UC Berkeley
UC Berkeley Previously Published Works

Title

Perfluoroalkyl Acids Inhibit Reductive Dechlorination of Trichloroethene by Repressing Dehalococcoides

Permalink

https://escholarship.org/uc/item/1b89d745

Journal

Environmental Science and Technology, 50(1)

ISSN

0013-936X

Authors

Weathers, Tess S
Harding-Marjanovic, Katie
Higgins, Christopher P
et al.

Publication Date

2016-01-05

DOI

10.1021/acs.est.5b04854

Peer reviewed

Perfluoroalkyl Acids Inhibit Reductive Dechlorination of Trichloroethene by Repressing *Dehalococcoides*

Tess S. Weathers[†], **Katie Harding-Marjanovic**[‡], **Christopher P. Higgins**[†], **Lisa Alvarez-Cohen**[‡], and **Jonathan O. Sharp**[*,†]

[†] Hydrologic Science and Engineering Program and Department of Civil and Environmental Engineering, Colorado School of Mines, Golden, Colorado 80401, United States

[‡] Civil and Environmental Engineering, University of California, Berkeley, California 94720, United States

*Phone: 303-273-3473; fax: 303-273-3413; e-mail: jsharp@mines.edu.

Cite this:*Environ. Sci. Technol.* 50, 1, 240-248   ⬇ RIS Citation   GO

Abstract
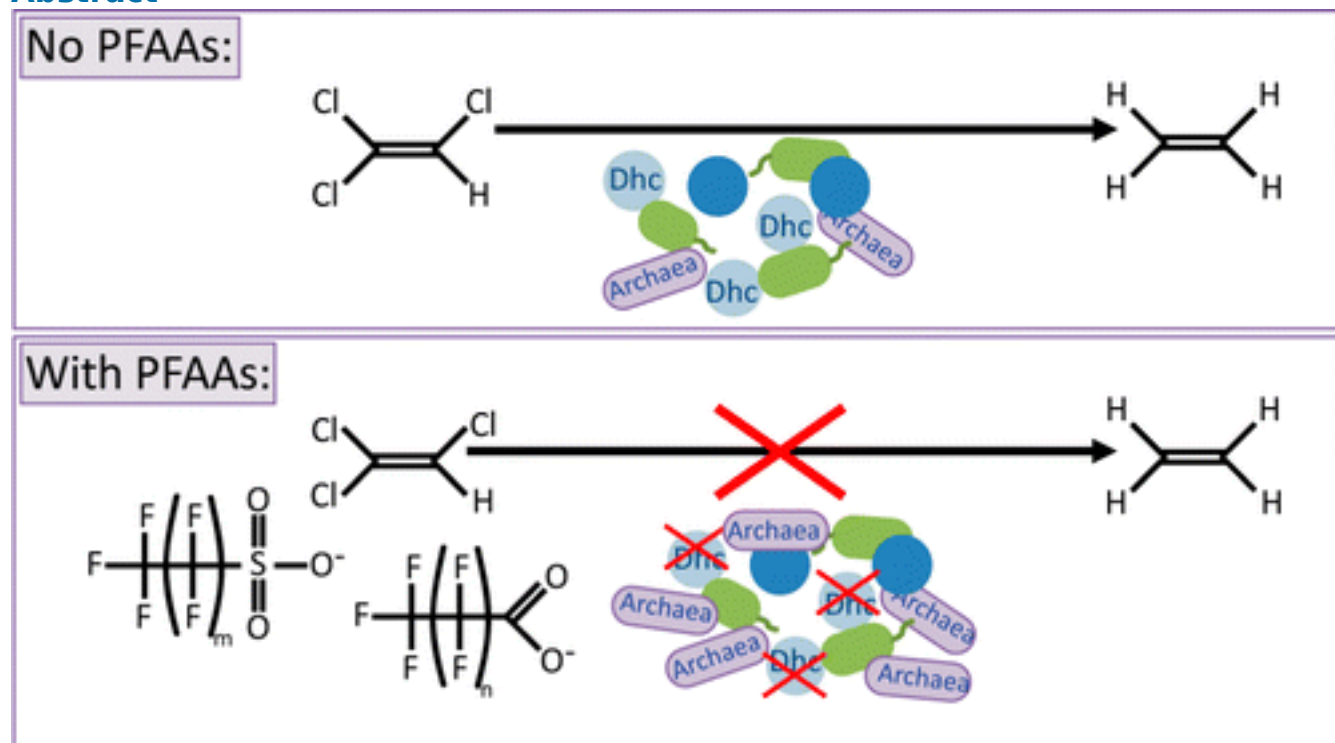
The subsurface recalcitrance of perfluoroalkyl acids (PFAAs) derived from aqueous film-forming foams could have adverse impacts on the microbiological processes used for the bioremediation of co-mingled chlorinated solvents such as trichloroethene (TCE). Here, we show that reductive dechlorination by a methanogenic, mixed culture was significantly inhibited when exposed to concentrations representative of PFAA source zones (>66 mg/L total of 11 PFAA analytes, 6 mg/L

each). TCE dechlorination, cis-dichloroethene and vinyl chloride production and dechlorination, and ethene generation were all inhibited at these PFAA concentrations. Phylogenetic analysis revealed that the abundances of 65% of the operational taxonomic units (OTUs) changed significantly when grown in the presence of PFAAs, although repression or enhancement resulting from PFAA exposure did not correlate with putative function or phylogeny. Notably, there was significant repression of *Dehalococcoides* (8-fold decrease in abundance) coupled with a corresponding enhancement of methane-generating Archaea (a 9-fold increase). Growth and dechlorination by axenic cultures of *Dehalococcoides mccartyi* strain 195 were similarly repressed under these conditions, confirming an inhibitory response of this pivotal genus to PFAA presence. These results suggest that chlorinated solvent bioattenuation rates could be impeded in subsurface environments near PFAA source zones.

•

Introduction

Poly- and perfluoroalkyl substances (PFASs) are contaminants of emerging concern found throughout the environment.(1) PFASs have been used in a diverse range of industrial, consumer, and commercial applications including pesticides, nonstick coatings, and in fire-fighting foams.(1-3) These compounds are environmentally recalcitrant, exhibit toxicity effects in primates and microbiota, and can bioaccumulate.(4-8) The prevalent use and environmental longevity, coupled with improvements in quantification, have led to widespread environmental detection in groundwater, surface water, soil, and air, as well as in humans, wildlife, and food crops.(6, 9-11)The U.S. Environmental Protection Agency has set provisional drinking water health advisories for two common PFASs: 0.4 μg/L for perfluorooctanoate (PFOA) and 0.2 μg/L for perfluorooctanesulfonate (PFOS).(2)

