Detection limits were one to two orders of magnitude lower than the injection concentration of CT, nitrate, or acetate.

Details of the operation of the automated system were presented by Hopkins et al. (1988) and Roberts et al. (1989) and will not be repeated here. All the analyses were performed simultaneously, with the DAC controlling the analysis and the collection of data through the Lab Master A/D board. A Spectra-Physics 4270 integrator processed the output from the GC-ECD and the GC-Hall analyses. An integrator programmed into the DAC system as a subprogram was used to process the output from the two ICs and the GC-FID.

TABLE 5.2. SUMMARY OF ANALYTICAL METHODS AND DETECTION LIMITS

Component	Method	Detection Limit
Dissolved Oxygen	Probe	0.1 mg/l
pH	Probe	NA
Anions (NO_2^-, Br^-, NO_3^-)	Ion Chromatography	0.5 mg/l
Acetate	Anion-Exclusion Chrom.	
Organic Solutes:		
(Freon-11, Freon-113, Chloroform, TCA, CT, TCE)	GC-ECD	0.5 µg/l
(Chloromethane, Chloroethane, Freon-11, Freon-113, 1,1-DCA, cis-DCE, Chloroform, TCA, CT, TCE)	GC-Hall	0.5 µg/l
Methane	GC-FID	1.0 mg/l

The data were stored in the system's database both as integrated peak areas and as computed concentrations. The storage as integrated peaks permitted recalculation of concentrations, if a calibration was in question or a peak was misidentified. The stored concentrations could then be plotted by the DAC system, providing real-time monitoring capabilities. Upon completion of the analysis, the DAC system automatically proceeded to the next sample. System interpretations could be made at this time in order to enter manual operation.

The system calibrations were performed in manual operation using external standards. The external standards for the GC calibrations required the preparation of a solution containing known concentrations of the compounds of interest in a 100-ml (Spectrum) gas-tight syringe. The chlorinated organics were then added to the syringe solution as standards dissolved in methanol. The standard syringe solution was processed in the same manner as a field sample. This was accomplished by attaching the syringe to the GC sample valves, where the sample manifold normally was connected. The standard solution was then pulled through the sampling loop by the Technicon Pump, in the same manner as the field sample. Similarly, the ICs were calibrated with a standard solution fed at the point where it normally connects to the sample manifold. During normal operation the system was calibrated several times a week. The system was also calibrated after maintenance of the analytical equipment.

The Analytical and Injection Systems Performance

The DAC system performed quite well during the field evaluation. The DAC system was operated approximately 112 days and produced approximately 74,000 individual data points.

The DAC system's performance depends on both the ability to make reproducible measurements and to maintain constant injection concentrations. Thus, an analysis of the injection concentrations during the experiments permits the injection system and the analytical system to be evaluated simultaneously. Table 5.3 presents statistics for the predominant compounds measured in the injected fluid during the initial transport experiment (Tracer14). In this test bromide and CT are being added to the injected fluid. The other compounds shown are background chemicals in the extracted groundwater that is reinjected. For completeness, acetate, which was added in the latter biotransformation experiment, is included in Table 5.3.

The inorganic compounds, bromide and nitrate, measured on the IC were very reproducible: sample coefficients of variation (CV) were less than 10% [(standard deviation/mean) × 100%]. A large number of measurements were collected during the experiment. Since the standard error of the mean is calculated by dividing the sample standard deviation by the square root of the number observations, the standard error of the mean for these observations would be a factor of 10 lower than the standard deviations shown, with an average CV of less than 1%.

The acetate measurement was obtained using a different separation column and detector. The CV is shown to be in the range of that for the bromide and nitrate analyses. The lower number of observations included in the analyses results from two factors: 1) the pulsed nature of its addition in the experiment, and 2) its controlled increase in concentration during the experiment.

The next group of compounds in Table 5.3 are the halogenated aliphatics, measured by GC using ECD detection. Carbon tetrachloride values are presented for two different injection concentration levels. The measurements are shown to be very reproducible (CV of 11% or less), which is quite exceptional for an automated GC analysis. The CV for CT, the spiked organic solute, is in the same range as Freon-113 and TCA, the native contaminants; this shows that the injection system is capable of producing stable injection concentrations for prolonged periods.

TABLE 5.3. REPRODUCIBILITY OF INJECTION CONCENTRATION AS MEASURED IN TRACER14 EXPERIMENTS

Compound	Mean	Std. Dev.	No. of Obs.	CV(%)
IC (mg/l):				
Bromide	67.5	3.3	124	5.01
Nitrate	24.7	2.46	185	9.96
Acetate	364	20	11	5.5
ECD (µg/l):				
Freon-113	5.91	0.65	192	11.0
TCA	50.2	4.38	196	8.22
CT	74.8	7.09	112	9.48
CT	45.3	2.90	67	6.40
Hall (µg/l):				
Freon-113	9.52	1.58	196	16.6
TCA	57.3	8.81	196	15.4
CT	87.2	12.6	126	14.4
CT	46.1	6.72	64	14.6

The third group is the same data set discussed above but produced by GC analysis with a Hall detector. The most striking difference in the two data sets is the higher CV obtained using the Hall detector. This probably results from the more complex series of steps required by the Hall detector, which entails chemical reactions. Thus the Hall detector is more difficult to maintain during continuous field operation.

The Hall measurements also tend be higher than the ECD. In the case of Freon-113, this results from the co-elution of Freon-113 and 1,1-DCE, both background contaminants in the field. The Hall detector is more sensitive to 1,1-DCE than the ECD. Hence, the tabulated values, which represent both Freon-113 and 1,1-DCE, are higher and, therefore, the ECD measurements are more representative of Freon-113 concentration. Comparison of means for ECD and Hall detection for TCA and CT at the high concentration, using a z-test at the 95% confidence level, shows the means are significantly different. At the lower CT concentration level, the means are not significantly different. These data indicate that the ECD detection was not linear at the higher concentrations. Instrument calibrations showed the detector response for CT was linear over the concentration range of 0–50 µg/l. Thus the CT biotransformation studies were performed at concentrations below 50 µg/l.

Normalizing Data

Some of the variability in measured concentrations at the field site results from instrument variations due to diurnal temperature fluctuations. These variations have been demonstrated by Hopkins et al. (1988) and Roberts et al. (1989). One method that has been demonstrated for reducing variations during the analysis of the data is to normalize the data at observation wells by dividing them by previously measured injection concentrations (C/C_0), where C_0 is the injection concentration. The normalization procedure provides a means of 1) showing the degree of fractional breakthrough at observation locations, and 2) determining the extent of transformation that results during transport through the biostimulated zone.

Table 5.4 compares the coefficients of variation (CV) at observation wells S1, S2, S3 for the measured concentrations (C) (raw data) and the normalized concentrations (C/C_0). Coefficients of variation are shown for Freon-113, TCA, and CT from both the ECD and the Hall detectors, and for bromide and nitrate from the IC analyses. Normalization results in significant reductions in the coefficients for all the analyses. Reductions in CV averaged 40% for ECD analysis, and 50% for the Hall analysis. For IC analysis, CV were reduced by approximately 20%. The reduction in the CV by normalization, combined with the large number of observations available for averaging, results in small errors when estimates of the degree of biotransformation of a chemical are made in the field evaluations.

TABLE 5.4. COMPARISON OF COEFFICIENTS OF VARIATION FOR RAW MEASURED CONCENTRATIONS (C) AND NORMALIZED CONCENTRATIONS (C/C_0)

	Well	Chemical	C CV (%)	C/C_0 CV (%)	No. of Obs.
ECD Detector					
	S1	Freon-113	7.91	5.16	39
	S2	Freon-113	7.81	5.81	39
	S3	Freon-113	7.36	6.79	36
	S1	TCA	9.34	2.83	39
	S2	TCA	9.02	4.03	38
	S3	TCA	8.36	2.64	36
	S1	CT	7.27	4.19	23
	S2	CT	7.49	4.70	23
	S3	CT	6.05	4.09	21
Hall Detector					
	S1	Freon-113	14.46	6.53	39
	S2	Freon-113	17.55	9.82	38
	S3	Freon-113	14.02	6.06	36
	S1	TCA	17.33	7.73	39
	S2	TCA	18.35	10.01	38
	S3	TCA	16.09	8.40	36
	S1	CT	14.82	7.89	23
	S2	CT	15.45	9.09	23
	S3	CT	12.59	5.08	21
I.C. Detector					
	S1	Br	5.44	3.95	41
	S2	Br	5.44	4.28	38
	S3	Br	4.75	4.36	36
	S1	Nitrate	10.13	7.20	95
	S2	Nitrate	10.37	8.83	88
	S3	Nitrate	10.68	8.47	86

SUMMARY

Methods are presented for the field evaluation consisting of a series of stimulus-response experiments. Modifications to the automated Data Acquisition and Control system were made, which permitted the controlled addition of the chemical of interest into the test zone, and real-time monitoring of their concentrations at the key sampling points. The quality assurance checks demonstrated that the automated Data Acquisition system was capable of providing a large number of measurements of high precision. The injection system used in the field tests delivered controlled amounts of chemicals, which were required in the series of stimulus-response experiments that were to be performed. Overall, the chemical delivery, sampling, and analysis procedures assured a degree of precision and accuracy that is adequate for the quantitative comparisons necessary to assess the representativeness of this biotransformation process.

The chemical delivery system was shown to be capable of maintaining constant injection concentrations of CT for extended periods of several weeks, or longer. The sampling and analysis procedures for the halogenated compounds resulted in coefficients of variation in the concentration measurements of 15% or less. This low coefficient of variation combined with the high frequency of analyses permitted assessments of minimum concentration changes of approximately 5% in the experiments. This insured us that the experimental system was capable of tracking the biotransformation of the halogenated compounds in the field experiments.

SECTION 6

RESULTS OF THE FIELD EXPERIMENTS

In this section the results of the field evaluation experiments are presented. As discussed in the experimental methodology (Section 5), the experiments were performed in a series of stages in order to provide a convincing and controlled evaluation of the proposed method of in-situ biotransformation of carbon tetrachloride (CT) and the other target compounds. The results from the four stages of the test are presented, along with an interpretation of the results, including comparisons with the results of the laboratory column studies (Section 4) and previous laboratory research.

Table 6.1 presents the experiments that were performed, the chemicals injected, and the processes studied. The first experiment involved a tracer test along the north experimental leg. The purpose of this experiment was to determine whether this leg, which had not been used in previous biostimulation experiments at the site, could be used for the evaluation. The results of this test indicated that in order to achieve effective capture of the injected fluid at the extraction well, high extraction rates were required that resulted in excessive drawdown at the extraction well. Based on this evidence, the south experimental leg that had been used in previous biostimulation experiments, was chosen for the present field evaluation due to its favorable hydraulic properties.

The Tracer14 test, and subsequent biostimulation-biotransformation tests, were performed using the south experimental leg that included the SI injection well, observation wells S1, S2, S3, and the extraction well P (Figure 5.1). In the Tracer14 test, bromide and CT were dissolved in recycled groundwater that was continuously injected under the induced gradient conditions of the subsequent biostimulation-biotransformation experiment. The transport of CT compared with bromide was studied. The experiment also served to saturate the test zone with CT, and thus served as a pseudo-control to determine whether biotransformation occurred in the absence of active biostimulation.

The Biostim4 experiment was the biostimulation-biotransformation test. The test zone was stimulated through the addition of acetate as a primary substrate for growth. Nitrate (present in the recycled groundwater) and CT were continuously injected into the test zone, while acetate was injected in short, high concentration pulses. The biostimulation of the test zone and the resulting biotransformation of the target compounds were monitored, along with the formation of intermediate products. In the latter stages of the Biostim4 experiment, nitrate was completely removed from the injected water by a surface bioreactor fed with acetate. The response of the target compounds in the test zone, when no nitrate was added, was monitored to observe whether enhanced transformation resulted.

TABLE 6.1. EXPERIMENTS CONDUCTED AND PROCESSES STUDIED

Experiment	Duration	Chemicals Injected	Average Concentration (mg/l)	Processes Studied
Tracer13	6/22-7/15/89 (560 hrs)	NO_3^-[a]	24 ± 5	Transport of fluid along the north experimental leg.
		Br^-	51±3	
Tracer14	8/9-8/31/89 (528 hrs)	NO_3^-	25±3	Transport of CT and bromide along the south experimental leg. Studied the processes of advection, dispersion, retardation, and transformation in the absence of biostimulation.
		Br^-	68±3	
		CT[b]	0.075±0.007	
		CT	0.045±0.003	
Biostim4 TEST1	9/09-11/15/89 (0-1260 hrs)	Acetate[c]	25 to 46	Biostimulation of a denitrifying population and biotransformation of CT, Freon-11, Freon-113, and TCA in response to biostimulation.
		Nitrate	22±3	
		Br	43±4	
		CT	0.039±0.012	
		Freon-11	0.0029±0.0003	
		Freon-113	0.0062±0.0005	
		TCA	0.051±0.004	
TEST2	(1260-1585 hrs)	Acetate[b]	12	Biotransformation in the absence of nitrate addition to the test zone.
		Nitrate	0	
		all others the same as TEST1		
Monitor1	4/ -4/ /90	None		Transformation of background contaminants 5 months after acetate addition was stopped.

[a]As NO_3^-.
[b]CT injected in two concentration steps.
[c]Pulse-averaged injection concentrations based on injecting a high-acetate concentration for only 1 hr of a 13-hr pulse cycle period.

In the final test, Monitor1, monitoring data from the test zone were collected five months after acetate addition was stopped. The goal of this monitoring was to determine whether transformation of background target contaminants continued after this prolonged period without addition of growth substrate.

RESULTS OF THE TRACER13 TEST

The Tracer13 test was performed as part of the test zone selection process. Results of past tracer tests at the field site (Roberts et al., 1989) revealed that a strong native flow component with a velocity of 1 to 2 m/d in a northerly direction at the field site. In past studies, in order to effectively capture the injected fluid at the extraction well, the fluid was injected upgradient at the

SI well and extracted at the P well 6 m downgradient. Over 95% of the injected fluid was captured by the extraction well when fluid was injected at a rate of 1.5 l/min and extracted at 10 l/min These operating conditions also resulted in the complete breakthrough of the injected fluid at the two closest monitoring wells, S1 and S2. Thus, the south experimental leg used in our previous experiments with methanotrophic bacteria had been biostimulated through the addition of methane and oxygen in three successive field seasons.

In the current evaluation it was desired to use an experimental leg that had not been previously biostimulated. The north experimental leg was available for this purpose. However, the injection at the NI well and extraction at the P well required injecting and extracting in the direction opposite to that of the natural gradient. The bromide tracer test (Tracer13) was performed to assess whether effective fluid capture could be achieved when operating in this manner.

The Tracer13 test was performed by injecting dissolved bromide tracer as a continuous pulse into the NI well at a rate of 1.0 l/m and extracting at a rate of 16 l/min at well P. The breakthroughs of the bromide tracer at the N1, N2, N3 observation wells and the extraction well were monitored.

The results of the Tracer13 test are summarized in Table 6.2. The residence times in the test zone, based on the time to achieve 50% breakthrough of the tracer, were fairly short, ranging from 4 to 20 hrs. The degrees of fractional breakthrough at the N1 and N2 wells were also unity, indicating complete permeation of the test zone in these areas by the injected fluid; however, significant dilution of the bromide tracer was observed at the N3 well. Mass balances on the amounts of bromide injected and extracted showed approximately 80% of the bromide was captured by the extraction well. It was desired for later experiments, when CT was added, that a recovery of 90% or greater be achieved.

With prolonged extraction at a rate of 16 l/min, drawdown below the extraction well screen occurred. This was first indicated by the presence of air bubbles in the extracted water and irregular extraction rates. The excess drawdown was later confirmed using a conductivity probe that measured the water level in the extraction well.

There are several possible reasons for the excessive drawdown at the extraction well. Pump tests performed in the initial characterization of the test zone (Semprini et al., 1988) did not show excessive drawdown when extraction rates of 20 l/min were applied. The following years of 1987 and 1988 were drought years in California. The piezometric level in the test zone in 1989 was approximately 50 cm lower than in earlier years. Also the biostimulation of the test zone in the

TABLE 6.2. SUMMARY OF RESULTS OF THE TRACER13 EXPERIMENT

Well	Residence Time[a] (hr)	Fractional Bromide Breakthrough
N1	4.2	1.00 ± 0.05
N2	12.0	1.00 ± 0.05
N3	14.5	0.85 ± 0.05
Extraction		0.090 ± 0.015

[a]Based on time to 50% fractional breakthrough.

previous methanotrophic studies may have resulted in some biological fouling of the area surrounding the extraction well, resulting in a loss of permeability. These combined factors may have resulted in the greater drawdown, prohibiting the use of the north experimental leg for the CT experiments.

The evaluation experiments were therefore performed along the south experimental leg. There are both advantages and disadvantages to using the south experimental leg. The main disadvantage is that this leg had been used in our previous experiments with methanotrophic bacteria. As a result, the zone was perturbed and was not representative of the initial concentrations of the native microorganisms. There are, however, distinct differences in the microbial processes involved in the two different studies. The methanotrophic process was an aerobic process, while the current process is an anaerobic one. The methanotrophs stimulated in the past experiments do not effectively degrade CT. They also require active methane utilization and oxygen in order to transform the chlorinated aliphatics, neither of which were present in the current evaluation. Thus methanotrophs *per se* should not contribute to CT transformation. The previous growth of methanotrophs might affect the evaluation since they would provide a source of substrate to other organisms via their decay. Microbial populations that could grow on the decay products might be enhanced over that present in the native aquifer. Thus, a more rapid biostimulation of the test zone upon the addition of the growth substrates might result, compared to that with the native aquifer.

It is unlikely that the stimulation of the methanotrophic bacteria eliminated microbes present in the native aquifer. If microbes were eliminated, they may have been reintroduced by the advective transport of groundwater through this highly transmissive test zone during the nine months between the two studies. During this period at least 50 pore volumes of groundwater flowed through the test zone, possibly permitting the reintroduction of native microbes.

There are several advantages to using the south experimental leg. The hydraulic characteristics were established in our previous work at the field site, permitting comparisons with the data collected in the present study. For instance, the transport of CT may be compared with that of other chlorinated aliphatics previously studied, such as TCE and vinyl chloride. Knowledge of results from previous studies also permits quick identification of spurious results. Another advantage in using the same experimental zone is that an evaluation can be made on whether different microbial processes could be applied in the same aquifer. This might be an effective means of remediating an aquifer contaminated by a mixture of chlorinated aliphatic compounds or by highly chlorinated compounds, where both anaerobic and aerobic processes are required for complete degradation.

RESULTS OF THE TRACER14 TEST

The Tracer14 test was initiated to study transport of CT and the conservative tracer (bromide). The experiment served to saturate the aquifer to CT injection concentrations before the start of the biostimulation experiment. The experiment established whether transformation of CT occurred in the absence of active biostimulation. The formation of chloroform (CF) as a potential intermediate product of CT transformation was tracked as an indicator of transformation. The concentrations of background contaminants, TCA and Freon-113, were also monitored to establish their concentration distribution in the test zone prior to biostimulation.

Groundwater amended with the chemicals of interest was continuously injected at a rate of 1.5 l/min into the SI injection well and extracted at a rate of 10 l/min at the extraction well P (Figure 5.1). The concentrations of the chemicals injected are given in Table 6.1.

Bromide was injected during the initial 350 hrs of the test, after which injection was stopped and bromide elution from the test zone was monitored in order to compute mass balances. Nitrate, contained in the native groundwater, served as the primary electron acceptor in the latter biostimulation experiments.

Figure 6.1 shows the breakthrough of bromide and CT at the S2 observation well during the first 300 hrs of the experiment. The delayed breakthrough of CT compared to the bromide tracer results from retarded transport due to sorption of CT onto the aquifer solids. The bromide tracer obtained complete breakthrough at the S2 well after 200 hrs of injection, demonstrating complete permeation of the injected fluid at the observation locations. The CT showed a slow approach towards complete breakthrough: extended tailing was observed at later times, as was observed in our previous transport studies with TCE and cis- and trans-DCE. In order to decrease the time required to achieve steady state, the injection concentration of CT was lowered from 75 to 45 µg/l after 350 hrs (Figure 6.2).

The breakthrough of CT at different observation locations, along with the response to the lowering of the injection concentration, is shown in Figure 6.2. The breakthrough during the first 300 hrs show the characteristic spatial dependence: the response at the nearest well, S1, precedes the farther wells, S2 and S3. The S3 well response was consistent with that in our previous studies; the initial breakthrough preceded that at the S2 well which is closer to the injection well but the S2 and S3 wells show similar degrees of breakthrough at longer times. A similar response was observed for the bromide breakthrough (Figure 6.3). These data and previous test data demonstrate that aquifer heterogeneities exist at the field site, and as a result the S3 well intercepts a zone that more rapidly transmits fluid than for the S2 well.

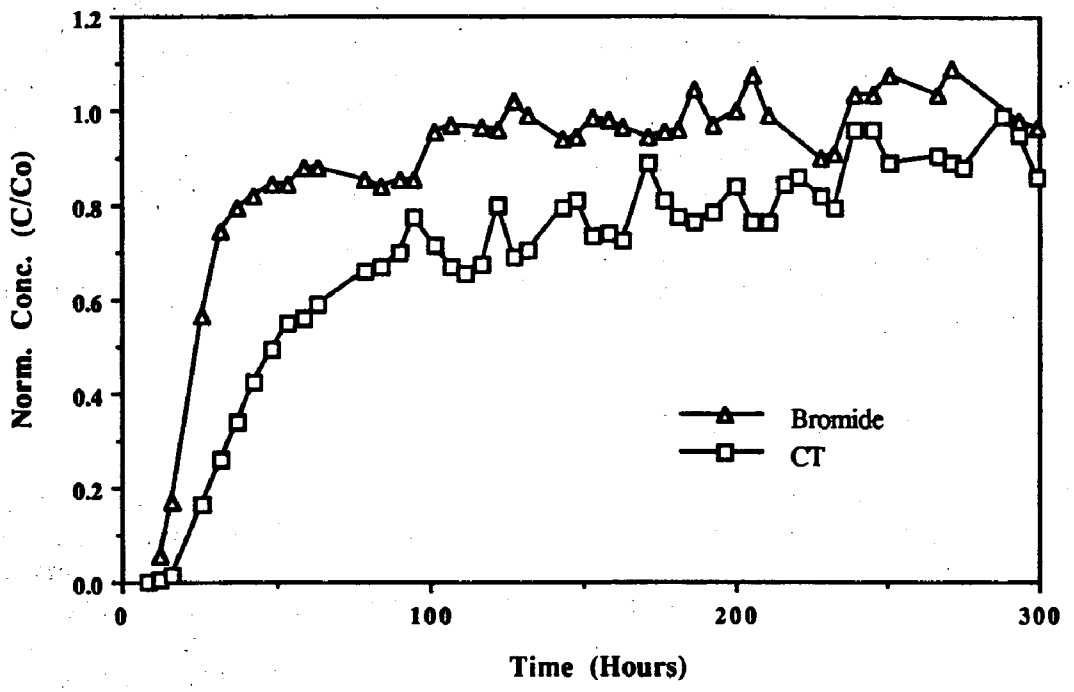

Figure 6.1. Normalized breakthroughs of CT and bromide at the S2 well in the Tracer14 experiment.

