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Field and Laboratory Studies of Carbon Tetrachloride Transformation in a Sandy Aquifer under Sulfate Reducing Conditions

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A field experiment was conducted in which carbon tetrachloride (CT) was found to transform to chloroform (CF) and carbon disulfide (CS_2) in a ratio of about 2:1. Subsequent laboratory work was undertaken to better define the conditions required for this product ratio and to investigate its use as a means of distinguishing biologically driven and abiotic transformations in aquifers. The field experiment was conducted to assess the efficacy of a bioremediation scheme for treating CT in groundwater. The test section of the aquifer was taken to sulfate reducing conditions by the periodic addition of acetate from a nutrient injection wall (NIW). Under these conditions, CT was observed to transform completely producing primarily CF and CS_2 at a ratio of approximately 2:1. In laboratory columns designed to mimic the field conditions, low-input concentrations of CT (<70 μg/L) resulted in complete dechlorination of the molecule, while higher input concentrations (>400 μg/L) led to the production of CF and CS_2 in the same ratio as observed in the field. It was determined that amorphous iron sulfide had precipitated on the sand grains during the column experiments. Stertile laboratory batch experiments were conducted to test CT reactivity with various iron sulfide solids. A narrow ratio of 2:1 ± 0.4 CF to CS_2 was only observed for CT reacting with freshly precipitated, amorphous FeS in near pH neutral solutions. The 2:1 ratio may be a useful tool for distinguishing abiotic transformations from biodegradation in sulfate reducing environments where FeS is actively precipitated.

Introduction

Carbon tetrachloride (CT) is a common groundwater contaminant that is known to be highly toxic to humans (*1*). Although it is easily transformed in many anaerobic environments (*2*−*5*), attempts at bioremediating this substance have met with limited success. The difficulties seem to result from the toxicity of CT to microorganisms as well as the production of hazardous intermediates, such as chloroform (CF) and methylene chloride (MC) (*6*). Laboratory investigations have demonstrated that CT can degrade along several competing pathways (*2*), not all of which produce chlorinated intermediates. In fact, several lead to the production of nonchlorinated substances, such as carbon dioxide (CO_2), carbon monoxide (CO), and formate. Under sulfate reducing conditions, carbon disulfide (CS_2), a compound with known toxicity to humans, has also been identified as a product (*6, 7*−*10*). Previous studies have indicated that CF and CS_2 are produced along mechanistically different pathways (*2, 8*).

The degradation of CT has been shown to occur both in biologically active systems, with direct biological involvement (*6, 7, 11*), and in abiotic systems (*6, 8, 9*). In the latter case, reactions in homogeneous solution, with compositions similar to those of typical groundwaters, are reported to be slow. Jeffers (*12*) reported a hydrolysis half-life of 40.5 years for a 10% saturated CT solution at pH 7. Kriegman-King and Reinhard reacted 1 μM CT with 1 mM HS^- at 25 °C and observed a half-life of 1400 days (*9*). Curtis and Reinhard conducted similar experiments using 2.5 μM CT and either 250 μM Fe^{2+} (pH 7.2) or 250 μM HS^- (pH 7.8) as reductants at 50 °C and observed half-lives of >138 and 33 days, respectively (*13*). They further found that in the presence of humic acid, which acted as an electron mediator, the reduction of CT was accelerated by a factor of 10.

Similar enhancements to the abiotic reduction rate of CT have been observed in heterogeneous systems containing sheet silicates and HS^- (*8*). Using the Arrhenius parameters reported by Kriegman-King and Reinhard, the 25 °C half-lives for 1 μM CT with 1 mM HS^- and 1.2 m^2/L vermiculite or biotite are calculated to be about 150 and 50 days, respectively. At 50 °C, reaction of CT with HS^- in the presence of these solids produced CS_2 as the predominant product. It was suggested that the ferrous iron in the sheet silicates may have played a role in the reaction with HS^-. However, it was also acknowledged that the reaction may have involved iron sulfides formed by iron dissolution and subsequent precipitation with sulfide (*14*).

In abiotic experiments conducted at 25 °C, Kriegman-King and Reinhard (*9*) showed that aqueous CT is reduced relatively rapidly when placed into contact with pyrite. The condition of the pyrite surface was found to be an important factor in determining both the rates of reaction and the product distributions. Pseudo-zero-order kinetics were observed with the fastest rates recorded for acid-washed and freshly exposed pyrite surfaces in anaerobic solution. The slowest rates corresponded to reactions on pyrite in aerobic solution. For the anaerobic reaction involving 13.5 mL of 1 μM CT, 1 mM HS^-, and 1.2−1.4 m^2/L freshly exposed pyrite, the CT concentration decreased by half over a period of about 9 days, corresponding to a zero-order rate of 0.056×10^{-6} mol L^{-1} day^{-1}. The principal product of this reaction was CF (48%) followed by CO_2 (10%), formate (5%), and CS_2 (2%). Approximately 12% of the carbon mass was considered sorbed to the solid, 1% remained unreacted, and 22% was unaccounted. In contrast, the acid washed pyrite, reacted under similar conditions at a rate of 0.082×10^{-6} mol L^{-1} day^{-1}, produced approximately equal amounts of CF and CS_2 (19−21% each), 17% CO_2, 4% formate, 9% adsorbed mass, and 22% unaccounted mass.

