Environmental Science & Technology

SUPPORTING INFORMATION

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Anaerobic Biodegradation of Ethylene Dibromide and 1,2-Dichloroethane in the Presence of Fuel Hydrocarbons

Number of Pages (including this cover sheet): 20

Number of Figures: 3

Number of Tables: 6

Supporting Information

Microcosm Sampling and Analytical Methods.

Microcosm Sampling. Volatile compounds in the microcosms were quantified using a headspace method. This required taking samples of the headspace by puncturing the septum with a syringe. Because of excessive diffusive losses of some of the volatile compounds (in particular, BTEX) during storage with punctured septa, a different procedure was used for sampling that allowed for storage of the microcosms with unpunctured septa. The next section of the Supporting Information provides a comparison of diffusive losses during the two methods of microcosm storage (i.e., with punctured versus unpunctured septa). This section describes how the headspace sampling was accomplished, followed by descriptions of the GC methods, CSIA, and methods for anions, iron and organic acids.

Headspace sampling began by shaking the microcosms to homogenize the sediment and groundwater and placing the microcosms in the anaerobic chamber in an upright position the night before samples were to be taken. At least one hour before sampling, the unpunctured septa that were on the bottles were quickly removed and replaced with septa that were designated for puncturing (i.e., they may have already been punctured several times). It took less than five seconds to exchange the septa. Duplicate headspace samples (0.5 mL) were then taken in separate syringes (1.0 mL series A-2 with a side-port needle, Precision Scientific) inside the chamber; the syringes were immediately removed and walked over to the GC. One sample was injected to the ECD, the other to the FID (see below). After confirming that the samples had been run successfully on the GC, the punctured septa on the microcosms were exchanged for unpunctured ones, the microcosms were removed from the chamber, shaken to homogenize the sediment and groundwater, and then stored in the inverted position until the next sampling event.

As needed, liquid samples were removed at the same time as headspace samples. Supernatant was removed using a 2 mL glass syringe.

GC Methods. As mentioned above, the two headspace samples were injected onto the GC, one immediately after the other. A single temperature program (40°C for 5 min, ramped at 10°C/min to 200°C, hold for 12 min) resolved all of the contaminants of interest. To quantify EDB, 1,2-DCA, bromoethane, and vinyl bromide, one of the headspace samples was injected onto an RTX 624 column (60-m, 0.53 mm inner diameter, 3.0 µm film thickness) connected to the ECD, with injector and detector temperatures set at 200°C and 260°C, respectively. Helium (3 mL/min) and nitrogen (33 mL/min) served as the carrier and make-up gases, respectively. A split flow rate of 220 mL/min (73.1:1 split ratio) was used for EDB concentrations greater than approximately 1.0 µg/L and a splitless mode was used thereafter (0.75 min splitless injections). To quantify hydrocarbons, the second headspace sample was injected onto an RTX-5 column (30-m, 0.53 mm inner diameter, 0.25 µm film thickness) connected to the FID was used, with injector and detector temperatures set at 250°C and 310°C, respectively. Helium (5.88 mL/min) and nitrogen (33.0 mL/min) served as the carrier and makeup gases, respectively. A split flow rate of 26.0 mL per minute was used, and injections were made in splitless mode (0.75 min).

Carbon Specific Isotope Analysis. Samples for CSIA were prepared by diluting 2 mL of groundwater from a microcosm tenfold in 25 mL vials with Teflon-backed septa to prevent volatilization losses. EDB and 1,2-DCA samples were preserved with HCl (3 drops). The analytes were extracted by a purge and trap (P&T model OI 4660) interfaced to a GC-IRMS instrument (Finnigan MAT 252 IRMS). Due to chromatographic complexity of the samples, satisfactory resolution of EDB and 1,2-DCA required a 2-dimensional chromatographic approach (separation on polar GC phase followed by separation on non-polar GC phase). The

P&T-GCIRMS interface described previously (*1*) was programmed for collecting 2 min heart-cuts of the sample eluting from the polar pre-column. The heart-cuts were directed onto a non-polar phase GC column for final separation followed by on-line combustion and analysis of isotope composition.

