Supporting Information for

Microbial dehalogenation of trichlorinated dibenzo-*p*-dioxins by a *Dehalococcoides*-containing mixed culture is coupled to carbon isotope fractionation

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I. Carbon isotope mass balance

The concentration and isotope composition of the substrate and metabolites were used to calculate a carbon isotope mass balance in order to obtain information on the transformation process and potential losses. In a close system the carbon isotope mass balance should be constant over the course of the experiment if all species are analysed without any losses.
Therefore, the isotope ratio (IR) was multiplied with the molar fraction of the individual compounds (MF), respectively to give the isotope mass balance:

Isotope mass balance = $(IR \times MF)_{TrCDD} + (IR \times MF)_{DiCDD(s)} + (IR \times MF)_{MCDD}$ (equation 1)

The δ value of the isotope measurement was used (see equation in the manuscript). The mole fraction (MF) is the fraction of dioxin congener relative to the total concentration (MF=1.0) of all dioxin congeners present in the sample. The absolute total concentration of dioxin congeners in the 1,2,3-TrCDD and 1,2,4-TrCDD-dechlorinating cultures was 15 and 30 µM, respectively.
The isotope balance was almost closed for the experiment with 1,2,4-TrCDD (Fig. S1). A slight enrichment of up to about one δ unit was observed in the experiment with 1,2,3 TrCDD indicating that some losses may have occurred or that not all metabolites were analysed quantitatively. However, taking into account an uncertainty of at least 0.5 δ units for individual isotope measurement, the isotope balance is adequate.

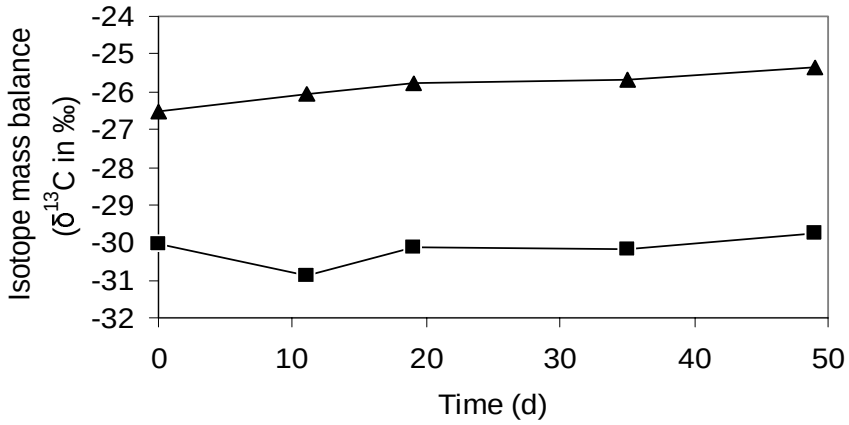

Fig. S1: Isotope mass balance of dioxin congeners during the course of reductive dehalogenation of 1,2,3-TrCDD (▲) and 1,2,4-TrCDD (■) as shown in Fig. 1. The values

represent the sum of the isotope ratios of all detectable dioxin congeners and their concentration. Each value represents the average of two sacrificed culture bottles.

II. Influence of the extraction method on the determined isotope ratios of trichlorinated dioxins

We examined the isotope composition of dioxin isomers and compared solvent extraction and solid-phase-micro-extraction (SPME). Gas chromatography-combustion isotopic ratio monitoring mass spectrometry (GC-C-IRMS) was used to assess the isotope composition. (see Analytical Methods for further details) (Table S1). The 1,2,3-TrCDD and 1,2,4-TrCDD obtained from AccuStandard (New Haven CT) were dissolved in hexane and had an isotope composition of - 27.28 ± 0.07 and - 32.43 ± 0.09, respectively. The hexane extraction of batch cultures at the beginning of the experiment gave nearly identical values taking the uncertainty of the isotope measurements into account. Thus, solvent extraction has no significant effect detectable with our method on the isotope composition. The extraction by the SPME technique used (Bunge et al. 2003) gave similar results taken the uncertainty of the isotope measurement into account. Thus, both methods give comparable results for the extraction of TrCDD congeners for isotope analysis. However, the uncertainty of the isotope measurement at concentrations of 15 and 30 µM used in the culture experiments for 1,2,3-TrCDD and 1,2,4-TrCDD, respectively, clearly increases, which may be a result of overall treatment of the sample as well as low concentration. The extraction of the non-inoculated controls at the end of the experiment did not show a significant enrichment in case of 1,2,3-TrCDD compared to the chemical dissolved in hexane. The experiment with 1,2,4-TrCDD showed a tendency for a slight enrichment in ^{13}C taking the analytical uncertainty into account. In the inoculated experiments 1,2,4 TrCDD was not enriched during the course of dehalogenation at 4 consecutive sampling points showing the reproducibility of the isotope measurements. The

isotope measurement of 1,2,3 TrCDD of the control after 70 days showed no significant change as expected compared to the inoculated degradation experiment at day 0.

Table S1: Comparison of $\delta^{13}C$ mean values and standard deviation (‰) of reference compounds dissolved in hexane or re-extracted from spiked (control) cultures (n: number of measurements).

Dioxin congener	Dissolved in hexane	Extraction of dioxins from the (control) cultures[a] at day 0 of the experiment by		Extraction of dioxins from the non-inoculated control at the end[b] of the experiment by
		hexane	SPME	SPME
1,2,3-TrCDD	- 27.28 ± 0.07 (n = 3)	- 27.19 ± 0.49 (n = 4)	- 28.72 ± 1.3 (n = 3)	- 27.44 ± 0.11 (n = 2)
1,2,4-TrCDD	- 32.43 ± 0.09 (n = 3)	- 32.93 ± 0.94 (n = 4)	- 32.65 ± 2.3 (n = 3)	- 31.34 ± 0.34 (n = 2)

[a]Data were obtained from one inoculated and two non-inoculated cultures extracted at time zero.
[b] Incubation for 70 d and 49 d in the experiments with 1,2,3-TrCDD and 1,2,4-TrCDD, respectively.

Reference

Bunge, M.; Adrian, L.; Kraus, A.; Opel, M.; Lorenz, W. G.; Andreesen, J. R.; Görisch, H.; Lechner, U. Reductive dehalogenation of chlorinated dioxins by an anaerobic bacterium. *Nature* **2003**, *421*, 357-360.