Supporting Information for

Mechanistic dichotomy in bacterial trichloroethene dechlorination revealed by carbon and chlorine isotope effects

Christina Lihl[†], Lisa M. Douglas[‡], Steffi Franke[§], Alfredo Pérez-de-Mora[†], Armin H. Meyer[†], Martina Daubmeier[†], Elizabeth A. Edwards[₸], Ivonne Nijenhuis[§], Barbara Sherwood Lollar[‡] and Martin Elsner[†,‖,*]

[†] Institute of Groundwater Ecology, Helmholtz Zentrum München, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany

[‡] Department of Earth Sciences, University of Toronto, Toronto, Ontario M5S 3B5, Canada

[§] Department for Isotope Biogeochemistry, Helmholtz-Centre for Environmental Research − UFZ, Permoserstrasse 15, 04318 Leipzig, Germany

[₸] Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, M5S 3E5, Canada

[‖] Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich, Marchioninistrasse 17, 81377 Munich, Germany

Supporting Information Summary: 9 pages, 2 Figure and 4 Tables

MATERIALS AND METHODS

Concentration measurements of *cis*-DCE and TCE. The concentrations of the substrates *cis*-DCE and TCE and the dechlorination products *cis*-DCE and VC were measured by gas chromatography coupled to a flame ionization detector (GC-FID, Varian CP-3800, Hewlett Packard 5980 Series II, Varian CP-3400). Samples were injected by a CombiPal autosampler (CTC Analytics). To achieve optimal separation appropriate columns (e.g.: GS-Q fused silica capillary column (30 m x 0.53 mm)) were used together with suitable GC temperature programs (e.g., start at 100 °C (1 min), increase to 225 °C at 50 °C/min and hold for 2.5 min). Injector and detector temperatures were between 200 °C and 250 °C. For TCE reductive dechlorination experiments changes in TCE, *cis*-DCE and partly VC concentrations over time are depicted in Figure S1.

