Kinetics of 1,2-Dichloroethane and 1,2-Dibromoethane Biodegradation in Anaerobic Enrichment Cultures

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1,2-Dichloroethane (1,2-DCA) and 1,2-dibromoethane (ethylene dibromide [EDB]) contaminate groundwater at many hazardous waste sites. The objectives of this study were to measure yields, maximum specific growth rates (μ), and half-saturation coefficients (K_s) in enrichment cultures that use 1,2-DCA and EDB as terminal electron acceptors and lactate as the electron donor and to evaluate if the presence of EDB has an effect on the kinetics of 1,2-DCA dehalogenation and vice versa. Biodegradation was evaluated at the high concentrations found at some industrial sites (>10 mg/liter) and at lower concentrations found at former leaded-gasoline sites (1.9 to 3.7 mg/liter). At higher concentrations, the *Dehalococcoides* yield was 1 order of magnitude higher when bacteria were grown with 1,2-DCA than when they were grown with EDB, while μ 's were similar for the two compounds, ranging from 0.19 to 0.52 day⁻¹ with 1,2-DCA to 0.28 to 0.36 day⁻¹ for EDB. K_s was larger for 1,2-DCA (15 to 25 mg/liter) than for EDB (1.8 to 3.7 mg/liter). In treatments that received both compounds, EDB was always consumed first and adversely impacted the kinetics of 1,2-DCA utilization. Furthermore, 1,2-DCA dechlorination was interrupted by the addition of EDB at a concentration 100 times lower than that of the remaining 1,2-DCA; use of 1,2-DCA did not resume until the EDB level decreased close to its maximum contaminant level (MCL). In lower-concentration experiments, the preferential consumption of EDB over 1,2-DCA was confirmed; both compounds were eventually dehalogenated to their respective MCLs (5 μ g/liter for 1,2-DCA, 0.05 μ g/liter for EDB). The enrichment culture grown with 1,2-DCA has the advantage of a more rapid transition to 1,2-DCA after EDB is consumed.

The Agency for Toxic Substances and Disease Registry maintains a prioritized list of the compounds most commonly found at Superfund sites that pose the most significant potential threat to human health. 1,2-Dibromoethane (ethylene dibromide [EDB]) ranks 6th among halogenated aliphatic compounds, while 1,2-dichloroethane (1,2-DCA) ranks 14th. EDB has the second lowest maximum contaminant level (MCL = 0.05 μ g/liter) in drinking water among all organic compounds after dioxin (1). The toxicity of 1,2-DCA is comparable to that of tetrachloroethene and trichloroethene, each with an MCL of 5.0 μ g/liter (1). 1,2-DCA is used as a precursor in the manufacture of vinyl chloride (VC). EDB was widely used as a pesticide and soil fumigant until these applications were banned in the 1980s. 1,2-DCA and EDB are among the most frequently detected contaminants in drinking water when an MCL is exceeded (2). 1,2-DCA has been found at 570 Superfund sites, while EDB has been detected at 27 (1).

The co-occurrence of EDB and 1,2-DCA in groundwater results mainly from environmental releases of leaded gasoline; these compounds were added as lead scavengers. Even though leaded gasoline has not been used for several decades, Falta et al. (3) demonstrated that EDB and 1,2-DCA persist at many sites that once had leaking storage tanks. Of 1,100 sites evaluated in South Carolina, 537 had EDB levels higher than its MCL. The predicted maximum concentrations of EDB and 1,2-DCA in groundwater from a leaded-gasoline release site are 1.9 and 3.7 mg/liter, respectively (4). The co-occurrence of 1,2-DCA and EDB has also been reported for at least one industrial site, where their concentrations in the vicinity of the source area were approximately 100 and 10 mg/liter, respectively (5).

Under low-redox anaerobic conditions, EDB and 1,2-DCA can

be used as terminal electron acceptors via organohalide respiration (6–13). The predominant pathway is dihaloelimination directly to ethene (see Section SM-1 in the supplemental material). Hydrogenolysis yields minor amounts of bromoethane from EDB and chloroethane from 1,2-DCA, both of which may be further reduced to ethane. Dehydrohalogenation yields minor amounts of vinyl bromide (VB) from EDB and VC from 1,2-DCA, both of which may undergo hydrogenolysis to ethene. Three types of microbes have been shown to use 1,2-DCA as a terminal electron acceptor, including *Dehalococcoides* (7, 9, 12, 13), *Dehalobacter* (8), and *Desulfitobacterium* (6, 10, 11). Dihaloelemination of EDB has been demonstrated only with *Dehalococcoides ethenogenes* strain 195 (12). Hydrogen serves as the electron donor for *Dehalococcoides* (9, 12, 13), *Dehalobacter* (8), and *Desulfitobacterium* (6); hydrogen can be provided directly or via a fermentable substrate in mixed cultures (7, 14). *Desulfitobacterium* also uses lactate and formate (6, 10, 11).

