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Enhanced Dechlorination of Carbon Tetrachloride and Chloroform in the Presence of Elemental Iron and *Methanosarcina barkeri*, *Methanosarcina thermophila*, or *Methanosaeta concillii*

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Previous experiments in our laboratory have demonstrated that the rate and extent of carbon tetrachloride (CT) and chloroform (CF) dechlorination were enhanced when a methanogenic enrichment culture and iron (Fe^0) were incubated together. Batch experiments with three pure cultures of methanogens, *Methanosarcina barkeri*, *Methanosarcina thermophila*, and *Methanosaeta concillii* were performed to determine how this enhanced transformation occurred. When hydrogen (H_2) was added as an electron donor, degradation of CT for all organisms and CF for *M. thermophila* was more rapid. H_2 was produced from the oxidation of iron, which therefore served as an H_2 source for the organisms, enhancing the transformation of CT and CF. Experiments with *M. thermophila* and *M. concillii*, which could not grow on H_2-CO_2 under the conditions tested, showed that H_2 could serve as an electron donor for dechlorination of CT and CF with these organisms as well. Experiments with supernatants from *M. thermophila* grown with and without iron indicated the presence of an excreted biomolecule active in the enhanced transformation of CT and CF.

Introduction

Halogenated aliphatics have been in widespread use for several decades in industrial applications such as degreasing, cleaning, and extraction. Many of these compounds are listed as priority pollutants by the United States Environmental Protection Agency and are known or suspected human carcinogens, mutagens, or toxins (*3*).

Elemental iron (Fe^0) has been shown to facilitate the abiotic hydrogenolysis of chlorinated aliphatics (*4*):

$$Fe^0 \rightarrow Fe^{2+} + 2e^- \quad (1)$$

$$H^+ + 2e^- + CCl_4 \rightarrow CHCl_3 + Cl^- \quad (2)$$

Several elemental metals, including Fe^0, can support the growth of methanogenic bacteria as the sole electron donor (*5, 6*). Electrons are made available through the production of hydrogen gas (H_2) from the corrosion of Fe^0 to Fe^{2+}.

$$4Fe^0 + 8H^+ \rightarrow 4Fe^{2+} + 4H_2 \quad (3)$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (4)$$

Methanogens, which under anaerobic conditions mediate the hydrogenolysis and hydrolytic reduction of halogenated aliphatics (*7*), appear to grow solely on CO_2 and the H_2 produced from the corrosion of Fe^0 (*5, 6*).

A combined system of elemental iron plus methanogens should provide a low-maintenance support for an anaerobic bacterial population in a treatment system, exploiting the dechlorinating abilities of both the biological and abiotic agents present. Preliminary studies have shown that, when iron is combined with a mixed population of methanogens, enhanced degradation (both in rate and extent) of carbon tetrachloride (CT) and chloroform (CF) (*1, 2*) occurred. This enhanced dechlorination was much greater than expected by simply adding the effects of biodegradation by resting cells with iron-mediated degradation.

From these experiments (*1, 2*), the question arose, how does this enhanced degradation take place? The research presented here addressed this question. Objectives included (a) the determination of whether H_2 from oxidizing Fe^0 could serve as an electron donor for biologically mediated dechlorination and (b) whether in the combined system a biological factor was excreted into the media that was active in the dechlorination of CT and CF. The model contaminants CT and CF were used with pure cultures of methanogens (*Methanosarcina barkeri* 227, *Methanosarcina thermophila*, and *Methanosaeta concillii*) for the experiments presented.

Experimental Section

Chemicals. High-pressure liquid chromatography (HPLC)-grade CT, CF, and dichloromethane (DCM) were obtained from Sigma. HPLC-grade methanol (MeOH) was used as a substrate (Fisher). A single bottle of elemental iron powder (Aldrich Chemical Co.) was used in all of the experiments. The iron powder (99.9% pure, 10 μm) was unwashed and had a specific surface area of 2.02 m^2/g. Methane gas (CH_4), H_2-CO_2 (80:20 vol/vol), and N_2-CO_2 (80:20 vol/vol) were obtained from Air Products.

Organism and Culture Conditions. Studies were performed with pure cultures of either *M. thermophila* (DSM 1825; Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany), *M. barkeri* 227 (DSM 1538), or *M. concillii* (DSM 3671). These three cultures were chosen for their varied abilities to utilize H_2-CO_2 for growth. *M. barkeri* will grow readily on H_2-CO_2 with no acclimation period, *M. thermophila* will only grow poorly on H_2-CO_2 after a long lag period (about 9 days) (*8, 9*), and *M. concillii* is unable to grow on H_2-CO_2. All three cultures were grown according to the directions provided by the DSM.

