Acetylene Inhibition of Trichloroethene and Vinyl Chloride Reductive Dechlorination

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Kinetic studies reported here have shown that acetylene is a potent reversible inhibitor of reductive dehalogenation of trichloroethene (TCE) and vinyl chloride (VC) by a mixed dehalogenating anaerobic culture. The mixed culture was enriched from a contaminated site in Corvallis, OR, and exhibited methanogenic, aceticogenic, and reductive dehalogenation activities. The H2-fed culture transformed TCE to ethene via cis-dichloroethene (c-DCE) and VC as intermediates. Batch kinetic studies showed acetylene reversibly inhibited reduction of both TCE and VC, and the levels of inhibition were strongly dependent on acetylene concentrations in both cases. Acetylene concentrations of 192 and 12 μM, respectively, were required to achieve 90% inhibition in rates of TCE and VC transformation at an aqueous concentration of 400 μM. Acetylene also inhibited methane production (90% inhibition at 48 μM) but did not inhibit H2-dependent acetate production. Mass balances conducted during the studies of VC inhibition showed that acetogenesis, VC transformation to ethene, and methane production were responsible for 52%, 47%, and 1% of the H2 consumption, respectively. The results indicate that halorespiration is the dominant process responsible for VC and TCE transformation and that dehalorespiring organisms are the target of acetylene inhibition. Acetylene has potential use as a reversible inhibitor to probe the biological activities of reductive dechlorination and methanogenesis. It can be added to inhibit reactions and then removed to permit reactions to proceed. Thus, it can be a powerful tool for investigating intrinsic and enhanced anaerobic remediation of chloroethenes at contaminated sites. The results also suggest that acetylene produced abiotically by reactions of chlorinated ethenes with zero-valent iron could inhibit the biological transformation of VC to ethene.

Introduction

Chlorinated aliphatic hydrocarbons (CAHs), such as tetrachloroethene (PCE) and trichloroethene (TCE), are major groundwater contaminants (1). Anaerobic biotransformation of PCE and TCE generates cis-dichloroethene (c-DCE), vinyl chloride (VC), and ethene as products. Incomplete transformation of PCE and TCE to c-DCE or VC is frequently observed in CAH-contaminated groundwater, and this limits the usefulness of anaerobic biotransformation as a natural attenuation process (2). Among these products, VC is the most undesirable as it is a human carcinogen and has the lowest drinking water standard (2 μg/L) (3).

A variety of biochemical tools have been used to probe the complexity of reductive dehalogenation processes in microbial systems. Using molecular approaches, Flynn et al. (4) showed that the structure of a dechlorinating mixed culture shifted as chlorinated ethenes were successively transformed. Terminal restriction fragment length polymorphism (T-RFLP) demonstrated that c-DCE- and VC-grown subcultures from PCE enrichments had distinct 16S rRNA genes and that subcultures grown on less chlorinated solvents lost their ability to degrade PCE. These results suggested that more than two dehalogenating microorganisms were responsible for the complete transformation of PCE to ethene (4). By examining anaerobic biotransformation of CAHs at the biochemical level, Magnuson et al. (5) partially purified two corrinoid-containing enzymes, PCE-reductive dehalogenase (PCE-RDase) and TCE-reductive dehalogenase (TCE-RDase), from Dehalococcoides mccainae strain 195 (6). The TCE-RDase was unable to transform PCE, a result that suggested that multiple enzymes were required to complete the transformation of PCE to ethene (5).

Inhibitors have also been widely used as mechanistic probes for individual microbial activities in complex microbial mixtures (7). For example, the role of methanogens in a wide variety of mixed anaerobic cultures has previously been investigated using 2-bromoethanesulfonate (BES). This compound is a structural analogue of coenzyme M and specifically inhibits methanogenesis because it competes with coenzyme M in the terminal methylation reaction in methane formation (8). Although BES is water-soluble and effective at low concentrations (~1 mM), it is often necessary to use much higher concentrations (10 mM) to fully inhibit methanogenesis and to overcome possible degradation, transport, and resistance problems (7).