Perfluoroalkyl acids (PFAAs), a subset of PFASs, are present in and can arise from components of aqueous film-forming foams (AFFF) used for fuel fire suppression.(12) Use of AFFF for military firefighter training has led to the introduction of PFAAs into groundwater in sites that are often contaminated with chlorinated solvents(3, 13-15) such as trichloroethene (TCE) and its toxic transformation products, cis-dichloroethene (cDCE) and vinyl chloride (VC).(16) Enhanced reductive dechlorination (ERD) is a bioremediation process for chlorinated solvents in which an electron donor, such as lactate (biostimulation), or potentially a known microbial consortium containing *Dehalococcoides* (bioaugmentation) is supplied.(16) Members of the

genus *Dehalococcoides* are known to completely degrade tetrachloroethene (PCE) and TCE to ethene, thus limiting the potential for toxic accumulation of vinyl chloride.[17] *Dehalococcoides* have been incorporated into ERD consortia in laboratory settings[17-19] and are often found in aquifers in which complete PCE and TCE dechlorination has been observed.[20, 21] It is yet unknown how PFAAs may impact microbial communities relevant to chlorinated solvent bioremediation. Although biodegradation of PFAAs is not expected,[2] there are concerns regarding the potential adverse effects from PFAA exposure on subsurface microbial communities and co-contaminant degradability. [1] Biodegradation of another commonly co-located contaminant, toluene, was not impacted in pure culture studies; however, the presence of PFAAs did correlate to increased formation of extracellular polysaccharides and enhanced transcription of stress-related genes.[7] Consequently, unanticipated impacts on PFAA or chlorinated solvent fate and transport in groundwater sources are possible, ranging from effects on co-contaminant degradation and microbial processes to changes in sorptive properties of PFAAs as a result of biostimulation or bioaugmentation.

The objective of this study was to assess potential impacts of these nontraditional contaminants, PFAAs, on reductive dechlorination of TCE. This was accomplished by querying for suppression of reductive dechlorination activity by a *Dehalococcoides*-containing methanogenic mixed community cultivated in the presence of varied concentrations of PFAAs. In parallel, we contrasted the community structure and putative functionality of these microcosms to understand ecological shifts and metabolic redundancy within this consortium that could be attributed to PFAA presence. Insights from the ecological profiles were then used to challenge an axenic culture of *Dehalococcoides* with PFAAs to understand the direct impact on this bacterial genus. Results revealed the suppression of *Dehalococcoides* growth and reductive dechlorination rates in both pure culture and mixed assemblages.

-

Materials and Methods

PFAA Preparation and Aqueous Analysis

Purity corrected stock solutions of an 11-analyte mixture were prepared as described elsewhere.[7, 14] PFAA salts (Sigma-Aldrich) were suspended in a 70/30 (v/v) methanol–water solution. Water used in this study was generated with a Milli-Q system (Millipore). Unless otherwise specified, each mixture contained perfluorobutanoate, perfluoropentanoate, perfluorohexanoate, perfluoroheptanoate, PFOA, perfluorononanoate, perfluorodecanoate, perfluoroundecanoate, perfluorobutanesulfonate, perfluorohexanesulfonate, and PFOS. These compounds represent a

range of carbon chain lengths and are commonly found at AFFF-impacted sites.[1, 11, 22] Empty culture bottles were spiked with the PFAA mixture while in an anaerobic chamber (90% nitrogen, 5% hydrogen, 5% carbon dioxide), and the methanol was evaporated as described elsewhere.[7] Final PFAA concentrations were verified using liquid chromatography–tandem mass spectroscopy with stable-isotope surrogate standards (Wellington Laboratories). Aqueous samples were centrifuged to remove particulates, sampled, and diluted as appropriate. A SCIEX 3200 mass spectrometer (MDS Sciex) was utilized with MultiQuant for quantitation to verify PFAA recovery.[7]

Cellular Preparation

Anaerobic experiments were inoculated with a *Dehalococcoides*-containing methanogenic mixed culture that was maintained in a stock bottle amended with exogenous cobalamin cofactors, such as vitamin B_{12}, to enable TCE dechlorination.[19, 23] This stable and robust culture maintained in Berkeley, CA was derived from TCE-contaminated groundwater.[23] The *Dehalococcoides* strains in this culture are most similar to *Dehalococcoides mccartyi* strain 195.[23] The described culture ferments lactate to produce hydrogen and acetate, the necessary electron donor and carbon source for *Dehalococcoides*. Pure-culture experiments using *D. mccartyi* strain 195 were maintained under similar conditions as the mixed culture but were amended with acetate and hydrogen instead of lactate.[17]

Reductive Dechlorination with the Mixed Culture

Batch systems containing TCE, lactate, and a PFAA mixture were designed to evaluate microbial degradation rates. ERD experiments contained triplicate sets of 22, 66, or 110 mg/L total PFAAs added to sterile 60 mL serum bottles as well as 0 mg/L controls spiked with non-PFAA containing 70/30 v/v methanol–water. These concentrations were chosen to reflect what may be observed near a PFAA source zone[3] (110 mg/L total at 10 mg/L each of 11 analytes) and to determine if there is a PFAA concentration threshold wherein biological effects are not observed. Following the PFAA addition and methanol evaporation described in the prior section, bottles were sealed with butyl rubber stoppers. Next, 48.5 mL of an autoclaved mineral salts medium[24] containing 20 mM lactate as electron donor and 100 µg/L vitamin B_{12} was added to stoppered bottles with a sterile syringe. During media addition, an exhaust needle was inserted into the stopper to avoid bottle pressurization, and low flow rates were used to minimize PFAA volatilization and flushing. After the media addition, the headspace was gently flushed with N_2/CO_2 (90:10) to remove residual hydrogen and oxygen. Each bottle was then amended with approximately 20 µmoles of TCE (Sigma-Aldrich, 99.9%) and allowed to sit for at least 24 h to facilitate TCE and PFAA equilibration. At time zero, the bottles were inoculated with 3% (v/v) of the previously grown methanogenic mixed community stock

culture and incubated in the dark at 34 °C for the duration of the experiment. All of the bottles were inverted several times at each sampling point to promote PFAA mixing. Abiotic controls devoid of microorganisms were prepared for the parallel pure-culture experiment. After TCE dechlorination profiles were generated, the bottles were sampled for phylogenetic sequencing and verification of PFAA concentrations.

Dechlorination Measurements

Chloroethenes, ethene, and methane were measured by injecting 100 μL of culture headspace into an Agilent 7890A gas chromatograph equipped with a flame ionization detector and 30 m × 0.32 mm J & W capillary column (Agilent Technologies). Hydrogen concentrations were measured by injecting diluted headspace samples into a gas chromatograph fitted with a reductive gas detector (Trace Analytical). Between 50 and 300 μL of culture headspace was withdrawn for each hydrogen measurement and diluted in 17 mL glass vials purged with N_2 to generate concentrations within the linear calibration range of the instrument. The total volume of extracted headspace was tracked throughout the incubation to ensure that the same approximate volume was removed from all bottles (1.5 to 1.9 mL). Initial TCE dechlorination rates were determined by obtaining the slope of a time-course regression through the linear portion of the degradation curve (days 1 through 4) after accounting for loss as a function of time generated from the abiotic control. Production rates for cDCE, VC, and ethene were also tabulated during the linear portion of the generation curve for each sample.

