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SUPPORTING INFORMATION

to

Does the number of cells of individual strains correlate with their contribution to the total substrate turnover within a microbial community?

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Pages: 10 Figures: 7, Tables: 1 Research Data

MATERIALS AND METHODS Sample Extraction and Analytical Methods

Sample Extraction: Samples for chlorinated ethene concentration analysis were prepared by dividing 1 mL liquid equally into two 10 mL crimp glass vials which were previously filled with 4.5 mL ultrapure water (Merck Millipore, Burlington, MA) and 100 μ L 1 M H₃PO₄. The latter was added to stop microbial activity. Sample vials were sealed with aluminum crimp caps containing silicone/PTFE septa and directly measured. For carbon and chlorine isotope measurements, 4 mL were extracted and distributed in portions of 1 mL into 1.5 mL-screw neck HPLC vials to which 50 μL of 10 M NaOH were added to stop microbial activity. Vials were closed with PTFE-lined screw caps, frozen and stored upside down at -20 °C for later isotope analysis. For molecular biological analysis, 4 mL were extracted and divided into portions of 2 mL in Eppendorf tubes for subsequent quantification of strain PCE1 and strain Y51 cell numbers. Samples were immediately stored on ice. Cell pellets were obtained by centrifuging the samples for 10 min at 10 600 *g* and 4 °C. The supernatant was discarded, and samples were stored at -20 °C.

Chlorinated Ethene Concentrations: Analysis of chlorinated ethenes was performed in duplicates on a GC-MS system (Agilent Technologies 7890A GC coupled to an Agilent Technologies 5975VL MS Detector, Santa Clara, CA) via static headspace injection of 500 μL using a Gerstel MultiPurpose Sampler MPS (Mülheim an der Ruhr, Germany). Injections were done in split mode 1:10 with a Split/Splitless injector operated at 240 °C. Chromatographic separation of cis-DCE, trans-DCE, TCE and PCE was achieved using an Rtx-VMS fused silica column (60 m x 0.25 mm, 1.4 μm film; Restek, Bellefonte, PA) applying the following temperature program: 40 °C held for 1 min, 30 °C/min to 110 °C and afterwards 25 °C/min up to a final temperature of 200 °C which was held for 2 min. Helium was used as carrier gas with a constant flow of 1 mL/min. For each measurement run, calibration curves were analyzed at the beginning and the end of the sequence.

Stable Carbon Isotope Analysis. Stable carbon isotope signatures of PCE, TCE and *cis*-DCE were determined using gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) comprising a Trace GC Ultra (Thermo Finnigan, San Jose, CA) coupled to a Delta Plus XP IRMS detector (ThermoFinnigan) via a GC Combustion III interface (ThermoFinnigan). For analysis, duplicate samples of selected time points (frozen in 1.5mL HPLC vials) were diluted with ultrapure water to a total volume of 3 mL in glass screw vials and sealed with magnetic screw caps containing a silicone/PTFE septum. Dilution was adjusted to obtain peak amplitudes of 2000 to 4000mV for precise δ^{13} C determination. Samples were injected using solid phase microextraction (SPME) via a StableFlex-Fiber covered with 85 μm Car/PDMS (Supelco, Bellefonte, PA) and a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland). Extraction was done at 40 °C for 20 min after which the sample was desorbed at 270 °C for 1 min in an OPTIC 3 PTV injector (GL Sciences, Eindhoven, Netherlands). For the chromatographic separation of PCE, TCE and cis-DCE, an Rtx-VMS capillary column (60 m x 32 mm; 1.8 μm film) and the following temperature program were applied: 40 °C held for 4 min, 8 °C/min to 180 °C, afterwards 20 °C/min up to 200 °C which was held for 2 min. Helium was used as carrier gas with an initial flow of 2 mL/min for 2 min during desorption and 1.5 mL/min for the rest of the measurement run. Compound specific standards with known carbon isotope signatures ($\delta^{13}C$) of -27.35 ‰, -26.68 ‰ and -25.35 ‰ for PCE, TCE and *cis*-DCE were measured in duplicates after every 10 samples and at the end of each measurement run. At the beginning of each measurement sequence, additional five standards were included.