Biodegradation of CT is a process that has been acknowledged for some time. Most studies have found substantial quantities of undesirable products resulting from the transformations of CT. Bouwer and McCarty (*3, 4*) demonstrated CT biodegradation in methanogenic and denitrifying environments with CF reported as a chief product. Bouwer and Wright (*5*) found, in column experiments, that CT biotransformed in a variety of redox environments with generally faster reactions occurring in the more reducing settings. Egli et al. (*11, 15*) showed that the methanogens and sulfate reducers in their systems were adversely affected by CT and

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CF but that the sulfate reducers could nevertheless transform CF to MC. In a controlled field experiment, Semprini established denitrifying conditions in a section of aquifer where he later injected CT (*16*). Approximately 30−60% of the mass was converted to CF, but the most rapid transformation rates were observed in the absence of nitrate, leading the researchers to suggest that the most active CT transforming microorganisms were not denitrifiers.

Criddle et al. (*17*−*20*) identified a denitrifying organism, *Pseudomonas* sp. strain KC, that is capable of transforming CT without producing significant quantities of chloroform. However, this organism is apparently sensitive to the concentrations of various metals (e.g., Fe, Cu, Co, Mo) (*18*) and may not be widely distributed in aquifers (*17*). Recently, a field demonstration project was undertaken in which strain KC was introduced to an aquifer containing CT. With subsurface pH adjustments, 60−80% CT removal was achieved and strain KC was reportedly assimilated into the aquifer community (*19*).

Carbon disulfide has been reported in a few studies of CT biodegradation, but has not been definitively shown to be a product of CT metabolism. Criddle et al. (*7*) observed it in experiments involving *Escherichia coli* K-12 under both fermenting and fumerate respiring conditions. However, they noted that CS_2 was detected in cultures that received CT and those that did not. They concluded that CS_2 production was a natural process and that CT or its derivatives might serve as a precursor to CS_2. Hashham et al. (*6*) and Freedman et al. (*10*) reported significant quantities of CS_2 production in batch experiments under sulfate reducing conditions. They found that the addition of cobalamine to the microcosms increased the rate of ^{14}CT transformation 100-fold and resulted in 73% conversion to $^{14}CS_2$. The mechanism leading to this rate increase was not determined. Carbon disulfide occurred in autoclaved and active microcosms, indicating that it was produced abiotically. In active bottles that received no cobalamine, CF was the chief degradation product (63%) with no CS_2 produced (*10*).

In this paper, we present field evidence of the degradation of CT along two competing pathways, producing CF and CS_2 intermediates, during a bioremediation experiment. We further present laboratory evidence that the abiotic production of CS_2 involved a reaction with freshly precipitated, amorphous FeS, possibly of biogenic origin.

Description of the Field Site and the Field Experiment

The C. F. B. Borden study site has been described in detail elsewhere (*21, 22*). This test was conducted in the upper 4 m of a surficial sand aquifer consisting of well-sorted fine to medium sand with a hydraulic conductivity between 7×10^{-5} and 1×10^{-4} m/s. The depth to the water table was on the order of 1 m, and the average ambient groundwater velocity was estimated to be about 0.1 m/day, based on previous work (*21*) and tracer tests, involving bromide and chloride, performed for this project.

A section of the test aquifer, normally containing 20 mg/L of background sulfate, was maintained in a sulfate reducing to methanogenic condition for 1.5 years with the periodic addition of acetate from a nutrient injection wall (NIW) (*22, 23*). Groundwater passing through the NIW and biostimulated zone was sampled using a monitoring network consisting of three fences of multilevel bundle piezometers located 1, 5, and 10 m from the NIW (Figure 1A). Details of the construction of the monitors have been given previously (*22, 24*). About 1 year after the first acetate injection, dissolved CT and TCE, at concentrations of about 1 mg/L each, were pumped into the aquifer through two wells, screened over a 2 m vertical distance and located about 0.4 m downgradient of the NIW (Figure 1B). In addition to the solvents, bromide ion was introduced to the aquifer at a concentration of about 275 mg/L to serve as a conservative tracer. The injections were accomplished by mixing 30 L of a concentrate (~20 mg/L CT and TCE), prepared in advance, with pristine groundwater collected previously from the site. The concentrate was prepared in PTFE bags (two 15 L bags were used) and was equilibrated 48 h prior to the injection. Flow meters were used to monitor the flow rates from the concentrate bags into each well so that the dilution factors could be maintained and adjusted during the injections. Following the introduction of the contaminant slug, the progress of the solutes was tracked at the three monitoring fences as it migrated through the biostimulated aquifer.

