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Kinetics of 1,1,1-Trichloroethane Transformation by Iron Sulfide and a Methanogenic Consortium

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To evaluate the effect of potential interactions between methanogenic bacteria and iron sulfide minerals during transformation of 1,1,1-trichloroethane (1,1,1-TCA), we measured the kinetics of 1,1,1-TCA transformation by mackinawite ($FeS_{(1-x)}$, but abbreviated as FeS) and a methanogenic consortium enriched on lactate (termed LEC). Results from batch kinetic experiments show that 1,1,1-TCA transformation by FeS and resting LEC can be described by second-order rate expressions, with rates depending on 1,1,1-TCA concentration (M), FeS surface area concentration (m^2 L^{-1}), and LEC concentration (as measured by mg L^{-1} volatile suspended solids (VSS)). In reactors containing FeS alone, 1,1-dichloroethane (1,1-DCA) and 2-butyne were identified as products, but only accounted for 6% of the 1,1,1-TCA transformed. In reactors containing LEC alone, the only identified product was 1,1-DCA, which accounted for 46 ± 8% of the 1,1,1-TCA transformed. Supernatant from LEC-alone reactors also transformed 1,1,1-TCA, suggesting that 1,1,1-TCA may be transformed by some non-cell component (such as a extracellular compound excreted by the organisms) that either reacts directly with 1,1,1-TCA or with the abiotic media to form a reactive species. Comparison of 1,1,1-TCA transformation rates from experiments with combinations of FeS (varying surface area concentrations) and LEC (varying VSS concentrations) to those with just FeS alone or LEC alone suggests some synergism occurs between the two reactive species. Observed enhancements took the form of faster 1,1,1-TCA transformation and faster 1,1-DCA appearance but less production of 1,1-DCA per unit of 1,1,1-TCA transformed. These observations suggest that the faster 1,1,1-TCA transformation in the combined systems (compared to the FeS-alone and LEC-alone experiments) is due to increased reactivity of both FeS and LEC, possibly due to production of soluble microbial products that make the FeS more reactive or less inhibition of LEC by 1,1,1-TCA due to FeS transformation of 1,1,1-TCA.

Introduction

Chlorinated aliphatic hydrocarbons (CAHs) comprise one of the main classes of soil and groundwater contaminants (*1*). Within this class, 1,1,1-trichloroethane (1,1,1-TCA) is one of the most predominant solvents encountered, with detection occurring in ~20% of the sites on the National Priority List (*2*). 1,1,1-TCA is used in a variety of industrial applications including adhesives, aerosols, textile processing, extraction solvents, industrial solvent blends, and metal cleaning (*3*). Due to the serious adverse health effects associated with 1,1,1-TCA exposure (*4, 5*), a maximum contaminant level (MCL) of 0.2 mg L^{-1} has been set for drinking water.

Transformation of CAHs has been shown to occur in the presence of iron sulfide minerals (*6–14*) and methanogenic microbes (*15–21*). Iron sulfide is a common soil mineral formed primarily through the microbial reduction of sulfate to sulfide and subsequent precipitation of sulfide with ferrous iron (*22*). Iron sulfide minerals have been shown to transform several CAHs, including carbon tetrachloride (CT) (*6, 7, 10, 14*), trichloroethene (TCE) (*9–11*), tetrachloroethene (PCE) (*9, 10*), and 1,1,1-TCA (*10*), as well as several other CAHs (*8, 10*). Transformation of CAHs by iron sulfide has been shown to occur primarily through nucleophilic substitution (*6, 7, 10, 14*) and electron transfer (*6, 7, 9, 10, 14*). The carbon recoveries observed in these studies range from around 10% to over 90%, and the half-lives are on the order of hours to a few days (*6, 7, 10, 14*). In the case of 1,1,1-TCA transformation by FeS, a half-life of several days and a carbon recovery of ~6% has recently been reported (*9, 10*). There are, however, a few studies that observed little to no transformation of CAHs, including 1,1,1-TCA, in the presence of iron sulfide (*12, 13*).

In contrast, the transformation of 1,1,1-TCA by both pure and mixed methanogenic microbes is well-characterized (*15–21*) and is believed to be a cometabolic process (*20*) resulting in the reductive dechlorination of 1,1,1-TCA to two common products: 1,1-dichloroethane (1,1-DCA) and chloroethane (CA) (*17, 23–25*). The degree of reductive dechlorination and resulting product distribution is dependent on the availability of an external electron donor. Lack of an external electron donor generally results in the transformation to and subsequent persistence of 1,1-DCA (*16, 17, 26*), whereas the presence of an electron donor results in further and more complete dechlorination to CA (*25*). The half-lives of these transformations range from several hours when an external electron donor is present to several days when no electron donor is present (*25*). The carbon recoveries have also been found to be dependent on the presence of an external electron donor, with ~40% observed with no external electron donor (*27, 28*) and near-complete recovery observed with an electron donor present (*17, 23–25*).

