Vinyl Bromide as a Surrogate for Determining Vinyl Chloride Reductive Dechlorination Potential

April Z. Gu,*,† H. David Stensel,† Jaana M. H. Pietari,† and Stuart E. Strand³

Department of Civil and Environmental Engineering, College of Forest Resources, University of Washington, Seattle, Washington 98195

Site evaluation for bioremediation of chlorinated ethenes may need treatability studies to assess the reductive dechlorination potential of vinyl chloride (VC). Dehalogenation of vinyl bromide (VB) was investigated as a surrogate measurement for the dechlorination potential of VC. VB dehalogenation rates and kinetics were studied and compared with those of VC by a methanogenic reductive dechlorinating enrichment culture that was dominated by Dehalococcoides species and by microcosms from two chloroethene-contaminated sites. The enrichment culture dehalogenated VB to ethene at higher rates than VC at similar concentrations. VB was dehalogenated with a higher enzyme affinity than was VC, as indicated by their half-velocity constants, 240 ± 150 and 21 ± 8 μM, for VC and VB, respectively. Cross-inhibition study exhibited some evidence for competitive inhibition between VC and VB, suggesting that their degradation might be catalyzed by the same enzyme in the culture. Laboratory microcosm studies using subsurface soil and groundwater from two contaminated sites demonstrated that the production of the reductive dehalogenation product (ethene) could be detected faster with VB as a substrate than with VC. As a result, a substantially shorter (up to 5–10 times) incubation time would be required to detect the same level of reductive dehalogenation activity using VB as a surrogate for VC in treatability assessments.

Introduction

Tetrachloroethene (PCE) and trichloroethene (TCE) are among the most abundant pollutants in the United States due to their widespread use in dry-cleaning and degreasing processes and past history of improper waste management. While dechlorination of chloroethenes can occur via reductive (1–8) and oxidative (1, 9, 10) processes, the sequential reductive dechlorination of PCE via TCE and cis-dichloroethene (cis-DCE) to vinyl chloride (VC) and ethene has been the most studied and applied remediation process. Both laboratory and field studies have shown that reductive dechlorination is an important mechanism for removal of the chloroethenes from polluted aquifers where low redox conditions prevail (2, 11–13).

The dechlorination of PCE to cis-DCE is a relatively fast process and can be easily stimulated, whereas the further reduction of cis-DCE to VC, and especially from VC to ethene, is significantly slower (14). The susceptibility and rate of reductive dechlorination decreases as the number of chlorine atoms in the chloroethene molecule decreases. The kinetic constants for the electron acceptors of each step of the sequential dechlorination of PCE to ethene are different (3, 6, 15–20). The relatively low maximum consumption rate of VC and the substantially higher half-velocity constant of VC compared to the same parameters for PCE, TCE, and cis-DCE partially account for the slower degradation rates of VC observed in aquifers and laboratory studies. Another reason is that while reduction of PCE or TCE to cis-DCE is carried out by relatively diverse groups of bacteria, further reductive dechlorination of cis-DCE to VC and ethene is catalyzed by a specific cluster of bacteria that is closely related to Dehalococcoides ethenogenes (14, 21–23). This Dehalococcoides group of bacteria may or may not be present depending on the site (24–27). In many laboratory studies with site samples or investigations in situ, cis-DCE and VC dechlorination did not occur or long time periods were necessary to stimulate significant reduction of VC to ethene. As a consequence, relatively low concentrations of reductive dechlorination intermediate products, such as cis-DCE and VC, often accumulate in sites that are contaminated with chloroethenes (28). Since vinyl chloride is a known human carcinogen and is more toxic than the other chlorinated ethenes, the ability of subsurface communities in contaminated aquifers to further reduce VC to ethene is of great interest.

