Supporting Information

# Dechlorination of commercial PCBs and other multiple halogenated compounds by a sediment-free culture containing *Dehalococcoides* and *Dehalobacter*

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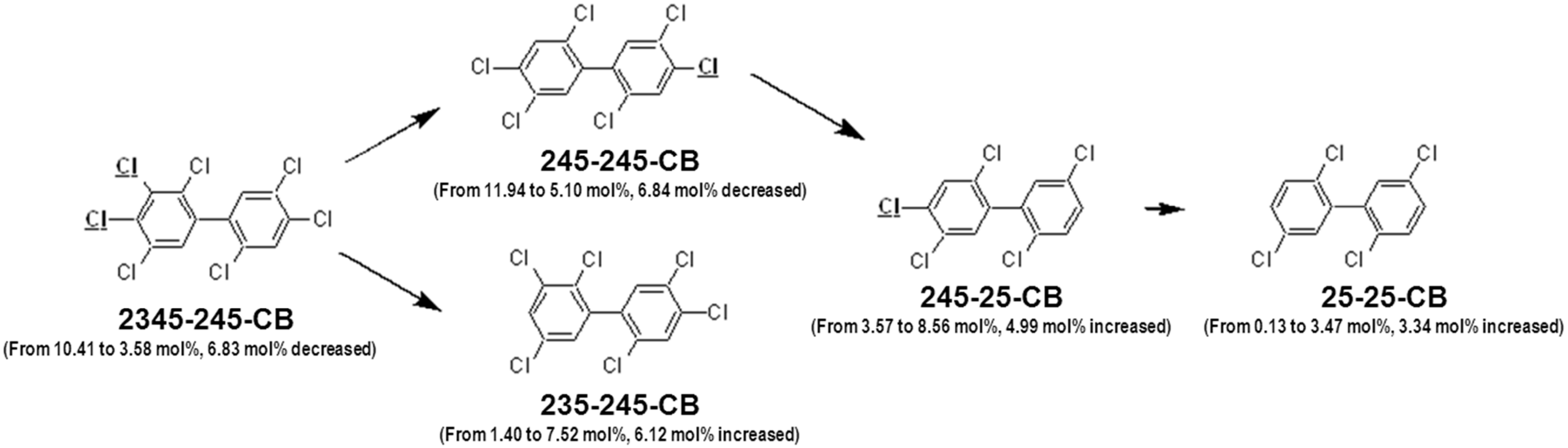
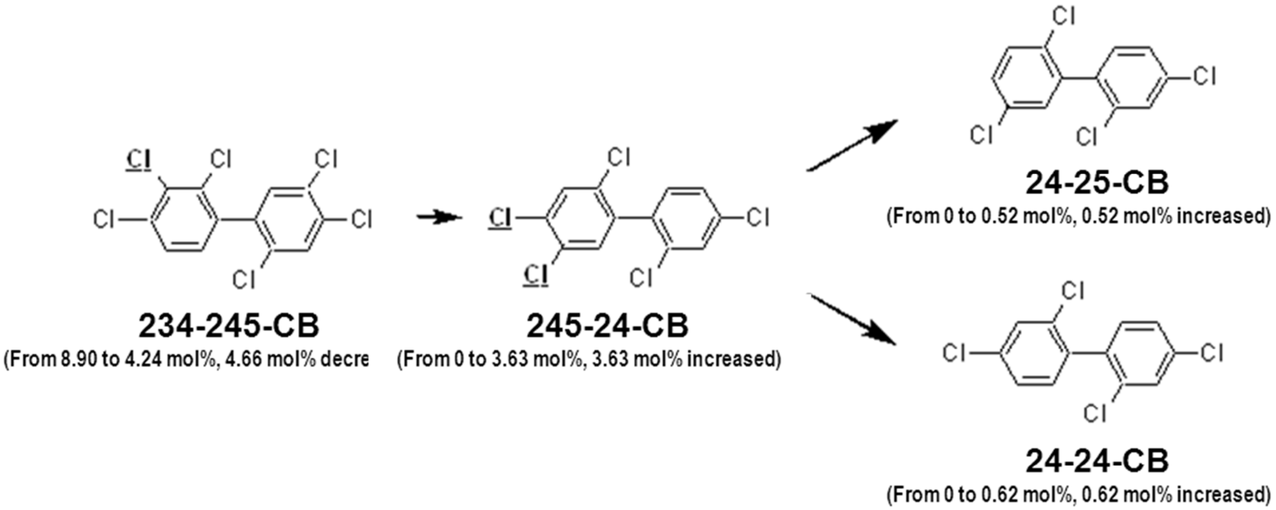
The 13 pages supplementary materials include 8 figures and 3 tables.