Given the metabolic diversity of anaerobic bacteria (e.g., ref *29*), it is likely that iron sulfide minerals and methanogens coexist in a wide variety of reducing environments, including anaerobic sediments and, perhaps, permeable reactive barriers (PRBs) containing zero-valent iron (Fe(0)) (*30*). Although transformation of CAHs in the presence of iron sulfide minerals and methanogenic consortia has been studied independently, relatively little is known about what interactions, if any, occur between these minerals and microbial species and whether such interactions are important in the fate of CAHs such as 1,1,1-TCA. To evaluate potential biogeochemical interactions, we have measured rates of 1,1,1-TCA transformation with synthesized mackinawite ($FeS_{(1-x)}$, but abbreviated as FeS) and a methanogenic consortium enriched on lactate (termed LEC). Mackinawite was selected because it is typically the initial iron sulfide

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form to precipitate (*22*) and has been identified in a number of reducing environments (*27, 28, 31*). Batch experiments have been conducted with FeS alone, LEC alone, and several combinations of FeS and LEC to assess transformation kinetics, product distributions, and potential interactions. The purpose of this work was to (i) quantify the rates of 1,1,1-TCA transformation by FeS and a methanogenic consortium as a function of iron sulfide surface area, microbial biomass, and 1,1,1-TCA concentrations and (ii) determine whether interactions between iron sulfide minerals and methanogenic consortia affect the rates and extent of 1,1,1-TCA transformation. Such interactions, if they exist, may be important to consider when the fate of 1,1,1-TCA in reducing environments is evaluated.

Experimental Methods

Lactate Enrichment Stock Culture. The source of organisms for our experiments was a 9-L stock methanogenic consortium enriched on lactate. The culture was originally started from a sample collected from a municipal anaerobic digester sludge in Iowa City, Iowa (*24*). A mixed culture enriched on lactate was used to provide a fairly diverse microbial population. The reactor was operated at 20 °C on an average 40-day hydraulic retention time such that 450 mL of the culture was removed every other day and replaced with fresh reduced nutrient media (*20*) and 180 mg L^{-1} lactic acid. The sodium bicarbonate in the reduced nutrient media served to maintain the pH of the LEC at ~7.5.

Iron Sulfide Synthesis. Mackinawite was synthesized using a method adapted from Butler and Hayes (*8*) by slowly adding 300 mL of 1.1 M Na_2S to 500 mL of 0.57 M $FeCl_2$ inside an anoxic chamber with a N_2/H_2 atmosphere (95/5% v/v). The resulting slurry was mixed for 3 days before centrifuging at 8000 rpm (for ~10 min) and replacing the supernatant with N_2-purged deionized water. The bottles were then resealed, shaken vigorously to resuspend the precipitate, equilibrated, and centrifuged again. This process was repeated over a total of eight centrifuge cycles. The black precipitate was then removed from the centrifuge bottles and freeze-dried under vacuum. The resulting powder was sieved through a 100-mesh brass sieve to ensure a more uniform particle size distribution. Samples of the sieved powder were identified as amorphous mackinawite with powder X-ray diffraction (XRD) on a Siemens diffractometer utilizing Cu Kα radiation. The powder samples were mixed with glycerol and spread to a thin layer on glass slides to protect the powder from oxidation during collection of the XRD spectra. An average specific surface area (A, $m^2\ g^{-1}$) of $0.14 \pm 0.03\ m^2\ g^{-1}$ (for five independently synthesized FeS batches) was measured under anoxic conditions with N_2 adsorption on a Quantachrome NOVA 1200 Brunauer–Emmett–Teller (BET) surface area analyzer.

Batch Experiments. All experiments were performed in 60-mL serum bottles with 50 mL of liquid volume. To maintain anoxic conditions, all serum bottles were prepared in the anoxic chamber. For LEC-alone experiments, LEC was first collected from the stock reactor and transferred to 125-mL serum bottles that had previously been capped with Teflon-lined rubber septa and sealed with aluminum crimp seals in an anoxic chamber. The serum bottles were then stored at 20 °C for 24 h to allow the biomass to settle. After 24 h, the serum bottles were placed in an anoxic chamber and unsealed. Biomass dilutions were made by adding an aliquot of full-strength LEC (240 mg L^{-1} VSS) and then diluting with supernatant from the serum bottles of settled biomass. No measurable lactate, acetate, or propionate was present in the reactors at the start of the experiments, and no lactate was added. Thus, the only available exogenous electron donor to these "resting" cells was soluble microbial products (bacterial exudates and products of cell death). For FeS-alone experiments, the desired mass of FeS powder was first added to each serum bottle, which was then followed by 50 mL of buffer solution prior to sealing the serum bottle. For combined LEC and FeS experiments, the desired mass of FeS powder was first added to each serum bottle, and then the desired concentration of LEC was prepared in the serum bottle using the dilution procedure described above.

Prior to the addition of 1,1,1-TCA, all reactors were removed from the anoxic chamber and purged with N_2/CO_2 (80/20% v/v) for a period of 5 min. This was done to strip any trace gases that might have been present in the anoxic chamber. An aliquot of 1,1,1-TCA was then injected through the rubber septa using a glass, gastight syringe from a saturated stock solution of 1540 mg L^{-1} 1,1,1-TCA (prepared by adding ~5 mL of the individual neat compound to 100 mL of deionized water). The reactors were stored in a 20 °C climate-controlled room on a rotary shaker table set at 160 rotations min^{-1}. The solution pH was measured using pH strips, and the oxidation–reduction potential (ORP) was measured using an Accumet Basic AB15 ORP probe. Headspace and aqueous samples were periodically withdrawn for analysis of organic compounds.

