# Supporting Information: Effects of aqueous film-forming foams (AFFFs) on trichloroethene (TCE) dechlorination by a *Dehalococcoides mccartyi*–containing microbial community

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**Analytical Methods Used for This Study**

***Chloroethene, methane, and hydrogen measurement.*** TCE, cDCE, VC, ethene, and methane were regularly measured in all experiments by injecting 100 µL of culture headspace into an Agilent 7890A GC-FID equipped with a GS-GasPro capillary column (30 m x 0.32 mm; Agilent Technologies, Inc., Santa Clara, USA). The oven temperature was ramped up from 45 to 200 °C in 4 minutes, with a 1 minute hold at 200°C. The injector and detector temperatures were maintained at 220 and 250 ºC, respectively. Hydrogen concentrations were measured by injecting diluted headspace samples into a GC fitted with a reductive gas detector (Trace Analytical, Menlo Park, CA, USA) as previously described.[1,2] Between 50 and 300 µL of culture headspace was withdrawn for each hydrogen measurement and diluted in 17 mL glass vials purged with nitrogen to generate concentrations within the linear calibration range of the instrument.

***PFAS measurement.*** PFAS compounds were quantified on an Agilent 6410 LC-MS/MS operating in both positive and negative electrospray ionization modes using the MS parameters, ion transitions, and internal standards as previously described.[3,4] Diluted culture samples reserved for LC-MS/MS quantification were first vortexed for 30 minutes at room temperature and then centrifuged at 15000 x g for 10 minutes. The supernatant was diluted with HPLC-grade methanol and water and then amended with 50 µL of an internal standard stock containing 20 to 40 µg/L of various internal standards.[3,4] PFAS analytes for which a commercial analytical standard or quantified source material was available were directly quantified using isotope dilution. For the PFAS compounds in the 3M AFFF formulation used in this study: $C_4$, $C_5$, $C_6$, and $C_8$ perfluoroalkyl sulfonamide amino carboxylates (PFSaAmA) and $C_4$, $C_5$, $C_6$, and $C_8$ perfluoroalkyl sulfonamido amines (PFSaAm), the concentrations in the cultures were estimated according to the method described in the supporting information of Houtz *et al.* 2013, which first normalized the LC-MS/MS analyte responses of the sulfonamide compounds to the PFHxS internal standard response and then quantified a concentration utilizing an empirically-generated relative response coefficient for each PFSaAmA and PFSaAm compound.

***Organic acid quantification.*** Acetate, lactate, propionate, and butyrate were quantified with on an HPLC equipped with an Aminex HPX-87H ion exclusion column (300 x 7.8mm, Bio-Rad, Hercules, CA) and a photodiode array detector (PDA) set at 210 nm (Waters, Milford, MA). The solvent consisted of 5 mM $H_2SO_4$ and was provided at a constant flow rate of 0.6 mL/min. 200 µL of centrifuged culture sample was acidified with $H_2SO_4$ in a 1 mL amber glass vial, and all organic acids were quantified using external calibration curves as previously described.[1,5]

***Dehalococcoides 16S rDNA quantification.*** *Dehalococcoides* cell numbers were determined by quantifying *Dehalococcoides* 16S rRNA gene copies in the AFFF-amended cultures. Cells were collected from 1.5 mL of centrifuged culture sample (15000 x g for 10 minutes) taken from AFFF amended incubations on days 0, 12, 20, 31, 41, and 53. DNA was extracted from the cell pellets using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc, Carlsbad, CA, USA) according to the manufacturer's instructions. *Dehalococcoides* 16S rRNA gene copies were quantified with quantitative polymerase chain reaction (qPCR) using the protocol, primers, and standards described previously.[1] DNA extraction and 16S qPCR enumeration conducted on non-AFFF controls (data not shown) indicates that the AFFF surfactants had no significant effect on extraction and qPCR efficiency. It is assumed the *Dehalococcoides* strain in this enrichment culture contains one 16S rRNA gene copy per cell.[1]

**Table S1**. The AFFF and DGBE amendment regime to live *D. mccartyi*-containing enrichment cultures (AFFF amendment experiments).

**Experimental setup:** Growth medium: 100 mL; TCE: 20 to 30 μmoles

| Culture | Amendment 1 | | Amendment 2 | | Amendment 3 | | Amendment 4 | | Amendment design |
|---|---|---|---|---|---|---|---|---|---|
| | Formulation (vol) | day | Formulation (vol/mass) | day | Formulation (vol) | day | Formulation (vol) | day | |
| 3M AFFF-amended live cultures | 3M (300 μL) | 0 | DGBE (250 μmoles)* | 13 | 3M (300 μL) | 18 | 3M (300 μL) | 40 | To investigate whether DGBE was the constituent in the 3M AFFF responsible for the hydrogen and acetate production |
| Ansul AFFF-amended live cultures | Ansul (300 μL) | 0 | 3M (300 μL) | 33 | | | | | To investigate whether the 3M AFFF contained fermentable substrates that could promote TCE dechlorination |
| National Foam-amended live cultures | National Foam (300 μL) | 0 | 3M (300 μL) | 33 | | | | | |

*Note: The DGBE mass added was chosen to approximate the concentration that would be expected with a 300-μL AFFF amendment with approximately 20% (w/w) DGBE, according to reported MSDS information on AFFF foams.