Methanogens were cultured in 140-mL bottles (Supelco), with thick butyl rubber stoppers (Bellco) or in a 1-L Pyrex bottle fitted with a constructed stopper. The constructed stoppers consisted of the following. A 20-mL headspace autosampler vial (Kimble) with the bottom of the vial removed was sealed with a thick butyl rubber stopper (Bellco). This vial was fit into a large butyl rubber stopper with the center removed, and the entire unit was then fit into a drilled hole in the plastic media bottle cap. In all cases, freshly prepared media was added to the bottles; the bottles were then sealed, autoclaved, and allowed to cool. Sterile techniques were

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then used to inoculate the media with the appropriate organism. Organism growth was monitored via methane concentration in the bottles. Periodically, the samples were checked under a microscope (Zeiss) to assess culture purity. All cultures were grown near their optimal temperatures (35 °C for *M. barkeri* and *M. concillii* and 50 °C for *M. thermophila*) and incubated quiescently on their sides.

Experimental Procedure. Triplicate reactors were used for each treatment in all experiments. CH_4 and H_2 were monitored over time to quantify growth and H_2 production and/or utilization. Biomass was measured [as mg/L volatile suspended solids (VSS)] before and after each experiment to monitor organism growth. All experiments were performed in 38-mL crimp-top bottles sealed with a thick butyl rubber stopper lined with Teflon. Experiments were performed at 50, 35, or 35 °C for *M. thermophila*, *M. barkeri*, and *M. concillii*, respectively.

Saturated aqueous stock solutions were prepared by equilibrating neat CT and CF (3 mL each) with NanoPure water. The solutions were shaken vigorously for several hours and then allowed to settle for several days at 20 °C before use. Primary standards for calibration were prepared gravimetrically with neat CT, CF, and DCM in methanol. Calibration standards were prepared by adding specific quantities of primary standards to 38-mL bottles containing 25 mL of NanoPure water.

Dechlorination Experiments. Reactors for these studies were prepared and incubated in the following manner. The bottles were filled with iron plus N_2-CO_2, or only H_2-CO_2 or N_2-CO_2. The bottles were then capped, sealed, and autoclaved. Once the bottles had cooled, they were filled with either 25 mL of medium or 25 mL of medium and cells in the stationary phase. Approximately 1 h after filling, CT or CF (approximately 5−10 μM, total concentration) was added to the bottles to begin the experiment. Bottles were incubated quiescently on their sides.

The following treatments, with an N_2-CO_2 headspace unless otherwise stated, were used (in triplicate) for the dechlorination experiments: a medium control consisting of medium only; a cells-only control consisting of resting cells and medium; an iron-only control consisting of iron and medium; a cells-plus-H_2 system consisting of resting cells and medium with a H_2-CO_2 headspace; and an iron-plus-cells system consisting of iron, resting cells, and medium. For experiments with *M. thermophila*, there was also a MeOH-fed system consisting of cells, medium, and added MeOH (100 mM). For *M. concillii*, there was an acetate-fed system containing excess acetate and no resting cell system. For experiments with *M. concillii*, growth of the cells took approximately 3 months. The added acetate (83 mM) was never completely used. At the end of the experiment, approximately 8−16 mM (or 480−960 mg/L) acetate remained; therefore, true resting cell treatments could not be investigated without risking general loss of cell viability.

CT, CF, and DCM were monitored by gas chromatography (GC) over time, as were H_2 and CH_4. Experiments with *M. thermophila* were of a 24−30 h duration, experiments were 1−4 days in length for *M. barkeri*, and experiments were 5 days in length for *M. concillii*.

Supernatant Exchange Experiments. The reactors for these experiments were prepared as follows. Thirty-eight-milliliter bottles contained iron plus N_2-CO_2 or only N_2-CO_2. After autoclaving, the bottles were filled with 25 mL of cell suspension entering the exponential growth phase (as monitored by CH_4 production). For each experiment, there were two iron-plus-cells treatments and three cells-only treatments (with each in triplicate). Once cells had reached the stationary phase (approximately 3 days), the reactors were decanted into centrifuge vials in the glovebag and capped. Vials were spun at 10 000 rpm (5700*g*) for 15 min in a centrifuge outside of the glovebag.