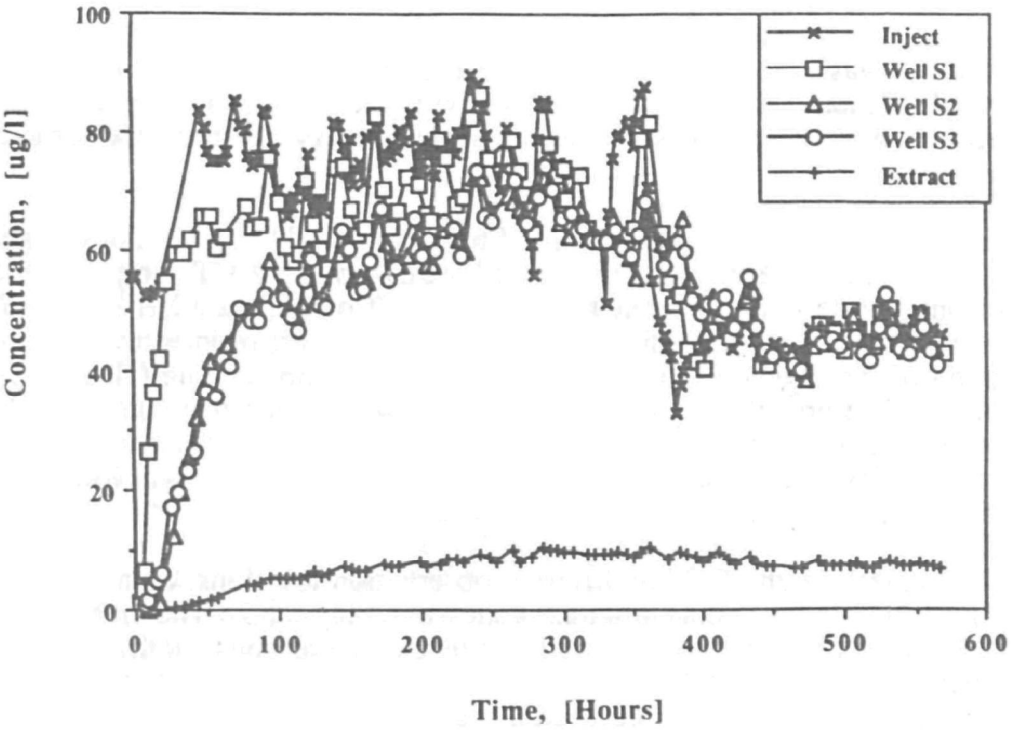

Figure 6.2. Concentration response at the observation wells with 75 µg/l CT added initially, followed by reduction in injection concentration to 45 µg/l after 350 hrs.

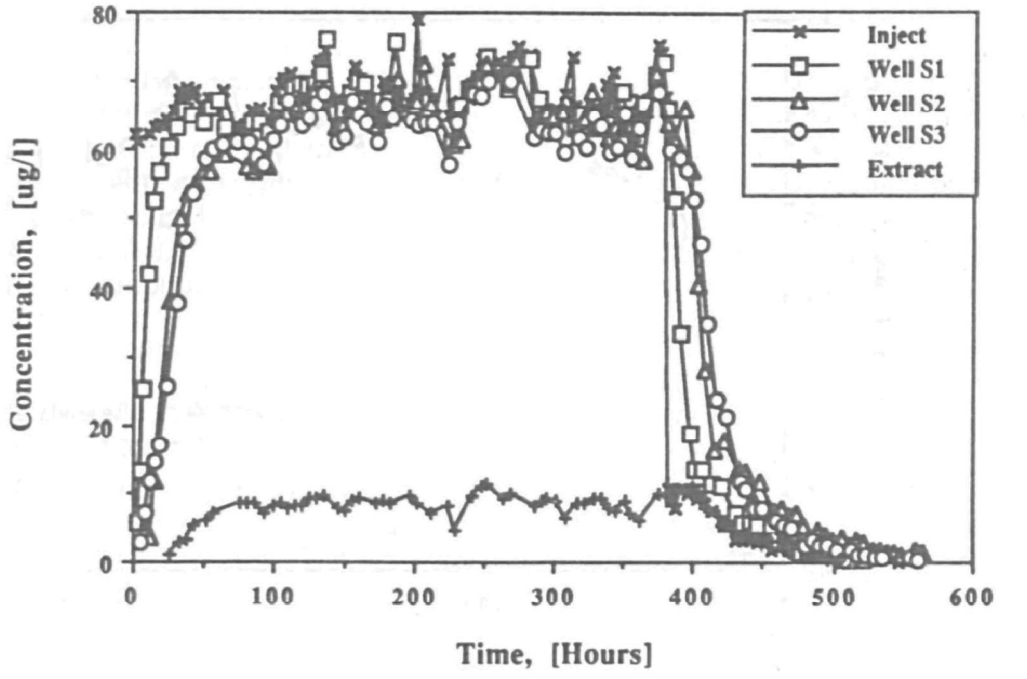

Figure 6.3. The Tracer14 bromide tracer breakthrough and elution response at the observation wells.

The wells responded similarly to the reduction in CT injection concentration; all observation well concentrations rapidly decrease towards the lowest injection concentration between 400 and 530 hrs. Thus, the two-step equilibration method used was effective for rapidly saturating the aquifer to the injected concentration levels. The lower observed concentration at the extraction well results from dilution by the native groundwater, due to the extraction rate being seven times greater that the injection rate.

The CT data in Figure 6.2 show the effects of diurnal temperature fluctuations on instrument performance, with diurnal cyclic variations, causing measured concentration variations of about 10 µg/l. These variations are dampened by the normalization procedure used (described in Section 5), as indicated by the normalized concentration profiles shown in Figure 6.1.

The bromide response at the observation wells is shown in Figure 6.3. Bromide achieved fractional breakthroughs near unity at the observation wells, indicating complete permeation of the injection fluid in these areas. The breakthrough response for the S2 and S3 wells showed the earlier arrival at the S3 well, similar to that observed for CT breakthrough. The elution of bromide through the test zone, when injection was stopped, was fairly rapid with some extended tailing. Part of this tailing resulted from the recycling of extracted bromide into the injected fluid, as illustrated in model simulations by Chrysikopoulos et al. (1990).

Bromide measurements in the injected and extracted water permitted mass balances calculations for the amount of bromide captured by the extraction well. These balances indicated that 90 ± 2% of the injected bromide was captured, which is somewhat lower than recoveries achieved in previous years under the same operating conditions. The reason for this difference is not known. Previous biostimulations of the test zone may have changed the flow characteristics. The recent drought may have also reduced the regional flow, such that injected fluids may have been less directed towards capture by the extraction well. Nevertheless, recovery of 90% of the injected fluid was sufficient to allow the evaluation tests to proceed.

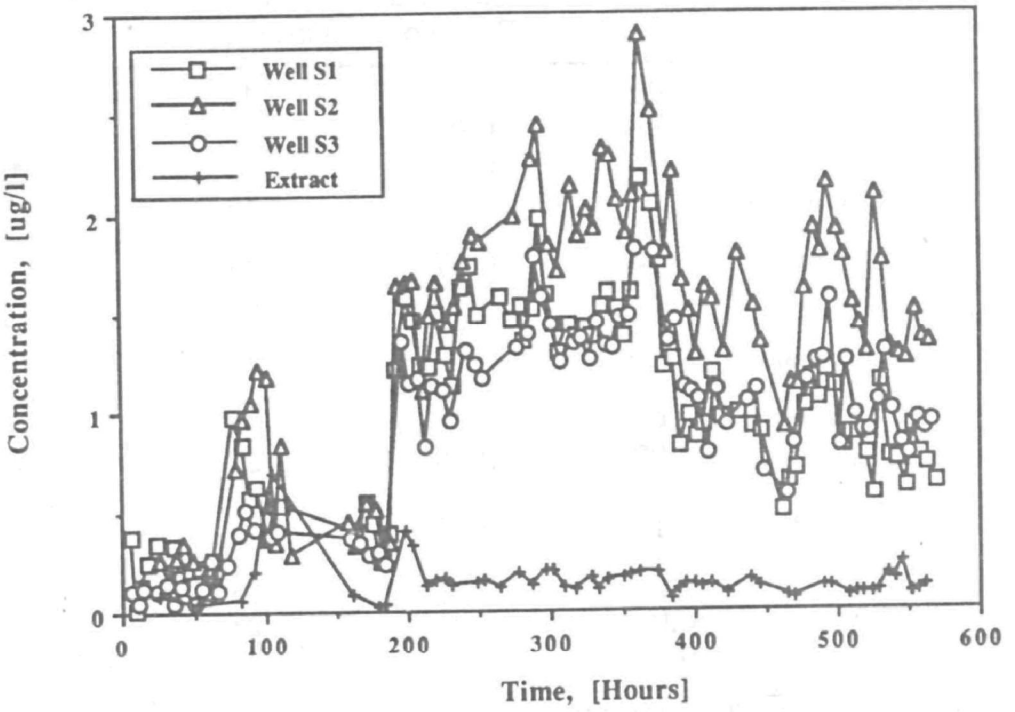

Figure 6.4. CF formed as a CT transformation product during the Tracer14 test.

During the Tracer14 experiment some CF production was observed in the test zone, as shown in Figure 6.4. Maximum CF concentrations were 2 µg/l, compared to the 45 µg/l of CT injected. During the early stages of the test (first 150 hrs), the CF data have greater associated error, since some CF was present in the water used to rinse the sampling manifold. This problem was corrected after approximately 150 hrs. Despite this earlier problem, the latter data confirm the production of CF in the test zone. The concentration at the S2 well was greater than at the S1 and S3 wells, indicating that the reaction proceeded to a greater extent the longer the CT resided in the test zone. The CF concentration was also observed to be dependent on the CT concentration. Upon reducing the CT injection concentration, the concentration of CF in the test zone decreased in a similar manner, as shown in Figure 6.2. The decrease was shown to be significant at the 5% confidence level, using a t-test for uncorrelated means.

The CF produced represents, on a molar basis, approximately 3 to 4% of the CT added; thus the extent of the formation was small but significant. The production of CF suggests that some of the CT was transformed during this period, but the amount was too small to be quantified from observations of CT decreases. In addition, we cannot distinguish between biological transformations and abiotic transformations. Nitrate concentrations in the test zone during the Tracer14 test (Table 6.3) showed a 10% reduction compared to the injection concentration, indicating approximately 3 mg/l of nitrate utilization. The degree of nitrate consumption was consistent with CF formation, with more nitrate utilized and more CF formed at the S2 well than at the S1 and S3 wells. The nitrate consumption indicated some biological activity was present, which may have been sufficient to promote a minor amount of CT transformation.

Table 6.3 contains the normalized breakthrough data for bromide, nitrate, and CT during periods of the experiment when near steady-state breakthrough concentrations were achieved at the monitoring locations.

TABLE 6.3. NORMALIZED VALUES FROM TRACER14

Chemical	Well	Mean	Standard Deviation	No. of Observations	Coefficient of Variance (%)
Bromide[a]	S1	1.00	0.04	41	4.0
	S2	0.98	0.04	38	4.3
	S3	0.94	0.04	36	4.4
	Extraction	0.13	0.02	35	14
Nitrate	S1	0.94	0.07	88	
	S2	0.91	0.08	81	
	S3	0.96	0.08	79	
	Extraction	1.03	0.07	72	
CT[b]	S1	0.98	0.04	23	4.2
	S2	0.99	0.05	23	4.7
	S3	0.98	0.04	21	4.1
	Extraction	0.16	0.01	22	8.6
CF[c]	S1	0.03	0.01	23	18
	S2	0.05	0.01	23	19
	S3	0.04	0.01	21	14

[a]Bromide averages over 120 to 380 hrs.
[b]CT averages over 340 to 565 hrs.
[c]Mole fraction of CF observed compared to injected CT.

Nearly complete bromide breakthroughs were observed at the S1 and S2 monitoring wells, with some dilution by native groundwater observed at the S3 monitoring well. Breakthroughs of CT were also near unity for the S1, S2, and S3 observation wells, suggesting minimal transformation of CT during transport through the test zone. The CF fractional breakthroughs shown have been normalized by dividing the molar concentrations of CF observed by the molar injection concentration of CT. The addition of the CF values to the CT values yielded mass balances slightly greater than 100%. The mass balances of greater than 100%, however, are within the measurement uncertainty.

Table 6.4 summarizes the transport characteristics of the test zone. Bromide tracer tests indicate fluid residence times in the range of 8 hrs for the S1 monitoring well, located one meter from the injection well, to 24 hrs for the second monitoring well, located 2.2 m from the injection well. These residence times agree with those of earlier tests (Roberts et al., 1989). Carbon tetrachloride residence times are longer, due to sorption onto the aquifer solids. Retardation factors were estimated by dividing the CT residence time by the bromide residence time. Carbon tetrachloride is estimated to be retarded by factors ranging from 1.5 to 2. These retardation factors are considered to be lower estimates of the degree of retardation since they were based on the initial residence times required to achieve 50% fractional breakthrough, and therefore they do not account for the effect of slow sorption and extended tailing in the breakthrough curve.

TABLE 6.4. TRACER14 ESTIMATED RESIDENCE TIMES AND RETARDATION FACTORS

Location	Time to 50% Bromide Breakthrough (hr)	Time to 50% CT Breakthrough (hr)	Estimated Retardation Factor (T_{CT}/T_{Br})
S1	8	12	1.5
S2	24	44	1.8
S3	28	57	2.0

The Tracer14 CT retardation values can be compared to values estimated from batch sorption studies performed with aquifer solids. Figure 6.5 shows the results of batch sorption studies with pulverized solid samples performed using the method described by Curtis (1984) and Ball (1989). The slope of the isotherm gives a K_d of 1.0 l/kg. The retardation factor can be estimated based on the measured K_d using the following relationship of Hashimoto et al. (1964):

$$R = 1 + \frac{\rho_b}{\theta} K_d \qquad (6\text{-}1)$$

based on an aquifer porosity (θ) of 0.33, and a bulk solids density (ρ_b) of 1.7 kg/l, a retardation factor of 6.0 is estimated. The laboratory estimated K_d is thus a factor of 3 greater than that estimated from the field test.

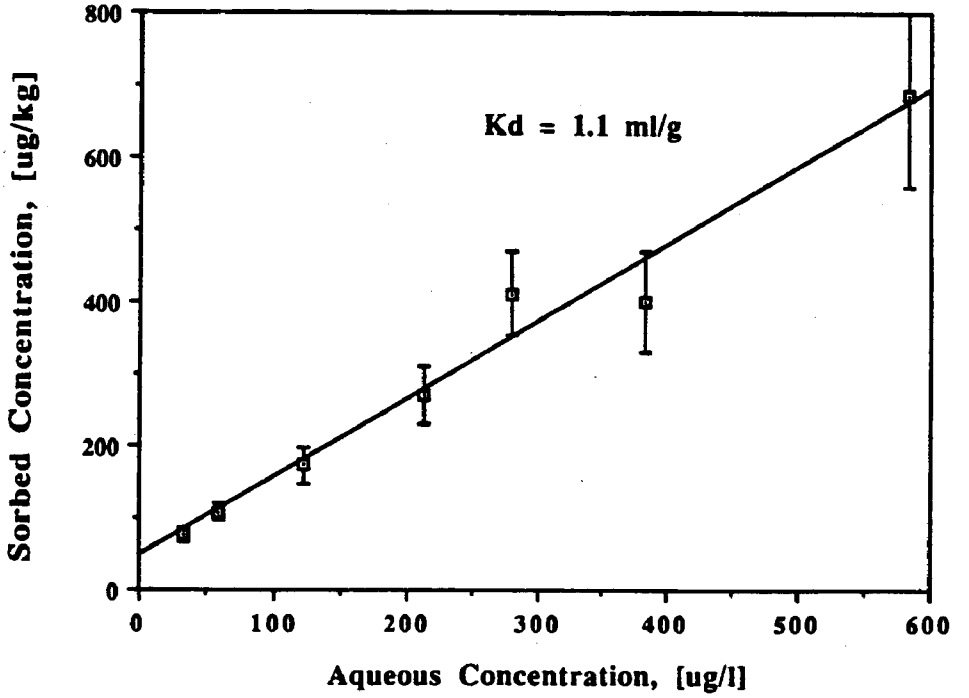

Figure 6.5. Ten-day batch sorption isotherm for CT onto Moffett aquifer solids.

There are several possible reasons for the difference. The solids used in the laboratory study may not truly represent those of the test zone. This partly results from limitations in the laboratory method, where particle sizes studied are restricted to 2 mm or less. Particle sizes up to several centimeters are present in the test zone. In the laboratory study the samples were pulverized to make the measurement, thus making all the sorption sites accessible. Previous studies with the test zone aquifer solids and TCE showed continued sorptive uptake with time that is consistent with a slow diffusional processes (Harmon and Roberts, 1989). The apparent K_d for TCE sorption onto aquifer solids increased by a factor of two for a 10-day K_d compared to a 1-day K_d. The CT residence time based on the 50% fractional breakthrough method used to estimate the field retardation factor represents equilibration times of one to two days. Lower laboratory K_d, and thus lower laboratory-estimated retardation factors, would be expected for solids that are not pulverized and equilibrated for similar times as in the field test.

The field-estimated CT retardation is within the range of values measured for vinyl chloride in our earlier study. The value is much lower than that observed for TCE, which was retarded by a factor of approximately 6, based on the 50% fractional breakthrough estimation method. Batch sorption studies predicted that TCE should be more strongly sorbed that CT, consistent with our field results. The batch sorption retardation estimates for TCE were also greater than the field estimate, probably due to the same factors outlined above.

SUMMARY OF THE TRACER14 TEST

The Tracer14 test supplied important information on transport in the test zone before biostimulation with acetate addition. CT was observed to be retarded due to sorption onto the aquifer solids. CT concentrations at the monitoring locations after breakthrough indicated minimal transformation of CT in the test zone. Perhaps a few percent of the CT was transformed to CF

during transport, which is consistent with overall mass balances. Longer residence times in the aquifer resulted in greater CF production. Nitrate utilization in the test zone indicated some biological activity was present that may have promoted the minor CT transformation.

The tracer tests demonstrated that controlled experiments could be performed with CT in the test zone. The ability to saturate the test zone to near injection concentrations, and the ability to track CF as a transformation product indicated that transformation resulting from enhanced biostimulation could be monitored readily in the biostimulation-biotransformation experiment described in the following.

THE BIOSTIM4 BIOSTIMULATION-BIOTRANSFORMATION EXPERIMENT

The biostimulation-biotransformation experiment (Biostim4) was performed as outlined in Section 5. Stimulation of the test zone was accomplished by feeding acetate as a primary substrate for growth. Nitrate was present in the test zone as a potential electron acceptor at a concentration of 24 mg/l. Sulfate was also present as a potential electron acceptor at a concentration of 700 mg/l. Methods used to prepare the injected fluid are outlined in Section 5.

In the initial stages of the biostimulation experiment (TEST1) nitrate was continuously reinjected at an average concentration of 23 mg/l. Potential clogging of the area surrounding the injection well might result from the continuous injection of acetate as the growth substrate together with nitrate as the electron acceptor. A method of pulse injection of acetate was therefore employed where acetate was added at high concentrations for a short period of time of a repeated pulse cycle. Model simulations (Section 7) indicated that this pulsing would help to distribute the microbial growth and might help prevent clogging near the injection well.

The concentrations of the injected chemicals are presented in Table 6.1. Acetate was injected for 1 hr of a 13-hr pulse cycle, and was not held constant during the course of the experiment, but was intentionally varied. Figure 6.6 shows the concentration history of acetate during the course of the experiment. Acetate concentrations over the pulse-period when added ranged from 330 to 600 mg/l in the first 1260 hrs, to 150 mg/l after 1260 hrs, when nitrate was removed from the injection water through use of the surface bioreactor. The pulse-averaged injection acetate concentration ranged from 25 mg/l to 46 mg/l, in the initial stages to 12 mg/l after nitrate removal.

The induced hydraulic gradient conditions in the aquifer were the same as used in the Tracer14 experiment. Groundwater was extracted at 10 l/min and injected at 1.5 l/min. During the complete experimental period CT was continuously injected into the test zone. Thus, as time progressed, the experiment allowed evaluation of the amount of CT biotransformed through the biostimulated zone. The native background contaminants -- Freon-11, Freon-113, and TCA -- were also continuously reinjected. Since 85% of the extracted groundwater was native groundwater, concentrations of injected nitrate and the native contaminants in the injected water were not changed greatly by transformation in the test zone.

Biostimulation (TEST1)

The response of acetate, nitrate, and nitrite at the S1 well to biostimulation of the test zone is shown in Figure 6.7. Acetate increased within the first 10 hrs, and then decreased. Pulses in acetate concentration were observed resulting from the pulsed input at the injection well. At 100 hrs a decrease in acetate resulted primarily from a decrease in the injection concentration (Figure 6.6). The pulse heights after the change were attenuated largely due to to the decrease in the

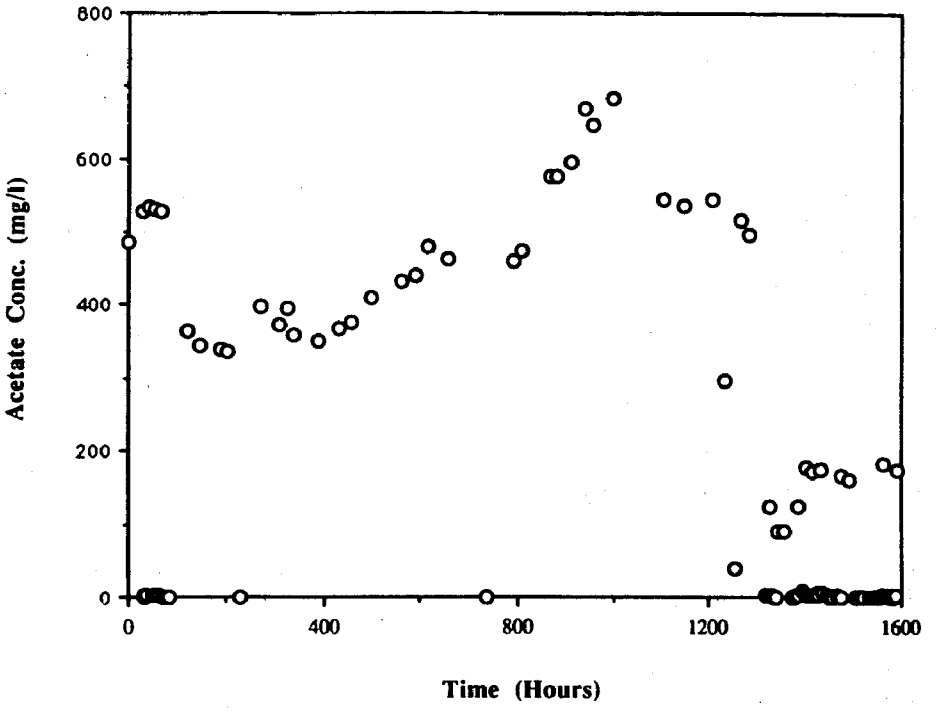

Figure 6.6. Injection concentrations of acetate over the pulse-period when added during the Biostim4 experiment.

