Supporting Information

$\delta^{13}C$ and $\delta^{37}Cl$ isotope fractionation to characterize aerobic vs. anaerobic degradation of trichloroethylene

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Methods

Culture incubation

Two pure strains were used for the batch experiments: *Pseudomonas putida* F1 and *Methylosinus strichosporium* OB3b, strains were kindly provided by Prof. Lawrence P. Wackett from the University of Minnesota and Dr. Jeremy Semrau from the University of Michigan. The growth media for F1 was prepared as described previously[1] and amended with 1 ml of trace element solution C [2]. *Methylosinus trichosporium* OB3b was cultivated in liquid media as described previously [3] with or without using $CuSO_4$ to express for either pMMO or sMMO respectively. Cultures were grown in 1 l glass bottles containing 300 ml of growth media and capped with Teflon-lined septa. As a carbon source, F1 cultures were amended with 700 µl of toluene (Daejung, ≥99.7%) and OB3b were grown with a gas phase containing 20% methane (Maxima, 99.95%). Cultures were incubated in the dark at 30°C on an orbital shaker (180 rpm). Before the experiments started, cultures where daily amended with a fresh carbon source. For strain F1, one set of experiments was used for carbon isotope measurements and a second set was used for chlorine isotope measurements. For strain OB3b a single experiment was analyzed for both chlorine and carbon isotope measurements. When expressing sMMO, a second experiment (experiment B) was analyzed for chlorine isotopes only.

Experimental setup

Pure cultures were harvested by centrifugation (3500 rpm for 15 min) and re-suspended in fresh growth medium to obtain an optical density of ~ 0.2, with the exception of experiment B (OD ~ 0.4). Experimental growth media (500 ml) was prepared as described above with the exception that it contained also resazurin (1 mg/l) as redox indicator, TCE (5.8 mg/l) (Sigma–Aldrich, ≥99.5% or Bio-Lab ltd, ≥99.5%) and either ethanol (158 mg/l) or potassium formate (0.02 M) as NADH source for F1 or OB3b cultures, respectively. After preparation, experimental growth medium was allowed to equilibrate overnight on a magnetic stirrer. For initiating the experiments, one milliliter of the concentrated bacterial culture was immediately transferred into 60 ml autoclaved serum bottles. Growth media was then added to each of the bottles (1:5 liquid to air) and the bottles were immediately crimped with Viton septa (20 mm Straight Plug OV P/N 13235). Bottles were shaken upside down to prevent leakage of the gas phase through the septa.

Sampling

All experiments were conducted in triplicates. Each experiment was accompanied by abiotic controls (no bacterial culture) to account for evaporation (TCE evaporation <5%), and biotic controls. Additionally, to

avoid piercing the bottle septa during incubation, the process of degradation was evaluated by additional pre-experiment bottles (Con A and Con B). The pre-experimental bottles where set up as explained above, Con A was haltered immediately by adding Phosphoric acid (98%), and the concentration of both Con A and Con B where monitored throw time. This was done due to several reasons i) in order to determine the degradation rate for each culture individually, ii) to avoid piercing the bottle septa and iii) avoid altering the liquid to head space ratio between experimental repetitions. Sampling of the experiment was conducted by sacrificing 3 replicate bottles at each time step, while the actual concentration in each bottle was validated only after the sacrificial sampling. TCE concentrations were quantified using a GC/MS (Trace 1310 coupled to a ISQ LT, Thermo Fisher Scientific). Phosphoric acid (98%) was added to each bottle (to reach pH≤ 2) for halting the degradation process. Once the experiment was completed, 0.5 ml of each repetition was used for concentration measurements to determine the remaining fraction of TCE (*f*, eq. 2). The rest of the liquid was then divided into 20 ml vials capped with Teflon-lined septa and preserved in frozen form [4]. Frozen samples, from F1 and sMMO experiments, were distributed between the Geological Survey of Israel (GSI) for chlorine isotope measurements, and the Helmholtz Zentrum München for carbon and chlorine isotope measurements.

Preparation of $\delta^{37}Cl$ standards

Pure TCE (Sigma – Aldrich, ≥99.5%) was allowed to freely evaporate in a fume hood to obtain isotopically distinct TCE standards. Samples were collected along the evaporation process producing three standards: AG-0, AG-1 and AG-2 which were isotopically characterized relative to EIL-1 and EIL-2 [5] by IRMS at the Helmholtz Zentrum München. The calibrated $\delta^{37}Cl_{SMOC}$ values of the standards were -0.24 ± 0.01‰, 1.18 ± 0.12‰ and 2.94 ± 0.11‰, respectively. These standards were used for calibrating the $\delta^{37}Cl$ measurements by GC/MS to the SMOC scale.