Once spun, supernatant exchange took place. One iron-plus-cells treatment (iron-stay) and one cells-only treatment (cells-stay) were shaken to resuspend the biomass and iron and were placed back into their original 38-mL bottles. Alternatively, the pellets from one iron-plus-cells treatment and one cells-only treatment were placed back into their original 38-mL bottles. The supernatants from the cells-only systems were placed in the bottles containing the iron-plus-cells pellets (iron-swap), and the iron-plus-cells supernatants were placed in the bottles containing the cells-only pellets (cells-swap). The supernatant from the final cells-only treatment was decanted into sterile, empty 38-mL bottles (supernatant control). After the 38-mL bottles were filled with the appropriate supernatants and pellets, the bottles were capped, and the experiment was started with the amendment of CT (5−10 μM). The reactors were incubated statically on their sides for the duration of the experiment.

Data Analysis. Because all of the various treatments did not contain organisms or iron, it was thought that the best way to compare transformation rates between treatments was first-order rate coefficients. Each system was expected to be heterogeneous; however, a complicated transformation rate model would not facilitate a better comparison between treatments. Because the different treatments were handled in as similar a manner as possible, a first-order rate coefficient calculation provides the most suitable means by which comparisons could be made. First-order rate coefficients were calculated as follows. Degradation of the contaminant (C) is represented by

$$\frac{dC}{dt} = -k'C$$

where k' is the first-order rate coefficient and t is time. This expression can be integrated, yielding the equation

$$-\ln \frac{C}{C_0} = k'(t - t_0)$$

where C_0 is the initial contaminant concentration at time zero (t_0). When $-\ln (C/C_0)$ is plotted against time, t, the initial slope is equal to k'.

The figures show average concentration versus time profiles for the triplicate reactors for each treatment. Error bars in the figures represent the standard deviation between the concentrations in each of the triplicate reactors for a given treatment. For each treatment, the rate of transformation and standard deviation (given in the tables) represent initial rates of CT or CF transformation averaged over triplicate reactors. The standard deviation was calculated from the individual rates of transformation in each of the triplicate reactors. For data that contained an initial lag period, rates of transformation are based on the transformation rate after the lag.

CT, CF, and DCM Measurement. Headspace samples (100 μL) were taken with a gastight locking syringe (Precision Sampling Corp., Baton Rouge, LA). These were directly injected into a gas chromatograph (Hewlett-Packard 5890 series II) equipped with an electron capture detector (GC-ECD) for CT and CF measurement. A fused silica capillary column (J&W) with a DB-5 stationary phase was used with nitrogen as the carrier gas. The oven temperature was held at 35 °C for a run length of 2.5 min. Method detection limits were 0.00034 and 0.025 μM for CT and CF, respectively, as determined by the method outlined in Standard Methods (Method 1030 E; *10*). DCM was measured by direct headspace injection into a GC-ECD equipped with a 60 m capillary

TABLE 1. First-Order Rate Coefficients for CT and CF Degradation by *M. barkeri*, *M. thermophila*, and *M. concillii* (day^{-1})

treatment	*M. barkeri*		*M. thermophila*		*M. concillii*
(parent compd)	CT	CF	CT	CF	CT
cells-only	2.13 ± 0.30	0.39 ± 0.14	8.31 ± 0.33	7.45 ± 1.58	NA[a]
Cells-plus-H_2	5.38 ± 0.43	0.64 ± 0.74	14.0 ± 0.92	11.1 ± 1.74	0.44 ± 0.03
MeOH-fed cells	NA[a]	NA[a]	11.9 ± 3.62	11.7 ± 3.76	0.31 ± 0.07[b]
iron-plus-cells	9.84 ± 1.09	0.76 ± 0.43	18.6 ± 1.64	16.2 ± 0.47	8.25 ± 2.06
iron-only	4.74 ± 0.15	0.21 ± 0.13	10.3 ± 0.62	0.31 ± 0.03	10.0 ± 1.61
medium control	0.29 ± 0.01	0.16 ± 0.05	0.27 ± 0.25	0.13 ± 0.01	0.20 ± 0.05

[a] Not applicable. [b] Acetate fed.

column. The column had a 1.8 μM DB-VRX stationary phase (Supelco). Nitrogen was the carrier gas. The oven temperature was held constant at 35 °C for a 10-min run length. Detection limits were approximately 0.412 μM.