Anions, Iron, and Organic Acids. Liquid samples were filtered (0.45 µm PVDF, Pall Life Sciences) and analyzed for bromide, chloride, nitrate and sulfate on a Dionex DX-100 Ion Chromatograph using a AS5A-5µ (4 x 150 mm) column and 0.01 N H_2SO_4 as eluant (0.6 mL/min). Lactate, acetate and propionate were quantified by high performance liquid chromatography using an anion exchange column (Aminex HPX-87H, BioRad) (*2*). Iron (II) was analyzed using the ferrozine method (*3*).

The effect of incubation method on losses of volatile compounds during storage of the microcosms. Prior microcosm experiments (*4*) suggested that loss of BTEX compounds may be significant when the microcosms are incubated with septa that have been repeatedly punctured. An alternative approach is to incubate the test bottles with unpunctured septa, as described above. The hypothesis was that the losses that occur during the brief time when swapping the unpunctured and punctured septa are smaller in comparison to not changing the septa and incubating the bottles for extended periods (i.e., months) with septa that have been punctured. To test this hypothesis, two sets of triplicate water controls were prepared using the same type of bottles as the microcosms, with 1.7 L of distilled deionized water present. Both sets were spiked with the same amounts of EDB, 1,2-DCA and BTEX. One set ("punctured septa") was sampled and incubated without changing the septa. For the other set ("unpunctured septa"), the septa were exchanged prior to and after sampling, as described above.

Data were collected for 120 days. Results are shown in Figure S-1. The data were fit to a first order model. For the unpunctured treatment, none of the trend lines were statistically significant (α=0.05), so no trend line is shown. This confirmed the lack of diffusive losses with this method of microcosm operation. A summary of the pseudo first order rates of loss for the punctured treatment is given in Table S-1. It is evident that diffusive losses were significantly greater for EDB, benzene, toluene, ethylbenzene and *o*-xylene in the bottles that were incubated with punctured septa. There was virtually no difference for 1,2-DCA. These results confirm the importance of incubating the microcosms with unpunctured septa rather than punctured ones, and that the process of exchanging septa just before and after sampling resulted in minimal (if any) losses of the volatile compounds.

