

# Effects of Aqueous Film-Forming Foams (AFFFs) on Trichloroethene (TCE) Dechlorination by a *Dehalococcoides mccartyi*-Containing Microbial Community

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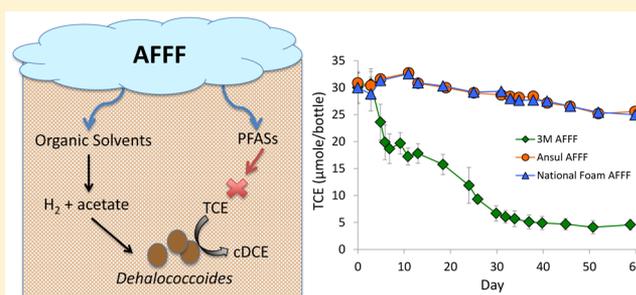
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## Supporting Information

**ABSTRACT:** The application of aqueous film-forming foams (AFFFs) to extinguish chlorinated solvent-fueled fires has led to the co-contamination of poly- and perfluoroalkyl substances (PFASs) and trichloroethene (TCE) in groundwater and soil. Although reductive dechlorination of TCE by *Dehalococcoides mccartyi* is a frequently used remediation strategy, the effects of AFFF and PFASs on TCE dechlorination are not well understood. Various AFFF formulations, PFASs, and ethylene glycols were amended to the growth medium of a *D. mccartyi*-containing enrichment culture to determine the impact on dechlorination, fermentation, and methanogenesis. The community was capable of fermenting organics (e.g., diethylene glycol butyl ether) in all AFFF formulations to hydrogen and acetate, but the product concentrations varied significantly according to formulation. TCE was dechlorinated in the presence of an AFFF formulation manufactured by 3M but was not dechlorinated in the presence of formulations from two other manufacturers. Experiments amended with AFFF-derived PFASs and perfluoroalkyl acids (PFAAs) indicated that dechlorination could be inhibited by PFASs but that the inhibition depends on surfactant concentration and structure. This study revealed that the fermentable components of AFFF can stimulate TCE dechlorination, while some of the fluorinated compounds in certain AFFF formulations can inhibit dechlorination.



## INTRODUCTION

Aqueous film-forming foams (AFFFs) are used for the suppression of liquid fuel fires, such as those from jet fuel or flammable solvents.<sup>1,2</sup> These water-based formulations typically contain fluorinated and hydrocarbon surfactants and one or more glycol ether-based solvents.<sup>1</sup> Many AFFF formulations contain diethylene glycol butyl ether (DGBE) and are at least 8% DGBE by weight. AFFFs have been optimized for rapid fire suppression by the addition of poly- and perfluoroalkyl substances (PFAS), key constituents that reduce surface tension and facilitate foam spreading and fire smothering.<sup>1</sup> Studies that have characterized PFASs in various AFFF formulations show that PFAS composition can vary significantly according to AFFF manufacturer and year of production.<sup>3,4</sup> AFFF sold by National Foam, Ansul, Chemguard, and Buckeye generally contain fluorotelomer-based PFASs, which are polyfluorinated compounds consisting of a perfluorinated chain connected to two or three nonfluorinated carbons and an ionic functional group of varying length and structure.<sup>3</sup> AFFF manufactured and sold by 3M typically contained perfluorinated sulfonates (PFSAs), perfluoroalkyl

sulfonamide amino carboxylates (PFSaAmA) and perfluoroalkyl sulfonamido amines (PFSaAm).<sup>3</sup>

The repeated use of AFFF at military, industrial, and municipal sites has led to groundwater and soil contamination by multiple classes of PFASs.<sup>5–10</sup> Firefighter training exercises conducted on U.S. Air Force bases historically released AFFF and fuels into the environment.<sup>1,8,9</sup> Because chlorinated solvents were frequently ignited as the flammable component in the training exercises, the infiltrated water often consisted of AFFF and chlorinated ethenes, such as trichloroethene (TCE).<sup>1,10</sup> This has led to contaminated sites containing both TCE and PFASs. Furthermore, the biotransformation of AFFF PFASs, such as fluorotelomer thioether amido sulfonate (FtTAoS), which produces perfluorinated carboxylic acids (PFCAs), may serve as a source of perfluoroalkyl acids (PFAAs) in contaminated environments.<sup>2,3</sup> Some field studies have also suggested that perfluorosulfonamide compounds,

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such as PFSaAm, are converted to perfluorosulfonic acids (PFSAs) in situ.<sup>8,10</sup>

Reductive dechlorination of trichloroethene (TCE) by *Dehalococcoides mccartyi* is a frequently employed in situ remediation strategy at many groundwater-contaminated sites.<sup>11,12</sup> The hydrogen, acetate, and vitamin B<sub>12</sub> required for *D. mccartyi* growth and dechlorination are often generated by fermentation of labile reduced organic compounds injected into the subsurface to stimulate the activity of microbial populations coexisting with *D. mccartyi* in complex subsurface communities.<sup>13–15,17</sup> Historic and repeated infiltration of the reduced carbon sources contained in AFFF have the potential to create a reducing subsurface environment conducive to TCE bioremediation by *D. mccartyi*. However, the effects of AFFF on dechlorination by *D. mccartyi*, and the metabolism of microorganisms fermenting organic compounds in AFFF are not well-described. Because AFFF is a complex mixture of chemicals, one or more of its components could adversely impact dechlorination. Remediation at sites with chloroethene and AFFF contamination has so far focused primarily on chlorinated solvent remediation with little regard to the effects that PFAS presence and concentration may have on remediation success.<sup>10</sup> The development of analytical techniques to detect and quantify more PFAS compounds specific to AFFF formulations has also allowed for the characterization of an increased number of PFASs in environmental samples.<sup>3,16</sup> Better characterization of co-contaminated aquifers coupled to an improved understanding of the effects of AFFF on in situ microbial communities will lead to more effective TCE remediation at these sites.

This study first evaluates the effects of various AFFFs on TCE dechlorination by exposing an enriched anaerobic microbial community containing *D. mccartyi* to three formulations: 3M Light Water (3%), National Foam Aer-O-Water 3EM (3%), and Ansul Ansulite (3%). These formulations represent AFFF products that were applied during fire-training exercises at various U.S. military bases beginning as early as 1976.<sup>3</sup> The goals of this study were to determine whether dechlorination can occur in the presence of high concentrations of AFFF and whether microbial communities are capable of fermenting the organics in AFFF to produce the hydrogen and acetate necessary to drive TCE dechlorination by *D. mccartyi*. The high concentrations of AFFF used in this study would be representative of concentrated source zones and would be relevant to the repeated exposure of microbial communities to the foams and the cumulative impacts of persistent AFFF components,<sup>31</sup> such as PFCAs and PFSAs (collectively referred to as PFAAs), on the activity of *D. mccartyi*.<sup>8,10</sup> The effects of individual AFFF components on the communities, such as reduced organics, PFASs, and PFAS breakdown products (i.e., PFAAs), were then examined to explain the differences in dechlorination observed among the AFFF-amended cultures.