Application of the reductive dechlorination process for bioremediation often requires treatability tests to evaluate the potential for and extent of reductive dechlorination by natural attenuation or with stimulation, especially when site information is insufficient. Current protocols include microcosm studies with amendments of electron donors and nutrients to observe dechlorination potential. The low degradation rates of VC make treatability assessments time-consuming and difficult. Microcosm studies might require incubation times as long as several months or even over a year.

In this study, we investigated the use of vinyl bromide (VB) as a surrogate to facilitate the evaluation of VC degradation ability. The use of a chemical surrogate has preceded in chloroethene dechlorination characterization. Vancheeswaran et al. (29) used trichlorofluoroethene (TCFE) as a surrogate to evaluate the reductive dechlorination activity of PCE and TCE to cis-DCE. TCFE is an unregulated analogue to PCE and TCE, which degraded at rates comparable to those observed with PCE and TCE. VB has a chemical structure similar to VC; thus, it was expected to serve as a chemical analogue. Brominated compounds have been shown to have higher dechlorination rates than their chlorinated analogues (30, 31). In vitro study of TCE reductive dehalogenase extracted from mixed culture containing Dehalococcoides ethenogenes was able to dehalogenate VB faster than VC, suggesting VB might be a useful analogue to evaluate VC reductive dechlorination activity (32). In addition, bromide ions can be easily monitored as an indicator of VB dehalogenation, since the background concentration of bromide is usually very low or even absent in groundwater of inland regions, compared to the frequently high concentrations of chloride ions.

The objectives of this research were to evaluate and compare the reductive dechlorination of VB and VC and to investigate the use of VB as a surrogate for VC in treatability tests of the potential of reductive dechlorination for VC.
remediation in site samples. First, VB and VC dehalogenation were investigated using an established laboratory enrichment culture that dechlorinates cis-DCE to VC and ethene. Second, microcosm studies were carried out to compare VB and VC degradation rates in site samples from two chloroethene-contaminated sites.

Methods

Chemicals. Vinyl chloride (99% purity) was purchased from Quality Standards & Research Gases Inc. (Laporte, TX); vinyl bromide (98% purity), acetic acid (99.7% purity), and NaBr (analytical grade) were purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI); lactic acid (85% solution) was purchased from Fisher Scientific (Fair Lawn, NJ); ethene (99.5% purity) was purchased from Scott Specialty Gases (Plumsteadville, PA), and methane (99% purity) was purchased from Praxair, Inc. (Danbury, CT). All chemicals were used as received.

Laboratory Enrichment Culture. The methanogenic reductive dechlorinating culture used for this study was from a continuously fed, fluidized-bed reactor (FBR), which was originally seeded from an anaerobic digester, and has been operated in our laboratory for over 6 years (33). The FBR reactor (325 mL of liquid, 100 mL gas volume) was operated at 35 °C with a 0.5-day hydraulic retention time. Celite biocatalyst carrier (30 g) R633 (Celite Co.) was used as the support media in the reactor, providing an expanded bed volume of 100 mL. The reactor was provided with reduced anaerobic mineral media (RAMM, 33). Propionate (3.38 mM/day) and cis-DCE (0.2 mM/day) were provided to the FBR semi-continuously using peristaltic pumps. The removal efficiency of cis-DCE was greater than 99.9% (no detectable cis-DCE in the effluent). Approximately 90–95% of the influent cis-DCE was recovered as ethene, indicating that the FBR contained an active VC-dechlorinating culture. Phylogenetic analysis of the mixed culture revealed the dominant population to be Dehalococcoides species (unpublished data).