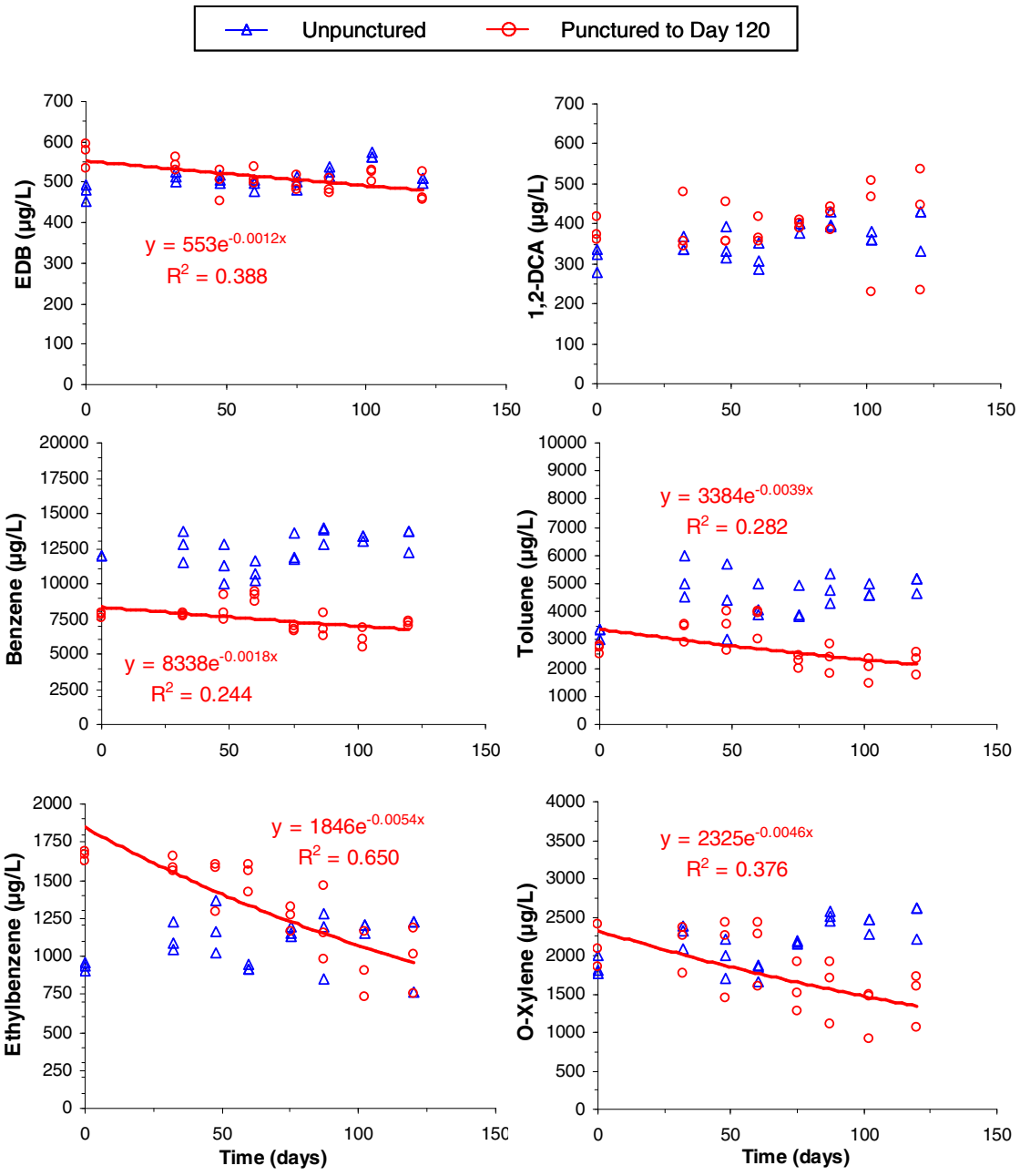

Figure S-1. Behavior of EDB, 1,2-DCA and BTEX in triplicate water control microcosms that were incubated with unpunctured septa (blue symbols) and punctured ones (red symbols). Trend lines are shown only when the first order regression line was statistically significant; for the compounds that had a slope that was not different from zero, no trend line is shown.

Table S-1. Summary of First Order Loss Rates from Water Controls with Punctured and Unpunctured Septa.

	Unpunctured		Punctured		
Compound	**Rate (yr^{-1})[a]**	**R^2**	**Rate (yr^{-1})**	**R^2**	**Comparison[b]**
EDB	0[c]	-	0.428 ± 0.115	0.388	Significant
1,2-DCA	0	-	0	-	Insignificant
Benzene	0	-	0.650 ± 0.244	0.244	Significant
Toluene	0	-	1.42 ± 0.483	0.282	Significant
Ethylbenzene	0	-	1.99 ± 0.311	0.650	Significant
o-Xylene	0	-	1.67 ± 0.458	0.376	Significant

[a] Rates were determined based on regression of pooled data from triplicate bottles.

[b] Student's *t*-tests ($\alpha = 0.05$) were performed to compare the rates for the unpunctured and punctured bottles. Where statistical differences were observed, "significant" was entered. If no statistical difference was observed, "insignificant" was entered.

[c] The slope was not statistically different from zero or was positive.

Comparison of EDB quantification by EPA method 8011 and headspace analysis. Care was taken prior to beginning the experiments to develop an analytical method capable of attaining EDB's very low MCL of 0.05 µg/L. EPA's method 8011 was compared to quantification by headspace analysis, on the basis of the amount of EDB delivered in a sample to the GC when 0.05 µg/L is present in the aqueous phase of the microcosms. Method 8011 analyzes for EDB and 1,2-dibromo-3-chloropropane by extracting an aqueous sample into hexane (*5*). The headspace method is based on 0.5 mL samples from the gas phase of the microcosms.