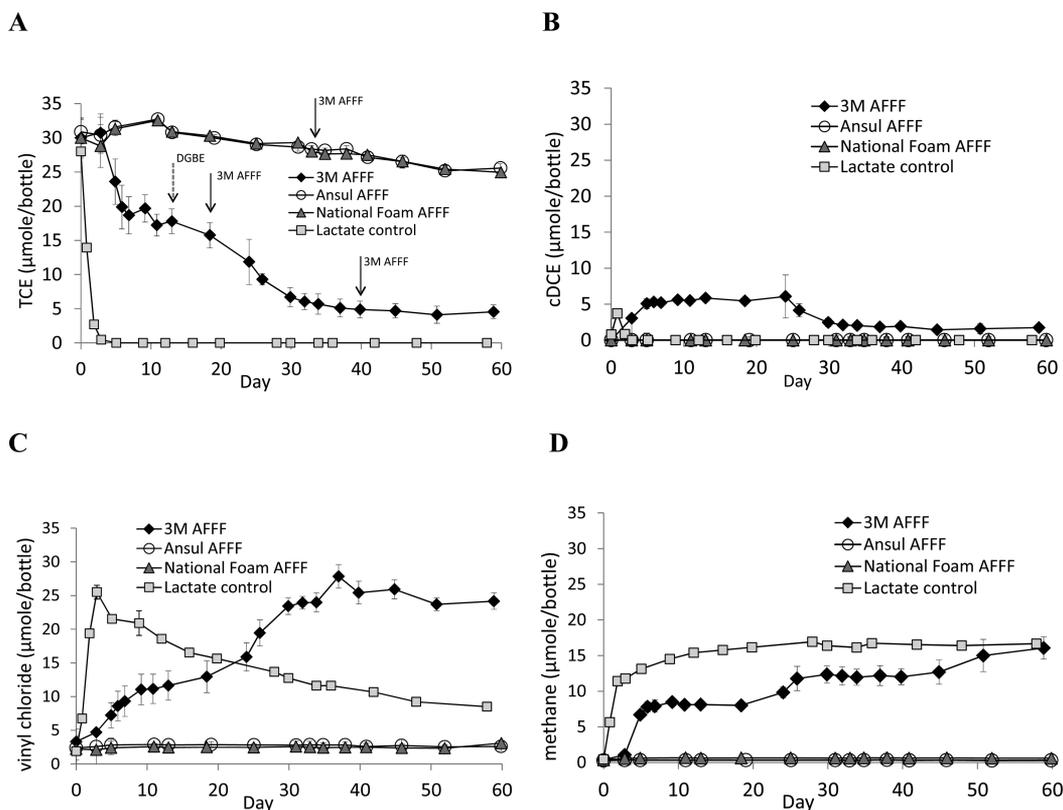
## MATERIALS AND METHODS

**Chemicals.** AFFF 3% formulations sold or produced by Ansul (Ansulite), 3M (Light Water), and National Foam (Aer-O-Water 3EM) with estimated manufacture dates of 2008, 1998, and 2008, respectively, were provided courtesy of Professor Jennifer Field's lab at Oregon State University as described elsewhere.<sup>3,8</sup> Each AFFF was stored in a sealed polyethylene tube at room temperature in the dark, and the same stock solutions were used throughout the study to ensure

consistency in PFAS and solvent composition. The composition of the foams, as reported in the most related material safety data sheet (MSDS), is summarized in Table S2. Commercial source materials containing fluorotelomer thioether amido sulfonate (FtTAoS), fluorotelomer sulfonamido betaine (FtSaB), fluorotelomer sulfonamido amine (FtSaAm), perfluoroalkyl sulfonamido amine (PFSaAm), and perfluoroalkyl sulfonamido amino carboxylate (PFSaAmA) were obtained from the Fire Fighting Coalition, and the concentrations of 6:2 FtTAoS, 6:2 FtSaB, and 6:2 FtSaAm in various stock solutions were previously determined by Backe et al.<sup>9</sup> All PFCAs, PFSAs, fluorotelomer sulfonates (FtSs), and isotopically labeled standards used for PFAS quantification by LC-MS/MS were purchased from Wellington Laboratories (Guelph, Ontario, CA). TCE and diethylene glycol butyl ether (DGBE) were purchased from Sigma-Aldrich (St. Louis, MO), and all other chemicals were purchased from either Fisher Scientific (Waltham, MA) or Sigma-Aldrich at the highest purity possible.

**Culture and Growth Conditions.** The AFFF and DGBE amendment experiments were conducted in 160 mL glass serum bottles with 100 mL of a reduced basal medium, containing a vitamin solution resulting in 100 µg/L vitamin B<sub>12</sub>, and a N<sub>2</sub>/CO<sub>2</sub> (90:10) headspace. The vitamin B<sub>12</sub> was supplied as cofactor necessary for reductive dehalogenase (RDase) activity. A total of 20 to 30 µmoles of neat TCE were added as the electron acceptor and equilibrated 24 h prior to culture inoculation. Approximately 5% (v/v) of an active *D. mccartyi*-containing enrichment culture was inoculated at day zero into medium representing each of the tested growth conditions. The culture had been grown for approximately 14 days under lactate and TCE amendment before it was inoculated into the experimental bottles used in this study. PFAA amendment experiments were performed in 60 mL serum bottles with 50 mL of medium as described above and a N<sub>2</sub>/CO<sub>2</sub> (90:10) headspace; 3% (v/v) of the *D. mccartyi* enrichment culture was inoculated at day zero. The smaller growth volume of the PFAA experiments was used to minimize the quantity of PFAAs added (while still achieving desired concentration). All serum bottles were incubated at 34 °C in the dark without shaking for the duration of the experiment. The *D. mccartyi* enrichment culture used for this study was seeded from a TCE-contaminated site and has been maintained in batch culture at UC Berkeley for over five years.<sup>15</sup> The culture has been propagated by sequential subculturing during this period with lactate as electron donor and repeated low amendments of TCE (0.2 mM) as electron acceptor to enable methanogenic activity. The culture contains a *D. mccartyi* strain similar to strain 195 in genomic content and function. The strain similarly employs an RDase for the metabolic dechlorination of TCE to *cis*-dichloroethene (cDCE) and vinyl chloride (VC) and cometabolic dechlorination of VC to ethene.<sup>15</sup>

**AFFF Amendment Experiments.** In experiments designed to examine the effects of AFFF on TCE dechlorination and to test the ability of AFFF to support dechlorination as a carbon source and electron donor, 300 µL of either Ansul, 3M, or National Foam AFFF (neat product) were amended to 100 mL of growth medium containing approximately 25–30 µmoles of TCE. The AFFF was subsampled from aerobic stock tubes and added to the medium with a sterile polypropylene syringe (BD Luer-Lok disposable syringe). A clean needle was ensured before putting aliquots into bottles to minimize the transfer of extra AFFF. Although the AFFF stock was aerobic, the small

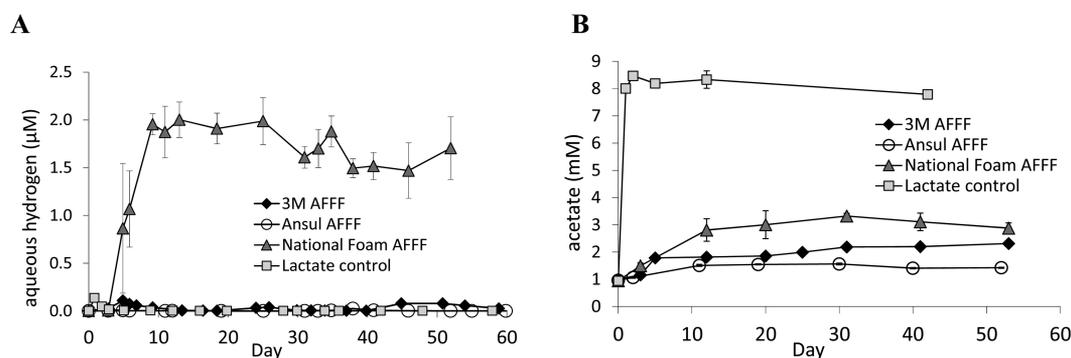


**Figure 1.** Reductive dechlorination in enrichment cultures amended with different AFFF formulations. Concentrations of TCE (A) cDCE (B), vinyl chloride (C), and methane (D) in cultures amended with 3M AFFF, National Foam AFFF, Ansul AFFF, or lactate. Arrows on (A) denote additional 3M AFFF (solid) and DGBE (dotted) amendments to cultures. Error bars represent the standard deviation of triplicate biological bottles.