Field Sample Sources. Groundwater Samples from Portland, OR. Groundwater samples were obtained from a chloroethene-contaminated site in Portland, OR. The groundwater was collected from a monitoring well by pumping through a sterilized glass jar. The sample was shipped overnight on ice and stored at 4 °C until used. The main contaminants and their highest concentrations observed on site (in mg/L) were PCE (50–100), TCE (50–100), 1,1,1-trichloroethane (50–100), methylene chloride (200), toluene (10–20), xylene (10–20), and ethylbenzene (5–10). The site was dominated by anaerobic methanogenic conditions throughout, with a redox potential that ranged from −50 to −200 mV. The pH value of the groundwater was 6.6–7.0. Reductive dechlorination daughter products detected included cis-DCE (20–30 mg/L), 1,1-DCE (5–10 mg/L), VC (5–10 mg/L), 1,1-dichloroethene (5–10 mg/L), chloroethene (1 mg/L), ethene (1 mg/L), and ethane (1 mg/L). The relatively high concentrations of methane and ethene detected in the groundwater samples we obtained indicated methanogenic conditions and the reduction of VC to ethene at the site.

Sediment and Groundwater Samples from Fort Lewis, WA. Field sediment and groundwater samples were obtained from the East Gate Disposable Yard (EGDY) remediation site at Fort Lewis, WA. The sediment was collected from 6.1 m below the surface using the split spoon method with a rotary-sonic drill rig. The groundwater was collected from 7.2 m below the surface using low-flow peristaltic pumps. Both the sediment and the groundwater were stored at 4 °C until used. The texture of the sediment was mainly gravel. The organic content of the sediment, excluding the gravels, was 1.02% (w/w). The pH value of the groundwater was 6.6, and Cl−, SO4 −2, and NO3 − concentrations were 0.035, 0.20, and 0.013 mM, respectively. The contaminant concentrations in the monitoring wells ranged from 0.33 to 1.2 µg/L for PCE, 7.1 to 2760 µg/L for TCE, 0.5 to 231 µg/L for cis-DCE, and 0.13 to 21.3 µg/L for VC. Other contaminants that were detected at the sampling area included 1,2-dichloroethane, toluene, and 1,4-bromofluorobenzene. The distribution of chloroethenes at the EGDY site indicates limited dechlorination with the formation of cis-DCE and a small amount of VC.

Biodegradation Experiments with Laboratory Enrichment Culture. The reductive biodegradation of VB and VC was examined in batch tests using the FBR culture. To investigate the substrate utilization kinetics of reductive dehalogenation, a series of cultures with varying VB or VC concentrations were prepared in 27-mL Balch tubes in duplicate and dosed with 11–610 µM VC per tube or with 9–830 µM VB per tube. To study the inhibition effect between VC and VB, two additional series of tubes were prepared: one series had the same initial concentration of VC (120 µM) with varying initial concentrations of VB (24–161 µM), and the other had the same initial concentration of VB (60 µM) and with varying initial concentrations of VC (16–191 µM). To optimize the growth conditions during the experiments by providing the unknown growth factor(s) produced by FBR microbial consortium (if needed) (7), filtered (0.22 µm syringe filter, Millipore Co.) FBR effluent (10 mL, purged with 20% CO2, 80% N2 gas mixture) was used as media instead of fresh RAMM. No acetate or propionate was detected in the FBR effluent. An aliquot of the mixed culture (0.2 mL of culture attached to Celite containing 1.96 mg of protein as biomass) was transferred from the FBR to the Balch tubes while purging the tubes with oxygen-free anaerobic gas. To compare the effects of different electron donors on reductive dehalogenation rates in the kinetic tests, VC and VB degradation tests were conducted in parallel, using lactate (2.2 mM) versus hydrogen (0.22 mmol) as the electron donor. Acetate (1.7 mM) was added to the test tubes that had H2 as electron donor. In the inhibition tests, only lactate (2.2 mM) was used as the electron donor. VC and/or VB gases were injected into the test tubes through a gastight syringe (VWR). The tubes were crimp-sealed using Teflon-lined gray-butyl septa (Wheaton) and were shaken vigorously in the dark at 35 °C. The dehalogenation of VC and/or VB was monitored by analyzing the headspace VC and/or VB gas concentrations as well as the ethene production. In a separate test using 165 mL serum bottles with the same culture, bromide ion in the liquid was analyzed in addition to the headspace analysis of VC and ethene gas concentrations. Killed controls were prepared as described above and sterilized by autoclaving twice at 121 °C for 25 min. Uninoculated control samples also were prepared in the same manner, except that 0.2 mL of filtered FBR effluent was substituted for the FBR culture.

