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Comparative Evaluation of Chloroethene Dechlorination to Ethene by *Dehalococcoides*-like Microorganisms

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Reductive dehalogenation of tetrachloroethene (PCE), trichloroethene (TCE), *cis*-1,2-dichloroethene (DCE), and vinyl chloride (VC) was examined in four cultures containing *Dehalococcoides*-like microorganisms. Dechlorination and growth kinetics were compared using a Monod growth-rate model for multiple electron acceptor usage with competition. Included were the Victoria mixed culture containing *Dehalococcoides* species strain VS (from Victoria, TX), the mixed culture KB-1/VC (from southern Ontario), the Pinellas mixed culture (from Pinellas, FL), and *D. ethenogenes* strain 195. All cultures, with the exception of *D. ethenogenes* strain 195, grew with VC as catabolic electron acceptor. A dilution method was developed that allows a valid comparison to be made of dehalogenating kinetics between different mixed cultures. Using this procedure, maximum growth rates on VC were found to be similar for strain VS and KB-1/VC (0.42−0.49 ± 0.02 d^{-1}) but slower for the Pinellas culture (0.28 ± 0.01 d^{-1}). The 16S rRNA gene sequences were determined to ensure that no cross contamination between cultures had occurred. Following enrichment of the VC dechlorinating microorganisms on VC, the cultures were amended with DCE, TCE, or PCE. The three mixed cultures failed to dechlorinate PCE or did so very slowly. However, the dilution technique indicated that all experienced growth on TCE and DCE as well as on VC. Maximum growth rates on DCE alone were quite similar (0.43−0.46 d^{-1}), while the Pinellas culture grew faster on TCE alone (0.49 d^{-1}) than did the other two mixed cultures (0.33−0.35 d^{-1}). Half-velocity and inhibition constants for growth on TCE were also determined for the three mixed cultures; both constants were found to be essentially equal and the same for the different cultures, varying between only 8.6 and 10.5 μM. The ability of the strain VS, KB-1/VC, and Pinellas cultures to utilize TCE rapidly with conversion to ethene is quite different from that of any other reported microorganism. It was separately confirmed with more traditional cell-counting techniques that strain VS coupled TCE, as well as DCE and VC, utilization with growth. This is the first report of an organism obtaining energy for growth through every step in the reduction of TCE to ethene. Also, as suggested by the dilution technique, the dehalogenating organisms in the KB-1/VC and Pinellas cultures appear to obtain growth from TCE utilization as well. Such ability to grow while dehalogenating TCE to ethene will be an important advantage for their use in bioaugmentation.

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Introduction

Tetrachloroethene (PCE) and trichloroethene (TCE) are among the most common organic groundwater contaminants. Under anaerobic conditions, they may be converted biologically through reductive dehalogenation to *cis*-1,2-dichloroethene (DCE) (*trans*-dichloroethene or 1,1-dichloroethene may also be formed; *1*), vinyl chloride (VC), and then ethene. As yet, no microorganism has been reported that can grow through every step in the reductive dechlorination of PCE or TCE to ethene. Several phylogenetically different bacteria have been identified with the ability to convert PCE and TCE to DCE, but the reduction of DCE to VC and ethene appears to be carried out for the most part by organisms of the genus *Dehalococcoides*. The first such organism identified, *Dehalococcoides ethenogenes* strain 195 (*2*), obtains energy for growth from reduction of PCE, TCE, and DCE to VC. It can convert VC to ethene but does so only co-metabolically. Two recently reported *Dehalococcoides* microorganisms, *Dehalococcoides* species strain VS (*3, 4*) and *Dehalococcoides* isolate BAV1 (*5*), can derive energy from both DCE and VC reduction to ethene. *Dehalococcoides* isolate BAV1 uses TCE and PCE only co-metabolically (*5*). The possible ability of strain VS to use TCE or PCE has not previously been indicated.

In the field, complete transformation to ethene, the desired end point, often does not occur naturally because of insufficiency in required electron donor, reaction kinetics, the absence of necessary dehalogenating bacteria, or other environmental factors. Engineering efforts to enhance complete microbial reductive dehalogenation at contaminated sites generally involve adding electron donor. Mixed cultures, such as Pinellas (*6*) and KB-1 (*7, 8*), containing *Dehalococcoides*-like strains have been successfully used in field bioaugmentation demonstrations to bring about complete dehalogenation to ethene. Other cultures with the ability to reductively dechlorinate VC to ethene rapidly may be found. The question of which culture is best for use arises when considering bioaugmentation. We present here a method that can be used to make such a judgment together with a comparative study of the utilization of VC, DCE, TCE, and PCE by four of the above-mentioned cultures or strains.