amended volumes did not cause the growth medium to become aerobic (cysteine sulfide was present in the growth medium as a reducing reagent, and resazurin served as an oxygen indicator, as previously described<sup>15</sup>). The growth medium, TCE, and AFFF were equilibrated for 24 h before culture inoculation. 5 mL of *D. mccartyi* enrichment culture was then inoculated into the medium, inverted several times to mix, and incubated at 34 °C for 5 h before the first sample was taken. A set of sterile controls was prepared by inoculating previously-autoclaved culture into the medium containing AFFF and TCE, and a set of inoculum-free medium controls was prepared by substituting 5 mL of sterile medium for the microbial culture. The autoclaved and medium controls were used to quantify any abiotic reactions occurring in the cultures as well as to assess potential PFAS losses due to sorption to biomass or the containers. Triplicate live controls containing 100 mL of growth medium, 2 mmol lactate, 25–30 μmoles of TCE, and no AFFF were also established to ensure vitality of the inoculum culture. The culture is typically grown and propagated under lactate amendment.<sup>15</sup> The AFFF and DGBE amendment regimes for each condition are described in Table S1. All of the bottles were inverted periodically throughout the incubation to promote PFAS mixing. Each time a sample was collected, between 1 and 1.5 mL of well-mixed culture was removed with a sterile syringe and stored in a 2 mL polypropylene centrifuge tube. Approximately 200 μL of the culture was immediately diluted in an equal volume of HPLC-grade methanol and reserved for LC-MS/MS analysis, and the remaining culture was centrifuged at 15000g for 10 min and stored at –20 °C for future analyses. All experiments were performed in triplicate.

**DGBE Amendment Experiments.** To determine the ability of DGBE to support TCE dechlorination as a fermentable carbon and energy source, 5 mL of *Dehalococcoides* enrichment culture was inoculated into triplicate experimental bottles containing 100 mL of growth medium, 25 μmoles of TCE, and either (1) 250 μmoles of DGBE, (2) 250 μmoles of DGBE and 2 mmol lactate, or (3) 2 mmol lactate. The DGBE concentration was chosen to approximate the concentration that would be expected for a 300 μL AFFF amendment with approximately 20% (w/w) DGBE, according to reported MSDS information on AFFF foams,<sup>19–21</sup> and to provide a similar total mass of carbon as achieved with a 2 mmol lactate amendment. DGBE was amended as a sterile stock solution previously-prepared in the same anaerobic growth medium. Bottles initially amended with DGBE received four additional 250 μmole amendments of DGBE on days 28, 36, 92, and 118, and bottles initially amended with lactate + DGBE received one additional 250 μmole amendment of DGBE on day 92. No additional DGBE was added to the lactate + DGBE bottles on day 118 because TCE dechlorination was observed during that time, indicating there was sufficient electron donor. All bottles were incubated at 34 °C for 200 days and reamended with 25 μmoles of TCE on day 92. All DGBE experiments were sampled periodically by extracting 1 to 1.5 mL of well-mixed culture into a polypropylene centrifuge tube, centrifuging at 15000g for 10 min, and storing at –20 °C for future use. All experiments were performed in triplicate.

**PFAS and AFFF Component Amendment Experiments.** To determine the impacts of certain AFFF components, including PFASs, on TCE dechlorination by *D. mccartyi*, we prepared triplicate cultures with basal medium amended with 2 mmol



**Figure 2.** Hydrogen production (A) and acetate production (B) in dechlorination enrichment cultures amended with 3M AFFF, Ansul AFFF, National Foam AFFF, or lactate. Error bars represent the standard deviation for biological triplicates.

lactate, 25  $\mu$ moles of TCE, and either 18 mg/L ethylene glycol, 12 mg/L 1-propanol, 45 mg/L FtTAoS, 16 mg/L 6:2 FtSaB, or 32 mg/L 6:2 FtSaB in 100 mL of growth medium. Lactate-only controls containing no additional AFFF components were also constructed in parallel. The concentrations of the amendments were intended to match the expected concentrations of each compound in the AFFF-amended experiments to facilitate a comparison of observed TCE dechlorination among the experiments. Amendment concentrations in the AFFF experiments were determined by either measuring concentrations in the foams directly (i.e., for 6:2 FtTAoS and 6:2 FtSaB) or estimating concentrations from the closest published MSDS information for each foam type (i.e., for ethylene glycol and 1-propanol), which typically provides a specific gravity of the AFFF and percent by weight of each foam constituent.<sup>19–21</sup> All bottles were incubated for 5 days at 34 °C and inverted periodically to promote mixing.

To further assess the impact of PFSA on TCE dechlorination, we tested three different PFSA amendments with enrichment cultures fed with 2 mmol lactate and 22  $\mu$ moles of TCE. These amendments contained 7.3, 22, and 36.7 mg/L of PFBS, PFHxS, and PFOS each, yielding three tested conditions with total PFSA concentrations of 22, 66, and 110 mg/L, respectively. These experiments were conducted in 60 mL serum bottles containing 50 mL of growth medium. These results were compared to a suite of 11 PFAAs at three concentrations: 2, 6, and 10 mg/L of each PFAA, yielding total PFAA concentrations of 22, 66, and 110 mg/L, respectively, described elsewhere.<sup>32</sup> This suite of PFAAs consisted of perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnA), perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), and perfluorooctanesulfonate (PFOS). A PFAA or PFSA methanol stock solution containing each respective set of tested compounds was first evaporated in 60 mL serum bottles<sup>35</sup> in an anaerobic chamber and then stoppered and amended with 48.5 mL of anaerobic growth medium, 1 mmol lactate, and 22  $\mu$ moles of TCE. The medium was equilibrated with the lactate, PFAAs or PFSA, and TCE for 48 h before inoculating with 1.5 mL of *D. mccartyi* enrichment culture and incubating for 7 days at 34 °C. Bottles were inverted several times once a day to promote mixing. Triplicate bottles without PFAAs were also constructed in parallel to serve as controls.

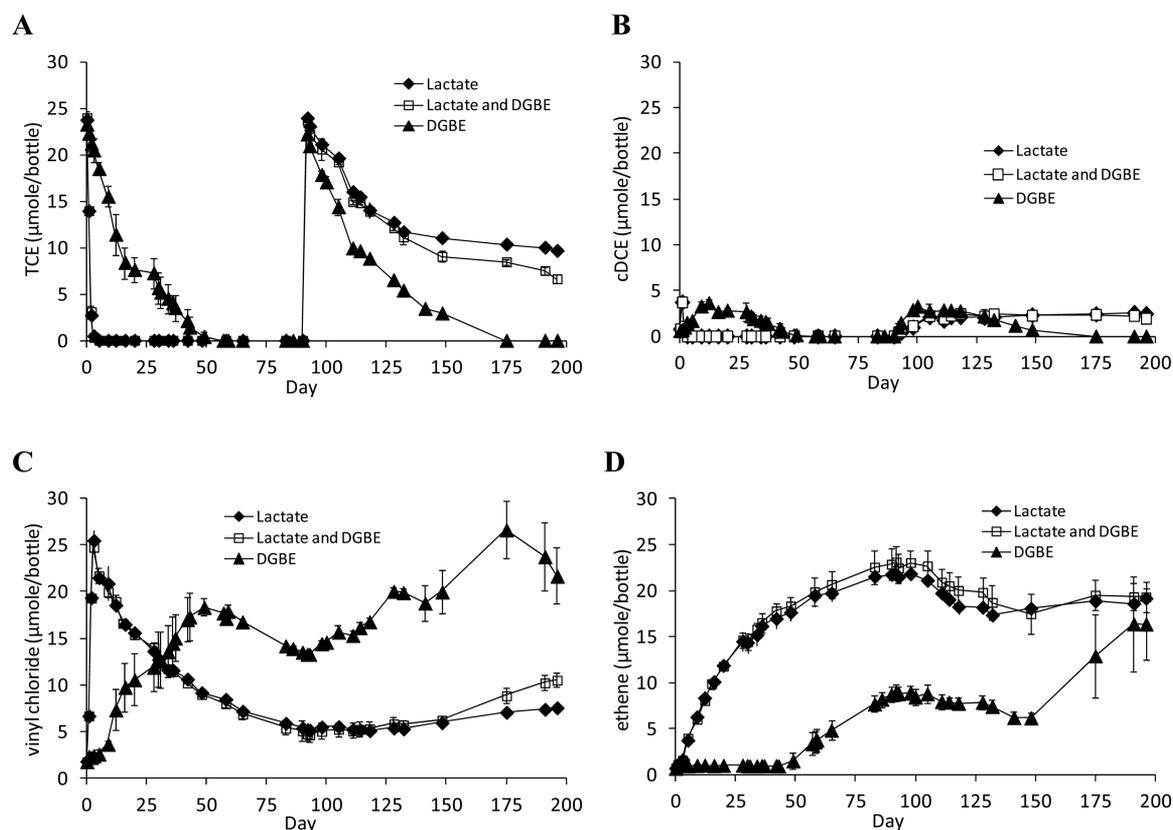
**Analytical Methods.** Details regarding the analytical methods used to quantify chloroethenes, methane, and