Phylogenetic Sequencing

Every sample containing the methanogenic mixed culture was extracted after dechlorination profiles were complete (~8 days). DNA was extracted with the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc.). Extracted DNA was quantified with a Qubit 2.0 Fluorometer and dsDNA HS Assay Kit (Life Technologies). Each sample was amplified using a TC-412 Thermocycler (TECHNE) with 2 μL DNA template, 2 μL each forward and reverse primers at 10 μM each, 6.5 μL PCR grade water, and 12 μL Phusion 2× MasterMix (New England BioLabs, Inc.). Dual-indexed primers (515F and 805R) target the V4 region of the 16s rRNA gene (Integrated DNA Technologies) and are described in the Supporting Information along with the amplification protocol. Select samples were run via gel electrophoresis to confirm that the amplicons were of a desired length (roughly 300 base pairs). Samples were purified and normalized with the SequalPrep Normalization Kit (Invitrogen) according to the manufacturer's protocol and pooled into a 2 mL RNase- and DNase-free sterile microcentrifuge tube. Pooled samples were concentrated with ULtra-.05 30K centrifugal filter devices (Amicon) according to the provided protocol. Pooled samples were quantified using the Qubit

Fluorometer as described above and diluted and requantified if necessary to normalize concentrations. Half of the final pooled sample volume was archived at –80 °C, with the balance sent for MiSeq sequencing using the V2 250 Cycle Paired-End kit at BioFrontiers Institute (Boulder, CO).

Sequence postprocessing was performed with MacQIIME version 1.9.0[25] with operational taxonomic unit (OTU) picking and chimera screening using Usearch61[26, 27] and alignment with PyNAST.[28, 29] Taxonomy was assigned with Greengenes reference database version 12_10[30] using RDP Classifier 2.2.[31] FastTree 2.1.3 was used to generate the phylogenetic tree[32] and was further manipulated in FigTree.[33] Weighted, nonrarefied β diversity was visualized with EMPeror,[34] and adonis and ANOSIM test statistics were calculated using a comparison of categories.[35] Samples were filtered so the minimum total observed fraction per OTU was 0.001. Taxa were summarized such that the OTU identifier with the greatest total abundance was retained: if the lowest classification for multiple OTU identifiers resulted in the same taxonomic assignment, the specific OTU identifier with the greatest total abundance was retained. The abundance of the resulting merged OTU reflects the sum of each OTU with the same taxonomic lineage. Differential abundances were calculated with the DeSeq2 package; the adjusted p values are reported herein.[36] The relative abundances represent the average ± standard deviation between the three experimental replicates per condition. Reporting of results and analysis within this work reflects the lowest identified taxonomic level of each OTU.

Functionality was assigned on the basis of the class-level information for the non-dechlorinating members of the consortium according to the comparative metagenomics study performed by Hug et al.[19] The methanogenic mixed culture used herein is similar to the ANAS culture maintained in Berkeley, California (described in Hug et al.),[18, 19] which also uses lactate as an electron donor. [18, 37] The functions discussed by Hug et al.[19] have been applied to the culture within this study. Sequence data have been submitted to the National Center for Biotechnology Information Sequence Read Archive database (BioProject PRJNA302232). Postprocessing scripts can be found in the Supporting Information.

-

Results and Discussion

Effect of PFAAs on Reductive Dechlorination by a Mixed Culture

Batch microbial incubations across a range of PFAA concentrations revealed that TCE dechlorination was severely inhibited when the methanogenic mixed culture was grown in the

presence of 110 mg/L PFAA ([Figure 1](#)). This corresponded with reduced cDCE production as well as limited VC and ethene production. Inhibition effects on TCE dechlorination were also observed at 66 mg/L manifested by a decrease in dechlorination rates of TCE as compared to the case without PFAAs ($p < 0.01$), along with a decrease in cDCE, VC, and ethene production rates ([Table 1](#)). Dechlorination of TCE, production and dechlorination of cDCE and VC, and production of ethene occurred similarly between cases with no PFAAs and those with 22 mg/L. Analogous inhibitory behavior has also been observed in the presence of select AFFF formulations containing a range of PFASs.[38] The 11-component PFAA mixture appeared to exert this selective pressure; systems with equivalent concentrations of only perfluoroalkyl sulfonates were not affected.[38]
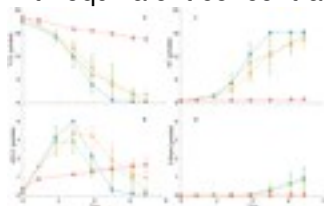

Figure 1. Reductive dechlorination by the mixed culture is inhibited by 110 mg/L total PFAAs (□) with slower rates of dechlorination for 66 mg/L total PFAAs (○), while 22 mg/L total PFAAs (△) is not significantly different from no PFAAs (×) ($p > 0.2$ for each sampling time and compound). (A) TCE profiles; (B) cDCE; (C) VC; and (D) ethene. Error bars represent the standard error of triplicate incubations.

Table 1. Dechlorination (−) and Production (+) Rates by the Mixed Culture

	rates (μmol/day)[*]			
	TCE	**cDCE**	**VC**	**ethene**
no PFAAs	−5.8 ± 0.2	+1.4 ± 0.0	+5.5 ± 0.2	+0.3 ± 0.3
22 mg/L	−4.3 ± 2.9	+1.5 ± 0.7	+3.5 ± 2.7	+0.3 ± 0.4
66 mg/L	−3.3 ± 1.0	+1.0 ± 0.2	+4.5 ± 1.3	+0.0 ± 0.1

rates (µmol/day)[*]			
	TCE	cDCE	VC	ethene
110 mg/L	−0.5 ± 0.1	+0.1 ± 0.1	+0.1 ± 0.1	0.0 ± 0.0

[*]

Values represent triplicate averages ± standard deviation.

Effects of PFAA Exposure on Community Structure

To understand how the 11-component PFAA mixture herein affected the dechlorination process, we explored relative abundance, community dynamics, and putative functionalities via next-generation sequencing. Phylogenetic sequencing of the methanogenic assemblage resulted in 17 total OTUs after filtering to 0.1% as described in the Methods and Materials section (Figure 2). This core microbiome was observed regardless of PFAA concentration; however, the relative abundance of select taxonomic units varied with increasing PFAA exposure.
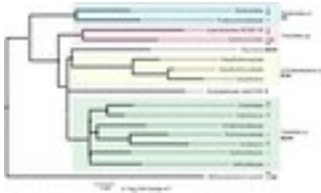

Figure 2. Phylogenetic tree of the unique OTUs depicting trends for putative function while showing that changes in abundance upon exposure to PFAAs do not correlate with phylogeny. Arrows represent the $\log_2$ fold-change in abundance between no PFAAs and 110 mg/L PFAAs with arrow size correlating to the magnitude of $\log_2$ fold-change. An upward-pointing arrow represents an increase in abundance when grown with PFAAs; a downward-pointing arrow represents a decrease in abundance when grown with PFAAs. Putative functions within the community are annotated as methionine synthesis (red square), fermenters (green triangle), corrinoid synthesis (orange circle), and oxygen scavenging (gray ×). Nodes represent

The functions and taxonomy described herein reflect what is typically observed in dechlorinating methanogenic mixed-cultures,(19, 23) and are thus extrapolated to this particular system. These functions are hypothetical, as data are being compared to a similar culture that is cultivated in semi-batch (versus batch) and likely contains a different strain of *Dehalococcoides* than the methanogenic mixed-culture used herein.(18, 23) This comparison is useful for assigning putative functionality considering the overall taxonomic similarities between the two analogous cultures.