Chemical Analyses. A gas chromatograph (GC) equipped with an electrolytic conductivity detector and a J&W Scientific 60-m column with a DB-VRX stationary phase was used to measure 1,1,1-TCA, 1,1-DCA, and 1,1-dichloroethene (1,1-DCE). Helium was the carrier gas at 4.6 mL min^{-1}, and the oven, inlet, and detector temperatures were 70, 250, and 900 °C, respectively. All other volatile organic compounds were measured using GCs with flame ionization detectors. Nitrogen was the carrier gas at 2–3 mL min^{-1}, and the oven, inlet, and detector temperatures were 35, 150, and 250 °C, respectively. Methane, ethane, ethene, acetylene, 2-butyne, and *cis*-2-butene were measured using a J&W Scientific 30-m column with a GSQ stationary phase, whereas CA, vinyl chloride (VC), and acetaldehyde were measured using a J&W Scientific 30-m column with a 3.00-μm DB1 stationary phase. Detection limits were at or near 0.05 μM for all these compounds except acetaldehyde, which had a detection limit of ~0.25 μM. Concentrations of lactate, acetate, propionate, and butyrate in the LEC were determined using direct liquid injections into an HPLC. At least 1 mL of LEC sample was filtered through a 0.45-μm Millipore syringe filter into a 2-mL sample vial. These sample vials were then crimp-sealed and stored for later analysis. The HPLC flow rate was 1 mL min^{-1} with 0.001 N H_2SO_4 as the eluent. The compounds were separated and detected using an Alltech anion exclusion column (300 mm long, 7.8 mm inner diameter) and a Hewlett-Packard 1100 Series UV/visible detector at 210 nm, respectively. Detection limits were ~0.1 mM for each of these compounds. Biomass concentrations were measured as mass of volatile suspended solids (VSS) per liter of liquid (*32*).

Results and Discussion

Transformation of 1,1,1-TCA in the Presence of FeS Alone. In the presence of FeS alone, complete removal of 175 μM 1,1,1-TCA was observed after 14 days in pH 7.5 $NaHCO_3$ buffer (Figure 1). Similar behavior was observed in 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer at pH 7.5 (data not shown). No removal was observed in $NaHCO_3$ or MOPS buffer alone. The rate of 1,1,1-TCA transformation was first order with respect to 1,1,1-TCA concentration and can be described by

$$d[1,1,1\text{-TCA}]/dt = -k_{obs}[1,1,1\text{-TCA}] \qquad (1)$$

where k_{obs} (d^{-1}) is the pseudo-first-order rate coefficient and [1,1,1-TCA] (mol L^{-1}) is the aqueous concentration of 1,1,1-TCA. Similar rates of 1,1,1-TCA transformation were observed

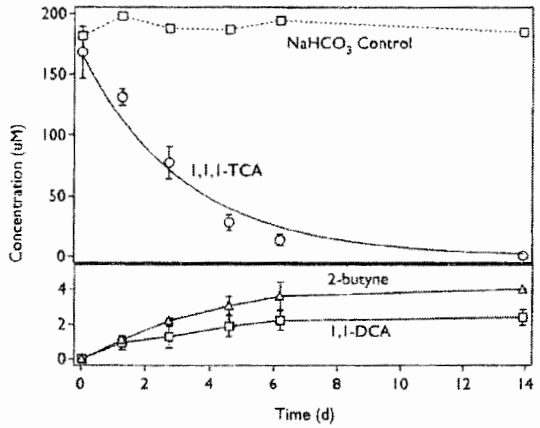

FIGURE 1. Disappearance of 1,1,1-TCA and appearance of 1,1-DCA and 2-butyne in the presence of FeS. Reactors were buffered with 100 mM $NaHCO_3$ (pH 7.5) and contained 10 g L^{-1} (1.4 m^2 L^{-1}) FeS and an initial 1,1,1-TCA concentration of 175 μM. Error bars represent ± one standard deviation based on triplicate reactors.

among five independently synthesized FeS batches with an average k_{obs} of 0.28 ± 0.03 d^{-1}, corresponding to a half-life of 2.5 days ($t_{1/2} = 0.693/k_{obs}$).

Minor amounts of 1,1-DCA (2%) and 2-butyne (4%) were observed as products, resulting in a carbon mass recovery of ~6% of the initial 1,1,1-TCA (Figure 1). A similar amount of 1,1-DCA was observed as the sole product in a previous study of 1,1,1-TCA transformation by FeS (*10*). On the basis of products previously observed from the transformation of 1,1,1-TCA via hydrolysis, elimination, rat metabolism (*33*), and reduction by $CrSO_4$ (*34*) and Fe(0) (*35*), we also analyzed for acetic acid, 1,1-DCE, acetylene, ethane, ethene, acetaldehyde, *cis*-2-butene, and VC. None of these potential products were detected. In addition, hexane extractions of the reactors recovered no 1,1,1-TCA, indicating negligible sorption of 1,1,1-TCA. The low mass recovery may be due to formation of other products for which we did not measure, such as ethanol or some volatile sulfur-containing compounds similar to those that have been observed in highly reducing groundwaters in the presence of H_2S (*36*).