2345-245-CB
(From 10.41 to 3.58 mol%, 6.83 mol% decreased)
245-245-CB
(From 11.94 to 5.10 mol%, 6.84 mol% decreased)
235-245-CB
(From 1.40 to 7.52 mol%, 6.12 mol% increased)
245-25-CB
(From 3.57 to 8.56 mol%, 4.99 mol% increased)
25-25-CB
(From 0.13 to 3.47 mol%, 3.34 mol% increased)


234-245-CB
(From 8.90 to 4.24 mol%, 4.66 mol% decre
245-24-CB
(From 0 to 3.63 mol%, 3.63 mol% increased)
24-25-CB
(From 0 to 0.52 mol%, 0.52 mol% increased)
24-24-CB
(From 0 to 0.62 mol%, 0.62 mol% increased)

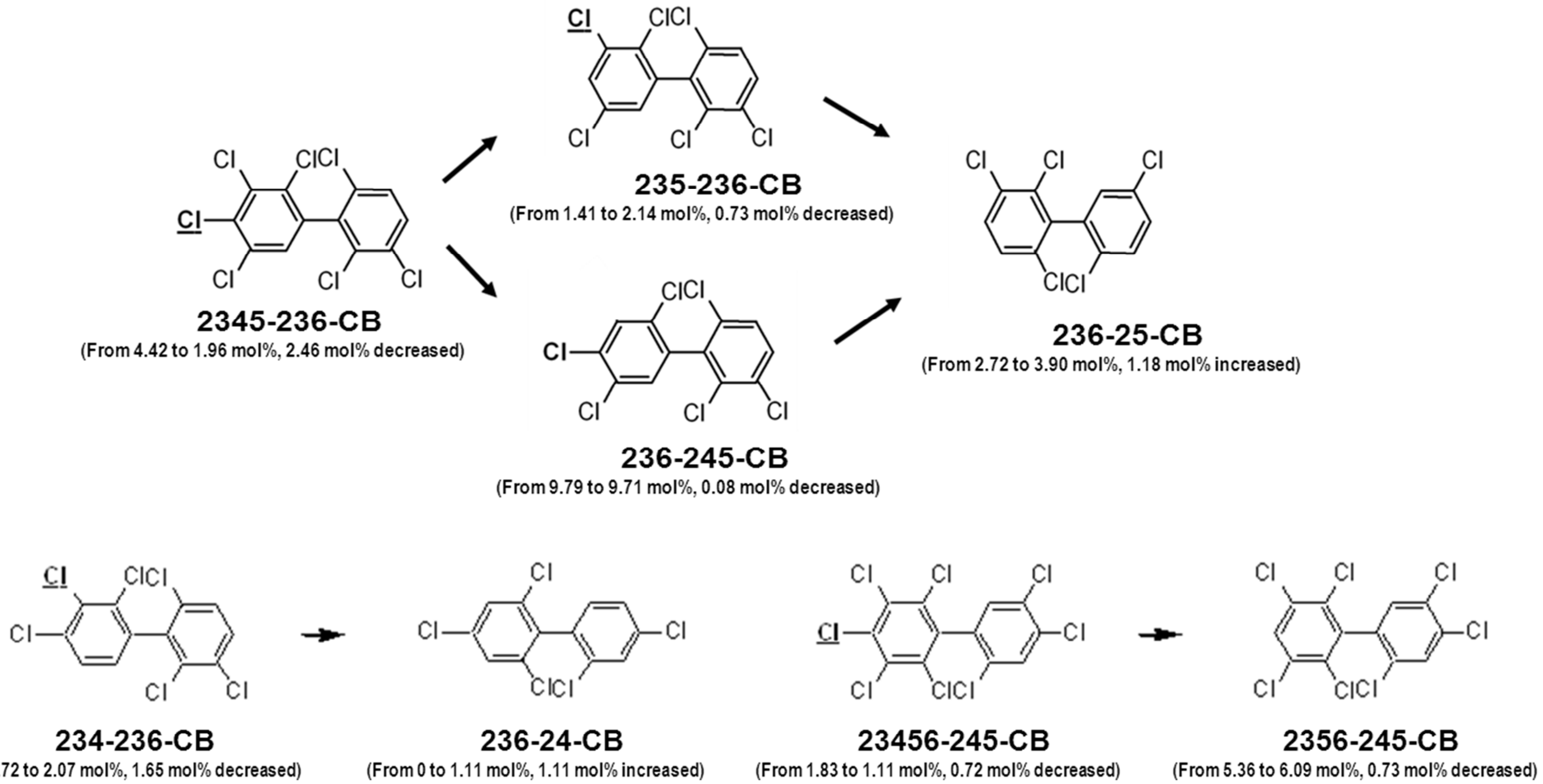


**Figure S1.** Inferred dechlorination pathways and products for most congeners dechlorinated by AD14 culture after four months of incubation. The dechlorination pathways for 2345-245-CB and 234-245-CB were confirmed by studying the dechlorination of individual congeners in AD14 cultures.