The role of methanogens in PCE degradation has been investigated using BES. In the absence of BES, an anaerobic sewage sludge system reduced PCE to TCE concurrently with methane production. In the presence of BES, both PCE transformation and methane production were inhibited, suggesting that methanogens were responsible for PCE degradation (9). This conclusion was supported by evidence showing PCE reduction by pure cultures of methanogens (10). However, later studies of the effects of BES on reductive dehalogenation have shown that some dechlorinating microorganisms are not methanogens. For example, DiStefano et al. (11) and Chiu and Lee (12) found that dechlorinators were not inhibited by BES.

Acetylene is another compound that has been used to inhibit methanogenesis. Micromolar concentrations of acetylene have been shown to strongly inhibit methane production in lake sediments (13, 14), marine sediments (15), and fish intestines (16). Acetylene has also been shown to selectively inhibit acetate-dependent methanogenesis during acetone degradation by a mixed culture of an acetogenic eubacterium and a Methanothrix sp. (17). The effects of acetylene have also been examined with several pure cultures of methanogens (18). Growth of Methanospiillum hungatei was completely and reversibly inhibited by 8 μM dissolved acetylene. Studies with cell free extracts indicated that acetylene did not inhibit several key enzyme activities including H2 uptake by hydrogenases, NADP reductase, and 16S rRNA methylase (M) (18). However, later studies of the effects of BES on reductive dehalogenation have shown that some dechlorinating microorganisms are not methanogens. For example, DiStefano et al. (11) and Chiu and Lee (12) found that dechlorinators were not inhibited by BES. In the absence of BES, an anaerobic sewage sludge system reduced PCE to TCE concurrently with methane production. In the presence of BES, both PCE transformation and methane production were inhibited, suggesting that methanogens were responsible for PCE degradation (9). This conclusion was supported by evidence showing PCE reduction by pure cultures of methanogens (10). However, later studies of the effects of BES on reductive dehalogenation have shown that some dechlorinating microorganisms are not methanogens. For example, DiStefano et al. (11) and Chiu and Lee (12) found that dechlorinators were not inhibited by BES.
methyl CoM reductase, and ATP hydrolase. However, the presence of acetylene prevented cells of M. hungatei from maintaining a transmembrane pH gradient, and this resulted in a loss of ATP synthesis activity and energy-dependent Ni2+ uptake (18).

While the precise mechanism of acetylene inhibition of methanogenesis remains unknown, the potency of the effects of acetylene on methanogenesis provoked our interest in evaluating this compound as a potential inhibitor and probe of reductive dechlorination activity in a mixed anaerobic culture. Acetylene is potentially useful as an inhibitor for this purpose because acetylene is highly water-soluble, but unlike BES, this inhibitor can be removed from incubations by sparging with an anaerobic gas. In this study, acetylene was evaluated as an inhibitor of TCE and VC transformation by a mixed methanogenic/acetogenic/dehalogenetic culture. The effect of acetylene concentration on the dechlorinating activities was defined in terms of both the concentration and the reversibility of the effects.

Materials and Methods

**Chemicals.** PCE (99%) and c-DCE (97%) were purchased from Acros (Fisher Scientific, Pittsburgh, PA), and TCE (99.9%), 1,1-DCE (99%), and trans-DCE (98%) were purchased from Aldrich (Milwaukee, WI). Gas standards, ethene (1000 ppm ± 5%), and VC (996 ppm ± 2%) in nitrogen were purchased from Scott Specialty Gases (Alltech Associates, Inc; Deerfield, IL). Methane (99%), CO2 (99%), acetylene (99.6%), and H2 (99%) were obtained from AIRCO (Vancouver, WA). Yeast extract was purchased from Fisher Scientific (Fair Lawn, NJ).