It is unclear whether the transformation of 1,1,1-TCA by FeS is a result of reaction with ferrous iron or sulfide species. Separate control experiments run with 2 μM 1,1,1-TCA in pH 7.5 $NaHCO_3$ buffer showed no appreciable transformation of 1,1,1-TCA over 100 days in the presence of 0.57 M ferrous iron (added as $FeCl_2$) or 1.1 M sulfide (added as Na_2S) (data not shown). The lack of 1,1,1-TCA transformation in the presence of aqueous ferrous iron or sulfide suggests that the ferrous iron or sulfide associated with the FeS structure (i.e., either as part of the structure or as an adsorbed species) is responsible for the observed 1,1,1-TCA transformation. Previous studies have suggested that the transformation of TCE to acetylene by FeS could be due to ferrous iron (*9, 11*), whereas others have suggested that sulfide species are responsible for the transformation of CT to carbon disulfide and carbon dioxide (*6, 7*).

Effect of FeS Concentration. At higher FeS surface area concentrations, faster rates of 1,1,1-TCA transformation were observed (Figure 2). The linear relationship between transformation rates, as quantified by k_{obs}, and FeS surface area concentration indicates that the reaction is first order with respect to FeS surface area for the 1,1,1-TCA and FeS surface area concentrations tested. Thus, the overall reaction can be characterized by a second-order rate expression of the form

$$d[1,1,1\text{-TCA}]/dt = -k_{FeS}\{FeS\}[1,1,1\text{-TCA}] \qquad (2)$$

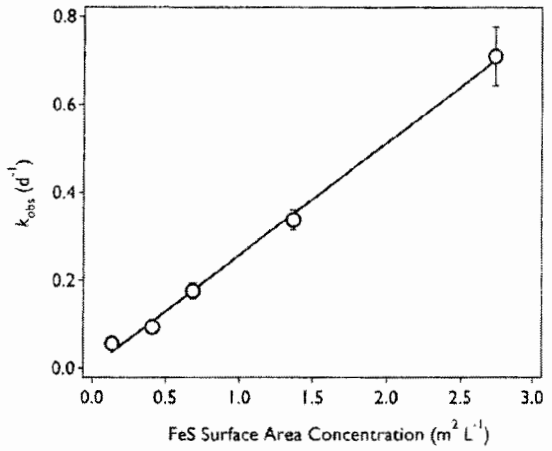

FIGURE 2. Effect of surface area concentration on the first-order rate coefficient for 1,1,1-TCA transformation by FeS. Initial 1,1,1-TCA concentration was ~2 μM. Reactors were buffered with 100 mM $NaHCO_3$ at pH 7.5. Solid line represents linear regression of the data ($n = 5$, $R^2 = 0.99$), resulting in k_{obs} = (0.26 ± 0.01 L m^{-2} d^{-1}){FeS} + 0.002 ± 0.011 d^{-1}. Error bars represent ± one standard deviation based on triplicate reactors.

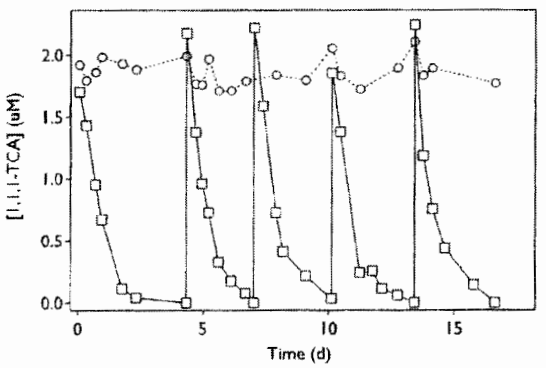

FIGURE 3. Disappearance of 1,1,1-TCA in the presence of 5.3 m^2 g^{-1} FeS in 100 mM MOPS (pH 7.5) over repeated exposures to 1,1,1-TCA. Each exposure resulted in a new initial 1,1,1-TCA concentration of ~2 μM. Open circles represent a control in MOPS buffer with no FeS present.

where the second-order rate coefficient, k_{FeS} (L m^{-2} d^{-1}), is defined as

$$k_{FeS} = k_{obs}/\{FeS\} \qquad (3)$$

and {FeS} is the surface area concentration as given by the product of the mass concentration (S, g L^{-1}) and specific surface area of FeS. Linear regression of the data in Figure 2 resulted in a k_{FeS} of 0.26 ± 0.01 L m^{-2} d^{-1} for the transformation of 1,1,1-TCA by FeS ($n = 5$, $R^2 = 0.99$). This value agrees reasonably well with a k_{FeS} value of 0.47 L m^{-2} d^{-1} calculated from single values of k_{obs} and {FeS} reported by Butler and Hayes (*10*). The slight difference is most likely due to variations in experimental parameters (e.g., we used sieved FeS in pH 7.5 $NaHCO_3$ buffer, whereas Butler and Hayes used 50 mM tris(hydroxymethyl)aminomethane buffer at pH 8.3 and did not report sieving).