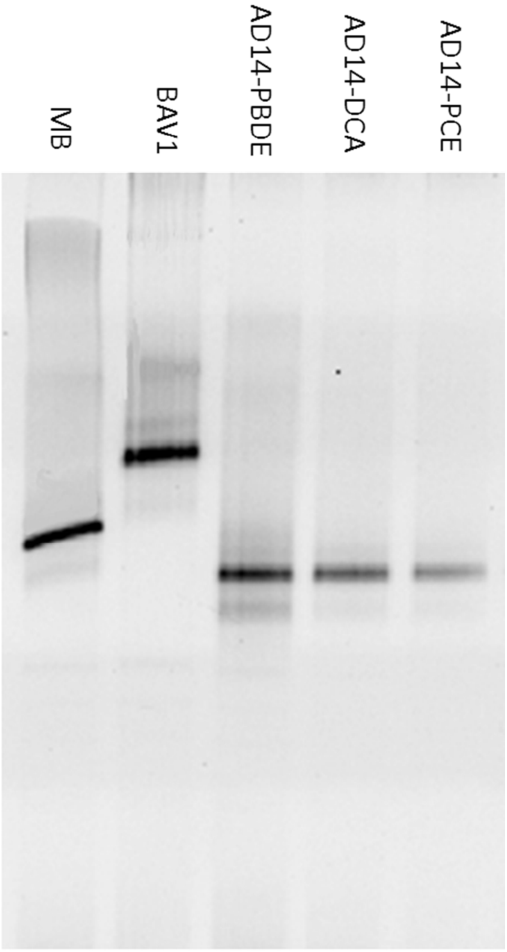


**Figure S2.** DGGE analysis of amplified 16S rRNA gene sequences from highly-enriched subcultures with *Dehalococcoides*-specific 1FGC and 259R primer set (Duhamel et al., 2004, AEM).

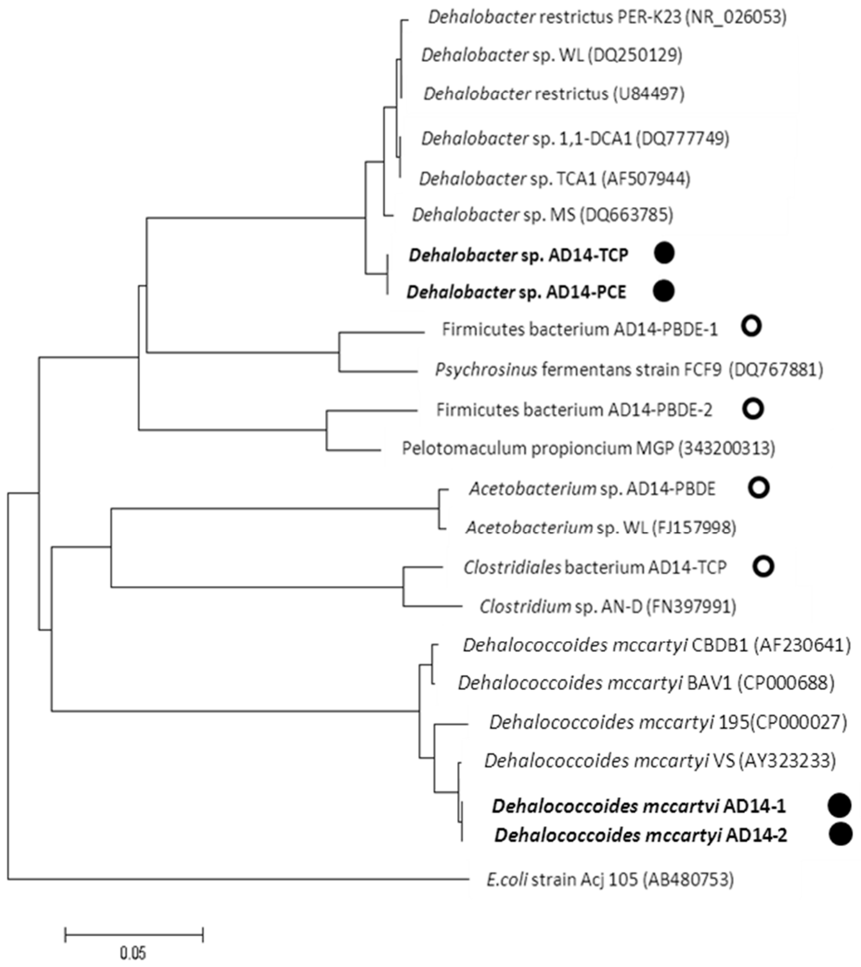


**Figure S3**. Phylogenetic tree of bacterial 16S rRNA genes from enriched subcultures (Dechlorinators and other non-dechlorinating bacteria were labeled with closed and open circles, respectively) and their closest relatives. Phylogenetic analyses was conducted via neighbor-joining with MEGA4.[27]

