Supporting Information

Concurrent Ethene Generation and Growth of *Dehalococcoides* Containing Vinyl Chloride Reductive Dehalogenase Genes During an Enhanced Reductive Dechlorination Field Demonstration

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1. Tracer Tests.

Methods. Two separate pulse input tracer tests were conducted to estimate flow velocity, residence times, influence area, and dilution in the PTA. Lithium bromide was used in the first test (Day 0), while sodium bromide was used in the second test (Day 69). Both tests involved a 14-day pulse period in which tracer solution (ca. 222 g L^{-1} LiBr or NaBr) was metered into the injection stream in 6.7 mL aliquots every 5 minutes, achieving a time-weighted average (TWA) concentration of 60 to 90 mg Br L^{-1}. Performance monitoring wells were sampled with varying frequencies to detect tracer breakthrough. Results of the first tracer test were used to calibrate the flow model.

PTA Hydraulics results. The results of the tracer tests indicated that the average groundwater pore velocity in the PTA over the 201 day operational period was relatively constant at app. 0.50 m d^{-1} (data not shown). The average hydraulic residence time between injection and wells B119, M101, M2 and M3 was 7 d, 20 d, 37 d and 68 d, respectively. The average hydraulic residence time between the injection wells and extraction well was approximately 62 days, indicating that approximately three aquifer pore volumes were recirculated during the 201 day test. *A priori* particle tracking predicted that the PTA system would capture > 90% of the recirculated groundwater within a treatment area of 1120 m^2; however, the tracer test results indicated that the actual treatment zone area was significantly larger (1500 m^2 to 1800 m^2 [data not shown]).

2. Procedures for Analysis of Biotreatability Study and PTA Samples.

Samples for the determination of chloroethenes, ethene and ethane were analyzed with an Agilent 6890N gas chromatograph (GC) equipped with an Agilent 5973 mass spectrometer (MS). Aqueous samples (1 mL) were injected into sealed vials, acidified (with 0.5 mL 4% H_2SO_4), heated to 80^{o}C, and the headspace was sampled and injected via autosampler into the GC. Separation was performed on a 25.0 m × 320 µm × 1.00 µm (nominal) capillary column (J&W GSQ) with helium (class 2) as carrier gas. Chloroform was used as internal standard (0.5 mL of a 10 ppmv aqueous solution). Detection limits for the chlorinated ethenes and ethene were below 1 µg L^{-1} whereas for ethane it was 2.6 µg L^{-1}. Samples for analysis of short-chained organic acids (lactate, acetate, formate, and propionate) were filtered through 0.45 µm nylon filter membrane acidified with 50 µL 17% H_3PO_4 per mL of sample, and frozen until analysis by suppressed ion chromatography (IC) using a Dionex IonPac ICE AS1 (9 × 250 mm) ion exclusion column, Dionex AMMS ICE II suppressor, and WATERS 432 conductivity detector (eluent: 4 mM heptafluorobutyric acid in 2% vol isopropanol; chemical suppression: 5 mM tetrabutyl ammonium hydroxide). Detection limits for the short-chained organic acids were below 0.01 mM. Anions were analyzed by IC (Dionex DX-120) using a two column set-up (Dionex Ion Pac AS14 (4 × 250 mm) column and an AG 14 (4 × 250 mm) column) with 3.5 mM Na_2CO_3 / 1 mM $NaHCO_3$ as eluent. Detection limits for anions were below 0.2 mg L^{-1}. Samples for analysis of cations (iron and lithium) were filtered through 0.45 µm nylon filters, acidified with 1M HNO_3 (4 drops per 20 mL sample), refrigerated at 4^{o}C until analysis, and analyzed by atomic absorption (Perkin Elmer Instruments Analyst 200 AAS 5000) with flame detection at wavelengths of 248.33 nm and 670.8 nm for iron and lithium, respectively. Detection limits were below 0.01 mM. To avoid potential bias of sampling artifacts, samples for H_2 analysis were collected prior to all other samples using a peristaltic pump without downhole metallic components. Gaseous samples were collected from bubbles off-gassing from a constant water flow pumped through a glass tube for trapping bubbles (~0.1 mm). When sufficient gas volume (~ 2 mL) had accumulated, a sample was collected from the bubble through a septum on the glass tube and analyzed immediately with a Trace Analytical RGD2 GC with two columns in series: a 6' Carbosieve pre-column with back flush and a 3' 13X molsieve column. Samples (up to 5) were analyzed