**Analytical Methods.** Gas-phase PCE, TCE, c-DCE, trans-DCE, 1,1-DCE, VC, acetylene, ethene, and methane concentrations were measured by injecting 100 μL of a headspace sample into a HP-5890 gas chromatograph (GC). The GC was equipped with a 30-m megabore GSQ-Plot column (J&W Scientific, Folsom, CA) connected to a flame-ionized detector (FID). H2 and CO2 were analyzed by injecting 100 μL of headspace sample into a HP-5890 GC. Chromatographic separation was achieved using argon as a carrier gas with a Supelco 60/80 Carboxen 100 column (15 ft x 0.125 in.; Bellefonte, PA), followed by quantification using a thermal conductivity detector (TCD).

**Culture Growth.** The mixed anaerobic culture was obtained from a TCE-contaminated groundwater and sediment at the Evanite site in Corvallis, OR. The area from which the samples were obtained had shown TCE transformation to VC with methane present. The culture was first grown in batch serum bottles on benzoate and PCE in RAMM media (19). This mixed culture is capable of completely transforming PCE through TCE, c-DCE, and VC to ethene with a conversion to ethene of nearly 100%. For the current study, the cells were grown in a 4-L batch reactor on benzoate, PCE, and anaerobic RAMM mineral medium that contained yeast extract. The media (400 mL) was exchanged weekly, resulting in a hydraulic residence time of about 70 d. The media contained 1.8 g of benzoate, 0.9 g of neat PCE, and 0.02 g of yeast extract that contributed approximately 1% of the carbon, while benzoate contributed 99%. No additional vitamins were added as nutrients. Cells were harvested during the weekly batch exchange.

To achieve rapid VC transformation, the harvested cells were further enriched in a 1000-mL batch serum bottle reactor (Wheaton, Millville, NJ). The harvested cells were purged with nitrogen to remove the residual CAHs. The purged gas was furnace-treated to remove residual oxygen by in-line contact with copper filings at 600°C. The cells were incubated in the 1000-mL batch reactor; with 5 mL of H2 (200 μmol), 20 mL of VC (800 μmol), and 20 mL of CO2 added to the reactor headspace. Additional H2 (5 mL) was added when H2 levels fell below 0.5% v/v gas phase. Ethene production was monitored to determine how well VC was transformed. The incubated cells were capable of transforming both TCE and VC. The VC concentration for cell incubation was double the concentration used in the inhibition studies (TCE and VC at 400 μM). This concentration was chosen to be much greater than reported Ks values (1.4 μM TCE and 2.6 μM VC) of dehalogenated cultures (20) and to maintain high concentrations throughout the incubation. Cells were harvested when the batch reactor reached a VC removal rate of 80 μmol/d.

**Inhibition Tests.** The tests were performed in 125-mL batch bottles (Wheaton; Millville, NJ) capped with butyl rubber septa (Wheaton; Millville, NJ) to allow temporal sampling. Cells (50 mL) were transferred from the VC-containing reactor to the 125-mL batch reactor in an anaerobic glovebox. The reactors were purged with furnace-treated nitrogen for 10 min to remove the glovebox H2 and any residual VC or ethene. H2 (5 mL), CO2 (20 mL) (furnace-treated), and VC (2 mL) were then added to the reactor headspace. Alternatively, for studies following TCE reduction, TCE-saturated liquid solution (2 mL) was added instead of VC. The initial H2 and CO2 concentrations in the headspace of each reactor were about 5% and 20% (v/v), respectively. The reactors were incubated in at 20 °C and shaken in an inverted position at 200 rpm on a rotary shaker table. The H2 and CO2 concentrations were maintained at excess levels for this kinetic study. H2 was resupplied during the experiment when the headspace H2 concentrations fell below 0.5% (v/v) by adding 5 mL of H2.

The acetylene inhibition experiments consisted of three stages: an initial rate-determination stage, an inhibition stage, and a recovery stage. In the first stage, the initial transformation rates of TCE or VC were determined in the absence of acetylene inhibition by monitoring their removal and accumulation of their transformation products (c-DCE, 1,1-DCE, and ethene). In the second stage, the transformation rates were determined in the presence of the acetylene. Prior to addition, the acetylene gas was scrubbed with 10% (w/v) CuSO4·5H2O to remove trace amounts of acetone, as described in Hyman and Wood (21). Finally, in the third stage the rates were remeasured. The initial VC or TCE and H2 concentrations were the same at the start of each stage, while the second stage had various amounts of acetylene added to the different reactors. Between stages, the reactors were purged with furnace-treated nitrogen gas for 15 min to remove all the CAHs. This sequence enabled the determination of transformation rates with and without acetylene present and in the presence of different CAHs.