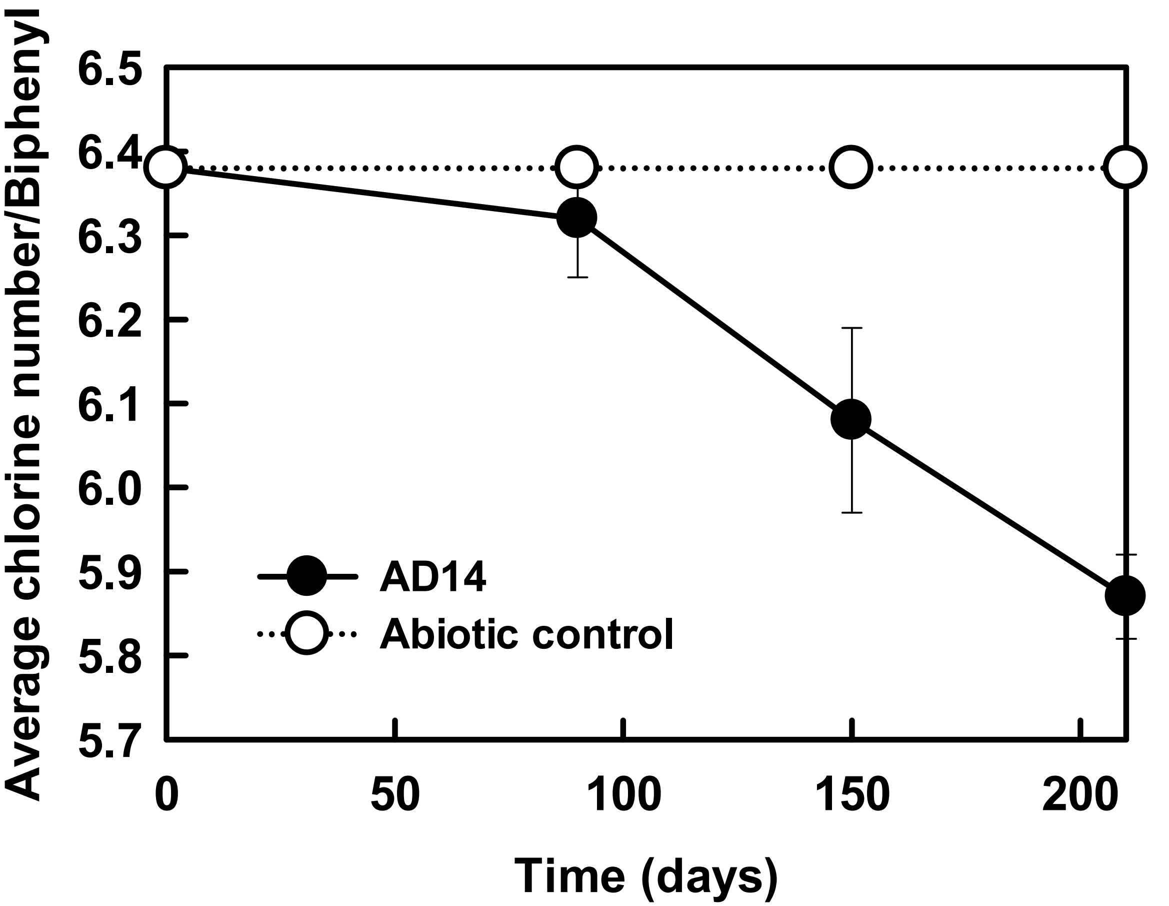
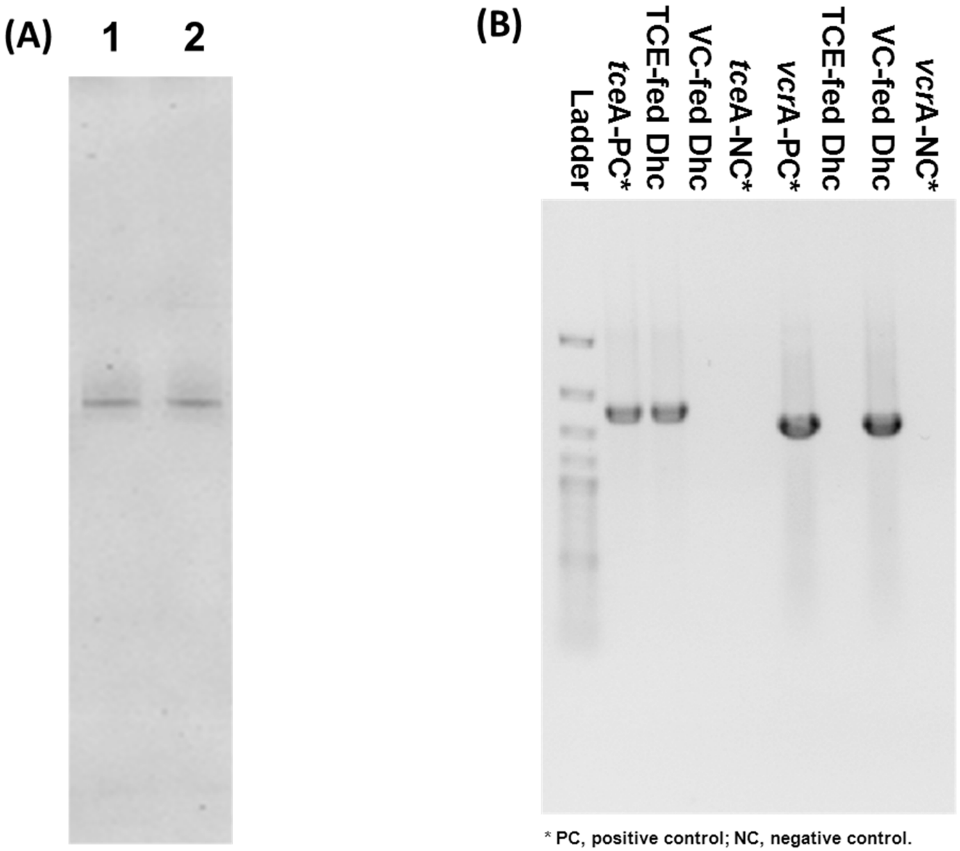