This mixed-culture is fermentative and methanogenic. The general pathway of reductive dechlorination within this community begins with conversion of lactate, the electron donor, to hydrogen by a set of fermenters.(39) In this study, fermentation has been putatively assigned to 11 OTUs, including members from the classes of Bacteroidia(40) and Clostridia and single members from Spirochaetes and Synergistia (Figure 2).(19, 41) The hydrogen can then be used by *Methanobacterium bryantii* to produce methane or to complex with chlorine as it is removed from the electron acceptors (TCE, cDCE, or VC) by *Dehalococcoides*, eventually producing ethene.(19, 42) Most of the remaining OTUs in this culture, along with those found within Clostridia and Spirochaetes, can be functionally assigned to a combination of methionine or corrinoid synthesizers (two functions that *Dehalococcoides* lacks) or can fulfill the niche of oxygen scavengers.(19) These hypothesized interactions only address functionality as it relates to *Dehalococcoides* vitality; it is likely that additional processes are occurring within the system; however, they are not included in this analysis. This general community composition is similar to what has been described elsewhere for reductive dechlorinating cultures sustained on lactate, which includes orders such as Clostridiales, Bacteroidales, and Spirochaetales, along with δ-proteobacteria, *Dehalococcoides*, and archaeal methanogens.(19, 37, 39)

The presence of PFAAs at varied concentrations tended to shift the relative abundance of many of these organisms. Analysis of β diversity depicted two distinct groups: the samples with 110 mg/L total PFAAs clustered independently from the 0, 22, and 66 mg/L samples (Figure 3). This is also observed statistically; both adonis and ANOSIM metrics exhibit statistical significance ($p = 0.013$ and $p = 0.011$, respectively) when the samples are grouped by concentration. The diversity of samples grown with 66 mg/L PFAAs is not significantly different from 22 mg/L or those without PFAAs, despite a reduction in dechlorination rate as discussed previously. TCE dechlorination rates in both the 22 and 66 mg/L samples is, however, well correlated to the relative abundance of *Dehalococcoides* in each sample, with Pearson correlation coefficients of –0.72 and –0.91,

respectively. This correlation further supports that *Dehalococcoides* abundance is a driving factor for both differences in community structure and inhibition of TCE dechlorination upon PFAA exposure.
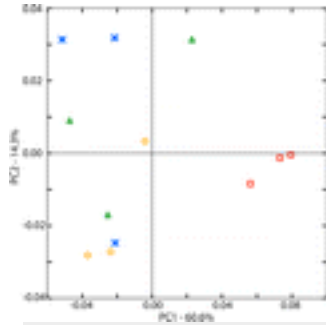

Figure 3. Principle component analysis of weighted nonrarefied taxon abundance shows distinct clustering of cultures grown in the presence of 110 mg/L PFAAs (□) on an axis that explains 60.6% of the variability. The rest of the cultures, grown either in the presence of 22 mg/L (△), 66 mg/L (○), or without PFAAs (×), do not exhibit any significant clustering between samples.

The most extreme shift in community structure occurred when comparing samples without PFAAs and those grown in the presence of 110 mg/L total PFAAs. Samples that effectively dechlorinated TCE without PFAAs had an average abundance of 4.5% ± 0.2% of *Dehalococcoides*, compared to an average abundance of 0.5% ± 0.2% for samples grown in the presence of 110 mg/L total PFAAs. This is an 8.4-fold decrease in abundance ($p < 0.01$) for communities grown in the presence of 110 mg/L PFAAs. An inverse shift in the relative abundance of methanogenic Archaea was also observed (Figure 2): without PFAAs, the *M. bryantii* abundance was 0.5% ± 0.3% versus 3.8% ± 0.9% with 110 mg/L PFAAs, resulting in an 8.5-fold increase ($p < 0.01$). This relationship is expected; *Dehalococcoides* and methanogens actively compete for the available hydrogen to either produce HCl through the dechlorination reaction or to produce methane, respectively.(19, 42) The relative abundance of *Dehalococcoides* and *M. bryantii* correlate well with TCE dechlorination rates (R^2 = 0.68 for both comparisons), as seen in Figure 4.
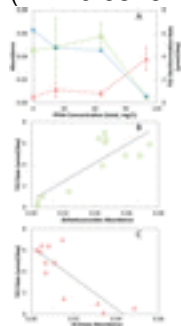

Figure 4. Abundances of *Dehalococcoides* (□) and *M. bryantii* (△) exhibit correlations with PFAA concentration (×) (panel A). TCE dechlorination rates also correlate with *Dehalococcoides* abundance (panel B) (R^2 = 0.68) and with Archaea abundance (panel C) (R^2 = 0.68).

Although the inverse correlation between *Dehalococcoides* and Archaea is anticipated in light of the interplay between TCE reduction and methane consumption,[42] our understanding of shifts and corresponding relationships between non-dechlorinating members of the community is limited. A consistent trend was observed in all members of δ-proteobacteria: no significant change in relative abundance was observed upon exposure to PFAAs. Members of this class (such as an unspecified *Desulfovibrionaceae* and *Desulfovibrio* sp.) are hypothesized to scavenge oxygen and synthesize corrinoid cofactors necessary for *Dehalococcoides*.[19, 42] Members of δ-proteobacteria also encode hydrogenases necessary for the conversion of hydrogen cations to H_2, along with the ability to produce exogenous methionine. Reductive dechlorination by *Dehalococcoides* irreversibly loses functionality upon exposure to oxygen; thus, *Dehalococcoides* may rely on oxygen-scavenging organisms, such as δ-proteobacteria, within the mixed community.[19] Another OTU that was not affected by PFAA presence was *Treponema*, a genus known for corrinoid and methionine synthesis, oxygen scavenging, and the ability to ferment lactate to produce H_2 (Figure 2).[19, 43] Because these organisms are not significantly impacted by PFAA presence, they are likely not contributing to the inhibition of *Dehalococcoides* vitality and activity.

Conversely, 11 OTUs exhibited statistically different abundances ($p < 0.05$) when grown in the presence of 110 mg/L PFAAs. To understand the potential effects of PFAAs on putative functions within the methanogenic mixed culture, we will focus on the scenario wherein the largest shifts in relative abundance occurred between samples without PFAAs and those with 110 mg/L PFAAs, although similar trends were observed when comparing between 22, 66, and 110 mg/L (Table S4). Taxa that may be responsible for corrinoid synthesis and oxygen scavenging exhibited varied responses to PFAA presence; thus, the inhibition of *Dehalococcoides* is not definitively connected to repression of organisms capable of corrinoid or methionine synthesis, or to oxygen scavenging within the community. For example, we hypothesize that members of the order Clostridiales are fermenters[44] and can synthesize methionine within this system.[19] Many of these OTUs were enhanced in the presence of 110 mg/L PFAAs: an unspecified Clostridiales increased by a fold-change of 4.7, *Coprococcus* increased by 2.4-fold, *Oscillospira* was enhanced 3.4-fold, and Veillonellaceae increased with a fold-change of 1.5. Others capable of fulfilling these niches were inhibited at 110 mg/L PFAAs: *Sedimentibacter* decreased 1.5-fold, and an unspecified Ruminococcaceae decreased 1.7-fold. Christensenellaceae, another member of Clostridiales, was not significantly impacted by PFAA presence at all. The reason some members of Clostridiales are enhanced while others are repressed or unaffected is yet to be understood.