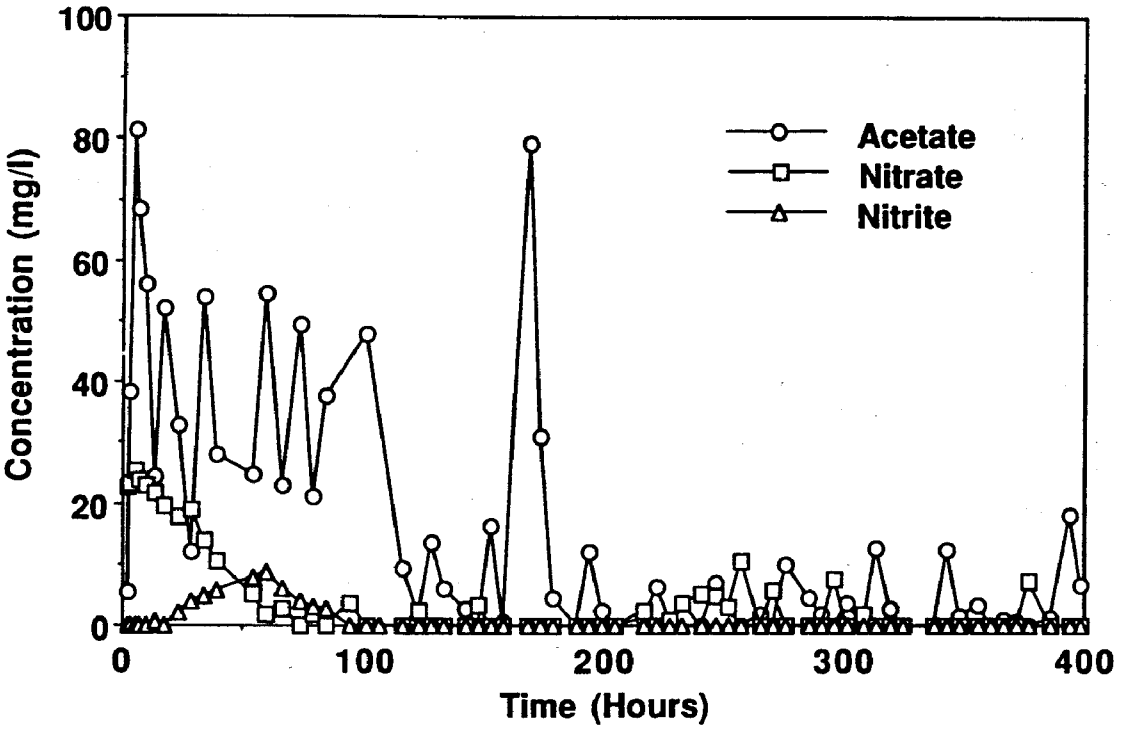

Figure 6.7. Response of acetate, nitrate, and nitrite at the S1 well, resulting from the biostimulation with acetate.

injection concentration. A spike in acetate concentration was observed at 160 to 180 hrs, owing to a malfunction in the pulse timer that permitted high acetate concentrations to be added for several hours, instead of the regular period of 1 hr.

The biostimulation of the test zone is best demonstrated by the nitrate response: a decrease in nitrate began immediately after acetate addition was started, with complete removal observed after 100 hrs. Nitrate was detected in the test zone occasionally during periods when acetate pulse concentrations were near zero. The cyclic variation in nitrate concentrations resulted from the pulse injection of acetate, as predicted by model simulations (Section 7). Another indicator of biostimulation of denitrifiers is the appearance of nitrite in the test zone (Figures 6.7 and 6.8). The production of nitrite as an intermediate in nitrate utilization was transitory. During its concentration increase in the first 60 hrs of acetate addition, nitrite represented over 50% of the stoichiometric quantity of nitrate respired. The nitrite concentrations then decreased to below the detection limit after 80 hrs, and was then most likely respired to N_2 (not measured).

The biostimulation response at the S2 well, shown in Figure 6.8, is similar to that observed at the S1 well. Pulses in acetate concentration, however, are not clearly evident here, as they were attenuated by dispersive transport in the test zone with the longer distance traveled. Such attenuation is also indicated by the decrease in concentration and the broadening of the acetate spike that occurred between 170 and 190 hrs. The appearance of this peak was delayed by 10 hrs due to the longer fluid residence time, consistent with values given in Table 6.3. The initial nitrate and nitrite responses are shown to agree very well with those observed at the S1 well. Distinct nitrate pulses were not observed, as a result of dispersive mixing, which is consistent with the acetate response at the well.

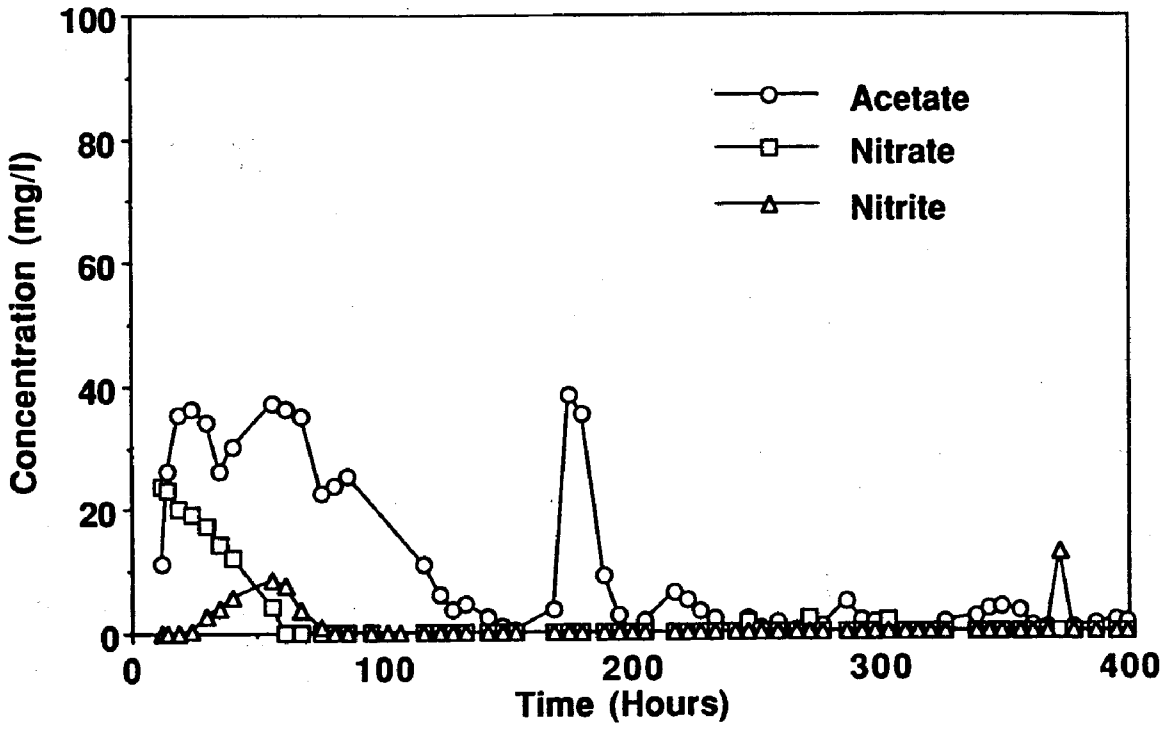

Figure 6.8. The acetate, nitrate, and nitrite response at the S2 well resulting from the biostimulation with acetate.

Fractional breakthroughs after 300 hrs of acetate addition indicated over 80% of the acetate and over 90% of the nitrate was consumed within the first meter of transport, indicating that the microbial population grew fairly rapidly. The stoichiometric ratios of nitrate to acetate consumption were approximately 1.0 mg NO_3^- per mg acetate. This value is lower than the ratio of 1.65 calculated for the complete respiration of nitrate to nitrogen gas. The lower ratio observed in the field test results from the incorporation of an estimated 40% of the acetate into cell biomass during biostimulation, which is typical (McCarty et al., 1969).

Nitrogen gas formation with distinct bubbles, however, was not observed in samples at the surface, since the complete respiration of 25 mg/l of NO_3^- would produce approximately 11 mg/l of N_2 gas, which is a factor of 2 lower than its solubility in water at atmospheric pressure and the aquifer temperature of 18°C. Nitrogen was also stripped from the injected fluid by helium gas (Figure 5.3). Thus we do not feel that gas-phase bubbles were formed to any great extent in the aquifer.

The responses presented in Figures 6.7 and 6.8 demonstrate rapid stimulation of denitrifying bacteria. The initial population of denitrifying bacteria must have been sufficient to produce the immediate uptake of nitrate observed, with the rapid decrease in nitrate concentration resulting as the denitrifying bacterial population quickly increased with time. The transitory appearance of nitrite in initial stages is typical (McCarty et al., 1969), before the increase in the denitrifying population with time results in the complete utilization of both nitrate and nitrite formed.

Biotransformation of CT (TEST1)

Figure 6.9 shows the nitrate, CT, and CF biostimulation response at the S2 well. CT transformation was not observed during the first 350 hrs despite the rapid stimulation of the denitrifying bacteria. Initial evidence of significant CT biotransformation appeared after 350 hrs of acetate addition. Over the 1250-hr period shown, CT concentrations gradually decreased, accompanied by a concomitant increase in CF concentration . Chloroform represented a significant fraction of the CT degraded. Over the period of 1160 to 1260 hrs, approximately 80% reduction of the CT was observed, with approximately 50% being converted to CF.

Figure 6.10 shows the response at the S1 well. As was the case at the S2 well, evidence of CT transformation was not observed until after acetate addition for 350 to 400 hrs. CT concentrations decreased at a much slower rate than at the S2 well, and the corresponding increase in CF was also much slower. Over the period of 1160 to 1260 hrs, approximately 31% of the CT injected was transformed during transport to the S1 well, with 66% appearing as CF.

There was a great difference between the transformation responses at the S1 and S2 wells. Transformation proceeded at faster rates at locations more distant from the injection well. This difference is illustrated in Figure 6.11, where the CT responses at all the observation locations are shown. The more rapid rates of decrease at the S2 and S3 wells, compared to the S1 well, are apparent. The S3 well response is shown to be similar to that of the S2 well. The extraction well also shows reduction of CT to the detection limit, indicating that the transformation of CT was occurring throughout the treatment zone.

The CT response indicates that the most rapid rates of transformation did not occur in the zone where most of the acetate and nitrate were consumed, but in the zones further away, where significantly less acetate and nitrate were consumed. The results indicate that the main denitrifying population stimulated in the first meter of the test zone did not participate in the transformation process to the same extent as microbes stimulated further away. This result is also supported by

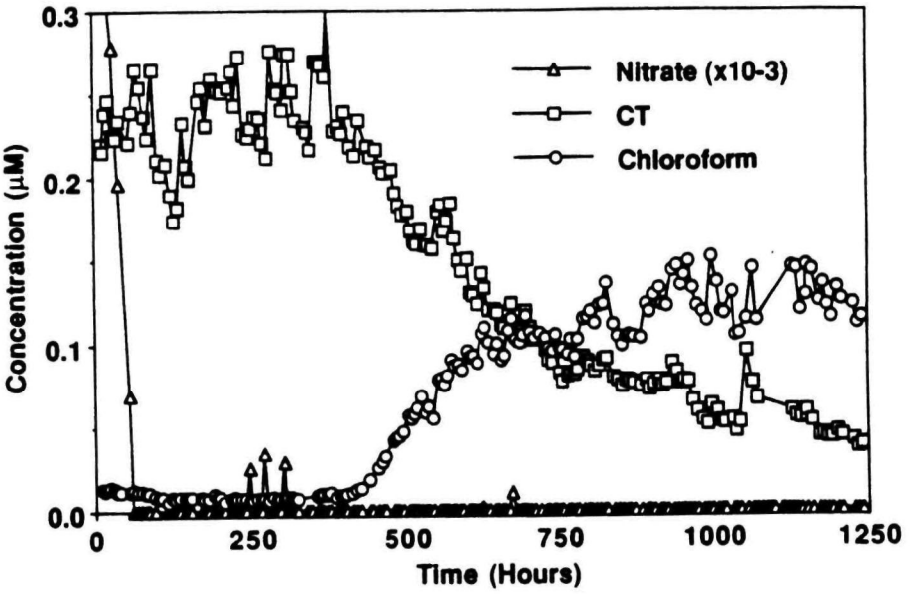

Figure 6.9. Nitrate, CT, and CF response at the S2 well for the first 1250 hrs of biostimulation with acetate.

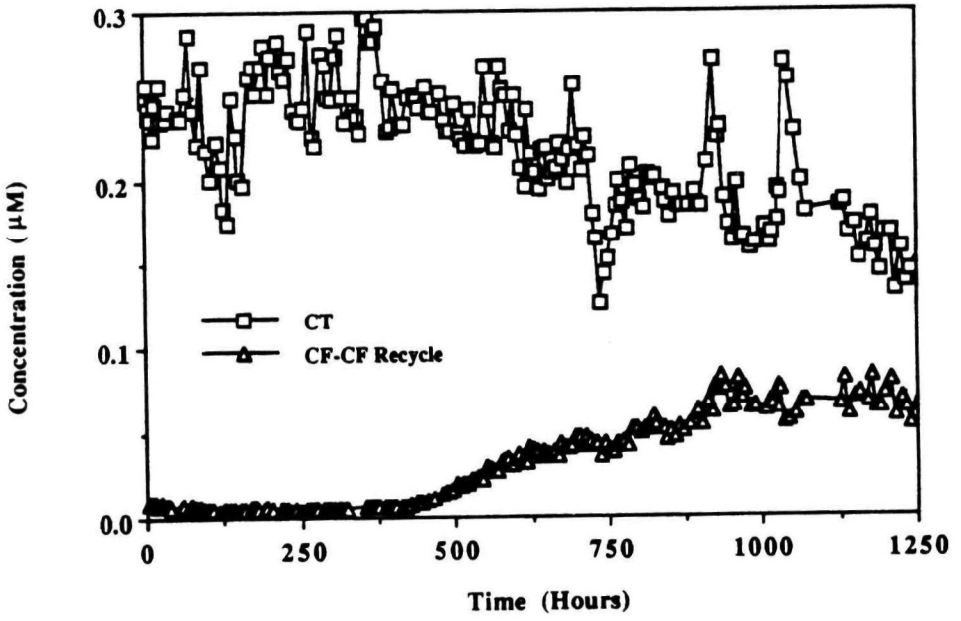

Figure 6.10. CT and CF response at the S1 well for the first 1250 hrs of biostimulation with acetate.

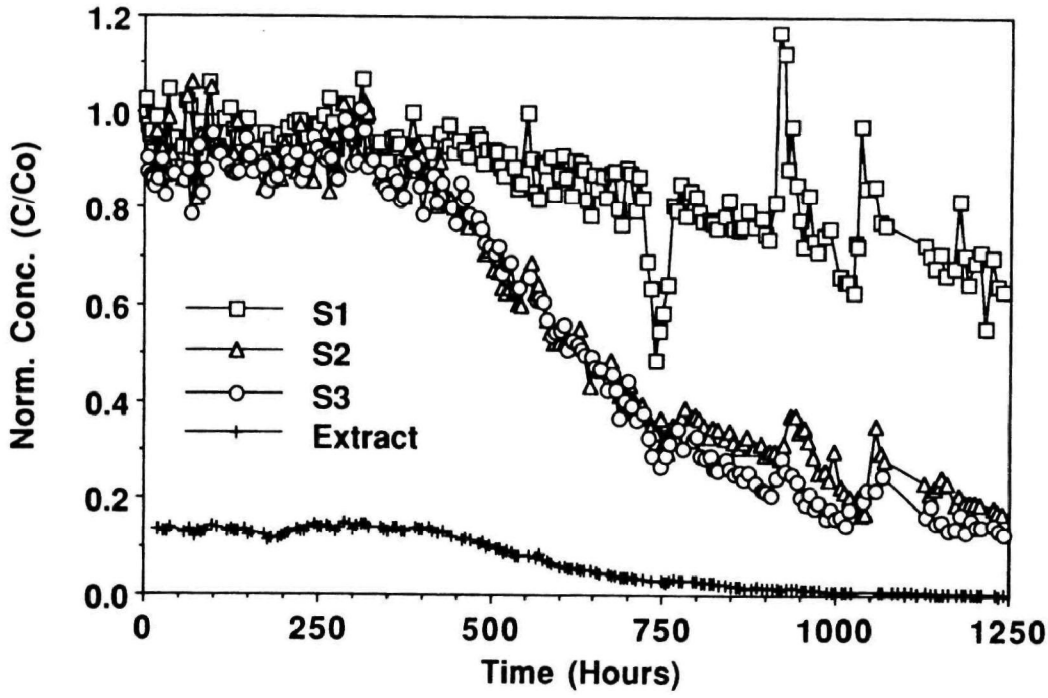

Figure 6.11. CT response to biostimulation at all of the observation wells.

the rate of nitrate utilization compared to rates of CT transformation. The much faster nitrate utilization rate indicates that transformation was not directly associated with the main population of the denitrifiers biostimulated. If this were the case, then transformation would have been observed much sooner and to a much greater extent in the first meter of transport. This observation is supported by the model simulations presented in Section 7.

One possible reason for the slow, but steady, decrease in CT concentrations is the growth of a CT-transforming microbial population that was originally at a much lower concentrations in the test zone compared to the population of denitrifiers. Biostimulation of a secondary population would have tended to occur in zones farther from the injection well. The substrate for growth of this microbial population may have been either acetate or products from the decay of the stimulated denitrifying population. The transformation of CT indicates that the growth of such a secondary population occurred in zones where nitrate was not present in high concentrations, or else that CT transformation rate was higher in the absence of NO_3^-. Perhaps the presence of nitrate inhibited CT transformation, or else nitrate prevented the growth of a secondary population for CT transformation. It may also be that redox conditions played an important role in the transformations, and the absence of nitrate resulted in more reducing conditions, that effected better CT transformation. Model simulations presented in Section 7 show field observations are better matched with the secondary population being mainly responsible for the transformation, and not by the stimulated denitrifiers.

BIOTRANSFORMATION IN THE ABSENCE OF NITRATE (TEST2)

The effect that nitrate had on the biotransformation of CT was evaluated in experiments where nitrate was removed completely from the injected fluid through use of a surface bioreactor. The goal of the experiments was to determine whether more effective transformation of CT could

be achieved in the first meter of the test zone, and whether a change might occur in the fraction of the CT transformed to CF. Nitrate removal at the surface was achieved using a bioreactor fed acetate. A detailed description of the bioreactor operation is presented in Section 5. Analyses for halogenated aliphatics showed no evidence for their transformation in the surface bioreactor. This may also have resulted from the slow growth of a secondary population in the bioreactor.

The transient nitrate removal experiment (TEST2) was initiated after 1260 hrs of acetate injection. Upon removing nitrate from the injected fluid the pulse-injected acetate concentration was lowered to 150 mg/l, while maintaining the previous pulse duration of 1 hr of acetate addition in a 13-hr pulse cycle. The pulse-averaged injection concentration here was approximately 12 mg/l. During the transient experiment, CT was continuously injected at the same concentration as in the previous experiment.

Figure 6.12 shows the response at the S1 well for CT and CF to the removal of nitrate from the injected fluid at 1260 hrs. A significant decrease in CT concentration was observed over the 300-hr period from 1280 to 1580 hrs. This decrease indicates a significant enhancement in CT transformation when nitrate was omitted. The CF concentration did not increase to the same extent as the CT decreased. This indicates either that less CF was being formed, or that CF was also being degraded at significantly in the test zone.

The response at the S2 well to nitrate removal is shown in Figure 6.13. A continued decrease in CT concentration occurred over the period from 1300 to 1580 hrs. The concentration appeared to decrease at a faster rate than during the period prior to nitrate removal. Part of the decrease resulted from the reduction in concentration within the first meter, resulting from the increase in the rate of transformation in that area of the test zone. The CF concentration shows a slight decrease in concentration with time despite the greater amount of CT being transformed.

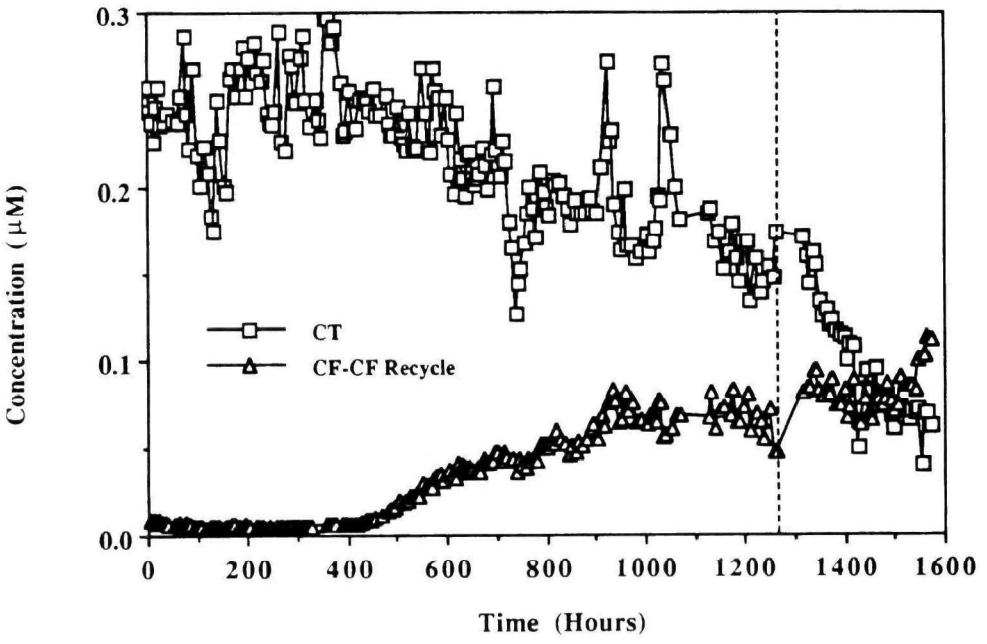

Figure 6.12. Response of CT and CF at well S1 to nitrate removal from the injected fluid at 1260 hrs. Chloroform values shown in Figures 6.10 and 6.11 have had the recycled CF of the injected fluid subtracted from the observed concentrations (CF-CF recycle).

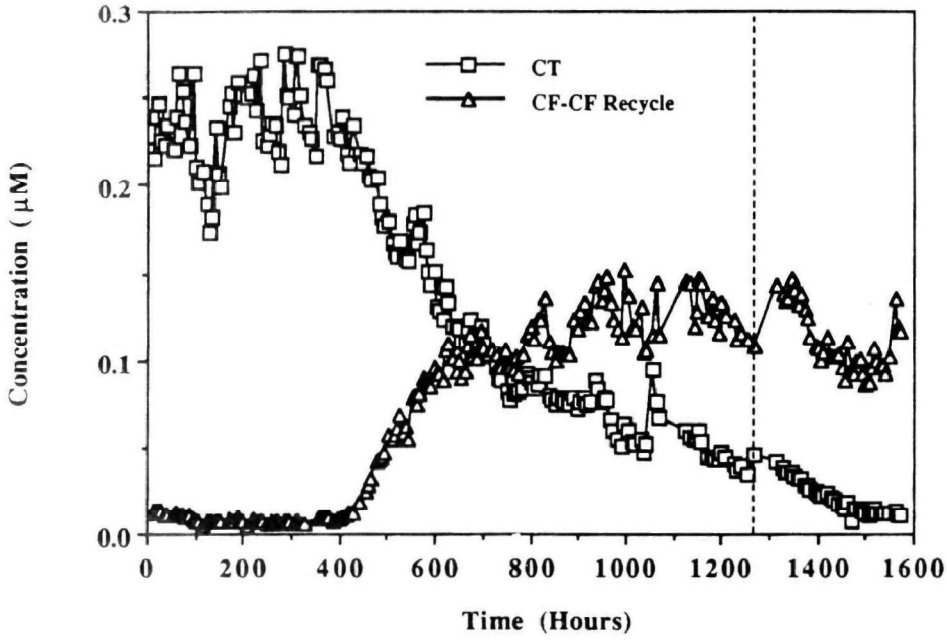

Figure 6.13. Response of CT and CF at well S2 to nitrate removal from the injected fluid at 1260 hrs.