In the kinetic tests, the consumption of VC and VB was monitored for about 165 h. Because the amount of VC or VB added to the tubes was very small (<25 µmol), based on the estimated yield of 0.003 mg protein/µmol Cl (33), the minor change (<0.075 mg protein) in biomass of dechlorinators during this period of time was neglected. The degradation rates (Xkmax) of VC and VB at different initial concentrations were calculated using the ethene production data for the kinetic tests and using haloethene depletion data for the cross-inhibition tests in the linear range of the substrate concentration versus time plots using a linear regression at 95% confidence intervals. The reaction kinetic coefficients were determined by least-squares nonlinear regression of the Michaelis–Menten equation to the experimental data using SPSS 10.0.5 (SPSS Inc.).

Microcosm Experiments with Field Samples. Reductive dechlorination of VC and VB in field samples was studied
using groundwater samples from Portland, OR, and sediment and groundwater samples from Fort Lewis, WA. For site samples from Portland, OR, a series of microcosms were prepared in 27 mL Balch tubes in duplicate with groundwater (10 mL) in an anaerobic glove chamber. Hydrogen (0.22 mmol) was added as the electron donor, and acetate (0.7 mM) was added as the carbon source. No other growth supplements were added. A colorimetric redox indicator (resazurin, 0.2 mg/L) was added to verify that anaerobic conditions were maintained during the incubation. The fluid in all of the tubes except killed controls turned from brown to clear within 1 month after setup and remained clear during the rest of the incubation. The microcosms were incubated inverted and quiescent, in darkness at 20 °C. The concentrations of VC, VB, ethene, methane, and acetate were monitored. Killed controls were prepared in the same manner but were sterilized by autoclaving twice at 121 °C for 25 min. For the sediment and groundwater samples from Fort Lewis, WA, a series of microcosms were prepared in 165 mL serum bottles, in duplicate, employing hydrogen as the electron donor (1.11 mmol) and acetate (1.7 mM) as the carbon source, and amended with vitamin mix (1 mL of vitamin mix/100 mL of sample). The vitamin solution contained p-aminobenzoic acid 50 μg/L, folic acid 20 μg/L, biotin 50 μg/L, nicotinic acid 50 μg/L, 1,4-naphthoquinone 50 μg/L, pantothenic acid 50 μg/L, riboflavin 50 μg/L, thiamine 50 μg/L, pyridoxine hydrochloride (B6)100 μg/L, cyanocobalamin (B12) 100 μg/L, and thioctic acid 50 μg/L. All experimental manipulations were performed in an anaerobic glove chamber. After removal of all the large-sized (>5 mm diameter) gravel particles, the subsurface sediment was mixed with the groundwater at a ratio of 1 part sediment to 2 parts groundwater (v:v). An aliquot of the mixed slurry (75 mL) was transferred to each bottle. A colorimetric redox indicator (resazurin, 0.2 mg/L) was added to all bottles. The microcosms were incubated inverted and quiescent, in darkness at 10 or 20 °C. The concentrations of VC, VB, ethene, methane, and acetate were monitored. Killed controls were prepared in the same manner as described above. In addition to killed controls, controls with the following amendments were included: VC or VB only, VC and vitamin mix, VC, lactate and RAMM, VC, RAMM and vitamin mix, and no amendments. All controls were incubated at 20 °C. Additional hydrogen gas (about 0.1 mmol) was injected every 2 months into the bottles that had H2 as electron donor to ensure sufficient electron donor in the bottles.