Transformation rates for each CAH were determined for each stage by measuring product formation. The rate was determined by linear regression of at least 5 data points. The rates of product gain were determined by mass balances assuming equilibrium between gas and liquid phases based on Henry’s law. Published Henry’s law constants of Gossett (22) were used for these determinations. The constant mixing of the reactors at 200 rpm, over the time scale of the experiments (hours) assured that phase equilibrium was achieved.

The various experiments examining the effects of acetylene on VC and TCE reduction are summarized in Table 1. Variation of initial transforming rates was likely due to differences in concentration of the dehalogenating microorganisms. Test 1 investigated the reversibility of acetylene inhibition on VC transformation, while in tests 2 and 3 the effect of acetylene concentration on VC transformation was studied. Acetylene inhibition of TCE transformation was studied in tests 4 and 6. Test 6 was conducted over a broader range of acetylene concentrations and at a higher initial TCE concentration than test 4. Only tests 4 and 5 were conducted.
were purged on day 10 and then VC, H2, and CO2 were added (data not shown). On day 36, acetylene was purged and methane production continued in control reactors with no acetylene addition. The reactors were purged on day 10 and then VC, H2, and CO2 were again added. Ethene production resumed achieving a maximum rate of ~83% of that achieved prior to the acetylene inhibition test, indicating that acetylene inhibition was largely reversible. A higher concentration of acetylene (1920 μM) was added to the reactors on day 40 to determine whether the effects of acetylene were irreversible at higher concentrations. As expected, ethene production was completely inhibited by acetylene, but slower rates of ethene production were subsequently observed after the acetylene was removed on day 45. The maximal rate of ethene production on day 45 was about 30% of the maximal rate observed prior to the first addition of acetylene. We do not know whether the rate decrease is associated with the exposure to the high acetylene concentration or other processes, such as the competition for hydrogen by acetogens as a result of the 45-d incubation. A third test with 120 μM acetylene showed VC transformation was fully inhibited at this lower concentration.

The second set of experiments examined the inhibitory effect of lower acetylene concentrations on VC transformation and methane production by the mixed culture. For these and all subsequent tests, cell mass was increased from that achieved in the mother reactor by an additional growth step in a batch reactor fed VC, as described in the Methods section. Figure 2 shows the effect of varying acetylene concentrations (0–48 μM) on VC transformation to ethene and methane production. Initial ethene production rates in stage I were similar in all the batch reactors. In stage II, varying concentrations of acetylene were introduced into duplicate reactors. Ethene production was completely inhibited with the highest acetylene concentration tested (48 μM) and was strongly inhibited (> 75%) at the lowest acetylene concentration tested (6 μM). The effects of acetylene on methane production were distinctly different than those observed for VC transformation. While methane production was essentially completely inhibited at the highest concentration of acetylene tested (48 μM), it was only inhibited by ~50% with 24 μM acetylene. Little or no inhibition of methane production occurred with the lower concentrations of acetylene tested (6 and 12 μM). In stage III, both ethene and methane production were rapidly re-instated after the removal of acetylene. As shown in earlier experiments, the ethene-generating activity was generally constant during stage III, and the rate of ethene production from most acetylene-treated reactors was indistinguishable from the untreated control reactors. The stage III recovery phase verified the

![FIGURE 1. Successive inhibition of VC transformation to ethene at three different aqueous acetylene concentrations and recovery upon the removal of acetylene.](Image)

