

SUPPORTING INFORMATION

Chlorinated Electron Acceptor Abundance Drives Selection of *Dehalococcoides mccartyi* (*D. mccartyi*) Strains in Dechlorinating Enrichment Cultures and Groundwater Environments

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Table S1. Summary of cultures investigated for the presence of both *Dehalococcoides* and *rdhA* genes.

Culture/KB1 enrichment	Main dechlorinating organism*	Sampling dates	e- donor	e- acceptor	Main reaction	Feeding	Target conc. Of electron acceptor/ Vol. liquid/ Vol. headspace
TCE/M_1998_Parent	<i>Dhc & Geo</i>	11-Jan-11	M	TCE	TCE to ETH	400 µL 5:1 stock (M/TCE)	0.68 mmol L ⁻¹ 1.6L/0.4L
TCE/M_1999	<i>Dhc & Geo</i>	25-Oct-11	M	TCE	TCE to ETH	400 µL 5:1 stock (M/TCE)	0.68 mmol L ⁻¹ 1.6L/0.4L
TCE/M_2010	<i>Dhc & Geo</i>	spring 2011	M	TCE	TCE to ETH	30 µL 5:1 stock (M/TCE)	0.68 mmol L ⁻¹ 0.2L/0.05L
TCE/ME_2001_SiREM	<i>Dhc & Geo</i>	2004, 05, 06, 07 and 09	ME	TCE	TCE to ETH	4 mL 5:1 (M/TCE) 1mL E	0.15-0.19 mmol L ⁻¹ 99L/1L
TCE/H ₂ _2001	<i>Dhc & Geo</i>	11-Jan-11/ 25-Oct-11	H ₂	TCE	TCE to ETH	60 mL- 100 mL H ₂ /15µL TCE	0.19 mmol L ⁻¹ 0.8L/0.2L
cDCE/M_2001	<i>Dhc</i>	7-Jun-11/ 25-Oct-11	M	cDCE	cDCE to ETH	110µL 5:1 stock (M/cDCE)	0.46 mmol L ⁻¹ 1.7L/0.3L
cDCE/M_2003	<i>Dhc</i>	07-Jun-11	M	cDCE	cDCE to ETH	50 µL 5:1 stock (M/cDCE)	0.46 mmol L ⁻¹ / 0.65L/0.35L
VC/M_2001	<i>Dhc</i>	25-Oct-11	M	VC	VC to ETH	53 µL/20 mL VC	0.69 mmol L ⁻¹ 1L/0.2L
VC/H ₂ _2003-1	<i>Dhc</i>	11-Jan-11	H ₂	VC	VC to ETH	20 mL H ₂ /4 mL VC	0.72 mmol L ⁻¹ 0.2L/0.25L
VC/H ₂ _2003-2	<i>Dhc</i>	11-Jan-11/ 25-Oct-11	H ₂	VC	VC to ETH	5 mL H ₂ /1 mL VC	0.16 mmol L ⁻¹ / 0.2L/0.05L
VC/M_2004	<i>Dhc</i>	25-Oct-11	M	VC	VC to ETH	5 µL/2 mL VC	0.69 mmol L ⁻¹ / 0.12L/0.06L
1,2DCA/M_2008_UT	<i>Dhc</i>	11-Jan-11/ 25-Oct-11	M	1,2-DCA	1,2-DCA to ETH	46 µL 5:1 stock (M/1,2-DCA)	0.2 mmol L ⁻¹ 1.5L/0.5L
1,2DCA/ME_2010_SiREM	<i>Dhc</i>	7-Jun-11/ 25-Oct-11	ME	1,2-DCA	1,2-DCA to ETH	50 µL 5:5:1 stock (M/E/1,2-DCA)	1.26 mmol L ⁻¹ / 0.2L /0.05L

cDCE = cis-dichloroethene; DCA = dichloroethane; *Dhc* = *Dehalococcoides*; E = ethanol; ETH = Ethene; *Geo* = *Geobacter*; M = methanol; ME = methanol/ethanol; TCE = trichloroethene; VC = vinyl chloride. *based on PCR, clone libraries and metagenome analysis. The name format for the enrichment cultures indicates electron acceptor amended/donor used_year created (e.g. VC/M_2001 is a VC and methanol enrichment culture first established in 2001 and VC/H₂_2003-2 is a second enrichment culture established in 2003 with VC and hydrogen). Cultures in grey were not used in this study, but are provided for completeness, as they are available for research and have been discussed in other publications.

Table S2. Compiled reductive dehalogenase sequences identified in the dechlorinating enrichment culture KB-1.