Effect of Multiple 1,1,1-TCA Exposures to FeS. The rate of 1,1,1-TCA transformation in the presence of 1.4 m^2 L^{-1} FeS remained relatively constant over five sequential exposures to 2 μM 1,1,1-TCA (Figure 3), with an average k_{FeS} of 0.24 ± 0.04 L m^{-2} d^{-1} for the five exposures. Similar values of k_{FeS} were found for multiple exposures of 1,1,1-TCA to

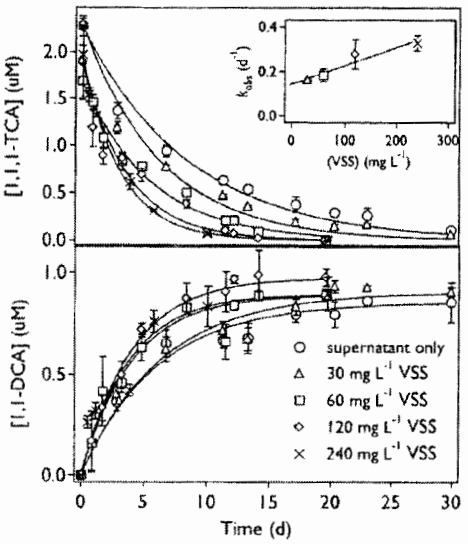

FIGURE 4. Effect of LEC biomass concentration on the disappearance of 1,1,1-TCA and appearance of 1,1-DCA. Initial 1,1,1-TCA concentration was ~2 μM. Reactors were buffered with 100 mM $NaHCO_3$ at pH 7.5. Solid lines represent a first-order kinetic model. Error bars represent ± one standard deviation based on triplicate reactors. Inset: Effect of LEC biomass concentration on k_{obs}. Solid line represents linear regression of the data.

higher (2.8 m^2 L^{-1}, k_{FeS} = 0.21 ± 0.03 L m^{-2} d^{-1}) and lower (0.7 m^2 L^{-1}, k_{FeS} = 0.28 ± 0.05 L m^{-2} d^{-1}) FeS surface area concentrations (data not shown). Assuming all of either the Fe(II) or S(−II) was available for reaction with 1,1,1-TCA, the number of electrons available from the FeS at these concentrations far exceeded (by 5 orders of magnitude) the electrons needed to reduce the total amount of 1,1,1-TCA to ethane. Based on these calculations, it appears that the rate of 1,1,1-TCA transformation was not limited by the amount of FeS. Note that, in these experiments, MOPS buffer was used instead of $NaHCO_3$ due to its stronger buffering capacity at pH 7.5. To evaluate buffer effects on the kinetics of 1,1,1-TCA transformation by FeS, two sets of reactors were run, one buffered with 100 mM $NaHCO_3$ and one with 100 mM MOPS (both at pH 7.5), containing 1.4 m^2 L^{-1} FeS. The difference in the transformation rates of 1,1,1-TCA was insignificant (i.e., $k_{FeS,\ NaHCO_3}$ = 0.26 L m^{-2} d^{-1} versus $k_{FeS,\ MOPS}$ = 0.27 L m^{-2} d^{-1}), suggesting that the change in buffer solution had little influence on 1,1,1-TCA transformation rates.

Transformation of 1,1,1-TCA by Methanogenic Consortium. In the presence of four different concentrations of LEC biomass (VSS), as well as supernatant containing negligible biomass, complete removal of 1,1,1-TCA was observed after 30 days (Figure 4). At higher LEC concentrations, slightly faster rates of 1,1,1-TCA transformation were observed. The linear relationship between the 1,1,1-TCA transformation rates, as quantified by k_{obs}, and LEC concentration indicates that the reaction is first order with respect to the LEC (Figure 4 inset). Unlike the FeS experiments, however, the linear relationship between k_{obs} and VSS includes a significant *y*-intercept term, suggesting that systems containing no LEC biomass (i.e., supernatant only) are capable of transforming 1,1,1-TCA. Additional experiments with reactors containing only supernatant confirmed that 1,1,1-TCA transformation occurs in the absence of significant biomass (Table 1). The overall reaction rate can be described by

$$d[1,1,1\text{-TCA}]/dt = -[k_{VSS}(\text{VSS}) + k_0][1,1,1\text{-TCA}] \quad (4)$$

where k_{VSS} (L mg^{-1} d^{-1}) represents a second-order rate coefficient and k_0 (d^{-1}) is a first-order rate coefficient accounting for the contribution of the supernatant to the overall rate coefficient. Linear regression of the data in the inset of Figure 4 resulted in a k_{VSS} of (8.0 ± 1.0) × 10^{-4} L mg^{-1} d^{-1} and a k_0 of 0.14 ± 0.02 d^{-1} (n = 4, R^2 = 0.92). A proportional relationship between 1,1,1-TCA reaction rate coefficient and microbial biomass concentration has been previously reported for the transformation of 1,1,1-TCA under low-biomass conditions, and the rate coefficient equation describing the relationship also included a positive, nonzero intercept (*18*).