**Table S2.** Composition of three AFFF formulations (3% concentrates) according to published MSDS information. Empty cells indicate the ingredient was not reported on the formulation's MSDS.

| | **Composition of formulation (% by weight)** | | |
|---|---|---|---|
| **Ingredient** | 3M Lightwater FC-203CF (3%)[a] | Ansul Ansulite AFC-5-A (3%)[b] | National Foam Aero-O-Water 3EM (3%)[c] |
| Water | 69 - 71 | 75 - 80 | 62 - 79 |
| Diethylene glycol butyl ether (DGBE) | 20 | 17 | 8 - 12 |
| Ethylene glycol | | | 6 - 8 |
| Hexylene glycol | | 0.5 | |
| 1-propanol | | 0.4 | |
| Perfluoroalkyl sulfonate salts (*i.e.* PFSAs) | 0.5 - 1.5 | | |
| Hydrocarbon surfactants (proprietary) | 1 – 5[d] | 5 - 10 | 6 - 11 |
| Fluorinated surfactants (proprietary) | 1 – 5[e] | | 0.5 - 2 |
| Triethanolamine | 0.5 – 1.5 | | |

[a][FC-203CF, MSDS No CKQCB]
[b][Ansulite 3% AFFF AFC-5-A, MSDS]
[c][Aer-O-Water 3EM, MSDS No. MNS210]
[d]Reported as "alkyl sulfate salt"
[e]Reported as "amphoteric fluoroalkylamide derivative"

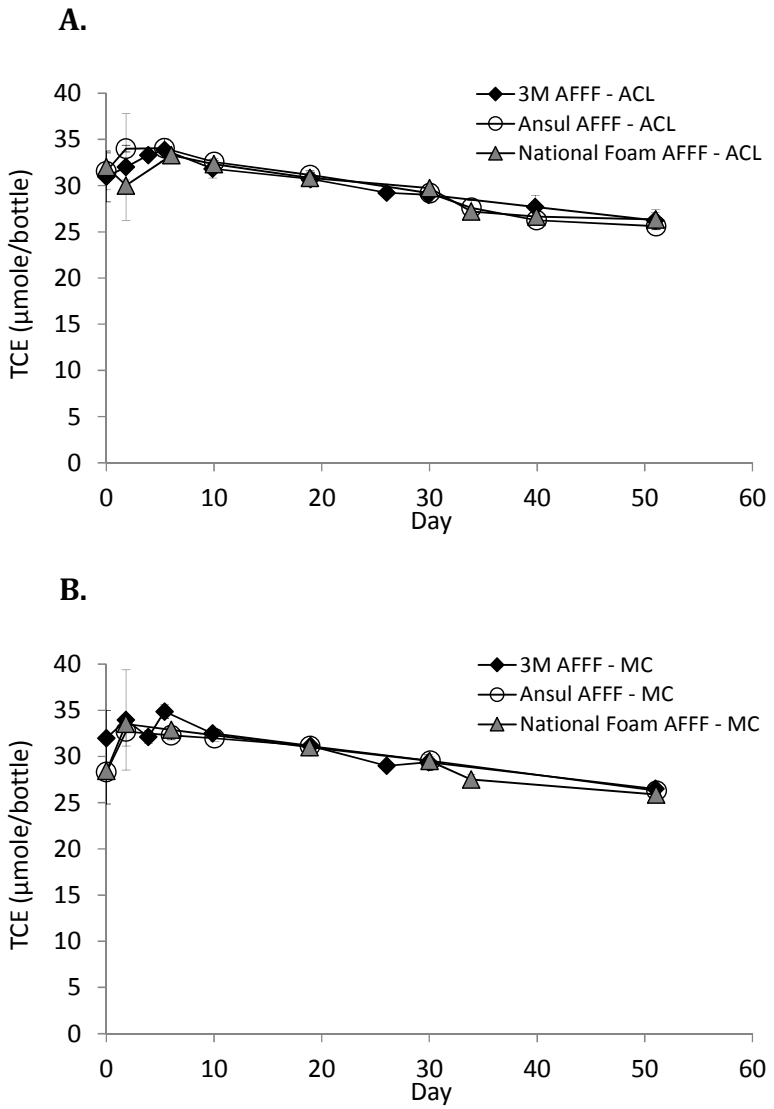


**Figure S1.** TCE dechlorination in AFFF-amended autoclaved (ACL) cultures (A) and medium controls (MC) (B). Error bars represent the standard deviation of biological triplicates. No cDCE, VC, ethene, hydrogen, or methane production was detected in the autoclaved or medium control cultures.

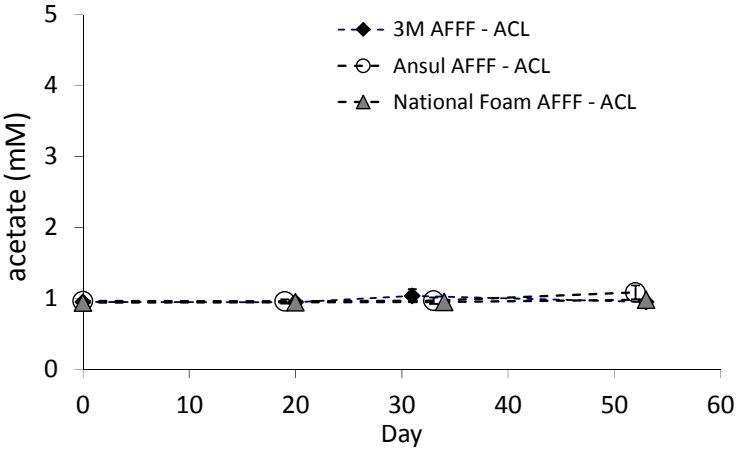


**Figure S2.** Acetate concentration in autoclaved cultures (ACL) amended with 3M AFFF, Ansul AFFF, or National Foam AFFF. Error bars represent the standard deviation of triplicate biological bottles.

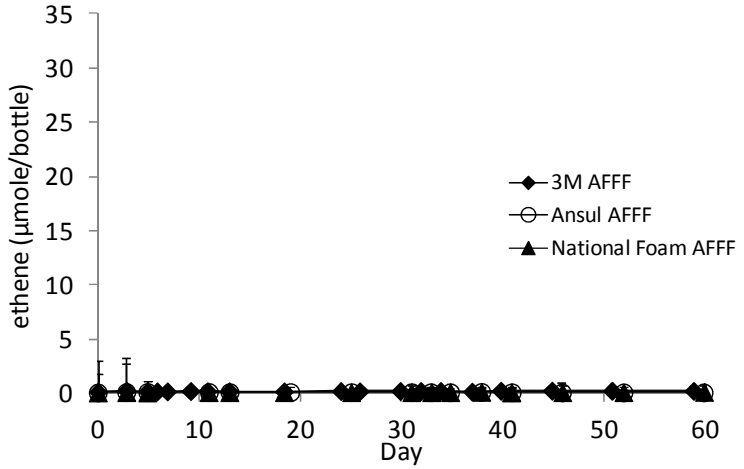


**Figure S3.** Ethene concentrations in cultures amended with a 3M, Ansul, or National Foam AFFF. Error bars represent the standard deviation of triplicate biological bottles.