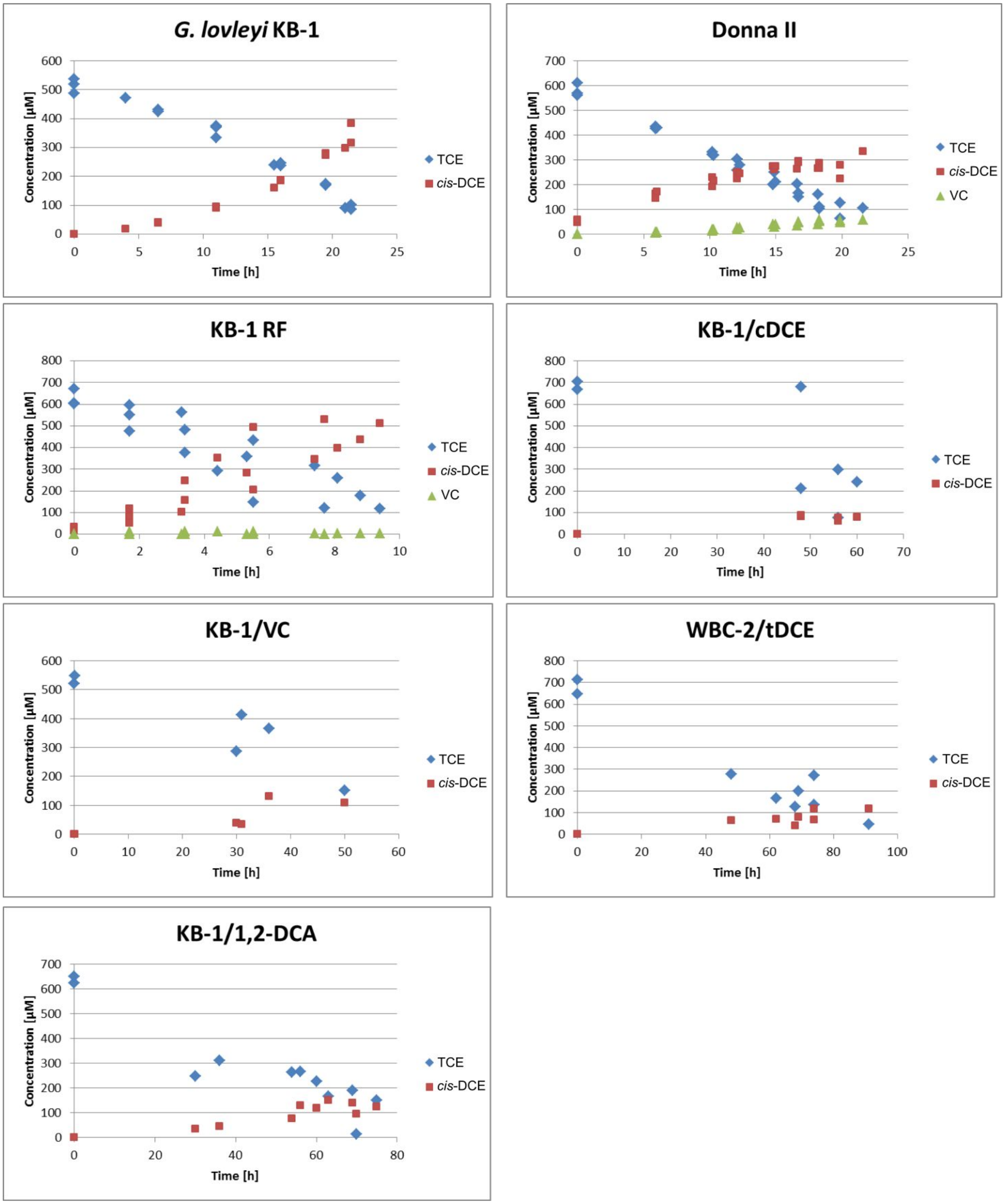

Figure S1. Concentration measurements during TCE reductive dechlorination experiments.

Stable carbon isotope analysis of *cis*-DCE and TCE. *cis*-DCE samples were transferred into a 40 ml purge & trap vial with a PTFE coated septum. For preconcentration the samples were taken up by a purge & trap system (Teledyne Tekmar, Velocity XTP Purge & Trap). TCE samples were thawed at room temperature and transferred into 10 ml headspace vials. Carbon isotope analysis was performed on a gas chromatograph (Thermo Scientific, Trace GC Ultra) coupled to an isotope ratio mass spectrometer (Thermo Scientific, Finnegan MAT 253 IRMS) via a GC/C III combustion interface at 940 °C. To achieve optimal separation a Vocol column (Supelco, 30 m x 0.25 mm, 1.5 µm film thickness) was used. For *cis*-DCE the injection into the GC was performed automatically by the purge & trap system at a split ratio of 1:10. TCE samples from the headspace (1 ml) were injected by a Concept autosampler (PAS Technology) at a split ratio of 6:1. Suitable GC temperature programs were used (e.g.: start at 60 °C (4 min), increase to 80 °C at 25 °C/min and hold for 1 min, increased to 200 °C at 30 °C/min and hold for 1 min). The analytical uncertainty 2σ of carbon isotope analysis was ±0.5 ‰.

Stable chlorine isotope analysis of *cis*-DCE and TCE. Samples were thawed and transferred into a 10 ml headspace vial which were closed and crimped with PTFE coated septa. The method for chlorine isotope analysis was adapted from Shouakar-Stash et al.[1]. Measurements were performed on the same GC-IRMS system as described above with the exception that the GC/C combustion interface was bypassed with a transfer line so that *cis*-DCE and TCE were directly transferred from the GC to the IRMS in a He carrier stream. There, the compounds were ionized and fragmented for isotope ratio analysis at the masses m/z 96/98 (*cis*-DCE) and m/z 95/97 (TCE), respectively. To achieve optimal separation a Vocol column (Supelco, 30 m x 0.25 mm, 1.5 µm film thickness) was used. Samples from the headspace (1 ml) were injected into the GC at a split ratio of 1:10. A typical GC oven temperature program was run (e.g.: start at 65 °C (2 min), increase to 92 °C at 10 °C/min and increase to 175 °C at 60 °C/min). *cis*-DCE and TCE reference gas pulses were injected via a dual inlet system at the beginning and end of each measurement as described in Bernstein et al.[2]. External two-point calibrations were performed with characterized standards of *cis*-DCE ("cisF" ($\delta^{37}Cl$ = -1.52 ‰) and "IS63" ($\delta^{37}Cl$ = +0.07 ‰)) and TCE ("Eil-1" ($\delta^{37}Cl$ = -2.7 ‰) and "Eil-2" ($\delta^{37}Cl$ = +3.05 ‰) (Department of Earth Sciences, University of Waterloo)) and used to convert measurements to $\delta^{37}Cl$ values relative to Standard Mean Ocean Chloride (SMOC)[3].