hydrogen by GC-FID,<sup>15,22</sup> PFASs by LC-MS/MS,<sup>8,23</sup> organic acids (i.e., lactate, acetate, butyrate, and propionate) by HPLC,<sup>14</sup> and *D. mccartyi* 16S rDNA genes with quantitative polymerase chain reaction (qPCR)<sup>15</sup> are provided in the [Supporting Information](#).

## RESULTS

**AFFF Amendment Experiments.** When 300  $\mu$ L AFFF was amended to TCE dechlorinating communities as the carbon source and electron donor, active reductive dechlorination was observed in 3M AFFF-amended cultures, but no dechlorination was observed in Ansul and National Foam-amended cultures (Figure 1A–C). Aqueous hydrogen concentrations in the 3M and Ansul cultures remained below 0.12  $\mu$ M throughout the incubation period, and hydrogen concentrations in the National Foam-amended cultures increased to 2  $\mu$ M within 10 days and remained approximately constant for the duration of the incubation (Figure 2A). The complete dechlorination of TCE to ethene and production of methane and hydrogen in the lactate-amended control culture confirmed the vitality of the inoculated stock culture. To determine whether the organic carbon in the 3M AFFF facilitated dechlorination by providing additional fermentable growth substrates, DGBE and 3M AFFF was amended to each of the cultures. When dechlorination stalled in the cultures originally fed 3M AFFF, DGBE was added on day 13, and additional 3M AFFF was added on days 18 and 40 (Figure 1A). Approximately 10  $\mu$ moles of TCE were dechlorinated from days 13 to 30, and no further dechlorination was observed after day 30 (Figure 1A), yielding a total of 25  $\mu$ moles of TCE that were dechlorinated to cDCE and vinyl chloride. Although no ethene production was observed in the 3M-amended culture, this is consistent with the dechlorination activity of the *D. mccartyi* strain in this culture.<sup>15</sup> The strain cometabolically reduces vinyl chloride to ethene, and it typically begins to dechlorinate vinyl chloride once all of the available TCE and cDCE have been depleted. Approximately 5  $\mu$ moles of TCE remained in the 3M AFFF-amended cultures at the end of the incubation period. Because dechlorination had been observed in 3M-amended cultures by day 13, and DGBE is known to be a constituent of 3M AFFF, DGBE was added to the 3M cultures (on day 13) to determine if it was, in fact, the constituent responsible for the observed acetate and hydrogen production.

Between 0.04 and 0.12  $\mu$ M aqueous hydrogen was produced after each 3M AFFF amendment, but no observable hydrogen production occurred following DGBE amendment (Figure S4).



**Figure 3.** Concentrations of TCE (A), cDCE (B), VC (C), and ethene (D) in enrichment cultures amended with lactate, lactate and DGBE, or DGBE-only as the carbon source and electron donor. Error bars represent the standard deviation of biological triplicates.

Increases in methane production similarly corresponded to AFFF amendment, yielding a total of 16  $\mu\text{moles}$  of methane production during the 60 day incubation, and no increase in methane was observed in the 5 days following DGBE amendment (Figure 1D). This finding confirms that hydrogen limitation after DGBE amendment was responsible for the slow dechlorination rate and lack of methanogenic activity of the culture.

Because DGBE fermentation produced low concentrations of hydrogen, and dechlorination rates were slower under DGBE amendment than under 3M AFFF amendment, it indicated that 3M AFFF may contain other ethylene glycol or hydrocarbon compounds that are fermentable to acetate and hydrogen. For these reasons, 3M AFFF was amended to the National Foam and Ansul AFFF cultures on day 33 to test whether fermentable organics in 3M AFFF could promote dechlorination. However, no dechlorination was observed in the National Foam- and Ansul-amended cultures, even after the amendment of 3M AFFF. Additionally, no significant methane production was detected (Figure 1D) in National Foam or Ansul-amended live cultures. No TCE dechlorination, methane, or hydrogen was detected in autoclaved cultures or medium control bottles under any AFFF amendment condition (Figure S1).

Acetate production was greatest in live cultures fed National Foam ( $\sim 2$  mM), followed by the 3M cultures ( $\sim 1$  mM) and Ansul cultures ( $\sim 0.5$  mM) (Figure 2B). Approximately 1 mM acetate was detected at time 0 in both live and autoclaved cultures due to carryover from the inoculation culture stock (Figure 2B and Figure S2). Lactate and butyrate were not detected in any of the live, autoclaved, or medium control cultures, and approximately 1 mM propionate was present at

time 0 in both live and autoclaved cultures due to inoculation carryover. The propionate concentration remained unchanged in all cultures throughout the incubation. No significant *D. mccartyi* growth, as measured by 16S rRNA gene copy numbers, was detected in the AFFF amendment cultures (Figure S5). Although the average 16S rRNA gene copy numbers increased from approximately  $4.7 \times 10^6$  copies/mL on day 0 to  $1.6 \times 10^7$  copies/mL on day 53 in the 3M AFFF-amended cultures ( $n = 3$ ), the increase (3.4 fold) was not statistically significant ( $p = 0.19$ ).

During a given AFFF amendment, the concentrations of all measured PFASs remained unchanged, indicating that the fluorosurfactant compounds in the three tested formulations were not transformed (Figures S6–S9). Each AFFF formulation contained PFASs that were characteristic of products sold by the corresponding manufacturer. Fluorotelomer compounds were present in the National Foam and Ansul formulations, while perfluorosulfonates and perfluorosulfonamide compounds were present in the 3M formulation. PFOS was the most abundant PFAS in 3M-amended cultures, and PFBS, PFHxS, PFHpS,  $C_4$ – $C_6$  PFSaAm, and  $C_4$ – $C_6$  PFSaAmA were also detected (Figure S6). The concentration of each PFAS compound approximately doubled and then tripled following the second and third respective 3M AFFF amendments to these cultures, as expected (Figure S6A–B). These amendments yielded total PFAS concentrations of approximately 40, 80, and 120 mg/L after each successive 3M AFFF dose. Similar concentrations of PFASs were present in cultures initially amended with National Foam and Ansul AFFF, and an increase in PFASs was observed after an addition of 3M AFFF was made. Approximately 30 mg/L of total PFASs, consisting

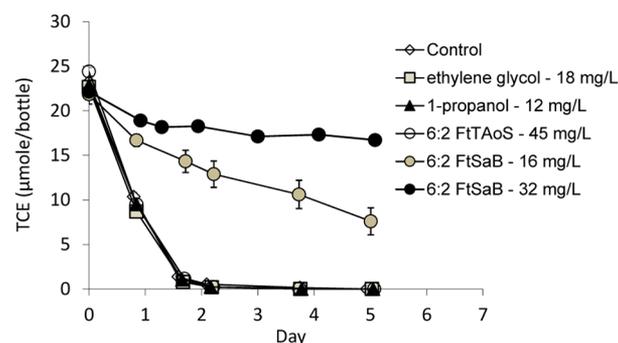
of 6:2 FtSaB, 6:2 FtSaAm, 6:2 FtS, and 8:2 FtS, was initially present in National Foam-amended cultures, and this concentration increased to 70–80 mg/L after 3M AFFF was added on day 33 (Figure S8A). The only PFAS detected in Ansul-amended cultures was 6:2 FtTAoS, and it was present at approximately 70 mg/L. After 3M AFFF was amended on day 33, the PFAS concentration increased to 110–120 mg/L (Figure S9A). Total PFAS concentrations in autoclaved and medium controls remained relatively constant throughout the incubation of all AFFF formulations, indicating that no significant abiotic reactions or physical losses occurred in the experiments.