With EPA Method 8011, a 35 mL aqueous sample is extracted into 2.0 mL hexane, concentrating the sample by a factor of 17.5 (*5*). The extraction procedure was modified for the purposes of this study in order to conserve aqueous volume, by reducing the aqueous and solvent volumes seven fold; i.e., 5.0 mL of microcosm water was extracted into 0.3 mL pentane. (Pentane was used rather than hexane, since pentane elutes faster than hexane and the hexane peak overlapped with 1,2-DCA, which elutes faster than EDB). Assuming 100% extraction efficiency (i.e., all mass in the aqueous phase is extracted by the solvent) and 0.05 µg/L EDB in the water, the mass injected onto the GC in a 1 µL sample is 8.33E-4 ng.

With the headspace method, the amount injected is based on the concentration in the gas phase that is in equilibrium with 0.05 µg/L EDB in the aqueous phase. This concentration is obtained based on a mass balance for the microcosm:

$$M_T = C_l V_l + C_g V_g \tag{S1}$$

where M_T is the total amount of EDB (µg/bottle); C_l is the concentration of EDB in the aqueous phase (µg/L), V_l is the aqueous volume (L), C_g is the gas phase concentration (µg/L), and V_g is the gas volume (L). Using Henry's law constant ($H_c = C_g/C_l$) and substituting C_l for C_g yields:

$$M_T = C_l V_l + H_c C_l V_g \tag{S2}$$

When V_l = 1.5 L, V_g = 0.3 L, C_l = 0.05 µg/L, and H_c = 0.0251, then M_T = 7.54E-2 µg.

Equation S1 may also be solved in terms of C_g by substituting for C_l:

$$C_g = \frac{M_T}{\frac{V_l}{H_c} + V_g} \tag{S3}$$

Using the value calculated for M_T from equation S-2 and the values above for V_l, V_g, and H_c, equation S3 yields a value of 1.26E-3 µg/L for C_g. The amount of EDB injected onto the GC in a 0.5 mL headspace sample is 6.28E-4 ng. This amount is approximately 75% of the amount injected based on the modified version of Method 8011. Since the assumption regarding complete extraction efficiency for Method 8011 is unrealistic and the headspace method delivers an amount to the GC sufficient to allow detection to below the MCL for EDB, the headspace method was selected for quantification. This approach avoids the need to perform extractions and does not disturb the amount of liquid in the microcosms.

EDB and 1,2-DCA results for individual microcosms. Figure 1 in the manuscript shows average results for EDB and 1,2-DCA in triplicate microcosms. Data for each bottle are presented in Figure S-2 for the source zone and Figure S-3 for the midgradient zone, in order to reveal the extent of variability among the replicates. Especially noteworthy is the rapid biodegradation of EDB and 1,2-DCA in NA source zone replicate #3 (also described in the manuscript). EDB was added a second time to this microcosm to confirm its biodegradation activity. NA source zone replicate #4 also needed to be respiked with EDB, although it did not consume the second addition of EDB as rapidly as replicate #3. The BST and AC replicates in the source zone behaved more similarly. All of the midgradient replicates behaved similarly, both with respect to EDB and 1,2-DCA (Figure S-3) and BTEX (data not shown).