Materials and Methods

Chemicals. Liquid *cis*-1,2-dichloroethene (97% Aldrich Chemical Company, Milwaukee, WI), trichloroethene (99+% Aldrich Chemical Co.), tetrachloroethene (99.9+% Sigma-Aldrich), and gaseous vinyl chloride (99.5%, Fluka, Switzerland) were used neat to prepare stock solutions and analytical standards. In addition, ethene (1000 and 100 ppm; Scott Speciality Gases, Alltech Associates) and vinyl chloride (1000 and 10 ppm; Scott Speciality Gases, Alltech Associates) were used as analytical standards, benzoate (sodium salt, 99%, Aldrich Chem. Co.) was used as a substrate, and sodium sulfide (Aldrich Chem. Co.) was used as a reducing agent.

Analytical Methods. Analyses of ethene, VC, DCE, TCE, and PCE were performed with a temperature program (40−

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220 °C) using a Hewlett-Packard model 5890 series II gas chromatograph equipped with a flame ionization detector (Hewlett-Packard) and a GS-Q fused-silica capillary column (length, 30 m; i.d. 0.53 mm; J&W Scientific, Folsom, CA). A reduction gas analyzer (Trace Analytical, Inc., Menlo Park, CA) was used to measure hydrogen. Solution concentrations and total mass were calculated using Henry's law constants (*9*).

Stock Cultures. The Victoria mixed culture containing strain VS was maintained in a closed continuously stirred tank reactor (CSTR) as previously described (*3*). A VC enrichment of this culture, maintained over 5 yr, was also used in these investigations. Liquid cultures of the three other cultures evaluated (Pinellas, KB-1/VC, and *D. ethenogenes* strain 195) were kept in anaerobic bottles and stored in an anaerobic chamber (10%H_2, 10% CO_2, 80% N_2). The bottles were amended with TCE (1−3 μL of neat liquid), VC (250 μL of gas), and PCE (1−3 μL of neat liquid) being added to each, respectively, approximately every 2 weeks.

Batch Studies. For the experimental portion of this research, two studies were undertaken: one a comparative VC growth study and the other a comparative growth and substrate utilization study with different chlorinated ethenes. These studies compared the dechlorination abilities of *D. ethenogenes* strain 195 and the *Dehalococcoides*-like microorganisms in the three mixed cultures (Victoria, KB-1/VC, and Pinellas). For the first study, a VC-enriched inoculum was first developed for each of the three mixed cultures. Here, bottles (120 mL) were filled with 60 mL of anaerobic media in an anaerobic chamber as previously described (*3*) and inoculated with 200 μL of each stock culture. Gaseous VC (99.5%) was added with a gastight syringe (200 μL). The cultures were then moved to a shaker (60−80 rpm) in an anaerobic chamber (20 ± 2 °C). Immediately after 5 μmol of ethene was formed in a bottle, 200 μL of the culture was transferred to fresh media (60 mL), in triplicate, and VC was again added (200 μL). The inoculum for the growth study was that formed after exactly 4 μmol of ethene was produced following this second transfer. To compare growth rates on VC, a range in concentration of each inocula (0.1, 0.3, 1, 3, and 9 mL) was transferred to fresh media (as above) to result in a liquid volume of 60 mL, and gaseous VC (200 μL) was added to each. An abiotic control with media and VC was included in all batch studies to demonstrate the cultures were required for dechlorination. Hydrogen served as the electron donor and was nonlimiting (>3%) in all studies. Headspace samples were periodically removed by syringe (250 μL) for chlorinated ethene analyses.

The purpose of the second study was to compare dechlorination rates of PCE, TCE, and DCE between the three different cultures that grew on VC (Victoria, KB-1/VC, and Pinellas). The development of inocula here was performed with VC, just as in the first study. For the growth and substrate utilization experiments, triplicate bottles were set up for each culture containing 1 mL of inoculum and either neat PCE (0.4 μL), neat TCE (0.3 μL), saturated DCE solution (34 μL), or gaseous VC (200 μL).

To further investigate TCE removal, bottles (120 mL) were filled with 20 mL of a highly enriched culture of strain VS made from the Victoria culture. Duplicate bottles were supplied with gaseous VC (50 μL on day 0, then 150 μL on day 3) or TCE (0.4 μL of neat liquid). Dechlorination was followed until all VC or TCE was converted to ethene (day 12). The change in strain VS concentration during VC and TCE utilization was then determined both by competitive PCR and by an activity procedure evaluated previously for strain VS (*3*, *10*). In the activity procedure a nongrowth limiting concentration of VC (150 μL) along with excess hydrogen is added to a culture, and the initial dechlorination rate is measured (over <1 d). A good estimate of strain VS concentration can then be obtained using the previously determined (*3*) strain VS maximum VC utilization rate (7.8 × 10^{-10} μmol of Cl^- (cell d)$^{-1}$). The increase in strain VS concentration during TCE or VC utilization was determined with these two procedures on the culture before and after TCE and VC utilization.

