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Robert S. Kerr Environmental
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Project Summary

In-Situ Biotransformation of Carbon Tetrachloride Under Anoxic Conditions

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This project evaluated the potential for enhanced in-situ biotransformation of chlorinated aliphatic solvents under anoxic conditions. The target test compound was carbon tetrachloride (CT). The transformation of 1,1,1-trichloroethane (TCA) and two chlorofluorocarbons (Freon-11 and Freon-113) present as background contaminants in the test zone groundwater was also evaluated. Laboratory column studies were performed initially and confirmed that transformation of CT was likely under the conditions of the proposed field tests, and indicated that chloroform was a product likely to result from the transformation. In the field experiments, biostimulation of a native microbial population in a shallow confined aquifer was accomplished through the introduction of acetate as the electron donor and substrate for growth, in the absence of oxygen and the presence of nitrate, which was used as the electron acceptor. Acetate and nitrate utilization commenced within a few days upon the addition of acetate. The disappearance of CT commenced 2 weeks after active denitrification began, and the rate accelerated following nitrate depletion. The appearance of chloroform as an intermediate product coincided with the disappearance of CT in the 10-week test and represented approximately 30% of the CT transformed. The laboratory studies suggested that the other major product of CT transformation by an alternate pathway was CO_2. The other halogenated solvents were also significantly transformed, but at slower rates than CT. The percent transformation within 2 meters of travel in the test zone was as follows: TCA, 15%; Freon-113, 20%; Freon-11, 68%; and CT, 95%. With all the halogenated aliphatics 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. A mathematical model which included the transport and transformation processes thought to be important successfully mimicked the behavior observed in the field study. The model results supported the hypothesis that the growth of a secondary population was responsible for the biotransformation, and that different compounds were transformed by the same process, but at different rates. This research demonstrates that it is possible to promote in-situ biotransformation of halogenated aliphatics in the subsurface under anoxic conditions. A problem confronting the use of anoxic bioremediation processes is the formation of halogenated intermediate products.

This Project Summary was developed by EPA's Robert S. Kerr Environmental Research Laboratory, Ada, OK, to announce key findings of the research project that is fully documented in a separate report of the same title (see

Project Report ordering information at back).

Introduction

Chlorinated aliphatic compounds with one or two carbon atoms are widely used as solvents, degreasing agents, and intermediates in chemical synthesis. Their widespread use and uncontrolled disposal has resulted in the contamination of groundwater supplies. There is an urgent need to better 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 may lead to complete mineralization to non-toxic end products and/or may create intermediate products that are less harmful, are more easily removed from the aquifer, and are more readily treated by other processes.

This project 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 were provided to enhance microbial growth. 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 CT, TCA, Freon-11, and Freon-113, was assessed by controlled addition, frequent sampling, quantitative analysis, and mass-balance comparisons. To provide guidance for the field work, laboratory studies were also performed to obtain a more basic understanding of key microbial and physical processes involved.

Objectives

The specific objectives of this project were the following: 1) to demonstrate in a controlled field experiment the ability to biostimulate an indigenous population of denitrifying bacteria under conditions representative of groundwater environments; 2) to quantify the extent of enhanced biodegradation of CT, 1,1,1-TCA, Freon-11, and Freon-113 in the biostimulated zone, and the formation of intermediate products; 3) to determine how to modify biostimulation conditions to achieve more complete mineralization of the halogenated aliphatics; 4) to evaluate laboratory procedures for simulating field results; and 5) to use mathematical models that incorporate key microbial and transport processes for interpreting the results of laboratory and field experiments.

Field Demonstration Methodology

A methodology was developed to evaluate objectively and quantitatively the effectiveness of the approach for stimulating anoxic microbial growth in order to transform the target organic compounds under natural conditions at the field site. The methodology entails creating a flow field dominated by pumping from an extraction well, while introducing solutes in known amounts at a nearby injection well and by measuring concentrations regularly at the injection, extraction, and intermediate observation points. Evidence of transformation was then assessed by quantitative examination of the concentration histories of the various solutes at the several monitoring points, and comparing results under biostimulation conditions with results obtained under similar conditions in the absence of biostimulation measures. A specially designed automated data acquisition and control system provided continuous records of high-accuracy data over sustained periods, which enabled mass balances to be made with relative errors of only a few percent.