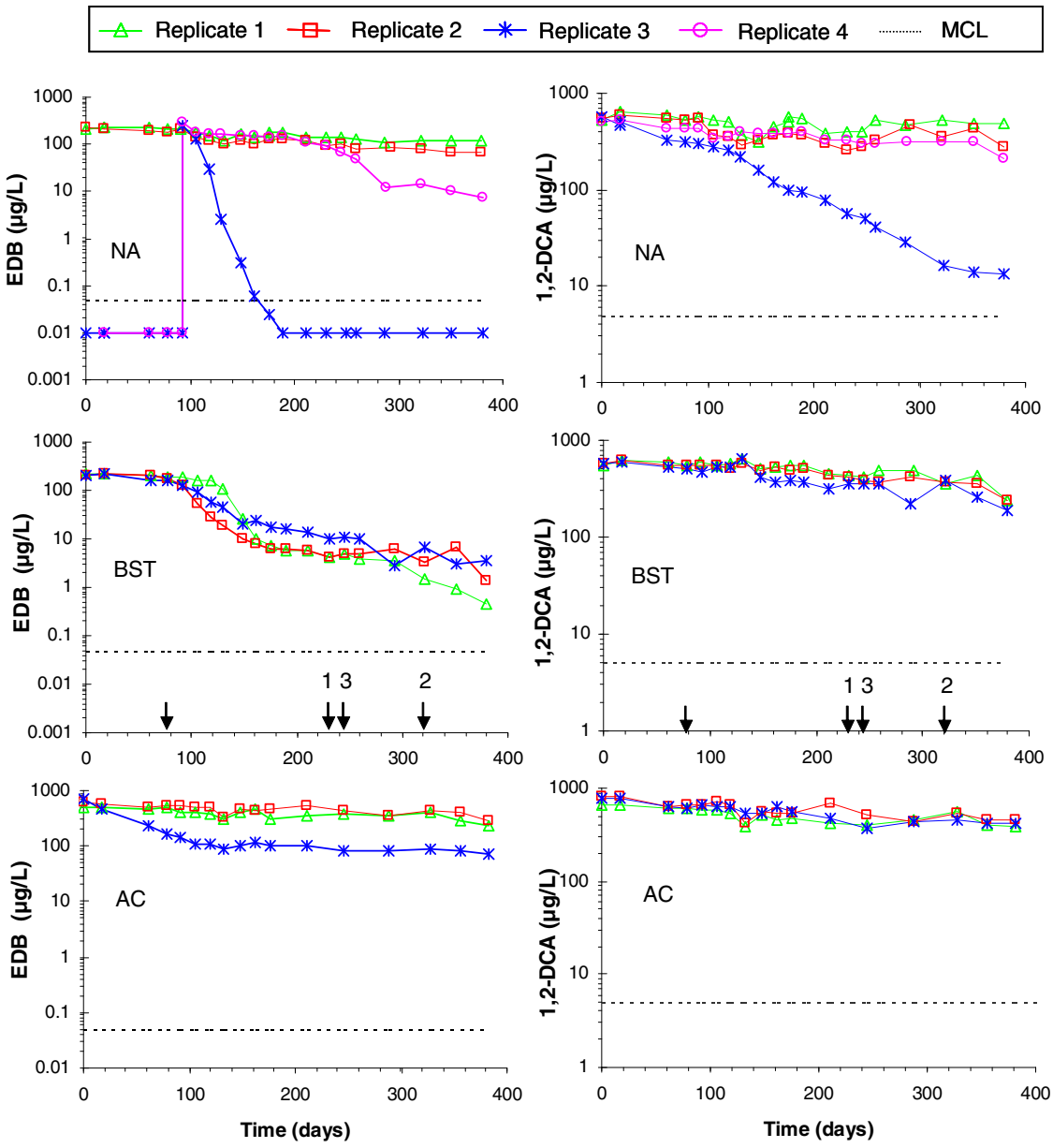

Figure S-2. Source zone EDB and 1,2-DCA microcosm replicates; arrows (↓) indicate when lactate was added to all replicates within a BST treatment. An arrow with a number above it indicates that lactate was added only to that replicate. New lactate additions were made only when the previous addition was completely consumed. Dashed horizontal lines indicate the MCL for EDB and 1,2-DCA.