**DGBE Amendment Experiments.** To determine whether DGBE can serve as a fermentable electron donor capable of driving TCE dechlorination, multiple amendments of 250  $\mu$ moles DGBE were applied to *D. mccartyi* enrichment cultures as the carbon source and electron donor (amendments made on days 0, 28, 36, 92 and 118) (Figure 3A–D). Two amendments of 25  $\mu$ moles of TCE were dechlorinated to vinyl chloride and ethene by DGBE-fed cultures with dechlorination rates that were slower than those fed 2 mmol lactate. Although the number of electron equivalents available from the fermentation of 250  $\mu$ moles of DGBE differs from that available in 2 mmol lactate (7.8 mmol eeq and 24 mmol eeq available in DGBE and lactate, respectively), both substrates were added in excess of what is required to dechlorinate 25  $\mu$ moles of TCE to ethene (0.15 mmol eeq), indicating that the cultures were not limited in fermentable substrate. There was little difference between cultures amended with lactate + DGBE and those amended with lactate only (Figure 3A), suggesting that lactate was more labile for fermentation, as expected. All conditions received a second amendment of TCE on day 90, while only the lactate + DGBE and DGBE-only conditions received additional carbon at that time (250  $\mu$ moles DGBE). TCE dechlorination stalled in both the lactate + DGBE and lactate conditions after approximately 125 days (Figure 3A). The dechlorination stall in the lactate condition was not unexpected as lactate was not re-amended to the culture to sustain the hydrogen concentrations necessary for dechlorination (aqueous hydrogen concentrations were below 0.2 nM after day 125). The DGBE + lactate condition was amended with less total DGBE than the DGBE condition and similarly possessed low aqueous hydrogen concentrations (below 0.46 nM after day 125).

Approximately 15–20  $\mu$ moles of methane were produced in the lactate and lactate + DGBE conditions during the first 25 days (Figure S10A), while no methane was initially produced in the DGBE-only condition. Methane was produced (2  $\mu$ moles) after day 60 in the DGBE-only condition and again after day 150 when the TCE was completely removed (Figure S10A). Aqueous hydrogen concentrations in the lactate and lactate + DGBE conditions increased rapidly to 0.15  $\mu$ M in the first 2 days, after which the hydrogen was consumed, leaving a residual concentration of 0.05  $\mu$ M until the second TCE amendment, when it decreased to nearly 0.0005  $\mu$ M (Figure S10C,D). In the DGBE-only condition, hydrogen remained just under 0.001  $\mu$ M throughout most of the incubation, with slight increases after days 60 and 150 when the TCE was completely removed. No significant acetate production was measured in the DGBE-only condition, while approximately 7 mM acetate was produced in the lactate and lactate + DGBE conditions, as expected with the standard growth of this culture on lactate (Figure S10B).<sup>15</sup> All conditions contained approximately 1 mM

acetate and 1 mM propionate at time 0, as the organic acids were present in the culture inoculum stock. Lactate, butyrate, and propionate production were not observed in DGBE-only amended cultures.

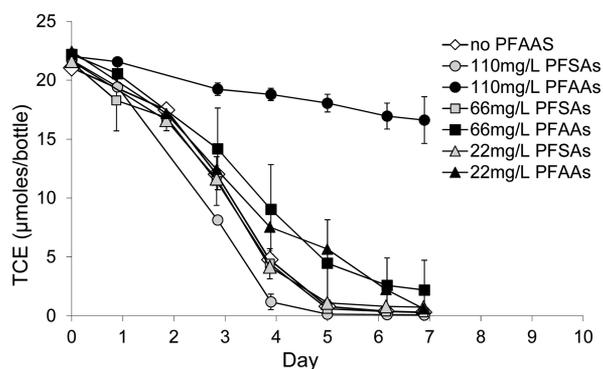
**AFFF Component and PFAS Amendment Experiments.** Experiments were performed to provide insight into the substances in National Foam and Ansul AFFFs that inhibited TCE dechlorination in the enrichment cultures. The principal fluorinated surfactants, 6:2 FtSaB and 6:2 FtTAoS, as well as ethylene glycol and 1-propanol, which are present in the National Foam and Ansul AFFF formulations at 6–8% and 0.4%, respectively,<sup>20,21</sup> were amended to lactate-fermenting cultures. The tested concentrations of each of the compounds were intended to reproduce concentrations present in the AFFF-amended experiments. TCE dechlorination was not affected by the presence of 18 mg/L ethylene glycol, 12 mg/L 1-propanol, or 45 mg/L 6:2 FtTAoS in comparison to a non-amended control; however, dechlorination was slowed by the presence of 16 mg/L 6:2 FtSaB and stalled completely in the presence of 32 mg/L FtSaB (Figure 4). Production of



**Figure 4.** TCE dechlorination by enrichment cultures in the presence of 18 mg/L ethylene glycol, 12 mg/L 1-propanol, 45 mg/L 6:2 FtTAoS, and 16 or 32 mg/L 6:2 FtSaB. Error bars represent the standard deviation of biological triplicates.

dechlorination products was not observed in the 32 mg/L FtSaB condition, while cDCE dechlorination to vinyl chloride was slower in the 16 mg/L FtSaB condition compared to the nonamended control (Figure S11). No difference in cDCE, vinyl chloride, and ethene production was observed between the control culture and the cultures amended with ethylene glycol, propanol, or FtTAoS (Figure S11). The concentration of both FtSaB and FtTAoS were stable over the incubation period, indicating the PFASs did not biotransform or bind significantly to biomass.

Dechlorination was not significantly affected by the presence of 110 mg/L total PFASs (Figure 5). Conversely, insignificant TCE dechlorination was shown to occur with the same quantity of PFAAs (110 mg/L).<sup>32</sup> The presence of 22 and 66 mg/L total PFASs and 22 mg/L total PFAAs also did not impact TCE dechlorination compared to a non-amended control.<sup>32</sup> The production of cDCE, vinyl chloride, and ethene in the 110 mg/L PFSA condition remained similar to the control culture, whereas no production of the compounds was observed in the 110 mg/L PFAA condition (Figure S13A–C).<sup>32</sup> The methane produced in all PFSA conditions was similar to the control culture (0–2  $\mu$ moles) and 22 and 66 mg/L PFAA conditions, while slightly more methane was produced in the 110 mg/L PFAA-amendment condition than the no-PFAA control (5  $\mu$ moles) (Figure S14A,B). However, little methane



**Figure 5.** TCE dechlorination by enrichment cultures in the presence of various concentrations of PFSAs (open gray symbols) and PFAAs (PFCAs and PFSAs [data from Weathers et al. 2016],<sup>32</sup> closed black symbols). Error bars represent the standard deviation of biological triplicates.

was produced overall in all of the conditions, including the control (Figure S14A).