Chlorine isotope measurements

Helmholtz Zentrum München: a TRACE GC instrument (Thermo Fisher Scientific, Milan, Italy) coupled to a Finnigan MAT 253 IRMS instrument (Thermo Fisher Scientific, Bremen, Germany) was used. Two most abundant fragment ions (m/z 95 and 97) were measured. All analyses involved injections of 1 mL headspace from 10 mL vials that contained 5 mL of solution (~0.2 mg/l), vails were agitated at 50°C for 5 min before injection and injector temp was held at 180°C. Peak separation was achieved using a DB-5 column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies). The column flow was 1.4 ml/min with a split flow of 14 ml/min. To avoid introduction of water to the IRMS, an additional wax column was used (60 m × 0.25 mm × 0.50 μm; Supelcowax™ 10) with a VALCO valve between the two columns. The GC

oven started at 70ºC (2 min), followed by a ramp of 10°C/min to 100°C and a second ramp of 60°C/min to 172°C. A volume of 1-2 µL of TCE was introduced the to the dual-inlet bellow to serve as a reference gas. Three reference peaks were introduced at the beginning and at the end of each analysis run.

Geological Survey of Israel: 6890N GC (Agilent) coupled to a 5975B qMS (Agilent) was used with an Rxi-5HT capillary column (30 m, 0.25 mm ID, 0.25 µm; Restek USA). Purge&Trap (OI Analytical) was used for TCE extraction and pre-concentration. Ten ml of water containing ~200 mg/l TCE were purged with He flow 40 ml/min for 11 min, following by desorption at 220^0C for 4 min; transfer line was held at 140°C. The following conditions were applied for GC run: injector was held at 220°C; split ratio 1:10; He was used as a carrier with flow 1 ml/min; the GC oven started at 40^0C and heated with 6^0C/min to 120°C. Ions defined by the single ion mode were m/z 60,62,95,97,130 and 132, and a dwell time of 0.02 sec was defined for all measurements. Electron energy was set to 70 eV and tuning was performed before each sequence.

Carbone isotope measurements

Helmholtz Zentrum München: Headspace injection was performed following a TRACE GC instrument (Thermo Fisher Scientific, Milan, Italy) coupled via a GC combustion interface to a Finnigan MAT 253 IRMS instrument (Thermo Fisher Scientific, Bremen, Germany). This analysis was done for samples of strain OB3b expressing sMMO enzyme. Analyses involved injections of 2 ml headspace from 10 ml vials that contained 5 ml of solution. Vails were agitated at 50°C for 5 min before injection. Injector temp was held at 150°C. Peak separation was achieved using a DB-5 column (30 m × 0.25 mm × 0.25 µm; Agilent Technologies). The column flow was 1.4 ml/min with a split flow of 7 ml/min. Three reference peaks of CO_2 calibrated against international standards were introduced at the beginning ant at the end of each analysis run.

Geological Survey of Israel: Carbon isotope analysis was done by Purge&Trap (Atomx; Teledyne Tekmar) connected to a GC/IRMS (GC Trace 2000; Delta V Plus; Thermo Scientific). TCE was purged from 10 ml of water sample with helium flow (40 ml/min) for 11 min, following by dry purge of the trap for 0.5 min with helium flow of 200 ml/min, and desorption at 245°C for 2 min. Peak separation was achieved using a DB-VRX column (30 m, 0.25 mm ID, 1.4 µm; Agilent USA). Injector was held at 220°C; oven started at 40°C and heated 6°C/min to 120°C. Three reference peaks of CO_2 calibrated against international standards were introduced at the beginning ant at the end of each analysis run.

Ben Gurion University: Carbon isotope analysis was done for strain OB3b expressing pMMO enzyme. Samples were introduced by Purge&Trap (Atomx; Teledyne Tekmar) connected to a GC/IRMS instrument (GC Trace ultra; Delta V Plus; Thermo Scientific). TCE was purged from 10 ml of water sample with helium flow (40 ml/min) for 11 min, following by dry purge of the trap for 2 min with

helium flow of 200 ml/min, and desorption at 280°C for 2 min. Peak separation was achieved using a Rxi®-5Sil MS column (30 m × 0.25 mm × 0.25 µm; RESTEK). Injector was held at 250°C with a 40 ml/min split and a constant flow of 0.8, oven started at 35°C (3 min), followed by a ramp of 10°C/min to 90°C and a second ramp of 30°C/min to 250°C held for 1 min. Three reference peaks of CO_2 calibrated against international standards were introduced at the beginning ant at the end of each analysis run.