The bar graph shown in Figure 6.14 shows the percentage transformation of CT based on the aqueous-phase concentration measurements at the S1 and S2 wells and the injected CT concentration. Also shown is the percentage of injected CT that appeared as CF. The graph shows the differences in the degrees of transformation before and after nitrate removal from the test zone. The dramatic increase in the degree of CT transformation at the S1 well when nitrate was removed is apparent. The percentage of injected CT being transformed to CF increases only slightly during the period of no nitrate addition, while at the S2 well the percentage actually decreases. Before nitrate addition, approximately 55 to 67% of the CT transformed appeared as CF. During the latter stages of no nitrate addition, this fraction decreased to 30-40% of the CT transformed.

The response to ceasing nitrate addition demonstrates that the reason for less CT transformation noted previously in zones where nitrate was present is either that nitrate *per se* inhibited the transformation of CT, or else nitrate inhibited the growth of secondary CT-transforming microorganisms. Less CF was observed as an intermediate product in the absence of nitrate. This may have resulted either from less being formed in a parallel pathway, or from CF itself being degraded as the transformation rates increased in the test zone.

During the transient test, acetate was injected into the test zone at a pulse-averaged concentration of approximately 12 mg/l. The average acetate concentrations in the observation wells over the time period from 1300 to 1384 hrs were: S1, 8.23 mg/l; S2; 3.60 mg/l; and S3, 3.57 mg/l. The data indicate approximately 4 mg/l of acetate utilization within both the first meter and the second meter of transport. Since nitrate was completely removed from the injected fluid, another electron acceptor was needed for microbial growth. One potential electron acceptor is sulfate, which was present at a concentration of 700 mg/l in the test zone.

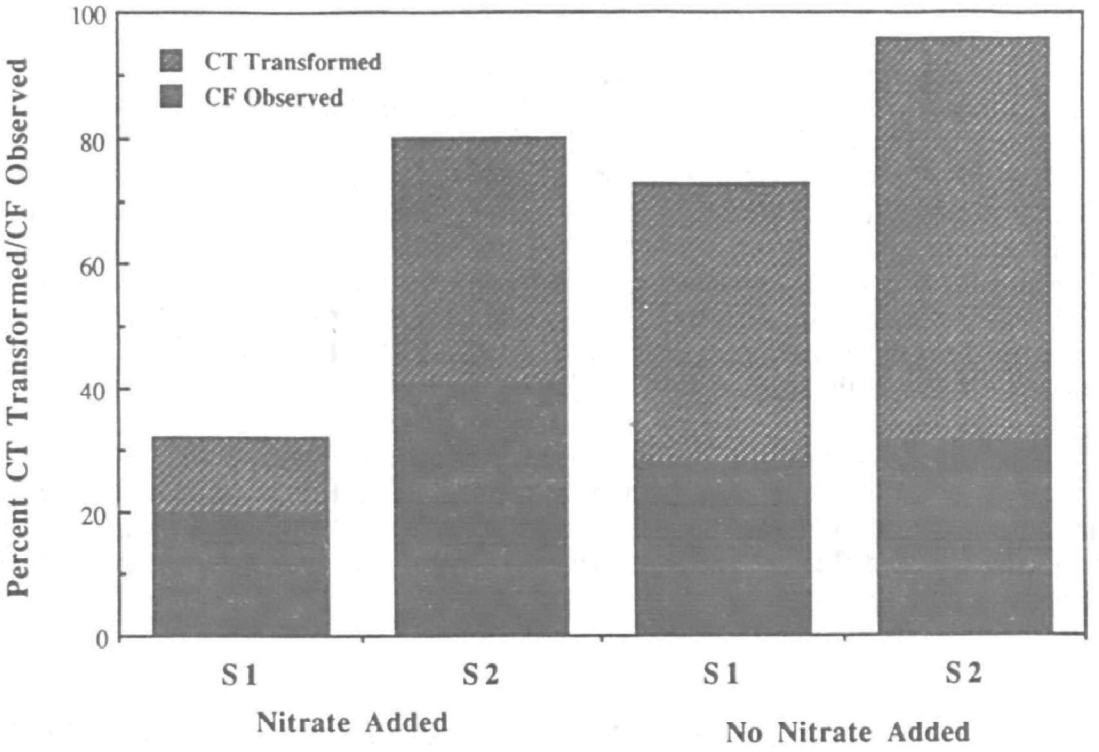

Figure 6.14. A bar graph showing the percentage transformation of CT and fraction appearing as CF during periods with and without nitrate addition.

There was, however, no evidence of sulfate-reduction in samples from the well. A silver wire placed in the sample line did not tarnish during the course of the experiments, suggesting that sulfide ion was not present in the groundwater samples. However, the lack of sulfide in aquifer water brought to the surface does not by itself demonstrate that sulfate reduction was not occurring in the test zone. Sulfide, if produced, may have quickly reacted with minerals present in the subsurface, thus being scavenged from solution. For instance, sulfide would react with iron minerals to form iron sulfide. This was found to be the case in anaerobic column-microcosm studies with Moffett aquifer samples in which sulfide was added to exchange water (McCarty et al., 1986). No sulfides were ever found present in the column effluent, suggesting removal in this manner. This has also been observed during sulfate reduction in marine systems (Widdel, 1988). The aquifer solids present would provide an abundant source of metals to complex with sulfide.

Analyses were also performed to detect methane in the test zone groundwater samples. The methane detection limit was 1 mg/l. Methane was not detected at this level in any of the groundwater samples from the test zone. The results indicated that methanogenic conditions were not established in the biostimulated zone, which is consistent with other column-microcosm studies with the Moffett aquifer materials (McCarty et al., 1986).

Transformation of Native Contaminants

During the Biostim4 experiment, the background contaminants, Freon-11, Freon-113, and TCA, were reinjected into the test zone. Their injection concentrations (Table 6.1) remained fairly constant throughout the Biostim4 experiment, thus permitting an evaluation of their response to biostimulation.

Presented in Table 6.5 are the normalized concentrations of the injected halogenated aliphatics along with the bromide tracer during the initial 350 hrs of the Biostim4 experiment. The normalized concentrations at the S1, S2, S3 observation wells show complete breakthrough to the injected values. CT is the only compound showing normalized concentrations of less than unity, indicating some transformation of CT occurred during this initial period. The normalized concentrations of the native contaminants at the extraction well were greater than unity. This resulted because of a slight reduction of these volatile compounds in the injection concentration used for normalization as the extracted water was trickled through the N_2 stripping column (Figure 5.4). Since the normalized concentrations of the halogenated organics were near unity at the S1, S2, and S3 observation wells, an evaluation of this transformation during biostimulation was possible.

The response at the S1 well to biostimulation is shown in Figure 6.15. The responses were similar to that observed for CT, but with transformation of Freon-11, Freon-113, and TCA occurring to lesser extents. The compounds also responded similarly to CT as a result of removal of nitrate from the injected fluid at 1260 hrs with greater decreases in concentration, compared to the prior period with nitrate addition.

TABLE 6.5. NORMALIZED CONCENTRATIONS DURING THE FIRST 350 HRS OF THE BIOSTIM4 EXPERIMENT

Chemical	Well	Mean	Standard Deviation	No. of Observations	Coefficient of Variance (%)
Bromide	S1	1.01	0.06	12	5.6
	S2	1.02	0.056	115	5.3
	S3	0.98	0.05	109	5.4
	Extract	0.117	0.025	98	21.3
Freon-11	S1	1.02	0.05	57	5.3
	S2	1.00	0.06	52	5.7
	S3	1.03	0.04	56	4.2
	Extract	1.18	0.04	46	3.6
Freon-113	S1	1.03	0.10	57	9.4
	S2	1.03	0.10	52	9.8
	S3	1.08	0.08	56	7.5
	Extract	1.51	0.06	46	4.2
TCA	S1	1.00	0.03	57	3.0
	S2	0.99	0.04	52	4.0
	S3	1.00	0.03	56	2.7
	Extract	1.02	0.04	46	4.2
CT	S1	0.96	0.04	57	4.3
	S2	0.92	0.06	52	6.2
	S3	0.89	0.04	56	4.4
	Extract	0.134	0.008	46	6.1

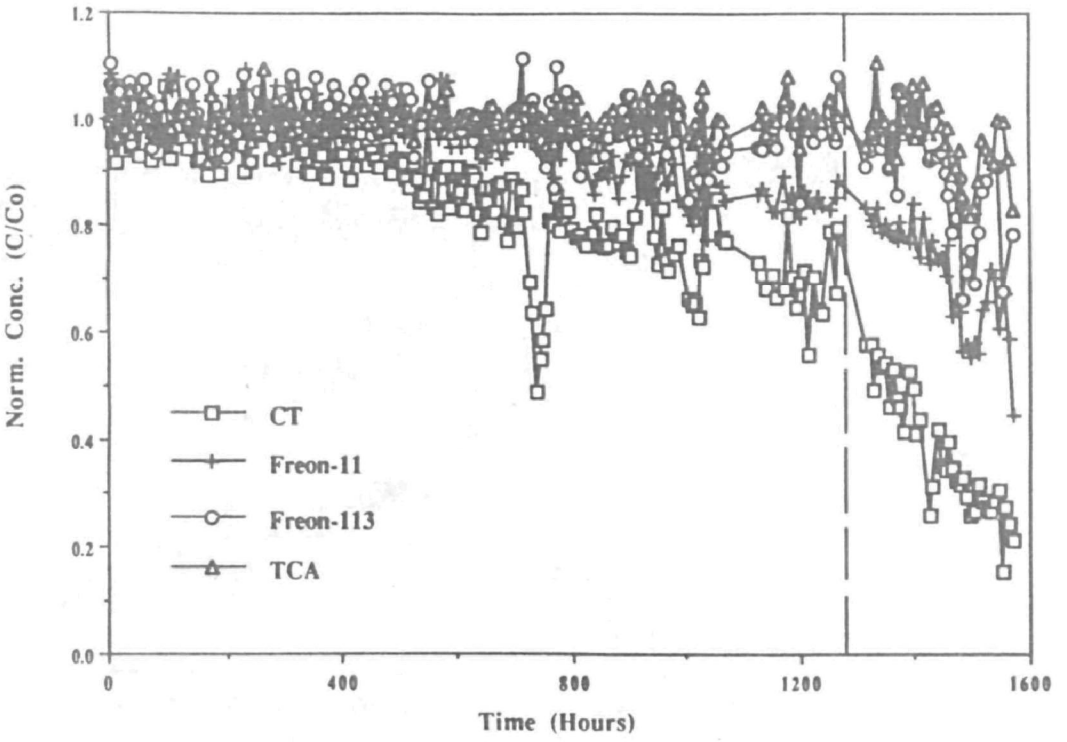

Figure 6.15. Response of the halogenated aliphatics at the S1 well due to biostimulation. Nitrate removed from the injected fluid at 1260 hr.

The response of the halogenated aliphatics at the S2 well is shown in Figure 6.16. The response of the Freons and TCA again is similar to that of CT, but with slower rates of transformation. This is best indicated by comparing the Freon-11 and CT responses. Modeling studies presented in Section 7 will show how these two responses can be explained with CT having a faster biotransformation rate compared to Freon-11. Based on the concentration responses, the rank in transformation rates are as follows: CT > Freon-11 > Freon-113 > TCA.

The S2 results indicate that steady-state concentrations were not achieved by the end of the Biostim4 experiment. The concentration trend indicates that greater extents of transformation would be achieved with prolonged biostimulation. For comparison purposes, estimates were made of the degree of transformation achieved by the end of the Biostim4 experiment, even though steady state had not yet been achieved. The estimated removals along with 95% confidence intervals are summarized in Table 6.6. In the 2- and 3-m biostimulated zone (wells S2 and S3), the mean degrees of transformation were: CT, 95%; Freon-11, 68%; Freon-113, 20%; and TCA, 15%, while within 1 m (well S1), they were: CT, 74%; Freon-11, 46%; Freon-113, 8%; and TCA, 9%.

IDENTIFICATION OF TRANSFORMATION PRODUCTS

As previously discussed, CF was the major CT transformation intermediate identified. Another possible intermediate product of CT transformation is dichloromethane, which can be produced from the reductive dehalogenation of CF (Figure 1.1). This compound could not be easily monitored in the field since it elutes very rapidly during GC analysis. Grab samples were therefore collected for analysis at the end of the Biostim4 test, when maximum concentrations were expected. Analyses were performed by a commercial laboratory using EPA's Standard Method 601. Dichloromethane was not detected in the samples at a detection limit of 1 µg/l. These results indicate that dichloromethane was not a significant intermediate. Chloromethane was also below the detection limit of 1 µg/l.

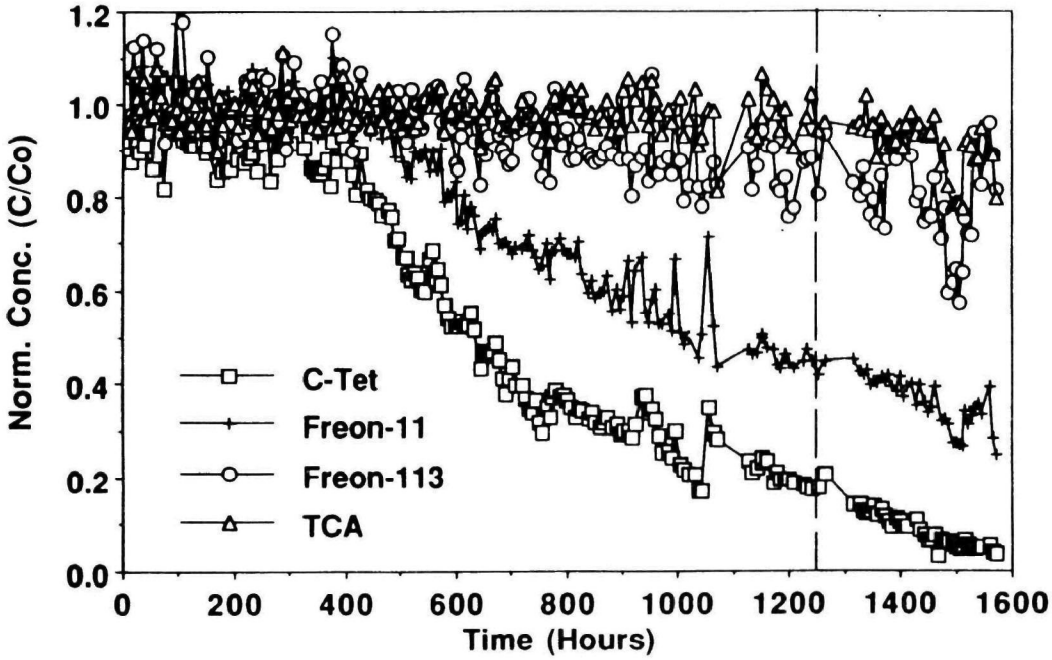

Figure 6.16. Response of the halogenated aliphatics at the S2 well due to biostimulation.

TABLE 6.6. ESTIMATES OF THE DEGREE OF TRANSFORMATION IN BIOSTIM4 BASED ON MEAN CALCULATED VALUES FROM 1450-1550 HRS

Chemical	Well	Percent Biotransformation	
		Average	95% Confidence Interval
CT	S1	74	70-78
	S2	95	94-96
	S3	96	95-97
	Extraction	93	89-96
Freon-11	S1	46	42-50
	S2	68	65-71
	S3	72	69-75
Freon-113	S1	8	0-16
	S2	20	10-30
	S3	18	8-27
TCA	S1	9	5-13
	S2	15	11-19
	S3	9	2-16

1,1-dichloroethane (1,1-DCA) was an expected transformation product of 1,1,1-TCA (Figure 1.1). 1,1-DCA was present as a background contaminant in the test zone at a concentration of 5.0 µg/l. Its presence may result from the biological transformation of TCA during transport in the subsurface from the contaminant source to the test zone. During Biostim4, the concentrations increased slightly by 0.3 µg/l at well S1 and 1.4 µg/l at well S2, indicating an increase with distance, consistent with TCA transformation (Table 6.6). However, here, the differences are small compared to the amount initially present in the groundwater, and thus we cannot statistically conclude for that 1,1-DCA was formed in the test zone.

Products of Freon-11 and Freon-113 transformation would be at low concentrations in the test zone due to the low concentration of the parent compounds (Table 6.1). A possible intermediate of Freon-11 (trichlorofluoromethane) is Freon-21 (dichlorofluoromethane, Cl_2CHF), in which one chlorine atom of Freon-11 is replaced by hydrogen. Freon-21 would be expected to be a fast eluter in GC analysis, and would not be resolved on the field site GC. The analysis preformed by the analytical laboratory did not detect Freon-21 in the groundwater samples. Two possible transformation products of Freon-113 (trichlorotrifluoroethane) are Freon-123 (1,2-dichlorotrifluoroethane) and chlorotrifluoroethylene. If Freon-113 behaved like hexachloroethane (Criddle et al., 1986), then di-halo-elimination might be favored and the halogenated ethene formed. However, these compounds were not identified in the field nor in the analytical laboratory. Identification of these compounds by GC-MS analysis was beyond the scope of this work.

The Biostim4 experiment was terminated after 1585 hrs of acetate addition as it became increasingly difficult to maintain fluid injection rates, with higher injection pressures required to maintain a constant rate of fluid injection. This most likely resulted from significant biogrowth in the region of the injection well. Thus, despite the pulsed addition of acetate, biofouling of the aquifers still appeared to occur. Biofouling may present a significant practical limitation in applying this bioremediation process.

RESULTS OF MONITORING SUBSEQUENT TO ACETATE ADDITION

Five months after acetate addition was stopped a survey of the field was conducted to determine if transformation of the native contaminants was still occurring. Over the 5-month period, injection and extraction of groundwater had been terminated, and the test zone was subjected to natural flow conditions. During the survey, the extraction well was turned off, and the SI injection well was converted into a sampling well by connecting the injection line to the sampling manifold. During sampling, the approximately 5 liters per sample that were withdrawn represented the well volume and a fraction of the volume of the sandpack surrounding the well.

The first sample drawn from the injection well immediately indicated that reducing conditions had evolved around the injection well, with a strong smell of hydrogen sulfide in the sample. The chromatogram from initial GC analysis with ECD detection was void of all peaks, and the Hall detector chromatogram had peaks only in the leading end (fast eluters) that did not represent any previous identified compounds. Thus, 1,1,1-TCA, Freon-11, and Freon-113 were not detected in the first sample, indicating virtually complete transformation of these compounds in the anaerobic zone that had developed around the injection well. The presence of fast eluters, measured by the Hall detector, suggests the presence of some dehalogenated transformation products.

Continuous sampling (5 liters per sample) of the area surrounding the injection well was conducted over a 48-hr period. A total of approximately 250 liters were removed during the period. Samples were also obtained from the S1, S2, and S3 monitoring wells. Samples were

also obtained along the north experimental leg, outside the influence of biostimulation, to serve as background concentration measurements. Measured concentrations in the zone of biostimulation could then be compared with the north-leg concentrations in order to evaluate the degree of biotransformation.

Figure 6.17 shows the concentrations of Freon-11 and Freon-113 as a function of time at the SI injection well. Concentrations increased with time to steady-state levels that were significantly lower than those along the north leg, indicating continued transformation. Figure 6.18 shows the TCA and 1,1-DCA response. The TCA concentration increased with time, the concentration of 1,1-DCA (an expected intermediate product) decreased. Based on the north-leg concentration measurements, the extents of transformation estimated during the steady-state period were: Freon-11, 58%; Freon-113, 31%; and TCA, 9%.

Restricted flow through the zone near the injection well was probably occurring under the existing natural gradient conditions before the sampling was started, possibly due to biofouling. Thus the contaminants had longer residence times to react, and thus were transformed more completely. Laboratory studies of Rittmann et al. (1988) have demonstrated this effect. The increase in concentration with time probably resulted from a decrease in residence time of the native contaminants in the biostimulated zone. Another possibility is that the redox conditions gradually changed and became less reduced, as native groundwater was forced through the biostimulated zone. Despite the increases, the measurements demonstrated that transformation was continuing in the treatment zone long after acetate addition was stopped.

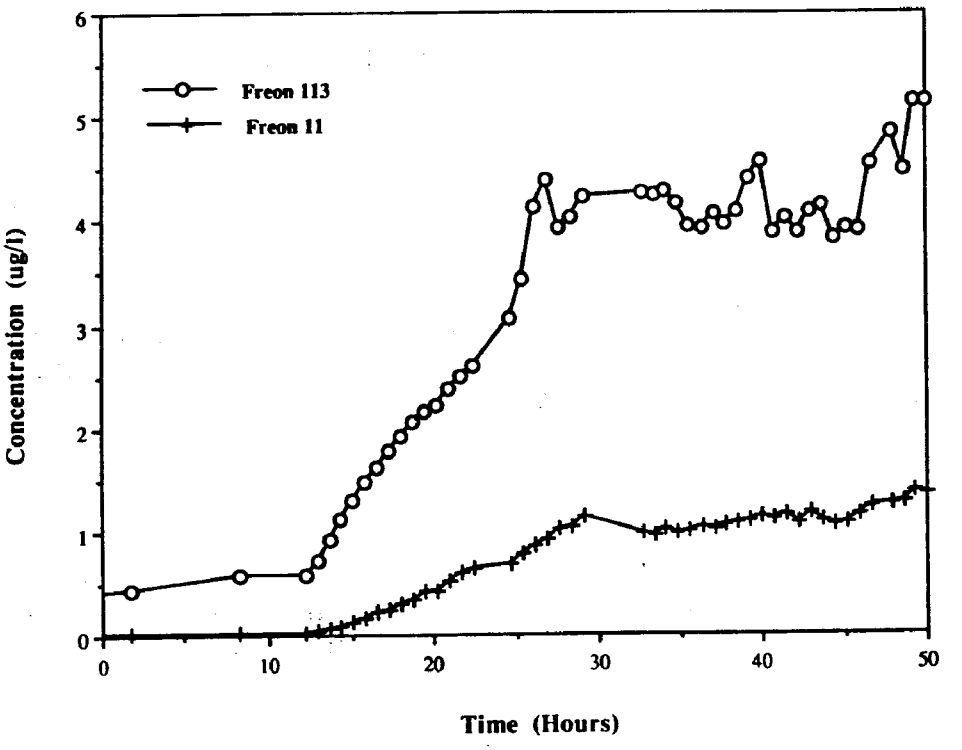

Figure 6.17. Freon-11 and Freon-113 concentration responses at the SI injection well during Monitor1, five months after active biostimulation.