**Analytical Methods.** VC and VB were analyzed by manual injection of headspace gas onto an SRI 8600 series gas chromatograph equipped with a flame ionization detector (FID) and a capillary column (Supelco SPB628, 60 m × 0.5 mm i.d.), helium as carrier gas (11.2 mL/min), using the following temperature program: 40 °C for 2 min, followed by 5 °C/min ramping to 65 °C, then 25 °C/min to 190 °C. Ethene was determined by manual injection of the headspace gas onto a Hewlett-Packard 5890A GC, equipped with a FID and a 6-ft HayeSep Q packed column (Supelco, PA), isothermal at 95 °C, with N2 as the carrier gas (50 mL/min). Concentrations were calculated using external standards.

Aqueous concentrations of VC and VB were calculated based on the measured headspace concentrations, by applying Henry’s constants and assuming the headspace and aqueous phases were in equilibrium. The assumption of equilibrium during the kinetic tests is based on vigorous shaking of the Balch tubes and the fact that measurements were made over a longer time scale (up to 165 h) than what is typically required to reach equilibrium. The Henry's constants (gas concentration in mM/aqueous concentration in mM) used were 0.91 at 20 °C and 1.49 at 35 °C for VC (34) and 0.52 at 20 °C and 1.16 at 35 °C for VB, respectively. The Henry’s constants for VB were determined using the modified EPICS procedure (35). The total mass of ethene in the tubes or bottles was determined by comparing the headspace measurement to standards that were prepared by adding a known amount of ethene to containers with the same headspace-to-liquid ratio as the samples being analyzed.

Acetate was determined on a Waters 712 WISP high-pressure liquid chromatograph (HPLC) equipped with an Aminex HPX-87H ion exclusion column (300 mm × 7.8 mm), using H2SO4 (4 mM at 0.6 mL/min) as eluent. The absorbance of the column effluent was measured with a Waters 486 UV detector (Millipore, MA) at 210 nm wavelength. Bromide ion was analyzed on a Dionex DX-120 ion chromatograph (IC) equipped with a suppressed conductivity detector, an AS14 analytical column (4 mm i.d.), and an AG14 guard column (4 mm i.d.). The eluent was 1 mM NaHCO3 and 3.5 mM Na2CO3 at a flow rate of 1.2 mL/min.

**Results**

Reductive Dehalogenation Kinetics of VC and VB. Figure 1a shows a typical observation of the disappearance of halogenes in tubes exposed to either VC or VB and the corresponding production of ethene by the cis-DCE-acclimated, laboratory FBR culture. Reductive dehalogenation of both VC and VB occurred immediately in all tubes without
any lag. The VB degradation rate was significantly faster than the VC degradation rate at similar initial concentrations. The recovery of ethene ranged from 80% to 95% of the dosed haloethenes. Figure 1b shows the disappearance of VB and the stoichiometric formation of the reductive dehalogenation products, ethene and bromide ion. The recoveries of bromide ion and ethene were 92% and 95%, respectively.

The reductive degradation rates of VC and VB as functions of the initial haloethene concentrations are shown in Figure 2. On the basis of the Michaelis–Menten model fit, the half-velocity constants ($K_v$) and the maximum substrate utilization rates ($X_{k_{max}}$) determined for VC and VB are shown in Table 1. The half-velocity constant of VB is approximately 10 times lower than that of VC.

When the initial concentrations of haloethenes were less than 400 μM, the removal rate of VB was significantly higher than that of VC. VB degradation rates were approximately 5–10 times higher than VC degradation rates when the initial haloethene concentration ranged from 5 to 50 μM. Substrate inhibition of VB dehalogenation was observed when the VB concentrations were greater than 500–600 μM (Figure 2). The VB dehalogenation rates at concentrations within the noninhibition range (<500 μM) were used in the modeling shown in Figure 2. This range is much higher than VC concentration ranges most commonly found in the field (<50 μM) (36) as well as those that would be used in most treatability tests. Substrate inhibition of VB was not included in the model because more data would be required to accurately model the substrate inhibition of VB, which is not the intention of this study. For both VC and VB, the dehalogenation rates did not appear to be affected by the choice between using either lactate or H₂ as electron donor.