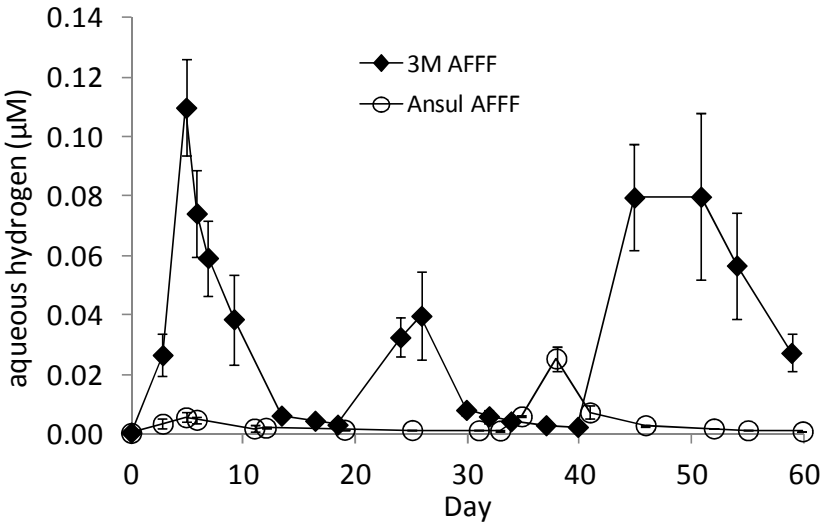


**Figure S4.** Hydrogen production in 3M and Ansul AFFF-amended cultures. This figure is related to text Figure 2A: the y-axis scale has been decreased in order to zoom in on the measured hydrogen concentrations in these conditions. Error bars represent the standard deviation of triplicate biological bottles.

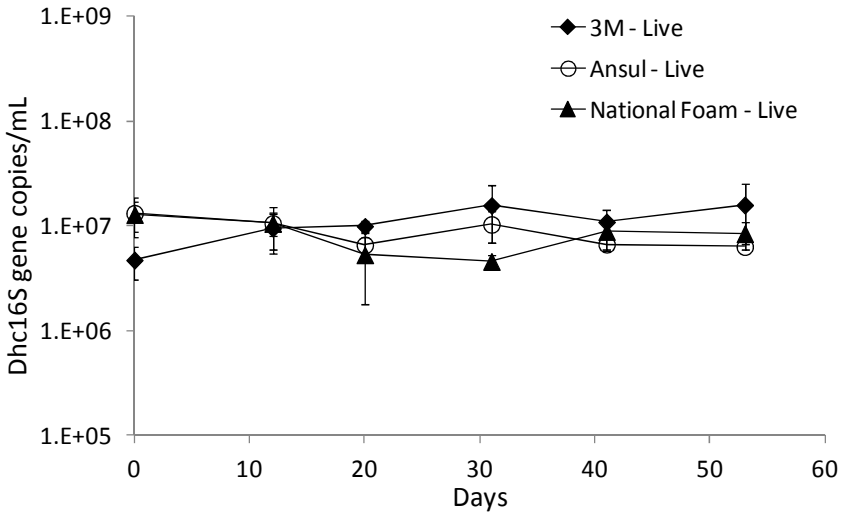


**Figure S5.** *Dehalococcoides* 16S rDNA copies in 3M, Ansul, and National Foam-amended TCE-dechlorinating cultures. Error bars represent the standard deviation of triplicate biological bottles.