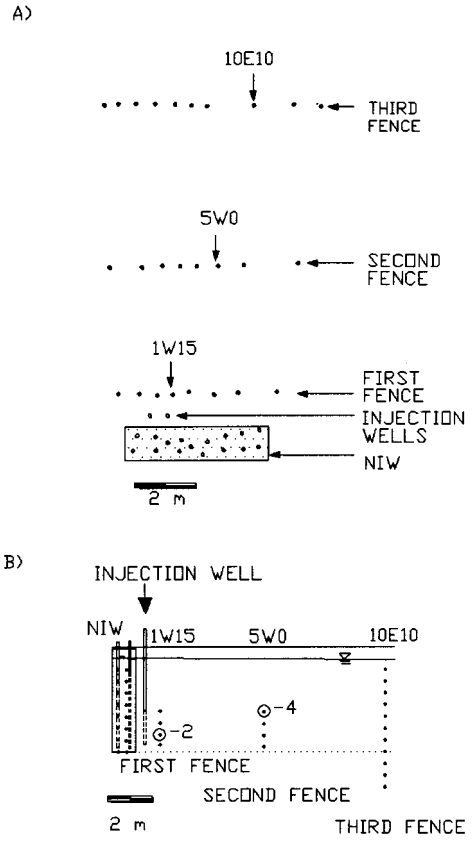

FIGURE 1. (A) Plan view of the field test site showing the locations of the CT injection wells and the monitoring fences. (B) The test site in cross-section showing the locations of the monitoring points.

Laboratory Methods

All chemicals used in this work were obtained in the highest grades available from various commercial suppliers and were used without additional purification.

Column Experiments. Column experiments were performed in two 25 cm long, 7.5 cm diameter, and one 50 cm long, 9 cm diameter, Plexiglas columns with four equispaced sampling ports located along their lengths. The columns were packed with aquifer material collected in core from the field site according to the methods of Zapico et al. (*25*). Immediately prior to core collection, the core barrels were rinsed with ethanol followed by Nanopure water to limit the introduction of surface bacteria to the samples. The core material originated from between 2.1 and 3.6 m below the ground surface (bgs) and varied in redox character from aerobic (dissolved oxygen concentrations of about 2 mg/L in pore water) to anoxic (dissolved oxygen in pore water < 0.15 mg/L). Only the aquifer material recovered from the core centers was used in packing the columns; material that came into direct contact with the core barrels was discarded. All column parts that came into contact with aquifer sand

were either autoclaved or precleaned with methanol and allowed to air-dry in a Laminair hood under positive atmospheric pressure. The two shorter columns were packed in a Laminair hood to minimize contact of the materials with airborne microorganisms. The third, longer, column was too large to handle in the hood, so it was packed under a stream of carbon dioxide. No attempt was made to maintain strictly anaerobic conditions during the packing procedures. Groundwater, collected from the field site and amended with about 500 mg/L acetate, made up the column influent. Sulfate was naturally present in the groundwater at 20 mg/L, but nitrate and phosphate were not detectable (<0.1 mg/L). Despite these potential inorganic nutrient limitations, no inorganic amendments to the groundwater were made.

Once packed, the columns were flushed with carbon dioxide gas for a couple of hours and then flooded. Water was passed upward through the columns at about 0.3 mL/min continuously, corresponding to an average linear velocity of 20−25 cm/day (depending on the column). Samples were collected from the sampling ports by temporarily closing the effluent line valve to divert flow through the desired port. Samples were collected by filling 100 mL glass syringes from which aliquots were dispensed for the various analysis required.

Within a few weeks of exposure to the acetate solution, three of the columns became anaerobic. In two cases (a 25 cm and a 50 cm long column), the anaerobic activity that developed was sufficient to completely deplete the 20 mg/L of sulfate being fed to the column, and bubbles of methane could be seen to develop on the inside walls of the Plexiglas columns. Although the bubbles appeared to be stationary, gas bubbles were continuous in the effluent, raising the possibility of gas movement within the column. In the third case, anoxic conditions were achieved, but sulfate reduction was slow to begin. So, the column activity was augmented by injecting (through the sampling ports) an inoculum consisting of groundwater taken from active microcosms prepared with the same core material as used in the columns. Sulfate reduction and methanogenesis were subsequently indicated by the disappearance of sulfate from the groundwater and the appearance of dissolved methane in the effluent.