## DISCUSSION

The ethylene glycol compounds in all three AFFF formulations were fermentable by the anaerobic microbial community used in this study, despite the fact that dechlorination was observed in only one of the three formulations (3M). The varying concentrations of hydrogen and acetate produced from each AFFF likely reflect the foams' differing initial fermentable organics compositions, the culture's ability to ferment those organics, and any effects other foam constituents may have had on fermenting microbial populations. The reported MSDS information indicates that although organic solvents typically account for approximately 20% (w/w) of an AFFF formulation, foams can contain varying amounts of DGBE and ethylene glycol (Table S2). National Foam AFFF typically contains 6–8% (w/w) ethylene glycol, a known microbial carbon and energy source that could be responsible for the higher concentrations of acetate and hydrogen observed in National Foam-amended cultures (Figure 2A,B).

Acetate production in the AFFF amendment experiments could have been produced through either homoacetogenesis (from carbon dioxide and hydrogen) or from the direct fermentation of ethylene glycol compounds. Acetate generation from homoacetogenesis was previously shown to occur when these cultures were grown on lactate.<sup>15</sup> Because hydrogen consumption was observed in the Ansul AFFF-amended cultures without concomitant methane production (Figures 1D and 2S), the acetate produced may have been generated through homoacetogenesis (Figure 2B) (TCE dechlorination did not occur in Ansul-amended cultures). The direct fermentation of ethylene glycol to acetate has also been previously reported and may have occurred in the AFFF cultures in this study. The anaerobic degradation of ethylene glycol to acetate, hydrogen, and ethanol by a variety of microorganisms has been observed previously,<sup>24–27</sup> including its fermentation by a *Desulfovibrio* strain,<sup>26</sup> a genus commonly found in dechlorinating microbial communities and present in the culture used for this study.<sup>15</sup> Although the addition of 18 mg/L ethylene glycol to cultures fermenting lactate did not produce more acetate and hydrogen than cultures given only lactate in this study (Figure S10B), it was likely the microorganisms preferentially consumed the lactate before

the ethylene glycol, as the cultures were maintained in a lactate-containing growth medium prior to the experiments. To our knowledge, anaerobic microbial degradation of DGBE has not been reported. However, a variety of polyethylene glycol compounds (PEGs), including diethylene glycol (DEG) and triethylene glycol (TEG), have been shown to biodegrade under anaerobic conditions to acetate and hydrogen, which can then be used to produce methane when degraded by methanogenic consortia.<sup>25,26</sup> In this study, dechlorination of TCE coupled with the low hydrogen concentrations and the absence of acetate accumulation observed in cultures provided with DGBE as the carbon source and electron donor indicated that the fermentation of DGBE was able to support slow reductive dechlorination. The low concentrations of hydrogen produced during DGBE fermentation may have limited the rate of the TCE dechlorination reaction. The aqueous hydrogen concentrations in the DGBE-amended cultures between days 1 to 50 ranged from 0.13 to 1.5 nM. These hydrogen concentrations are within or below the previously published threshold range of 1–2 nM for dechlorination,<sup>28</sup> although some *D. mccartyi* strains have been shown to have a consumption threshold <1 nM.<sup>29,33,34</sup> The fermentation of DGBE may favor the production of alternate products (e.g., ethanol, which was reported as a product of ethylene glycol fermentation<sup>24</sup>). Methane and hydrogen concentrations began to increase in these cultures immediately following the disappearance of each aliquot of TCE on day 50 and after day 150, suggesting that the thermodynamic equilibrium and energetics of the system may have shifted once a dechlorination electron sink was removed. Because low concentrations of aqueous hydrogen were maintained in the DGBE-amended cultures during the first 50 days of incubation (average of 0.5 nM), dechlorination may have been favored over methane generation during this period. Dechlorination is typically favored over methanogenesis at low hydrogen concentrations.<sup>28</sup> Alternatively, TCE may have inhibited the complete fermentation of DGBE to hydrogen, methane, and acetate. This may also have been the case in cultures initially amended with 3M AFFF, as the methane concentrations were inversely correlated with TCE concentrations. However, the inhibition would have been unidirectional because 2.5 mM DGBE (250 µmol) did not inhibit TCE dechlorination in the presence of 20 mM lactate (2 mmol) (Figure 3A). Although DGBE fermentation supported TCE dechlorination by producing small amounts of hydrogen electron donor (Figures 3 and 4), all AFFF-amended cultures produced greater concentrations of both hydrogen and acetate than the DGBE-fed cultures, even though nearly 40 mmol of electron equivalents were supplied to the DGBE cultures over the incubation period (which is greater than the dose supplied in 2 mmol of labile lactate, 24 mmol eeq). This suggests that lower concentrations of the other carbon sources in AFFF could have been responsible for the hydrogen, acetate, and methane production in the AFFF-amended experiments (Figures 1 and 2). These organics may include ethylene glycol in the National Foam formulation, 1-propanol and hexylene glycol in Ansul AFFF, or other organic surfactants that are reported to be present in all three AFFFs at 1–11% (w/w) (Table S2).<sup>19–21</sup> However, due to the proprietary nature of many AFFF foams, the identity and concentrations of specific components are not well-known.

TCE dechlorination occurred in the presence of 3M AFFF (0.3% v/v) but was inhibited in the presence of the same dilution of National Foam and Ansul AFFF formulations. The

dechlorination in 3M-amended cultures appears to have been supported primarily by the fermentation of 3M AFFF carbon sources to hydrogen and acetate that promoted dechlorination by *D. mccartyi*, although it is unclear why dechlorination stalled after 30 days (Figures 1 and 2). It is possible that the concentration of total PFASs after the second AFFF amendment (approximately 80 mg/L) caused inhibition during this period and prevented the complete dechlorination of TCE. It is also possible that the degradation of DGBE or other materials in the 3M AFFF generated fermentation products in quantities that were toxic to *D. mccartyi*. Although sufficient hydrogen and acetate were also produced in the Ansul and National Foam-amended cultures to support dechlorination (aqueous hydrogen concentration remained above the approximate minimum threshold range for *D. mccartyi*, 1–2 nM),<sup>28,29</sup> no TCE degradation was observed, suggesting that chemicals unique to Ansul and National Foam formulations may have inhibited *D. mccartyi* dechlorination but not microbial fermentation. Because methane production was also not observed in Ansul and National Foam-amended cultures, the same chemicals inhibitory to TCE dechlorination might also suppress methanogenic activity. The lack of methane production following the amendment of 3M AFFF to Ansul- and National Foam-containing cultures confirms the presence of compounds inhibitory to methanogenesis in those formulations.

The inhibition of TCE dechlorination in National Foam amended cultures may have been due to the presence of its principal PFAS compound, 6:2 FtSaB. Interestingly, higher concentrations of the principle PFAS in Ansul AFFF, 6:2 FtTAoS, had no effect on TCE dechlorination (Figure 4). The tested concentration of 6:2 FtTAoS in the PFAS-amended experiment (45 mg/L) was lower than the measured concentration in the Ansul-amended experiment (60 mg/L), and dechlorination inhibition in the presence of Ansul AFFF could have been due to higher concentrations of FtTAoS in these experiments. The total initial concentration of PFASs in cultures with 3M AFFF was 33% higher (by mass) than the concentration in cultures with National Foam AFFF (40 and 30 mg/L, Figures S6 and S8, respectively); however, TCE dechlorination occurred even after two doses of 3M AFFF had been amended (Figure 1A). This suggests that dechlorination inhibition may not only depend on PFAS concentration but on the type and structure of the non-fluorinated functional groups.