No information was found in the literature on Monod kinetic parameters (maximum specific growth rate and half-saturation coefficient) for the use of 1,2-DCA or EDB as a terminal electron acceptor. First-order degradation rates of 0.44 to 18 year⁻¹ for

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1,2-DCA and 1.5 to 110 year⁻¹ for EDB have been reported (15). Wilson et al. (16) estimated a half-saturation coefficient for EDB of 490 to 1,000 µg/liter on the basis of its solubility; however, experimental measurements were not made. In addition to a lack of information on the kinetics of 1,2-DCA and EDB utilization, no studies were found that evaluated the effect of each compound on the rate of biodegradation of the other when they are present together. A microcosm study with soil and groundwater from a site contaminated with leaded gasoline demonstrated anaerobic biodegradation of EDB at a higher rate, and to a greater extent, than that of 1,2-DCA (17). However, it was not clear if EDB inhibited the use of 1,2-DCA or if it was simply consumed at a higher rate. At an industrial site where EDB and 1,2-DCA were present at levels above 10 mg/liter, reductive dehalogenation of both compounds was observed (5). However, there was no indication that the co-occurrence of 1,2-DCA and EDB had an effect on the rate of dehalogenation of either compound.

The objectives of this study were to measure yields, maximum specific growth rates, half-saturation coefficients, and lag times in enrichment cultures that use 1,2-DCA and EDB as terminal electron acceptors and lactate as the electron donor and to evaluate the effect of each compound on the biodegradation rate of the other at the high concentrations that may be found at industrial sites (e.g., >10 mg/liter) and at the lower concentrations (1.9 to 3.7 mg/liter) reported at leaded-gasoline sites.

MATERIALS AND METHODS

Chemicals and medium. The chemicals (purity, sources) used were EDB (99%, Acros Organics), 1,2-DCA (99%, Mallinckrodt), VC (99.5%, Fluka), polymer grade ethene (99.9%, Airgas), ethane (99.95%, Matheson), methane (99%, Matheson), bromoethane (99%, EMD Chemicals), chloroethane (99.7%, Sigma-Aldrich), and VB (98%, Pfaltz & Bauer). Sodium lactate syrup was obtained from EM Science (58.8 to 61.2% sodium lactate; specific gravity = 1.31). All other chemicals were reagent grade. Enrichment cultures were grown in a mineral salts medium (see Section SM-2 in the supplemental material).

Analytical methods. 1,2-DCA, EDB, chloroethane, bromoethane, VC, VB, ethane, ethene, and methane were monitored by headspace analysis as previously described (18). Aqueous phase detection limits ranged from 0.2 to 6.0 µg/liter for the halogenated compounds, except for EDB, which had a detection limit of 23 µg/liter. To quantify EDB at lower concentrations, headspace samples were analyzed using a Hewlett-Packard 5890 Series II Plus gas chromatograph equipped with an electron capture detector (17); the detection limit was 0.05 µg/liter. Different syringes were used for the flame ionization and electron capture detector measurements of EDB to minimize the carryover of compound adsorbed to the syringe. The gas chromatograph response to a headspace sample (0.5 ml) was calibrated to give the total mass of the compound in that bottle (18), which was then converted to an aqueous-phase concentration using Henry's law constants (19).

Enrichment cultures. Two anaerobic enrichment cultures were developed; one was provided with 1,2-DCA as the terminal electron acceptor, and the other was provided with EDB. The inoculum for both was an enrichment culture that uses all of the chlorinated ethenes as terminal electron acceptors, with *Dehalococcoides* yields ranging from 7.9×10^7 to 1.7×10^9 gene copies/µmol Cl⁻ released (20). Morris et al. (21) compared reductive dehalogenases in the parent culture (referred to as the SRNL enrichment) to those found in two pure strains of *Dehalococcoides* and the KB1 enrichment culture. When provided with 1,2-DCA or EDB, the enrichment started dehalogenating these compounds at increasing rates. Sodium lactate was used as the electron donor. Performance of the enrichment cultures over the 16 to 26 months they were used for this study is provided in Section SM-3 in the supplemental material. Ethene was the

TABLE 1 Treatments evaluated during high-concentration experiments^a

Treatment	Inoculum grown with:	Compound(s) added
A	1,2-DCA	1,2-DCA
B	1,2-DCA	1,2-DCA + EDB
C	EDB	1,2-DCA
D	EDB	1,2-DCA + EDB
E	1,2-DCA	EDB
F	EDB	EDB

^a 9.2 to 110 mg/liter 1,2-DCA, 17 to 86 mg/liter EDB.

principal product from 1,2-DCA and EDB; trace levels of VC and VB were also present. Sodium hydroxide was added periodically to keep the pH between 6.7 and 7.1.