Multiple measurements of these standards were performed before, during and at the end of each sequence, in order to calibrate the obtained values of the samples. The analytical uncertainty 2σ of chlorine isotope analysis was ±0.2 ‰.

qPCR analysis of KB-1/1,2-DCA, KB-1/VC, KB-1/cDCE and WBC-2/tDCE.

The following Tables S1 – S3 contain detailed information regarding the qPCR analysis.

Table S1: Primer sets, sequences and annealing temperatures for qPCR analyses.

Target	Primer	5' → 3' Sequence	Annealing Temp. [°C]	Reference
vcrA	VcrA 670F VcrA 440R	GCCCTCCAGATGCTCCCTTTAC TGCCCTTCCTCACCACTACCAG	60	Molenda et al. 2016[4]
tceA	TceA_500F TceA_795R	TAATATATGCCGCCACGAATGG ATCGTATACCAAGGCCCGAGG	64	Fung et al. 2007[5]
bvcA	Rdh6_318F Rdh6_555R	ATTTAGCGTGGGCAAAACAG CCTTCCCACCTTGGGTATTT	60	Waller et al. 2005[6]
tdrA	TdrA1404F TdrA1516R	GCCTCTCGCCCCCACTAAACC GCCATCCTTCATAACCACTCACGCA	62.5	Molenda et al. 2016[4]
Dehalococcoides	Dhc 1F Dhc 264R	GATGAACGCTAGCGGCG CCTCTCAGACCAGCTACCGATCGAA	60	Grostern & Edwards 2009[7]
Dehalogenimonas	Dhg273F Dhg537R	TAGCTCCCGGTCGCCCG CCTCACCAGGGTTTGACATGTTAGAAG	59	Manchester et al. 2012[8]
Total Archaea	Arch 787F Arch 1059R	ATTAGATACCCGBGTAGTCC GCCATGCACCWCCTCT	60	Yu et al. 2005[9]
Total Bacteria	Bac 1055F Bac 1392R	ATGGCTGTCGTCAGCT ACGGGCGGTGTGTAC	55	Dionisi et al. 2003[10]

Table S2: Quality details for qPCR analyses of *rdhA* genes, *Dehalococcoides* 16S rRNA and *Dehalogenimonas*16S rRNA gene copies.

Reaction	Highest Std. (copies/µl)	Lowest Std. (copies/µl)	Highest Blank (copies/µl)	Efficiency (%)	R^2	Detection Limit (copies/ml)
vcrA	4.00E+07	4.00E+02	4.00E+03	93.4	0.981	2.50E+05
bvcA	1.07E+06	1.07E+00	1.07E+00	92.5	0.997	6.69E+01
tceA	3.86E+07	3.86E+04	3.86E+02	95.9	0.998	2.41E+06
tdrA	4.66E+07	4.66E+01	4.66E+02	87	0.996	2.91E+04
Dehalococcoides	6.90E+07	6.90E+02	6.90E+02	81.7	0.996	4.31E+04
Dehalogenimonas	4.59E+07	4.59E+02	4.59E+03	96.1	0.997	2.87E+05
General Archaea	1.08E+07	1.08E+03	1.08E+03	81.6	0.997	6.75E+04
General Bacteria	4.59E+07	4.59E+02	4.59E+02	88.9	0.996	2.87E+04

Table S3: qPCR analyses of *rdhA* genes, *Dehalococcoides* 16S rRNA and *Dehalogenimonas* 16S rRNA gene copies in *Dehalococcoides*-containing mixed cultures KB-1/1,2-DCA, KB-1/VC, KB-1/cDCE, and WBC-2/tDCE following TCE dehalogenation experiments.