Comparative Dechlorination and Growth Kinetics. With mixed cultures, the concentration of organisms carrying out a particular reaction is generally unknown and difficult to determine. Thus, reaction rates with mixed cultures are generally reported in units that cannot be used for comparative purposes. For example, dechlorination rates have been reported in mass removal rate per microcosm or bottle (*11*), per unit liquid volume (*7*), per unit of total biomass (*12*), or per unit of total protein (*13*). However, the approach described here allows us to make direct comparisons of basic dechlorination and growth kinetics between cultures. The main parameter used in this comparison is organism maximum growth rate, which is directly related to dechlorination kinetics. The maximum growth rate for each culture was determined from a least-squares model fit to experimental data (*14*). For this purpose, the dechlorination of TCE, DCE, and VC was modeled using Monod kinetics with competitive kinetics between the multiple electron acceptors involved in dechlorination (for the case when electron donor is non-rate-limiting):

$$-\frac{d[VC]}{dt}=\left(\frac{\left(\frac{\mu_V}{Y}\right)X[VC]}{[VC]+K_V\left(1+\frac{[DCE]}{K_{ID}}+\frac{[TCE]}{K_{IT}}\right)}\right)-\left(\frac{\left(\frac{\mu_D}{Y}\right)X[DCE]}{[DCE]+K_D\left(1+\frac{[VC]}{K_{IV}}+\frac{[TCE]}{K_{IT}}\right)}\right) \quad (1)$$

$$-\frac{d[DCE]}{dt}=\left(\frac{\left(\frac{\mu_D}{Y}\right)X[DCE]}{[DCE]+K_D\left(1+\frac{[VC]}{K_{IV}}+\frac{[TCE]}{K_{IT}}\right)}\right)-\left(\frac{\left(\frac{\mu_T}{Y}\right)X[TCE]}{[TCE]+K_T\left(1+\frac{[VC]}{K_{IV}}+\frac{[DCE]}{K_{ID}}\right)}\right) \quad (2)$$

$$-\frac{d[TCE]}{dt}=\left(\frac{\left(\frac{\mu_T}{Y}\right)X[TCE]}{[TCE]+K_T\left(1+\frac{[VC]}{K_{IV}}+\frac{[DCE]}{K_{ID}}\right)}\right) \quad (3)$$

$$\mu=\left(\frac{\mu_V[VC]}{[VC]+K_V\left(1+\frac{[DCE]}{K_{ID}}+\frac{[TCE]}{K_{IT}}\right)}+\frac{\mu_D[DCE]}{[DCE]+K_D\left(1+\frac{[VC]}{K_{IV}}+\frac{[TCE]}{K_{IT}}\right)}+\frac{\mu_T[TCE]}{[TCE]+K_T\left(1+\frac{[VC]}{K_{IV}}+\frac{[DCE]}{K_{ID}}\right)}\right)-b \quad (4)$$

Here μ_V, μ_D, and μ_T are the maximum growth rates (d^{-1}) on VC, DCE, and TCE respectively; b is the cell decay rate (d^{-1}); X is the concentration (cells L^{-1}) of dechlorinating cells; and Y is the yield (cells μmol^{-1}). [VC], [DCE], and [TCE] are the

TABLE 1. Kinetic Coefficients and Growth Rates, along with 95% Confidence Intervals, for the Reductive Dechlorination of DCE and VC Developed Previously with the Victoria Culture and Used in This Study

coefficients	units	DCE	VC
half-velocity (K_D, K_V)	μM	3.3 ± 2.2^a	2.6 ± 1.9^a
competitive (K_{ID}, K_{IV})	μM	3.6 ± 1.1^b	7.8 ± 1.5^b

[a] Ref *12*. [b] Ref *10*.

solution concentrations (μM) of VC, DCE, and TCE, respectively. K_V, K_D, and K_T are the half-velocity coefficients (μM) for VC, DCE, and TCE, respectively. K_{IV}, K_{ID}, and K_{IT} are the competitive coefficients (μM) for VC, DCE, and TCE, respectively. As summarized in Table 1, these coefficients for VC and DCE were determined previously (*10*, *12*), while the coefficients for TCE were determined as part of this study.