Yields. To determine yields, the enrichment cultures were grown by repeated additions of 1,2-DCA or EDB in 2.6-liter glass bottles. As chloride or bromide concentrations increased by approximately 0.5 to 1.0 µmol/ml (based on the amount of 1,2-DCA or EDB consumed), liquid samples were removed from the bottles, the DNA was extracted, and quantitative PCR (qPCR) was used to quantify the number of *Dehalococcoides* 16S rRNA gene copies in the enrichment cultures. qPCR was also used to evaluate the presence of *Dehalobacter* and *Desulfotobacterium*. Details are provided in Section SM-4 in the supplemental material.

Batch kinetics at high concentrations. For this study, 9.2 to 110 mg/liter of 1,2-DCA and 17 to 86 mg/liter of EDB were considered to be high concentrations. The treatments evaluated for high-concentration experiments are summarized in Table 1. Two received 1,2-DCA, two received EDB, and two received a mixture of the two compounds. One-half of the treatments were inoculated with the 1,2-DCA enrichment culture, and the others were inoculated with the EDB culture.

Kinetic coefficients were determined in batch tests in 160-ml serum bottles. Experiments were initiated in an anaerobic chamber by combining 2 ml from one of the enrichment cultures with 98 ml of medium. The serum bottles were sealed with Teflon-faced red rubber septa (VWR) and crimp caps. Outside the chamber, the headspaces were purged (70% N₂, 30% CO₂), the bottles were resealed, and the pH was adjusted (with NaOH) to 6.9 to 7.1. Serum bottles were incubated in an inverted position (liquid in contact with the septa) on a shaker table at room temperature (22 to 24°C). Lactate was added so that the initial ratio of electron equivalents of the donor to the equivalents needed for dehalogenation was higher than 5 (assuming 2 mol of H₂ per mol of lactate [22]); over the full incubation period, the ratio ranged from 113 to 228 for different treatments (see Section SM-5 in the supplemental material). Repeat additions of lactate were made to ensure that it remained in excess throughout the incubation period. Acetate and propionate were the principal organic acids that accumulated (see Section SM-5 in the supplemental material); accumulation of propionate is an indicator of an excess of hydrogen (23).

The initial amount of 1,2-DCA added (using a water-saturated solution or undiluted compound) was 9.6 to 26 µmol/bottle (9.2 to 25 mg/liter). When it became apparent that a rapid rate of consumption had begun, repeat additions of 1,2-DCA were made in order to maintain a maximum rate of growth. Dehalogenation of 1,2-DCA was coupled to an increase in the number of *Dehalococcoides* 16S rRNA gene copies, as indicated in the results of the yield experiment. The highest level of 1,2-DCA reached was 115 µmol/bottle (110 mg/liter). Once the rate of 1,2-DCA consumption reached a maximum, no more was added; the rate of consumption was monitored to determine kinetic parameters. Similarly, the amount of EDB added was 9.1 to 21 µmol/bottle (17 to 39 mg/liter) initially and was increased to a maximum of 46.8 µmol/bottle (86.2 mg/liter). This pattern of 1,2-DCA and EDB addition was adopted after trying other strategies, such as the addition of a single large dose, which were found to be inhibitory (e.g., EDB at >100 mg/liter; see Section SM-6 in the supplemental material). When we started at a lower initial concentration

and increased the amounts added over time, the cultures were able to biodegrade higher concentrations of EDB without apparent inhibition.

Batch kinetic experiments were performed in triplicate and repeated at least three times over a 17-month period. A stepwise approach to determine the maximum specific growth rates (μ , day⁻¹) and half-saturation constants (K_S , mg/liter of 1,2-DCA or EDB) was used, as described by Chang and Criddle (24). Maximum specific growth rates were determined first, followed by K_S ; this avoided the collinearity problems that can occur when both parameters are determined simultaneously.

Maximum specific growth rates in 1,2-DCA and EDB were determined by using a method adapted from respirometry (25). Assuming that endogenous decay is negligible during periods of exponential growth, the substrate concentration (in this case, 1,2-DCA or EDB) is high relative to K_S , and the initial biomass concentration is low relative to the new amount formed, maximum growth rates were determined by using the following equation:

$$\frac{dS_u}{dt} = \mu S_u \quad (1)$$

where S_u is the cumulative uptake of the terminal electron acceptor over time (mg/liter of 1,2-DCA or EDB). When the integrated form of this equation is plotted ($\ln S_u$ versus time), the slope equals μ . Averages were calculated from two to eight serum bottles per treatment (Table 1). During the interval when data were collected to determine μ , the ratio of 1,2-DCA and EDB to *Dehalococcoides* biomass (expressed as electron equivalents, assuming 4.2×10^{-15} g cell/gene copy [26]) was above 20; this corresponds to unrestricted growth or “intrinsic” conditions (27).