Similarly, two OTUs in the order Bacteroidales, which contribute to fermentation and potentially methionine synthesis, were observed:(19, 45) one unidentified Bacteroidales was repressed in the presence of 110 mg/L PFAAs by a fold-change of 7.1, while Porphyromonadaceae was enhanced 1.8-fold. Also repressed at 110 mg/L was WCHB1–05 in the family Anaerolinaceae, which decreased by a fold-change of 4.8. This OTU was the most closely related to *Dehalococcoides* as a member of the phylum Chloroflexi. Fold-changes and statistical significance for each OTU and concentration comparison can be viewed in Table S4. This exercise demonstrates that the selective pressures as a result of PFAA presence are highly variable; in general, enhancement and repression do not trend with putative functionality or with phylogeny.

Reductive Dechlorination of Axenic *Dehalococcoides*

Because the previously described experiments were performed with mixed cultures, it is impossible to determine whether *Dehalococcoides* was directly affected by PFAAs or if another subset of the community upon which *Dehalococcoides* is dependent was affected. For example, it is not clear whether *Methanobacterium* excels in the presence of PFAAs or if *Methanobacterium* fills the niche resulting from *Dehalococcoides* inhibition. To understand whether *Dehalococcoides* is directly affected by PFAA exposure, we repeated the TCE dechlorination experiment with *Dehalococcoides* in pure culture. With *Dehalococcoides mccartyi* strain 195 maintained axenically, the inhibitory effects of PFAAs on TCE dechlorination behavior were similar to those observed in the more complex methanogenic community (Figure 5). The rates of production and dechlorination of each solvent with *Dehalococcoides* in pure culture further illustrate increasing degrees of inhibition with increasing PFAA content (Table 2). Pronounced inhibition was observed at 110 mg/L, with a significant reduction in TCE dechlorination rates for this concentration as compared to dechlorination rates devoid of PFAAs ($p < 0.01$) (Table 2, Figure 5). Correspondingly, minimal cDCE and VC production occurred with 110 mg/L. Ethene generation was not observed above the background from inoculation carry-over in any of the treatments, which may be explained by the inefficiency of *Dehalococcoides* in pure culture,(24) coupled with TCE presence across these incubations. The dechlorination of TCE is more energetically favorable than that of VC; therefore, significant generation and subsequent dechlorination of VC is not expected to occur if TCE remains in the system.(46) TCE dechlorination rates with 66 mg/L PFAAs were also significantly inhibited when contrasted with systems without PFAAs ($p < 0.01$). A delayed rate of cDCE and VC production was observed at 66 mg/L, although rates of VC production were similar between 66 mg/L and the case without PFAAs. At 22 mg/L total PFAAs, the average TCE dechlorination rate was lower than the system lacking PFAAs, yet the inhibition of the rate was not statistically significant (Figure 5).

Correspondingly, both cDCE and VC production rates were slightly retarded at 22 mg/L, although the difference did not maintain statistical significance.
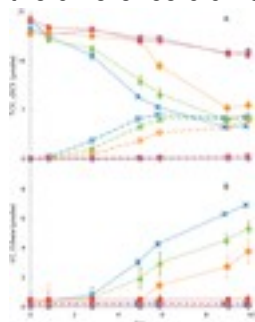

Figure 5. Dechlorination by *D. mccartyi* strain 195 in the presence of PFAAs. Panel A shows TCE profiles with solid symbols and cDCE with open symbols. Panel B shows VC (solid) and ethene (open). Inhibition of dechlorination is observed with 110 mg/L total PFAAs (red squares) and behaves similarly to the abiotic control (purple diamonds). Slower rates of dechlorination and production were observed for 66 mg/L total PFAAs (orange circles) with mild inhibition at 22 mg/L total PFAAs (green triangles) compared to no PFAAs (blue ×). Error bars represent the standard error of triplicate incubations.

Table 2. Dechlorination (−) and Production (+) Rates by Axenic *Dehalococcoides*

	rates (µmol/day)[*]			
	TCE	**cDCE**	**VC**	**ethene**
	(day 3–6)	**(day 1–6)**	**(day 5–10)**	**(day 0–10)**
no PFAAs	−2.5 ± 0.2	+1.3 ± 0.1	+0.8 ± 0.1	0.0 ± 0.0
22 mg/L	−2.1 ± 0.6	+1.1 ± 0.1	+0.7 ± 0.1	0.0 ± 0.0

	rates (μmol/day)[*]			
	TCE	**cDCE**	**VC**	**ethene**
	(day 3–6)	**(day 1–6)**	**(day 5–10)**	**(day 0–10)**
66 mg/L	−1.2 ± 0.4	+0.5 ± 0.1	+0.6 ± 0.3	0.0 ± 0.0
110 mg/L	−0.6 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
abiotic	−0.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

[*]

Values represent triplicate averages ± standard deviation.

-

Environmental Implications

Understanding reductive dechlorination in the presence of PFAAs is of environmental concern due to the common co-location of PFAAs and chlorinated solvents and the reliance on microbiological processes for chlorinated solvent attenuation. Concentrations up to 7 mg/L for PFOA in groundwater have been measured; a choice of 10 mg/L of each analyte as investigated here may be representative of a source zone or subsurface worst-case scenario,[(3)] especially considering the increases in environmental detection and measurement sensitivity of a variety of PFASs. [(11)]Additionally, increased PFAA sorption in the presence of non-aqueous phase liquids (NAPLs) has been observed, particularly at higher PFAA concentrations.[(14, 15)] This may result in an

accumulation of PFAAs where TCE is present, even outside of source zones. It is also known that PFAA precursors found in AFFF may generate additional PFAAs in groundwater over time. (47)Because the depth of knowledge regarding the concentration and distribution of PFAAs is continually growing, this is a current approximation of what may be observed near a source zone *in situ*. On the basis of the results from this study, it is clear that dechlorination of TCE is significantly inhibited both within the dechlorinating mixed community and with *Dehalococcoides* in pure culture at these high concentrations reflective of PFAA source zones or areas of NAPL co-contamination. Phylogenetic investigations revealed that the presence of PFAAs at high concentrations directly impacts *Dehalococcoides*' relative abundance and has a varied effect on the composition of the methanogenic mixed community.