To determine the maximum growth rates from these equations from measured concentrations of TCE, DCE, and VC, values for Y, X, b, K_i, and K_{ij} are needed where i and j are designators for v, d, t and D, T, respectively. The values Y and X, in particular, are difficult to measure with mixed cultures. However, this difficulty can be surmounted with the batch technique used. The inoculum approach used resulted from growth of a very small seed concentration on a known amount of VC. The concentration of VC dehalogenating organisms in the inocula is thus equal to YS_m/V_i, where S_m is the mass (μmol) of VC consumed and V_i is the culture volume of the seed vessel. When x mL of this inocula is transferred to 60 mL of media in the batch bottles, the concentration is reduced by the dilution factor (D), which here equals $x/60$. This gives an initial organism concentration, X^o, in the batch culture, which is related to the dilution used:

$$X^o = DYS_m/V_i \text{ or } \frac{X^o}{Y} = DS_m/V_i \quad (5)$$

Organism growth rate is related to the change in organism concentration as follows:

$$\mu_i = \frac{dX/dt}{X} \text{ or } \frac{dX}{Y} = \mu_i \frac{X}{Y} dt \quad (6)$$

In the numerical approach used here, the value X/Y in eqs 1−3 from one time step in the numerical model is increased by dX/Y as indicated in eq 6 for the next time step. In other words:

$$\frac{X_k}{Y} = \frac{DS_m}{V_i}[(1 + \mu_1\Delta t)(1 + \mu_2\Delta t) \ldots (1 + \mu_k\Delta t)] \quad (7)$$

where X_k is the microorganism concentration after k time steps. With the dilution procedure, the value on the right side of eq 7 is substituted into eqs 1−3, resulting in the cancellation of X and Y so that neither need be known to solve the system of equations (eqs 1−4).

While the values of K_i are also an unknown, except for strain VS, this was relatively unimportant in the determination of maximum growth rates since the initial substrate concentrations used were well above this value so that growth rates were highly insensitive to K_i.

Modeling Approach. The above procedure was utilized to compare VC, DCE, and TCE maximum growth rates between the *Dehalococcoides*-like microorganisms in the three cultures (strain VS, KB-1/VC, and Pinellas). Previously determined VC and DCE half-velocity and competitive coefficients were included in this modeling approach (Table 1). Also, a previously determined (*3*) decay coefficient (0.05 d^{-1}) for cells growing under optimal conditions (nonlimiting electron acceptor and donor) was included. This leaves the maximum growth rates on individual chlorinated ethenes (μ_V, μ_D, or μ_T) as the only fitting parameters, which were determined (together with the 95% confidence interval) using nonlinear least-squares fit (*14*) to the experimental data.

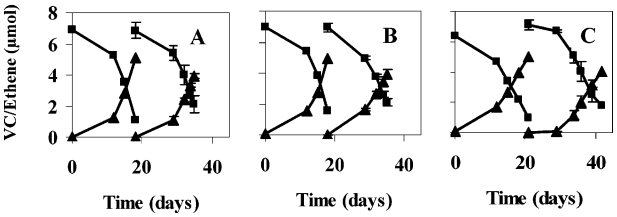

FIGURE 1. VC (squares) and ethene (triangles) by Victoria (A), KB-1 (B), and Pinellas (C) cultures. Following the formation of 5 μmol of ethene (day 18 for panels A and B; day 21 for panel C), culture (200 μL) was transferred to triplicate media bottles and VC was added (200 μL) with the results shown. Error bars, following these transfers, represent standard deviations for triplicate samples. Lines do not represent model simulations.

DNA Isolation and Competitive PCR. For the Victoria, Pinellas, and KB-1/VC cultures, genomic DNA was extracted (1 mL) from the inoculum used in the VC growth comparison study and also from cultures in the comparative substrate utilization study following complete TCE dechlorination. DNA was extracted using a DNeasy tissue system (Qiagen Inc, Valencia, CA), following the manufacture's instructions (final volume 200 μL). DNA was extracted from the *D. ethenogenes* strain 195 culture following dechlorination of DCE. Competitive PCR was carried out, as previously described (*3*), on DNA extracted from the VC enrichment culture of strain VS following TCE and VC dechlorination (day 12) to determine cell concentration.

Amplification and Cloning of16S rRNA Genes. PCR with forward primer DeF and reverse primer DeR, as previously described (*3*), was carried out on DNA extracted from all cultures. Aliquots of the PCR products were resolved by electrophoresis in 2% (wt/vol) agarose gel (Sigma) in TBE buffer, stained in ethidium bromide solution (5 μg/mL) for 20 min, followed by destaining in water for 40 min. The bands, visualized upon UV excitation, were found to be of the appropriate size with a 1-kb DNA ladder (Gibco). The remaining PCR products were cloned into *Escherichia coli* with a TOPO TA cloning kit (Invitrogen Corporation, Carlsbad, CA) following the manufacturers instructions. Plasmids were extracted from the cloned cells using a QIAprep miniprep system (Qiagen Inc), and the inserts were sequenced (Stanford University's Protein and Nucleic Acid Facility, PAN).