Name in paper	OG**	Previous name	Name in Hug et al., 2013 ¹	Accession number	NCBI GI	JGI locust Tag	JGI/IMG name	metagenome contig ID
KB1_1	6	KB1_1	KB1_1 & KB13109_4*	DQ177506	77176847	DCKB1_110270	none	none
KB1_2	11	KB1_2	KB1_2	DQ177507	77176850	DCKB1_110450	2013897470	C3109
KB1_3	12	KB1_3	KB1_3	DQ177508	77176853	DCKB1_11560	2013887593	C299
KB1_4	13	KB1_4	KB1_4	DQ177509	77176856	DCKB1_110180	2013897443	C3109
KB1_5	15	KB1_5 (DET 1545)	KB1_5	DQ177510	77176859	DCKB1_110110	2013897436	C3109
KB1_6 (<i>bvcA</i>)	28	KB1_6 (<i>bvcA</i>)	KB1_6	DQ177511	77176862	none	none	FOFA22070
KB1_7	19	KB1_7	KB1_7 & KB13241_7*	DQ177512	77176865	DCKB1_114830	none	none
KB1_8	17	KB1_8	KB1_8	DQ177513	77176868	DCKB1_11540	2013887591	C299
KB1_9	17	KB1_9	KB1_9	DQ177514	77176871	DCKB1_110210	2013897446	C3109
KB1_10	34	KB1_10	KB1_10 & KB13109_9*	DQ177515	77176874	DCKB1_110480	2013897473	C3109
KB1_11	37	KB1_11	KB1_11 & KB13240_1*	DQ177516	77176877	DCKB1_114790	none	C3240
KB1_12	18	KB1_12	KB1_12	DQ177517	77176880	DCKB1_110630	2013897488	C3109
KB1_13 (<i>pceA</i>)	30	KB1_13 (<i>pceA</i>)	KB1_13	DQ177518	77176883	DCKB1_110540	2013897479	C3109
KB1_14 (<i>vcrA</i>)	8	KB1_14 (<i>vcrA</i>)	KB1_14	DQ177519	77176886	DCKB1_96900	2013896112	C2841
KB1_15	39	C3241_1	KB13241_1	KP085015	733372930	DCKB1_115000	2013897921	C3241
KB1_16	50	C3241_2	KB13241_2	KP085016	733372933	DCKB1_115150	2013897936	C3241
KB1_17	49	C3241_3	KB13241_3	KP085017	733372936	DCKB1_115210	2013897942	C3241
KB1_18	14	C3241_4	KB13241_4	KP085018	733372939	DCKB1_115020	2013897923	C3241
KB1_19	36	C3241_5	KB13241_5	KP085019	733372942	DCKB1_115090	2013897930	C3241
KB1_20	38	C3241_6	KB13241_6	KP085020	733372945	DCKB1_114910	2013897912	C3241
KB1_21	32	C3241_8	KB13241_8	KP085021	733372948	DCKB1_114860	2013897907	C3241
KB1_22	40	KB1_22	KB1_22 & KB13109_7*	JX081249	393716494#	DCKB1_110600	none	none
KB1_23	29	C3107_1	KB13107_1	KP085022	733372951	DCKB1_107470	2013897169	C3107
KB1_24	22	C3107_2	KB13107_2	KP085023	733372954	DCKB1_107520	2013897174	C3107
KB1_25	56	C1024_1	KB11024_1	KP085024	733372957	DCKB1_37290	2013890159	C1024
KB1_26	23	C3108_1 (cons.synt)	KB13108_1	KP085025	733372960	DCKB1_107910	2013897212	C3108
KB1_27 (<i>tceA</i>)	5	C338_1 (<i>tceA</i>)	KB1338_1	KP085026	733372963	DCKB1_14890	2013887924	C338
KB1_28	26	KB1_group26	n/a	KP085027	733372966	DCKB1_13760	2013887811	C338
KB1_29 (partial)	n/a	KB1_F43012	n/a	KP085028	733372969	DCKB1_297220	2013916109	FOFA43012
KB1_30 (partial)	n/a	KB1_F51719	n/a	KP085029	733372971	DCKB1_319470	2013918333	FOFA51719
Geo_rdh	41	KB-1_Geo_rdase	n/a	JX081248	393716492#	DCKB1_87010; DCKB1_86980	none	Geo_RD

*possibly two highly similar sequences (>99%) from different sequencing efforts as presented in reference (1) - Hug *et al.*, 2013.

**OG: ortholog group number, as defined in reference (1)

cons.synt. = conserved syntetic; OG = Ortholog Group

#accession numbers reported in reference (2) - Tang *et al.*, 2013. Note that accession number for Geo_RD in (2) was listed erroneously as 393716494, instead of 393716492.

Table S3. List of rdhA targets with corresponding OG and primer pairs for qPCR employed in this study. Unless specified otherwise, primers were specifically designed for this study.

OTU/functional gene (accession #) Ortholog Group Number	Primer pair	sequence	Annealing (Celsius)	fragment length (bp)
<i>Dehalococcoides 16S rRNA</i> (AY146779.1)	Dhc 1f ⁽³⁾ Dhc 264r ⁽³⁾	5'-GATGAACGCTAGCGCG-3' 5'-CCTCTCAGACCAGCTACCGATCGAA- 3'	60	264
KB1_1 (DQ177506) OG10	KB1_1_246f ⁽⁴⁾ KB1_1_336r ⁽⁴⁾	5'-ATCGGAGCTGCACAAGTAGG- 3' 5'-TCTTGTGAGCGGTGTCTTG- 3'	60	91
KB1_4 (DQ177509) OG13	KB1_4_310f KB1_4_642r	5'-GCTCTTAACGCAGGGCAA- 3' 5'-GCGGCTGGCATCTACAGG- 3'	63	333
KB1_5 (DQ177510) OG15	KB1_5_1017f ⁽⁴⁾ KB1_5_1137r ⁽⁴⁾	5'-GATGCAGGCATTACCGTTT- 3' 5'-GTCTCTTGCCTTCGGTCAG- 3'	60	121
KB1_6 (<i>bvcA</i>) (DQ177511) OG28	KB1_6_318f ⁽⁴⁾ KB1_6_555r ⁽⁴⁾	5'-ATTTAGCGTGGGCAAAACAG- 3' 5'-CCTTCCCACCTGGGTAT TT- 3'	60	238
KB1_11 (DQ177516) OG37	KB1_11_723f KB1_11_943r	5'-CCACCAATATCACCATACCTAA- 3' 5'-ACCGGTGGGAATGAAACG- 3'	59	221
KB1_12 (DQ177517) OG18	KB1_12_804f KB1_12_1041r ⁽⁴⁾	5'-TTCATCACGACAGCTTGGTG- 3' 5'-TTTGGGGGTCTAACTGCTC- 3'	60	238
KB1_14 (<i>vcrA</i>) (DQ177519) OG8	KB1_14_642f ⁽⁴⁾ KB1_14_846r ⁽⁴⁾	5'-GAAAGCTCAGCGATGACTC- 3' 5'-TGGTTGAGGTAGGGTGAAGG- 3'	60	205
KB1_15 (KP085015) OG39	KB1_15_491f KB1_15_847r	5'-GTCGGGAAGAGACTACCGCT- 3' 5'-TGCCAAGACCTGATAGCCT- 3'	66	357
KB1_16 (KP085016) OG50	KB1_16_727f KB1_16_955r	5'-GCCAGCCCCGATACGTGAACTG- 3' 5'-GGAACCGCGCTGAACATGG- 3'	65	229
KB1_17 (KP085017) OG49	KB1_17_259f KB1_17_590r	5'-GTCCTTATAGAACATGCCAGA- 3' 5'-GTACCAATACAAGTCTCTCAG- 3'	63	332
KB1_18 (KP085018) OG14	KB1_18_414f KB1_18_556r	5'-GGCTTATCCTGGTATCGTA- 3' 5'-AACCGATAAACATGGCAC- 3'	57.5	143
KB1_19 (KP085019) OG36	KB1_19_1086f KB1_19_1283r	5'-CCTAACCAACCGGGCAAT- 3' 5'-CGCCAGCCGAGAAAACC- 3'	54	198
KB1_23 (KP085022) OG29	KB1_23_431f KB1_23_636r	5'-TTGGTCAGGGTCGGTCA- 3' 5'-TTCACCAACATTCAAGGACTC- 3'	57	206
KB1_25 (KP085024) OG56	KB1_25_397f KB1_25_613r	5'-TGC GAAATGGCGGGATG- 3' 5'-GCACTGAAGGCCGAATGTAC- 3'	60	216
KB1_27 (<i>tceA</i>) (KP085026) OG5	tceA500f ⁽⁵⁾ tceA795r ⁽⁵⁾	5'-TAATATATGCCGCCACGAATGG- 3' 5'-ATCGTATACCAAGGCCGAGG- 3'	64	296
<i>Geobacter 16S rRNA</i>	Geo_73f ⁽³⁾ Geo_485r ⁽³⁾	5'-CTTGCTTTCATTTAGTGG- 3' 5'-AAGAAAACC GGTTATTAA CC- 3'	66	413
<i>Geobacter_rdhA</i> (JX081248)	GeordhA_812f GeordhA_1045r	5'-AGCTGAGTCAGGGTTTGAGCC- 3' 5'-CCATACGACTGCCCTCACCCAT- 3'	66	234