Following the onset of anaerobic activity, the influent solutions were spiked with CT at concentrations between 50 and 1500 μg/L. Approximately 15 L batches of these spiked solutions were prepared in clear PTFE reservoir bags. The spikes were performed by injecting appropriate quantities of the neat chemicals into the reservoir, with microliter syringes, to establish the desired concentrations. Solutions were allowed to equilibrate at least 48 h prior to being used for experimentation. Concentrations in these reservoirs were found to be constant for several days, after which time the reservoirs were replenished and respiked.

Batch Experiments. Batch experiments to investigate CT reactions with various iron sulfide phases were conducted in a glovebox under a nitrogen atmosphere with 3% hydrogen. Millipore (18 MΩ) water was autoclaved and deaerated for several hours with nitrogen prior to use in the experiments. All equipment that came into contact with the water and solids was either autoclaved or sterilized with anhydrous ethanol prior to use. Freshly precipitated iron sulfide was prepared by spiking 50 mL of deaerated, deionized water in a 60 mL hypovial with 1 mL each of 0.5 M sodium sulfide and 0.5 M of ferrous ammonium sulfate. A black precipitate formed immediately. The solution was then spiked with 15 μL of an 8000 mg/L (53 mM) solution of CT in methanol to a final concentration of about 1.5 mg/L. The vial was quickly topped off with deaerated deionized water and then sealed with a PTFE-lined septum and crimp cap. The vials were shaken to ensure good mixing and then were allowed to stand for 24 h in the glovebox. Batch experiments involving commercially obtained iron sulfides were prepared in a fashion similar to that described above, except that 300 mg of solid was added directly to the vials containing 50 mL of deaerated, deionized water. Following the standing period of 24 h, the vials were opened and about 14 mL of supernatant solution were transferred to a GC vial for analysis.

Analytical Methods. All analyses for CT, CF, MC, and CS_2 were performed by the headspace technique using a Varian Genesis autosampler and a Hewlett-Packard 5890 GC equipped with a split injection port split 12:1 at 150 °C and a capillary column DB-VRX 30 m by 0.32 mm internal diameter (i.d.), 1.8 μm maintained isothermally at 32 °C. Helium was used as the carrier gas at 3.5 mL/min, and a photoionization detector (PID) was employed with a 11.7 eV lamp (detection limit (DL) = 5−10 μg/L except MC (20 μg/L), precision (P) = ±15−20%).

Sulfate was analyzed by ion chromatography at the University of Waterloo Water Quality Laboratory (DL = 1 mg/L, P = ±10%) or by turbidimetric analysis of barium sulfate precipitate, using a Milton Ray Company Spectronic 20 spectrophotometer set to a wavelength of 450 nm (DL = 5 mg/L, P = ±25−50%).

Iron was analyzed by atomic absorption spectrophotometry (AA) using a Varian model 1475 AA (DL = 0.05 mg/L, P = ±5%).

Methane analyses were performed on headspace gas using a Fischer Hamilton model 29 gas partitioner equipped with a 6 ft by 1/4 in. packed aluminum column containing 30% DEHF on 60−80 mesh chromosorb P. The column was in series with 40/60 mesh molecular sieve 13X (DL = 0.3 mmol/L, P = ±10−20%).

Dissolved oxygen (DO) was analyzed immediately following collection using a Jenway 3410 electrochemical analyzer probe (DL = 1 mg/L, P = ±10−20%) and/or Winkler titrations as described in Greenberg et al. (*26*) (DL = 0.15 mg/L, P = ±5%).

Scanning electron microscopy (SEM) for the characterization of iron sulfide solids was performed by the Surface Science Western Institute, University of Western Ontario, using a Hitachi S-4500 field emission SEM with an EDAX CDU LEAP detector at a beam energy of 15 kV. X-ray diffraction analyses were performed at the University of Waterloo using a Siemens Kristalloflex X-ray diffractometer. Samples were analyzed under a stream of nitrogen in a Plexiglas hood with X-ray transparent foil windows to minimize oxidation during the analyses.