Site Characterization

The Moffett Field Naval Air Station, Mountain View, CA, site chosen for this demonstration was used earlier to study in-situ restoration of chlorinated aliphatics by methanotrophic bacteria (EPA/600/S2-89/033), and has been well characterized. The site is characteristic of typical groundwater contamination, where a shallow sand-and-gravel aquifer is contaminated by chlorinated compounds widely used as solvents. Drilling logs revealed that the shallow aquifer of the test site consisted of a layer of sand and gravel, approximately 5 m below the surface and 1.2 m thick, well confined above and below by a silty clay layer of low permeability. The transmissivity of the test zone is high (approximately 100 m^2/day), which permits extraction of water at the design rate (approximately 10 l/min) without excessive drawdown at the extraction well.

The formation groundwater was also of appropriate composition for the field experiments. The dissolved oxygen concentration was below detection. Nitrate and sulfate, two potential electron acceptors, were present at concentrations of 25 mg/l (as nitrate) and 700 mg/l (as sulfate). The groundwater was contaminated with TCA (50 µg/l), Freon-113 (6 µg/l), and Freon-11 (3 µg/l). The target compound, CT, was not present and therefore was continuously added in a controlled manner to the injected water. The other halogenated aliphatics that were present in the extracted groundwater were reinjected along with CT into the test zone. There were no appreciable amounts of toxic metals. Both nitrate and phosphorus, naturally present in the subsurface, served as sources of N and P so that their addition was not required during biostimulation of the test zone.

The schematic of the test zone, including the injection, extraction, and monitoring wells, is shown in Figure 1. Tracer experiments were performed along the two legs to determine whether the north leg (NI, N1, N2, N3, P) or the south leg (SI, S1, S2, S3, P) was best suited for the biostimulation-biotransformation experiments. Under the induced gradient conditions of injection and extraction, only 80% of the bromide was recovered at the extraction well when injected into the NI well, while over 90% was recovered when it was injected into the SI well. A strong regional flow from north to south caused the lower recovery with the north leg, and so the south leg was used for the biostimulation experiments.

The south experimental leg had been used previously for bioremediation studies using methanotrophic bacteria, an aerobic treatment process. Thus, in using the same experimental leg, a determination was possible of whether both aerobic and anoxic transformation processes could be enhanced in the same test zone.

A tracer test was performed along the south experimental leg to study the relative rate of transport of CT and a bromide tracer under the induced gradient conditions created by continuous injection and extraction. The test determined the extent to which CT was retarded in its transport, and also served to indicate whether substantial losses of CT occurred in the test zone before it was biostimulated. This was necessary to assure the validity of the experimental approach and to quantify the extent of biotransformation before and after the test zone was biostimulated. The hydraulic residence times (Table 1) between the injection and the three observation wells, S1, S2, and S3, were found to be in the range of 8 to 28 hrs. CT residence times were longer due to sorption onto the aquifer solids and ranged from 12 to 57 hrs. The resulting retardation factors ranged from 1.5 to 2.0. CT was much less strongly sorbed than cis- and trans-dichloroethylene (DCE) and trichloroethylene (TCE), whose retardation