For DNA extracted from the KB-1/VC and Pinellas cultures (used for inoculum in the VC growth comparison study) partial sequences (1091−1320 base pairs) from nine clones each were obtained. For DNA extracted from *D. ethenogenes* strain 195 and for DNA extracted from the Victoria inoculum (used in the VC growth comparison study), partial sequences (ranging from 421 to 930 base pairs) from five clones each were obtained. For DNA extracted from cultures following TCE dechlorination, partial sequences (960−1140 base pairs) from four (Pinellas) or five (Victoria and KB-1/VC) clones each were obtained. The sequences were compared to known sequences using BLAST in GenBank (*15*) and were analyzed with DNASTAR MegAlign software.

Results and Discussion

VC Dechlorination. Dechlorinating microorganisms in the Victoria, KB-1/VC, and Pinellas cultures dechlorinated VC to ethene, forming 5 μmol of ethene in 18 (Victoria and KB-1/VC) or 21 d (Pinellas) (Figure 1). This conclusion is corroborated by recent research demonstrating the expression of the same VC-reductase gene in all three cultures (*4*).

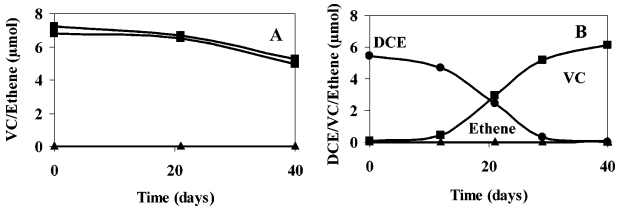

FIGURE 2. DCE (circles), VC (squares), and ethene (triangles) mass change with time in *D. ethenogenes* strain 195 cultures. Cultures were supplied with either VC (A) or DCE (B). Lines do not represent model simulations.

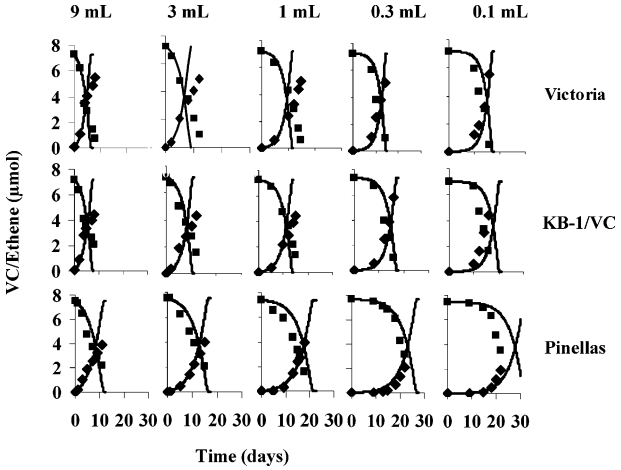

FIGURE 3. VC (squares) and ethene (diamonds) by Victoria, KB-1/VC, and Pinellas cultures inoculated with 9, 3, 1, 0.3, or 0.1 mL of culture. Lines represent model simulations as discussed in the text.

TABLE 2. Maximum Growth Rates and 95% Confidence Intervals for Reductive Dehalogenation of TCE, DCE, and VC by Three Different Mixed Cultures and One Highly Enriched Culture

culture	maximum growth rate (d^{-1})		
	μ_T	μ_D	μ_V
VS mixed	0.35 ± 0.07	0.46 ± 0.04	0.49 ± 0.02
highly enriched	0.35 ± 0.13		
KB-1/VC	0.33 ± 0.06	0.44 ± 0.02	0.42 ± 0.03
Pinellas	0.49 ± 0.03	0.43 ± 0.00	0.28 ± 0.01

As expected of cells growing on VC, the initial dechlorination rate was low following inoculation and increased exponentially with time. Standard deviations from the second transfer (triplicate cultures) illustrate the level of reproducibility typical of these experiments (Figure 1). *D. ethenogenes* strain 195 did not dechlorinate VC, indicating no growth on VC, but did dechlorinate DCE to VC (with no further reduction to ethene) as expected (Figure 2).

The results of the VC-fed serial dilution study for each of the three mixed cultures are illustrated in Figure 3. The μ_V values and 95% confidence intervals obtained by least-squares fitting of eqs 4−7 to the Figure 3 results are summarized in Table 2. The growth rates of the dehalogenators in the Victoria and KB-1/VC cultures are similar (0.49 and 0.42 d^{-1}, respectively), while that of the Pinellas culture is lower (0.28 d^{-1}). The value of 0.49 d^{-1} compares with that of 0.4 d^{-1} determined previously using the more traditional method of cell counting (*3*). The dilution method used here is believed to be more precise because of the normal errors in cell counting methods, especially with mixed cultures. In summary, evidence for growth of dehalogenating bacteria in these three cultures on VC provided by the current study includes the repeated transfer of VC dehalogenating microorganisms, the increased rate of VC consumption following each transfer, increased VC dechlorination in cultures with greater inocula, and their similarity in VC dechlorination kinetics. Since several *Dehalococcoides* strains have now been shown to grow on VC, this capability may be more typical than was once thought.