**Figure S4.** Decrease in average chlorine number per biphenyl in AD14-PCE subculture and its abiotic control.



* PC, positive control; NC, negative control.

**Figure S5.** (A) DGGE analysis (16S rRNA gene sequences were amplified with universal bacterial primers 8FGC and 518R) confirm the culture purity of *Dehalococcoides mccartyi* strain AD14-1 (TCE-fed, lane 1) and strain AD14-2 (VC-fed, lane 2); (B) PCR amplification with tceA-/vcrA-gene specific primers suggest that TCE- and VC-fed *Dehalococcoides* (Dhc) exclusively possess tceA and vcrA gene, respectively.

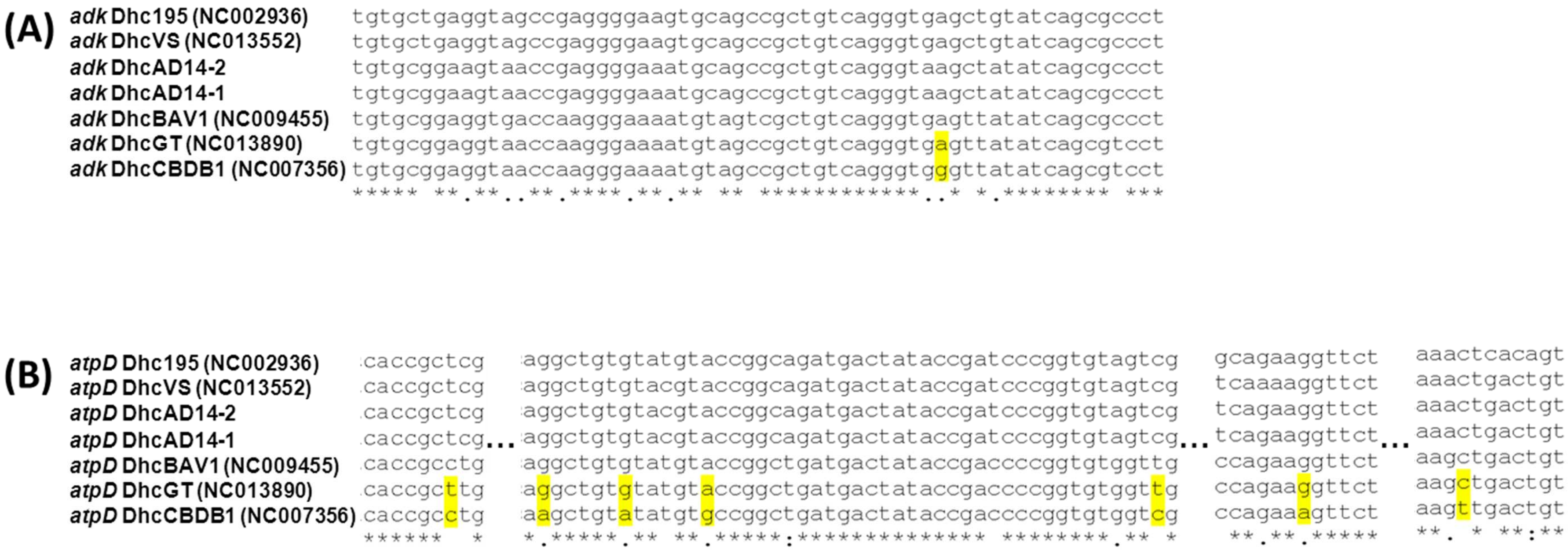