	KB-1/1,2-DCA	KB-1/VC	KB-1/cDCE	WBC-2/tDCE
***vcrA* (copies/ml)**	1.20E+08 5.30E+07	5.60E+07 5.00E+07	8.30E+07 9.60E+07	1.30E+08 5.40E+07
***bvcA* (copies/ml)**	6.00E+03 5.50E+03	- -	6.00E+03 5.50E+03	2.20E+04 1.20E+03
***tceA* (copies/ml)**	1.10E+08 5.70E+07	- -	2.90E+07 1.50E+07	8.70E+06 5.80E+06
***tdrA* (copies/ml)**	1.10E+05 1.60E+05	1.90E+05 2.60E+05	1.90E+05 2.60E+05	6.90E+07 5.10E+07
***Dehalococcoides* (copies/ml)**	7.40E+07	3.70E+07 2.60E+07	4.30E+07 3.70E+07	5.60E+07 3.90E+07
***Dehalogenimonas* (copies/ml)**	- -	- -	- -	1.40E+07 1.30E+07
General Bacteria (copies/ml)	1.40E+08 7.70E+07	8.60E+07 7.50E+07	1.50E+08 9.50E+07	9.90E+07 3.60E+07
General Archea (copies/ml)	2.70E+06 2.50E+06	1.40E+06 2.70E+06	7.90E+06 2.90E+06	2.30E+06 2.30E+06

* Samples shaded in grey were below the detection limit.

RESULTS

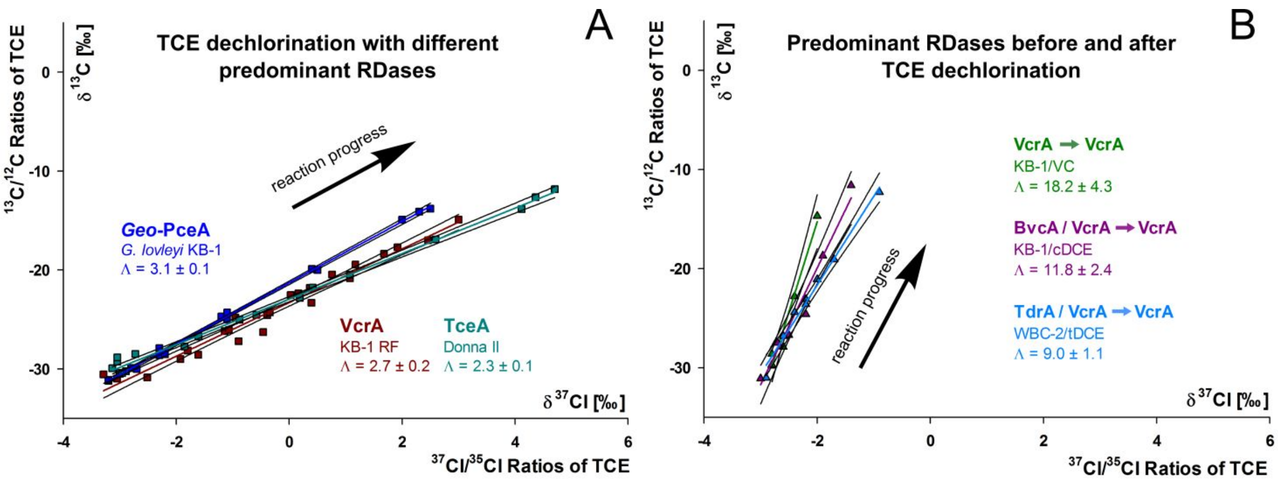

Figure S2. Carbon and chlorine isotope effects in TCE dechlorination by (A) *G. lovleyi* KB-1 (blue), KB-1 RF (brown) and Donna II (cyan) and (B) KB-1/VC (green), KB-1/cDCE (purple) and WBC-2/tDCE (light blue) with special regards to predominant RDases. (95 % confidence intervals are given as values and as black lines next to the regression slopes).

Table S4: Data of previous studies used for Figure 5.