TABLE 1. First-Order Rate Coefficients (k_{obs}) of 1,1,1-TCA Transformation for Various Supernatant Treatments and Reduced Media Control

treatment	k_{obs} (d^{-1})
unfiltered supernatant collected from settled LEC	0.12 ± 0.02[a]
filtered supernatant collected from settled LEC	0.11 ± 0.03
unfiltered supernatant collected from settled LEC and killed with $HgCl_2$	0.11 ± 0.01
chemically reduced media[b]	0.003 ± 0.001
supernatant collected from FeS/$NaHCO_3$ buffer solutions	0.0002 ± 0.0009

[a] Plus/minus values in the observed rate coefficients represent ± one standard deviation based on triplicate reactors. [b] Composition of abiotic reduced media is available in ref *20*.

The k_{obs} values measured for 1,1,1-TCA transformation by supernatant collected from LEC biomass and prepared by several methods are given in Table 1. The measured k_{obs} with unfiltered supernatant (k_{obs} = 0.12 ± 0.02 d^{-1}) is similar to k_0 estimated from the *y*-intercept of the linear relationship between k_{obs} and VSS (Figure 4 inset). To eliminate the possibility of colloidal reactive species in the LEC supernatant (such as colloidal biomass or precipitates), additional experiments were conducted with supernatant (i) filtered through Whatman glass fiber filters and (ii) amended with the biocide $HgCl_2$. Both treated LEC supernatant experiments resulted in rates of 1,1,1-TCA transformation (k_{obs} = 0.11 ± 0.03 and 0.11 ± 0.01 d^{-1}, respectively) similar to untreated LEC supernatant (Table 1). Chemically reduced media controls and supernatant from FeS-alone experiments showed negligible 1,1,1-TCA transformation.

The consistency of rates among the three LEC supernatant controls and lack of reaction with FeS supernatant suggests that 1,1,1-TCA is transformed by some non-cell component, such as an excreted biomolecule. Excreted biomolecules that may be capable of dechlorination have been observed in several other anaerobic cultures (*37–42*). For example, reactive biomolecules have been identified in cultures of *Methanosarcina thermophila* (*39*) and a *Pseudomonas* species (*40, 42*) as active agents in the dechlorination of CT. Characterization of the biomolecules excreted by *M. thermophila* suggested that the biomolecules are most likely porphorinogens (*37, 38*), whereas the biomolecule excreted by *Pseudomonas stutzeri* strain KC has been identified as pyridine-2,6-bis(thiocarboxylate) (*42*).

The only product detected from the transformation of 1,1,1-TCA by the LEC was 1,1-DCA (Figure 4). For all LEC biomass concentrations, the amount of 1,1-DCA produced was relatively constant, accounting for 46 ± 8% of the initial 1,1,1-TCA. Similar mass recoveries of 1,1-DCA in the presence of methanogens have been reported by others (*24, 26*). The two products most commonly reported from biologically mediated transformation of 1,1,1-TCA are 1,1-DCA and CA, as a result of cometabolic reductive dechlorination (*17, 23–25*). In this study, the 1,1-DCA was persistent under the conditions tested even after complete removal of 1,1,1-TCA, and no CA was detected in the reactors. Other researchers

TABLE 2. First-Order Observed (k_{obs}) and Predicted (k_{pred}) Rate Coefficients of 1,1,1-TCA Transformation and Observed and Predicted First-Order Rate Coefficients of 1,1-DCA Appearance (k_{app}) for Various Combinations of FeS Surface Area Concentrations and LEC Biomass Concentrations[a]

FeS (m^2 L^{-1})	LEC supernatant[c]	VSS (mg L^{-1}) 55	100	200
	Observed First-Order Rate Coefficient, k_{obs} (d^{-1})[b]			
0.42	0.30 ± 0.05	0.33 ± 0.03	0.61 ± 0.10	0.74 ± 0.08
0.98	0.48 ± 0.04	0.55 ± 0.03	0.69 ± 0.08	1.08 ± 0.08
1.40	0.56 ± 0.03	0.77 ± 0.13	0.80 ± 0.06	1.17 ± 0.02
	Predicted First-Order Rate Coefficient, k_{pred} (d^{-1})[d]			
0.42	0.25	0.29	0.33	0.41
0.98	0.39	0.44	0.47	0.55
1.40	0.50	0.55	0.58	0.66
	Observed First-Order Appearance Rate Coefficient, k_{app} (d^{-1})[b]			
0.42	0.30 ± 0.04	0.29 ± 0.07	0.34 ± 0.01	0.23 ± 0.02
0.98	0.35 ± 0.01	0.31 ± 0.02	0.37 ± 0.00	0.30 ± 0.02
1.40	0.42 ± 0.05	0.29 ± 0.02	0.41 ± 0.04	0.43 ± 0.12
	Predicted First-Order Appearance Rate Coefficient, k_{app} (d^{-1})[e]			
0.42	0.18	0.21	0.22	0.26
0.98	0.18	0.21	0.22	0.26
1.40	0.18	0.21	0.22	0.26

[a] Reactors were buffered with 100 mM $NaHCO_3$ at pH 7.5. [b] Based on triplicate reactors. [c] Filtered supernatant collected from settled LEC (VSS ≈ 0 mg L^{-1}). [d] Values calculated using eq 7 with k_{FeS} = 0.26 ± 0.01 L m^{-2} d^{-1}, k_{VSS} = (8.0 ± 1.0) × 10^{-4} L mg^{-1} d^{-1}, and k_0 = 0.14 ± 0.02 d^{-1}. [e] Values calculated using k_{app} = 0.0004 (VSS) + 0.1833 derived from the linear regression of the k_{app} vs VSS data generated from Figure 4.

have observed a similar persistence of 1,1-DCA (*16, 17, 26*), and to date, only one study reported 1,1,1-TCA being transformed to CA in near-stoichiometric amounts (*25*).