until successive measurements were within 10%. The detection limit was 0.008 nM H_2. Groundwater samples (3 mL) for analysis of methane were injected into a 5 mL evacuated sample vial (Exetainer® Vial 819W, Labco Ltd., UK) containing 4 drops of H_2SO_4 (0.1 M). Headspace samples were injected manually as direct on-column injections into a Shimadzu 14A GC equipped with a packed column (3% SP1500 Carbopack B) and a flame ionization detector. Methane was analyzed with an isothermal column temperature of 100 °C. The detection limit was 0.003 mM. Microcosm samples for analyis of methane and H_2 were collected by extracting headspace samples (200 µL and 400 µL, respectively) followed by on-column injection for GC analysis. The headspace concentrations were converted to aqueous concentrations by using Henry's law (Syracuse Research Corporation Environmental Science PhysProp Database: http://www.syrres.com/esc/physdemo.htm. Accessed July 2008).

3. Pre-Design Biotreatability Study.

Methods. A pre-design biotreatability study was conducted to assess the dechlorination capabilities of the indigenous bacteria and to measure parameters for design of the ERD system (i.e., electron donor longevity, treatment rates, etc). The study compared two electron donor treatments (lactate and propionate) and included live and deactivated controls to quantify intrinsic biodegradation capacity and abiotic losses, respectively. Laboratory batch microcosms were assembled using aquifer material collected from three well locations in PTA (M1, M2, and M4). Three sets of microcosms were constructed in parallel for the three sample locations, with each set of microcosms employing the same control and treatment conditions. Duplicate microcosms were assembled for each treatment and control using 320 mL borosilicate glass bottles containing approximately 100 g of sediment, 200 mL of groundwater, and 80 mL headspace. The microcosms were prepared inside an anaerobic glovebox (COY Anaerobic Chamber, COY Laboratory Products Inc., Michigan, US) containing N_2 (98%) and H_2 (2%) and sealed with Teflon™-coated butyl rubber septa and aluminum screw caps. The final set up was performed outside the glove box and to remove H_2, the groundwater was purged with a N_2/CO_2 mixture (80%:20%). To homogenize chloroethene concentrations across treatments, all microcosms were spiked with 1,500 µg L^{-1} TCE by injection of 1 mL TCE aqueous stock solution (0.3 g L^{-1}). Treatment microcosms initially received either 6 mM lactate or 4.2 mM propionate from concentrated stock solutions. To evaluate the effect on bioaugmentation with *Dehalococcoides-vcr* on treatment performance, 200 µL of the dehalorespiring culture KB-1 (10^7-10^8 total *Dehalococcoides* cells mL^{-1}) was added to half the treatment microcosms after 57 days. Deactivated control microcosms were sterilized via amendment with $HgCl_2$ (2 mL 5%-solution). All the microcosms were incubated at 10°C for 320 days, and sampled periodically to analyze for anions (nitrate, and sulfate), dissolved iron, hydrogen gas, short-chained organic acids (lactate, acetate, formate, and propionate), chloroethenes, and dissolved hydrocarbon gases (methane, ethene, and ethane).

Results. Figure S1 and Table S1 summarize the results from the treatability test. TCE was conserved in deactivated control microcosms without significant loss (data not shown). Example of TCE degradation results (for well M1) in the intrinsic control and treatment microcosms are shown in Figure S1. In the intrinsic controls, TCE was dechlorinated to cDCE within approximately 48 days, but dechlorination subsequently stalled and did not proceed past cDCE during the remainder of the 328 day incubation (Figure S1A). The addition of lactate accelerated the rate of TCE dechlorination, with complete stoichiometric conversion to cDCE within 27 days. Subsequent dechlorination of cDCE in the lactate-amended microcosms stalled for approximately 100 days (Figure S1B), and then proceeded slowly to

VC and subsequently ethene. Significant ethene generation was evident at 173 days, with mass balance conversion to ethene by 250 days. In microcosms amended with both lactate and KB-1 (Figure S1C), cDCE was converted stoichiometrically to ethene within approximately 45 to 80 days after bioaugmentation (approximately 3 times faster than with lactate alone). Chloroethene degradation and ethene-generation trends in the propionate-amended microcosms (data not shown) resembled trends in the lactate-amended microcosms. However, in the propionate-amended microcosms, dechlorination generally occurred more slowly in comparison with the lactate-amended.