References: Duhamel and Edwards 2006; Waller *et al.* 2005; Fung *et al.* 2007; OG – ortholog group

Table S4. Mismatches between primers and sequences in Ortholog Groups (OG). Note that primers were designed to target KB1 sequences and not the OG groups.

OTU/functional gene	Primer pair	primer specificity to OG group number of primer-sequence mismatches
KB1_1 OG10 (7 sequences)	KB1_1_246f ⁽⁴⁾	4 mismatches – VS_1421, GY50_1427; 1 mismatch (rest of sequences)
	KB1_1_336r ⁽⁴⁾	3 mismatches – AY374245, btf_1481; 5 mismatches VS_1421, GY50_1427
KB1_4 OG13 (9 sequences)	KB1_4_310f	4 mismatches – VS_1430, GY50_1436
	KB1_4_642r	1 mismatches – VS_1430, GY50_1436
KB1_5 OG15 (10 sequences)	KB1_5_1017f ⁽⁴⁾	4 mismatches – DET_1545
	KB1_5_1137r ⁽⁴⁾	1 mismatches – DET_1545, 2 mismatches – btf_1497, GY50_1443
KB1_6 (<i>bvcA</i>) OG28 (2 sequences)	KB1_6_318f ⁽⁴⁾	0 mismatches
	KB1_6_555r ⁽⁴⁾	0 mismatches
KB1_11 OG37 (3 sequences)	KB1_11_723f	0 mismatches
	KB1_11_943r	0 mismatches
KB1_12 OG18 (3 sequences)	KB1_12_804f	5 mismatches GY50_1408
	KB1_12_1041r ⁽⁴⁾	3 mismatches GY50_1408
KB1_14 (<i>vcrA</i>) OG8 (4 sequences)	KB1_14_642f ⁽⁴⁾	0 mismatches
	KB1_14_846r ⁽⁴⁾	1 mismatches all four sequences
KB1_15 OG39 (5 sequences)	KB1_15_491f	0 mismatches
	KB1_15_847r	0 mismatches
KB1_16 OG50 (3 sequences)	KB1_16_727f	0 mismatches
	KB1_16_955r	0 mismatches
KB1_17 OG49 (3 sequences)	KB1_17_259f	0 mismatches
	KB1_17_590r	0 mismatches
KB1_18 OG14 (6 sequences)	KB1_18_414f	0 mismatches
	KB1_18_556r	0 mismatches
KB1_19 OG36 (3 sequences)	KB1_19_1086f	0 mismatches
	KB1_19_1283r	0 mismatches
KB1_23 OG29 (4 sequences)	KB1_23_431f	1 mismatch – DET_0235; 3 mismatches – GY50_0087
	KB1_23_636r	4 mismatches – GY50_0087; 7 mismatches - DET 0235
KB1_25 OG56 (2 sequences)	KB1_25_397f	0 mismatches
	KB1_25_613r	0 mismatches
KB1_27 (<i>tceA</i>) OG5 (3 sequences)	tceA500f ⁽⁵⁾	0 mismatches
	tceA795r ⁽⁵⁾	0 mismatches
<i>Geobacter_rdhA</i>	GeordhA_812f	0 mismatches
	GeordhA_1045r	0 mismatches

References: Waller *et al.* 2005; Fung *et al.* 2007; OG – ortholog group

Table S5. Details of the standard curves generated for qPCR with the different primer pairs, including average slopes, Y-intercepts, R^2 and their corresponding standard deviations.

Target	m		n		R		Eff		N
	average	stdev	average	stdev	average	stdev	average	stdev	
Dhc 16S	-3.462	0.030	35.179	0.596	0.998	0.002	94.5	1.1	4
KB1_1	-3.225	NA	34.180	NA	0.997	NA	104	NA	1
KB1_4	-3.479	0.201	34.930	1.39	0.999	0.000	94.2	7.8	3
KB1_5	-3.515	NA	35.063	NA	0.999	NA	92.5	NA	1
KB1_6 (bvcA)	-3.488	0.016	35.520	0.373	0.9972	0.002	93.5	0.6	2*
KB1_11	-3.427	0.082	35.215	0.596	0.999	0.000	95.8	3.1	3
KB1_12	-3.350	0.322	36.177	0.327	0.996	0.006	99.2	14	2*
KB1_14 (vcrA)	-3.381	0.142	35.280	1.727	0.998	0.001	97.7	5.7	2*
KB1_15	-3.328	0.136	35.231	0.386	0.994	0.004	99.8	5.6	2*
KB1_16	-3.353	0.126	35.405	1.000	0.998	0.001	96.2	4.7	3
KB1_17	-3.464	0.001	35.759	0	0.997	0.000	94.4	0.0	2*
KB1_18	-3.333	0.043	34.086	1.829	0.998	0.002	99.5	1.8	2*
KB1_19	-3.174	0.001	34.069	0.02	0.998	0.000	107	0.1	2*
KB1_23	-3.442	0.094	34.936	0.109	0.997	0.001	95.3	3.5	3
KB1_25	-3.473	0.167	35.540	0.163	0.997	0.000	94.2	6.2	2*
KB1_27 (tceA)	-3.512	NA	35.087	NA	0.996	NA	92.6	NA	1
Geobacter 16S	-3.520	NA	35.579	NA	0.994	NA	92.3	NA	1
Geobacter_rdhA	-3.394	0.2382	35.161	0.784	0.997	0.0029	99.4	9.8	2*

avg = average; Dhc = *Dehalococcoides*. Eff = efficiency; m = slope; n = Y-intercept; R² = coefficient of determination; N = number of standard curves used for the calculations; NA = not applicable; stdev = standard deviation.