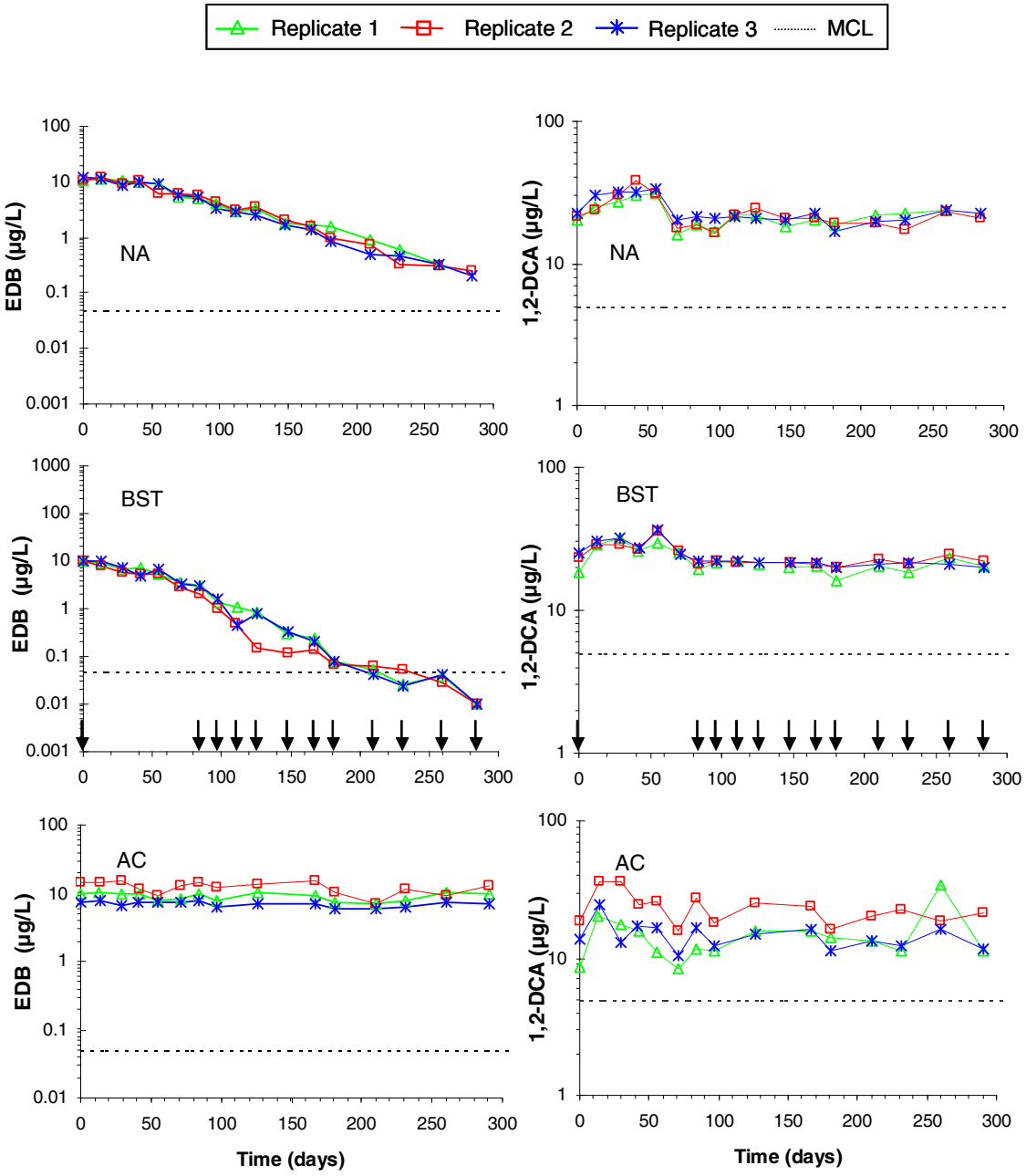

Figure S-3. Midgradient zone EDB and 1,2-DCA microcosm replicates; arrows (↓) indicate when lactate was added to all replicates within a BST treatment. An arrow with a number above it indicates that lactate was added only to that replicate. New lactate additions were made only when the previous addition was completely consumed. Dashed horizontal lines indicate the MCL for EDB and 1,2-DCA.

Characteristics of soil from the source and midgradient zones. Table S-2 shows various properties of the soil taken from the source and midgradient zones at the Clemson, South Carolina UST site sampled for this study. The soils contain a lower level of total iron than would be expected for clays that are native to the area. However, the soil at the site consists of clays along with poorly-sorted fill material consisting of a mixture of sand, silt and clay.

Table S-2: Soil Characteristics[a]

Compound	Source Zone	Midgradient Zone
Phosphorus (mg/kg)	2.5	1.0
Potassium (mg/kg)	20	20
Calcium (mg/kg)	350	41
Magnesium (mg/kg)	23	14
Zinc (mg/kg)	5.2	2.3
Manganese (mg/kg)	27	22
Copper (mg/kg)	0.30	0.35
Boron (mg/kg)	0.1	0.05
Sodium (mg/kg)	9.0	9.0
Nitrate Nitrogen (mg/kg)	2.0	1.0
pH	5.1	4.8
Iron (mg/kg)	35	16
Carbon (%)	0.30	0.01

[a] Analyses performed by the Agricultural Services Laboratory at Clemson University.