Once the μ and yield (Y) were known, the maximum specific rates of 1,2-DCA and EDB utilization ($\hat{q}$, $\mu\text{mol Cl}^-$ or Br^- /gene copy/day for 1,2-DCA or EDB) were calculated on the basis of the following equation:

$$\hat{q} = \frac{\mu}{Y} \quad (2)$$

As shown below, biodegradation of 1,2-DCA and EDB slowed substantially at concentrations well below their respective half-saturation coefficients. To describe this behavior, the Monod equation was modified as follows:

$$-\frac{dS}{dt} = \frac{\mu}{Y} \cdot \frac{X(S - S_i)}{K_S + (S - S_i)} \quad (3)$$

$$\frac{dX}{dt} = -\frac{dS}{dt}Y - bX \quad (4)$$

where X represents the *Dehalococcoides* concentration (number of gene copies/liter), S is the aqueous-phase concentration of 1,2-DCA or EDB (mg/liter), K_S is the Monod half-saturation coefficient (mg/liter of 1,2-DCA or EDB), S_i is a “transition concentration” (mg/liter 1,2-DCA or EDB) at which biodegradation proceeded at a low rate, and b is the endogenous decay coefficient for *Dehalococcoides*, the value of which (0.05 day⁻¹) was from Cupples et al. (28). The values of μ and Y were determined as described above; Y values were converted to numbers of *Dehalococcoides* gene copies per mg of 1,2-DCA or EDB consumed. As with μ , K_S values were determined under intrinsic conditions (27); i.e., the ratio of 1,2-DCA and EDB to *Dehalococcoides* biomass was above 20 when data collection for K_S determination started.

Values of K_S and S_i were determined by fitting equations 3 and 4 to substrate depletion data from the various treatments (Table 1) using AQUASIM (19). μ and Y were allowed to vary within their 95% confidence levels, the initial value of S was restricted to within 10% of the measured value, b was allowed to vary $\pm 10\%$ of its reported value (28), and the initial value of X was not restricted. Each data point was weighted with the inverse of S . The fitting process was initiated by the simplex optimization method and then fully optimized by the secant method, which reports the standard deviation of each parameter (19). The effect of mass transfer on equilibrium between the headspace and liquid was evaluated by incorporating a volumetric mass transfer coefficient (K_La) into

equation 3 (19), using specific mass transfer coefficients for 1,2-DCA and EDB from the literature (29). In all instances, the model fits with and without K_La were identical, indicating that it was appropriate to use headspace measurements to represent the aqueous-phase concentrations.

The lag time for each bottle was the time interval from day zero to the onset of exponential growth; averages and standard deviations were calculated on the basis of the lag times in individual bottles.

As shown below, the presence of EDB consistently inhibited the onset of 1,2-DCA dechlorination. To verify this, a separate experiment was performed in which three sets of duplicate serum bottles were prepared with 1,2-DCA as the substrate and inoculated with the 1,2-DCA enrichment culture (2%, vol/vol) (i.e., the same as treatment A in the high-concentration experiments). A comparatively small amount EDB was added to two of the sets (i.e., 370 and 645 $\mu\text{g/liter}$) when the 1,2-DCA amount remaining from the third addition was at least 36.3 mg/liter; the third set received no EDB.

Biodegradation of 1,2-DCA and EDB at lower concentrations. For this study, 1,2-DCA and EDB concentrations of ≤ 4.5 and ≤ 2.2 mg/liter, respectively, were considered to be representative of the levels found near the source zones at leaded-gasoline release sites (4). Separate experiments were performed at these lower concentrations in the manner described above, except that only one addition of 1,2-DCA and/or EDB was made and only one set of triplicates was evaluated. As with the higher-concentration experiments, lactate was added in excess of the electron donor required for dehalogenation; the initial ratio of electron equivalents of the donor to the equivalents needed for dehalogenation was higher than 82.

RESULTS

Yields. Yields were measured for three of the four treatments (A, C, and F; Table 1) in which 1,2-DCA or EDB was provided. qPCR was used to measure increases in *Dehalococcoides*, *Dehalobacter*, and *Desulfotobacterium*, the three genera currently known to respire 1,2-DCA. Increases over time were observed only for *Dehalococcoides* (see Section SM-7 in the supplemental material) and were used to calculate yields (Table 2). Results for 1,2-DCA were similar when it was dechlorinated by the 1,2-DCA enrichment culture (treatment A) and by the EDB enrichment culture (treatment C); at the end of the incubation period, *Dehalococcoides* concentrations ranged from 7.3×10^{10} to 1.7×10^{11} gene copies/liter. With EDB, a yield was determined only for treatment F and it was significantly lower than for 1,2-DCA (based on copy numbers per μmol of Cl^- or Br^- released); at the end of the incubation period, *Dehalococcoides* concentrations ranged from 1.8×10^{10} to 2.5×10^{10} gene copies/liter. Because of the lower yield with EDB and the higher *Dehalococcoides* population in the 1,2-DCA enrichment culture, the smaller increase in copy numbers over time above the starting concentration made it more difficult to determine a *Dehalococcoides* yield for treatment E. For treatments A, C, E, and F, *Dehalobacter* levels were measured after the final additions of 1,2-DCA and EDB were consumed; results ranged from 4.4×10^5 to 3.1×10^7 gene copies/liter. *Desulfotobacterium* was not amplified in any of the treatments.