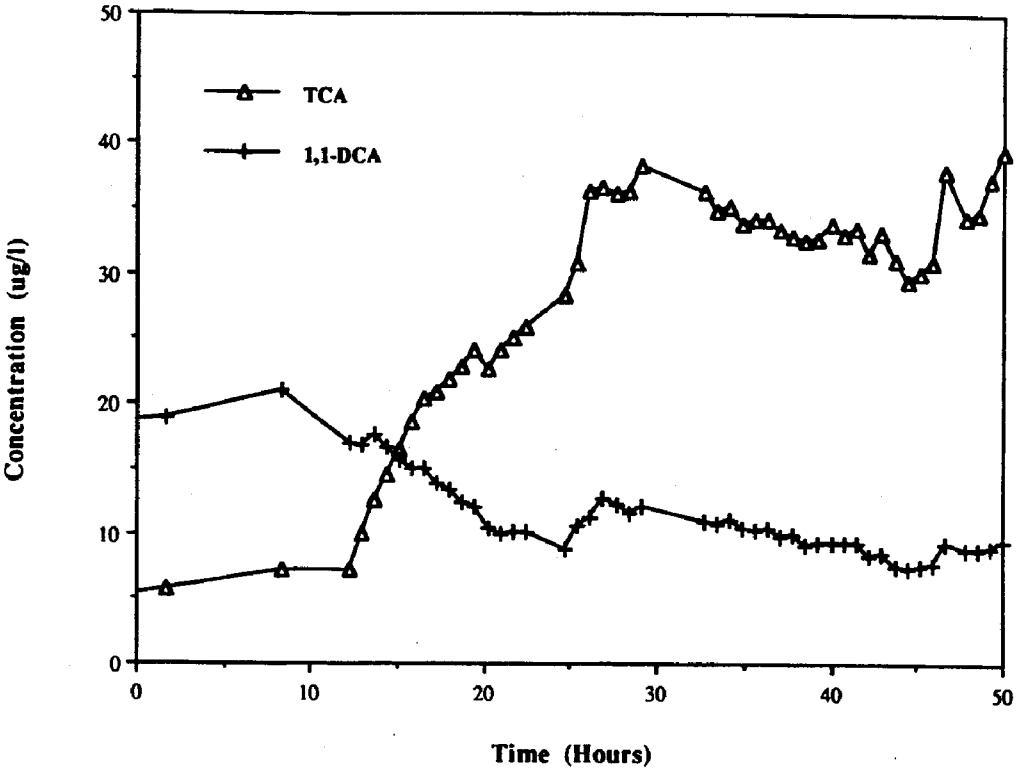

Figure 6.18. Trichloroethane and 1,1-DCA concentration responses at the SI injection well during Monitor1, five months after active biostimulation.

Table 6.7 summarizes estimated transformation at the downgradient monitoring wells. The degree of transformation is shown to decrease with distance from the injection well. The flow conditions here, however, were natural gradient, and thus the flow was probably not aligned with the observation wells, as occurs under induced flow conditions. Thus, the influx of native groundwater may have caused the increase in concentration with distance from the injection well. The data indicates that the most active zone was near the injection well, where the microbial biomass was the highest under active biostimulation conditions.

TABLE 6.7. PERCENT TRANSFORMATION FIVE MONTHS AFTER ACTIVE BIOSTIMULATION

Well	Freon-11 (%)	Freon-113 (%)	TCA (%)
Injection	57.5	31.2	9.12
S1	43.4	26.4	1.8
S2	19.6	12.1	2.0
S3	5.4	3.1	1.8

DISCUSSION OF RESULTS

The biostimulation and biotransformation evaluation conducted at the Moffett Field site demonstrated the feasibility of stimulating a microbial community in the subsurface to transform CT and some other halogenated aliphatics under anoxic conditions. Denitrifying bacteria were easily stimulated through the addition of acetate as a primary substrate for growth into the test zone. The initiation of the transformation of CT was delayed, with respect to the biostimulation of the denitrifying population. The gradual decrease in concentration of the CT and other halogenated aliphatics suggest that microbial population(s) other than the main population of denitrifiers were slowly stimulated, and were responsible for the biotransformation of the target compounds. Transformation also appeared to be inhibited by the presence of nitrate. This was indicated both by the spatial transformation of the target compounds when nitrate was being injected along with acetate, and the enhanced rates of transformation that were observed when nitrate was removed from the injected fluid. Chloroform was formed as an unwanted product of CT transformation. The lower fraction of CT transformed was observed as CF, when nitrate was removed from the test zone, suggesting more favorable transformations under more reducing conditions. Monitoring of the background contaminants in the test zone showed transformation continued up to 5 months after active biostimulation although acetate addition was terminated. This suggests that transformation may have been associated with secondary microorganisms growing on decaying products of organisms grown when acetate was added.

In the Tracer14 test, a few percent of the CT added was transformed to CF. Nitrate concentration was also reduced by approximately 3 mg/l during transport through the biostimulated zone. In the subsequent Biostim4 experiment, very rapid stimulation of denitrifying bacteria was observed. These results suggest that an active population of bacteria existed in the test zone prior to biostimulation with acetate. This is not surprising, since the south experimental leg had been stimulated to grow methanotrophic bacteria in previous field seasons. Decay of the methanotrophic biomass probably provided a source of substrates for growth of a CT-transforming population. At other field sites, the microbial population might be lower than at Moffett. This might result in longer lag times before transformation is observed.

The response to NO_3^- removal was too fast for the transformation of CT to be directly related to the growth of denitrifiers. There are several possibilities for the lag in transformation of the target compounds compared with the rapid biostimulation of the denitrifying bacteria. The most likely is that the bulk of the transformation was being provided by microorganisms other than the main population of denitrifiers. The response indicates a low population of these bacteria initially in the test zone, and a slow growth of this population with time. The biostimulation response also suggests that the growth of this CT-transforming population was inhibited by the presence of nitrate.

No attempt was made to isolate the bacteria in the test zone responsible for the transformations. Early attempts to isolate denitrifiers with CT transforming ability in the solid columns failed (Section 4). Attempts to isolate CT-degrading denitrifiers from Moffett groundwater samples taken from the test zone prior to biostimulation also failed (Criddle, 1989). A more detailed characterization of the microbial communities involved in the transformations was beyond the scope of this one-year project. We can only hypothesize about the microbial population that was responsible for the transformation. No methane production was observed in the test zone; therefore, we are fairly confident that methanogenic conditions were not induced in the test zone. The high sulfate concentrations in the test zone (700 mg/l) might have inhibited the establishment of methanogenic conditions.

One possibility is transformation under sulfate-reducing conditions. Egli et al. (1988) demonstrated CT transformation by *Desulfobacterium*, with approximately 75% conversion to CF. Bouwer and Wright (1988) observed faster rates of transformation in column studies operated under sulfate-reducing conditions, compared to nitrate-reducing conditions. Recently Bagley and Gossett (1990) have reported the reductive dechlorination of PCE to TCE and cis-dichloroethylene under sulfate-reducing conditions. The causative organism or group, however, was not identified. As previously discussed, sulfate-reducing conditions may have been promoted, even though sulfide production during active biostimulation was not observed. In the post-stimulation monitoring, five months after active biostimulation was stopped, hydrogen sulfide production in the region of the injection well was observed. This may have resulted from the fermentative decay of proteins in the stimulated biomass, or from the stimulation of sulfate-reducing bacteria. Sulfate reducers might also have grown on decay products of the denitrifiers, both after and during active biostimulation. Sulfate reducers might also have grown on acetate. Eight mg/l of acetate was consumed in the test zone after nitrate was completely removed from the injected fluid. Sulfate reducers may have been responsible for this consumption.

Another possibility is that organisms such as *Clostridium* growing on decay products of the stimulated denitrifiers were carrying out the transformation. Galli and McCarty (1989) found *Clostridium* sp. isolated from a halogenated aliphatic transforming methanogenic culture and grown on amino acids converted CT quantitatively to CF. The *Clostridium* also transformed 1,1,1-TCA to 1,1-DCA, but at a much slower rate than CT was transformed. The observation of some initial CT transformation associated with decay of a previously stimulated methanotrophic culture and continued transformation five months after acetate addition was stopped suggests that microbes growing on microbial decay-products were at least partly responsible for the transformations observed.

The ability of denitrifiers to promote the transformation is not strongly supported by our results. Criddle (1989) did isolate a denitrifying pseudomonad from an aquifer in Orange County, CA, that transformed CT. However, he also tested several denitrifying cultures from the American Type Culture collection, and found none were capable of transforming CT. Thus, the ability of denitrifiers to degrade CT does not appear to be a common trait among such bacteria.

Criddle (1989) also found that transformation of CT by the pseudomonad in Moffett groundwater was inhibited at pH 7. After raising the pH to 8.0, he observed enhanced CT transformation. By raising the pH, a precipitate formed that was later shown to inhibit transformation. Through a series of tests, Criddle (1989) found iron in solution was the most likely cause of this inhibitory effect. Criddle (1989) postulated that under iron-limiting conditions, the denitrifying culture may have produced bioagents, possibly a siderophore, that would make iron available to the cell and may have promoted CT transformation.

In the field experiment changes in pH were not observed. However, changes in redox conditions could have changed iron availability. Sulfate reduction may have caused iron deficiency through the production of iron sulfides. If a denitrifying culture was present that behaved like Criddle's (1989) pseudomonad, then these conditions may have promoted CT transformation.

The rates of transformation increased when nitrate was removed from the treatment zone. The results indicated more rapid rates of transformation under more strongly reducing conditions. These results agree with those of Bouwer and Wright (1988) and Criddle (1989). We do not know if rapid rates of transformation would have been observed if nitrate had been removed from the injected fluid at the start of the experiment. The question remains of whether the biostimulation

of denitrifiers in the test zone was required to provide a source of growth substrate, in the form of their respiration products, for other bacteria, such as *Clostridium*, that promote the transformation.

The field tests demonstrated that the rates of transformation were compound-specific. The progression of the extents of transformation shows a similar pattern among the compounds, with greater extents of transformation the greater the distance traveled. The response indicates that the same process(es) were transforming the compounds, only at different rates. This will be developed in more detail in the modeling section.

However, with some simplifying assumptions, this hypothesis can be tested. A basic assumption is that the transformation is described by equation (1.1), the pseudo-first-order form of the Monod equation. At steady-transformation conditions equation (1.1) can be integrated to yield:

$$\ln\left(\frac{C}{C_0}\right) = -\left(\frac{k}{K_S}\right)Xt \qquad (6\text{-}1)$$

Assuming also that Xt is constant with distance, i.e., the same active cell population transforms all the compounds, but with a different value of k/K_S for each compound. Ratios of the $\ln(C/C_0)$ for one compound at two different distances can be compared to that for other compounds, where C/C_0 is the fractional breakthrough for each compound. This should yield a constant ratio, if constant ratios were obtained, thus indicating that the main difference between chemicals is the reaction rate constant, k/K_S, and that the active microbial population was the same for all of the chemicals.

The analysis discussed above was performed for the S1 and S2 wells using the fractional transformations provided in Table 6.6. The C/C_0 ratio is given by (1 – fractional transformation). Results of the analysis are presented in Table 6.8. The ratios are shown to be fairly constant, ranging from 1.72 to 2.68, with a mean value of 2.11, with a coefficient of variation less than 20%. The agreement is quite good considering the variability in the estimates, especially of Freon-113 and TCA. The constant ratio indicates that the differences in the responses for the different compounds result primarily from the same process(es) responsible for the transformations, but with different rates. The ratio of 2 also indicates that the first meter and second meter of the test zone contributed equally to the transformation at the end of the Biostim4 experiment.

TABLE 6.8. COMPARISON OF FIRST-ORDER MODEL FOR EXTENTS OF TRANSFORMATION

Chemical	Well	C/C_0	$\ln C/C_0$	$\frac{\ln C/C_{0\ S2}}{\ln C/C_{0\ S1}}$
CT	S1	0.26	–1.35	2.22
	S2	0.05	–2.99	
Freon-11	S1	0.54	–0.62	1.85
	S2	0.32	–1.14	
Freon-113	S1	0.92	–0.083	2.68
	S2	0.80	–0.223	
TCA	S1	0.91	–0.094	1.72
	S2	0.85	–0.163	
				Average = 2.11
				CV = 0.177

The chemical dependence on transformation rates is consistent with previous reports. Rates of reductive transformations in general are faster the more oxidized the molecule (Vogel et al., 1987). In dehalogenation reactions Br is a better leaving group than Cl, and Cl is a better leaving group than F. Our results agree with these general trends. CT was observed to be degraded at a faster rate than Freon-11 (trichloro-fluoro-methane). In the case of Freon-11, the substitution of one fluoride on the molecule results in a significant reduction in the rate of transformation. Comparison of the rates of transformation for the two halogenated ethanes shows slightly greater rates for Freon-113 (1,1,2-trichloro-1,2,2-trifluoro-ethane) compared to TCA. Here, the differences are complicated by the differing degree of substitution and the different substituting groups.

Our observations of more rapid CT than TCA transformation agree with laboratory observations. Galli and McCarty (1989) observed CT to be transformed 13 times faster than TCA by *Clostridium* sp. Bouwer and McCarty (1983b) observed complete transformation of CT in a denitrifying mixed culture, but found no evidence of TCA transformation by the same culture. Rittmann et al. (1988) observed much greater extents of CT transformation compared to TCA in column with successive denitrifying zones. In one experimental run with long column residence times they observed 96% transformation of CT but only 10% transformation of TCA.

CF was observed as a major intermediate product of CT transformation. Less CF was observed under the more reducing conditions created by removing nitrate from the injected fluid. Bouwer and Wright (1988), however, observed the opposite response: more CF was observed under sulfate-reducing conditions, compared to denitrifying conditions. There does not appear to be a clear trend for determining what conditions minimize CF formation. Criddle (1989) proposed a range of transformation products that might be formed as a result of the formation of a trichoromethyl radical (Figure 6.19). He discussed the ability of parallel pathways to form a range of intermediate products. In his work, he observed the formation of CO_2, CS_2, and CF by *E. coli* over a range of redox conditions. Less CF was observed under fumarate-respiring conditions than fermenting conditions, which represented a more reducing environment.

CF is the main transformation product, and the only transformation product that was identified in the field investigation. Transformation via parallel pathways was most likely occurring in the test zone. However, it is not known whether changes in the fraction of CF formed during the course of the field experiments resulted from shifts in the parallel pathways, or from transformation of CF, with enhanced rates of CF transformation observed in the latter stages of the experiment. CF is an unwanted intermediate of CT transformation. Thus, finding the conditions that will minimize its formation is important. More basic research is required to determine under what environmental and microbial and chemical conditions CF formation is minimized and degradation to CO_2 is maximized.

Sorption of the halogenated aliphatics did not have a strong influence on the results of the field evaluation. Carbon tetrachloride and the other halogenated aliphatics were not strongly sorbed onto the aquifer solids in tests performed before the test zone was biostimulated. Thus, transformation was probably not limited by sorption interactions. CT and TCA were retarded to similar extents, judging from a comparison of the TCA transport experiment conducted earlier at the Moffett site (Roberts et al., 1989) and the CT transport experiments performed in this study. Thus the differences in the CT and TCA responses do not result from sorption interactions. Based on model simulations presented in Section 7, the carbon associated with the increased microbial mass in the test zone represents only a small fraction (approximately 3%) of the carbon present, with 97% associated with aquifer solids. Thus, increases in the sorption capacity of the test zone, resulting from biostimulation, is not a process likely to affect our field observations.

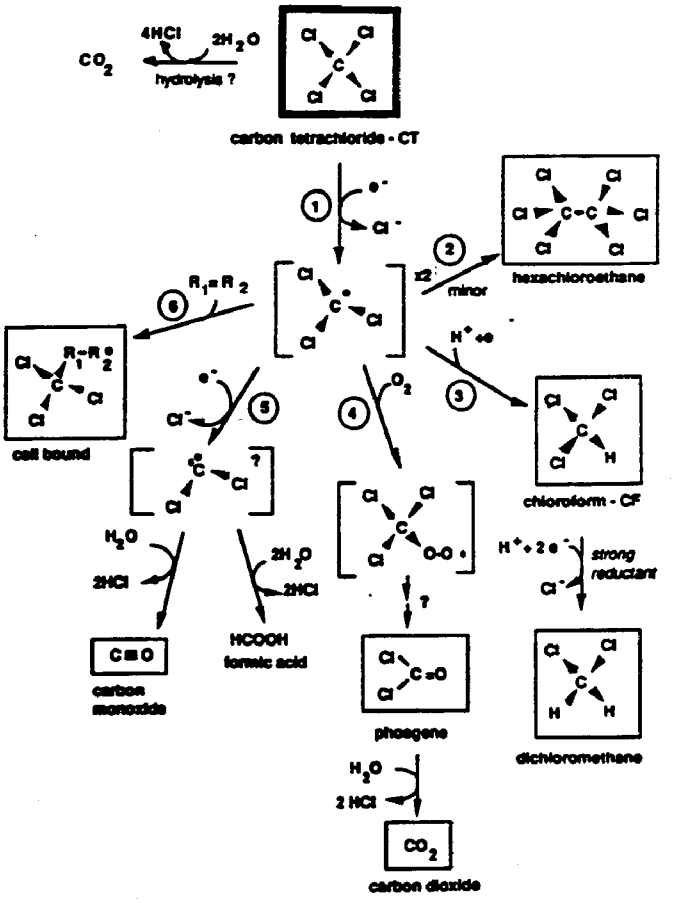

Figure 6.19. Known abiotic and biotic transformations of CT. Products that have been detected are shown in boxes (from Criddle, 1989).

Abiotic transformation processes could also be contributing to the overall transformations that were observed. Abiotic transformations may have been induced by the biostimulation of the test zone, which created the appropriate environmental conditions for abiotic reactions. The recent abiotic transformation studies of Kriegman and Reinhard (in press) and Reinhard et al. (1990) with sulfide minerals, and of Curtis (1990) with humic acids in the presence of Fe^{2+} and HS^-, demonstrated CT reduction at rates of environmental significance. Curtis (1990) indicated microbes can create the environmental conditions to produce reduced iron and/or Hs^-, which might interact with humics to produce an agent capable of reducing CT. Here more basic work is required in this complex interface between microbial processes and transformations, and abiotic transformations in the presence of aquifer solids.

The laboratory column studies discussed in Section 4 generally agree very well with the results of the field evaluation and demonstrated that denitrifying conditions could be promoted rapidly by the addition of acetate as a substrate for growth. The columns also showed the lag in transformation of CT, with a gradual decrease in CT concentration observed with time. In the columns, a period of approximately 60 days (1500 hrs) was required to achieve an estimated CT transformation of 80 to 90%, similar to that observed in the field evaluation. This slow response

in the columns first indicated that the biotransformation was most likely caused by secondary microorganisms, and not by the main population of denitrifiers. The column studies also showed continued transformation months after acetate addition was stopped, similar to the limited field observations.

Less CF production was observed in the columns than in the field. This may have resulted from longer residence times in the columns compared to field conditions. Similar degradation of CF may have occurred in the field in the latter stages of the experiments. The column studies also demonstrated that significant amounts of the CT were degraded to CO_2. Some complete mineralization to CO_2, therefore, was probably occurring in the field study.

The results of this field study differed greatly from our previous study performed at the field site with aerobic methanotrophic bacteria. The present study demonstrated that more highly substituted compounds were transformed more rapidly under anoxic conditions. The reverse was true for the aerobic transformation progress. Both studies agree with results of laboratory studies and estimates based on theoretical considerations.

In the earlier study with methanotrophs, most of the transformation occurred in the first meter, where most of the biological mass was stimulated. Transformation also responded immediately to methane utilization. Here a specific microbial population was stimulated to initiate the cometabolic transformation through the addition of methane as a specific growth substrate. When methane addition was stopped, transformation rapidly ceased. Thus transformation was strongly linked to methane consumption and occurred in the regions where that substrate was consumed.

In the current study the biostimulation with acetate was much less substrate-specific, and the main population stimulated apparently was not likely the main transforming population. Hence, the spatial and temporal responses were very different from those observed in the methanotrophic study. Transformation was not strongly linked to acetate uptake, and most of the transformation initially occurred in zones more distant from the injection well, where little acetate was being consumed. Transformation was still occurring five months after acetate addition was stopped. Although a microbial transformation mediated by a secondary population and not by the main population of denitrifiers has been proposed, abiotic processes resulting from chemical conditions created by the biostimulation may also be contributing to the transformation of the halogenated compounds.

Performing the two studies in the same experimental zone demonstrated that aerobic and anoxic processes can be carried out in the same aquifer zone. Here, it would be of interest to determine whether methanotrophs could again be stimulated since that would be the sequence most likely to be used in practice. For instance, reductive processes could be used as a first stage of transformation, followed by aerobic processes to degrade less chlorinated intermediates that were formed. In the case of CT transformation, the aerobic methanotrophic process could be used to degrade CF that was formed by the anoxic process.

SECTION 7

BIOTRANSFORMATION SIMULATIONS OF THE FIELD EVALUATION

INTRODUCTION

A non-steady-state simulation model of the field experiments was developed in order to evaluate knowledge of processes affecting CT transformation, and to identify processes which are still poorly understood. The model accounts for the basic processes of microbial growth, electron donor and electron acceptor utilization, and the biotransformation of the chlorinated aliphatics. Transport processes of advection, dispersion, and sorption in porous media are included in the model formulation. Model simulations provided a quantitative means of evaluating our understanding of processes affecting field results.

MODEL DEVELOPMENT

The basic features included in the model are summarized in Table 7.1. The model simulated the stimulation of two microbial populations. The first population stimulated, X_1, is a denitrifying population that uses acetate as a primary substrate (i.e. electron donor) and nitrate as an electron acceptor. The second microbial population, X_2, grows on the decay products of the denitrifiers.

TABLE 7.1. BASIC FEATURES OF THE NON-STEADY-STATE BIOTRANSFORMATION MODEL

1-D Transport
Advection, Dispersion, Sorption
Growth of Dual Microbial Populations 1 and 2
Monod Kinetics for Growth Populations 1 and 2, and Electron Donor and Acceptor Utilization
Shallow Biofilm of Microorganisms
Population 2 Grows on Decay Products of Population 1 and is Inhibited by Nitrate
Equilibrium
Non-Equilibrium
Contaminant Biotransformation Kinetics
Monod Kinetics for Populations 1 and 2
Formation and Transformation of CT Intermediates
Boundary Conditions Which Permit Cyclic Pulsing of Acetate and Nitrate

The growth of this population is assumed to be inhibited by the presence of nitrate. The transformation of carbon tetrachloride (CT) and the other halogenated aliphatics is assumed to be governed by Monod kinetics. Both the denitrifying population and the secondary microbial population are taken to be capable of transforming CT, but at different rates. The formation of intermediate products from CT transformation is also included in the model formulation. The intermediate products can also be transformed by either of the two microbial populations. The model is presented for a linear (uniform) flow geometry. Previous modeling of the methanotrophic experimental results (Semprini and McCarty, 1989) indicated that 1-D uniform flow modeling was adequate for the induced flow conditions of the test zone.

The model incorporates the basic microbial rate processes into the partial differential equations describing solute transport in porous media. Microbial growth and electron donor and acceptor utilization are modeled using Monod kinetics, assuming that the rates are functions of aqueous substrate concentrations. The biomass is assumed to be an attached shallow biofilm that is fully penetrated by the substrate, i.e., there are no substantial concentration gradients within the film. Sorption of the components is modeled as either an equilibrium or non-equilibrium process.