Results and Discussion

Field Experiment. Bromide breakthrough curves were obtained from six points along the first sampling fence, and five points along the second fence, located about 0.5 and 4.5 m from the injection wells, respectively. One-dimensional analytical solutions to the advection dispersion equation (*22*) were fitted to the data using a least-squares optimizer (*27*). Velocity estimates ranged from 0.065 m/day to 0.161 m/day, and dispersivities were found to range between 0.014 and 0.12 m. Additional details concerning hydraulic conditions at the site are provided by Devlin and Barker (*22*). The bromide data were used to calculate a mass balance for the organic solutes (CT, CF, and CS_2). About 71% of the CT mass injected could be accounted for passing the first monitoring fence. Unfortunately, due to changes in the flow direction over time, the bromide pulse separated from the organic pulse during the experiment, so a reasonable mass balance could not be calculated for the second fence. To estimate pseudo-first-order rate constants for the organic transformation processes, breakthrough curve data from the multilevel samplers were fit to the following equations which were solved numerically by finite differences (*28*). Numerical

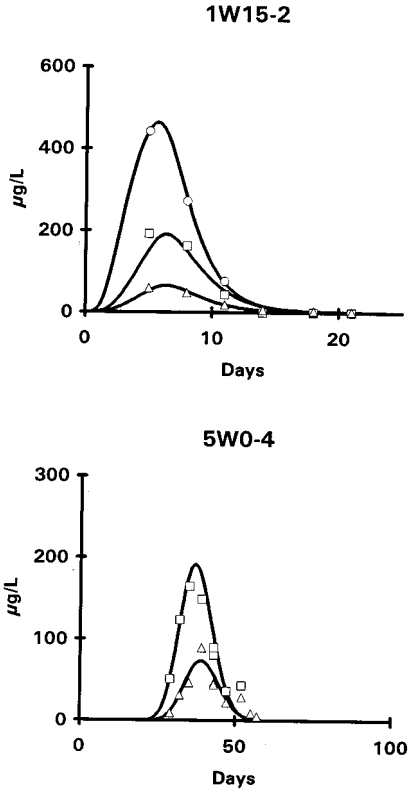

FIGURE 2. Breakthrough curves for CT, CF, and CS_2 at the first (1W15-2) and second (5W0-4) monitoring fences. Circles are CT, squares are CF, and triangles are CS_2 data points. Lines are the model fits using eqs 1, 2, and 3 (see text). Note that CT transforms completely before reaching the second monitoring fence.

dispersion was avoided by strict adherence to the Peclet and Courant number criteria in all simulations (*29*).

$$\frac{\partial C_1}{\partial t} = \frac{D}{R_1}\frac{\partial^2 C_1}{\partial x^2} - \frac{v}{R_1}\frac{\partial C_1}{\partial x} - \lambda_1 C_1 \quad (1)$$

$$\frac{\partial C_2}{\partial t} = \frac{D}{R_2}\frac{\partial^2 C_2}{\partial x^2} - \frac{v}{R_2}\frac{\partial C_2}{\partial x} - \lambda_2 C_2 + a\lambda_1 C_1 R_1/R_2 \quad (2)$$

$$\frac{\partial C_3}{\partial t} = \frac{D}{R_3}\frac{\partial^2 C_3}{\partial x^2} - \frac{v}{R_3}\frac{\partial C_3}{\partial x} - \lambda_3 C_3 + b\lambda_1 C_1 R_1/R_3 \quad (3)$$

where D is the dispersion coefficient (L^2/T) (note, the dispersion coefficient can be approximated by $D = \alpha v$ in the environment under study here), α is the dispersivity (L), v is the average linear groundwater velocity (L/T), R is the retardation factor, λ is the pseudo-first-order rate constant, and C is concentration. The subscripts 1, 2, and 3 refer to CT, CF, and CS_2, respectively. The coefficients a and b are mass fractions that dictate the distribution of CT mass in the two products, CF and CS_2. The ratio of these two coefficients, a:b, will be called the pathway ratio throughout this paper.

Following its injection into the aquifer, CT was found to transform predominantly to CF and CS_2, representing approximately 71% of the injected mass at the first monitoring fence. The above model was found to describe all six breakthrough curves generated from the first monitoring fence and the four complete breakthrough curves produced at the second fence (two examples are provided in Figure 2). Five breakthrough curves were incomplete at the second fence, due to locally low groundwater velocities, and could not be modeled with confidence—the detection of MC was limited to a few of these locations. It was determined that CT had transformed with a half-life of 3−7 days, and the pathway ratio that produced the best visual fit of the model to the data was 2:1 (CF:CS_2) (Figure 2). In contrast to this, previous work at the site indicated CT was recalcitrant in the aquifer under natural conditions (*28*).

The possibility of CS_2 hydrolysis was considered because CS_2 depletion, at a significant rate, could profoundly affect the product ratios. Using the data from Kriegman-King and Reinhard (*8*) and Adewuyl and Carmichael (*29*), half-lives for CS_2 hydrolysis were estimated to be in the range of 188 ± 88 days at 12 °C (the approximate temperature of the Borden groundwater), well in excess of the residence time between the injection wells and the first monitoring fence (5−10 days for CS_2) and considerably larger than the residence time to the second fence (40−70 days). On this basis, CS_2 hydrolysis was assumed negligible for the purposes of this analysis. Our modeling excercise appears to support this assumption. However, CS_2 hydrolysis will not be negligible in all field situations, particularly in cases where travel distances and residence times are large, so CS_2 hydrolysis should be reconsidered in each case.