Stable Chlorine Isotope Analysis: Stable chlorine isotope signatures of PCE and TCE were analyzed using an Agilent Technologies 7890B GC coupled to an Agilent Technologies 5977A MSD and a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland). For details see Supporting Information. The gas chromatograph was equipped with a Rtx-VMS fused silica column (60 m x 0.25 mm, 1.4 μm film; Restek, Bellefonte, PA) and operated with a constant flow of 1mL/min helium as carrier gas. Sample enrichment was conducted at 40°C for 20min with SPME technique using a StableFlex-Fiber covered with 85 μm Carboxen/Polydimethylsiloxan (Supelco). The samples were desorbed in a Split/Splitless injector for 2 min at 260 °C with a split ratio of 1:10. For each sample, analysis was conducted in quintuplicates which were bracketed by five replicates per concentrationadjusted external standards (EIL-2 and PCE_{enriched}). Bulk chlorine isotope ratios were determined as previously described by $\frac{1}{1}$ using the modified multiple ion method after Jin et al. $\frac{2}{3}$ A two-point calibration curve with external standards (PCE: δ^{37} Cl_{EIL−2} = -2.52 ‰; δ^{37} Cl-PCE_{enriched} = 10.8 ‰ (recently developed by Buchner et al.⁴), TCE: $\delta^{37}Cl_{EIL2} = -2.7\%$, $\delta^{37}Cl_{EIL1} = 3.05\%$) was used to calculate δ^{37} Cl isotope values relative to the SMOC scale as recommended in previous studies. $5, 6$

DNA Extraction. Genomic DNA was extracted from living-replicates using the DNeasy® UltraClean® Microbial Kit (Qiagen GmbH, Hilden, Germany) following the manufacturers instruction. To increase DNA yields, samples were kept in a water bath at 70°C for 10 min prior to bead treatment.

Quantification of prdA- and pceA-genes. For each sample, the qPCR assay was performed in triplicates in 10µL reaction volume containing 1 µL of template DNA, 250 nM of forward (prdA:5'- CTGGTCTTGGAGAGTTGGGC- 3'; *pceA*: 5'-GCCGGCGTTCAAGGCCTCAT- 3') and reverse (*prdA*: 5'-TCTGCGGCTCCAAAACTGAT- 3'; *pceA*: 5' GGGGAAAGACCTGCCCACGC3') primer and 5 µL Sso AdvancedTM Universal SYBR® Green Supermix (Bio-Rad Laboratories, Inc. Hercules, CA). Amplification and detection were conducted using an iCycler (Bio-Rad Laboratories, Inc. Hercules, CA) with an iQTM5 qPCR detection system and Optical System software (Bio-Rad Laboratories, Inc. Hercules, CA). Temperature settings for *prdA*-gene were initial 95°C for 3 min, followed by 40 cycles of 95°C for 30 s and 60°C for 15 s. Temperature settings for *pceA* were initial 95°C for 3 min, followed by 40 cycles of 95°C for 30 s and 65°C for 15 s. Specificity of product formation was ensured at the end of each assay run by melt curve analysis using the following temperature program: 1 min at 95°C, 1 min at 55°C and stepwise increase by 0.5°C every 10 s up to 95°C. Specific amplification of the desired gene fragments was additionally checked by performing gel electrophoresis after each qPCR analysis. Quantification of *prdA*- and *pceA*-gene copies was enabled by simultaneous analysis of plasmid standards containing either a target gene fragment (*prdA*) or the complete target gene (*pceA*) (for details see $(7, 8)$). For comparability of total cell numbers, gene abundances of the re-spike experiments were corrected for dilution due to the addition of spike solutions.

Error of Molecular Biological Analysis. Errors of measured gene abundances reflect the percentage standard deviation of each sample accounting for introduced biases during sample extraction, removal of the supernatant, DNA extraction and sample preparation for the qPCR assay. The error was determined by processing five replicate samples extracted from a pure culture of strain PCE1 for the *prdA*-gene and strain Y51 for the *pceA*-gene which were both grown on pyruvate and PCE. DNA concentrations were measured after extraction using the Qubit® 2.0 Fluorometer and the Qubit® dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA) and statistically tested for outliers using the two-sided Grubbs test. Since no outliers were detected, gene abundances were determined for the five replicates of each strain using the qPCR assay. Measured gene abundances (3 wells per sample \times 5 samples = 15 measurement replicates each) were tested again for outliers using two-sided Grubbs test. No outliers were detected, and samples were corrected for the elution volume and extraction volume. For measured gene abundances, the average and standard deviation were calculated using the individual 15 replicates. Based on this, the percentage standard deviation was calculated which amounted to 21 % for the *prdA*-gene and 18% for the *pceA*-gene. Primer specificity and potential cross-amplification of gene fragments from both strains were tested by analyzing two replicate samples comprising either *pceA* primer and genomic DNA of strain PCE1 or *prdA* primer and genomic DNA of strain Y51. No amplification or bands in subsequent gel electrophoresis analysis were detected.