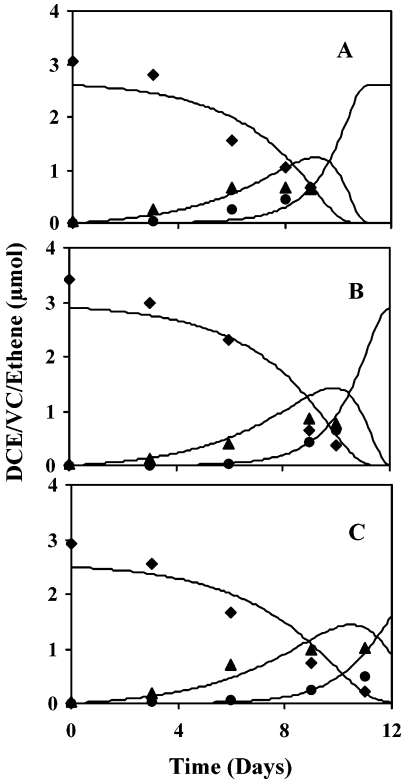

FIGURE 4. Reductive dehalogenation of DCE to ethene by (A) Victoria culture, (B) KB-1/VC culture, and (C) Pinellas culture. Symbols represent DCE (diamonds), VC (triangles), and ethene (circles). Lines represent model simulations as discussed in the text.

DCE, TCE, and PCE Dechlorination. In the second study, triplicates of each individual mixed culture grown on VC were supplied with DCE, TCE, or PCE. Using the dilution procedure, the dehalogenation results from cultures fed DCE were used to determine the maximum growth rate on DCE alone (μ_D) using least-squares analysis and the values for μ_V from Table 2. The maximum growth rates found for growth on DCE alone (μ_D) are similar for the three mixed cultures (0.43−0.46 d^{-1}) and are also similar to that on VC by the Victoria and KB-1/VC cultures (0.42−0.49 d^{-1}) (Figure 4). In agreement with this, recent research indicates that the purified VC-reductase from strain VS from the Victoria culture dehalogenates DCE and VC at similar rates (*4*).

Figure 5 illustrates a representative model fit for TCE removal (Pinellas culture). An unexpected finding was that all three mixed cultures dehalogenated TCE. Since the half velocity and inhibition coefficients (K_T and K_{IT}), and maximum growth rate (μ_T) for TCE were unknown, the three coefficients were used as fitting parameters along with previously determined values for μ_V and μ_D from Table 2 in a least-squares fit to the TCE utilization data. The maximum growth rate found for TCE utilization alone was again the same for the Victoria and KB-1/VC cultures (0.23 d^{-1}), but lower than that for the Pinellas culture (0.49 d^{-1}). Also of interest is that the half velocity and inhibition coefficients for TCE utilization by the three cultures (K_T and K_{IT}) are equal to each other and about the same for the three cultures varying between 8.6 and 10.5 μM (Table 3). The near equality between the half-velocity and inhibition coefficients was found previously for DCE. With VC on the other hand, the

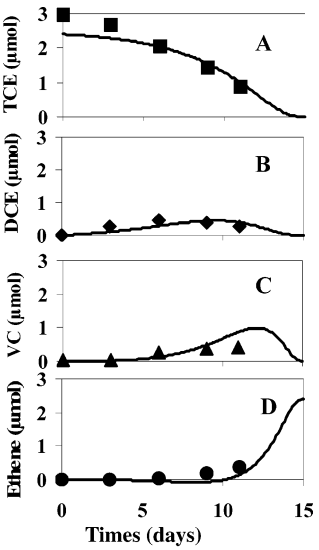

FIGURE 5. Reductive dehalogenation of TCE to ethene by the Pinellas culture. Symbols represent TCE (squares), DCE (diamonds), VC (triangles), and ethene (circles). Lines represent model simulations as discussed in the text.

TABLE 3. Half-Velocity and Inhibition Coefficients for Reductive Dehalogenation of TCE by Three Different Mixed Cultures and One Highly Enriched Culture

	coefficient (μM)	
culture	K_T	K_{IT}
VS mixed	9.0 ± 0.4	8.6 ± 0.4
highly enriched	12.4 ± 0.6	6.8 ± 1.4
KB-1/VC	10.0 ± 2.0	10.0 ± 0.6
Pinellas	10.5 ± 0.7	10.5 ± 0.7

inhibition coefficient (found from an earlier study, *10*) is significantly higher than the half-velocity coefficient, the higher value meaning the presence of VC is less inhibitory to the dehalogenation of DCE and TCE.