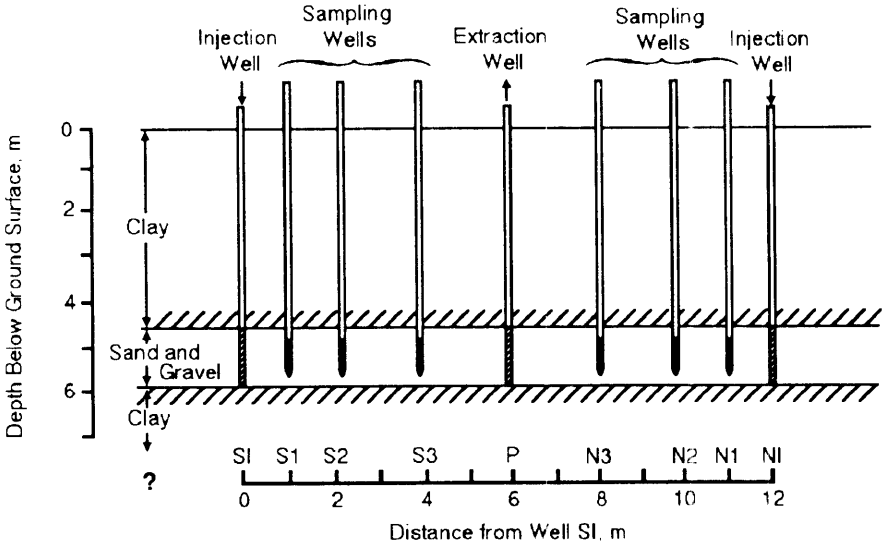

Figure 1. *Schematic of the injection system.*

Table 1. *Results of Tracer 14 Test*

	Well S1	Well S2	Well S3
Normalized Br Breakthrough (C/C_0)	1.00	0.98	0.94
Normalized CT Breakthrough (C/C_0)	0.98	0.99	0.98
Time to 50% Br Breakthrough (hr)	8	24	28
Time to 50% CT Breakthrough (hr)	12	44	57
Estimated Retardation Factor (T_{CT}/T_{Br})	1.5	1.8	2.0

factors in previous studies ranged from 6 to 12.

The tracer test also confirmed that the injected fluid completely permeated the test zone around the S1 and S2 wells, as indicated by the normalized breakthroughs of near unity (Table 1). CT also reached a normalized concentration near unity, indicating minimal transformation and sorption losses with prolonged injection. A minor amount of chloroform (CF) production was observed early upon CT addition, with the maximum CF concentration representing 3 to 4% of the CT added. Thus, minor CT transformation was observed before biostimulation of the test zone through acetate addition.

Laboratory Studies

Sorption

Batch sorption studies were performed on pulverized aquifer solid samples. A linear sorption isotherm was measured that yielded a K_D estimate of 1.0 l/kg. The estimated retardation factor based on the laboratory measured K_D value was 6.0, a factor of 3 greater than that estimated in the field test. There are several possible reasons for the higher laboratory estimate. Pulverization limited diffusional processes that were likely occurring in the field. Diffusional limited sorption would have resulted in low estimated values of retardation based on the time to 50% breakthrough of CT and bromide used in the field retardation estimates. The samples used in the laboratory tests may not have been representative of those of the test zone, due to aquifer heterogeneities and the inability to obtain intact aquifer cores from the test zone's highly permeable zone.

Laboratory Column Studies

Batch exchange soil column experiments were performed to determine the applicability of laboratory results to field studies. The experiments showed conclusively that CT could be transformed to a significant extent under anoxic conditions, biostimulated through the addition of a primary substrate for growth. The columns were batch-fed a range of primary substrates for growth (ethanol, acetate, methanol, and glucose) that were added to groundwater from the field site along with unlabeled and ^{14}C-labeled CT.

Rapid biostimulation of the columns was observed upon addition of the growth substrates, with complete nitrate removal occurring within 10 days. The decreases in aqueous CT concentrations were more gradual and occurred over a period of 60 days. CT concentrations were most reduced in columns fed acetate or ethanol, with 80 to 90% removal observed compared to a non-sterile control column to which no growth substrate was added.

The ^{14}C-labeled CT studies confirmed the transformation of the CT; 35 to 50% of the CT added was completely mineralized to CO_2, while 30 to 40% was transformed to CF. Denitrifying strains from the column effluent strains did degrade CT. These pure culture studies suggested that denitrifiers were not the microbes responsible for the transformations in the laboratory columns.

The column studies proved useful as a means of assessing the effect of biostimulation as a means of facilitating transformation of CT under controlled laboratory conditions. The tests indicated that acetate would be an appropriate, nontoxic growth substrate for the field test; the test zone should be rapidly biostimulated, but CT transformation was expected to significantly lag behind the uptake of nitrate and acetate. Partial mineralization of CT to CO_2 might be realized in the field; however, the formation of CF

as an intermediate product was also predicted. The lag in time before transformation of CT was observed, combined with the lack of CT transformation by denitrifying cultures, indicated that the main population of denitrifiers was not likely to be responsible for CT transformation.