The rates of microbial growth and decay of the denitrifiers (population 1) were assumed to be functions of both electron donor and acceptor:

$$\frac{dX_1}{dt} = X_1 Y_1 k_1 \left(\frac{C_{D1}}{K_{SD1} + C_{D1}}\right)\left(\frac{C_{A1}}{K_{SA1} + C_{A1}}\right) - X_1 b_{11}\left[\frac{C_{A1}}{K_{SA1} + C_{A1}}\right] - X_1 b_{12} \qquad (7\text{-}1)$$

where X_1 = cell concentration (mg/l), k_1 = maximum utilization rate (g donor/g cell-d), Y_1 = yield coefficient (g cells/g donor), K_{SD1} = donor saturation constant (mg donor/l), K_{SA1} = acceptor saturation constant (mg acceptor/l), b_{11} = cell decay coefficient (d^{-1}) accounting for respiration in the presence of nitrate, b_{12} = cell decay coefficient (d^{-1}) accounting for respiration in the presence or absence of nitrate, and C_{D1} and C_{A1} are the concentrations of the acetate (electron donor) and nitrate (electron acceptor) (mg/l), respectively. The values of C_{D1} and C_{A1} are identical to local concentrations in the advecting pore water, owing to the assumption of a shallow biofilm.

Rates of utilization of electron donor and acceptor by population 1 are given by equations (7-2) and (7-3), respectively:

$$\frac{dC_{D1}}{dt} = -k_1 X_1 \left(\frac{C_{D1}}{K_{SD1} + C_{D1}}\right)\left(\frac{C_{A1}}{K_{SA1} + C_{A1}}\right) \qquad (7\text{-}2)$$

$$\frac{dC_{A1}}{dt} = -k_1 F X_1 \left(\frac{C_{D1}}{K_{SA1} + C_{D1}}\right)\left(\frac{C_{A1}}{K_{SA1} + C_{A1}}\right) - d_c f_d b_{11} X_1 \left(\frac{C_{A1}}{K_{SA1} + C_{A1}}\right) \qquad (7\text{-}3)$$

where F is the ratio of electron acceptor to electron donor utilization for the biomass synthesis (g acceptor/g donor), d_c = cell decay oxygen demand (g NO_3/g cells), and f_d is the fraction of cells that is biodegradable (McCarty, 1975).

The second microbial population, X_2, grows on the decay products of the denitrifiers and is assumed to be inhibited by the presence of nitrate. Its rates of growth and decay are given in equation (7-4).

$$\frac{dX_2}{dt} = X_2 Y_2 k_2 \left(\frac{C_{D2}}{K_{SD2} + C_{D2} + \frac{C_{A1}}{KI}} \right) - X_2 b_{21} \left[\frac{C_{A1}}{K_{SA2} + C_{A1}} \right] - X_2 b_{22} \qquad (7\text{-}4)$$

where the subscript 2 represents the second population and is used for the associated parameters. The electron donor, C_{D2}, on which the second population grows, is produced from the decay of the denitrifying population. The coefficient KI is a growth-inhibition factor linked to the presence of nitrate, C_{A2}. Decreases in KI and increases in nitrate concentration will decrease the rate of growth of the second population.

The rate of production and utilization of the electron donor C_{D2} is given by equation (7-5):

$$\frac{dC_{D2}}{dt} = X_1 b_{11} \left[\frac{C_{A1}}{K_{SA2} + C_{A1}} \right] F_{D2} + X_1 b_{12} F_{D2} - X_2 k_2 \left[\frac{C_{D2}}{K_{SD2} + C_{D2} + \frac{C_{A1}}{KI}} \right] \qquad (7\text{-}5)$$

where F_{D2} is the fraction of the degraded biomass that goes into the production of the second electron donor C_{D2}.

The kinetic model used for the transformation of contaminants is the simple Monod Model (McCarty, 1984):

$$\frac{dC_C}{dt} = -X_1 k_{C1} \left(\frac{C_C}{K_{SC1} + C_C} \right) \qquad (7\text{-}6)$$

where k_{C1} = maximum transformation rate of the contaminant (g contam./g cell-d) for, K_{SC1} = contaminant substrate saturation coefficient (mg contam./l), and C_C is the contaminant concentration. The subscript (C_1) here represents the transformation by population 1. The same rate equation applies for population 2, with parameters represented by subscripts (C_2). Transformation of intermediate products is also governed by the same equation and is represented by subscripts (I1) and (I2) for transformation by populations 1 and 2, respectively.

The formation of the intermediate products is based on kinetics for formation by parallel pathways, as discussed by Criddle (1989), where a fraction of the parent compound forms the intermediate. The intermediate can be formed and subsequently transformed by both of the microbial populations, with the overall rate being the sum of formation and transformation by both populations given by:

$$\frac{dC_I}{dt} = X_1 k_{C1} \left(\frac{C_C}{K_{SC1} + C_C} \right) FR_{I1} + X_2 k_{C2} \left(\frac{C_C}{K_{SC2} + C_C} \right) FR_{I2}$$

$$- X_1 k_{I1} \left(\frac{C_I}{K_{SI1} + C_I} \right) - X_2 k_{I2} \left(\frac{C_I}{K_{SI2} + C_I} \right) \qquad (7\text{-}7)$$

where C_I is the intermediate concentration and FR_{I1} and FR_{I2} are the fractions converted to the intermediate by populations 1 and 2, respectively.

The rate equations presented above must be incorporated into equations that describe transport in the subsurface. The 1-D uniform transport of the electron donors 1 and 2, electron acceptor, and the contaminant is governed by advection, dispersion, and sorption given by:

$$\frac{\partial C}{\partial t} + \frac{\rho_b}{\theta}\frac{\partial \bar{C}}{\partial t} = D_h \frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial t} \tag{7-8}$$

where C is the concentration in the liquid phase (mg/l), $\bar{C}$ is the concentration of the sorbed solute on the solid phase (mg/kg), D_h is the hydrodynamic dispersion coefficient (m^2/d), v is the average interstitial fluid velocity (m/d), x is the spatial coordinate (m), ρ_b is the bulk density of the solid matrix (kg/l), and θ is the porosity. Based on our laboratory studies, sorption onto the aquifer solids was modeled as linear and reversible, with the equilibrium sorbed-phase concentration given by:

$$\bar{C} = K_d C \tag{7-9}$$

where K_d is the partition coefficient (l/kg).

For the case of equilibrium sorption, substitution of equation (7-9) into (7-8) leads to the following transport equation in terms of the liquid-phase concentration:

$$R\frac{\partial C}{\partial t} = D_h \frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial t} \tag{7-10}$$

where R is the retardation factor for the solute (Hashimoto et al., 1964):

$$R = 1 + \frac{\rho_b}{\theta} K_d \tag{7-11}$$

For the non-equilibrium case, the simple first-order linear nonequilibrium model was used:

$$\frac{d\bar{C}}{dt} = \alpha (K_d C - \bar{C}) \tag{7-12}$$

where α is the rate coefficient for mass transfer between the phases (d^{-1}). This simple model represents a reasonable approximation of more complex sorption models that include diffusive transfer between mobile and immobile zones (van Genuchten, 1985).

Substituting equation (7-12) into equation (7-8) yields:

$$\frac{\partial C}{\partial t} = D_h \frac{\partial^2 C}{\partial x^2} - v\frac{dC}{dx} - \frac{\rho_b}{\theta}\alpha (K_d C - \bar{C}) \tag{7-13}$$

Equations (7-12) and (7-13) must be solved to completely describe transport with non-equilibrium sorption.

The kinetic expressions presented in equations (7-1) through (7-7) are added to the transport equations. Microbial populations 1 and 2 are considered to be immobile, and thus the rate equations (7-2) and (7-4) are applied. The rate equations with transport are presented in Table 7.2, which recapitulates the resulting expressions for the primary electron donor (eq. 7-14), electron acceptor (eq. 7-15), the second (product) electron donor (eq. 7-16), the transformation of the target

TABLE 7.2. RATE EQUATIONS WITH TRANSPORT

$$\frac{\partial C_{D1}}{\partial t} = D_h \frac{\partial^2 C_{D1}}{\partial x^2} - v \frac{\partial C_{D1}}{\partial x} - k_1 X_1 \left(\frac{C_{D1}}{K_{SD1} + C_{D1}}\right)\left(\frac{C_{A1}}{K_{SA1} + C_{A1}}\right) \tag{7-14}$$

$$\frac{\partial C_{A1}}{\partial t} = D_h \frac{\partial^2 C_{A1}}{\partial x^2} - v \frac{\partial C_{A1}}{\partial x} - k_1 F X_1 \left(\frac{C_{D1}}{K_{SA1} + C_{D1}}\right)\left(\frac{C_{A1}}{K_{SA1} + C_{A1}}\right) - d_c f_d b_{11} X_1 \left(\frac{C_{A1}}{K_{SA1} + C_{A1}}\right) \tag{7-15}$$

$$\frac{\partial C_{D2}}{\partial t} = D_h \frac{\partial^2 C_{D2}}{\partial x^2} - v \frac{\partial C_{D2}}{\partial x} + X_1 b_{11} \left[\frac{C_{A1}}{K_{SA1} + C_{A1}}\right] F_{D2} + X_1 b_{12} F_{D2} - X_2 k_2 \left[\frac{C_{D2}}{K_{SD2} + C_{D2} + \frac{C_{A1}}{KI}}\right] \tag{7-16}$$

$$\frac{\partial C_C}{\partial t} = D_h \frac{\partial^2 C_C}{\partial x^2} - v \frac{\partial C_C}{\partial x} - X_1 k_{C1} \left(\frac{C_C}{K_{SC1} + C_C}\right) - X_2 k_{C2} \left(\frac{C_C}{K_{SC2} + C_C}\right) - \frac{\rho_b}{\theta} \alpha_C (K_{dC} C_C - \bar{C}_C) \tag{7-17}$$

$$\frac{dC_I}{dt} = D_h \frac{\partial^2 C_I}{\partial x^2} - v \frac{\partial C_I}{\partial x} + X_1 k_{C1} \left(\frac{C_C}{K_{SC1} + C_C}\right) FR_{I1} + X_2 k_{C2} \left(\frac{C_C}{K_{SC2} + C_C}\right) FR_{I2} - X_1 k_{I1} \left(\frac{C_I}{K_{SI1} + C_I}\right) - X_2 k_{I2} \left(\frac{C_I}{K_{SI2} + C_I}\right) - \frac{\rho_b}{\theta} \alpha_I (K_{dI} C_I - \bar{C}_I) \tag{7-18}$$

contaminant (eq. 7-17), and the intermediate product of the transformation (eq. 7-18). To simplify the equations, sorption is presented only for the contaminant and the intermediate. This simplification is consistent with the simulations of the field evaluation experiments were the electron donors and accceptors are considered not to sorb (retardation factor of 1), while the contaminants and intermediates do sorb. The sorption case presented is for non-equilibrium sorption. Along with equations (7-17) and (7-18), two additional equations for the solid-phase concentration, as represented by equation (7-12), must be solved.

For a semi-infinite system, the following initial and boundary conditions are applied:

$$C(x, t = 0) = f(x) \tag{7-19a}$$

$$-D_h \frac{\partial C}{\partial x} + vC = vg(x = 0, t) \tag{7-19b}$$

$$\frac{\partial C}{\partial x}(x = \alpha, t) = 0 \tag{7-19c}$$

where f(x) can take several forms: a constant value spatially, or a value that varies with distance. To specify initial conditions, values must be estimated for the concentrations of microbial mass, electron donor, electron acceptor, and secondary substrate. The inlet is represented by a third- or flux-type boundary condition for mobile components in equation (7-19b), where the parameter g(t) can take several forms, such as a constant value in time (as continuous feed), a pulse-type distribution, or a variable concentration distribution. Since the microbial mass is assumed immobile, a constant concentration boundary condition (first type) was used at $x = 0$. The outlet boundary condition used is a transmissive boundary condition.

This model was formulated using the finite-difference method that was solved by numerical integration. The method used and its verification are discussed by Semprini and McCarty (1989; in press).

MODEL SIMULATIONS OF BIOSTIMULATION EXPERIMENTS

Model simulations were compared with the results of biostimulation and biotransformation experiments presented in Section 6. The ability of the model to simulate the transient uptake of acetate and nitrate observed in the field experiments was tested. Since the biotransformation of the chlorinated aliphatics depends on the biostimulation of the two microbial populations, simulations of the biotransformation of the chlorinated organics were attempted only after good matches were obtained to the biostimulation portions of the experiments.

Model Inputs

Model simulations of transient acetate responses at the S1 and S2 observation wells assumed that the flow between the injection and monitoring wells can be represented by 1-D uniform flow. This assumption has been supported in 2-D simulations under the induced-flow conditions of the tests by Semprini and McCarty (1989; in press) and the 1-D analysis of tracer test results by Chrysikopoulos et al. (1990).

Breakthroughs of injected bromide and CT during the Tracer14 test were used to estimate the average interstitial fluid velocity and to determine sorption parameters for CT. Shown in Figure

7.1 is the model match of field observations at the S2 well. The fits were obtained using dispersion and fluid velocities consistent with those used in previous model simulations (Roberts et al., 1989). The first-order non-equilibruim sorption model adequately fits the retarded breakthrough of CT at the S2 well. The K_d value used in the simulation is lower than that derived from the laboratory study. Possible reasons for this difference are discussed in Section 6. The results indicate that the basic transport model used in the biostimulation and biotransformation simulations does a reasonable job of simulating transport in the field.

Table 7.3 lists the model input parameters, which were obtained by independent estimation to the extent possible, including: 1) measurement in the field or laboratory, 2) estimation based on literature values, or 3) adjustment within a range of literature values to obtain a good model fit. A heuristic fitting procedure was used: adjusted values were constrained within a reasonable range based on literature or theoretically derived values. As indicated in Table 7.3, dispersion coefficients were lower than those inferred from the fit to the complete breakthrough (Figure 7.1). The lower dispersion coefficients were required to match the field response to the short 1-hr pulse high concentration of acetate, as discussed in Section 6.

Table 7.4 contains the operational data used in the model. The operational data for chemical injection conditions were those used in the field experiments presented in Section 6. In these initial simulations 12 nodes over an interval of 2.4 meters were used. Time steps on the order of 0.001 d were required to maintain stability for simulations of 80 days.

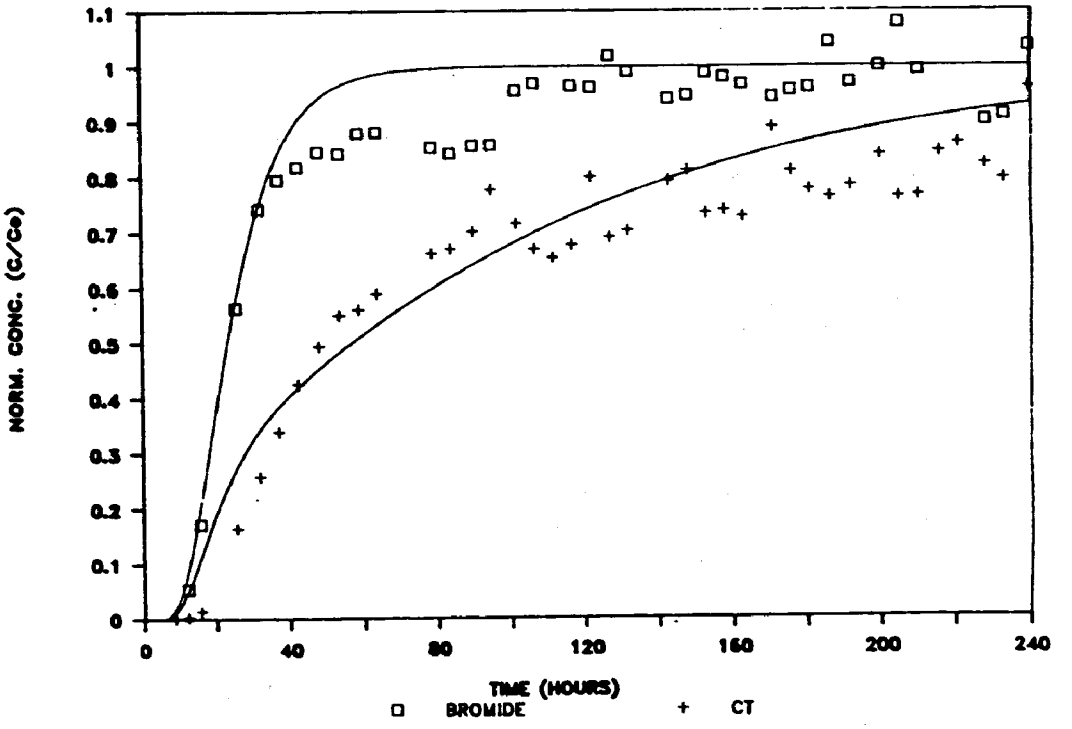

Figure 7.1. Bromide and CT breakthrough at the S2 well, and the corresponding simulation using the nonequilibrium sorption model. Model Parameters were: $v = 2.7$ m/d; $D_h = 0.17$ m^2/d; $K_d = 0.5$ l/kg; and $\alpha = 0.5$ d^{-1}.

TABLE 7.3. OPERATIONAL DATA USED IN THE BIOSTIMULATION MODEL

	Parameter	Biostim4	Value Basis[b]	Literature Values	Reference
	Parameters - Population 1				
Growth and Decay	k_1 (mg/mg-d)	3.5[a]	L	5.5	Rittmann et al. (1988)
	Y_1 (mg/mg)	0.27	L	0.27	Rittmann et al. (1988)
	K_{SD1} (mg/l)	1.0	F	0.55-2.0	Rittmann et al. (1988)
	K_{SA1} (mg/l)	1.0	F	0.145	Rittmann et al. (1988)
	b_{11} (d^{-1})	0.06	L	--	--
	b_{12} (d^{-1})	0.02	L	0.07	Rittmann et al. (1988)
	F	0.88	F	1.14	Rittmann et al. (1988)
	f_d	0.8	L	0.8	McCarty (1975)
	d_c	1.2	L	1.2	McCarty (1975)
	F_{D2} (mg/mg)	0.50	F		
CT Transformation	k_{C1} (mg/mg-d)	0.0005	F		
	k_{I1} (mg/mg-d)	0.0001	F		
	K_{SI1} (mg/mg-d)	1.0	F		
	FR_{I1}	0.50	F		
	Parameters - Population 2				
Growth and Decay	k_2 (mg/mg-d)	4.0	F		
	Y_2 (mg/mg)	0.15	F		
	K_{SD2} (mg/l)	1.0	F		
	K_{SA2} (mg/l)	1.0	F		
	KI	0.2			
	b_{21} (d^{-1})	0.15	F		
	b_{22} (d^{-1})	0.02	F		
CT Transformation	k_{C2} (mg/mg-d)	0.40	F		
	K_{SC2} (mg/l)	1.00	F		
	FR_{I2} (mg/mg)	0.50	F		
	k_{I2} (mg/mg-d)	0.06	F		
	K_{IS2} (mg/l)	1.00	F		
	Transport Parameters				
	v (m/d)	2.7	M		
	D (m^2/d)	0.32	F		
	R_{D1}	1	F		
	R_{A1}	1	F		
	R_{D2}	1	F		
	K_{DC} (1/kg)	0.50	F		
	K_{DI} (1/kg	0.25	F		
	α_C (d^{-1})	0.50	F		
	α_I (d^{-1})	1.0	F		

[a]Parameters adjusted for temperature differences.

[b]L = laboratory; F = fitted; M = measured in field tests.

TABLE 7.4. INPUT PARAMETERS USED IN THE BIOSTIMULATION MODEL SIMULATIONS

Parameter	Biostim4
Total Simulation Length (m)	2.4
Num. Nodes	12
Dx (m)	0.2
Dt (d)	0.001
Initial Conditions	
X_{1i} (mg/l)	1.9[a]
X_{2i} (mg/l)	0.2
C_{D1} (mg/l)	0.0
C_{A1} (mg/l)	0.0
Injection Conc.	
C_{D10} (mg/l)	600 (t = 0 to 96 hrs) 320 (t = 0 to 1900 hrs)
C_{A10} (mg/l)	26
Pulse Interval	
C_{D10} = (600 or 320 mg/l)	0.042 d
C_{D10} = 0 mg/l	0.50 d
C_{A10} = 26 mg/l	0.592 d

[a]Average value for the distributed concentration over the distance of 2.4 m.

Simulation of Acetate and Nitrate Utilization

Figure 7.2 illustrates the simulation match for the nitrate concentration response at the S1 well during the first 400 hrs of Biostim4. A good simulation match to the rapid uptake of nitrate was obtained. The match indicates that the nitrate response resulted from the biostimulation of denitrifying bacteria in the test zone. The simulations also predict regular pulses in nitrate concentration at the S1 well after 100 hrs of biostimulation, in response to the injection of acetate in short high concentration pulses. The field observations do not provide as consistent a record of pulsing as predicted by the model simulations. This results partly from the difficulty of obtaining samples at a sufficiently high frequency to capture the breakthrough of pulses in the field. Several pulses, however, were observed in the nitrate experimental record that are consistent with those predicted by the model.

The response of acetate at the S1 well to the injection of acetate in short high concentration pulses, shown in Figure 7.3, is captured fairly well by the model. During the first 100 hrs of the experiment, acetate was injected at a concentration of 550 to 600 mg/l, after which the pulse concentration was lowered to 320 mg/l. These changes in the injection concentration were incorporated in the simulation through the inlet boundary (equation 7-19b). The model response is consistent with the field observations, predicting lowering of the observed peak heights of acetate after 100 hrs in response to biostimulation and the change in injection concentration. The acetate simulations also match fairly well the general response to pulsing with regard to the frequency, duration, and attenuation of the pulses. Here again, the frequency of data collection in the experiment does not provide a sufficiently complete record to quantify accurately the pulse

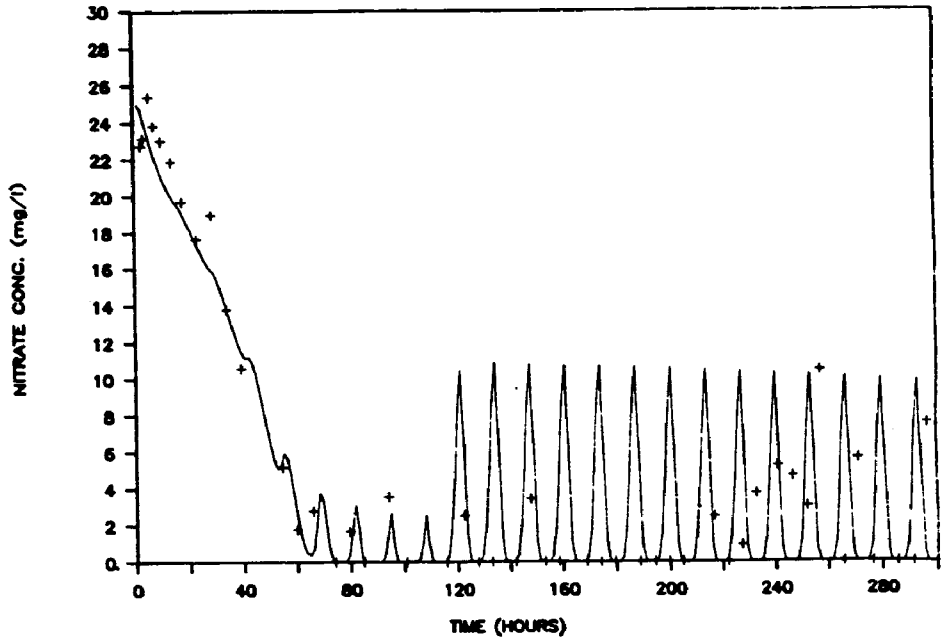

Figure 7.2. Simulated and observed nitrate responses at the S1 well due to biostimulation with acetate.