Kinetics at high initial concentrations of 1,2-DCA and EDB. When 1,2-DCA was added to the DCA enrichment culture (treatment A; Table 1), consumption started after 10 days and ethene was the predominant product (Fig. 1a). VC accumulation reached 0.61% of the 1,2-DCA consumed (see Section SM-8 in the supplemental material). When EDB was added to the EDB enrichment culture (treatment F), consumption started sooner (Fig. 1b) and ethene was the predominant product. VB accumulation reached 0.052% of the EDB consumed (see Section SM-8 in the supplemental material). Methanogenesis was suppressed at all times (in

TABLE 2 Summary of kinetic parameters based on the high-concentration experiments^a

Treatment	Dehalococcoides yield (copy no./μmol Cl ⁻ or Br ⁻)		μ _d (day ⁻¹)		q̂ (μmol Cl ⁻ or Br ⁻ /copy/day)		K _S (mg/liter)		S _p (mg/liter)		Lag time (days)	
	1,2-DCA	EDB	1,2-DCA	EDB	1,2-DCA	EDB	1,2-DCA	EDB	1,2-DCA	EDB	1,2-DCA	EDB
A	4.6 × 10 ⁷ ± 0.74 × 10 ⁷	— ^b	0.48 ± 0.026	—	1.0 × 10 ⁻⁸ ± 0.10 × 10 ⁻⁸	—	21.7 ± 0.02	—	2.14 × 10 ⁻² ± 0.08 × 10 ⁻²	—	15 ± 3.0	—
B	—	—	0.41 ± 0.049	0.28 ± 0.019	9.0 × 10 ⁻⁹ ± 1.19 × 10 ⁻⁹	3.9 × 10 ⁻⁸ ± 0.47 × 10 ⁻⁸	25.2 ± 0.04	1.81 ± 0.013	2.54 × 10 ⁻² ± 0.09 × 10 ⁻²	4.76 × 10 ⁻⁴ ± 1.78 × 10 ⁻⁴	32 ± 13	11 ± 3.7
C	2.6 × 10 ⁷ ± 0.26 × 10 ⁷	—	0.52 ± 0.026	—	2.0 × 10 ⁻⁸ ± 0.15 × 10 ⁻⁸	—	15.8 ± 0.03	—	9.98 × 10 ⁻³ ± 0.62 × 10 ⁻³	—	43 ± 8.9	—
D	—	—	0.19 ± 0.008	0.35 ± 0.028	7.4 × 10 ⁻⁹ ± 0.60 × 10 ⁻⁹	4.9 × 10 ⁻⁸ ± 0.62 × 10 ⁻⁸	14.3 ± 0.04	2.64 ± 0.015	1.10 × 10 ⁻¹ ± 0.02 × 10 ⁻¹	9.81 × 10 ⁻⁴ ± 1.12 × 10 ⁻⁴	75 ± 7.1	16 ± 5.7
E	—	NM ^c	—	0.32 ± 0.016	—	4.5 × 10 ⁻⁸ ± 0.54 × 10 ⁻⁸	—	3.65 ± 0.022	—	1.76 × 10 ⁻³ ± 0.92 × 10 ⁻³	—	15 ± 5.7
F	—	7.1 × 10 ⁶ ± 1.3 × 10 ⁶	—	0.36 ± 0.020	—	5.1 × 10 ⁻⁸ ± 0.60 × 10 ⁻⁸	—	2.80 ± 0.007	—	3.35 × 10 ⁻⁴ ± 1.06 × 10 ⁻⁴	—	15 ± 4.9

^a Average values ± 95% confidence intervals are shown.

^b —, the compound was not added to this treatment.

^c NM, not measured; see text.

comparison to the enrichment culture; see Fig. S-3 in the supplemental material). Results of treatment C (1,2-DCA added to the EDB culture) and treatment E (EDB added to the 1,2-DCA culture) were similar to those shown in Fig. 1a and b, respectively (see Section SM-9 in the supplemental material). This indicated that the 1,2-DCA enrichment culture was able to switch to EDB as the terminal electron acceptor and vice versa. Simulated concentrations of *Dehalococcoides* increased initially when most of the 1,2-DCA and EDB was consumed and then declined as decay became predominant (see Section SM-10 in the supplemental material).

When 1,2-DCA and EDB were added together and inoculated with the 1,2-DCA culture (treatment B), EDB consumption started first (Fig. 1c). 1,2-DCA consumption did not start until EDB decreased close to its detection limit. The pattern of EDB and 1,2-DCA additions was similar to those used when the individual compounds were added (Fig. 1a and b). Ethene was the predominant product. VB accumulation reached 0.072% of the total EDB consumed; VC reached 0.40% of the 1,2-DCA consumed (see Section SM-8 in the supplemental material). Methanogenesis was suppressed throughout (in comparison to the enrichment culture; see Fig. S-2 in the supplemental material).