The kinetics of 1,1-DCA appearance can be modeled by modifying a general appearance kinetic model to account for incomplete mass recovery of 1,1,1-TCA.

$$d[1,1\text{-DCA}]/dt = k_{app}[1,1,1\text{-TCA}] \quad (5)$$

The initial concentration of 1,1,1-TCA was adjusted to represent the amount of 1,1,1-TCA accounted for by the final measured concentration of 1,1-DCA (i.e., $[1,1,1\text{-TCA}]_0$ was set equal to $[1,1\text{-DCA}]_f$). Here, k_{app} (d^{-1}) is the 1,1-DCA appearance rate coefficient quantified by using the adjusted initial 1,1,1-TCA concentration (see Supporting Information for examples of kinetic calculations). Similar corrections to appearance kinetic models have been used to describe the ultimate 1,1-DCA formation capacity by a *Clostridium* sp. transforming 1,1,1-TCA (*23*). For all LEC concentrations, the observed rate coefficients for 1,1-DCA appearance were relatively constant, with an average k_{app} value of 0.22 ± 0.06 d^{-1}.

Transformation of 1,1,1-TCA in the Presence of FeS and LEC Combined. The kinetics of 1,1,1-TCA transformation in the presence of both FeS and LEC can be modeled as two parallel reactions by combining eqs 2 and 4 and incorporating a lumped first-order term, k_{int} (d^{-1}), to account for interactions between FeS and LEC:

$$d[1,1,1\text{-TCA}]/dt = -k_{FeS}\{FeS\}[1,1,1\text{-TCA}] - [k_{VSS}(VSS) + k_0][1,1,1\text{-TCA}] \pm k_{int}[1,1,1\text{-TCA}] \quad (6)$$

To evaluate the interaction between FeS and LEC, we measured the rate of 1,1,1-TCA transformation in the presence of different combinations of FeS and LEC biomass (Table 2) and compared the observed rate coefficient, k_{obs}, to the predicted rate coefficient, k_{pred} (d^{-1}), which assumes no interaction between the FeS and LEC biomass:

$$k_{pred} = k_{FeS}\{FeS\} + k_{VSS}(VSS) + k_0 \quad (7)$$

Comparison of k_{obs} versus k_{pred} for all combinations of FeS surface area and LEC concentrations is shown in Figure 5. All data points lie above the 1:1 line, which represents the relationship between k_{obs} and k_{pred} if the transformation of 1,1,1-TCA by FeS and LEC occurred independently of one another. Figure 5 shows that in the presence of FeS and LEC combined, 1,1,1-TCA was transformed faster than predicted using rate coefficients determined from FeS- and LEC-alone experiments and eq 7, suggesting that there is some synergistic interaction occurring between FeS and LEC.

Linear regression of the data in Figure 5 results in the following relationship between k_{obs} and k_{pred}:

$$k_{obs} = (1.90 \pm 0.36)k_{pred} - 0.15 \pm 0.15\ d^{-1} \qquad n = 12,\ R^2 = 0.80 \quad (8)$$

The slope of 1.90 ± 0.36 indicates that the measured reaction rate in these combined systems is almost twice the predicted transformation rate calculated on the basis of independent, parallel reactions. Enhanced transformation of chlorinated hydrocarbons in the presence of both abiotic and biotic reactive species has been observed previously. Combining Fe(0) and a mixed methanogenic consortia resulted in about a 2-fold increase in chloroform transformation rate relative to the sum of the rate coefficients for the separate Fe(0) and methanogenic consortia treatments (*43*).

To further understand the nature of the synergistic interaction between FeS and LEC, we compared (i) observed and predicted 1,1-DCA appearance rate coefficients (k_{app}), (ii) observed rate coefficients for 1,1,1-TCA transformation (k_{obs}) and observed rate coefficients for 1,1-DCA appearance (k_{app}), and (iii) observed and predicted 1,1,1-TCA mass recoveries as 1,1-DCA ($[DCA]_f/[TCA]_0$). Since the formation of 1,1-DCA can be attributed primarily to LEC activity (i.e., 1,1-DCA accounted for only 2% of the 1,1,1-TCA transformed by FeS alone compared to 46 ± 8% of the 1,1,1-TCA transformed by LEC biomass alone), a comparison of the rate and extent of 1,1-DCA formation provides insight into whether the synergism is due to enhanced activity of LEC. In all but one combination of FeS surface area and LEC biomass concentrations tested, a slight increase in 1,1-DCA appearance rates (represented here by k_{app} as determined from eq 5) was observed relative to the predicted appearance rate coefficient (Figure 6). The faster appearance of 1,1-DCA suggests that LEC is transforming 1,1,1-TCA faster in the presence of FeS. The presence of FeS also resulted in more reducing conditions (ORP measurements changed from −90 mV with no FeS present to −135 mV with 1.40 m^2 L^{-1} FeS). Previous studies have found that, under certain conditions, rates of CAH transformation by methanogens are slower at higher redox potentials (*44*). The slower rates observed at higher redox potentials suggest that one explanation for the larger observed values of k_{app} may be that the presence of FeS alters the solution or surface redox chemistry so as to enhance the activity of LEC.