**TABLE 1. Concentrations Used in Inhibition Experiments and Initial Rates of Dehalogenation**

<table>
<thead>
<tr>
<th>experiment</th>
<th>initial acetum conc (μM)</th>
<th>initial avg rates of product prodn (μmol/h)</th>
<th>acetylene conc (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>test 1 VC 380</td>
<td>ethene (0.13)</td>
<td>120, 480, 1920</td>
<td></td>
</tr>
<tr>
<td>test 2 VC 450</td>
<td>ethene (0.34)</td>
<td>0, 6, 12, 24, 48</td>
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<tr>
<td>test 3 VC 440</td>
<td>ethene (0.23)</td>
<td>0, 0.75, 1.5, 3, 6, 12</td>
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<tr>
<td>test 4 &amp; TCE 380</td>
<td>c-DCE (0.85)</td>
<td>0, 12, 24, 48, 96, 192</td>
<td></td>
</tr>
<tr>
<td>test 5 VC 470</td>
<td>ethene (0.16)</td>
<td>0, 2.4, 6, 12, 24, 48</td>
<td></td>
</tr>
<tr>
<td>test 6 TCE 850</td>
<td>c-DCE (4.67)</td>
<td>2.4, 12, 48, 120, 180, 240</td>
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*Tests 4 and 5 were conducted with the same set of harvested cells.

Acetylene Inhibition of VC Transformation. The initial study (test 1) evaluated acetylene inhibition of VC transformation at three different acetylene concentrations and investigated the reversibility of the inhibition. The experiments were conducted in duplicate at an aqueous VC concentration of 380 μM. Figure 1 shows the time course of ethene accumulation from VC transformation. Over the first 9 d, small amounts of ethene accumulated in the reactors. The reactors were purged on day 10 and then VC, H2, and CO2 were replenished. This procedure was repeated at days 14 and 17, and rapid rates of ethene generation were established after day 20. The increase in ethene production rates was consistent with exponential growth of a dehalogenating culture. On day 27, the reactors were again purged and replenished with H2, CO2 and VC. The rate of ethene production was ~83% of maximum rate prior to purging, a result that demonstrates that purging did not substantially impact the culture’s dehalogenating activity. When rapid dehalogenating activity had been confirmed, acetylene (480 μM) was added on day 31. Acetylene completely inhibited ethene production over a period of 4 d, while ethene production continued in control reactors with no acetylene added (data not shown). On day 36, acetylene was purged from the reactors, and VC, H2, and CO2 were again added. Ethene production resumed achieving a maximum rate of

<table>
<thead>
<tr>
<th>cummulative ethene mass (μmol)</th>
<th>cumulative ethene mass (μmol)</th>
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<tr>
<td>10</td>
<td>15</td>
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<td>20</td>
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<td>55</td>
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<tr>
<td>60</td>
<td>65</td>
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with same set of harvested cells. Other studies were performed with cells harvested at different times.

Results

Acetylene Inhibition of VC Transformation. The initial study (test 1) evaluated acetylene inhibition of VC transformation at three different acetylene concentrations and investigated the reversibility of the inhibition. The experiments were conducted in duplicate at an aqueous VC concentration of 380 μM. Figure 1 shows the time course of ethene accumulation from VC transformation. Over the first 9 d, small amounts of ethene accumulated in the reactors. The reactors were purged on day 10 and then VC, H2, and CO2 were replenished. This procedure was repeated at days 14 and 17, and rapid rates of ethene generation were established after day 20. The increase in ethene production rates was consistent with exponential growth of a dehalogenating culture. On day 27, the reactors were again purged and replenished with H2, CO2 and VC. The rate of ethene production was ~83% of maximum rate prior to purging, a result that demonstrates that purging did not substantially impact the culture’s dehalogenating activity. When rapid dehalogenating activity had been confirmed, acetylene (480 μM) was added on day 31. Acetylene completely inhibited ethene production over a period of 4 d, while ethene production continued in control reactors with no acetylene added (data not shown). On day 36, acetylene was purged from the reactors, and VC, H2, and CO2 were again added. Ethene production resumed achieving a maximum rate of

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The results presented in Figure 2 showed that VC reduction was more acetylene-sensitive than methanogenesis. We therefore investigated the effects of a lower range of acetylene concentrations (0–12 μM) on VC transformation (Figure 3). All batch tests had similar initial rates of VC transformation in stage I. In stage II, the rate of ethene production decreased with increasing acetylene concentration. Duplicate values showed nearly identical results. An acetylene concentration of 6 μM inhibited the rate of VC transformation by greater than 50%. The reversibility of acetylene inhibition was again demonstrated in stage III after acetylene was removed. The results clearly demonstrated the dependence of the inhibition of the rates of VC transformation on acetylene concentration.