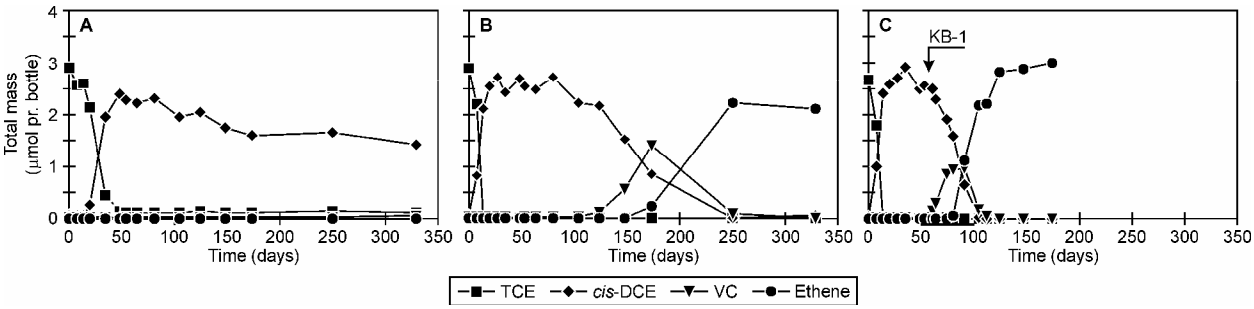

FIGURE S1. TCE dechlorination in microcosms prepared using aquifer material from well M1. A. Intrinsic control; B. Lactate-amended; and C. Lactate and KB-1®-amended.

TABLE S1. Treatability Study Results Summary.

Sample I.D.	Treatment	Terminal Product	Days to Complete Conversion to cDCE	Days to VC Formation	Days to Mass-Balance Ethene	Days to Mass-Balance Ethene After KB-1
M1	Intrinsic	cDCE	48	-	-	-
	Lactate	ETH	27	116	250	-
	Lactate+KB-1	ETH	30	60	123	66
	Propionate	VC/ETH	48	161	>251	-
	Propionate+KB-1	ETH	35	60	136	79
M2	Intrinsic	VC	8	173	-	-
	Lactate	VC	8	148	-	-
	Lactate+KB-1	ETH	8	60	136	79
	Propionate	VC	14	174	-	-
	Propionate+KB-1	ETH	13	60	148	91
M4	Intrinsic	cDCE	80	-	-	-
	Lactate	ETH	35	173	250	-
	Lactate+KB-1	ETH	20	60	100	43
	Propionate	ETH	114	230	290	-
	Propionate+KB-1	ETH	74	64	112	55