* Note that when N = 2 the range, that is, the difference between the two values available was calculated instead of the standard deviation.

Table S6. Absolute qPCR gene copy numbers per L of culture for each *rdhA* gene used for the calculation of the *rdhA/Dhc* ratios of Figure 2a of the main manuscript. Note that values for the 16S rRNA of *Geobacter* strain KB-1 and the *Geobacter rdhA* gene for some of the cultures are also included, although they do not appear on Figure 2.

	TCE/M 1999 jan11	TCE/H2 2001 jan11	TCE/ME 2001 SiREM oct09	TCE/M 1998 parent oct11	TCE/M 2010 mar11	VC/H2 2003-1 jan11	VC/H2 2003-2 jan11	VC/H2 2003-2 oct11	cDCE/M 2003 jun11	cDCE/M 2001 jun11	cDCE/M 2001 oct11	1,2DCA/ M 2008 UToct11	1,2DCA/ M 2008 UTjan11	1,2DCA/ ME 2010 SiREM
<i>Dhc 16S</i>	1.7E+8	1.5E+9	7.1E+8	4.4E+10	2.3E+10	3.9E+9	1.3E+9	1.3E+10	1.2E+9	1.3E+9	6.9E+9	1.2E+10	5.5E+8	2.2E+9
KB1_25	1.4E+6	1.4E+6	≤8.2E+5	1.6E+9	5.4E+8	≤8.2E+5	≤8.2E+5	≤8.2E+5	≤8.2E+5	≤8.2E+5	≤8.2E+5	2.2E+10	3.5E+8	1.0E+9
KB1_16	1.9E+6	1.8E+6	≤8.7E+5	1.0E+9	6.6E+8	≤8.7E+5	≤8.7E+5	≤8.7E+5	≤8.7E+5	≤8.7E+5	≤8.7E+5	1.4E+10	3.8E+8	1.2E+9
KB1_17	≤5.0E+5	5.0E+5	≤5.0E+5	3.4E+8	2.8E+8	≤5.0E+5	≤5.0E+5	≤5.0E+5	≤5.0E+5	≤5.0E+5	≤5.0E+5	3.9E+9	1.5E+8	5.3E+8
KB1_19	2.6E+6	2.5E+6	9.1E+5	1.7E+9	6.4E+8	9.8E+6	3.2E+6	2.3E+07	≤8.3E+5	≤8.3E+5	≤8.3E+5	1.9E+10	5.7E+8	1.2E+9
KB1_27	3.5E+7	3.9E+7	3.7E+8	1.8E+10	1.0E+6	2.5E+6	8.5E+5	NA	7.0E+5	5.3E+5	NA	NA	4.1E+8	1.1E+9
KB1_6	1.1E+6	3.0E+6	≤1.0 E+6	≤1.0E+6	3.9E+6	≤1.0 E+6	≤1.0 E+6	≤1.0 E+6	5.5E+8	4.1E+8	4.1E+9	1.2E+9	2.1E+7	5.3E+8
KB1_15	3.5E+6	2.7E+6	8.9E+5	1.0E+9	1.2E+9	1.3E+10	2.5E+9	6.0E+9	3.8E+8	5.9E+8	2.4E+9	1.3E+10	7.4E+8	2.2E+9
KB1_18	3.7E+6	1.3E+6	≤1.0E+6	1.2E+9	7.5E+8	6.5E+9	2.4E+9	4.6E+9	1.4E+8	3.0E+8	1.3E+9	1.5E+10	6.7E+8	1.2E+9
KB1_5*	4.1E+8	1.6E+9	7.9E+8	5.0E+10	1.6E+9	1.0E+10	2.9E+9	NA	6.6E+8	1.3E+8	NA	NA	3.4E+8	1.3E+9
KB1_23	3.1E+8	9.4E+8	5.3E+8	3.5E+10	2.2E+10	2.5E+9	5.0E+8	8.6E+9	3.5E+7	5.1E+5	2.9E+6	4.9E+8	9.9E+6	2.0E+8
KB1_12	4.2E+7	6.0E+8	2.2E+9	5.4E+10	6.0E+9	6.3E+8	6.8E+7	1.4E+10	2.2E+8	4.3E+5	9.1E+6	1.8E+9	3.6E+6	6.5E+7
KB1_4	3.1E+8	8.6E+8	5.0E+8	5.0E+10	NA	8.3E+9	3.1E+9	1.4E+10	4.1E+8	2.9E+8	1.3E+9	1.0E+10	NA	NA
KB1_11	4.6E+7	7.2E+8	7.1E+7	1.8E+9	1.2E+9	1.2E+10	5.1E+9	6.3E+09	2.5E+8	5.0E+8	1.8E+9	2.7E+8	1.2E+7	1.3E+7
KB1_14	7.9E+7	5.8E+8	7.1E+8	4.7E+10	8.7E+9	7.1E+9	9.7E+8	2.8E+10	7.4E+8	7.4E+8	8.0E+9	7.1E+9	7.9E+7	6.2E+8
KB1_1*	5.7E+8	3.2E+9	1.0E+9	1.1E+11	2.1E+9	1.9E+10	5.4E+9	NA	2.8E+8	4.8E+8	8.9E+9	NA	5.2E+8	2.4E+9
<i>Dhc</i> <i>16S*</i>	4.2E+8	2.5E+9	1.5E+9	1.1E+11	2.2E+9	2.1E+10	4.7E+9	NA	2.7E+8	4.9E+8	1.3E+9	NA	4.4E+8	2.2E+9
<i>Geo 16S</i>	NA	NA	1.6E+8	5.9E+9	NA	NA	≤1.1E+6	≤1.1E+6	NA	NA	≤1.1E+6	≤1.1E+6	NA	NA
<i>Geo_rdh</i>	3.3E+7	1.0E+9	1.5E+8	7.8E+9	2.0E+9	<3.6E+5	≤4.6E+5	≤4.6E+5	≤4.6E+5	≤4.6E+5	≤4.6E+5	≤4.6E+5	≤4.6E+5	≤4.6E+5

*Absolute quantification for KB1_1, KB1_5 and KB1_27 was conducted on a different date than for the rest of *rdhA* genes and thus values for these genes were referred to *Dhc* values of the corresponding date. *Dhc* = *Dehalococcoides*; NA = not analyzed; *Geo* = *Geobacter*; ≤ MDL value is equal to or lower than the method detection limit. KB1_27 (*tceA*); KB1_6 (*bvcA*); KB1_14 (*vcrA*).