Breakthrough curves for CF and CS_2 could be fit with pseudo-first-order rate constants of zero for these intermediates. Thus, the modeling gave no indication that either CF or CS_2 underwent significant transformations between the injection wells and the second monitoring fence. However, as mentioned above, traces of MC were detected at some locations along the second fence, so at least one CF transformation was demonstrated in the aquifer.

A third monitoring fence, 9.5 m from the injection wells, only yielded a single breakthrough curve for CS_2, i.e., breakthrough was only observed at one multilevel monitoring point. There was no corresponding appearance of CF or MC, suggesting that these compounds were degraded before arriving there. However, due to the complexities of the variable flow system, the relatively small size of the contaminant slug and the difficulties in monitoring such a system, it is difficult to draw firm conclusions regarding the fates of these two chlorinated intermediates in this test.

Laboratory Columns. To investigate the nature of the transformations in more detail, three-column experiments were conducted with sediment and groundwater from the site. As in the field experiment, the groundwater was amended with acetate (200−500 mg/L) and allowed to develop an anaerobic character. In all three columns DO fell below detection limits (0.15 mg/L) between the column inlet and the first port, about 5 cm into the column, within several days of initiating the experiment. Sulfate was also removed from solution in two of the three columns within a few weeks. Sulfate disappearance was soon followed by a darkening of the column sediment until the entire column (except a few millimeters at the influent end) was black. This change was assumed, and later confirmed, to be due to the accumulation of an iron sulfide coating on the grains. A pronounced sulfide odor was present in the effluent water (no quantitative sulfide analyses were performed), while Fe^{2+} concentrations were always below 0.1 mg/L and usually undetectable (i.e., <0.05 mg/L).

Excavation of one of the columns at the end of the experiment confirmed that the black color was nearly uniform in its occurrence vertically and laterally. The pronounced odor in the effluent water was further evidence that sulfate reduction was taking place. A third column was inoculated with water from sulfate reducing microcosms prepared from the same core material used in packing the columns and subsequently also began reducing sulfate. In all columns, at an input concentration of about 70 µg/L or less CT was nearly completely transformed to CF and MC, with no detectable CS_2 production. In the 50 cm column, CF and MC were also apparently transformed by dechlorination to unknown

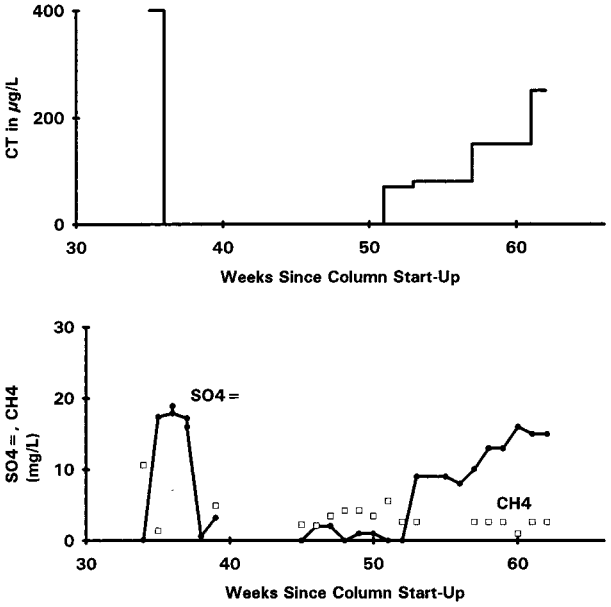

FIGURE 3. Sulfate and methane variations in column effluent as a function of CT input concentrations.

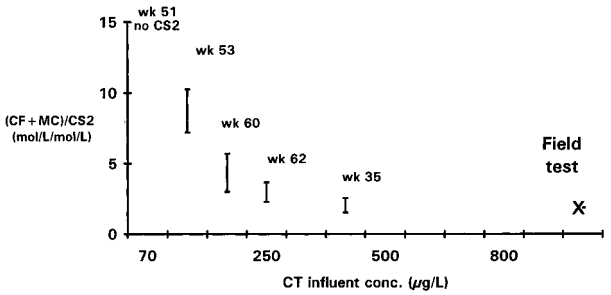

FIGURE 4. Pathway ratios as a function of CT input concentration. Vertical bars represent the range of ratios observed along the column length.

products. This result strongly supports the hypothesis that microorganisms naturally present in the Borden aquifer are capable of completely dechlorinating CT. However, complete dechlorination was not observed at all CT influent concentrations.