Methane and H_2 Measurement. Headspace samples of 100 μL were directly injected into a gas chromatograph (Hewlett-Packard 5890) with a thermal conductivity detector (GC-TCD) for CH_4 and H_2 measurement. A 6-ft stainless steel-packed column with Haysep Q packing was used. Nitrogen was the carrier gas. The oven temperature was held at 120 °C for a run length of 2.5 min. Detection limits were approximately 10 μL/reactor for each gas.

VSS Measurement. The methodology was followed as outlined in Standard Methods (Method 2540 G; *10*).

Results and Discussion

Direct comparisons of the CT and CF first-order transformation coefficients for the three different species should be viewed with caution. However, relationships between treatments, product distribution, and transformation patterns can be compared. In addition, because these experiments were conducted at the optimal growth conditions for each organism, the rates of transformation are very high and should not be extrapolated to field applications. Replicate experiments were performed and, when statistically compared with a two-way analysis of variance (ANOVA) test with interaction, were found to behave in the same manner as those experiments reported in the paper. These results are not included for brevity.

In the experiments described here, *M. thermophila* was not exposed to H_2-CO_2 until introduced to the bottles containing iron or an H_2 headspace (approximately 1 h before CT or CF addition). Because experiments with *M. thermophila* generally lasted less than 2 days, no acclimation to growth on H_2-CO_2 was expected. Indeed, methane and biomass measurements showed no growth in the *M. thermophila* and *M. concillii* treatments containing iron or an H_2 headspace. H_2 was produced in all iron-containing treatments with time (data not shown). The CH_4 and H_2 profiles showed the expected trends for each treatment based on the catabolic abilities of these three organisms (data not shown).

Two-sided, paired *t*-tests over the first-order transformation coefficients were determined between various treatments at the 95% confidence level. The results from these tests were used to establish significance; this significance is mentioned in the text.

Dechlorination Experiments. First-order rate coefficients for CT and CF transformation by *M. thermophila*, *M. barkeri*, and *M. concillii* are listed in Table 1 for each of the treatments studied. Transformation rate coefficients were calculated as described when either CT or CF was added. Coefficients were not calculated for daughter products.

M. thermophila. CT transformation profiles and concomitant CF formation and transformation profiles are shown in Figure 1 for *M. thermophila*. In experiments with *M. thermophila*, the iron-only system produced a nearly stoichiometric amount of CF from CT; this CF underwent slow subsequent transformation. In all treatments containing organisms, less CF was formed (ranging from 20 to 50% of initial CT concentration). CF was very quickly transformed in the treatments containing cells-plus-H_2 and iron-plus-cells (Figure 1). In treatments of cells-only and MeOH-fed cells, less CF was formed, and it was subsequently transformed more slowly.

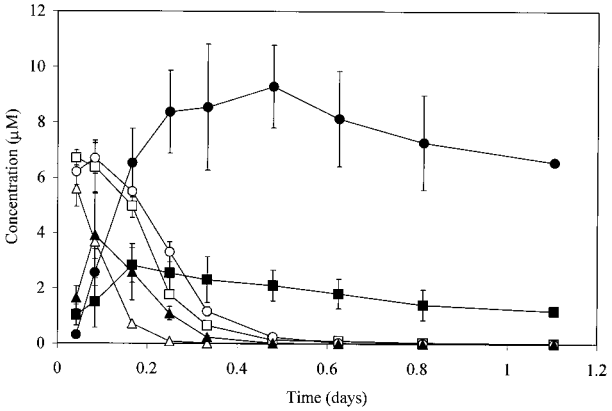

FIGURE 1. CT transformation profiles and subsequent CF formation and transformation profiles in experiments with *M. thermophila* and added CT. Error bars represent ± one standard deviation. Symbols are □, cells-only CT; ■, cells-only CF; △, iron-plus-cells CT; ▲, iron-plus-cells CF; ○, iron-only CT; ●, iron-only CF. The cells-plus-H_2, MeOH-fed cells, and medium control treatments have been deleted for clarity.

In experiments where CF was added, very little transformation occurred in the iron-only and medium systems (Table 1), while systems containing *M. thermophila* transformed CF readily. In the systems where all the CF was transformed (the systems containing organisms), approximately 50% (on a molar basis) was transformed to DCM; Figure 2 shows DCM production by *M. thermophila*. Cells-only treatments produced DCM but were unable to degrade it (Figure 2). However, in biological systems containing added iron, H_2, or MeOH, DCM was formed and subsequently degraded.