Note: The difference between duplicate measurements was usually around 5% or less of absolute value of each duplicate measurement.

Table S7. Absolute qPCR gene copy numbers per L of culture for each *rdhA* used for Figure 2b of the main manuscript.

	TCE/ME 2001 Sir Feb 2004	TCE/ME 2001 Sir Jan 2005	TCE/ME 2001 Sir Mar 2006	TCE/ME 2001 Sir Apr 2007 SaBRE inoculum	TCE/ME 2001 Sir Oct 2009 ISSO inoculum
Dhc 16S	6.8E+10	2.0E+11	5.3E+10	3.5E+10	7.1E+08
KB1_25	≤8.2E+05	≤8.2E+05	≤8.2E+05	≤8.2E+05	≤8.2E+05
KB1_16	≤8.7E+05	≤8.7E+05	≤8.7E+05	≤8.7E+05	≤8.7E+05
KB1_17	≤5.0E+05	≤5.0E+05	≤5.0E+05	≤5.0E+05	≤5.0E+05
KB1_19	8.9E+07	1.5E+08	1.2E+08	1.3E+08	9.1E+05
KB1_6/bvcA	7.0E+07	8.5E+09	2.3E+09	3.2E+08	≤1.0 E+06
KB1_15	4.5E+10	1.9E+10	6.7E+08	7.6E+08	8.9E+05
KB1_18	7.9E+10	2.0E+10	3.9E+08	6.3E+08	≤1.0E+06
KB1_23	6.0E+10	2.6E+11	4.7E+10	3.7E+10	5.3E+08
KB1_12	6.8E+10	3.6E+11	7.2E+10	5.6E+10	2.2E+09
KB1_4	6.0E+10	2.1E+11	NA	4.0E+10	5.0E+08
KB1_11	9.6E+10	1.3E+11	1.5E+10	2.6E+10	7.1E+07
KB1_14/vcrA	2.7E+11	6.1E+11	9.7E+10	7.5E+10	7.1E+08
Geobacter 16S	4.3E+09	3.3E+10	5.0E+09	4.6E+09	1.6E+08
Geo_rdhA	2.1E+10	7.8E+10	6.6E+09	3.9E+09	1.5E+08

Dhc = 16S rRNA gene of *Dehalococcoides*; *Geo_rdhA* = *Geobacter rdhA*; *Geobacter* = 16S rRNA gene of *Geobacter*; NA = not analyzed. ≤ MDL value is equal to or lower than the method detection limit. Note: The difference between duplicate measurements was usually around 5% or less of the absolute value of each duplicate measurement.

Table S8. Absolute qPCR gene copy numbers per L of groundwater or per L of culture (for KB-1 inoculum) for each *rdhA* gene from which *rdhA/Dhc* ratios for Figure 4 of the main manuscript were calculated.

site	ISSO ¹	ISSO ¹	ISSO ¹	ISSO ¹	ISSO ¹	ISSO ^{1,2}	SABRE ¹	SABRE ¹	SABRE ¹	SABRE ¹	SABRE ^{1,3}	SABRE ^{1,3}
sample	prebiost month 1	pre-bioaug month 18	KB-1 inoc.	post-bioaug month 22	postbioaug month 30	SW70 month 0	KB-1 inoc.	SW70 month 2	SW70 month 10	SW70 month 15	effluent month 15	influent month 15
Reme-diation status	2.5 mos. before start of biostim. and 1 mo. before bioaug..	15 mos. of biostim. and 3 mos. after bioaug..		19 mos. of biostim. and 11 mos. after bioaug..	27 mos. of biostim. and prior to biostim. and bioaug.			2 weeks after bioaug. and 1 mo. after biostim.	8.5 mos. after bioaug. and 9 mos. after biostim.	13.5 mos. after bioaug. and 14 mos. after biostim.	13.5 mos. after bioaug. and 14 mos. after biostim.	13.5 mos. after bioaug. and 14 mos. after biostim.
Dhc 16S	1.1E+6	1.5E+6	7.1E+8	1.2E+6	5.9E+6	≤6.2E+5	3.5E+10	1.4E+8	2.6 E+6	5.3 E+7	6.4 E+6	3.4 E+7
KB1_4	3.8E+5	7.4E+5	5.0E+8	7.3E+5	2.5E+6	≤8.3E+5	4.0E+10	3.1E+8	4.4E+6	4.6E+7	8.4E+6	5.0E+7
<i>bvcA</i>	4.4E+5	5.0E+5	≤1.0 E+6	1.1E+5	1.6E+6	≤3.4E+5	3.2E+8	≤3.4E+5	2.1E+6	1.1E+8	2.1E+7	6.5E+7
KB1_11	≤2.4 E+5	7.7E+5	7.1E+7	3.6E+5	2.9E+6	≤9.6E+5	2.6E+10	9.2E+7	9.7E+5	6.4E+6	≤9.7E+5	≤9.7E+5
KB1_12	≤1.8 E+5	9.0E+4	2.2E+9	1.3E+5	5.1E+4	≤4.6E+5	5.6E+10	1.2E+8	≤4.6E+5	5.9E+6	≤4.6E+5	1.8E+6
<i>vcra</i>	≤6.2 E+4	1.4E+6	7.1E+8	7.9E+5	1.1E+6	≤4.2E+5	7.5E+10	3.2E+8	2.E+6	4.4E+7	≤8.1E+3	1.1E+7
KB1_15	≤6.0 E+4	7.7E+5	8.9E+05	3.5E+5	1.9E+6	≤2.8E+4	7.6E+8	6.5E+4	≤2.8E+4	2.1E+6	≤2.8E+4	8.8E+5
KB1_16	≤1.8 E+5	1.3E+5	≤8.7E+5	7.1E+4	3.8E+5	≤5.8E+5	≤8.7E+5	≤5.8E+5	≤5.8E+5	3.1E+6	≤5.8E+5	≤5.8E+5
KB1_17	≤2.4 E+5	4.7E+4	≤5.0E+5	2.3E+4	2.5E+5	≤3.2E+4	≤5.0E+5	≤3.2E+4	≤3.2E+4	2.4E+6	≤3.2E+4	6.5E+5
KB1_18	≤6.0 E+4	4.8E+5	≤1.0E+6	2.1E+5	8.9E+5	≤4.8E+5	6.3E+8	≤4.8E+5	≤4.8E+5	2.3E+6	≤4.8E+5	3.2E+6
KB1_19	≤1.7 E+5	2.1E+5	9.1E+05	1.0E+5	2.6E+5	≤6.1E+5	1.3E+8	4.2E+6	1.5E+6	2.9E+6	1.2E+6	1.2E+7
KB1_23	≤1.8 E+5	7.0E+4	5.3E+8	3.6E+4	3.3E+5	≤7.3E+5	3.7E+10	2.6E+8	≤7.3E+5	≤7.3E+5	≤7.3E+5	≤7.3E+5
KB1_25	≤7.0E+4	3.0E+3	≤8.2E+5	3.0E+3	2.0E+5	≤3.2E+4	≤8.2E+5	≤3.2E+4	1.9E+5	5.8E+6	1.6E+6	3.6E+6
<i>Geo 16S</i>	NA	NA	≤1.1E+6	NA	NA	≤4.1E+4	5.0E+9	7.7E+6	≤4.1E+4	≤4.1E+4	≤4.1E+4	3.0E+5
<i>Geo rdhA</i>	NA	NA	≤4.6E+5	NA	NA	≤2.1E+4	6.6E+9	4.0E+7	≤2.1E+4	1.7E+5	≤2.1E+4	≤2.1E+4