**Figure S6.** Sequence alignment of *adk* (A) and *atpD* (B) genes from *Dehalococcoides mccartyi* strains 195 (Dhc195), VS (DhcVS), BAV1 (DhcBAV1), GT (DhcGT), CBDB1 (DhcCBDB1), AD14-1 (DhcAD14-1) and AD14-2 (DhcAD14-2). Sequence differences between strains GT and CBDB1 were highlighted with yellow colour.

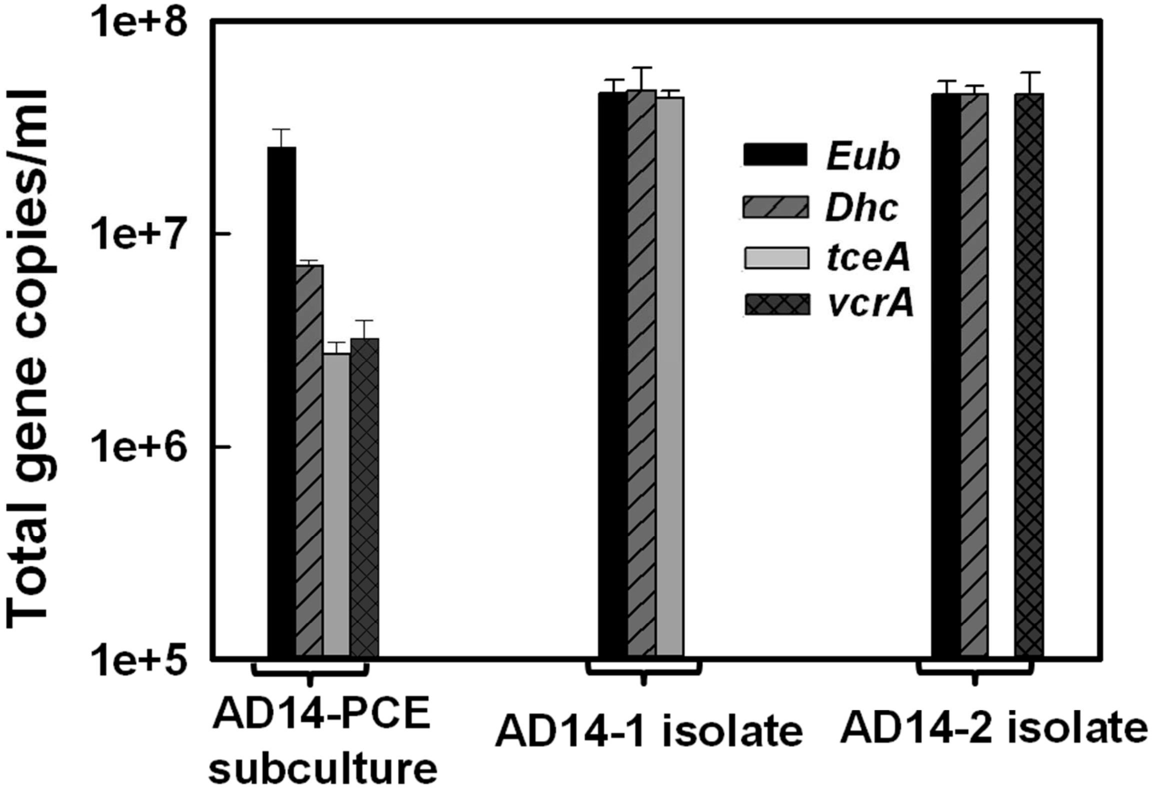


**Figure S7**. qPCR analysis of total bacteria and *Dehalococcoides* during isolating strains AD14-1 and AD14-2 from subculture AD14-PCE with 16S rRNA gene- and functional gene-targeted primers. Eub, *Eubacteria*; Dhc, *Dehalococcoides*.