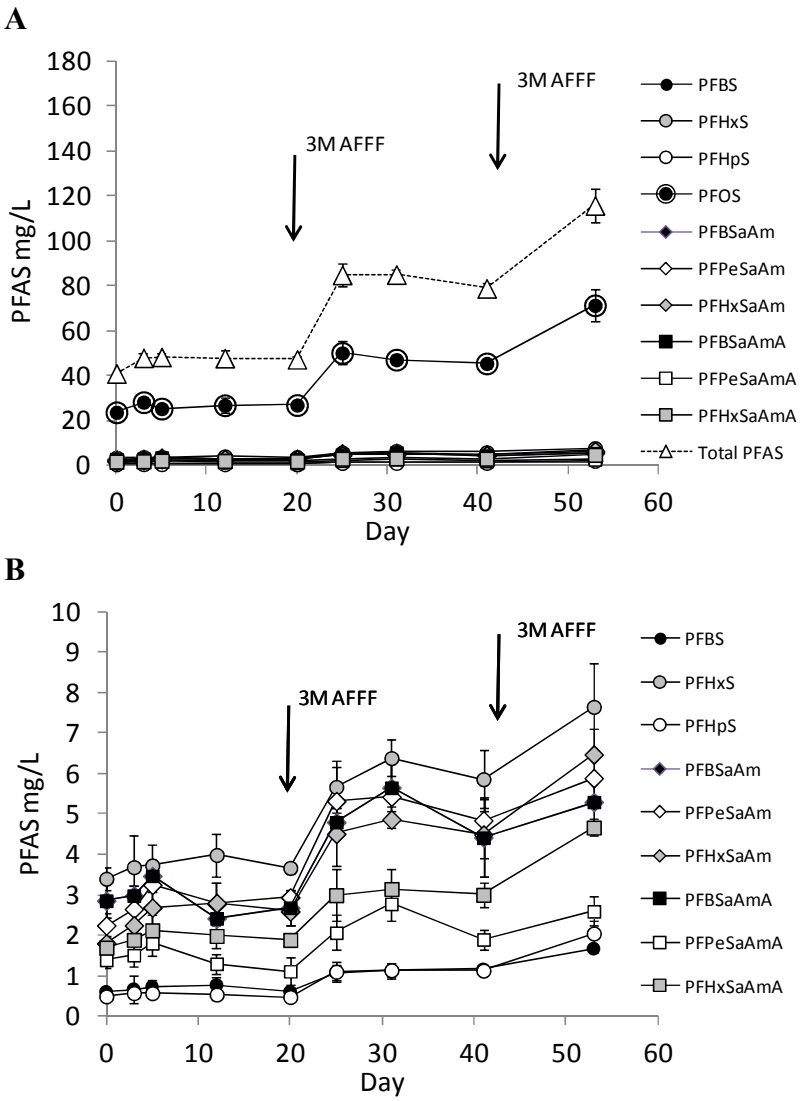


**Figure S6.** Concentrations of measured PFAS compounds in live TCE-dechlorinating cultures initially amended with 3M AFFF (A, upper). The arrows denote two additional 3M AFFF amendments that were made to the live cultures. The "Total PFAS" concentration (A) represents the sum of all PFAS compounds plotted in A and B. Plot B (lower) is related to plot A: the y-axis scale has been decreased to zoom in on measured PFAS compounds. Error bars represent the standard deviation of triplicate biological bottles.

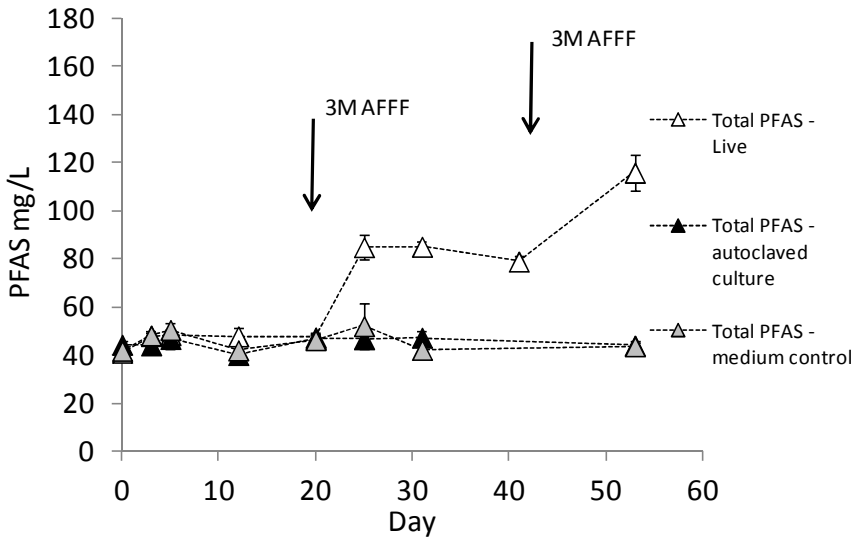


**Figure S7.** The total PFAS concentrations measured in live cultures, autoclaved cultures, and medium controls initially amended with 3M AFFF. The two additional 3M AFFF amendments were made to the live cultures only. Error bars represent the standard deviation of triplicate biological bottles.

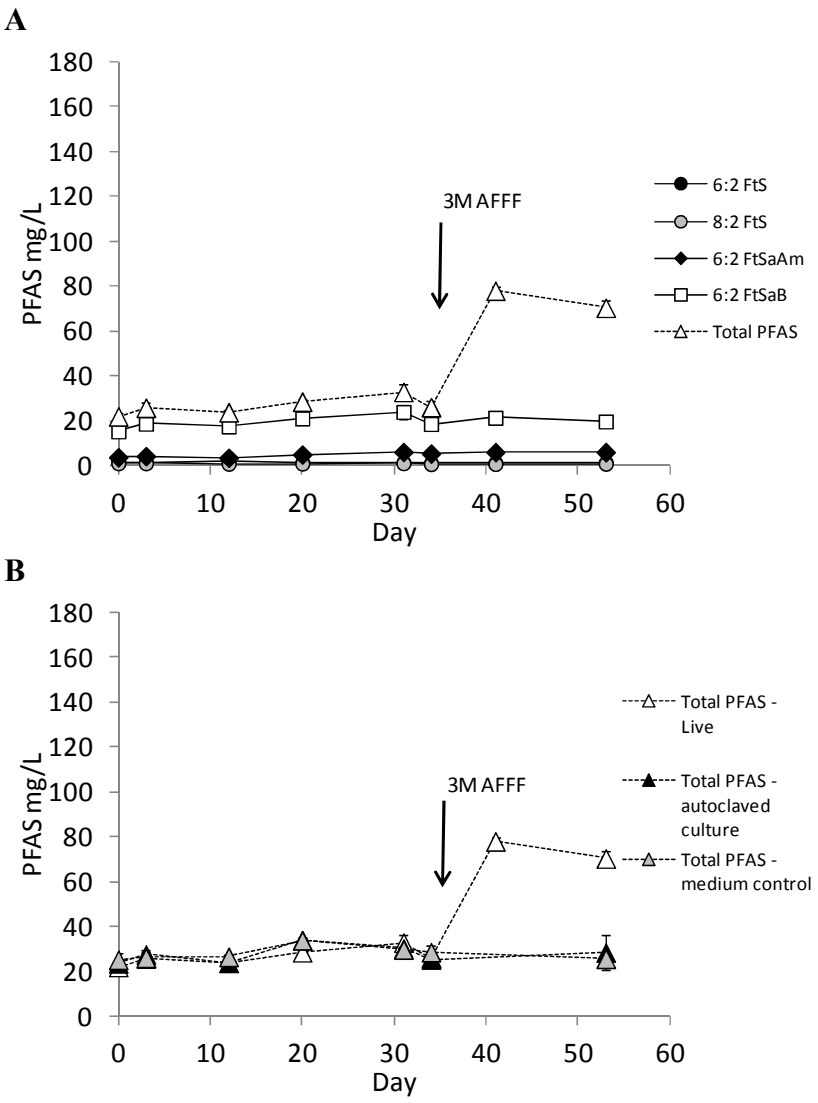


**Figure S8.** Concentrations of all measured PFAS compounds in live cultures initially amended with National Foam AFFF (A, upper). The arrow denotes a 3M AFFF amendment that was made to live cultures only. The "Total PFAS" concentration in A represents the sum of all PFAS compounds measured in the live cultures, while plot B (lower) shows the total PFAS concentration measured in the autoclaved cultures and medium control. Error bars represent the standard deviation of triplicate biological bottles.

