

*Supplementary Material to*

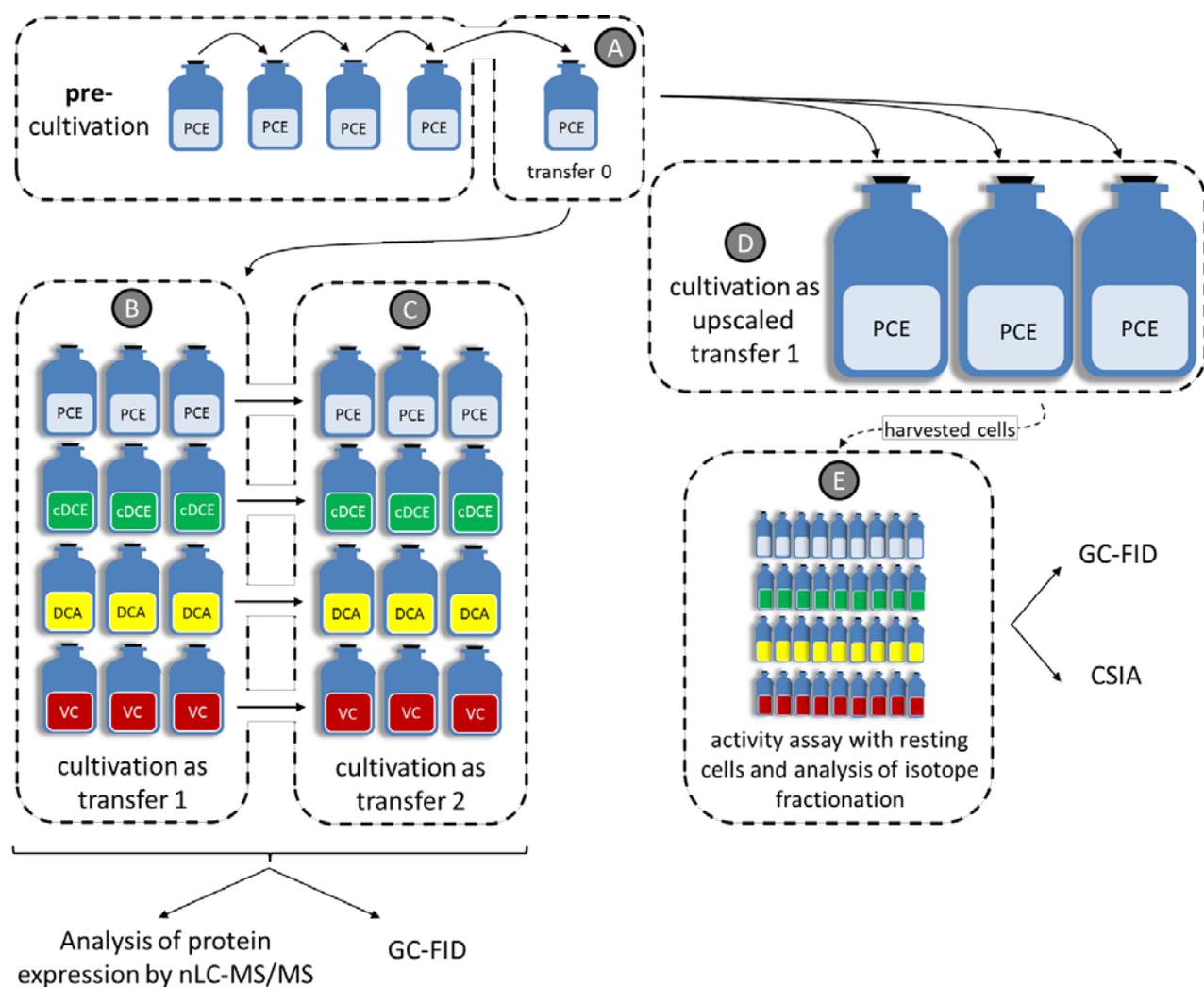
**Dual element (C/Cl) isotope analysis indicates distinct mechanisms of reductive dehalogenation of chlorinated ethenes and dichloroethane in *Dehalococcoides mccartyi* strain BTF08 with defined reductive dehalogenase inventories**