Comparison of Gibbs free energies for dehalogenation of EDB, 1,2-DCA, and associated daughter products. Table S-3 compares Gibbs free energy values for hydrogenolysis, dihaloelimination, and dehydrohalogenation of EDB, 1,2-DCA and their associated daughter products under standard and actual conditions for the source and midgradient microcosms. Table S-4 lists Gibbs free energy of formation values, Henry's law constants, and aqueous concentrations that were used in calculations for Table S-3. Transformations of EDB and its potential brominated daughter products are more thermodynamically favorable than for 1,2-DCA and its chlorinated daughter products in all reactions, with one exception: hydrogenolysis of vinyl chloride is slightly more favorable than vinyl bromide. It should be noted, however, that vinyl chloride and vinyl bromide did not accumulate in any of the microcosms in this study.

Table S-3. Comparison of Gibbs Free Energies for Transformation of EDB, 1,2-DCA and Associated Daughter Products.

Transformation Process	Reaction	$\Delta G^{o'}$ (kJ/mol)[a]	ΔG (kJ/mol), Source Zone[b]	ΔG (kJ/mol), Midgradient Zone[b]
Dihaloelimination	EDB + H_2 –> ethene + $2Br^-$ + $2H^+$	-195.0	-244.9	-259.8
	1,2-DCA + H_2 –> ethene + $2Cl^-$ + $2H^+$	-188.3	-225.5	-247.9
Hydrogenolysis	EDB + H_2 –> bromoethane + Br^- + H^+	-153.5	-169.9	-177.4
	1,2-DCA + H_2 –> chloroethane + Cl^- + H^+	-152.3	-162.4	-154.9
	bromoethane + H_2 –> ethane + Br^- + H^+	-140.4	-156.8	-164.3
	chloroethane + H_2 –> ethane + Cl^- + H^+	-134.9	-145.0	-137.5
	vinyl bromide + H_2 –> ethene + Br^- + H^+	-148.4	-151.3	-158.7
	vinyl chloride + H_2 –> ethene + Cl^- + H^+	-149.8	-159.8	-152.4
	ethene + H_2 –> ethane	-99.0	-99.4	-100.0
Dehydrohalogenation	EDB –> vinyl bromide + Br^- + H^+	-46.6	-63.0	-70.4
	1,2-DCA –> vinyl chloride + Cl^- + H^+	-38.5	-48.5	-41.1

[a] Calculated using the aqueous Gibbs free energies of formation in Table S-4. Temperature = 25°C; all reactants and products at 1 M or 1 atm except H^+, pH = 7.0.

[b] ΔG calculated from $\Delta G^{o'}$ using the Nernst equation and the field conditions specified in Table S-4.

Table S-4. Data Used for Gibbs Free Energy Calculations Presented in Table S-3.

	$\Delta G^o_{f(g)}$		Henry's law constant[a]		$\Delta G^o_{f(aq)}$		Field Concentrations	
Compound	**kJ/mol**	**Source**	**atm·m^3/mol**	**Source**	**kJ/mol**	**Source**	**Source Zone**	**Midgradient Zone**
EDB	-10.60	(*6*)	0.0006664	(*7*)	-11.82[b]	-	1.33E-06 M	6.67E-08 M
1,2-DCA	-73.90	(*6*)	0.0014400	(*7*)	-73.22[b]	-	1.00E-05 M	4.00E-07 M
Bromoethane	-26.33	(*6*)	0.0075006	(*8*)	-21.34[b]	-	1.00E-06 M	1.00E-06 M
Chloroethane	-60.00	(*6*)	0.0104458	(*9*)	-54.20[b]	-	1.00E-06 M	1.00E-06 M
Vinyl bromide	81.06	(*10*)	0.0062300	(*11*)	23.83[b]	-	1.00E-06 M	1.00E-06 M
Vinyl chloride	51.54	(*6*)	0.0263497	(*7*)	59.46[b]	-	1.00E-06 M	1.00E-06 M
Ethene	68.16	(*6*)	0.1771136	(*12*)	80.97[b]	-	2.75E-02 M	6.67E-03 M
Ethane	-32.95	(*6*)	0.4232135	(*12*)	-18.04[b]	-	2.41E-02 M	5.00E-03 M
Bromide	-	-	-	-	-103.97	(*13*)	1.33E-06 M	6.67E-08 M
Chloride	-	-	-	-	-131.30	(*13*)	1.73E-05 M	3.47E-04 M
H^+ (pH = 7)	-	-	-	-	-39.83	(*14*)	6.40E-07 M	6.40E-07 M
H_2	-	-	-	-	0.00	(*14*)	1.00E-03 atm	1.00E-03 atm