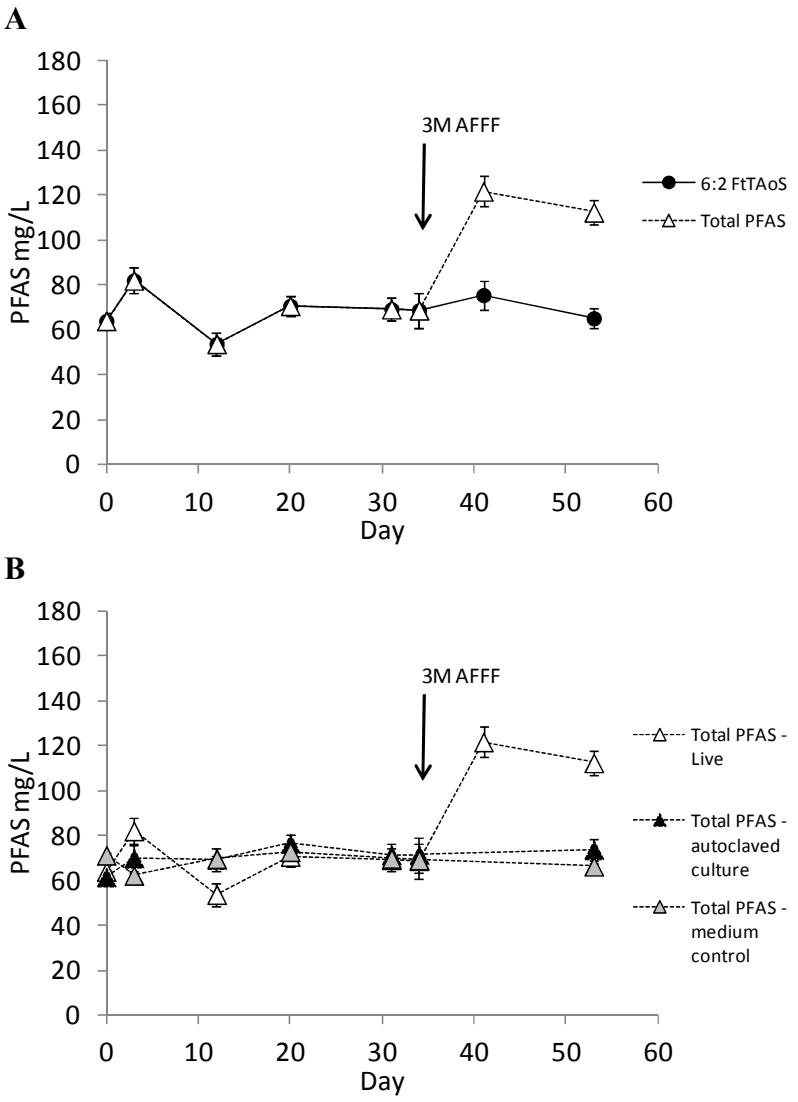


**Figure S9.** Concentrations of all measured PFAS compounds in live cultures initially amended with Ansul AFFF (A, upper). The arrow denotes a 3M AFFF amendment that was made to live cultures only. The "Total PFAS" concentration in A represents the sum of all PFAS compounds measured in the live cultures, while plot B (lower) shows the total PFAS concentration measured in the autoclaved cultures and medium control. Error bars represent the standard deviation of triplicate biological bottles.