Error propagation for the calculation of cell fractions: Errors for the cell fractions (**Eq. 3** in the main article) were calculated by Gaussian error propagation considering the measured cell abundances (cells $_A$) and cells_B) and the corresponding errors described above:

$$
e(f_A) = \sqrt{\left(\frac{\partial f_A}{\partial cells_A} \times e(cells_A)\right)^2 + \left(\frac{\partial f_A}{\partial cells_B} \times e(cells_B)\right)^2}
$$

= $\sqrt{\left(\frac{cells_B}{(cells_A + cells_B)^2} \times e(cells_A)\right)^2 + \left(-\frac{cells_A}{(cells_A + cells_B)^2} \times e(cells_B)\right)^2}$ (S1)

Error propagation for the strain-specific substrate turnover based on CSIA data: Errors for the strain specific turnover (**Eq. 2** in the main article) were calculated by Gaussian error propagation considering the measured enrichment factors in the mix culture and the pure cultures and the corresponding confidence intervals:

$$
e(F_A) = \sqrt{\left(\frac{\partial F_A}{\partial \epsilon_A} \times e(\epsilon_A)\right)^2 + \left(\frac{\partial F_A}{\partial \epsilon_B} \times e(\epsilon_B)\right)^2 + \left(\frac{\partial F_A}{\partial \epsilon_{mix}} \times e(\epsilon_{mix})\right)^2}
$$

=
$$
\sqrt{\left(\frac{\epsilon_B - \epsilon_{mix}}{(\epsilon_A - \epsilon_B)^2} \times e(\epsilon_A)\right)^2 + \left(\frac{\epsilon_{mix} - \epsilon_A}{(\epsilon_A - \epsilon_B)^2} \times e(\epsilon_B)\right)^2 + \left(\frac{1}{\epsilon_A - \epsilon_B} \times e(\epsilon_{mix})\right)^2}
$$
(S2)

Error propagation for the calculated enrichment factors based on qPCR data: Errors for the calculated isotope enrichment factors (**Eq. 4** in the main article) were calculated by Gaussian error propagation considering the measured cell fractions and enrichment factors in the pure cultures and the corresponding confidence intervals:

$$
e(\varepsilon_{\text{calc}})
$$

= $\sqrt{\left(\frac{\partial \varepsilon_{\text{calc}}}{\partial \varepsilon_{\text{A}}} \times e(\varepsilon_{\text{A}})\right)^{2} + \left(\frac{\partial \varepsilon_{\text{calc}}}{\partial f_{\text{A}}} \times e(f_{\text{A}})\right)^{2} + \left(\frac{\partial \varepsilon_{\text{calc}}}{\partial \varepsilon_{\text{B}}} \times e(\varepsilon_{\text{B}})\right)^{2} + \left(\frac{\partial \varepsilon_{\text{calc}}}{\partial f_{\text{B}}} \times e(f_{\text{B}})\right)^{2}}$ (S3)
= $\sqrt{(f_{\text{A}} \times e(\varepsilon_{\text{A}}))^{2} + (\varepsilon_{\text{A}} \times e(f_{\text{A}}))^{2} + (f_{\text{B}} \times e(\varepsilon_{\text{B}}))^{2} + (\varepsilon_{\text{B}} \times e(f_{\text{B}}))^{2}}$

RESULTS AND DISCUSSION

Chlorinated Ethene Concentration Profiles for the re-spike experiment

Fig. S1 Fraction of chlorinated ethenes (PCE: \circ , TCE: \Box , cis-DCE: \circ) versus time of the 1st spike for the binary mixed culture containing *Desulfitobacterium dehalogenans strain PCE1 and Desulfitobacterium hafniense strain Y51*. Shown are the results of the two microcosm replicates (MC1 and MC2) as well as the cell free control. Error bars represent standard deviation of calculated fractions from GC-MS concentrations measurements $(n = 2)$.