To determine which microbial process were inhibited by acetylene in the reactions described in Figure 3, mass balances for H2 utilization, methane production, and acetate formation were performed for each reactor during stage II of the tests. Average values for all the reactors indicated methane production was responsible for less than 1% of the H2 consumed; VC transformation to ethene was responsible for about 47%, and the acetogenesis was responsible for about 52%. Figure 4 shows measured H2 utilization versus acetylene concentration for each reactor during the stage II period of reactions (Figure 3). Higher acetylene concentrations resulted in a substantial reduction in H2 utilization for reductive dechlorination, while H2 utilization mainly for acetogenesis was largely unaffected by concentrations of acetylene that fully inhibited VC reduction. H2 consumption decreased at high acetylene concentrations due to the inhibition of VC transformation. The amount of acetate produced during the three stages of the experiment was a factor of 3 greater than that predicted for stage II, indicating that acetogenesis was occurring throughout the experiment.

**Acetylene Inhibition on TCE Transformation.** Acetylene inhibition of TCE transformation by the mixed culture was also investigated. Since the culture transformed TCE at a faster rate than VC, it was hypothesized that higher acetylene concentrations might be required to inhibit TCE transformation. Inhibition of TCE transformation was therefore studied at concentrations of acetylene up to 192 μM. The aqueous TCE concentration for these tests was 400 μM.

Figure 5 shows the concentration history of TCE and its transformation products over the range of acetylene concentrations ranging from 0 to 48 μM. Methane inhibition results are shown in panel B. The experiments were carried out in three stages: initial (I), inhibited (II), and recovery (III) of methane and ethene production. (The average and range of duplicates tests are presented.)

Additionally, reversibility of VC transformation over a range of the acetylene inhibitions. In contrast, the methane-generating activity was not constant over time, and there is evidence that the effects of the higher concentrations of acetylene were not fully reversible after removal of the inhibitor.
centrations from 0 to 192 μM for three stages of the inhibition tests. Both c-DCE and 1,1-DCE were produced from TCE transformation, with c-DCE accounting for more that 99% of the DCE products formed. Some VC was also formed, and its production increased after significant c-DCE accumulated. The controls (no acetylene added) showed similar DCE production rates for all stages. In stage II, the production c-DCE and 1,1-DCE decreased proportionally with increasing acetylene concentration. VC production was essentially completely inhibited at all acetylene concentrations. The results indicated that c-DCE transformation was more susceptible to acetylene inhibition than TCE transformation. After the removal of acetylene, similar rates of TCE transformation and c-DCE and 1,1-DCE product accumulation occurred as in stage I, indicating that TCE inhibition by acetylene was reversible for all acetylene concentrations tested.

The change in production rates of c-DCE and 1,1-DCE upon inhibition with acetylene were similar. The result possibly indicates the same enzyme is responsible for the production of both compounds. An interesting observation is that rates of VC production appear to increase through the test, with controls (no acetylene) accelerating at a faster rate in stage III, while rates of DCE accumulation are similar. The results might indicate growth of a dehalogenating population that transforms c-DCE to VC, with a faster rate of growth in controls that were not inhibited by acetylene. The results could indicate that growth of a DCE-dehalogenating culture was inhibited by acetylene during stage II.

Inhibition Patterns. Rates of TCE and VC transformation were determined on the basis of product production rates prior to and after acetylene inhibition. To normalize each reactor for small differences in the initial rates, the measured rates were normalized by taking a ratio of the inhibited rate \(V_{i=1}\) in stage II to the initial rate \(V_{i=0}\) in stage I. The normalized ratio represents the fractional decrease in rate due to acetylene inhibition. The ratio was then used to calculate the percent inhibition of the initial rate \(V_{i=0}\) as

\[
\%\text{ inhibition} = (1 - \frac{V_{i=1}}{V_{i=0}}) \times 100\% \tag{1}
\]

The percent inhibition was then plotted versus acetylene concentration, thus permitting a comparison between the overall inhibition patterns for TCE and the VC transformation.