Dhc = *Dehalococcoides*; inoc. = inoculum; mo. = month; mos. = months; NA = not analyzed; prebiost = prebiostimulation; prebioaug = prebioaugmentation; postbioaug = postbioaugmentation. ≤ MDL

Note 1: The difference between duplicate measurements was usually around 5% or less of the absolute value of each duplicate measurement.

Note 2: Prior to any treatment *Dhc* counts were below the method detection limit in all wells, except in effluent where they were 1E+5 gene copies per L.

Note 3: *Dhc* and *rdhA* counts for SW75 and EFF at month 10 were below the MDL (data not shown in Table above)

Table S9. Detection limits for the quantification via qPCR of the 16S rRNA genes of *Dehalococcoides*, *Geobacter* and the *rdhA* genes for different sample batches (field samples and lab cultures).

	SABRE	ISSO prebiostim	ISSO postbiostim and postbioaug	KB-1 cultures and inoculum
	Gene copies L ⁻¹	Gene copies L ⁻¹	Gene copies L ⁻¹	Gene copies mL ⁻¹
<i>Dhc 16S</i>	6.2E+5	7.1E+4	3.1E+3	8.9E+2
KB1_1	NA	NA	NA	3.6E+2
KB1_4	8.3E+5	2.1E+5	1.4E+4	1.1E+3
KB1_5	NA	NA	NA	7.4E+2
KB1_6	3.4E+5	4.8E+4	2.2E+3	1.0E+3
KB1_11	9.7E+5	2.4E+5	1.5E+4	1.1E+3
rdhA12	4.6E+5	1.8E+5	1.1E+4	4.2E+2
KB1_14	4.2E+5	6.2E+4	8.1E+3	6.8E+2
KB1_15	2.8E+4	6.0E+4	6.4E+3	8.9E+2
KB1_16	5.8E+5	1.8E+5	1.2E+4	8.7E+2
KB1_17	3.2E+4	2.4E+5	5.5E+3	5.0E+2
KB1_18	4.8E+5	6.0E+4	7.2E+3	1.0E+3
KB1_19	6.1E+5	1.7E+5	8.8E+3	8.2E+2
KB1_23	7.3E+5	1.8E+5	1.5E+4	9.5E+2
KB1_25	3.2E+4	7.0E+4	3.0E+3	8.2E+2
KB1_27	NA	NA	NA	4.6E+2
Geo 16S	4.1E+4	NA	NA	1.1E+3
Geo rdhA	2.1E+4	NA	NA	4.6E+2

NA = not analyzed; postbioaug = postbioaugmentation; postbiostim = postbiostimulation;
prebiostim = prebiostimulation.

Note: Detection limits reflect the extraction volumes used: L for groundwater samples and mL for cultures.