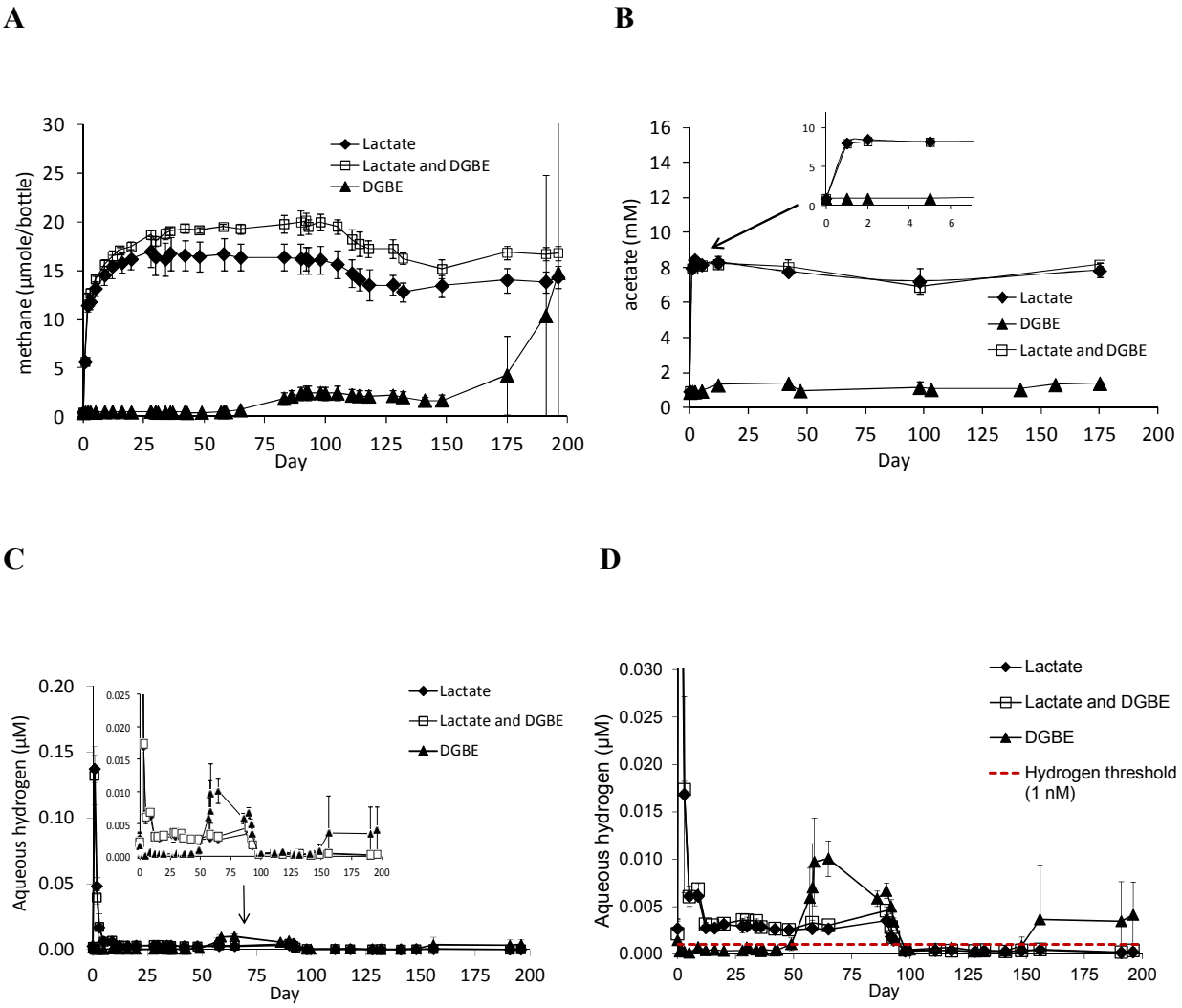


**Figure S10.** Concentrations of methane (A), acetate (B), and aqueous hydrogen (C, decreased y-axis scale: D) in cultures amended with either lactate, lactate + DGBE, or DGBE-only as a carbon and energy source. Inset on C shows reduced y-axis scale to show hydrogen concentrations, while plot D is a larger view of the inset with the *Dehalococcoides* (Dhc) previously-published minimum hydrogen threshold shown (1nM). Error bars represent the standard deviation of triplicate biological bottles.

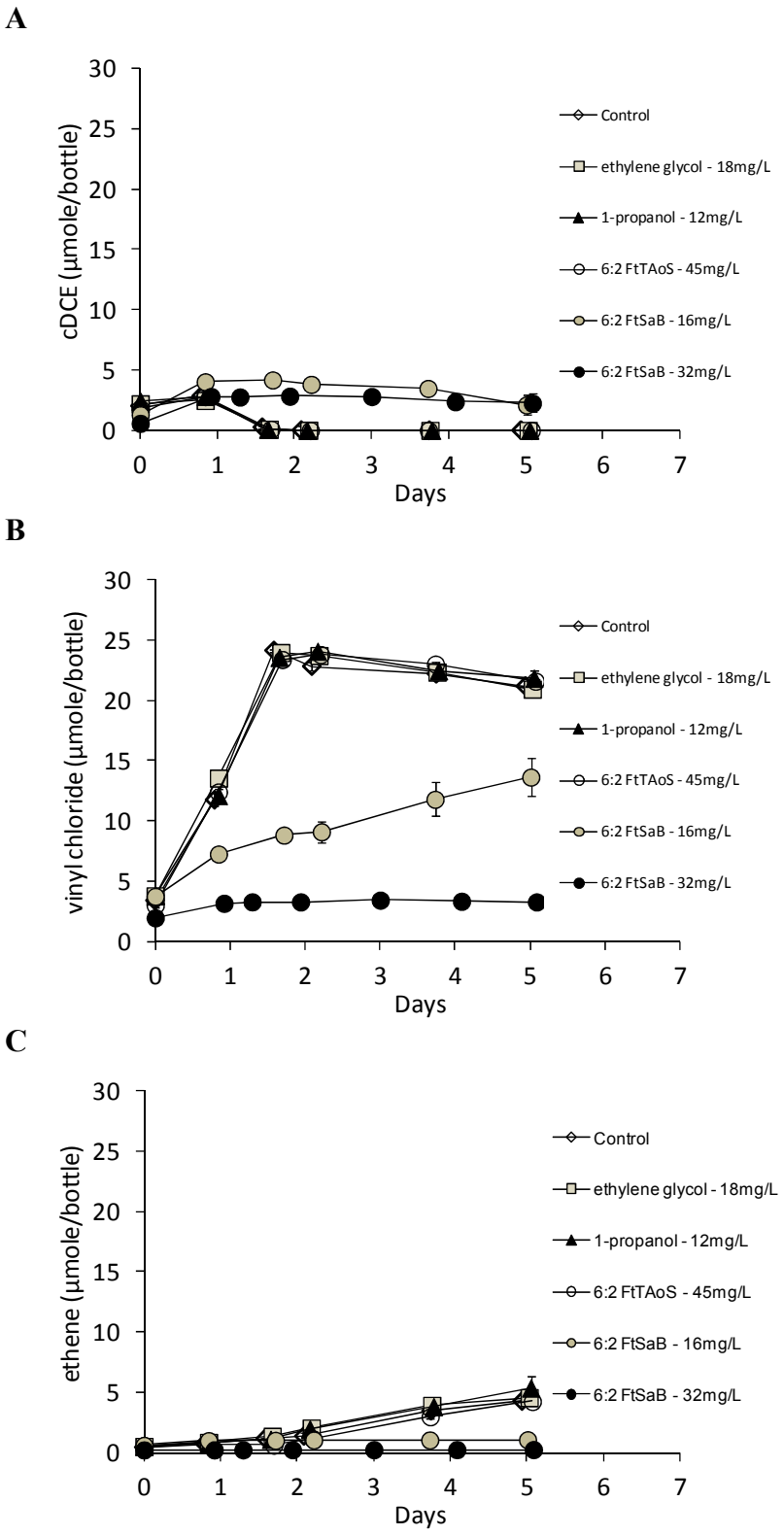


**Figure S11.** Production of cDCE (A), vinyl chloride (B), and ethene (C) following TCE dechlorination in a *Dehalococcoides* enrichment culture amended with various concentrations of AFFF constituents. Error bars represent the standard deviation of triplicate biological bottles.