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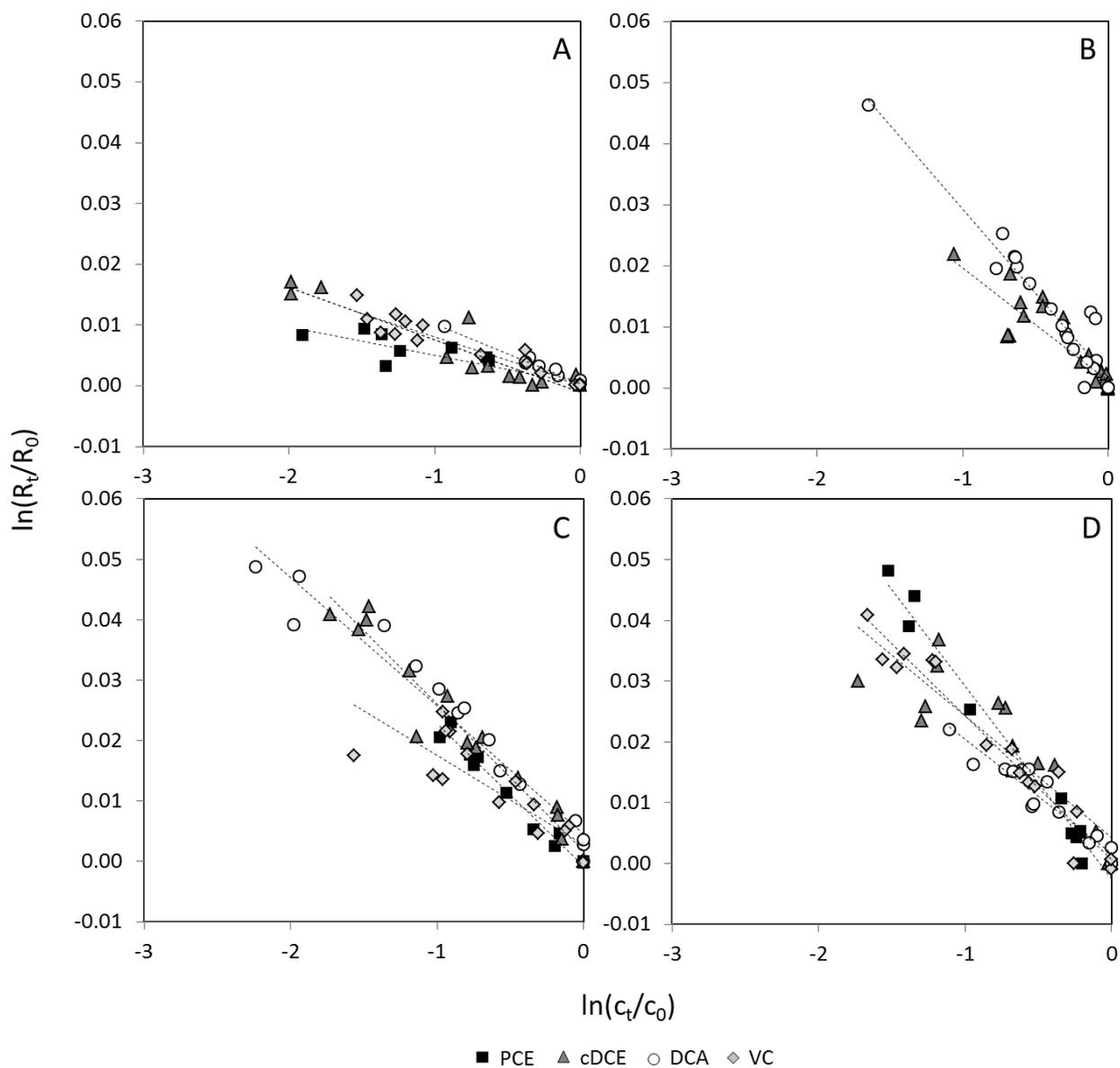


**Figure S1: Experimental setup exemplified for pre-cultivation with PCE.** Identical procedures were done for pre-cultivation with cDCE, DCA and VC.