The significant scatter in a plot of k_{obs} values (for 1,1,1-TCA transformation) versus observed k_{app} values (Figure S1 in Supporting Information), however, indicates that enhanced LEC activity in the combined systems does not fully account for the k_{obs} increase. The lack of a relationship between k_{obs} and k_{app} suggests that there are additional factors contributing to the larger rate coefficients for 1,1,1-TCA transformation. Lower mass recoveries of 1,1,1-TCA as 1,1-DCA (i.e., $[DCA]_f/[TCA]_0$) observed in the combined experiments (Figure S3 in Supporting Information) compared to those predicted from experiments with LEC alone and FeS provide some evidence that FeS is also more reactive in the presence of LEC (since more of the 1,1,1-TCA is presumably going to the unidentified

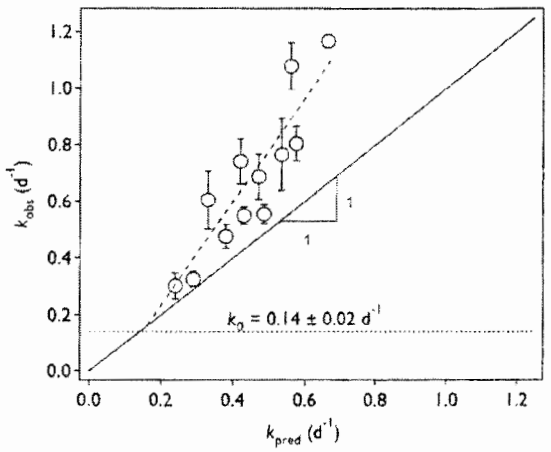

FIGURE 5. Comparison of k_{obs} versus k_{pred} for 1,1,1-TCA transformation by combinations of FeS surface area concentrations and LEC biomass concentrations. Reactors were buffered with 100 mM $NaHCO_3$ at pH 7.5. Error bars represent ± one standard deviation based on triplicate reactors. Dashed line represents linear regression of the data.

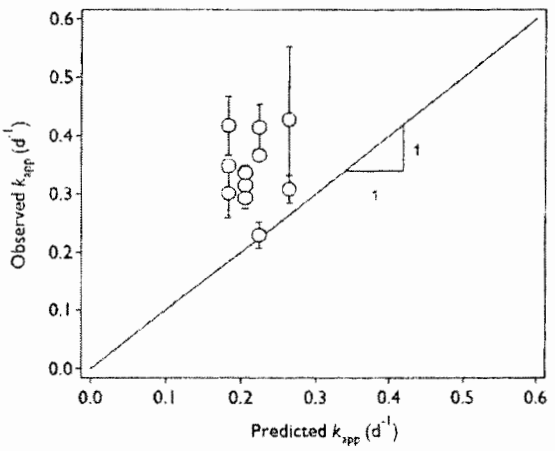

FIGURE 6. Comparison of observed k_{app} versus predicted k_{app} for 1,1-DCA appearance from the transformation of 1,1,1-TCA by combinations of FeS surface area concentrations and LEC biomass concentrations. Reactors were buffered with 100 mM $NaHCO_3$ at pH 7.5. Error bars represent ± one standard deviation based on triplicate reactors.

products from the FeS–TCA reaction). Previous observations that 1,1,1-TCA (at concentrations similar to those used here, i.e., 1–5 μM) has been shown to inhibit methanogenic consortia (*20, 21*) suggest that the enhanced reactivity of FeS (in the presence of LEC) may be an alternative explanation for the faster 1,1-DCA appearance. Specifically, the faster transformation of 1,1,1-TCA by FeS may mitigate any inhibitory effect of 1,1,1-TCA on the LEC biomass by decreasing the 1,1,1-TCA concentration within the system to noninhibitory levels, thereby allowing the LEC biomass to produce 1,1-DCA faster.

Based on the synergistic interactions observed in the presence of LEC and FeS (i.e., faster 1,1,1-TCA transformation and faster 1,1-DCA appearance, but less production of 1,1-DCA per unit of 1,1,1-TCA transformed) it appears that the reactivities of both FeS and LEC are enhanced in the combined systems. One explanation for the enhanced reactivity of FeS in the presence of LEC is the production of soluble microbial products that make the FeS more reactive (e.g., creating a reactive Fe(II) or S(−II) species on the FeS surface), whereas potential explanations for the enhanced reactivity of LEC in the presence of FeS are (i) mitigation of the inhibitory effect of 1,1,1-TCA (because it is transformed by FeS) or (ii) alteration of the solution or surface redox chemistry (as reflected in the lower ORP measurements) created by the presence of FeS. The synergistic interaction observed between FeS and LEC suggests that considering independently determined biotic and abiotic rates may not be sufficient for characterizing rates of CAH attenuation in anaerobic environments where FeS and methanogens may coexist (e.g., sediments and permeable reactive barriers containing Fe(0)).

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Supporting Information Available

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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