In one of the columns, CT was introduced at 4 different input concentrations, 400, 80, 150, and 250 μg/L, as summarized in Figure 3. Methane and sulfate concentrations in the effluent were found to be sensitive to the CT input concentration. This was expected since CT and its breakdown products are known to be toxic to aquifer microorganisms. As in the field experiment, the observed breakdown products consisted of CF, MC, and CS_2, accounting for up to 90% of the transformed mass (other breakdown products were not analyzed). CS_2 hydrolysis was again neglected since rates calculated for 25 °C (*8*) indicated a reaction half-life >45 days and greatly in excess of the residence time of solutes in the column (~1 day).

At lower CT input levels relatively small amounts of CS_2 were produced, but the relative amount of CS_2 production steadily increased with increasing CT input concentrations. This led to the declining pathway ratios shown in Figure 4 and the apparent asymptotic approach of the pathway ratios to about 2:1 CF:CS_2, the same ratio observed in the field. This result was duplicated in the 50 cm column with input concentrations of 70 and 1150 μg/L.

A possible explanation for the above data is that transformations resulting directly from biological activity predominated at low CT concentrations, but this activity was inhibited at higher CT concentrations so that normally less favored abiotic transformations became the dominant transformation avenue. This notion is supported by previous studies that have indicated CS_2 production to be an abiotic process (*7, 6, 10*).

It was hypothesized that any abiotic reductions that occurred in the columns and the aquifer probably involved iron sulfide phases precipitated as coatings on the sand grains. Reactions of this kind have been demonstrated for CT and pyrite (*9*) as well as hexachloroethane and mackinawite (*31*). Attempts were made to analyze the black coating on the grains, but this proved to be difficult because the coating was so thin. Instead, samples of the black precipitate were obtained from the inside of the PTFE column effluent line. These samples were examined by XRD and SEM/EDX and confirmed to be noncrystalline iron sulfide (FeS). This was considered to be the solid phase most likely to be coating the grains and reacting with CT.

Batch Experiments. If a continuously precipitating amorphous FeS was responsible for the reduction of CT in the columns and aquifer with a pathway ratio of 2:1 CF:CS_2, then it should be possible to reproduce this result in aseptic batch tests with freshly precipitated FeS. A series of experiments of this type were conducted, in which five replicate vials were prepared with several different iron sulfide phases, including freshly precipitated FeS, aged (1 month) amorphous FeS, a commercially acquired pyrrhotite, and a commercial pyrite. The identities of all solids were confirmed by XRD and SEM/EDX analysis. Controls were prepared in the same fashion as the other batch vials but contained only deionized water spiked with CT.

The reaction vials (five for each system tested) were shaken and then allowed to stand 24 h before they were sacrificed for analysis. In this time, between 20% and 60% of the CT in the vials transformed to CF, MC, or CS_2 (Table 1). Reaction kinetics were not examined in this work; it was assumed that either pseudo-zero- or pseudo-first-order kinetics would describe the reactions (*8, 9*). Butler and Hayes (*31*), in their experiments with hexachlorethene (HCE) and FeS, observed pseudo-first-order kinetics when they reacted 16 μM HCE with 5 g/L FeS. In our experiments, approximately 300−400 mg of FeS was precipitated or weighed into the 60 mL reaction vials, corresponding to a solid concentration of about 5−7 g/L, and CT was added at about 1.5 mg/L (10 μM). So, the assumption of pseudo-first-order kinetics would seem to be reasonable. The fact that the reactions were incomplete at the time of analysis does not affect the calculation of pathway ratios for either pseudo-zero- or pseudo-first-order reactions. Devlin (*24*) showed that for the first-order disappearance of a solute forming two products simultaneously (i.e., $A \rightarrow b\mathrm{B} + c\mathrm{C}$ where $b + c = 1$) the product ratios are constant in time, $\mathrm{B/C} = b/c$. The same is true when pseudo-zero-order kinetics apply, assuming the initial concentrations of B and C are zero.

Various pathway ratios were observed for the different systems, ranging from 1.0 (aged amorphous FeS) to undefined, due to the lack of CS_2 production (both commercial sulfide minerals) (Table 1). Of particular note was that the average pathway ratio for freshly precipitated FeS reacting with CT was 2.1:1 ± 0.4 (one standard deviation) CF:CS_2, in close agreement with the results of the column and field experiments.