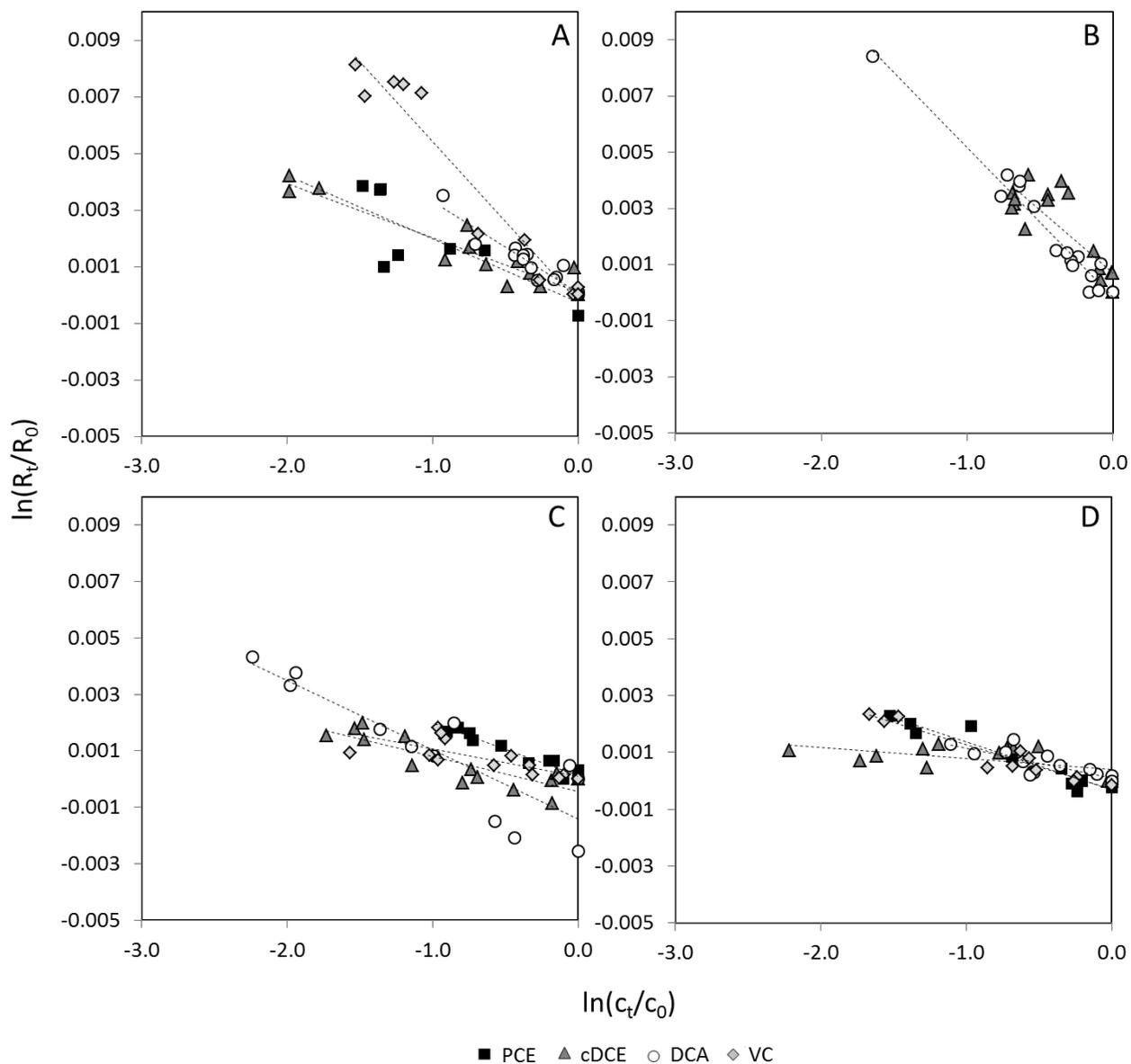
*D. mccartyi* strain BTF08 was cultivated with PCE, cDCE, DCA or VC for at least 4 passages to generate one single starter culture for the four electron acceptor (pre-cultivation, “transfer 0”, A). From these starter cultures we generated cultures for protein expression analysis (B and C) and cell material for activity tests (D and E).

For proteomic analysis the starter cultures were transferred twice in medium with one of the four electron acceptors (transfer 1 and 2, B and C) to monitor the effect of the electron acceptor on RdhA expression. Cells were transferred when the electron acceptor was completely dehalogenated. Proteomic analysis was done from cultures from both transfers (transfer 1 and transfer 2).