M. barkeri. In experiments with *M. barkeri*, CT was transformed in the treatments containing iron-plus-cells at a rate significantly faster than in all other treatments (Table 1, Figure 4). The cells-plus-H_2 treatments also transformed CT at a significantly faster rate than the cells-only treatment. Accumulation of CF followed a pattern similar in some ways to that observed for *M. thermophila*, with approximately stoichiometric CF formation in the iron-only system and only a transient CF accumulation in the iron-plus-cells reactors (Figure 3). However, unlike *M. thermophila*, reactors with cells-plus-H_2 and cells-only accumulated CF at levels similar to the iron-only reactors. Subsequent degradation of CF was very slow in all of the treatments except the iron-plus-cells reactors.

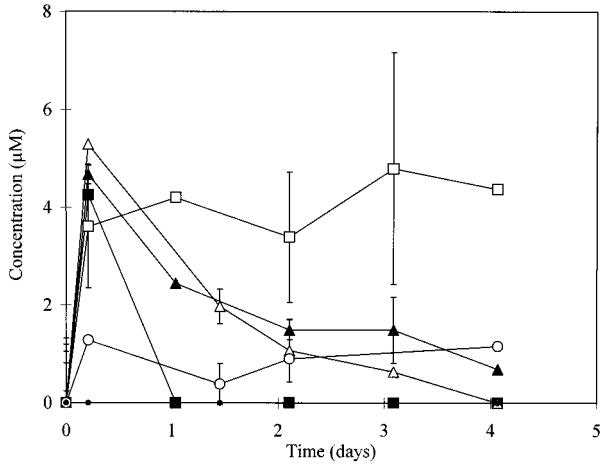

FIGURE 2. DCM formation and subsequent transformation profiles in experiments with *M. thermophila* and added CF. Error bars represent ± one standard deviation. Symbols are: □, cells-only; ▲, cells-plus-H_2; △, iron-plus-cells; ■, MeOH-fed cells; ○, iron-only treatments. No DCM was formed in the medium control treatments; therefore, these points are not shown.

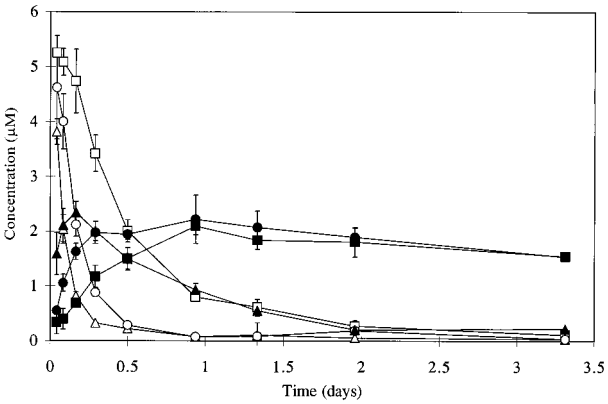

FIGURE 3. CT transformation profiles and subsequent CF formation and transformation profiles in experiments with *M. barkeri* and added CT. Error bars represent ± one standard deviation. Symbols are □, cells-only CT; ■, cells-only CF; △ iron-plus-cells CT; ▲, iron-plus-cells CF; ○, iron-only CT; ●, iron-only CF. The cells-plus-H_2 and medium control treatments have been deleted for clarity.

When CF was added to *M. barkeri* as the parent compound, very slow transformation of CF was observed in all of the treatments (Table 1). *M. barkeri* was sensitive to higher concentrations of CF. In the experiments where CT was added, less CF was formed than when CF was added directly (2.5 versus 5.5 μM, respectively). When less CF was present, it was transformed over two times faster than when the higher concentration of CF was added to the organisms. *M. thermophila* did not exhibit such sensitivity.

When CF was added at 5.5 μM, the transformation rate coefficient for CF was not significantly different in any of the treatments. DCM formed and increased in concentration over the experimental period in the cells-plus-H_2 and iron-plus-cells treatments. No DCM was formed in the other treatments (data not shown).

M. concillii. In experiments with *M. concillii,* transformation of CT occurred very slowly in the systems without iron (Table 1 and Figure 4). CT did degrade significantly faster in the cells-plus-H_2 reactors than in the acetate-fed reactors, but the difference was slight. However, unlike the results with *M. thermophila* and *M. barkeri,* CT transformation in the iron-plus-cells system was not faster than in the iron-only system. The rate of CT transformation by *M. concillii* was very slow, and the rate of iron-mediated