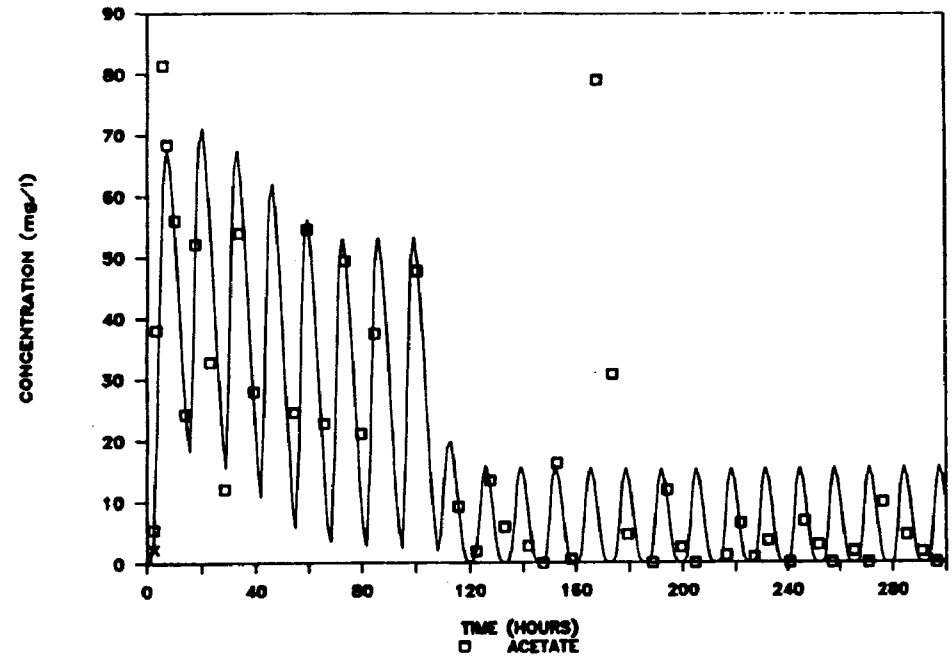

Figure 7.3. Simulated and observed acetate responses at the S1 well following a step change in the acetate input.

amplitude and frequency. The model was therefore fitted to the extreme values observed, presuming that these values circumscribe approximately the regular minimum and maximum values that were occurring. The response to stimulation of denitrifiers is not as clearly apparent in the acetate simulations as in the denitrifying simulation, due to the pulsing. The general decrease in acetate concentration is observed in the first 100 hrs, as indicated by the decrease in concentration of the minimum and maximum pulse values. The model does correctly simulate the observed periodicity of acetate and the lack of periodicity of nitrate during the first 100 hrs (Figure 7.2).

Figure 7.4 shows the simulation of acetate and nitrate responses at the S2 well. A good match was obtained using the same model parameters as for the S1 simulation (Figures 7.2 and 7.3). The model successfully predicts the uptake of nitrate and the maximum acetate values observed along with the attenuation in both the acetate and nitrate pulses due to transport and biological uptake. The ability of the model to match both responses at the S1 and S2 wells using the same input parameters is encouraging, since it indicates that the model adequately represents both the transport and microbial processes during the early stages of the experiment. The anomalous acetate data at approximately 180 hrs reflect the acetate input spike shown in Figure 6.7, which was not accommodated in the simulation input, as indicated in Figures 7.3 and 7.4.

The initial biomass concentration, X_{1i}, was unknown, and thus had to be treated as a major fitting parameter in the simulations. Since the test zone had been stimulated in the previous years, the initial microbial concentration was high, the best fitted value being 30 times that used in our initial simulations of biostimulation of methanotrophic bacteria (Semprini and McCarty, 1989). The bacterial population was also not uniform in space: the simulations indicate higher concentrations near the injection well, since the decaying methanotrophic biomass, which provides substrates for growth, was predicted previously to be higher in that region (Semprini and McCarty, 1989).

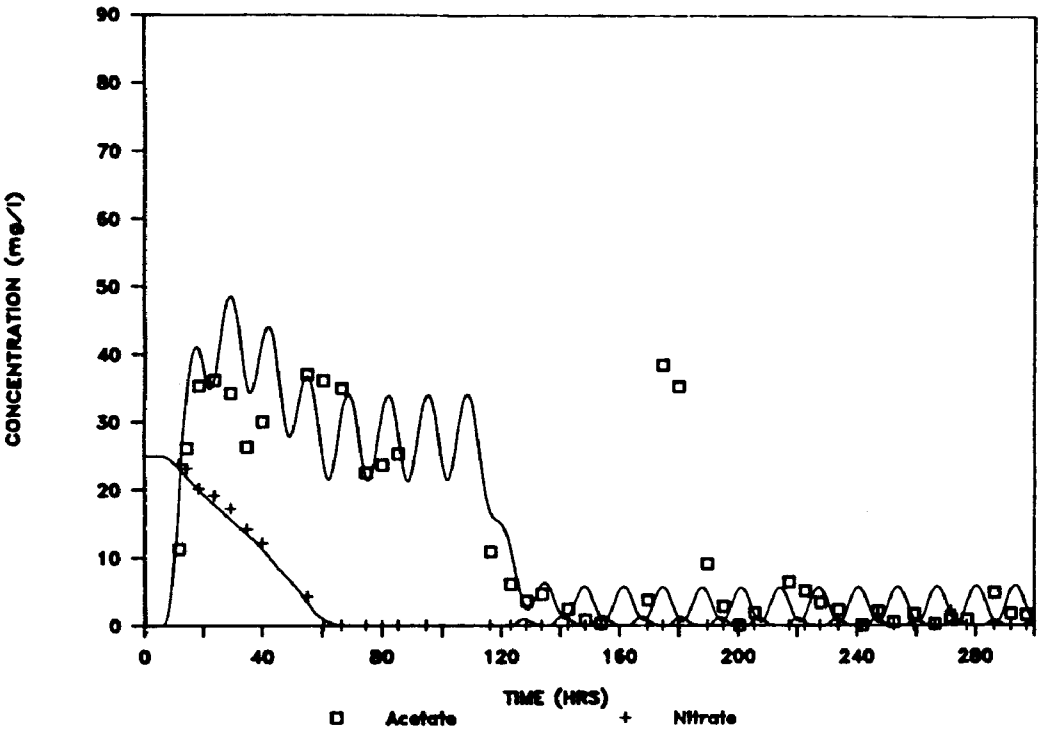

Figure 7.4. Simulated and observed acetate and nitrate responses at the S2 well.

Other parameters affecting the lag time are k_1, Y_1, b_{11}, b_{12}, K_{SD1}, and F. These are basic rate coefficients for which average literature values were used initially, but slight adjustments were made subsequently to improve model fit. The adjusted values indicated in Table 7.2 are consistent with values measured in the laboratory or determined from theoretical considerations. Values for d_c and f_d were not adjusted. The good fit between model simulations and field results, using coefficients largely derived from basic studies, is encouraging.

In order to match the pulses in acetate and nitrate observed in the field, due to pulsing of acetate at the injection well, the dispersion coefficient had to be adjusted from those obtained in model matches of bromide tracer tests (Figure 7.1). Dispersion coefficients were decreased by a factor of six, which is not a great reduction considering the known aquifer heterogeneities that would cause different responses among tests. The use of a lower dispersion coefficient is in agreement with the results of our previous methanotrophic modeling studies (Semprini and McCarty, 1989). The same dispersion coefficients were used for both the S1 and S2 well simulations. This yielded pulse heights of acetate and nitrate which were attenuated less at the S1 well compared to those at S2, which is consistent with field observations and the analytical solutions of Valocchi and Roberts (1983). Greater microbial uptake with the longer distance traveled also attenuates the pulse heights at the S2 well.

For a given pulsing strategy, biomass would reach some steady-state level after a sufficiently long time. The near-steady-state distribution of denitrifying biomass predicted after 1200 hrs of acetate addition is shown in Figure 7.5. Even though acetate was pulse-injected, the model nonetheless predicts that the denitrifying biomass grows close to the injection well. This results from the rapid growth kinetics of the denitrifiers and the presence of some nitrate in the acetate pulse. The model also assumes shallow biofilm kinetics, which may be invalid in the region close to the injection well: mass transport limitations, which would influence local concentrations within a deeper biofilm, are not considered in the model formulation. Biofilm modeling of the growth would result in more distributed growth than predicted here (Rittmann et al., 1988). Despite its limitations, the model does indicate that some clogging in the region of the wellbore was likely, as was observed in the latter stages of the biostimulation study.

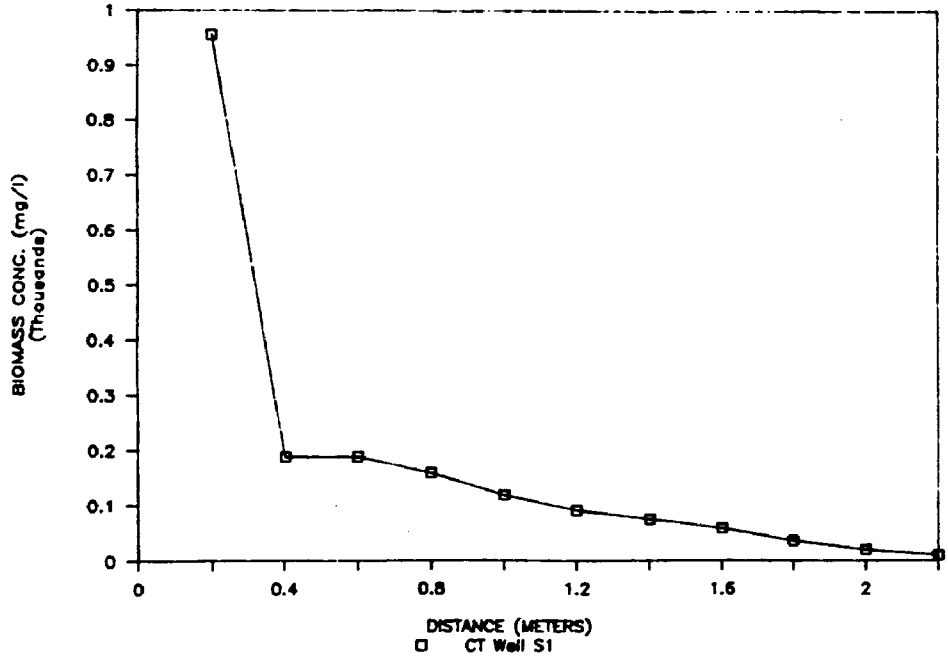

Figure 7.5. Simulation of the near-steady-state denitrifying biomass distribution.

Simulation of the Chlorinated Aliphatics Transformation

Model simulations of the biotransformation of chlorinated aliphatics were compared with the results of the field evaluation. The simulations were performed for two cases: 1) transformation by denitrifiers, and 2) transformation by both denitrifiers and a second population growing on the decay products of the denitrifiers. Model input parameters for the simulations appear in Table 7.2.

Figure 7.6 presents model simulations for CT transformation by denitrifiers for the conditions for the conditions of growth illustrated shown in Figures 7.2-7.4. The simulated responses at wells S1 and S2 do not provide a good match with the observed data. In order to obtain a reasonable fit to the transformation observed at later times, an excessively rapid initial decrease in CT concentration was required, compared to that observed in the field test. This results from the rapid growth of the denitrifiers predicted from the simulations of acetate and nitrate utilization. Most of the microbial population resides in the first meter of the test zone (Figure 7.5), and thus the simulation indicates that most of the transformation occurs there. This differs greatly from the field observations, which show that most of the transformation occurred in the zone between the S1 and S2 well. This anomaly suggests that the main population of denitrifying bacteria was not responsible for the transformation of CT.

Simulations were performed with the two-population model to determine whether responses similar to those obtained in the field test could be achieved. Since most of the input parameters for the model were not known, the simulations are considered as an exploratory exercise to evaluate 1) whether responses similar to those observed in the field can be obtained, 2) what processes and process parameters are required to achieve a similar response, and 3) whether the fitted process parameter values agree reasonably compared with laboratory-determined parameters. Both the transformation of CT and the formation of CF as an intermediate product were simulated. The response of the model to the period of nitrate addition and the sudden removal of nitrate after 1260 hrs of acetate addition was evaluated. Simulations of the transformation of the other halogenated aliphatics were also performed to determine the compound-specific rate coefficients required to match their respective responses.

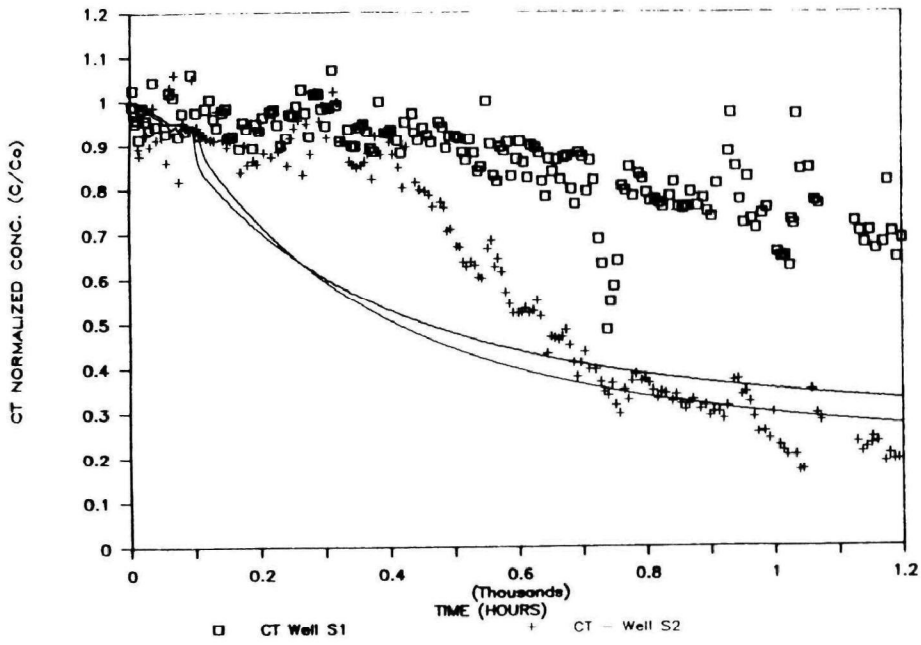

Figure 7.6. Simulation of CT transformation by denitrifiers.

Important guesstimates in the simulations are the initial concentration and rate of growth and decay for population 2; the extent of growth inhibition due to the presence of nitrate; the rates of cometabolic transformation; and the fraction of transformed CT that forms intermediates via the parallel pathway. The model assumed that population 2 was responsible for most of the CT transformation. Thus, the transformation rate coefficient, k_{C1}, for the denitrifiers (population 1) was set to a much lower value than the k_{C2} values of population 2 (Table 7.2).

The simulation of the CT transformation and CF formation at the S2 well is shown in Figure 7.7. A reasonable fit to the transformation of CT and the formation of CF was achieved. The simulation shows the gradual increase in the rate of transformation concomitant with the increase in population 2, as indicated by the decrease in CT and the increase in CF concentrations. The simulation of the CF concentration exhibits a plateau in concentration that is reached at 900 to 1000 hrs, and then a slight decrease in concentration after 1000 hrs; this results from the transformation of CF by population 2. The rate coefficient k_{C2} for CT transformation was a factor of 5 greater than the coefficient k_{I2} for CF transformation.

The simulation of the response at the S1 well using the same parameters as used for the S2 well is shown in Figure 7.8. A reasonable fit was obtained, in that the slower decrease in CT concentration compared to that observed at the S2 well was successfully simulated with a consistent input parameter set. Thus, when transformation by population 2 was included, a much better fit to the field observations was obtained, compared to that achieved by the denitrifying population alone (Figure 7.6). The chloroform match is not as good as that achieved for the S2 well. At an early time, less formation is predicted than was observed. The fraction of CT transformed to CF may have been greater in the first meter than in the second meter. In the simulations, the fraction of CT transformed via the parallel (i.e. CF-forming) pathway (FR_{I2}) was held constant; no attempt was made to change FR_{I2} to improve the fit.

The initial concentration of population 2 in the test zone prior to biostimulation was an important fitting parameter. In estimating this concentration, both the temporal response and the initial degree of transformation were considered. The initial population was considered to be large enough, so that an initial transformation of approximately 3% occurred in the test zone, consistent with the amount of CF production observed during the Tracer14 experiment. This initial population was assumed to be uniformly distributed in the test zone. The initial concentration of the secondary bacteria was assumed to be a factor of 10 lower than the initial population of denitrifying bacteria.

In order to simulate the slower rate of CT disappearance at the S1 well compared to that at the S2 well (i.e., consistent with the observed behavior), the growth of population 2 in the first meter had to be limited. This was accomplished in the model formulation by inhibiting the growth and enhancing the decay of population 2 in the presence of nitrate, as given by equation (7.4). Growth was strongly inhibited by using a growth inhibition factor, KI, of 0.2. Decay of population 2 was enhanced in the presence of nitrate by using a decay factor, b_{21}, of 0.15/d.

The response of the model to the removal of nitrate (at t = 1260 hrs; see Figure 6.12) from the injected fluid does not match closely the behavior observed in the field study. The simulations do not show the rapid decrease in CT concentration that was indicated by the field results (Figures 7.7 and 7.8). One possibility for the absence of transformation rate increases is that the simulated growth of population 2 required the production of the requisite growth substrate, C_{D2}, via the decay of the denitrifying population (equation 7-5). For the simulations shown in Figures 7.7 and 7.8, the degradation of the denitrifying population was strongly tied to the presence of nitrate, with decay coefficients in the presence of nitrate (b_{11}) of 0.06/d compared to a rate of 0.02/d in the absence of nitrate (b_{12}). Thus, when nitrate addition was stopped, less decay of the denitrifying

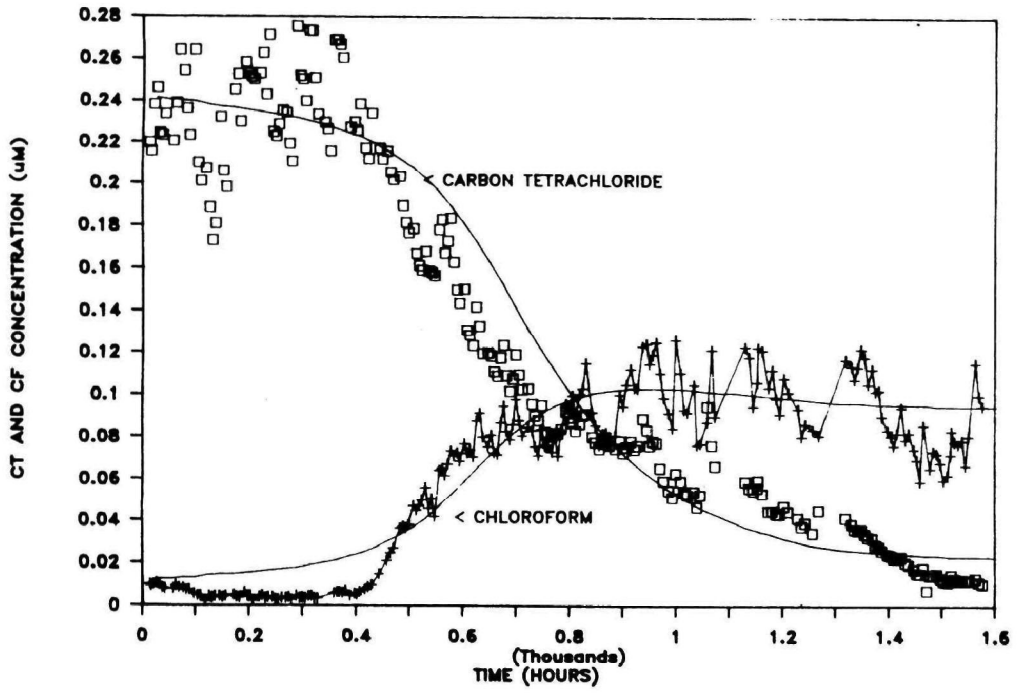

Figure 7.7. Simulated and observed CT and CF responses at the S2 well using the two-population model.

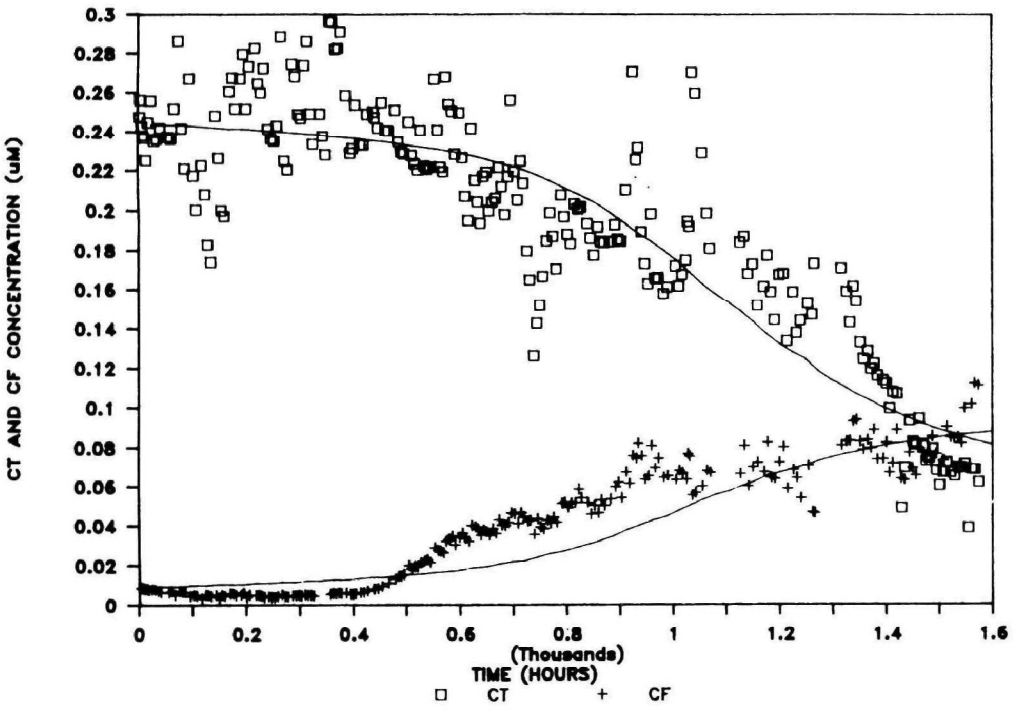

Figure 7.8. Simulated and observed CT and CF responses at the S1 well using the two-population model.

population occurred in the simulations, and less secondary substrate was available for growth of population 2 according to the simulation.

To study the effect of the mode of decay of the denitrifying population on the response to the termination of nitrate addition, a simulation was performed in which the decay rate coefficients were changed, with b_{11} being 0.02/d and b_{12} being 0.06/d. Figure 7.9 shows the effect of these parameter value changes on the simulated CT response at the S1 and S2 wells following nitrate termination at 1260 hrs: the response to the termination of nitrate addition at well S1 is similar to the field data, with enhanced rates observed, as shown by the kinks in the curves. However, the overall agreement between the model simulation and the data, especially for well S2, is not as good as that obtained before the parameter modification (Figures 7.7 and 7.8).

These simulation results illustrate the need for improved understanding of the relevant processes and the requisite rate parameters. Toward this end, additional model sensitivity analyses are being undertaken to determine the optimum set of model parameters that best matches all aspects of the data. In addition, several other processes should be considered for possible incorporation into the model formulation: namely, the growth of population 2 or another population, on acetate, as well as the direct inhibition of nitrate on CT transformation. However, better insight into the appropriate models to use for transformation kinetics and the populations involved in the transformation is required before these additional complexities are warranted.