It has been observed elsewhere that dechlorinating communities provided with acetate, lactate, or methanol as electron donors appear to be phylogenetically distinct from one another. These different communities, however, still exhibit conserved metabolic pathways, particularly for methionine and corrinoid synthesis, fermentation, and oxygen scavenging.(19) This implies that although community shifts may occur in the presence of PFAAs, the machinery required to facilitate *Dehalococcoides* growth and dechlorination may still be provided, suggesting that there exists a degree of functional redundancy within the methanogenic mixed culture. Exploring the putative functions of the mixed methanogenic communities may not necessarily provide the single key to the mechanisms behind the repression of *Dehalococcoides*; however, this is an important step in understanding the effects of PFAAs on environmentally relevant systems. To understand whether particular processes or functions are affected by PFAA presence, one may utilize additional exploration, such as proteomics or functional metagenomics, to understand the impacts of PFAA exposure.

The parallel observations of impacts on reductive dechlorination by *Dehalococcoides* both axenically and in community incubations confirm that source-level concentrations of PFAAs will have a direct impact on *Dehalococcoides.* However, due to the variation in community responses, it is difficult to ascertain the mechanisms affecting the repression of *Dehalococcoides.* This repression could potentially be attributed to a stress response(7) or inhibition of vitamin B_{12}uptake.(19, 24) For example, vitamin B_{12}, in the form of cyanocobalamin, is a required enzymatic cofactor for reductive dehalogenases(23) and contains both a cyano group and 5′6′-dimethylbenzimidazole as the lower ligand.(48) *Dehalococcoides* growth can be hindered by changing the type of lower ligand;(23, 24) one might hypothesize that if PFAAs chemically interact with this lower ligand, it may be converted to a form that is unusable for *Dehalococcoides*. Although we do not expect or observe any

defluorination, there is evidence of potential physicochemical interactions between PFAAs and vitamin B_{12}, though the coordination is not well understood.(49) At 110 mg/L PFAAs, there are 4 orders of magnitude more PFAAs present (3×10^{-4} mol/L) than vitamin B_{12} amendments (7×10^{-8} mol/L), which could result in a deficit for use by *Dehalococcoides*.(24, 48) Future work to examine the effects of increasing vitamin B_{12} dosage in the presence of high concentrations of PFAAs or the quantification of cobalamin lower ligand moieties could help elucidate this potential interaction and explore its relevance to subsurface settings.

In summary, this study addresses the impacts of PFAAs on the enhanced reductive dechlorination of TCE and a community responsible for that process. PFAAs and TCE are environmental pollutants, the former being recalcitrant and not easily remediated; the latter is often treated with bioremediation via reductive dechlorination. Interestingly, high concentrations, similar to what may be found near a PFAA source zone, significantly inhibit TCE dechlorination by directly repressing *Dehalococcoides.* Because of *Dehalococcoides*' role in reductive dechlorination, suppression of *Dehalococcoides* growth may result in an accumulation of VC, especially if other dechlorinators that are not capable of attenuating this carcinogen, such as *Geobacter*, are active and unaffected.(19) In sites that are co-contaminated with both chlorinated solvents and PFAAs, biostimulation or bioaugmentation to enhance reductive dechlorination processes may not be the most suitable option for solvent remediation. Additionally, if ERD is the current cleanup strategy for chlorinated solvents and is observed to be ineffective, the presence of coexisting PFAAs may be the cause of inhibition. These results underscore the need for complete site investigation, monitoring, and exploration to ensure continued chlorinated solvent bioremediation in locations that may contain PFAAs.

-

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b04854.

- Tables showing PFAA analytes used in this study and corresponding characteristics, observed taxa, abundance, fold changes, and statistical significance. Additional details on dual-indexed primer specifications, the amplification program, and sequencing postprocessing scripts. (PDF)

- **PDF**
  -

Perfluoroalkyl Acids Inhibit Reductive Dechlorination of Trichloroethene by Repressing Dehalococcoides

Author Contributions

This manuscript was written through contributions of all authors. Dechlorination experiments were prepared by T.S.W. at Colorado School of Mines, and the inoculation and dechlorination measurements were conducted by K.H.-M. at UC Berkeley. Analysis of dechlorination results, phylogenetic sequencing, postprocessing, and putative function assessments were performed by T.S.W. All authors have given approval of the final version of the manuscript.

The authors declare no competing financial interest.

-

Acknowledgment

ABBREVIATIONS

PFAS	poly- and perfluoroalkyl substances
PFAA	perfluoroalkyl acids
AFFF	aqueous film-forming foams
PFOS	perfluorooctanesulfonate
PFOA	perfluorooctanoic acid

PCE	tetrachloroethene
TCE	trichloroethene
cDCE	cis-dichloroethene
VC	vinyl chloride
ERD	enhanced reductive dechlorination
OTU	operational taxonomic unit

- [Reference QuickView](#)
-

References

This article references 49 other publications.

1. [1.](#)

   Key, B. D.; Howell, R. D.; Criddle, C. S. Fluorinated organics in the biosphere *Environ. Sci. Technol.* **1997**,31 (9) 2445– 2454 DOI: 10.1021/es961007c

   [[ACS Full Text ⬧](#)], [[CAS](#)]

2. [2.](#)

   Environmental Protection Agency. Emerging Contaminants - Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). http://www.epa.gov/sites/production/files/2014-04/documents/factsheet_contaminant_pfos_pfoa_march2014.pdf; **2014**.

3. [3.](#)

   Moody, C. A.; Field, J. A. Perfluorinated surfactants and the environmental implications of their use in fire-fighting foams *Environ. Sci. Technol.* **2000**, 34 (18) 3864– 3870 DOI: 10.1021/es991359u

   [[ACS Full Text ⬧](#)], [[CAS](#)]

4.   [4.](#)

Moody, C. A.; Martin, J. W.; Kwan, W. C.; Muir, D. C. G.; Mabury, S. A. Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etobicoke Creek *Environ. Sci. Technol.* **2002**, 36, 545– 551 DOI: 10.1021/es011001+

[ACS Full Text ⬥], [CAS]

5.   [5.](#)

Higgins, C. P.; McLeod, P. B.; Macmanus-Spencer, L. A.; Luthy, R. G. Bioaccumulation of perfluorochemicals in sediments by the aquatic oligochaete *Lumbriculus variegatus Environ. Sci. Technol.* **2007**, 41 (13) 4600– 4606 DOI: 10.1021/es062792o

[ACS Full Text ⬥], [CAS]

6.   [6.](#)

Blaine, A. C.; Rich, C. D.; Sedlacko, E. M.; Hundal, L. S.; Kumar, K.; Lau, C.; Mills, M. A.; Harris, K. M.; Higgins, C. P. Perfluoroalkyl acid distribution in various plant compartments of edible crops grown in biosolids-amended soils *Environ. Sci. Technol.* **2014**, 48 (14) 7858– 7865 DOI: 10.1021/es500016s

[ACS Full Text ⬥], [CAS]

7.   [7.](#)

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 (9) 5458–5466 DOI: 10.1021/es5060034

[ACS Full Text ⬥], [CAS]

8.   [8.](#)

Seacat, A. M. Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys *Toxicol. Sci.* **2002**, 68 (1) 249– 264 DOI: 10.1093/toxsci/68.1.249

[Crossref], [PubMed], [CAS]

9.   [9.](#)

Martin, J. W.; Whittle, D. M.; Muir, D. C. G.; Mabury, S. A. Perfluoroalkyl Contaminants in a Food Web from Lake Ontario *Environ. Sci. Technol.* **2004**, 38 (20) 5379– 5385 DOI: 10.1021/es049331s

[ACS Full Text ⬥], [CAS]

10.   [10.](#)

Higgins, C. P.; Field, J. A.; Criddle, C. S.; Luthy, R. G. Quantitative Determination of Perfluorochemicals in Sediments and Domestic Sludge *Environ. Sci. Technol.* **2005**, 39 (11) 3946– 3956 DOI: 10.1021/es048245p

[ACS Full Text ⬇], [CAS]

11. 11.