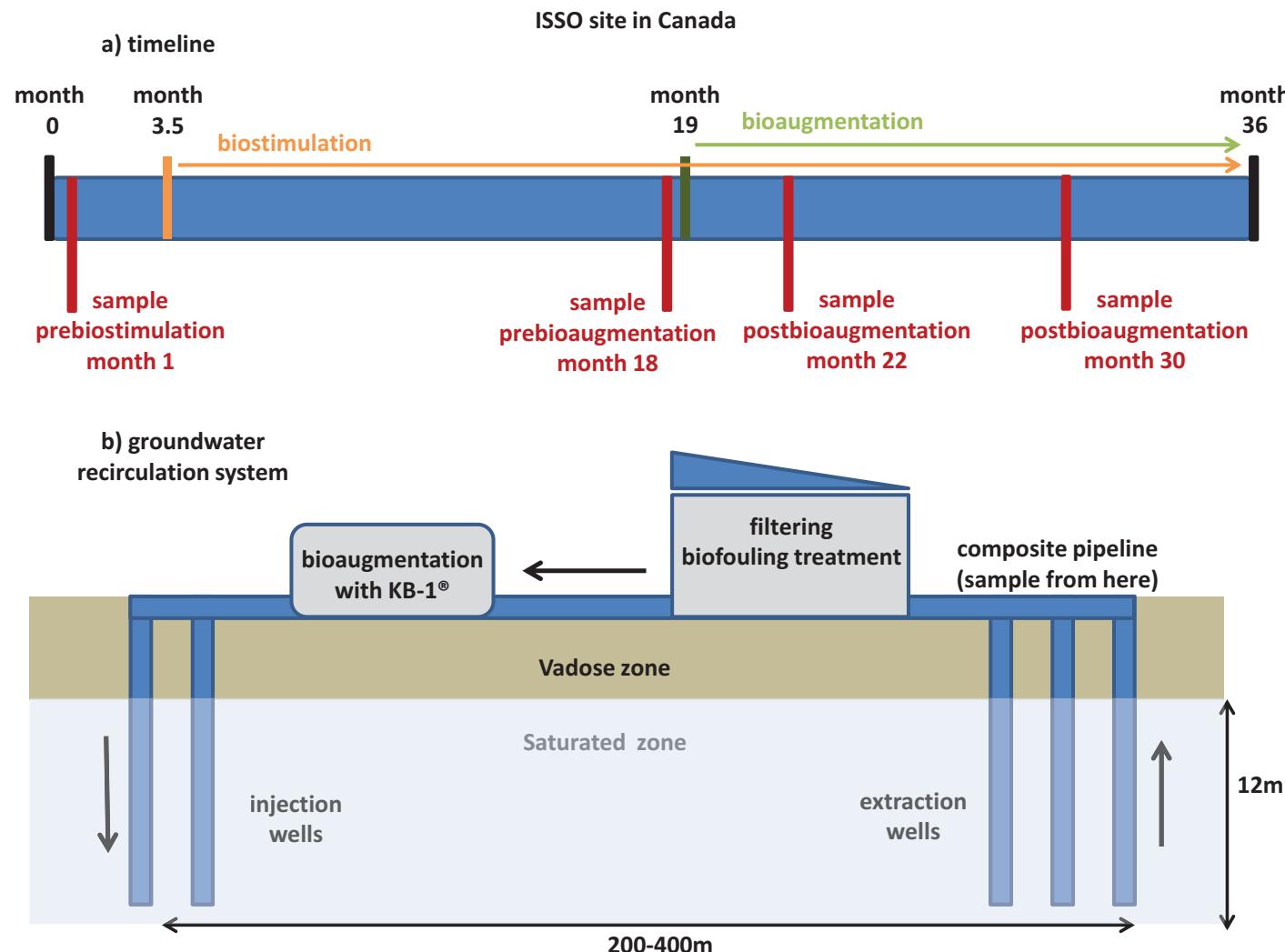


Figure S1. The ISSO site in Canada. a) Timeline including sampling dates and other relevant events such as the start of the biostimulation and bioaugmentation phases. b) Frontal section of the site depicting the direction of the groundwater within the recirculation system.

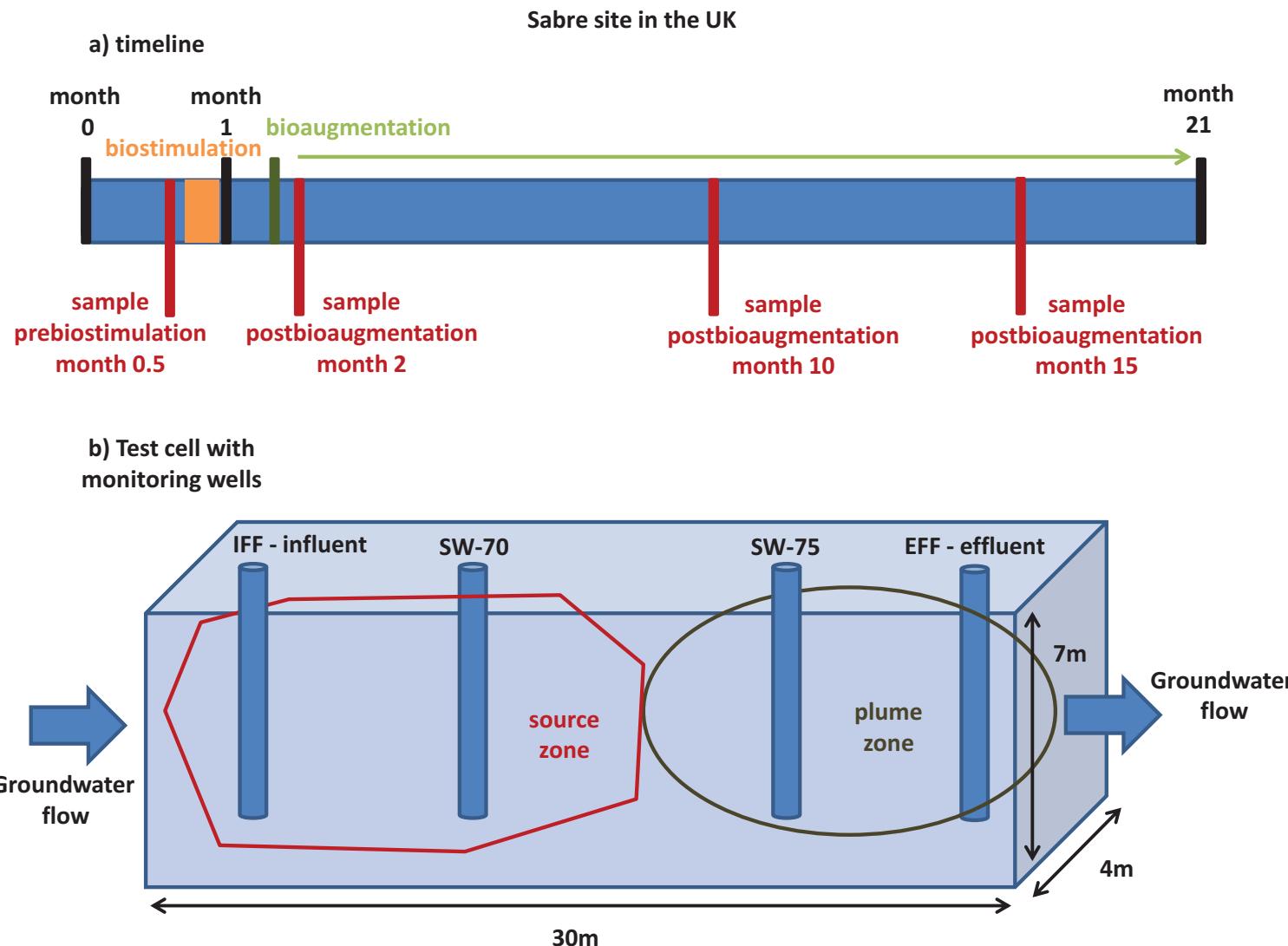


Figure S2. The Sabre site in the UK. a) Timeline including sampling dates and other relevant events such as the start of the biostimulation and bioaugmentation phases. b) Sketch of the pilot test cell with sampling locations including four monitoring wells: influent, SW-70, SW-75 and effluent.