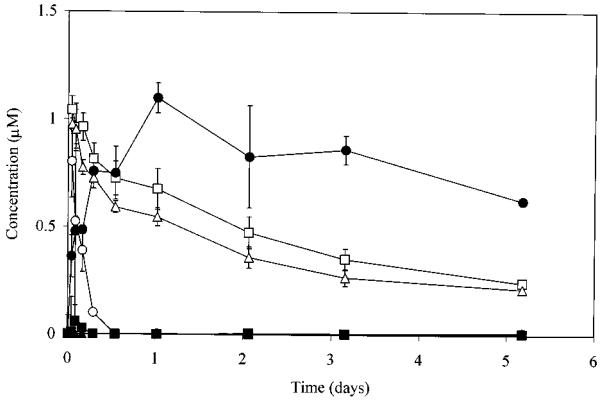

FIGURE 4. CT transformation profiles and subsequent CF formation and transformation profiles in experiments with *M. concillii* and added CT. Error bars represent ± one standard deviation. Symbols are □, acetate-fed cells CT; ■, acetate-fed cells CF; △, cells-plus-H_2 CT; ▲, cells-plus-H_2 CF; ○, iron-only CT; ●, iron-only CF. The iron-plus-cells and medium control treatments have been deleted for clarity.

transformation was quite fast; therefore, the rate of CT transformation in the iron-plus-cells system was dominated by the abiotic reaction, and no enhanced degradation was observed when organisms were added. Transformation profiles for CT and subsequent formation and transformation profiles for CF are given in Figure 4.

Discussion of Dechlorination Experiments. An H_2 headspace with cells enhanced CT and CF degradation by *M. barkeri* and *M. thermophila* as compared to cells-only systems. An H_2 headspace with cells also enhanced CT and CF degradation by *M. concillii* as compared to the acetate-fed cells system. For *M. barkeri*, when one adds the rate coefficient for CT transformation by cells-plus-H_2 to the iron-only transformation coefficient, it equals that of the iron-plus-cells systems. For *M. thermophila*, the CT transformation coefficient for cells-only plus iron-only approximates that of the iron-plus-cells systems. However, when one adds the CF transformation coefficients for the cells-plus-H_2 systems to that for the iron-only systems, it is a better approximation of the combined system than the cells-only plus the iron-only systems. These results show that H_2 was a key factor in the enhanced degradation of CT and CF for both organisms, but not the only factor of importance in the transformation of these contaminants by *M. thermophila*.

M. barkeri can use H_2-CO_2 for growth and through increased biomass should degrade CT and CF at a faster rate if H_2 was present. Also, H_2 would be expected to serve as an electron donor for dechlorination because *M. barkeri* contains hydrogenase, which would utilize the reducing equivalents from H_2 to reduce CT or CF. In other studies, H_2 has also been implicated as the electron donor for dechlorination of PCE (*11*).

Although H_2-CO_2 cannot be used as a growth substrate for *M. thermophila* under the conditions studied here, enhanced degradation of CT and CF occurred in the presence of an H_2 headspace with this organism. This was consistent with the observation that even though *M. thermophila* does not grow on H_2-CO_2, it does exhibit a low level of hydrogenase activity (*8, 12*). This demonstrates that H_2 can be used as an electron donor for dechlorination when the cells were unable to utilize H_2-CO_2 for growth. This has important implications for bioremediation since field conditions (such as pH, micronutrients, and temperature) are rarely ideal for organism growth. It can be inferred from these results that if H_2 is present (via the corrosion of iron) and methanogens, having active hydrogenase, are also present, the organisms will not need to be able to grow in order to effectively use the H_2 as an electron donor for dechlorination.

TABLE 2. First-Order Rate Coefficients for CT Degradation by *M. barkeri* and *M. thermophila* in the Supernatant Exchange Experiments (day^{-1})

treatment[a]	*M. barkeri*	*M. thermophila*
cells-stay	0.87 ± 0.16	10.8 ± 0.06
cells-swap	1.18 ± 0.24	15.7 ± 0.22
iron-swap	5.47 ± 0.58	15.6 ± 0.60
iron-stay	5.43 ± 1.14	34.7 ± 3.20
supernatant control	0.31 ± 0.28	16.2 ± 0.19

[a] Cells-stay contains pellet and supernatant not exposed to iron; cells-sway contains pellet not exposed to iron and exposed supernatant; iron-swap contains pellet exposed to iron and supernatant not exposed; iron-stay contains pellet and supernatant exposed to iron. The supernatant control is supernatant only from the system not exposed to iron.