Rotander, A.; Toms, L.-M. L.; Aylward, L.; Kay, M.; Mueller, J. F. Elevated levels of PFOS and PFHxS in firefighters exposed to aqueous film forming foam (AFFF) *Environ. Int.* **2015**, 82, 28– 34 DOI: 10.1016/j.envint.2015.05.005

[Crossref], [PubMed], [CAS]

12. 12.

Moody, C. A.; Field, J. A. Determination of perfluorocarboxylates in groundwater impacted by fire-fighting activity *Environ. Sci. Technol.* **1999**, 33 (16) 2800– 2806 DOI: 10.1021/es981355+

[ACS Full Text ⬇], [CAS]

13. 13.

McGuire, M. E.; Schaefer, C.; Richards, T.; Backe, W. J.; Field, J. A.; Houtz, E.; 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(12) 6644– 6652 DOI: 10.1021/es5006187

[ACS Full Text ⬇], [CAS]

14. 14.

Guelfo, J. L.; Higgins, C. P. Subsurface transport potential of perfluoroalkyl acids at aqueous film-forming foam (AFFF)-impacted sites *Environ. Sci. Technol.* **2013**, 47 (9) 4164– 4171 DOI: 10.1021/es3048043

[ACS Full Text ⬇], [CAS]

15. 15.

McKenzie, E. R.; Siegrist, R. L.; McCray, J. E.; Higgins, C. P. The influences of a non-aqueous phase liquid (NAPL) and chemical oxidant application on perfluoroalkyl acid (PFAA) fate and transport. Water Research**2015**, submitted for publication

16. 16.

Scheutz, C.; Durant, N. D.; Broholm, M. M. Effects of bioaugmentation on enhanced reductive dechlorination of 1,1,1-trichloroethane in groundwater: a comparison of three sites *Biodegradation* **2014**, 25(3) 459– 478 DOI: 10.1007/s10532-013-9674-x

[Crossref], [PubMed], [CAS]

17. 17.

Maymo-Gatell, X.; Chien, Y.-T; Gossett, J. M.; Zinder, S. H. Isolation of a Bacterium That Reductively Dechlorinates Tetrachloroethene to Ethene *Science* **1997**, 276 (5318) 1568– 1571 DOI: 10.1126/science.276.5318.1568

[Crossref], [PubMed], [CAS]

18. 18.

Holmes, V. F.; He, J.; Lee, P. K. H.; Alvarez-Cohen, L. Discrimination of Multiple *Dehalococcoides* Strains in a Trichloroethene Enrichment by Quantification of Their Reductive Dehalogenase Genes *Appl. Environ. Microbiol.* **2006**, 72 (9) 5877– 5883 DOI: 10.1128/AEM.00516-06

[Crossref], [PubMed], [CAS]

19. 19.

Hug, L. A.; Beiko, R. G.; Rowe, A. R.; Richardson, R. E.; Edwards, E. A. Comparative metagenomics of three *Dehalococcoides*-containing enrichment cultures: the role of the non-dechlorinating community *BMC Genomics* **2012**, 13 (1) 327 DOI: 10.1186/1471-2164-13-327

[Crossref], [PubMed], [CAS]

20. 20.

Yang, Y.; Zeyer, J. Specific Detection of Dehalococcoides Species by Fluorescence In Situ Hybridization with 16S rRNA-Targeted Oligonucleotide Probes *Appl. Environ. Microbiol.* **2003**, 69 (5) 2879– 2883 DOI: 10.1128/AEM.69.5.2879-2883.2003

[Crossref], [PubMed], [CAS]

21. 21.

Hendrickson, E. R.; Payne, J. A.; Young, R. M.; Starr, M. G.; Perry, M. P.; Fahnestock, S.; Ellis, D. E.;Ebersole, R. C. Molecular Analysis of Dehalococcoides 16S Ribosomal DNA from Chloroethene-Contaminated Sites throughout North America and Europe *Appl. Environ. Microbiol.* **2002**, 68 (2) 485– 495DOI: 10.1128/AEM.68.2.485-495.2002

[Crossref], [PubMed], [CAS]

22. 22.

Buck, R. C.; Franklin, J.; Berger, U.; Conder, J. M.; Cousins, I. T.; de Voogt, P.; Jensen, A. A.; Kannan, K.;Mabury, S. A.; van Leeuwen, S. P. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins *Integr. Environ. Assess. Manage.* **2011**, 7 (4) 513– 541 DOI: 10.1002/ieam.258

[Crossref], [PubMed], [CAS]

23. 23.

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 (14) 6439– 6450 DOI: 10.1007/s00253-013-4896-8

[Crossref], [PubMed], [CAS]

24. 24.

He, J.; 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 (9) 2847– 2853DOI: 10.1128/AEM.02574-06

[Crossref], [PubMed], [CAS]

25. 25.

Caporaso, J. G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F. D.; Costello, E. K.; Fierer, N.;Peña, A. G.; Goodrich, J. K.; Gordon, J. I.QIIME allows analysis of high-throughput community sequencing data *Nat. Methods* **2010**, 7 (5) 335– 336 DOI: 10.1038/nmeth.f.303

[Crossref], [PubMed], [CAS]

26. 26.

Edgar, R. C. Search and clustering orders of magnitude faster than BLAST *Bioinformatics* **2010**, 26 (19)2460– 2461 DOI: 10.1093/bioinformatics/btq461

[Crossref], [PubMed], [CAS]

27. 27.

Edgar, R. C.; Haas, B. J.; Clemente, J. C.; Quince, C.; Knight, R. UCHIME improves sensitivity and speed of chimera detection *Bioinformatics* **2011**, 27 (16) 2194– 2200 DOI: 10.1093/bioinformatics/btr381

[Crossref], [PubMed], [CAS]

28. 28.

Caporaso, J. G.; Bittinger, K.; Bushman, F. D.; DeSantis, T. Z.; Andersen, G. L.; Knight, R. PyNAST: a flexible tool for aligning sequences to a template alignment *Bioinformatics* **2010**, 26 (2) 266– 267 DOI: 10.1093/bioinformatics/btp636

[Crossref], [PubMed], [CAS]

29. 29.