Similar results were obtained for treatment D (1,2-DCA and EDB added simultaneously; the EDB enrichment culture served as the inoculum) (Fig. 1d). EDB consumption started first, and 1,2-DCA consumption started only after the EDB level had fallen below 15 μg/liter. Ethene was the predominant product. VC accumulation reached 0.64% of the 1,2-DCA consumed; VB reached 0.093% (see Section SM-8 in the supplemental material). Methanogenesis was suppressed while EDB was present.

Mineral medium control results demonstrated that consumption of 1,2-DCA and EDB was biotic. There was no statistically significant loss of either compound from controls over incubation periods similar to those shown in Fig. 1 (see Section SM-11 in the supplemental material).

Maximum specific growth rates were determined on the basis of the exponential increase in cumulative 1,2-DCA and EDB consumption. Figure 2a provides representative results; average results for all treatments are shown in Table 2. The maximum specific growth rate on 1,2-DCA was equivalent for the treatments inoculated with the 1,2-DCA enrichment culture, with or without EDB added (A and B, respectively), and in the treatment inoculated with the EDB culture that received EDB and 1,2-DCA together had a lower growth rate on 1,2-DCA (treatment D) (Student's *t* test, $\alpha = 0.05$). There was no significant difference among the maximum growth rates on EDB, i.e., treatments B, D, E, and F (Table 2) (Student's *t* test, $\alpha = 0.05$). Overall, the average growth rate on 1,2-DCA for all treatments ($0.40 \pm 0.15 \text{ day}^{-1}$) was not significantly higher than that on EDB ($0.33 \pm 0.04 \text{ day}^{-1}$) (Student's *t* test, $\alpha = 0.05$).

Batch depletion data were fitted to equations 3 and 4, providing K_S and S_p . Representative results for treatments A, B, D, and F are shown in Fig. 2b and c (for treatments C and E, see Section SM-12 in the supplemental material); average values are presented in Table 2. The K_S values for 1,2-DCA are an order of magnitude higher than for EDB. When both compounds were added, the K_S for 1,2-DCA increased in the treatment inoculated with the 1,2-DCA enrichment culture (B versus A) but decreased somewhat in the treatment with the EDB enrichment culture (D versus C). The lowest K_S value for EDB occurred when EDB was added along with

FIG 1 Representative results of the biodegradation of 1,2-DCA in treatment A (a), EDB in treatment F (b), 1,2-DCA and EDB in treatment B (c), and 1,2-DCA and EDB in treatment D (d). Each arrow indicates the addition of 0.31 mmol lactate. The y axes are expressed in μmol per bottle (btl) to directly reveal the stoichiometry of the parent compounds and daughter products.

1,2-DCA and the 1,2-DCA enrichment culture served as the inoculum (treatment B), while K_S was highest for EDB when EDB alone was present and the 1,2-DCA culture served as the inoculum (treatment E). The presence of 1,2-DCA along with EDB decreased the K_S for EDB (i.e., treatment B versus E and D versus F).

The transition concentrations (S_t) for 1,2-DCA were 1 to 2 orders of magnitude higher than those for EDB (Table 2). The most notable influence on 1,2-DCA use occurred in the EDB enrichment-inoculated cultures fed both compounds (treatment D), whose S_t was significantly higher than when only 1,2-DCA was added (treatment C). With EDB, the relative standard deviations for S_t were higher. For most of the treatments, once S_t was reached, little or no additional dehalogenation occurred. However, 2 of 11 bottles in treatment A and 3 of 9 bottles in treatment C did eventually reach the MCL of 1,2-DCA; 2 of 8 bottles in treatment F reached the MCL of EDB.

The maximum specific substrate utilization rates ($\hat{q}$) for 1,2-DCA and EDB were $(2.0 \pm 0.30) \times 10^{-8}$ and $(5.1 \pm 1.01) \times 10^{-8}$ $\mu\text{mol Cl}^-$ or $\text{Br}^-/\text{gene copy/day}$, respectively (Table 2). The co-occurrence of 1,2-DCA and EDB did not significantly change $\hat{q}$ for either compound, with one exception: when the EDB enrichment

culture served as the inoculum, the copresence of EDB decreased the $\hat{q}$ for 1,2-DCA by 63% (treatment D versus C).

In the presence of EDB, there was a significant increase in the lag times for 1,2-DCA (i.e., treatment B versus A and D versus C; Table 2). The lag time for 1,2-DCA was also longer in the treatments inoculated with the EDB enrichment culture than in those inoculated with the 1,2-DCA culture (i.e., treatment C versus A and D versus B). However, the lag time for EDB was not impacted by the presence of 1,2-DCA (regardless of the inoculum) and was equivalent to the lag time for 1,2-DCA in treatment A.