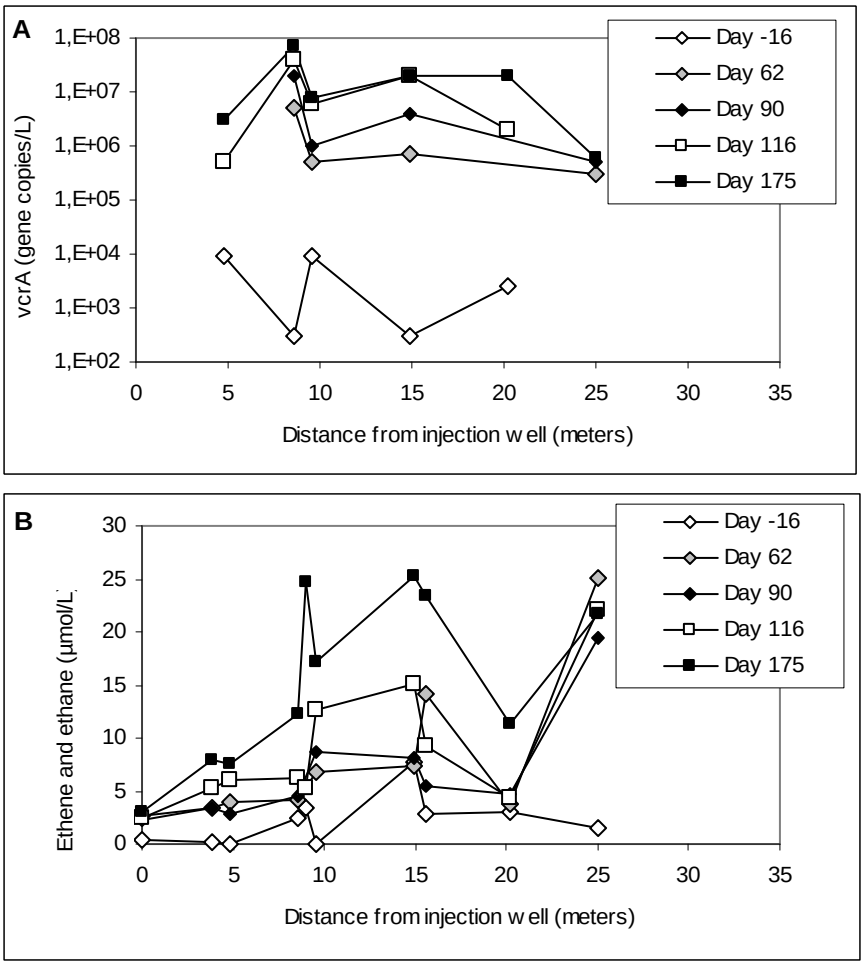

Figure S2. Concentrations of Dehalococcoides containing vcrA (A) and ethene (B) in the PTA on Day -16, Day 62, Day 90, Day 116 and Day 175.

Percent Identity

	1	2	3	4	5	6	7	8	
1		99.9	99.9	100.0	22.2	36.1	36.4	99.8	1
2	0.1		100.0	99.9	23.9	33.2	36.3	98.0	2
3	0.1	0.0		99.9	22.2	36.0	36.3	99.7	3
4	0.0	0.1	0.1		22.2	36.1	36.4	99.8	4
5	51.3	50.8	51.3	51.3		23.0	26.3	22.4	5
6	52.5	52.9	52.5	52.5	84.6		98.3	36.1	6
7	52.0	53.0	52.0	52.0	79.1	1.7		36.0	7
8	0.2	0.6	0.3	0.2	57.4	49.4	52.3		8
	1	2	3	4	5	6	7	8	

Divergence

1 DQ177519_ KB1RdhAB14.seq
2 KB 1 full.seq
3 GDNA 3331 VCR full comp_mod.seq
4 GDNA 3334 VCR full comp_mod.seq
5 AY553222_BAV 1 putative vinyl chloride.
6 AY 563562 BAV1 putative vinyl chloride.
7 AB268343 uncultured bacterium vcr.seq
8 AY 322364 Bacterium VS vinyl chloride dehalogenase.seq

FIGURE S3. Sequence distance matrix output DNASTAR Lasergene (MegAlign) indicating that M101 (Day 201) and B119 (Day 201) are closely related to each other (99.9%) and 100% related to either published KB-1 VCR (in the case of B119) or the KB-1 VCR sequence obtained in this study from KB-1 DNA (in the case of M101). The only position where a potential mismatch was identified between the M101 *vcrA* sequence and the published KB-1 *vcrA* sequence was at base pair position 601. This may indicate a real difference in the sequences or a sequencing error. The *vcrA* sequences from B119 and M101 were also aligned

against the sequence of *vcrA* from *Dehalococcoides* strain VS (GenBank Accession AY322364) and the *vcrA* sequence for B119 had three mismatches at consensus positions 427, 661 and 808, indicating that the indigenous *vcrA* was not as closely related to the strain VS *vcrA* as they were to the KB-1 *vcrA.* All other published VCR genes compared demonstrated lower homology to each other and to the sequences derived from the site.

TABLE S2. Field Demonstration Results Summary.

Well	Distance from injection well (m)	Average hydraulic residence time (days)	Days to initial dechlorination of cDCE to VC	Days to initial dechlorination of VC to ethene	Molar fraction (%) of ethene+ethane[#] at Day -16 and Day 201
I102	0	0	15	15	2 - 42
B103	3.8	3	25	25	1 - 94
B119d	4.8	7	15	25	2 - 78
M102	8.0	17	25	25	3 - 45
M1	9.0	19	116*	116*	12 - 93
M101	9.6	20	62	62	2 - 33
M2	15	37	62	76	5 - 76
B123	15.6	37	62	116	4 - 23
M103	19	63	116	132	1 - 22
M3	25	68	62	62	3 - 62
AV1	29	76	62	116	3 - 42

* No measurements between Day -16 and Day 116.

[#] In general ethane comprised <5% of the sum of ethene+ethane