	System	Ɛ(C) [‰]	95% CI	Λ	95% CI	Study
TCE	*Desulfitobacterium hafniense* Y51	-9.1	0.6	3.4	0.2	Cretnik et al. 2013[3]
	Geobacter lovleyi strain SZ	-12.2	0.5	3.4	0.2	
	Enrichment culture (*Desulfitobacterium aromaticivorans*)	-8.8	0.2	2.5		Wiegert et al. 2013[11]
	Desulfitobacterium hafniense Y51	-8.8	0.2	3.4	0.2	Buchner et al. 2015[12]
	Desulfitobacterium hafniense Y51	-9.0	0.2	2.8	0.3	
	Desulfitobacterium hafniense Y51	-9.0	0.2	3.5	0.2	
	Desulfitobacterium hafniense Y51	-8.6	0.0	3.2	0.2	
	Dehalococcoides culture (two species)	-16.4	0.4	4.5		Kuder et al. 2013[13]
	Aquifer microcosms	-12.2	1.0	3.4	0.1	Dogan-Subasi et al. 2017[14]
	RDase from *Sulfurospirillum multivorans* (norpseudo-B12)	-20.0	0.5	5.3	0.3	Renpenning et al. 2014[15]
	RDase from *Sulfurospirillum multivorans* (nor-B12)	-20.2	1.1	5.0	0.8	
	norpseudo-B12 (purified cofactor)	-18.5	2.8	4.5	0.8	
	nor-B12 (purified cofactor)	-15.1	2.7	3.7	0.3	
	dicyanocobinamid (purified cofactor)	-16.5	0.7	4.2	0.6	
	cyano-vitamin B12 (purified cofactor)	-15.0	2.0	4.4	0.7	
	Cyanocobalamin (purified cofactor)	-16.1	0.9	3.9	0.2	Cretnik et al. 2013[3]
	Cobaloxime (model system)	-21.3	0.5	6.1	0.5	
	Vitamin B12 pH 5.0 (model system)	-16.3	0.9	12.8	1.4	Heckel et al. 2018[16]
	Vitamin B12 pH 5.5 (model system)	-15.8	0.9	9.1	0.5	
	Vitamin B12 pH 6.5 (model system)	-16.3	1.1	5.2	0.2	
	Vitamin B12 pH 11 (model system)	-17.5	1.0	3.3	0.1	
	Geobacter lovleyi strain KB-1	-10.3	0.8	3.1	0.1	this study.
	KB-1 RF	-9.6	0.5	2.7	0.2	
	Donna II	-13.5	0.6	2.3	0.1	
	KB-1/cDCE	-8.3	3.4	11.8	2.4	
	KB-1/VC	-10.6	9.3	18.2	4.3	
	WBC-2/tDCE	-7.0	1.9	9.0	1.1	
	KB-1/1,2-DCA	-5.4	1.5	4.5	0.8	
cDCE	KB-1 (containing *Dehalococcoides*)	-18.5	1.8	11.6	0.9	Abe et al. 2009[17]
	Aquifer microcosms	-18.0	4.0	4.5	3.4	Dogan-Subasi et al. 2017[14]
	Vitamin B12 pH 6.5 (model system)	-28.4	1.1	18.2	2.2	Heckel et al. 2018[16]
	D. mccartyi 195	-23.2	4.1	10.0	0.4	this study
	D. mccartyi BTF08	-31.1	6.3	17.8	1.0	
PCE	*Desulfitobacterium sp.* strain Viet1	-19.0	0.9	3.8	0.2	Cretnik et al. 2014[18]
	Vitamin B12 pH 6.5 (model system)	-16.7	1.0	3.9	0.3	Heckel et al. 2018[16]
	Vitamin B12 pH 9.0 (model system)	-17.0	1.2	4.2	0.3	
	Vitamin B12 pH 11 (model system)	-16.6	2.7	3.9	0.4	
	Enrichment culture (*Desulfitobacterium aromaticivorans*)	-5.6	0.7	2.8	0.8	Wiegert et al. 2013[11]
	Sulfurospirillum (PceA-TCE)	-3.6	0.2	2.7	0.3	Badin et al. 2014[19]
	Sulfurospirillum (PceA-DCE)	-0.7	0.1	0.7	0.2	
	RDase from *Sulfurospirillum multivorans* (norpseudo-B12)	-1.4	0.1	2.2	0.7	Renpenning et al. 2014[15]
	RDase from *Sulfurospirillum multivorans* (nor-B12)	-1.3	0.1	2.8	0.5	
	norpseudo-B12 (purified cofactor)	-25.3	0.8	6.9	0.7	
	nor-B12 (purified cofactor)	-23.7	1.2	5.0	0.8	
	cyano-B12 (purified cofactor)	-22.4	0.8	4.6	0.2	
	dicyanocobinamid (purified cofactor)	-25.2	0.5	7.0	0.8	

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