Figure 6 summarizes the overall patterns of acetylene inhibition of TCE transformation to c-DCE (panel A), and 1,1-DCE (panel B) and on VC transformation to ethene (panel C) for the repeated tests (Table 1). The tests show very reproducible effects of acetylene on TCE transformation for both c-DCE and 1,1-DCE (Figure 6A,B) and VC transformation to ethene (Figure 6C). Essentially identical inhibition patterns were observed for TCE transformation to c-DCE and 1,1-DCE, possibly indicating that the same enzyme is involved in their production. The stronger inhibition of acetylene on VC transformation is apparent, as indicated by the lower acetylene concentrations.

Discussion

This study demonstrated that acetylene is a potent and reversible inhibitor of several reductive dechlorination reactions catalyzed by organisms in a mixed dehalogenating culture. Our results confirm several previous reports concerning the inhibitory effects of acetylene on methanogenesis (23, 24). For example, the experiment described in Figure 2B demonstrated an almost complete inhibition of methane production with 48 μM acetylene. We do not know whether both acetoclastic and H₂-utilizing methanogens are present in the culture. VC reduction was considerably more sensitive to acetylene inhibition than methanogenesis, with more than 90% inhibition of VC reduction achieved with 12 μM acetylene, a concentration that achieved less than 10% inhibition of methanogenesis. The selectivity of the effects of low concentrations of acetylene (< 12 μM) toward VC reduction rather than either methanogenesis or acetogenesis was further confirmed by the results presented in Figure 4. Given the characteristically large flux of H₂ utilization coupled to VC reduction (Figure 4) and the apparent lack of involvement of methanogenesis or acetogenesis in VC reduction (Figure 2), our results suggest that organisms capable of using chlorinated alkenes and likely VC as terminal electron acceptors are the main target of inhibition by low concentrations of acetylene.

Two different groups of organisms capable of dehalorespiration with chlorinated alkenes are currently recognized. One group of organisms including, among others, Dehalospirillum multivorans and Desulfobacterium strains can use PCE as an electron acceptor during H₂-dependent growth. These organisms can reduce PCE to TCE and DCE but do not further reduce DCE. The second group of chlorinated alkene-respiring organisms, currently containing only Dehalococcoides ethenogenes, can reduce PCE further to ethene (6). Both groups of organisms possess cobalamin- and Fe-S-containing reductase enzymes responsible for chlorinated alkene reduction. All PCE-respiring organisms contain a PCE reductase responsible for PCE reduction to TCE. These enzymes also reduce TCE further to DCE. The extended substrate range of D. ethenogenes is due to the activity of a second reductase enzyme, TCE reductase, that reduces TCE primarily to c-DCE and can also reduce c-DCE to VC and VC to ethene (5).

The mode of action of acetylene as an inhibitor of the reductive dechlorination reactions described here remains unclear. Acetylene has many effects on microbial processes and typically inhibits redox-active metalloenzymes (25). The inhibitory mechanisms underlying these effects are diverse.
and occur over a wide range of acetylene concentrations (25). Acetylene is perhaps best known as an inhibitor of Mo- and V-containing nitrogenases. In this instance, acetylene acts as poor alternative substrate for these enzymes ($K_M = 0.12\text{--}0.8\text{mM}$) and undergoes reduction to yield either ethene or ethane (26, 27). The potential for acetylene reduction was also monitored during our experiments and in control reactions containing acetylene and no TCE or VC additions. No significant ethene production was observed. In addition, in the test shown in Figure 1, ethene production was not evident when VC transformation was blocked at high acetylene concentration ($1920\text{M}$), indicating that acetylene was not being reduced. Acetylene concentrations also remained constant in all the tests (data not shown). Limited ethene inhibition studies were also performed along with the acetylene studies report here. Ethene aqueous concentration of 140, 350, and 575$\mu$M showed minimal inhibition of VC transformation. These results suggest that ethene accumulation during VC transformation tests did not affect VC transformation rates.