The culture was able to reduce multiple doses of VC and VB to ethene repeatedly in the absence of higher chlorinated ethenes, and the dechlorination rates increased significantly (8–10 times) over time (8 months) (data not shown). After more than 8 months enrichment with only VB, the VB-fed culture was able to dechlorinate VC immediately when exposed to VC. Similarly, the VC-fed culture was able to immediately degrade VB after 8 months of feedings with VC only. These results suggest that there were organisms present in the mixed culture that might be able to obtain energy from either VC or VB reduction.

The dehalogenation ability of an acclimated anaerobic sludge from a digester (fed with municipal waste primary and secondary sludge) to dehalogenate VC and VB was investigated (data not shown). Dehalogenation of either VC or VB by the digester sludge with no prior exposure to VC or VB was negligible (<0.0009 μmol/L/day) during the 14 days of this experiment.

Inhibition between VC and VB. If VC and VB were substrates for the same dehalogenating enzyme(s) in the cis-DCE dechlorinating cultures, they would be expected to exhibit competitive inhibitory kinetics (37). The dehalogenation rates of VC and VB in the presence of both haloethenes was investigated to examine the inhibitory effects between the compounds. For initial concentrations of VB ranging from 24 to 161 μM, VB dehalogenation was inhibited by the presence of an initial concentration of 120 μM VC (Figure 3a). The inhibitory effect of VC on VB dehalogenation decreased as the concentration ratio of VB to VC increased. For initial concentrations of VC ranging from 16 to 191 μM, reductive dechlorination of VC was significantly inhibited by the presence of 60 μM VB (Figure 3b). The concentration of inhibitor (VC or VB) was changing due to its degradation during the experiment, and the change was up to 13% for VC and up to 30% for VB, depending on the initial substrate (VC or VB) concentrations. The inhibition by VB of VC dehalogenation was more pronounced than the inhibition by VC of VB dehalogenation, which would be expected since VB had a lower half-velocity coefficient than VC.

Reductive Dehalogenation of VC and VB in Field Samples. Figure 4 shows the production of ethene from the reduction of VC and VB in the microcosms of groundwater samples from Portland, OR, incubated at 20 °C. After a lag of about 50 days, rapid ethene production was observed in the VB-fed tubes. At day 87, about 1.0 μmol of ethene was produced in the VB-fed tubes. After 106 days of incubation, about 80% of the VB fed (2.8 μmol) in the test tubes was reduced and recovered as ethene. In comparison, insignificant ethene production (about 0.05 μmol) was detected in VC-fed tubes after 85 days of incubation. A relatively small amount of ethene production (0.19 μmol, about 10% of 2.0 μmol VC fed) was observed in VC-fed tubes after 106 days of incubation. In contrast, clear evidence of reductive dehalogenation of VB was acquired in a shorter incubation time, clearly demonstrating the potential for haloethene reduction by the site material with electron donor addition. No ethene production was detected in the killed controls, suggesting that the degradation of VC and VB was biologically mediated.

Figure 5 shows the production of ethene from the reduction of VC and VB in the microcosm incubations of samples from Fort Lewis, WA, at 10 and 20 °C. Ethene was detected in VB-fed bottles after 5 days of incubation, and a marginal amount of ethene was also detected in the VC-fed bottles. The ethene production decreased and leveled off after 21 days. At the end of the incubation of 168 days, about 11% of the dosed VB (4.2 μmol) was recovered as ethene in VC-fed bottles while only 1.5% of the VC dosed (4.2 μmol) was recovered as ethene in VB-fed bottles. No ethene production was detected in the killed controls as well as other controls described previously (data not shown), indicating that the limited dehalogenation activity was biologically mediated and occurred only with amendments.