For experiments with *M. concillii*, H_2 was also able to serve as an electron donor for dechlorination at very low levels when it was unable to serve as an electron donor for growth. *M. concillii* is a relatively poorly studied organism; it is only able to utilize acetate for growth and is therefore not thought to contain hydrogenase. It is unclear how H_2 would be utilized as an electron donor for dechlorination without the presence of hydrogenase. It is possible that these organisms do contain such enzymes, but at a low enough level that they are unable to contribute to organism growth. In early characterization studies of this organism, a hydrogenase assay showed very slight activity (*13*). It is not clear whether this was an artifact or did in fact represent a very small quantity of this enzyme in the culture. In an in situ situation where H_2 is cathodically produced, organisms similar to *M. concillii*, present at a distance from the iron, could still contribute to the enhanced dechlorination by utilizing this H_2 for dechlorination.

The transformation patterns of CF (when CT was added) or DCM (when CF was added) indicated that the presence of H_2 gas increased the rate at which CF and DCM were subsequently degraded. Again, this indicates that the enhanced degradation of CT, CF, and DCM observed when elemental iron and methanogenic bacteria were present together was due, at least in part, to the H_2 gas produced from corroding iron serving as an electron donor for dechlorination.

In a mixed anaerobic culture, H_2 would be expected to serve as both an electron donor for growth as well as an electron donor for dechlorination. One would expect to find organisms that would fall into each of the categories investigated in this research: cells that grow readily on H_2-CO_2, cells that will grow poorly on H_2-CO_2 after an acclimation period, and cells that are completely unable to utilize H_2-CO_2 for growth. The results presented here demonstrate that each of these culture types will be able to use H_2 as an electron donor for dechlorination.

Research by Weathers and Parkin (*1*) and Weathers et al. (*2*) corroborates that H_2 was responsible for the enhanced transformation of CT and CF in a mixed anaerobic culture. Dechlorination occurred at a much faster rate when organisms were in the presence of iron rather than under an N_2-CO_2 headspace.

Supernatant Exchange Experiments. Supernatant exchange experiments were performed to determine whether an excreted biomolecule was responsible for all or a portion of the enhanced CT and CF transformation. The supernatants from treatments where organisms were grown in the presence of iron were exchanged with those where organisms were grown in media only. First-order transformation coefficients were calculated for CT for each treatment with *M. barkeri* and *M. thermophila* and are presented in Table 2. Treatment names are as described in the Experimental Section. For *M. thermophila*, there was a significant difference between the cells-swap and the cells-stay treatments (Table 2), with the cells-swap treatment transforming CT at a faster rate. The supernatant control also transformed CT very rapidly (Table 2). However, in experiments with *M. barkeri*, no statistical difference was seen between the cells-swap and cells-stay treatments (Table 2). There was also no significant difference between the iron-swap and iron-stay treatments (Table 2). In addition, no appreciable transformation of CT was observed in the *M. barkeri* supernatant control.

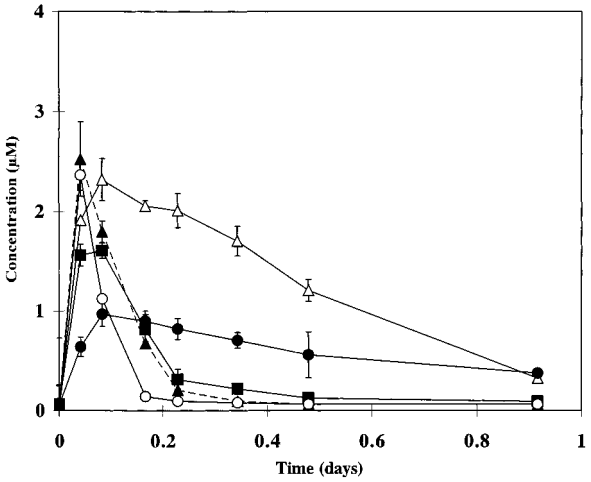

FIGURE 5. CF profiles in the supernatant exchange experiments with *M. thermophila* and added CT. Error bars represent ± one standard deviation. Symbols are △, cells-stay; ■, cells-swap; ▲, iron-swap; ●, supernatant control treatments; ○, iron-stay treatments. Treatment names are as defined in the Experimental Section.

In experiments with *M. thermophila*, the CF formation and subsequent degradation profile was very different for the cell systems with or without exchanged supernatants (Figure 5). A buildup of CF in the cells-stay reactors to about 50% of the initially added CT occurred, followed by slow degradation. In the cells-swap reactors, less CF was formed and was quickly transformed. In the iron-plus-cells systems with or without exchanged supernatants about 50% of the initially added CT was transformed to CF, which was quickly degraded. The rate of CF transformation appeared to be about the same in the three groups of reactors that contained iron or contained supernatant exchanged from the iron-plus-cells systems. CF was also formed in the supernatant control treatments, which was slowly transformed.