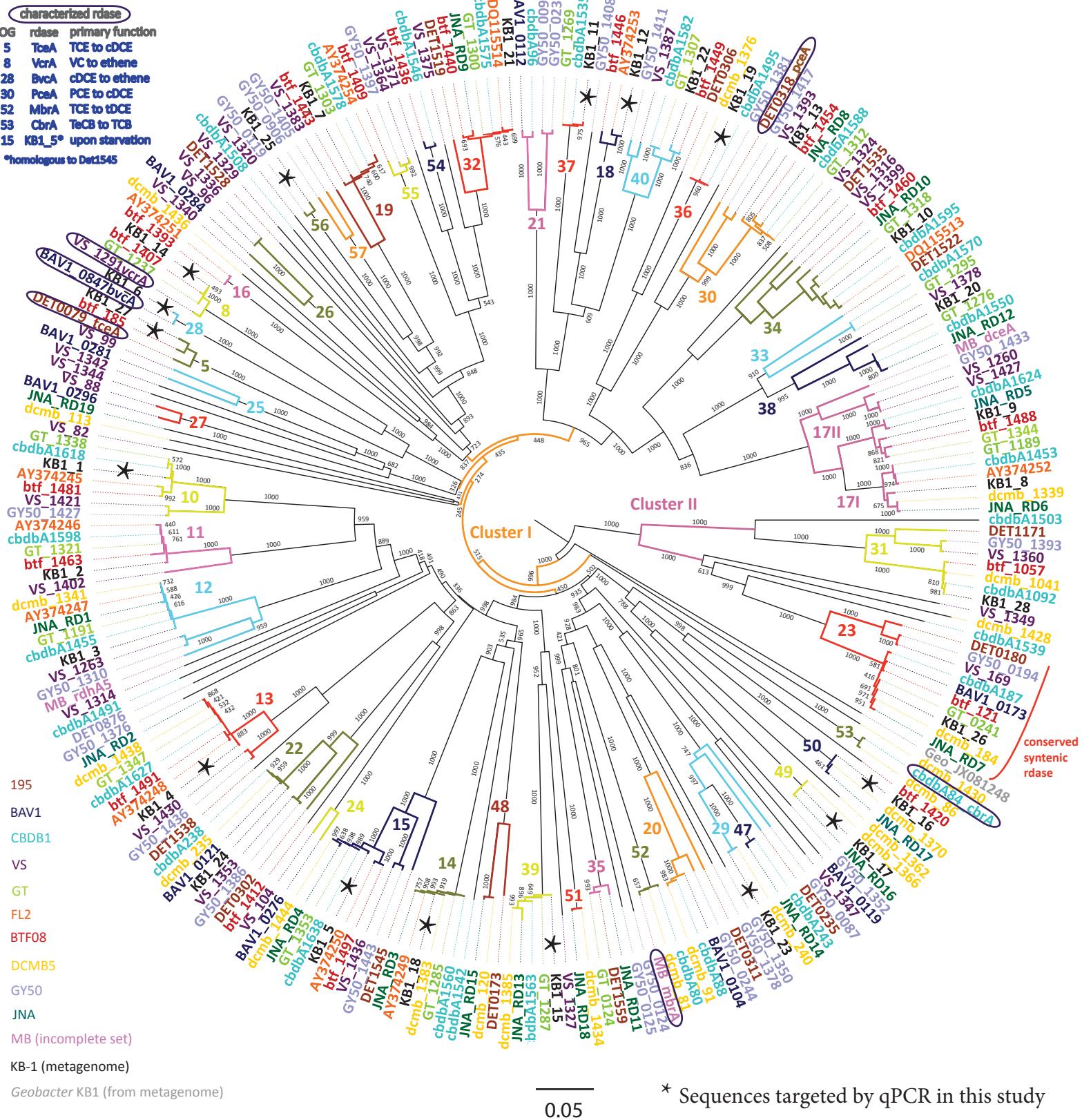


Figure S3. Phylogenetic tree with bootstraps based on the full nucleotide sequences of the *rdhA* genes of eleven isolated *D. mccartyi* strains as well as those found in the mixed dechlorinating culture KB-1, including an *rdhA* gene found in *Geobacter* KB-1. The scale at the bottom of the tree represents the number of substitutions per nucleotide. The numbers in colours represent *rdhA* ortholog groups (OGs). Genbank accession numbers for the nucleotide sequences of the MB strain are as follows: EU625401.1 for MB_rdhA5, GU120391.1 for mbrA and EU625402 for dceA1.

References cited in Supporting Information

1. Hug LA, Maphosa F, Leys D, Löffler FE, Smidt H, Edwards EA, Adrian L (2013). Overview of organohalide-respiring bacteria and a proposal for a classification system for reductive dehalogenases. *Phil Trans Royal Soc B: Biol Sci*, 368(1616): 20120322.
2. Tang S, Chan WW, Fletcher KE, Liang X, Seifert J, Löfller FE, Edwards EA. 2013. Functional characterization of reductive dehalogenases using blue native polyacrylamide gel electrophoresis. *Appl Environ Microbiol* 79(3):974–981.
3. Duhamel M, Edwards EA. Microbial composition of chlorinated ethene-degrading cultures dominated by *Dehalococcoides*. *FEMS Microbiol Ecol* 26(58): 538–549.
4. Waller AS, Krajmalnik-Brown R. Löfller FE, Edwards EA. Multiple reductive-dehalogenase-homologous genes are simultaneously transcribed during dechlorination by *Dehalococcoides*-containing cultures. *Appl Environ Microbiol* 71: 8257–8264.
5. Fung JM, Morris RM, Adrian L, Zinder SH. Expression of reductive dehalogenase genes in *Dehalococcoides ethenogenes* strain 195 growing on tetrachloroethene, trichloroethene, or 2,3-dichlorophenol. *Appl Environ Microbiol* 73: 4439-45.