[a] T = 25°C

[b] $\Delta G^o_{f(aq)} = \Delta G^o_{f(g)} + RT(\ln H)$

Comparison of EDB, 1,2-DCA and BTEX first order biodegradation rates. First order biodegradation rates observed in the microcosms (Figure 2) were compared to in situ rates of decay at the Clemson, South Carolina UST site sampled for this study. Rates for the UST site were estimated with the following first order decay model, assuming steady state conditions (*15*):

$$C(x) = C_o e^{\frac{-x}{v}\lambda_p} \quad \text{(S4)}$$

where *C(x)* is the contaminant concentration (µg/L) as a function of distance downgradient of the source, C_o is the source zone monitoring well concentration (µg/L), x is distance (m) between the source and midgradient monitoring wells, v is seepage velocity (m/yr), and λ_p is the pseudo-fist order rate of decay (yr^{-1}). The resulting in situ decay rates are quite similar to the source zone microcosm decay rates (Table S-5). In the case of EDB, the two rates are nearly identical; the 1,2-DCA microcosm rate is 30% higher than the field rate. It should be noted that this comparison is based upon concentrations trends between the source and midgradient monitoring wells (MW-1 and MW-3, respectively) from which soil and groundwater samples were taken to prepare the microcosms. Since only two wells were utilized for this comparison, calculated field decay rates may not be strictly representative of actual in situ decay rates. The BTEX microcosm rates are 1.5 to 2.6 times higher than the rates estimated from the Clemson UST field data.

Table S-5. Comparison of First Order Biodegradation Rates (yr^{-1}).

Compound	This Study[a]				Clemson UST Site[b]	Other Field Studies[c]
	Source Zone		Midgradient Zone			
	NA	BST	NA	BST		
EDB	1.5 ± 1.0	5.5 ± 1.2	5.4 ± 0.3	9.4 ± 0.2	1.3	1.2 - 137
1,2-DCA	1.3 ± 0.3	0.8 ± 0.1	0.3 ± 0.1	0.7 ± 0.2	0.9	0.73
Benzene	1.5 ± 0.2	2.3 ± 0.2	3.5 ± 0.8	3.1 ± 0.4	1.0	4.4
Toluene	2.7 ± 0.3	2.3 ± 0.3	15 ± 3.3	12 ± 1.0	1.1	83
Ethylbenzene	2.6 ± 0.3	1.7 ± 0.2	9.3 ± 1.2	11 ± 1.0	0.9	30
o-Xylene	2.3 ± 0.3	1.3 ± 0.1	9.5 ± 1.7	11 ± 1.2	0.6	4.4

[a] From Figure 2 in the manuscript.
[b] Calculated using equation S4, based on concentration data in Table S-6, x = 5.97 m, and v = 3.79 m/yr.
[c] From reference (*16*).

Table S-6. Field Concentration Data Used to Calculate First Order Biodegradation Rates for the Clemson UST Site.

Compound	Source Zone Concentration (µg/L)	Midgradient Zone Concentration (µg/L)
EDB	320	13
1,2-DCA	860	96
Benzene	35,578	2,669
Toluene	17,068	1,063
Ethylbenzene	2,581	243
o-Xylene	3,286	623

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