In experiments with *M. barkeri*, the CF profile in the cells-swap and cells-stay treatments was approximately the same (Figure 6). In both systems CF was formed from CT, but was not further transformed. Likewise, the CF profile in the iron-stay treatment was mimicked in the iron-swap treatment, with CF forming but failing to undergo further dechlorination. Nearly stoichiometric quantities of CF were produced from CT in the iron-containing systems. Little CF was formed in the supernatant control, and no further transformation of the CF was observed.

The degradation of CT by *M. barkeri* was not affected by exchanging the supernatants between the cells-only and iron-plus-cells treatments. This indicates that no extracellular compound was active in these systems. However, when the same experiments were run with *M. thermophila*, there was an enhanced degradation of CT and CF in the cells-only systems containing supernatant from the iron-plus-cells systems. There was no visual evidence of iron carryover in the exchanged supernatants. The enhanced transformation could therefore be due to an extracellular factor released from the organisms, which was active in CT and CF

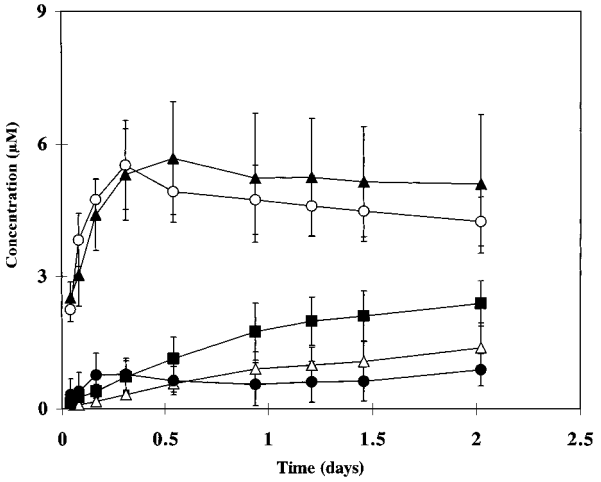

FIGURE 6. CF profiles in the supernatant exchange experiments with *M. barkeri* and added CT. Error bars represent ± one standard deviation. Symbols are △, cells-stay; ■, cells-swap; ▲, iron-swap; ●, supernatant control treatments; ○, iron-stay treatments. Treatment names are as defined in the Experimental Section.

transformation. The rapid transformation observed in the supernatant control indicates that the difference between the cells-only and cells-plus-iron supernatants could be that growth in the presence of iron results in the production of a greater quantity of a factor always released by the cells or the release of an additional factor.

Evidence for similar extracellular factors can be found in the literature. Dybas and co-workers (*14*) have found that a *Pseudomonas* species will degrade CT completely to CO_2 and a nonvolatile fraction under denitrifying conditions. This transformation capability was attributed to both intra- and extracellular factors and in particular to a small excreted biomolecule.

Methanogenic extracellular proteins have been found that are also excreted in response to environmental conditions. Kim et al. (*15*) found that when a copper-resistant methanogen, *Methanobacterium bryantii* BKYH, was exposed to high concentrations of copper, the organism responded by excreting 4-fold increased levels of three extracellular proteins. These proteins were thought to protect the organism from toxicity and allow it to grow in an environment where it would otherwise be unable to live. *M. thermophila* may respond in a manner similar to Fe^0 by excreting a protein that also happens to be active in the dechlorination of CT and CF.

Experiments conducted to assess the cause of enhanced CT and CF transformation when methanogens were in the presence of elemental iron allowed the following conclusions to be drawn. First, providing H_2 as an electron donor resulted in faster degradation of CT for all organisms and CF for *M. thermophila*; therefore, the enhanced dechlorination when organisms were incubated with iron was likely due to iron providing a source of H_2. Organisms did not need to be able to grow on H_2-CO_2 for H_2 to act as an electron donor for dechlorination (as for *M. thermophila* or *M. concillii*). Iron was thereby able to enhance the CT (and to some extent CF) dechlorination rate by serving as an electron donor for dechlorination. Finally, an excreted biomolecule from *M. thermophila* was implicated in the enhanced transformation of both CT and CF when grown in the presence and absence of elemental iron. No such biomolecule was detected from *M. barkeri*. Further studies were conducted to study this biomolecule and will be discussed in an additional paper (*16*).

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