Field Demonstration of Biostimulation and Biotransformation

The biostimulation and biotransformation evaluations conducted in the field were consistent in most major respects with expectations from laboratory results and theory. It was confirmed that a native bacterial community could be rapidly stimulated by introducing acetate as a growth substrate into an aquifer that contained nitrate and sulfate as potential electron acceptors, without any supplemental nutrients. In the initial biostimulation experiment, the utilization of acetate and nitrate rapidly commenced, with virtually complete nitrate utilization occurring after 100 hrs of acetate addition. A transitory buildup of nitrite concentration was observed within the first 60 hrs of addition, in response to the establishment of denitrifying conditions. Clogging of the injection well and borehole was effectively controlled by adding the acetate in a high concentration pulse for a period of one hour in a 13-hr pulse cycle, while continuously recycling nitrate in the native groundwater. More than 80 to 90% of the acetate was consumed within the first meter of transport. The stoichiometric ratios of nitrate to acetate consumption were approximately 1 mg NO_3 per milligram acetate, which is lower than the ratio calculated for complete respiration of nitrate to nitrogen gas, due to the incorporation of an estimated 40% of the acetate into cell biomass during biostimulation, consistent with literature reports.

In order to evaluate transformation of CT, the target organic compound, CT was continuously injected at a concentration of 40 µg/l until the soil was saturated, as evidenced by the complete breakthrough at the monitoring wells (Table 1), in the absence of acetate addition. CT injection into the test zone was continued upon the addition of acetate. CT transformation, as indicated by a decrease in its concentration at monitoring locations, and the formation of CF as an intermediate product significantly lagged behind the uptake of acetate and nitrate in the test zone (Figure 2). Decreases in CT concentration and increases in CF concentration were observed after approximately 400 hrs, with gradual decreases over the 1250-hr period that acetate and nitrate were injected into the test zone. Transformation of CT and the formation of CF as an intermediate product were more rapid and more complete at the S2 observation well, 2 meters from the injection well (Figure 2), compared to the S1 well, 1 meter from the injection well. The response indicated that the most rapid rates of transformation did not occur in the first meter of transport, where most of the acetate and nitrate were consumed, but in the zones further removed, where significantly less acetate and nitrate were consumed.

The results indicate that the main denitrifying population did not participate in the transformation process to the same extent as microbes stimulated further away. The transformation of CT by denitrifiers may have been strongly inhibited by the presence of nitrate in the test zone. Another possibility is that a secondary microbial population, living on acetate or decay products of the stimulated denitrifiers, were slowly growing and were responsible for the transformation. The growth of this population and/or its transformation of CT may have been inhibited by the presence of nitrate.

A transient experiment was performed to study the effect that nitrate had on the biotransformation, and to determine whether more effective CT transformation could be achieved in the first meter of transport. Nitrate was completely removed from the injected fluid through use of a surface bioreactor fed acetate. The transient test was initiated at 1260 hrs (Figure 3). A significant decrease in CT was observed over the 300-hr period of the test. Chloroform concentration increased to a lesser extent, indicating either that less was being formed in a parallel transformation pathway or CF was being degraded at higher transformation rates. Before nitrate was completely eliminated from the test zone, 55 to 67% of the transformed CT appeared as CF, while only 30 to 40% was observed after nitrate addition was terminated. Chloroform was the main chlorinated intermediate product found. Dichloromethane and chloromethane, possible intermediate products of CT transformation, were not detected at a detection limit of 1 µg/l.

There was no direct evidence for the stimulation of sulfate-reducing bacteria or methanogenic bacteria when nitrate was completely removed. Neither sulfide nor methane were detected in groundwater extracted from the test zone. If sulfate-reducing conditions were established, however, reactions with test zone minerals may have scavenged sulfide from the groundwater.

The transformation of background contaminants, including Freon-11, Freon-113, and TCA, was also observed in the biostimulated zone. The responses of the halogenated aliphatics were similar to that of CT, but with slower rates of transformation (Figures 4 and 5). Rates of transformation were also enhanced when nitrate was removed from the test zone. The degrees of transformation (Table 2), quantified by normalization to the bromide breakthrough, were as follows: CT, 70-97%; Freon-11, 42-75%; Freon-113, 0-30%; TCA, 5-19%. Of the values cited, the lower value represents the nearest observation well and the lower of the 95% confidence intervals, and the higher value represents the farther observation wells and the upper 95% confidence intervals. As indicated in Figures 4 and 5, steady-state transformation conditions had not been achieved by the end of the experiments. Thus these transformation extents are considered as conservative estimates.

Overall the field results confirmed the ability of indigenous bacteria to promote the biotransformation of CT, Freon-11, Freon-113, and TCA under anoxic conditions. Denitrification was readily accomplished through the addition of acetate to the test zone. The responses indicate, however, that the main population of denitrifying bacteria was not responsible for the CT transformation, but that a secondary population was responsible. CT transformations of 95% or greater were achieved in the test zone. Chloroform, however, was produced as an intermediate transformation product, and accounted for 30 to 40% of the CT transformed.