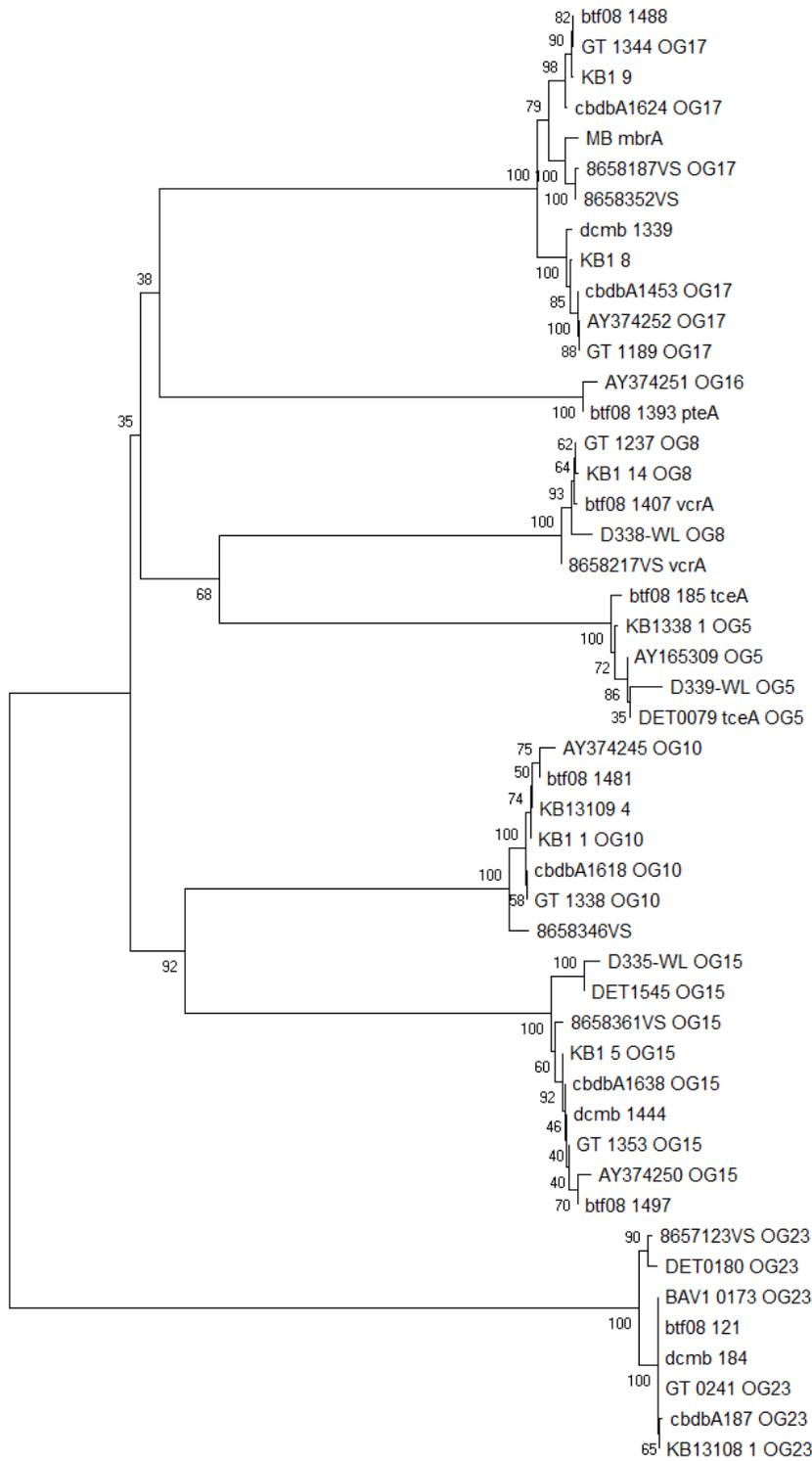
For activity test and isotope fractionation analysis, transfer 0 cultures were used to inoculate cultures with larger volumes for all four electron acceptors (shown only for PCE, D). This cell material was used for activity tests (E) which were analyzed after varying incubation times for dehalogenation products and isotopic signature of residual electron acceptors.



**Figure S2: Rayleigh-Plots for stable isotope carbon fractionation.** Activity assays using resting cells of *D. mccartyi* strain BTF08 with the electron acceptors (A) PCE, (B) 1,2-DCA, (C) cDCE or (D) VC in dependence of the electron acceptors used for growth in upscaled transfer 1. Linear fits are represented by dashed lines.



**Figure S3: Rayleigh-Plots for stable isotope chlorine fractionation.** Activity assays using resting cells of *D. mccartyi* strain BTF08 with the electron acceptors (A) PCE, (B) 1,2-DCA, (C) cDCE or (D) VC in dependence of the electron acceptors used for growth in upscaled transfer 1. Linear fits are represented by dashed lines.



0.20

**Figure S4: Evolutionary relationships of taxa representing the expressed RdhA proteins from *D. mccartyi* strain BTF08.** The evolutionary relationship was determined using the Minimal Evolution method (Rzhetsky and Nei, 1992) computed by MEGA7 (Kumar et al., 2016). The percentage of replicate trees in which the associated taxa are clustered together in the bootstrap test including 500 replicates are shown next to the branches and the tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Involving 48 amino acid sequences the final dataset comprised 344 positions, gaps and missing data were eliminated.

## References

- Kumar, S., Stecher, G., and Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol* **33**: 1870-1874.
- Rzhetsky, A., and Nei, M. (1992) A Simple Method for Estimating and Testing Minimum-Evolution Trees. *Mol Biol Evol* **9**: 945-967.