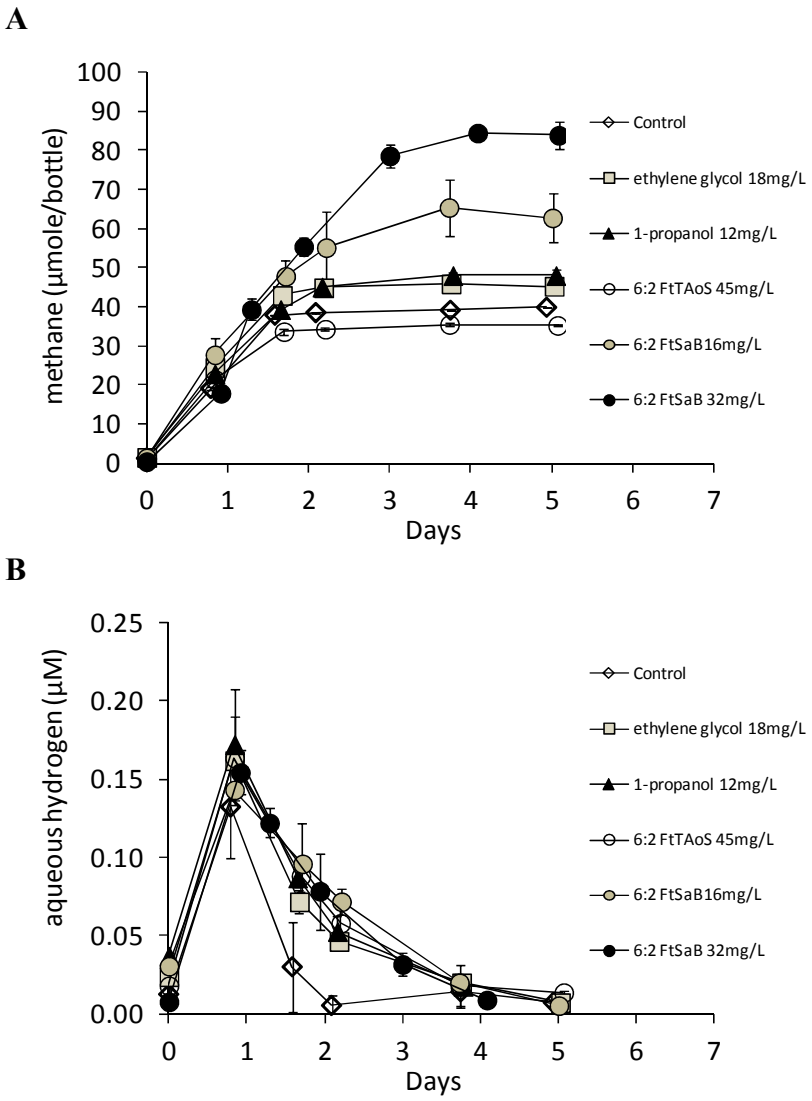


**Figure S12.** Methane (A) and hydrogen (B) production during TCE dechlorination in a *Dehalococcoides* enrichment culture amended with various concentrations of AFFF constituents. Error bars represent the standard deviation of triplicate biological bottles.

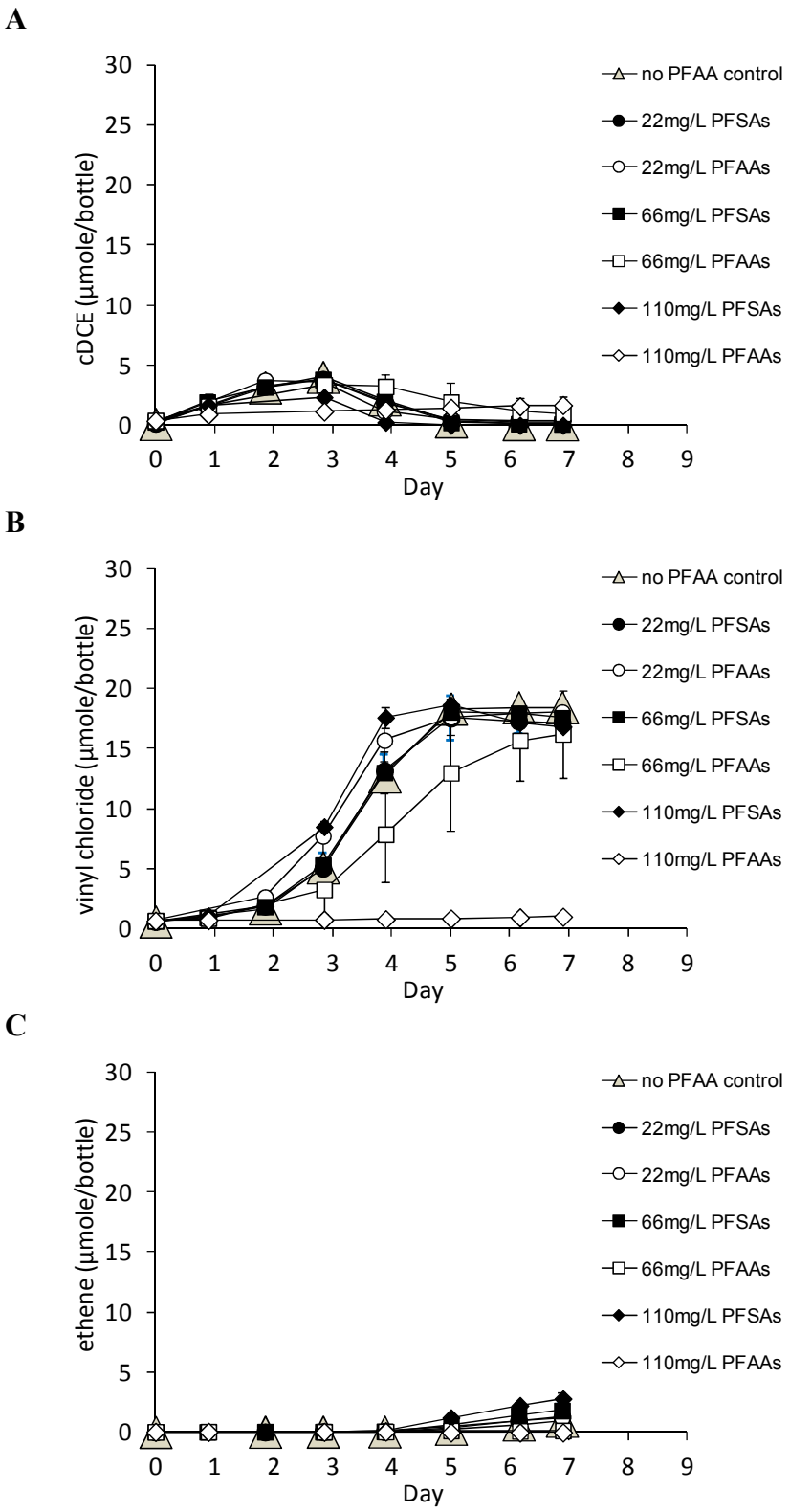


**Figure S13.** Concentration of cDCE (A), vinyl chloride (B), and ethene (C) following TCE dechlorination in a *Dehalococcoides* enrichment culture amended with various concentrations of PFAAs (PFSAs and PFCAs) and PFSAs only. Plots include data from Weathers et. al 2016.[6]