Mathematical Modeling

A non-steady-state model that was developed for simulating the biostimulation and biotransformation tests proved useful in interpreting the results of the field experiments. The model accounts for the basic phenomena of microbial growth, electron donor and electron acceptor utilization, biotransformation of the chlorinated compounds, and the formation of intermediate products. The model simulates 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 approach adequately simulated the transient decreases in CT concentration due to its transformation and the increase in CF concentration due to its formation as an intermediate product. Some parameter adjustments were necessary to achieve the model fits. The model also fit well the observed field transformation of other halogenated aliphatics (Figures 4

Table 2. *Estimates of the Degree of Transformation 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

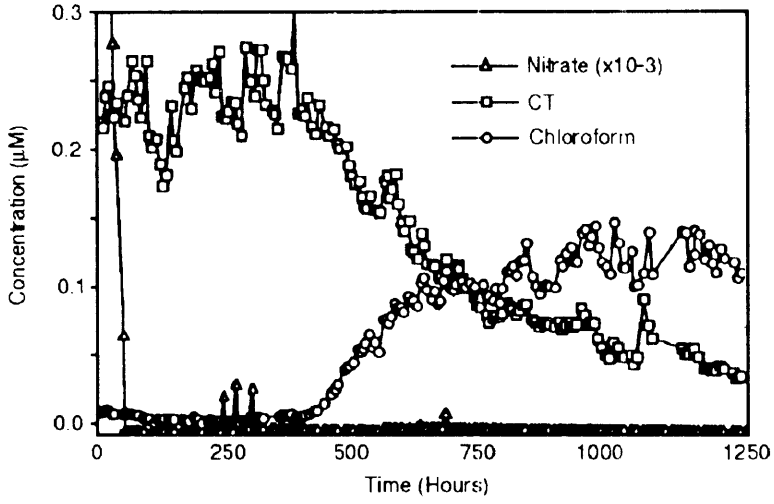

Figure 2. *Nitrate, CT, and CF concentration histories at the S2 well for the first 1250 hrs of biostimulation with acetate.*

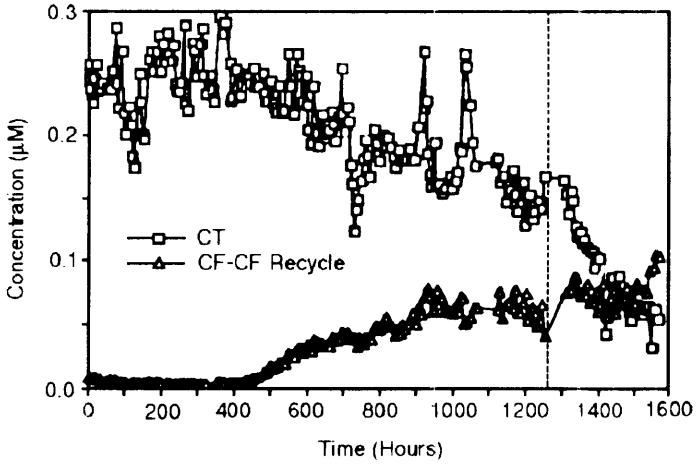

Figure 3. *Response of CT and CF at well S1 to nitrate removal from the injected fluid after 1260 hrs. The CF values represent net values after subtracting CF concentration present in the recycled injection water.*

and 5), indicating that these transformations were mediated by the same processes, but at different rates.

The rate coefficients determined from model fits to the field observations were in the range of those reported in the literature for microbial transformation under sulfate-reducing conditions, and for a pure *Clostridium* culture. Rate coefficients for the apparent specific first-order rate constants (in units of liter•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. There was a factor of 40 difference between the rate of CT (the most rapidly transformed) and TCA (the least rapidly transformed). CF was estimated to be degraded at a rate five times slower than CT. These differences in rates are consistent with those reported in the literature.

Summary

The results of this project showed that CT was transformed to a significant extent and at a rapid rate under subsurface conditions in the absence of dissolved oxygen, when a native population was biostimulated by the addition of acetate in the presence of nitrate. Chloroform was formed as an intermediate product. Laboratory column studies, conducted under similar conditions as the field tests, confirmed that a significant amount of CT was completely mineralized to CO_2. Laboratory soil column studies also predicted the responses that were later observed in the field experiments.