### Table 1. Determined Michaelis–Menten Model Half-Velocity Coefficient and Maximum Substrate Utilization Rate for VC and VB Dehalogenation by cis-DCE-dechlorinating FBR Culture (196 mg/L protein as biomass) at 35 °C

<table>
<thead>
<tr>
<th>substrate</th>
<th>$K_v$ (μM)</th>
<th>$X_{k_{max}}$ (μM/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VC</td>
<td>240 ± 150</td>
<td>33 ± 9</td>
</tr>
<tr>
<td>VB</td>
<td>21 ± 8</td>
<td>37 ± 3</td>
</tr>
</tbody>
</table>

* Parameters determined using least-squares nonlinear regression (SPSS 10.5) with 95% confidence level interval.
of H₂, acetate, and vitamin mix. The organic contents in the sediment, lactate, RAMM, or vitamin mix did not stimulate dehalogenation. In addition, no significant difference in ethene production at 10 and at 20 °C was observed.

**Discussion**

The dehalogenation experiments using our laboratory enrichment culture, which is dominated by dechlorinating bacteria closely related to *Dehalococcoides ethenogenes*, provide evidence that the same microorganism(s) that catalyzes VC reductive dechlorination also catalyzes the reductive dehalogenation of VB. The kinetics study shows that Michaelis–Menten enzyme kinetics can be applied to the reductive dehalogenation of VC and VB for the culture studied. The half-velocity constant of VC for the original FBR culture from which our culture was derived was reported to be 360 μM (33), which is within the margin of error of our determination (Kₜ = 240 ± 150 μM). The Kₜ for VC is comparable to the values reported in the literature (20). The significant lower Kₜ for VB compared to VC shows that the culture has a greater affinity for VB than VC. The rates of dehalogenation of VC and VB are influenced by the biomass concentration of the dehalogenators, the concentrations of the electron donor and acceptor, and the toxicity of the halogenated compounds. In all of our experiments, biomass was kept the same, the electron donor (hydrogen or lactate) was provided in excess, and only data in the nontoxic range was used for kinetic coefficient determination. Since the kinetic studies were conducted over a period of days, equilibrium between the headspace and the aqueous phases should have been achieved. For both VC and VB, the degradation rates determined using gaseous H₂ as the electron donor were consistent with the rates determined using lactate as electron donor. These results also indicate that there was no mass transfer limitation of H₂ from gas phase to liquid phase when H₂ was provided in the gas phase.

The cross-inhibition results suggested characteristics of competitive inhibition between VB and VC in the FBR culture. Thus, the same enzyme might be employed for dehalogenation of VC and VB. This hypothesis was also supported by capability of long-term VC- or VB-enriched subculture to degrade both VC and VB immediately and with similarly increased rates.
Microcosm studies with sediment and/or groundwater samples from the chloroethene-contaminated sites demonstrated that the test with VB showed dehalogenation activity in a manner similar to that for the test with VC. Moreover, due to its greater dehalogenation rate, the ethene production using VB as a surrogate was faster and the results were more clearly defined than with VC. Assuming that at least 15% ethene recovery from dehalogenation is necessary to conclude the presence of an active dechlorinating population for a site sample, the evaluation of VB reduction potential with site samples from Fort Lewis, WA, indicated that no significant and sustainable VC reduction to ethene could be achieved with electron donor and vitamin mix amendments. The results were consistent with the limited dechlorination of VC observed at the site. The ethene production from VB or VC reduction leveled off, and one possible explanation for this is that the initial dehalogenation was due to a co-metabolic process, which could not be sustained in the absence of highly chlorinated compounds (21, 38). The groundwater samples from Portland, OR, demonstrated a rapid and sustainable level of ethene production from VB dehalogenation in the microcosm studies. In addition, there was a continuous increase in the rate at which ethene was produced during the incubation, indicating that the population that dechlorinated VC and VB increased during the incubation. The results were consistent with the high ethene concentration observed at the site, indicating active VC degradation. Other contaminants (e.g., toluene) at the site might serve indirectly as electron donors for the reductive dechlorination process (39). The results suggest that there were active VC-reducing microbial populations at the site and effective VC bioremediation can be achieved with this site. The VB dehalogenation results provided relatively rapid and clear evidence in support of this conclusion.