The simplest PFAS functional group structures are represented in the PFSA and PFAA amendment experiments (Figure 5), where the effects of having a sulfonate or carboxylate group adjacent to the perfluoroalkyl chain are differentiated. Because the concentration of each PFSA in the 110 mg/L total PFSA condition was greater than the concentration of each PFSA in the 110 mg/L total PFAA condition (Figure 5), the PFCAs are either solely responsible for the inhibition of TCE dechlorination in this experiment or the combination of PFCAs and PFSA together cause inhibition. Weathers et al. 2016 showed that a decrease in the abundance of *D. mccartyi* occurred in the presence of the same 110 mg/L PFAA condition compared to the no-PFAA control.<sup>32</sup> The abundances of Archaea concomitantly increased because the organisms were not repressed by the PFAAs.<sup>32</sup> Increased methane generation was not observed in the 110 mg/L PFSA condition tested in this study, as repression of dechlorination did not occur, and *D. mccartyi* likely competed with the methanogens for the available hydrogen. Table S3

shows that the total molar concentration of PFAAs in the 110 mg/L PFAA and 110 mg/L PFSA-amended experiments was similar, suggesting that ionic strength may not have been a significant contributor to dechlorination inhibition in the 110 mg/L PFAA condition. The molar concentration of CF<sub>2</sub> groups was also similar between the 110 mg/L PFAA and PFSA conditions, indicating that the number of the carbon–fluorine groups in the experiment were not likely responsible for the dechlorination inhibition (Table S3). The C<sub>9</sub>–C<sub>11</sub> PFAAs (i.e., PFNA, PFDA, and PUnA) were present at approximately 59 μM in the 110 mg/L PFAA condition but were absent in the 110 mg/L PFSA condition, indicating that the longer-chained PFAAs may have contributed to TCE inhibition (Table S3). It is possible that the increased binding affinity of longer-chained PFAAs to the microbes interfered with their proper metabolism and dechlorination function. Weathers et al. (2016) also showed that the growth of pure culture *D. mccartyi* strain 195 was repressed in the presence of the same suite of 110 mg/L PFAAs that was tested in this study.<sup>32</sup> This suggests that direct inhibition of PFAAs on the activity of the cells is occurring.

Dechlorination activity still occurred with higher concentrations of PFAAs than 6:2 FtSaB, further suggesting that the inhibition in FtSaB-amended experiments was likely attributable to the PFAS's nonfluorinated functional group. FtSaB was the only tested PFAS in this study that contains a non-fluorinated and positively charged betaine functional group, which could potentially aid in the binding of the compound to negatively charged biomolecules, such as proteins and nucleic acids. This type of binding might interfere with normal cellular metabolic and replication processes. Alkyl betaines and other cationic surfactants have previously been shown to have various antimicrobial effects.<sup>36,37</sup> Although the mode of action between the betaines and cells is not clear in some cases, it is generally thought that the compounds interact with the lipid bilayer structure of the cell membrane.

Although the mechanism for dechlorination inhibition of *D. mccartyi* is not yet known, it is possible that the PFASs directly interact with *D. mccartyi* cells to prevent dechlorination, (e.g., by interfering specifically with RDases or preventing proper transport functions by binding to *D. mccartyi*'s proteinaceous S-layer). A previous study found that the hydrocarbon surfactant Tween-80 specifically inhibited dechlorination by *D. mccartyi* organisms but not other Gram-positive and Gram-negative dechlorinating isolates.<sup>30</sup> Surfactant sorption or partial diffusion through *D. mccartyi*'s S-layer might prevent the cells' proper metabolism and replication and could potentially explain the absence of observable *D. mccartyi* growth in the 3M AFFF-amended experiments (Figure S3).

## ENVIRONMENTAL IMPLICATIONS

The results of this study suggest that there is a complex interplay between AFFF and TCE in bioremediation communities that varies depending on the AFFF formulation and composition. The organics in the three tested AFFFs in this study were fermentable under anaerobic conditions and produced the hydrogen and acetate necessary to support dechlorination by *D. mccartyi*. Although the biodegradation of DGBE appears to produce lower concentrations of hydrogen than other carbon constituents in the foams (e.g., ethylene glycol), organic compounds in each of the AFFF formulations were capable of providing the hydrogen and acetate required for dechlorination by *D. mccartyi*.

The type and quantity of PFAS compounds present may determine whether dechlorination will be inhibited by the presence of AFFF, especially in highly concentrated source zones. Generally, TCE dechlorination by *D. mccartyi* appears to occur in the presence of relatively high concentrations of PFASs, and no impact on dechlorination activity is observed at PFAS concentrations that are higher than those found at most field sites.<sup>5</sup> However, it has also been revealed that major fractions of PFASs in the field are currently unidentified and may constitute a significant proportion of the total PFASs in the environment.<sup>8,10</sup> Additionally, there is increasing evidence that site remediation activities and in situ biotransformation could be responsible for the conversion of AFFF PFAS compounds, such as FtTAoS, into more water-soluble PFCAs,<sup>10,23</sup> potentially increasing the load of PFAAs to which microbial communities are exposed.

AFFF application at firefighter training sites was often heterogeneous with respect to time and formulation use. Foams from different manufacturers and production years were often used at the same site, leading to groundwater and soil contaminated with multiple classes of carbon solvents and PFASs.<sup>8–10</sup> Additional research is needed to describe the effects of a range of AFFF concentrations on microbial communities in the presence of soil and the potential combinatorial effects of multiple formulations types. A holistic understanding of the effects of AFFF on TCE-dechlorinating microbial communities will lead to better sequential treatment operations at AFFF and chloroethene-contaminated sites.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b04773.

Figures showing TCE dechlorination; acetate, ethene, and hydrogen concentrations; *Dehalococcoides* 16S rDNA copies; PFAS concentrations; Methane, acetate, and hydrogen in DGBE cultures; cDCE, vinyl chloride, and ethene in AFFF components experiments; methane and hydrogen in AFFF components experiments; cDCE, vinyl chloride, and ethene in PFAA experiments; and methane and hydrogen in PFAA experiments. Tables showing AFFF and DGBE amendment regime for enrichment cultures, composition of three AFFF formulations, and concentrations of -CF<sub>2</sub> groups and alkyl chain lengths in PFAA experiments. (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