The lack of CS_2 production in the reaction of CT with the commercial sulfides may have been due to an oxidized coating on the solids. No pretreatment of the mineral surfaces was undertaken in the preparation of the reaction vials, so some uncertainty concerning the condition of the surfaces exists. Nevertheless, low proportions of CS_2 production have previously been reported for CT reacting on oxidized pyrite in anaerobic systems (corresponding to pathway ratios from

TABLE 1. Summary of Results for the Batch Experiments

solid phase	% CT disappearance (corrected for control)[a]	% mass unaccounted (corrected for control)[a]	pathway ratio CF:CS_2 obsd range (replicates)	avg pathway ratio ±1 std dev
fresh amor. FeS	66	22	1.5−2.6 (4)	2.1 ± 0.4
aged FeS	72	42	1.0−5.9 (6)	2.9 ± 2.0
pyrite	20[b]	20[b]	nd[c]	nd
pyrrhotite	61	24	nd	nd

[a] Average of five vials. [b] Approximate value using average control performance from the other experiments. [c] nd = not determined due to lack of CS_2 production.

10:1 to undefined (*9*)), so the above explanation seems at least plausible. Interestingly, in that study CT reacted on fresh pyrite surfaces in anaerobic solution with a pathway ratio of about 24:1, suggesting that high ratios should indeed be expected with that phase under conditions typical of many aquifers.

The range of pathway ratios calculated for CT reacting with aged FeS was very large compared to that determined from the fresh precipitate (Table 1). These variations cannot be attributed to surface oxidation since the solids were maintained in an oxygen-free environment from the time of precipitation to the time of analysis. Rickard (*33*) reported the alteration of amorphous FeS, precipitated from near neutral pH solution, to ordered mackinawite within a few hours, raising the possibility that similar alterations were occurring in our aged FeS samples and that these might have affected the pathway ratios. However, if alterations of this kind were occurring, they could not be confirmed by XRD in the month-old samples. It was also noted in the preparation of the sulfide precipitates that the solids tended to flocculate differently from vial to vial. Although every attempt was made to prepare the solids identically, no pH buffer was used in the experiments and slight variations in the volumetric addition of stock solutions could have resulted in slight pH variations. Notably, although such variations in pH should have been present in the fresh as well as the aged FeS vials, the scatter in pathway ratios was limited to aged samples.

To investigate the effect of pH on the reactivity of CT with the fresh FeS, batches were prepared as previously described and then pHs were adjusted to values between 2 and 10 with either sodium hydroxide or hydrochloric acid. The pH-adjusted solutions were spiked with CT and allowed to sit for 24 h before being analyzed. The preliminary tests indicated that below pH 5 there was little reaction within the 24 h standing period. Above pH 6, both CF and CS_2 were produced and pathway ratios quickly climbed to about 2:1 CF:CS_2. This finding is consistent with the field and column data, in which the groundwater exhibited pH values in the range 6.1−7.4 and 6.8−8.4, respectively. As the pH rose above neutrality there was a tendency for the transformation of CT to proceed more quickly (based on increases in the percentage transformed within 24 h). Butler and Hayes (*31*) have reported a similar trend in reaction rates for hexachloroethane reduction rates by mackinawite, but further tests are needed to quantify these trends in CT−FeS systems.

The possibility of abiotic solution phase reactions between sulfide species (such as sulfide, bisulfide, and polysulfides) and CT were also considered in this study. Low levels of polysulfides were of particular concern since these species may be much more reactive toward chlorinated organics than monosulfide species. Such reduction reactions, involving hexachloroethane, have recently been shown to be relevant in environmental samples (*32*). In each of the experiments performed, vials were prepared that contained sodium sulfide solution (0.5 M—note that the sulfide concentrations in the heterogeneous systems were considerably less—theoretically undetectable if the amounts of Fe^{2+} and S^{2-} were exactly balanced as intended) but no ferrous ion or FeS solid phase. These controls indicated that homogeneous solution reactions were possible but at much lower rates than were observed in the heterogeneous systems. For example, 0.5 M total sulfide (polysulfides were presumed present but were not explicitly analyzed) resulted in the transformation of 3−7% (by moles) of the initial CT to CF within 24 h. This compares with the conversion of about 11−20% CT to CF and CS_2 in the presence of amorphous FeS (and relatively low levels of dissolved sulfide) over the same time period. No reactions were observed in controls containing Fe^{2+} without the presence of sulfide.

Implications

The data indicate that the 2:1 CF:CS_2 ratio is indicative of a reaction with amorphous FeS. The fact that the ratio was relatively constant in three independent experiments where the mass of available FeS (and the corresponding surface area) was unlikely to be the same raises the possibility that the ratio is determined by a surface reaction whose order is independent of the order of the overall system kinetics. This is an area that deserves further research.

Another, more practical, implication that follows from the consistency of the 2:1 CF:CS_2 ratio is that it may be useful as an indicator of abiotic CT transformations in sulfate reducing environments, when background levels of CF and CS_2 are low. Since CT is subject to both biodegradation and abiotic transformations, this distinction could be important in the interpretation and design of remediation programs.

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