DeSantis, T. Z.; Hugenholtz, P.; Larsen, N.; Rojas, M.; Brodie, E. L.; Keller, K.; Huber, T.; Dalevi, D.; Hu, P.;Andersen, G. L. Greengenes, a Chimera-Checked

16S rRNA Gene Database and Workbench Compatible with ARB *Appl. Environ. Microbiol.* **2006**, 72 (7) 5069– 5072 DOI: 10.1128/AEM.03006-05

[Crossref], [PubMed], [CAS]

30. 30.

McDonald, D.; Price, M. N.; Goodrich, J.; Nawrocki, E. P.; DeSantis, T. Z.; Probst, A.; Andersen, G. L.;Knight, R.; Hugenholtz, P. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea *ISME J.* **2012**, 6 (3) 610– 618 DOI: 10.1038/ismej.2011.139

[Crossref], [PubMed], [CAS]

31. 31.

Wang, Q.; Garrity, G. M.; Tiedje, J. M.; Cole, J. R. Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy *Appl. Environ. Microbiol.* **2007**, 73 (16) 5261– 5267 DOI: 10.1128/AEM.00062-07

[Crossref], [PubMed], [CAS]

32. 32.

Price, M. N.; Dehal, P. S.; Arkin, A. P. FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments *PLoS One* **2010**, 5 (3) e9490 DOI: 10.1371/journal.pone.0009490

[Crossref], [PubMed]

33. 33.

Rambaut, A. FigTree v1.4.2; University of Edinburgh: Edinburgh, Scotland, **2015**.

34. 34.

Vázquez-Baeza, Y.; Pirrung, M.; Gonzalez, A.; Knight, R. EMPeror: a tool for visualizing high-throughput microbial community data *GigaScience* **2013**, 2 (1) 16 DOI: 10.1186/2047-217X-2-16

[Crossref], [PubMed], [CAS]

35. 35.

Fierer, N.; Lauber, C. L.; Zhou, N.; McDonald, D.; Costello, E. K.; Knight, R. Forensic identification using skin bacterial communities *Proc. Natl. Acad. Sci. U. S. A.* **2010**, 107 (14) 6477– 6481 DOI: 10.1073/pnas.1000162107

[Crossref], [PubMed], [CAS]

36. 36.

Love, M. I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.*. **2014**, 15 (12). 550 DOI: 10.1186/s13059-014-0550-8

[Crossref], [PubMed], [CAS]

37. 37.

Richardson, R. E.; Bhupathiraju, V. K.; Song, D. L.; Goulet, T. A.; Alvarez-Cohen, L. Phylogenetic Characterization of Microbial Communities That Reductively Dechlorinate TCE Based upon a Combination of Molecular Techniques *Environ. Sci. Technol.* **2002**, 36 (12) 2652– 2662 DOI: 10.1021/es0157797

[ACS Full Text], [CAS]

38. 38.

Harding-Marjanovic, K. C.; Yi, S.; Weathers, T. S.; Sharp, J. O.; Sedlak, D. L.; Alvarez-Cohen, L. Effects of aqueous film-forming foam (AFFF) on trichloroethene (TCE) degradation by a *Dehalococcoides* enrichment culture *Environ. Sci. Technol.* **2015**, DOI: 10.1021/acs.est.5b04854

[ACS Full Text]

39. 39.

Freeborn, R. A.; West, K. A.; Bhupathiraju, V. K.; Chauhan, S.; Rahm, B. G.; Richardson, R. E.;Alvarez-Cohen, L. Phylogenetic Analysis of TCE-Dechlorinating Consortia Enriched on a Variety of Electron Donors *Environ. Sci. Technol.* **2005**, 39 (21) 8358– 8368 DOI: 10.1021/es048003p

[ACS Full Text], [CAS]

40. 40.

Cupples, A. M.; Spormann, A. M.; McCarty, P. L. Growth of a *Dehalococcoides*-Like Microorganism on Vinyl Chloride and cis-Dichloroethene as Electron Acceptors as Determined by Competitive PCR *Appl. Environ. Microbiol.* **2003**, 69 (2) 953– 959 DOI: 10.1128/AEM.69.2.953-959.2003

[Crossref], [PubMed], [CAS]

41. 41.

Sakamoto, M. The Family Porphyromonadaceae. In *The Prokaryotes*; Rosenberg, E.; DeLong, E. F.;Lory, S.; Stackebrandt, E.; Thompson, F., Eds.; Springer Berlin Heidelberg: Berlin, Heidelberg, **2014**; pp811– 824.

[Crossref]

42. 42.

Men, Y.; Feil, H.; VerBerkmoes, N. C.; Shah, M. B.; Johnson, D. R.; Lee, P. K. H.; West, K. A.; Zinder, S. H.;Andersen, G. L.; Alvarez-Cohen, L. Sustainable syntrophic growth of Dehalococcoides ethenogenes strain 195 with Desulfovibrio vulgaris Hildenborough and Methanobacterium congolense: global transcriptomic and proteomic analyses *ISME J.* **2012**, 6 (2) 410– 421 DOI: 10.1038/ismej.2011.111

[Crossref], [PubMed], [CAS]

43. 43.

Drennan, D. M.; Almstrand, R.; Lee, I.; Landkamer, L.; Figueroa, L.; Sharp, J. O. Organoheterotrophic bacterial abundance associates with zinc removal in lignocellulose-based sulfate-reducing systems *Environ. Sci. Technol.* **2015**, DOI: 10.1021/acs.est.5b04268

[ACS Full Text]

44. 44.

Biddle, A.; Stewart, L.; Blanchard, J.; Leschine, S. Untangling the Genetic Basis of Fibrolytic Specialization by Lachnospiraceae and Ruminococcaceae in Diverse Gut Communities *Diversity* **2013**, 5 (3) 627– 640DOI: 10.3390/d5030627

[Crossref]

45. 45.

Bergey, D. H.; Holt, J. G. *Bergey's Manual of Determinative Bacteriology*; Williams & Wilkins: Baltimore, Maryland, **1994**.

46. 46.

Aulenta, F.; Canosa, A.; Majone, M.; Panero, S.; Reale, P.; Rossetti, S. Trichloroethene Dechlorination and H_2 Evolution Are Alternative Biological Pathways of Electric Charge Utilization by a Dechlorinating Culture in a Bioelectrochemical System *Environ. Sci. Technol.* **2008**, 42 (16) 6185– 6190 DOI: 10.1021/es800265b

[ACS Full Text], [CAS]

47. 47.

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**, 130725144806004 DOI: 10.1021/es4018877

[ACS Full Text]

48. 48.

Yan, J.; Im, J.; Yang, Y.; Loffler, F. E. Guided cobalamin biosynthesis supports Dehalococcoides mccartyi reductive dechlorination activity *Philos. Trans. R. Soc., B* **2013**, 368 (1616) 20120320– 20120320 DOI: 10.1098/rstb.2012.0320

[Crossref], [PubMed], [CAS]

49.   49.

Ochoa-Herrera, V.; Sierra-Alvarez, R.; Somogyi, A.; Jacobsen, N. E.; Wysocki, V. H.; Field, J. A. Reductive Defluorination of Perfluorooctane Sulfonate *Environ. Sci. Technol.* **2008**, 42 (9) 3260– 3264 DOI: 10.1021/es702842q

[ACS Full Text], [CAS]