- (1) Moody, C. A.; Field, J. A. Perfluorinated surfactants and the environmental implications of their use in fire-fighting foams. *Environ. Sci. Technol.* **2000**, *34*, 3864–3870.
- (2) Tuve, R. L.; Jablonski, J. E. Method of extinguishing liquid hydrocarbon fires. U.S. Patent 3,258,423; June 28, 1966.
- (3) Place, B. J.; Field, J. A. Identification of novel fluorochemicals in aqueous film-forming foams used by the U.S. military. *Environ. Sci. Technol.* **2012**, *46*, 7120–7127.
- (4) D'Agostino, L. A.; Mabury, S. A. Identification of Novel Fluorinated Surfactants in Aqueous Film Forming Foams and Commercial Surfactant Concentrates. *Environ. Sci. Technol.* **2014**, *48*, 121–129.
- (5) Moody, C. A.; Hebert, G. N.; Strauss, S. H.; Field, J. A. Occurrence and persistence of perfluorooctanesulfonate and other perfluorinated surfactants in groundwater at a fire-training area at Wurtsmith Air Force Base, Michigan, USA. *J. Environ. Monit.* **2003**, *5*, 341–345.
- (6) Weiss, O.; Wiesmüller, G. A.; Bunte, A.; Göen, T.; Schmidt, C. K.; Wilhelm, M.; Hölzer, J. Perfluorinated compounds in the vicinity of a fire training area – Human biomonitoring among 10 persons drinking water from contaminated private wells in Cologne, Germany. *Int. J. Hyg. Environ. Health* **2012**, *215*, 212–215.
- (7) Schultz, M. M.; Barofsky, D. F.; Field, J. A. Quantitative determination of fluorotelomer sulfonates in groundwater by LC MS/MS. *Environ. Sci. Technol.* **2004**, *38*, 1828–1835.
- (8) Houtz, E. F.; Higgins, C. P.; Field, J. A.; Sedlak, D. L. Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil. *Environ. Sci. Technol.* **2013**, *47*, 8187–8195.
- (9) Backe, W. J.; Christensen, K. E.; Field, J. A. Newly-identified cationic, anionic, and zwitterionic fluorinated chemicals in groundwater at U.S. Military bases by non-aqueous large-volume injection HPLC-MS/MS. *Environ. Sci. Technol.* **2013**, *47*, S226–S234.
- (10) McGuire, M. E.; Schaefer, C.; Richards, T.; Backe, W. J.; Field, J. A.; Houtz, E. F.; Sedlak, D. L.; Guelfo, J. L.; Wunsch, A.; Higgins, C. P. Evidence of remediation-induced alteration of subsurface poly- and perfluoroalkyl substance distribution at a former firefighter training area. *Environ. Sci. Technol.* **2014**, *48*, 6644–6652.
- (11) Lovley, D. R. Cleaning up with genomics: applying molecular biology to bioremediation. *Nat. Rev. Microbiol.* **2003**, *1*, 35–44.
- (12) McCarty, P. L. Microbiology – breathing with chlorinated solvents. *Science* **1997**, *276*, 1521–1522.
- (13) Duhamel, M.; Edwards, E. A. Microbial composition of chlorinated ethene-degrading cultures dominated by *Dehalococcoides*. *FEMS Microbiol. Ecol.* **2006**, *58*, S38–S49.
- (14) He, J. Z.; Holmes, V. F.; Lee, P. K. H.; Alvarez-Cohen, L. Influence of vitamin B12 and cocultures on the growth of *Dehalococcoides* isolates in defined medium. *Appl. Environ. Microbiol.* **2007**, *73*, 2847–2853.
- (15) 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. Biotechnol.* **2013**, *97*, 6439–6450.
- (16) Houtz, E. F.; Sedlak, D. L. Oxidative conversion as a means of detecting precursors to perfluoroalkyl acids in urban runoff. *Environ. Sci. Technol.* **2012**, *46*, 9342–9349.
- (17) Lee, P. K. H.; Macbeth, T. W.; Sorenson, K. S.; Deeb, R. A.; Alvarez-Cohen, L. Quantifying genes and transcripts to assess the in situ physiology of “*Dehalococcoides*” spp. in a trichloroethene-contaminated groundwater site. *Appl. Environ. Microbiol.* **2008**, *74*, 2728–2739.
- (18) Lee, P. K. H.; Johnson, D. R.; Holmes, V. F.; He, J. Z.; Alvarez-Cohen, L. Reductive dehalogenase gene expression as a biomarker for physiological activity of *Dehalococcoides* spp. *Appl. and Environ. Microbiol.* **2006**, *72*, 6161–6168.
- (19) FC-203CF Lightwater (TM) AFFF 3%. MSDS No. CKQCB; 3M Company: St. Paul, MN, 1999.
- (20) Ansilite 3% AFFF AFC-5-A Foam Concentrate. Wormald/Tyco International: Rydalmere, Australia, 2008.

(21) *Aer-O-Water 3EM, Fire Fighting Foam Concentrate*. MSDS No. NMS210; National Foam, Inc: Exton, PA, 2010.

(22) Freeborn, R. A.; West, K. A.; Bhupathiraju, V. K.; Chauhan, S.; Rahm, B. G.; Richardson, R. E.; Alvarez-Cohen, L. 2005. Phylogenetic analysis of TCE-dechlorinating consortia enriched on a variety of electron donors. *Environ. Sci. Technol.* **2005**, *39*, 8358–8368.

(23) Harding-Marjanovic, K. C.; Houtz, E. H.; Yi, S.; Sedlak, D. L.; Alvarez-Cohen, L.; Field, J. A. Aerobic biotransformation of fluorotelomer thioether amido sulfonate (Lodyne) in AFFF-amended microcosms. *Environ. Sci. Technol.* **2015**, *49*, 7666–7674.

(24) Gaston, L. W.; Stadtman, E. R. Fermentation of ethylene glycol by *Clostridium glycolicum*, sp. n. *J. Bacteriol.* **1962**, *85*, 356–362.

(25) Dwyer, D. F.; Tiedje, J. M. Degradation of ethylene glycol and polyethylene glycols by methanogenic consortia. *Appl. Environ. Microbiol.* **1983**, *46*, 85–90.

(26) Dwyer, D. F.; Tiedje, J. M. Metabolism of polyethylene glycol by two anaerobic bacteria *Desulfovibrio desulfuricans* and a *Bacteroides* sp. *Appl. Environ. Microbiol.* **1986**, *52*, 852–856.

(27) Straß, A.; Schink, B. Fermentation of polyethylene glycol via acetaldehyde in *Pelobacter venetianus*. *Appl. Microbiol. Biotechnol.* **1986**, *42*, 25–37.

(28) Yang, Y. R.; McCarty, P. L. Competition for hydrogen within a chlorinated solvent dehalogenating anaerobic mixed culture. *Environ. Sci. Technol.* **1998**, *32*, 3591–3597.

(29) Loeffler, F. E.; Yan, J.; Ritalahti, K. M.; Adrian, L.; Edwards, E. A.; Konstantinidis, K. T.; Muller, J. A.; Fullerton, H.; Zinder, S. H.; Spormann, A. M. *Dehalococcoides mccartyi* gen. nov., sp. nov., obligately organohalide-respiring anaerobic bacteria relevant to halogen cycling and bioremediating, belong to a novel bacterial class, Dehalococcoidia classis nov., order Dehalococcoidales ord. nov and family Dehalococcoidaceae fam. nov., within the phylum Chloroflexi. *Int. J. Syst. Evol. Microbiol.* **2013**, *63*, 625–635.

(30) Amos, B. K.; Daprato, R. C.; Hughes, J. B.; Pennell, K. D.; Loeffler, F. E. Effects of the nonionic surfactant Tween 80 on microbial reductive dechlorination of chlorinated ethenes. *Environ. Sci. Technol.* **2007**, *41*, 1710–1716.

(31) Liou, J. S. C.; Szostek, B.; DeRito, C. M.; Madsen, E. L. Investigating the biodegradability of perfluorooctanoic acid. *Chemosphere* **2010**, *80*, 176–183.

(32) Weathers, T. S.; Harding-Marjanovic, K. C.; Higgins, C. P.; Alvarez-Cohen, L.; Sharp, J. O. Perfluoroalkyl acids inhibit reductive dechlorination of trichloroethene by repressing *Dehalococcoides*. *Environ. Sci. Technol.* **2016**, *50* (1), 240–48.

(33) Sung, Y.; Ritalahti, K. M.; Apkarian, R. P.; Loeffler, F. E. Quantitative PCR confirms purity of strain GT, a novel trichloroethene-to-ethene-respiring *Dehalococcoides* isolate. *Appl. Environ. Microbiol.* **2006**, *72*, 1980–1987.

(34) He, J.; Sung, Y.; Krajmalnik-Brown, R.; Ritalahti, K. M.; Loeffler, F. E. Isolation and characterization of *Dehalococcoides* sp. strain FL2, a trichloroethene (TCE)- and 1,2-dichloroethene-respiring anaerobe. *Environ. Microbiol.* **2005**, *7*, 1442–1450.

(35) Weathers, T. S.; Higgins, C. P.; Sharp, J. O. Enhanced biofilm production by a toluene-degrading *Rhodococcus* observed after exposure to perfluoroalkyl acids. *Environ. Sci. Technol.* **2015**, *49*, 5458–5466.

(36) Birnie, C. R.; Malamud, D.; Schnaare, R. L. Antimicrobial Evaluation of N-alkyl betaines and N-alkyl-N,N-Dimethylamine oxides with variations in chain length. *Antimicrob. Agents Chemother.* **2000**, *44*, 2514–2517.

(37) Lindstedt, M.; Allenmark, S.; Thompson, R. A.; Edebo, L. Antimicrobial activity of betaine esters, quaternary ammonium amphiphiles which spontaneously hydrolyze into nontoxic components. *Antimicrob. Agents Chemother.* **1990**, *34*, 1949–1954.