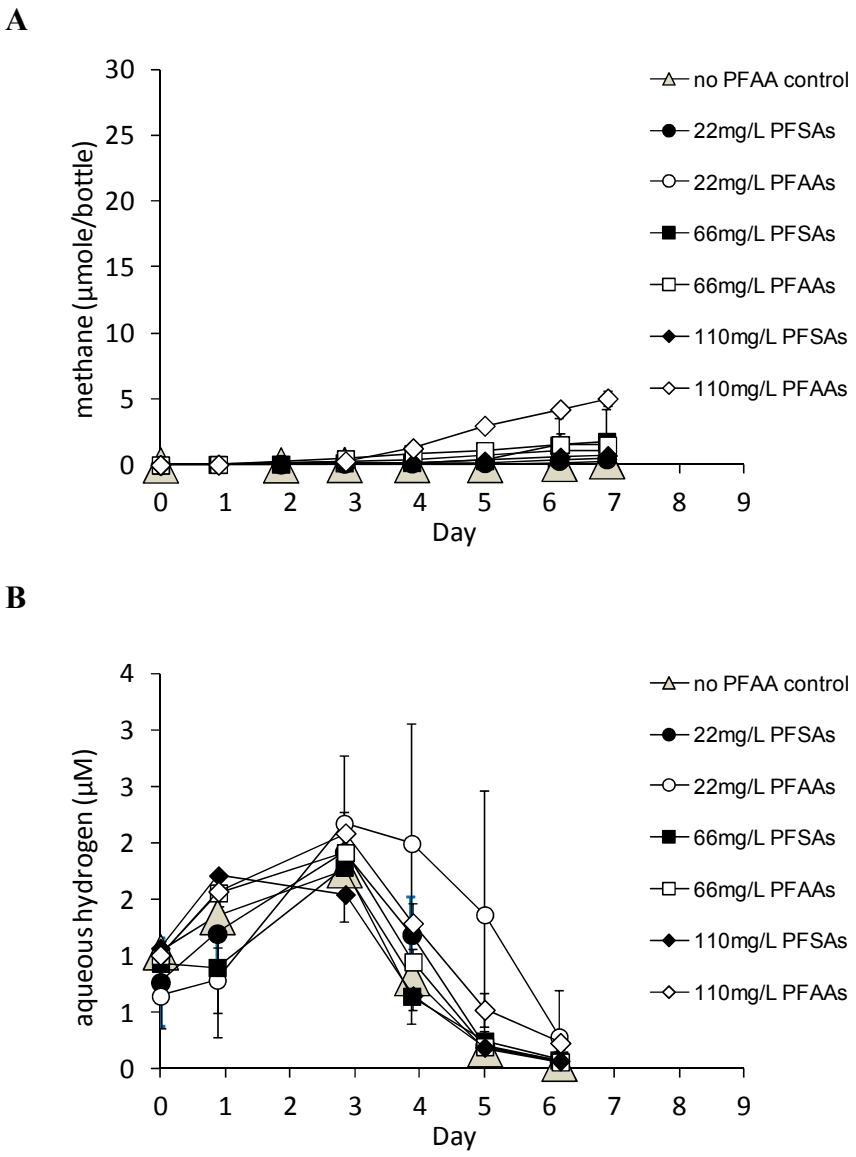


**Figure S14.** Methane (A) and hydrogen (B) production during TCE dechlorination in a *Dehalococcoides* enrichment culture amended with various concentrations of PFAAs (PFSAs and PFCAs) and PFSAs only.

**Table S3.** Molar concentrations of PFASs, -$CF_2$ groups, and various alkyl chain lengths in the PFSA and PFAA amendment experiments (PFAA data reported in Weathers et al. 2016[6]).

| Tested Experimental Condition | Did TCE Dechlorination Occur? | Total PFASs amended (µM) | –$CF_2$ groups (µM) | C4, C5, C6 alkyl chains (µM) | C8 alkyl chains (µM) | C9, C10, C11 alkyl chains (µM) |
|---|---|---|---|---|---|---|
| 22 mg/L total PFAAs[6] | Yes | 61 | 351 | 35 | 9 | 12 |
| 66 mg/L total PFAAs[6] | Yes | 183 | 1052 | 105 | 27 | 35 |
| **110 mg/L PFAAs[6]** | **No** | **305** | **1753** | **175** | **44** | **59** |
| 22 mg/L PFSAs | Yes | 57 | 324 | 43 | 15 | 0 |
| 66 mg/L PFSAs | Yes | 172 | 975 | 128 | 44 | 0 |
| **110 mg/L PFSAs** | **Yes** | **287** | **1627** | **214** | **73** | **0** |

## References


(1) Men, Y.; Lee, P.K.H.; Harding, K.C.; Alvarez-Cohen, L. Characterization of four TCE-dechlorinating microbial enrichments grown with different cobalamin stress and methanogenic conditions. *Appl. Microbiol. & Biotech*, **2013**. *97*, 6439-6450.

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(6) Weathers T.S.; Harding-Marjanovic K.C; Higgins C.P.; Alvarez-Cohen L.; Sharp, J.O. Perfluoralkyl acids inhibit reductive dechlorination of tricholorethene by repressing *Dehalococcoides*. *Environ. Sci. Technol*. **2016**, *50*, pp 240-48