The rapid utilization of TCE by the cultures came somewhat as a surprise because the recently isolated, VC-respiring *Dehalococcoides* isolate BAV1 (*5*) uses TCE only co-metabolically. This rapid utilization of TCE and the strong suggestion from the model that this utilization is coupled with growth (μ_T values of 0.35−0.49 d^{-1}) was further investigated using the VC enrichment of the Victoria culture. Here in a separate study using a highly enriched (>95% purity) strain VS culture developed from the Victoria culture, the separate utilization of TCE and VC was investigated. Figures 6 and 7 illustrate a comparison between experimental data and modeled results using Tables 1−3 coefficients, except for μ_T, K_T, and K_{IT}, which were determined using the dilution technique and the data in Figure 6. The value for μ_T was found to match that found with the mixed Victoria culture (0.35 d^{-1}). The values for K_T and K_{IT} were similar (Table 3). Growth yields on both TCE and VC were determined using competitive PCR, resulting in values of $4.7 \pm 0.3 \times 10^8$ cell/μmol of Cl^- when grown on TCE and $5.2 \pm 0.4 \times 10^8$ cell/μmol of Cl^- when grown on VC. A third method to confirm that growth occurred on TCE was obtained from a comparison of the estimated increase in cell numbers while growing on TCE and VC obtained by measuring maximum VC utilization rates initially and after utilization of TCE and VC was complete (see Batch Studies under Materials and Methods). The cultures dechlorinated the same chlorine mass during either TCE or VC dechlorination and at the same rate (Figure 8). Thus, if growth occurred on TCE, then the increase in cell numbers while growing on TCE should be the same as that while growing on VC. This was found to be the case (Table 4). Thus, three separate lines of evidence, one a modeling fit to the data and two others where increase in cell numbers was determined, confirm that growth of strain VS occurred from TCE dehalogenation alone.

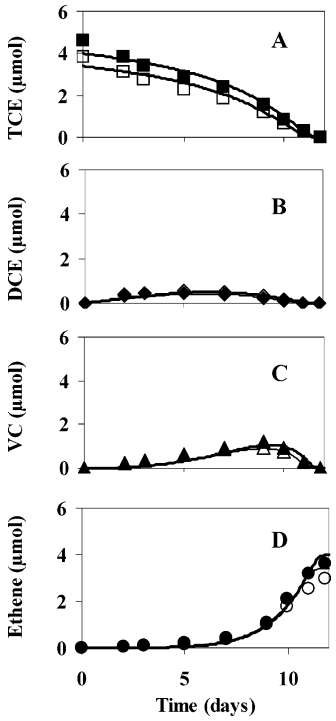

FIGURE 6. TCE (A), DCE (B), VC (C) dechlorination, and ethene formation (D) in duplicates of the VC enrichment of strain VS from the Victoria culture. Lines represent model simulations, as discussed in the text.

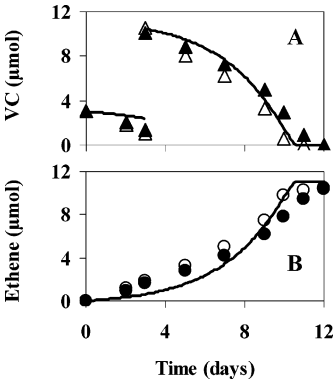

FIGURE 7. VC dechlorination (A) and ethene formation (B) in duplicates of the VC enrichment of strain VS. Lines represent model simulations, as discussed in the text.

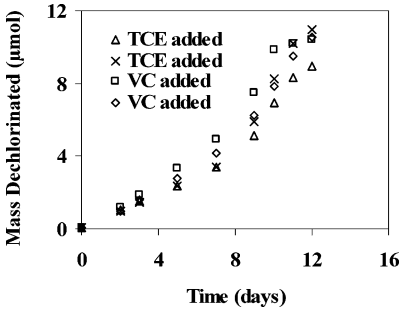

FIGURE 8. Comparison of mass dechlorinated (μmol of chloride produced) with time by the VC enrichment of strain VS supplied with either TCE (triangles and crosses) or VC (squares and diamonds).

TABLE 4. *Dehalococcoides* Species Strain VS Growth Yields on TCE and VC As Determined from VC Utilization Rate Measurements, Computed Increase in Cell Biomass, and Mass Dechlorinated

	VC	TCE
initial VC rate (μmol (d L)$^{-1}$)	28 ± 2^a	28 ± 2^a
initial cells (cell L^{-1})^b	$3.6 \pm 0.3^a \times 10^{10}$	$3.6 \pm 0.3^a \times 10^{10}$
final VC rate (μmol (d L)$^{-1}$)	228, 204	312, 204
final cells (cell L^{-1})^b	2.9×10^{11}, 2.6×10^{11}	4.0×10^{11}, 2.6×10^{11}
mass dechlorinated (μmol Cl$^-$)	10.39, 10.52	10.94, 8.97
yield (cell (μmol Cl$^-$)$^{-1}$)^c	$4.6 \pm 0.3^a \times 10^8$	$5.8 \pm 0.8^a \times 10^8$

[a] Average and range of replicates. [b] Determined by dividing VC utilization rate by standardized rate of 7.8×10^{-10} μmol ethene/cell·d [c] Yield = (20 mL sample/1000 mL)(final cells − initial cells)/mass dechlorinated.