Effect of low EDB concentrations on high 1,2-DCA concentrations. The effect of EDB on 1,2-DCA biodegradation was further assessed in a separate experiment. After initiation of the dechlorination of high concentrations of 1,2-DCA with inoculum from the 1,2-DCA culture, various amounts of low concentrations of EDB were added when at least 36.3 mg/liter of 1,2-DCA still remained. Addition of EDB immediately decreased the rate of 1,2-DCA consumption, while the EDB was consumed (Fig. 3; see Section SM-13 in the supplemental material). As the EDB level decreased close to its MCL, consumption of 1,2-DCA resumed at approximately the same rate prior to the addition of EDB.

FIG 2 Representative results of experiments with high concentrations of 1,2-DCA and EDB to determine $\hat{\mu}$ (a), K_S for 1,2-DCA (b), and K_S for EDB (c). Lines in panel a represent the fit to equation 1, and those in panels b and c represent the fit to equations 3 and 4. The treatments are described in Table 1.

Biodegradation of 1,2-DCA and EDB at lower concentrations. In bottles inoculated with the 1,2-DCA culture and started at lower concentrations of 1,2-DCA and EDB, EDB was consumed first, reaching its MCL in 11 to 22 days (Fig. 4a). The presence of 1,2-DCA along with EDB had mixed effects on the rate of EDB

FIG 3 Representative results for the 1,2-DCA enrichment culture biodegrading high concentrations of 1,2-DCA when no EDB was added (a), 370 µg/liter EDB was added (b), or 645 µg/liter EDB was added (c). Each arrow indicates the addition of 0.31 mmol lactate. EDB was added on day 16.5 and is shown on a log scale (MCL = 0.05 µg/liter).

biodegradation; in two of the three replicates, bottles with 1,2-DCA reached the MCL of EDB first, while in one of the bottles EDB consumption was slower than in the bottles with only EDB present. In the treatment with only 1,2-DCA, the MCL was reached by days 28 to 45, following a lag of 9 to 14 days. With EDB present, biodegradation of 1,2-DCA started shortly after EDB reached its MCL and 1,2-DCA was consumed at a rate equivalent to or slightly higher than that in the treatment with 1,2-DCA alone, suggesting that prior exposure to EDB increased the subsequent rate of 1,2-DCA biodegradation.

With the EDB enrichment culture as the inoculum, EDB was consumed before 1,2-DCA (Fig. 4b). The treatment with EDB alone reached the MCL first; however, the time required to reach the MCL was notably longer (34 to 38 days) than with the 1,2-DCA enrichment culture. The lag time for 1,2-DCA was 49 to 61 days, considerably longer than for the 1,2-DCA enrichment culture (Fig. 4a). In the treatment with both compounds present, biodegradation of 1,2-DCA started approximately when EDB reached its MCL. Overall, lag times for 1,2-DCA and EDB biodeg-

FIG 4 Biodegradation of lower concentrations of 1,2-DCA and/or EDB that are typical of leaded-gasoline spills in enrichment cultures grown with 1,2-DCA (a) or EDB (b) as the terminal electron acceptor. Triplicate bottles (numbered 1, 2, and 3) are shown for each treatment. Arrows indicate the addition of 0.25 mmol lactate.

radiation at the lower initial concentrations were equivalent to or shorter than those at the levels used in the higher-concentration kinetic tests (Table 2).

DISCUSSION

Of the three genera known to halorespire 1,2-DCA (i.e., *Dehalococcoides*, *Dehalobacter*, and *Desulfitobacterium*), only *Dehalococcoides* increased in this study in direct proportion to the amounts of 1,2-DCA and EDB consumed. The predominance of *Dehalococcoides* in the 1,2-DCA and EDB enrichment cultures is consistent with the fact that their origin is an enrichment culture that respire tetrachloroethene and trichloroethene. Notably, the *Dehalococcoides* yield was approximately 1 order of magnitude lower when EDB was used than when 1,2-DCA was used. This result was consistent with a preliminary study performed under similar conditions (20). No other studies were found for comparison of the EDB yield; the *Dehalococcoides* yield we measured for 1,2-DCA is approximately one-quarter of that reported for the KB-1/TCE enrichment culture (7). The reason for the lower yield with EDB than with 1,2-DCA is not known, although thermodynamics do

not offer a compelling explanation; dihaloelimination is slightly more favorable for EDB than for 1,2-DCA (17). A similar disparity in yields was reported for at least one other pair of chlorinated and brominated compounds; a methylotroph grown on dibromomethane produced approximately 26% less biomass than on dichloromethane (30). In this case, however, the compounds were used as sole carbon and energy sources under aerobic conditions.

EDB was used in preference to 1,2-DCA when both compounds were present, regardless of the inoculum. This is consistent with the microcosm study of Henderson et al. (17), based on a site contaminated with leaded gasoline. Reductive debromination is also more favorable than dechlorination for other types of halogenated organics (e.g., polybrominated biphenyls versus polychlorinated biphenyls [31]). This preference was especially apparent when dechlorination of 1,2-DCA was interrupted by adding EDB at a concentration more than 100 times lower than that of 1,2-DCA; use of 1,2-DCA did not resume until the EDB level decreased close to its MCL (Fig. 3). The inhibitory effect of EDB on 1,2-DCA utilization correlates with the order-of-magnitude lower K_S for EDB than for 1,2-DCA (Table 2). Yu and Sem-

prini (32) observed a similar correlation in mixed cultures that respire tetrachloroethene; i.e., inhibition of VC reduction by *cis*-1,2-dichloroethene was consistent with a much higher K_S for VC than for *cis*-1,2-dichloroethene. Additional studies are needed to establish if the dehalogenase used for 1,2-DCA dechlorination (e.g., see references 8 and 11) is also used for EDB debromination.