Fig. S2 Fraction of chlorinated ethenes (PCE: \circ , TCE: \Box , cis-DCE: \circ) versus time of the 4th spike for the binary mixed culture containing *Desulfitobacterium dehalogenans strain PCE1 and Desulfitobacterium hafniense strain Y51*. Shown are the results of the two microcosm replicates (MC1 Desuguobacter lam nations is strain 191. Shown are the results of the two interocosin repricates (NCT: and MC2) as well as the cell free control. Error bars represent standard deviation of calculated fractions from GC-MS concentrations measurements $(n = 2)$. and MC2) as well as the cell free control. Error bars represent standard deviation of calculated fraction

Fig. S 3 Fraction of chlorinated ethenes (PCE: \circ , TCE: \Box , cis-DCE: \circ) versus time of the 7th spike for the binary mixed culture containing *Desulfitobacterium dehalogenans strain PCE1 and* Desulfitobacterium hafniense strain Y51. Shown are the results of the two microcosm replicates (MC1

and MC2) as well as the cell free control. Error bars represent standard deviation of calculated fractions from GC-MS concentrations measurements $(n = 2)$.

Fig. S4 Fraction of chlorinated ethenes (PCE: \circ , TCE: \Box , cis-DCE: \circ) versus time of the 12th spike for the binary mixed culture containing *Desulfitobacterium dehalogenans strain PCE1 and Desulfitobacterium hafniense strain Y51*. Shown are the results of the two microcosm replicates (MC1 and MC2) as well as the cell free control. Error bars represent standard deviation of calculated fractions from GC-MS concentrations measurements $(n = 2)$. Fig. 34 Fraction of Chiorinated ethenes (FCE, \cup , TCE, \sqcup , Cis-DCE, \vee) versus time of the 12 Spike from

Fig. S5 Fraction of chlorinated ethenes (PCE: \circ , TCE: \Box , cis-DCE: \circ) versus time of the 15th spike for the binary mixed culture containing *Desulfitobacterium dehalogenans strain PCE1 and* Desulfitobacterium hafniense strain Y51. Shown are the results of the two microcosm replicates (MC1 and MC2) as well as the cell free control. Error bars represent standard deviation of calculated fractions from GC-MS concentrations measurements $(n = 2)$.

Fig. S6 Fraction of chlorinated ethenes (TCE: \Box , cis-DCE: \Diamond) versus time of $8th$ spike with TCE for the binary mixed culture containing *Desulfitobacterium dehalogenans strain PCE1 and Desulfitobacterium hafniense strain Y51*. Shown are the results of the two microcosm replicates (MC1 and MC2) as well as **Fig. S6** Fraction of chlorinated ethenes (TCE: \Box , cis-DCE: \Diamond) versus time of 8^{th} spike with TCE for the degradation of chlorinated ethenes (TCE: \Box , cis-DCE: \Diamond) versus time of 8^{th} spike with TCE fo

the cell free control. Error bars represent standard deviation of calculated fractions from GC-MS concentrations measurements $(n = 2)$.

Comparison of Strain Specific PCE turnover: CSIA vs. qPCR

Cell specific contribution to PCE turnover

The cell specific contribution to PCE turnover was calculated according to:

cell specific contribution
$$
A = \frac{\varepsilon_{\text{measured}} - \varepsilon_{\text{B}}}{\text{cells}_A \times (\varepsilon_{\text{B}} - \varepsilon_A)}
$$
(S4)

with the measured isotope enrichment factor $\varepsilon_{\text{measured}}$ of the binary synthetic culture, the isotope enrichment factors of the pure culture of each strain (ε_A and ε_B) and the cell number of strain A (cells_A). Cell specific contribution was calculated at the start of the experiment as well as and after complete PCE transformation for each PCE re-spike (PCE_end) (see **Fig. S7**).

Fig. S7 Cell specific contribution of Desulfitobacterium dehalogenans strain PCE1 and Desulfitobacterium hafniense strain Y51 within the binary synthetic culture. The contribution was calculated based on Eq. S4*.*

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Research data

Data-Table 1: Chlorine and Carbon Isotope Data for the Respike-Experiment

Data-Table 2 Carbon and Chlorine Isotope Date for Rayleighplot

Data-Table 3: Gene copy numbers

Data-Table 4 Calculated cell fractions