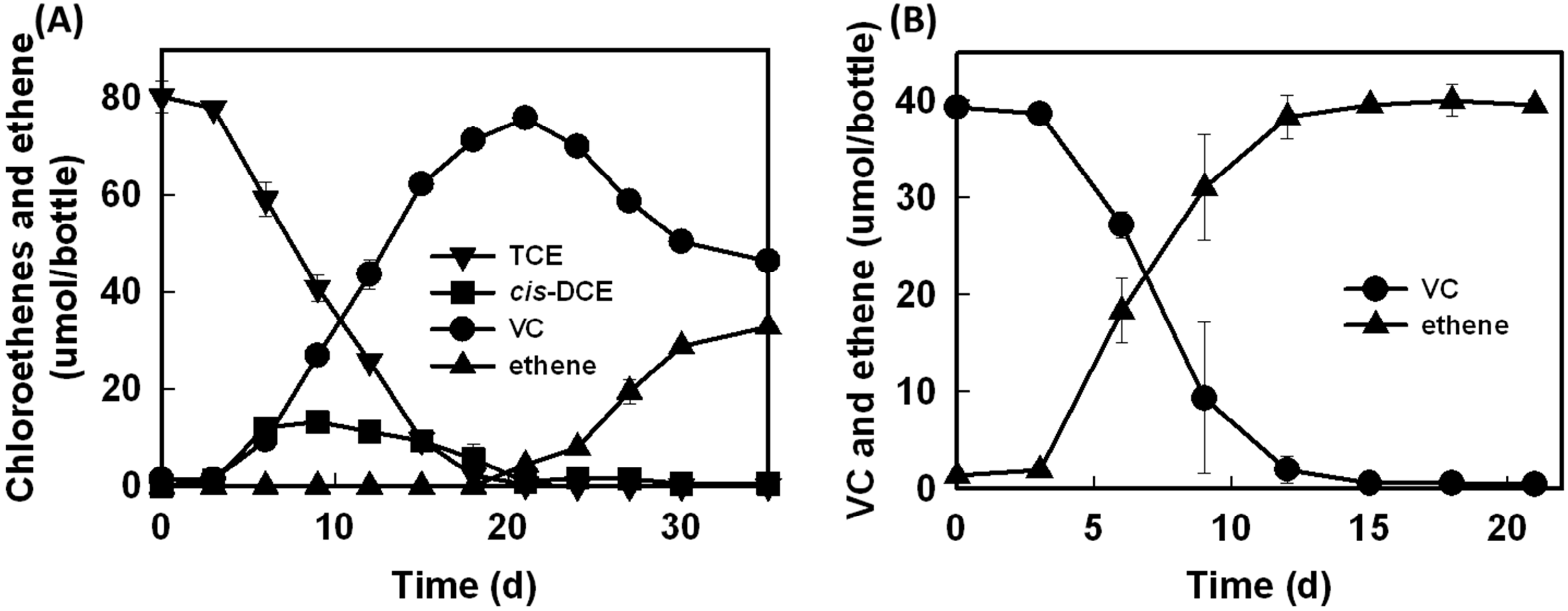


**Figure S8.** Dechlorination of (A) TCE by strain AD14-1 and (B) VC by strain AD14-2.

**Table S1.** Primers and probes used in this chapter

| Primer | Target | Orientation | Sequence (5' to 3') | Reference or source |
|---|---|---|---|---|
| 8F | *Bacteria* 16S rRNA genes | Forward | AGAGTTTGATCCTGGCTCAG | 1 |
| 1392R | *Bacteria* 16S rRNA genes | Reverse | ACGGGCGGTGTGTAC | 2 |
| BacF1 | *Bacteria* 16S rRNA genes | Forward | TCCTACGGGAGGCAGCAG | 3 |
| BacR1 | *Bacteria* 16S rRNA genes | Reverse | GGACTACCAGGGTATCTAATCCTGTT | 3 |
| BacR2 | *Bacteria* 16S rRNA genes | Reverse | GGACTACCAGAGTATCTAATTCTGTT | 3 |
| BacProbe | *Bacteria* 16S rRNA genes | Probe | FAM-CGTATTACCGCGGCTGCTGGCAC-TAMRA | 3 |
| DhcF | *Dehalococcoides* 16S rRNA gene | Forward | GGTAATACGTAGGAAGCAAGCG | 3 |
| DhcR | *Dehalococcoides* 16S rRNA gene | Reverse | CCGGTTAAGCCGGGAAATT | 3 |
| DhcProbe | *Dehalococcoides* 16S rRNA gene | Probe | VIC-ACATCCAACTTGAAAGACCACCTACGCTCACT-TAMRA | 3 |
| tceAF | *tce*A gene | Forward | ATCCAGATTATGACCCTGGTGAA | 4 |
| tceAR | *tce*A gene | Reverse | GCGGCATATATTAGGGCATCTT | 4 |
| tceAProbe | *tce*A gene | Probe | FAM-TGGGCTATGGCGACCGCAGG-TAMRA | 4 |
| vcrAF | *vcr*A gene | Forward | CTCGGCTACCGAACGGATT | 3 |
| vcrAR | *vcr*A gene | Forward | GGGCAGGAGGATTGACACAT | 3 |
| vcrAProbe | *vcr*A gene | Probe | FAM-CGCACTGGTTATGGCAACCACTC-TAMRA | 3 |
| DhcadkF | *adk* gene | Forward | GATGAGATTACCATTAGTGT | This study |
| DhcadkR | *adk* gene | Reverse | TTACCGCACTTTATAGCTGA | This study |
| DhcrpoBF | *rpo*B gene | Forward | CTGTTTGAGGTTATTGATAATGACC | This study |
| DhcrpoBR | *rpo*B gene | Reverse | GCCAGAGAACCGATAAGACCA | This study |
| DhcatpDF | *atp*D gene | Forward | TGCCTTTCCTTTACTCCTACCGAT | This study |
| DhcatpDR | *atp*D gene | Reverse | GCRTCATCAATAGTGCCTACCAT | This study |
| Deb433F | *Dehalobacter* 16S rRNA gene | Forward | ATACTGTTGTTAGGGAAGAACGGC | This study |
| Dre645R | *Dehalobacter* 16S rRNA gene | Reverse | CCTCTCCTGTCCTCAAGCCATA | 5 |
| GC-clamp[a] | - | Forward | CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG | 6 |

[a] The GC-clamp was attached to the 5´-end of the 8F and 519F to get primers 8FGC and 519FGC, respectively.