The maximum growth rates in 1,2-DCA (0.19 to 0.52 day⁻¹) are similar to the rate of *Desulfotobacterium dichloroeliminans* strain DCA1 (0.21 day⁻¹) on the basis of its yield and maximum utilization rate (6). The maximum growth rates in EDB were within the same range (0.28 to 0.36 day⁻¹); no previous studies were found for comparison.

K_S values for 1,2-DCA or EDB were not found in the literature. In general, the values we measured for 1,2-DCA (147 to 254 μ M) are notably higher than those for polychlorinated ethenes (e.g., 1.6 to 3.9 μ M for tetrachloroethene, 1.8 to 2.8 μ M for trichloroethene, and 1.8 to 1.9 μ M for *cis*-dichloroethene) but similar in magnitude to those for VC (63 to 602 μ M) (32). The K_S values we measured for EDB are considerably lower (9.6 to 19.4 μ M). Nevertheless, these concentrations are 4 orders of magnitude higher than the MCL of EDB (0.00027 μ M).

The use of a transition concentration (S_t) in equation 3 allowed for a better fit of the high-concentration data in the region where 1,2-DCA and EDB approached low concentrations (Fig. 2). When the 1,2-DCA and EDB depletion data were fitted without S_t , the K_S values for 1,2-DCA did not change substantially or increased somewhat; the K_S values for EDB were unchanged for treatment F and decreased 2.6- to 336-fold for treatments B, D, and E (see Section SM-14 in the supplemental material). Also, without S_t , there was a notable increase in the error associated with K_S . Overall, inclusion of S_t had a greater influence on the K_S value for EDB. Coleman et al. (33) used a similar modification of an equation for predicting oxygen consumption during the aerobic biodegradation of VC, although in their experiments a true threshold existed. In our study, S_t was not a true threshold, since 1,2-DCA and EDB levels continued to decrease at a low rate in some bottles. The onset of a lower rate of consumption and/or the cessation of dehalogenation appeared to be a consequence of the high concentrations of 1,2-DCA and EDB (perhaps because of a cumulative toxicity impact), since all of the replicates reached the MCLs of both compounds in the lower-concentration experiment (Fig. 4). This suggests that the MCL may not reliably be reached in the vicinity of a source zone for a release of undiluted material. As the concentration decreases away from the source area, the likelihood of reaching the MCL should improve. Huang and Becker (34) offer a different approach to modifying the Monod equation to capture the toxicity associated with biomass inactivation. However, modification of equations 3 and 4 by replacing S_t with a biomass inactivation term resulted in poor fits of the model to the EDB and 1,2-DCA data (results not shown).

One of the concerns about the anaerobic dechlorination of 1,2-DCA is the potential for VC accumulation. In this study, VC accumulation reached 7.6 μ M (0.40 to 0.68% of the total 1,2-DCA consumed), although the VC level decreased with continuing incubation (Fig. 1). No accumulation of VC was reported in laboratory studies with *D. dichloroeliminans* strain DCA1 (6, 35), although 20 to 30 μ M accumulation occurred in a pilot scale bioaugmentation test (10). The accumulation level of VB was lower than that of VC (1.3 μ M or 0.05 to 0.09% of the total EDB consumed), and it too decreased over time (Fig. 1).

Evidence continues to mount for the need to monitor 1,2-DCA and EDB contamination of groundwater, especially at former leaded-gasoline sites (16). Corresponding interest in remediation approaches is likely to increase. The kinetic coefficients obtained in this study can be used in groundwater models (e.g., REMChlor) to simulate the impact of biodegradation on the extent of plume migration, as well as enhanced bioremediation, including the inhibitory effect of EDB on 1,2-DCA. Previously, no kinetic information was available for EDB, and no side-by-side comparisons of EDB and 1,2-DCA existed. The strong inhibitory effect of EDB on 1,2-DCA identified in this study has important implications for our understanding of the possible persistence of 1,2-DCA. Bioaugmentation is a candidate approach for sites where monitored natural attenuation is infeasible. Although considerable information on cultures that can dechlorinate 1,2-DCA is available, most have not been tested for the ability to debrominate EDB. Of the two enrichment cultures evaluated in this study, the 1,2-DCA culture has the advantage of a more rapid transition to 1,2-DCA after EDB is consumed (Fig. 4). Additional information on the ability of enrichment cultures to dehalogenate 1,2-DCA and EDB in the presence of persistent fuel hydrocarbons is needed.

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