Chloral hydrate concentrations

Chloral hydrate analysis was done following liquid-liquid MTBE extraction modified from Nikolaou et al. [6]. Chloral hydrate formation was monitored on a separate experiment conducted in triplicates. Similarly to the explained above with minor changes, each vail contained one milliliter of bacterial culture and nine milliliter of experimental media using glass vails sealed with Teflon lined septa and incubated up-side-down at 30^0C. At each time step 1 ml of MTBE was add to three of the vails. Each vail was then vigorously shaken by hand for 1 min, then 2 ml of the top fraction where transferred to Two ml glass vails. In order to recover the organic phase and discard the water, the two ml glass vails where frozen in -20^0C and the organic unfrozen phase was collected to a clean 2 ml glass vail for further analysis. Chloral Hydrate standard was prepared in water 0.05 – 0.2 mg/l and a similar extraction process was conducted. Concentrations were determined using GC (1310 Trace; Thermo Scientific) coupled to a ISQ LT qMS (Thermo Scientific) with an Rxi-624Sil MS column (30 m × 0.25 mm × 0.25 µm; RESTEK). 1 µl of liquid sample was injected using a TriPlus RSH (Thermo Scientific) auto sampler. Injector was held at 175°C, and flow velocity was 1 ml/min, with a split ratio of 20 ml/min. The oven started at 35°C (0.5 min), followed by a ramp of 5°C/min to 85°C and a second ramp of 40°C/min to 230°C held for 2 min. Ions defined by the single ion mode were m/z 82 with a 0.2 (sec) dwell time defined for all measurements. Electron energy was set to 70 eV and auto-tuning was performed before each sequence.

Determining TCE concentrations

GC (1310 Trace; Thermo Scientific) coupled to a ISQ LT qMS (Thermo Scientific) was used with an Rxi-624Sil MS column (30 m × 0.25 mm × 0.25 µm; RESTEK). All analyses involved injections of 0.75 ml headspace using a TriPlus RSH (Thermo Scientific) auto sampler, twenty mL vials that contained ten ml of solution were agitated at 50°C for 15 min before injection. Injector was held at 200°C, flow velocity was 1 ml/min with a split ratio of 10 ml/min. The oven started at 40°C (1 min), followed by a ramp of 20°C/min to 60°C and a second ramp of 45°C/min to 250°C held for 1 min. Ions defined by the scan mode of 50-200 m/z with 0.2 (sec) dwell time defined for all measurements. Electron energy was set to 70 eV and auto-tuning was performed before each sequence.

RESULTS

Table S1: Measured chlorine isotopic enrichment values by two instruments

	$\varepsilon^{37}Cl$	
	IRMS	qMS
Methylosinus trichosporium OB3b, sMMO	-1.2±0.1[a]	-0.9±0.3 [a]
	-1.5±0.2[b]	-1.6±0.4 [b]
Pseudomonas. putida F1	0.3±0.2	0.1±0.3
[a] Experiment A [b] Experiment B		

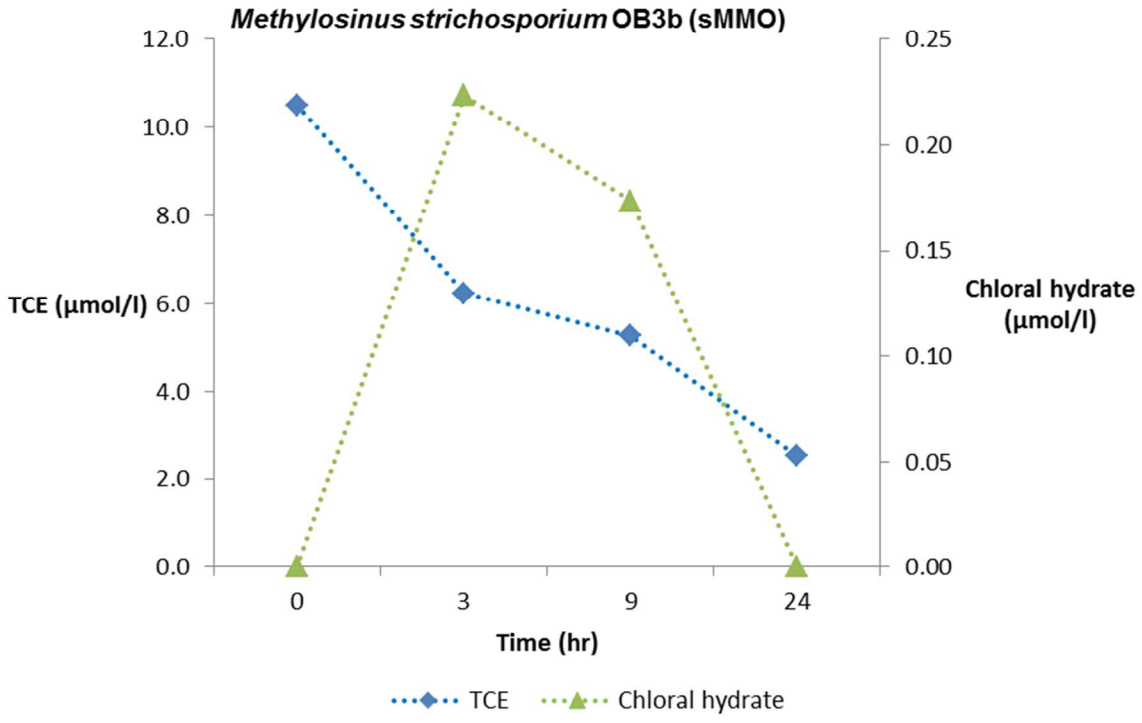

Figure S1: Chloral hydrate formation during TCE degradation using OB3b expressing sMMO enzyme. A similar experiment using pMMO enzyme did not show any chloral hydrate formation (data not shown).

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