Freon-11, Freon-113, and TCA, background contaminants in the test zone, were also transformed to significant extents in the field. Transformation was more complete after nitrate was completely removed from the test zone and in zones that lacked the main population of denitrifiers. The response observed in the field and in the laboratory columns indicated that a secondary microbial population, and not the main denitrifying population, was responsible for the transformation. Modeling studies supported the hypothesis of a secondary population being responsible for the transformation. The modeling results were consistent with the hypothesis that the halogenated aliphatics were transformed by a similar biological process as CT, but at slower rates. The rates of transformation determined from model fits to the field response were in the range of those reported in the literature.

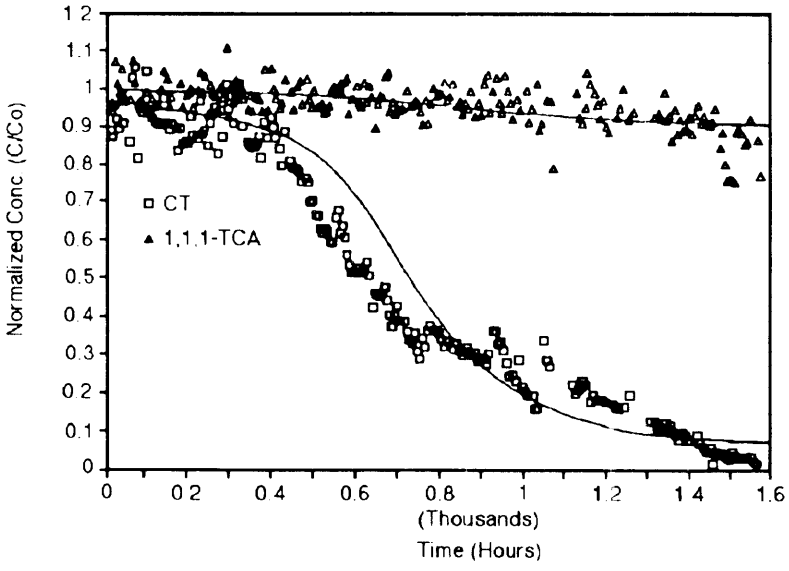

Figure 4. *Model simulations and field concentration histories of TCA and CT at the S2 observation well.*

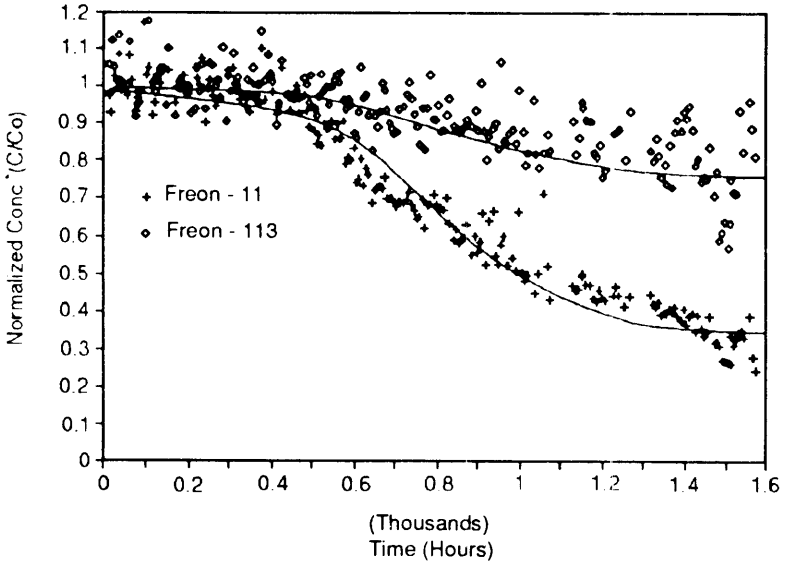

Figure 5. *Model simulations and concentration histories of Freon-11 and Freon-113 at the S2 observation well.*

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***Wayne C. Downs** is the EPA Project Officer (see below).*
The complete report, entitled "In-Situ Biotransformation of Carbon Tetrachloride Under Anoxic Conditions," (Order No. PB91-148 346/AS; Cost: $23.00, subject to change) will be available only from:
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