Acetylene also inhibits many Ni/Fe-containing uptake hydrogenases, and the inhibition process conforms to many of the characteristics of slow, tight-binding inhibition (28). While an effect of acetylene on hydrogenase activity could potentially account for the effects we have observed in this study, several factors suggest that this is unlikely. First, acetylene is a relatively weak inhibitor of Ni/Fe uptake hydrogenases ($K_I = 0.3\text{ M}$), and these enzymes are not expected to be impacted by the low concentrations of acetylene shown to be effective in our experiments (28). Second, $H_2$ is an extremely potent competitive protecting agent against acetylene inhibition ($K_D = 0.4\text{ M}$) (28). The

FIGURE 5. TCE transformation and c-DCE, 1,2-DCE, and VC production at acetylene concentrations ranging from 0 to 192$\mu$M. (The average and range of duplicates tests are presented.)
experiments described here were all conducted with high H₂ concentrations, conditions that would be expected to minimize acetylene inhibition of hydrogenases. Third, our results suggest that H₂ consumption coupled to either methanogenesis or acetogenesis was not inhibited by the low acetylene concentrations shown to be effective against VC reduction. It is also important to recall that, as outlined in the Introduction, acetylene inhibition of methanogenesis has previously been studied and could not be correlated with effects on hydrogenase activity (17).

Our study has provided evidence for an unusually potent inhibition of reductive dechlorination reactions. One possibility is that acetylene is directly inhibiting chlorinated ethene reductases required for TCE and VC reduction in this mixed culture. Like all other acetylene-inhibited enzymes, these are metalloenzymes, and unlike many other acetylene-sensitive enzymes, acetylene is a close structural analogue of the reductase substrates. It is also notable that as corrinoid enzymes these enzymes contain a cofactor that can readily undergo alkylation reactions (29). It would clearly be very interesting to examine the effects of acetylene on either pure cultures of dehalorespiring organisms or the purified reductases to test the validity of these conclusions and further define the inhibition mechanism and its kinetic characteristics.

Broader Implications. Irrespective of the precise site or mode of action of acetylene discussed above, it is important to recognize that the effects of acetylene we have characterized here have potentially broad implications. For example, acetylene is known to be produced from the abiotic reduction of TCE and PCE to chloroacetylene and acetylene (30-31). The results of our work would indicate that the abiotically produced acetylene could potentially inhibit the biotic transformation of TCE and VC in a combined process, such as in-situ treatment using zero-valent iron (ZVI) (32, 33). Lampron et al. (32) showed changes in the product distribution of TCE transformation resulting from microbial transformation around a permeable iron barrier. The result indicated a combination of abiotic and biotic transformations might result in VC accumulation. Luo and Sewell (33) found that PCE was completely degraded to ethene and ethane in a ZVI system that produced ethene and ethane but not acetylene. However, in systems where acetylene was produced, only partial transformation of PCE was observed.
may be that the production of acetylene inhibited these systems. If abiotic acetylene is produced and is a stable product, our results indicate that the biotic transformation of VC could be inhibited.

Since acetylene is a gaseous chemical, physically it can be easily applied and removed by purging unlike BES, which is not volatile. Acetylene can be easily purged by sparging with an anaerobic gas, as was demonstrated here, and dehalogenation was rapidly re-initiated with an addition of either TCE or VC and H2. Acetylene is a natural produced gas, and thus regulatory approval might be obtained for its use in field studies of intrinsic or enhanced anaerobic dehalogenation. For example, it might be used in push–pull tests described by Hageman et al. (34). Acetylene could be used to inhibit transformation rates of TCE and an added analogue, such as trichlorofluoroethene (TCFE), to provide indirect evidence of in-situ transformation (34, 35). Being a reversible inhibitor, it can be added to the subsurface to inhibit transformations, and upon its removal, transformations should again proceed. The inhibition of transformation of other CAHs including PCE and c-DCE is also of interest. Future studies are also needed with pure cultures such as D. ethenogenes (6).

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