The results of this study suggest that VB could serve as a chemical analogue probe for detecting and possibly quantifying the potential of subsurface microbial populations to reductively dechlorinate VC. For the site treatability tests in this study, the initial VC concentrations in the range of 30–80 μM were used, which were chosen based on the VC level observed at sites. Concentrations of VC less than 50 μM have been most commonly found in the field (36). Within these concentration ranges, the incubation time necessary to detect the same level of reductive dehalogenation activity could be up to 5–10 times shorter using VB as a surrogate for VC, compared to the same initial concentrations. Another advantage of using VB as a surrogate is that the concentration of bromide is usually very low or even absent in field backgrounds (except in coastal areas); thus, bromide can be more easily and accurately monitored as the reductive dehalogenation product of VB. Finally, though VB has advantage as a tracer to study subsurface dechlorination potential for VC, its application will be limited due to its toxicity. The impact of toxicity can be minimized at very low VB concentrations (<20 μM) and used for limited, in situ, and controlled pilot-scale evaluations of VC dechlorination potential. The VB degradation rates at a concentration of 20 μM would be comparable to the VC degradation rate at about 300 μM.

A feasible method for evaluating the potential of VC degradation at contamination sites is an important part of the process for determining whether natural attenuation or stimulated biodegradation is sufficient for remediation and for selecting the optimum treatment strategy. Other remediation methods such as bioaugmentation, aerobic oxidation, or anaerobic mineralization should be considered for sites in which microbial populations that catalyze reductive VC-dehalogenation are absent. Using VB as a surrogate for VC in treatability tests and VC-dehalogenation studies can offer faster results for site evaluation of VC reduction to ethene, adding to the techniques available for bioremediation site assessment.

This study examined the behavior of one laboratory enrichment that was dominated by an organism closely related (~99% identical of 16S rDNA) to Dehalococcoides ethenogenes. Dehalococcoides populations have been found to be associated with complete reductive dechlorination of chlorinated ethenes to ethene, and they have been frequently detected in anaerobic VC-degrading environments (22–27). Dehalococcoides species that exhibit close 16S rDNA phylogenetic relationships may differ in their dehalogenating activities and the presence/absence of certain dehalogenating enzyme(s) (22, 23). Thus, reductive dehalogenation of VB by other VC-reducing dechlorinators should be investigated in the future. In addition, microcosm studies with a greater variety of contaminated site samples are needed to determine whether the results obtained from this study can be generally applied to differing dechlorinating bacteria under varying site conditions.

Acknowledgments

This work was supported by the Consortium for Risk Evaluation with Stakeholder Participation (CRESP), funded by the Department of Energy and a grant from the National Institute of Environmental Health Science. Professor Russ Herwig, from the Department of Fishery at the University of Washington, and Steven Cox, from United States Geological Survey (USGS), are acknowledged for providing the sediment, groundwater material, and related site information from the EGDY site. We thank Dr. Loe Lehmec, from Exponent Co., and Brian O’Neal, from IT Corp., for providing the groundwater sample from Portland, OR, and related site information. We thank Professor John Ferguson for valuable discussions.

Literature Cited

(22) Ritalahti, K. M.; Brown, R. K.; Tiedje, J. M.; Löfler, F. E. Presented at The Sixth International In Situ and On-Site Bioremediation Symposium, San Diego, CA, June 2001; platform abstracts C1.
(28) Poster Abstracts for The Fifth International In Situ and On-Site Bioremediation Symposium, San Diego, CA, April 1999; abstracts C2.
(37) Grady, C. P. L., Jr.; Lim, H. C. In Biological Wastewater Treatment; Marcel Dekker: New York, 1980; pp 312–313.

Received for review June 17, 2002. Revised manuscript received June 16, 2003. Accepted June 18, 2003.

ES0207899