Model simulations were also performed for the transformations of Freon-11, Freon-113, and TCA. The simulations were all performed using the set of parameters given in Table 7.2, with only the rate parameter k_{C2} varied for the different compounds. The value of K_{SC2} was held constant at 1 mg/l. The simulations of CT and TCA (Figure 7.10), and Freon-11 and Freon-113 (Figure 7.11) at the S2 well respectively demonstrate reasonable matches to the field responses, indicating that the same biotransformation processes were occurring, but at different rates.

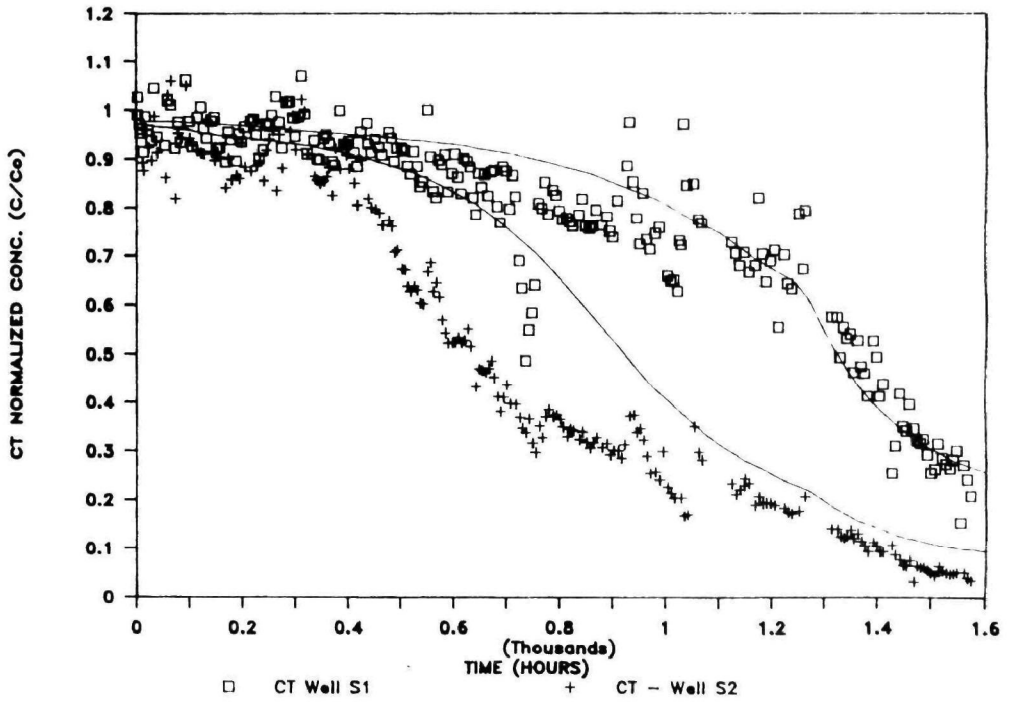

Figure 7.9. Sensitivity to denitrifiers' decay coefficients in response to no nitrate addition.

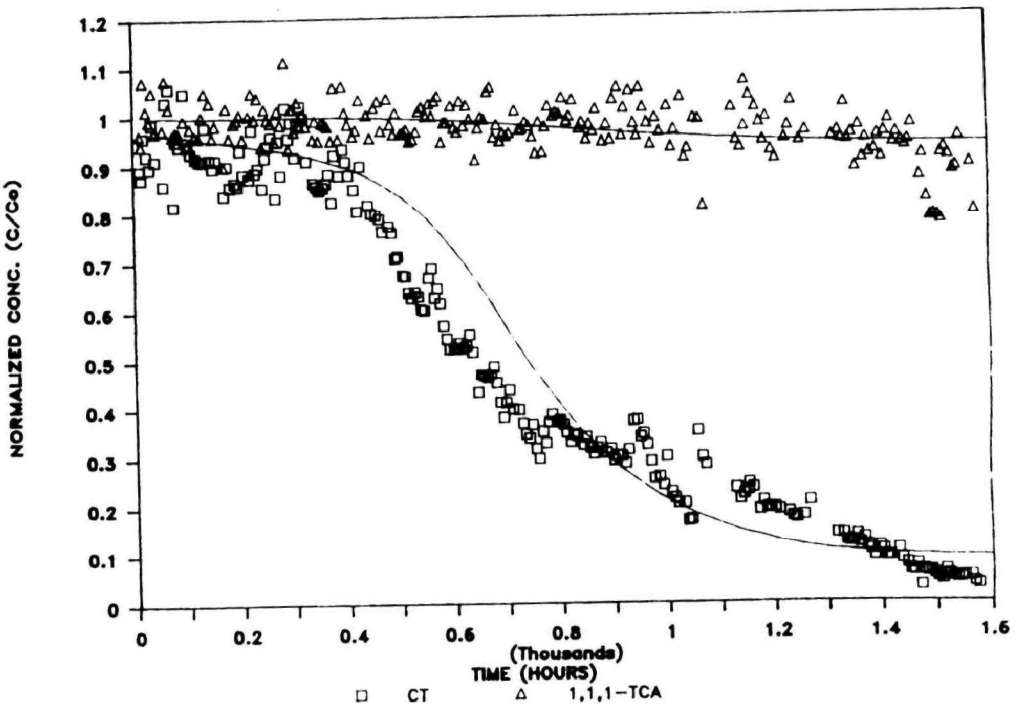

Figure 7.10. Simulation of transformation of CT and TCA at the S2 well.

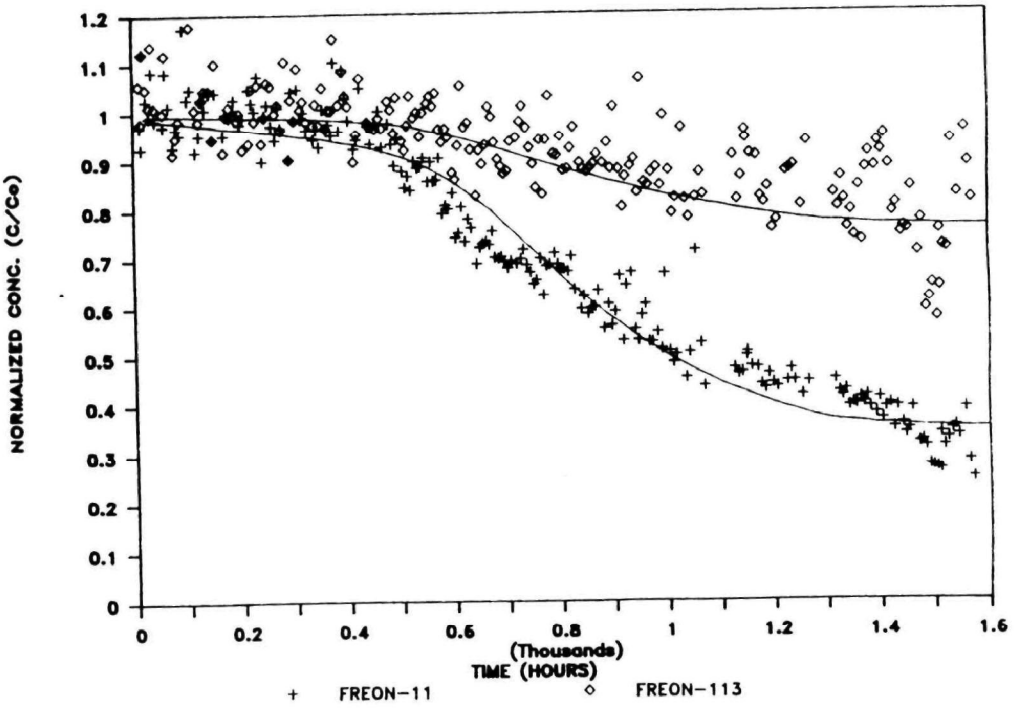

Figure 7.11. Simulation of transformation of Freon-11 and Freon-113 at the S2 well.

The values of the effective first-order transformation rate constants, k_{C2}/K_{SC2}, used in the simulations are given in Table 7.5. The rate constant for CT was forty times greater than for TCA, based on the field data. The simulations indicate that Freon-11 is transformed at less than half the rate of CT as a result of the substitution of one fluorine atom for a chlorine atom. The substitution of a hydrogen atom for a chlorine atom has an even greater effect: CF transforms at a rate only one fifth as great as does CT. The rate of transformation of Freon-113 ($C_2Cl_3F_3$) is four times greater than that of the less-substituted TCA ($C_2Cl_3H_3$).

TABLE 7.5. VALUES OF k/K_S FOR THIS STUDY

Compound	This Study $\frac{k}{K_S}$ $\left(\frac{1}{\text{mg-cells•d}}\right)$	Galli and McCarty (1989) $\frac{k}{K_S}$ [a] $\left(\frac{1}{\text{mg-cells•d}}\right)$	Bouwer and Wright (1988) $\frac{k}{K_S}$ [b] $\left(\frac{1}{\text{mg-cells•d}}\right)$
CT	0.40	0.12	0.20
Freon-11	0.16	--	--
CF	0.08	0.012	--
Freon-113	0.04	--	--
TCA	0.01	0.007	0.005

[a]Reported rates on protein basis, converted to cell based on protein being 65% of the cell mass.
[b]Under sulfate-reducing conditions.

The absolute rates and ratio of rates can be compared with laboratory studies of Galli and McCarty (1989) using *Clostridium* sp. and with mixed-culture column studies under sulfate-reducing conditions performed by Bouwer and Wright (1988). The absolute ratios of k/K_S from this field study are 2 to 3 greater than those from the earlier laboratory studies. The agreement is reasonable considering the many fitted parameters in the model, including the yield and rates of biological decay, which would affect both the estimated microbial concentrations and the resulting rate coefficients. The comparison of k/K_S ratios also agrees with Galli and McCarty (1989) and Bouwer and Wright (1988), who reported that TCA degraded at a rate 13 and 40 times lower than CT, compared to a factor of 40 reported here. Galli and McCarty (1989) found that CF degraded rates ten times slower than CT, while a ratio of 5 is reported here. Overall there appears to be a reasonable agreement between both the absolute and relative values of the rate coefficients from the laboratory and field studies, which is encouraging.

Summary of the Model Simulations

Overall the model simulations yield results similar to those observed in the field evaluation using a reasonable set of model parameters. The simulations indicate that a significant population of denitrifiers was initially present, but the hypothesis that the transformation of the CT is brought about mainly by denitrifiers is not supported by the model simulations. On the contrary, comparison between the simulations and the observations supports the hypothesis that a secondary population, the growth of which is inhibited by nitrate, was responsible for the transformation.

The simulations accounted for the transformation of CT and the formation of CF as an intermediate product. The simulations also indicate that the different halogenated aliphatics were biotransformed by the same process but at different rates. The rates derived from the model fits were in the range of values derived in laboratory studies.

Additional model sensitivity analysis is necessary to determine optimum parameters that best fit the overall data, including the transient test when nitrate was removed. It appears that the denitrifiers' decay rate in the absence of nitrate may be substantially higher than previously believed; this aspect deserves further study.

Model refinements should include 1) the growth of the secondary population on acetate; 2) the inhibition of transformation due to the presence of nitrate; and 3) mass transfer limitations when the growth exceeds that of a shallow biofilm.

More detailed laboratory studies are required to determine the appropriate contaminant transformation kinetic models. These studies should address 1) whether nitrate inhibits the growth of the secondary population, and if so, the appropriate model for the inhibition, 2) whether the presence of nitrates inhibit the rate of CT transformation, and if so, how the inhibition submodel should be formulated, 3) the role of redox conditions, and whether biotransformation models need to be coupled with geochemical models, and 4) whether complex biofilm models need to be considered, including microbial speciation within the biofilm.

The modeling presented here has been a useful tool in gaining a better understanding of the processes occurring in the field evaluation. The results demonstrate that models of this type are useful for integrating biotransformation processes into groundwater transport and in comparing laboratory and field results. The modeling also serves to determine important directions for further laboratory research and to sharpen the questions that must be addressed in future field work.

REFERENCES

Bagley D. M., and J. M. Gossett. 1990. Tetrachloroethylene Transformation to Trichloroethylene and cis-1,2-Dichloroethene by Sulfate-Reducing Enrichment Cultures. Appl. Environ. Microbiol. 56:2511-2516.

Ball, W. P. 1989. Equilibrium Sorption and Diffusion Rates Studies with Halogenated Organic Chemicals and Sandy Aquifer Material. Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA.

Barrio-Lage, G., F. Z. Parsons, R. S. Nassar, and P. A. Lorenzo. 1986. Sequential Dehalogenation of Chlorinated Ethenes. Env. Sci. Techn. 20:96-99.

Belay, N., and L. Daniels. 1987. Production of Ethane, Ethylene, and Acetylene from Halogenated Hydrocarbons by Methanogenic Bacteria. Appl. Environ. Microbiol. 53:1604-1610.

Bouwer, H. 1978. Groundwater Hydrology. McGraw-Hill Book Company, New York.

Bouwer, E. J., and P. L. McCarty. 1983a. Transformation of 1- and 2-Carbon Halogenated Aliphatic Organic Compounds under Methanogenic Conditions. Appl. Environ. Microbiol. 45:1286-1294.

Bouwer, E. J., and P. L. McCarty. 1983b. Transformation of Halogenated Organic Compounds under Denitrification Conditions. Appl. Environ. Microbiol. 45:1295-1299.

Bouwer, E. J., and P. L. McCarty. 1985. Utilization Rates of Trace Halogenated Organic Compounds in Acetate-Grown Biofilms. Biotechnol. Bioeng. 27:1564-1571.

Bouwer, E. J., and J. P. Wright. 1988. Transformation of Trace Halogenated Aliphatics in Anoxic Biofilm Columns. J. Contaminant Hydrol. 2:155-169.

Bouwer, E. J., B. E. Rittmann, and P. L. McCarty. 1981. Anaerobic Degradation of Halogenated 1- and 2-Carbon Organic Compounds. Env. Sci. Technol. 15:596-599.

Chrysikopoulos, C. V., P. V. Roberts, and P. K. Kitanidis. 1990. One-Dimensional Solute Transport in Porous Media with Well-to-Well Recirculation: Application to Field Experiments. Water Resour. Res. 26:1189-1195.

Criddle, C. S. 1989. Reductive Dehalogenation in Microbial and Electrolytic Model Systems. Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA.

Criddle, C. S., P. L. McCarty, M. C. Elliott, and J. F. Barker. 1986. Reduction of Hexachloroethane to Tetrachloroethylene in Groundwater. J. Contaminant Hydrol. 1:133-142.

Curtis, G.P. 1984. Sorption of Hydrophobic Organic Solutes onto Aquifer Materials: Comparisons Between Laboratory Results and Field Observations. M.S. Thesis, Department of Civil Enginelering, Stanford University, Stanford, CA .

Curtis, G. P., P. V. Roberts, and M. Reinhard. 1986. 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.

Curtis, G. P. 1990. Reductive Dehalogenation of Hexachloroethane and Carbon Tetrachloride by Aquifer Sand and Humic Acid. Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA.

Egli, C., R. Scholtz, A. M. Cook, and T. Leisinger. 1987. Anaerobic Dechlorination of Tetrachloromethane and 1,2-Dichloroethane to Degradable Products by Pure Cultures of *Desulfobacterium* sp. and *Methanobacterium* sp. FEMS Microbiol. Lett. 43:257-261.

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

Fogel, M. M., A. R. Taddeo, and S. Fogel. 1986. Biodegradation of Chlorinated Ethenes by a Methane-Utilizing Mixed Culture. Appl. Environ. Microbiol. 51:720-724.

Freeze, R. A., and J. A. Cherry. 1979. Groundwater. Prentice-Hall, Inc., Englewood Cliffs, NJ, p. 604.

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

Gossett, J.M. 1985. Anaerobic Degradation of C1 and C2 Chlorinated Hydrocarbons. U.S. Air Force Report ESL-TR-85-38. National Technical Information Service, Springfield, VA.

Harmon, T. C., and P. V. Roberts. 1989. Section 8 in P. V. Roberts, L. Semprini, G. D. Hopkins, D. Grbić-Galić, P. L. McCarty, and M. Reinhard. In-situ Aquifer Restoration of Chlorinated Aliphatics by Methanotrophic Bacteria. EPA/600/2-89/033. Department of Civil Engineering, Stanford University, Stanford, CA, 1989.

Hartsmans, S., J. A. M. de Bont, J. Tramper, and K. Ch. M. A. Luyben. 1985. Bacterial Degradation of Vinyl Chloride. Biotechnol. Letts. 7:383-388.

Hashimoto, I., K. B. Deshpande, and H. C. Thomas. 1964. Peclet Numbers and Retardation Factors for Ion Exchange Columns. Ind. Eng. Chem. Fund. 3(3):213-218.

Hopkins, G. D., L. Semprini, P. V. Roberts, and D. M. Mackay. 1988. An Automated Data Acquisition System for Assessing in-situ Biodegradation of Chlorinated Aliphatic Compounds. Proc. of Sec. National Outdoor Action Conference on Aquifer Restoration, Ground Water Monitoring and Geophysical Methods, NWWA, Las Vegas, Nevada, May 23-26, Vol. 1, pp. 201-203.

Horvath, A. L. 1982. Halogenated Hydrocarbons: Solubility and Miscibility in Water. M. Dekker, New York, p. 889.

Johns, R. A., L. Semprini, and P. V. Roberts. 1990. Estimating Aquifer Properties in Regression of Pump Test Response. Submitted to Ground Water.

Kriegman, M. R., and M. Reinhard. In press. Reduction of Hexachloroethane and Carbon Tetrachloride at Surfaces of Ferrous Iron Bearing Minerals. In: Organic Substances and Sediments in Water, R. A. Baker, ed. ACS Symposium Series, American Chemical Society, Washington, D.C.

Leo, A., C. Hansch, and D. Elkins. 1971. Partition Coefficients and Their Uses. Chem. Rev. 71(6):525-616.

McCarty, P. L. 1975. Stoichiometry of Biological Reactions. Progress in Water Tech. 7(1): 157-172.

McCarty, P. L. 1984. Application of Biological Transformations in Ground Water. Proc. 2nd International Conference on Groundwater Quality Research, Tulsa, March 1984, N. N. Durham and A. E. Redelfs, eds. University Center for Water Research, Oklahoma State University.

McCarty, P. L. 1988. Bioengineering Issues Related to in-situ Remediation of Contaminated Soils and Groundwater. In: Environmental Biotechnology, G. S. Omenn, Ed. Plenum Publishing Corporation, pp. 143-162.

McCarty, P. L., L. Beck, and P. St. Amant. 1969. Biological Denitrification of Wastewaters by Addition of Organic Materials. Proceedings, 24th Annual Industrial Waste Conference, Purdue University, pp. 1271-1285.

McCarty, P. L., H. Siegrist, T. M. Vogel, J. Nakai, and J. Peltola. 1986. Biotransformation of Groundwater Contaminants. Technical Report No. 298, Department of Civil Engineering, Stanford University, Stanford, CA.

Parsons, F. and G. Barrio-Lage. 1985. Chlorinated Organics in Simulated Groundwater Environments. J. Am. Water Works Assoc. 77(5):52-59.

Press, F., and R. Siever. 1974. Earth. Freeman & Co., San Francisco, p. 945.

Reinhard, M., G. P. Curtis, and M. R. Kriegman. 1990. Abiotic Reductive Dechlorination of Carbon Tetrachloride and Hexachloroethane by Environmental Reductants. Final Report, Robert S. Kerr Environmental Research Laboratory, Environmental Protection Agency, Ada, OK.

Rittmann, B. E., A. J. Valocchi, J. E. Odencrantz, and W. Bae. 1988. In-situ Bioreclamation of Contaminated Groundwater. Illinois Hazardous Waste Research and Information Center, HWRIC RR 031.121.

Roberts, P. V., M. N. Goltz, and D. M. Mackay. 1986. 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.

Roberts, P. V., L. Semprini, G. D. Hopkins, D. Grbić-Galić, P. L. McCarty, and M. Reinhard. 1989. In-situ Aquifer Restoration of Chlorinated Aliphatics by Methanotrophic Bacteria. EPA/600/2-89/033. Department of Civil Engineering, Stanford University, Stanford, CA.

Roberts, P. V., G. D. Hopkins, D. M. Mackay, and L. Semprini. 1990. A Field Evaluation of in-situ Biodegradation of Chlorinated Ethenes: Part 1, Methodology and Field Site Characterization. Ground Water 28:591-604.

Semprini, L., and P. L. McCarty. 1989. Section 13 in P. V. Roberts, L. Semprini, G. D. Hopkins, D. Grbić-Galić, P. L. McCarty, and M. Reinhard. In-situ Aquifer Restoration of Chlorinated Aliphatics by Methanotrophic Bacteria. EPA/600/2-89/033. Department of Civil Engineering, Stanford University, Stanford, CA, 1989.

Semprini, L., and P. L. McCarty. In Press. Comparison Between Model Simulations and Field Results for in-situ Biorestoration of Chlorinated Aliphatics. Part 1. Biostimulation of Methanotrophic Bacteria. Ground Water.

Semprini, L., P. V. Roberts, G. D. Hopkins, and D. M. Mackay. 1988. A Field Evaluation of in-situ Biodegradation for Aquifer Restoration. EPA/600/S2-87/096. NTIS No. PB 88-130 257/AS. Springfield, VA.

Siegrist, H., and P. L. McCarty. 1987. Column Methodologies for Determining Sorption and Biotransformation Potential for Chlorinated Aliphatic Compounds in Aquifers. J. Contaminant Hydrol. 2:31-50.

Valocchi, A. J. 1986. Effect of Radial Flow on Deviation from Local Equilibrium During Sorbing Solute Transport through Homogeneous Soils. Water Resour. Res. 22(12):1693-1701.

Valocchi, A. J., and P. V. Roberts. 1983. Attenuation of Groundwater Contaminant Pulse. J. Hydrol. Eng. (ASCE) 109(12):1665-1682.

van Genuchten, M. 1985. A General Modeling Approach for Modeling Solute Transport in Structure Soils. Proc. 17th International Congress, International Association of Hydrogeologists, Tucson, AZ, January 1985.

Vogel, T. M. 1988. Biotic and Abiotic Transformation of Halogenated Aliphatic Compounds. Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA.

Vogel, T. M., and P. L. McCarty. 1985. Biotransformation of Tetrachloroethylene to Trichloroethylene, Dichloroethylene, Vinyl Chloride, and Carbon Dioxide under Methanogenic Conditions. Appl. Environ. Microbiol. 49:1080-1083.

Vogel, T. M., and P. L. McCarty. 1987. Abiotic and Biotic Transformations of 1,1,1-Trichloroethane under Methanogenic Conditions. Env. Sci. Techn. 21(12):1208-1213.

Vogel, T. M., C. S. Criddle, and P. L. McCarty. 1987. Transformations of Halogenated Aliphatic Compounds. Env. Sci. Techn. 21(8):722-736.

Westrick, J. J., W. J. Mello, and R. F. Thomas. 1984. The Groundwater Supply Survey. J. Am. Water Works Assoc. 76:52-59.

Widdel, F. 1988. Microbiology and Ecology of Sulfate- and Sulfur-Reducing Bacteria. In: Biology of Anaerobic Microorganisms, A. J. B. Zehnder, Ed. John Wiley & Sons, New York, pp. 469-585.

Wilson, J. T., and B. H. Wilson. 1985. Biotransformation of Trichloroethylene in Soil. Appl. Environ. Microbiol. 29:242-243.

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