**Table S2.** Effect of AD14 culture on PCB homolog distribution after 120 days of incubation

| PCB homolog [a] | Mole Percent of Total PCBs | | | % Decrease |
|---|---|---|---|---|
| | Aroclor1260 [b] | Dechlorinated Aroclor 1260 [c] | SD | |
| Tri-CB | 0.07 | 0.10 | 0.03 | |
| Tetra-CB | 0.21 | 4.72 | 0.26 | |
| Penta-CB | 9.63 | 30.35 | 2.47 | |
| Hexa-CB | 47.75 | 38.23 | 1.34 | 19.94 |
| Hepta-CB | 36.26 | 21.86 | 1.63 | 39.71 |
| Octa-CB | 5.51 | 4.18 | 0.09 | 24.14 |
| Nona-CB | 0.57 | 0.56 | 0.01 | |

[a] No mono-, or dichlorobiphenyls were detected.
[b] Data are the means for two uninculated controls.
[c] Data are the means for the three AD14 cultures that showed extensive dechlorination of Aroclor 1260.

**Table S3.** Summary of halogenated compound utility profile by *Dehalococcoides mccartyi* strain AD14-1 and AD14-2

| **Isolates** | **Halogenated compounds reduced** | **End-products** |
|---|---|---|
| *Dehalococcoides mccartyi* AD14-1 | TCE | VC and ethene |
| | 1,2-DCA | Ethene |
| | 2,4,6-TCP | No activity[a] |
| | octa-BDE mixture | hexa-BDEs |
| | Aroclor 1260 | No activity[b] |
| *Dehalococcoides mccartyi* AD14-2 | PCE/TCE | No activity[a] |
| | *cis*-DCE | Ethene |
| | 1,2-DCA | Ethene |
| | 2,4,6-TCP | 2,4-DCP |
| | octa-BDE mixture | penta- and tetra-BDEs |
| | Aroclor 1260 | No activity[b] |

[a] the activities were assessed after 6 months of incubation.
[b] PCB dechlorination activity was assessed after 12 months of incubation. Aroclor 1260 dechlorination activity was conducted in defined medium inoculated with AD14-control culture which was obtained by transferring culture AD14 for three times (12 months) in defined media without amending Aroclor 1260.

## References

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(5) Smits, T. H.; Devenoges, C.; Szynalski, K.; Maillard, J.; Holliger, C. Development of a real-time PCR method for quantification of the three genera Dehalobacter, Dehalococcoides, and Desulfitobacterium in microbial communities. *J. Microbiol. Methods*. **2004,** *57*, 369-378.

(6) Muyzer, G.; De Waal, E. C.; Uitterlinden, A. G. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol*. **1993,** *59*, 695-700.