The three methods used above all indicate that growth yields with TCE dehalogenation to ethene are similar to that for VC dehalogenation to ethene. If the TCE degradation was co-metabolic, as has been found for the recently isolated, VC-respiring, *Dehalococcoides* isolate BAV1 (*5*), then the ethene formation rate and the growth yield for the TCE fed cells would be two-thirds of that found for the VC-fed cells. While such detailed analysis of growth yield through cell counting was not conducted for the KB-1/VC and Pinellas cultures, the similar maximum growth rates determined by the dilution procedure and their close similarities in TCE utilization to strain VS suggest that they too obtain energy from TCE dehalogenation.

The Pinellas and KB-1/VC cultures failed to dechlorinate PCE. PCE dechlorination did occur after a lag period of 6−12 d with the Victoria mixed culture, suggesting that the presence of an additional dechlorinating microorganism in the culture might be responsible for the eventual PCE dechlorination.

16S rRNA Gene Sequences. Since the Victoria, KB-1/VC, and Pinellas cultures all exhibited such similar dehalogenation kinetics, a question arises as to whether they all contain the same strain of *Dehalococcoides*. The Victoria culture contains a *Dehalococcodies* strain (strain VS) that is distinctly different from that in the other two cultures (*3*, *16*). However, the similarity in 16S rRNA sequence between the Pinellas and KB-1/VC cultures makes it difficult to ascertain if these two cultures contain the same or a different *Dehalococcoides* sp. To ensure no cross contamination occurred during this study, the 16S rRNA gene sequences for the three cultures were obtained, and the numbers of differences in base pairs from that of *D. ethenogenes* strain 195 (GenBank Accession No. AF004928) were determined. These sequences were 99.4−100% identical with those previously reported for these cultures (*16*). The 16S rRNA gene sequences were also determined from cultures taken at the end of the growth experiments to investigate whether contamination between cultures might have occurred during these experiments. All sequences so obtained agreed with those of the starting cultures, thus we conclude that no cross contamination occurred. It is of interest to note that while the nucleotide sequence for strain VS is quite different from that of the other mixed cultures; their activity toward PCE, TCE, DCE, and VC was very similar. This indicates that 16S rRNA-based methods alone cannot be used for characterizing a culture's potential for dechlorination.

In summary, a dilution procedure was developed that can be used for comparing maximum growth and dehalogenation rates in mixed cultures. This procedure was used to compare three mixed cultures and one pure culture containing *Dehalococcoides*-like microorganisms. *Dehalococcoides*-like bacteria within the three mixed cultures (Victoria, KB-1/VC, and Pinellas) could dehalogenate TCE, DCE, and VC at similar rates but could not grow on PCE, suggesting that they have similar mechanisms for dechlorination. Maximum growth rates for dehalogenating bacteria in the Victoria and KB-1/VC cultures were very similar and about the same on DCE and VC (0.42−0.49 d^{-1}) but lower on TCE (0.35 d^{-1}). The Pinellas culture had about the same growth rate on DCE (0.43 d^{-1}) but lower on VC (0.28 d^{-1}) and higher on TCE (0.49 d^{-1}). However, the three mixed cultures performed quite differently from *D. ethenogenes* strain 195, which does dechlorinate PCE with growth but dechlorinates VC only co-metabolically. They also differ from the isolate *Dehalococcoides* isolate BAV1, which grows with VC but uses TCE only co-metabolically. The most significant finding of this research is the confirmed ability of the strain VS to obtain energy from each step in the dehalogenation of TCE to ethene. Similar dehalogenation characteristics of *Dehalococcoides*-like strains in the KB1/VC and Pinellas cultures suggests they too have this ability. This should be an important advantage when using these cultures for bioaugmentation of TCE contaminated sites.

This study also provided additional evidence for the ability of the model represented by eq 4, or its equivalent given elsewhere for the case when electron donor is also limiting (*10*), to correctly simulate dehalogenation rates providing information on the parameters of importance. This equation indicates that the overall growth rate (μ) is equal to the sum of growth rates individually on TCE, DCE, and VC. This may suggest that the maximum growth rate would be greater if growing on all three electron donors rather than on one alone. However, this need not be true because the growth rate is modified by the competitive coefficients, which reduces the growth rate on one electron acceptor when others are present. Thus, to obtain correct growth and dehalogenation rates, the complete model with competitive coefficients as provided here is needed. Although we have not addressed here the biological basis for this model with competition, possible explanations include a competition of several chloroethenes for one enzyme or the competition of different, chloroethene-specific reductive dehalogenases for a common pool of reducing equivalents, or other mechanism(s).

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