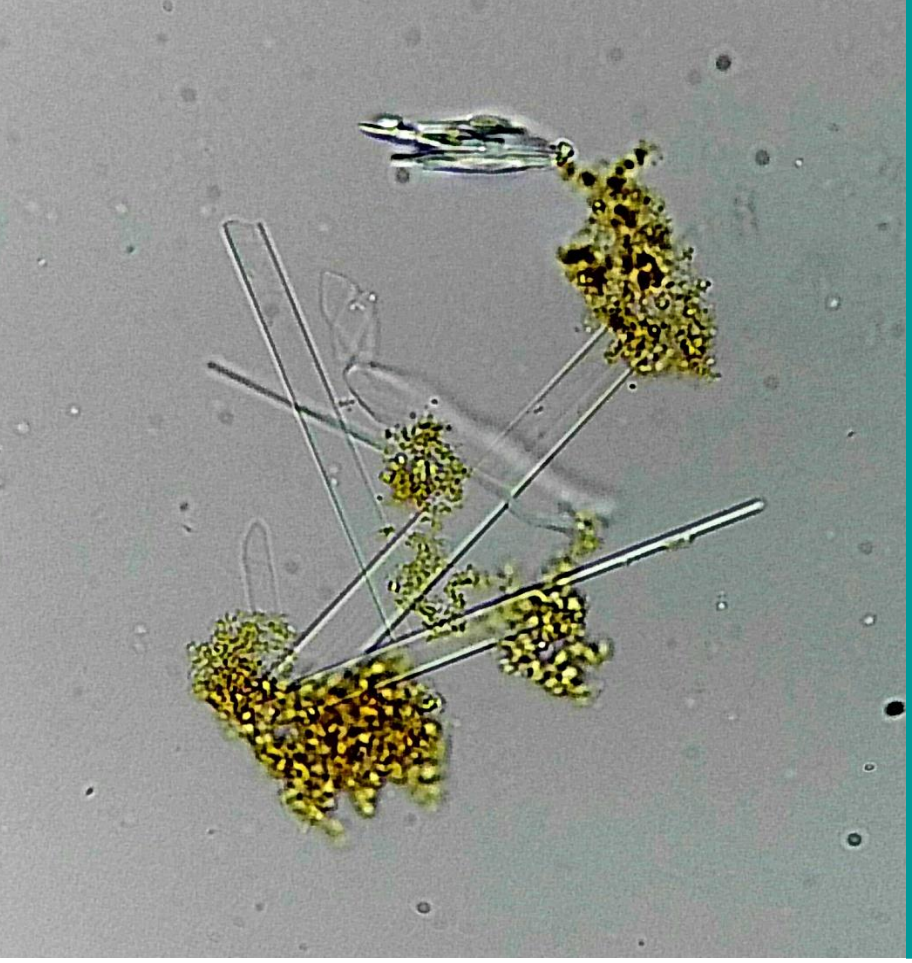

BIODEGRADATION OF PERFLUOROALKYL SUBSTANCES

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Better Microbes for a Cleaner Tomorrow

FIXED EARTH

BIODEGRADATION OF PERFLUOROALKYL SUBSTANCES

Contents

Introduction

Perfluoroalkyl substances (PFAS) are an emerging global contaminant. PFAS represents a family of compounds that are known to contain thousands of compounds that have been used in numerous industrial applications over the past several decades. As a result, PFAS contamination has been identified at thousands of sites globally and is believed to exist in the bloodstream of the majority the human population.

Of particular concern are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) which have been widely used in aqueous film forming foams (AFFFs) used in firefighting efforts. In December 2019 the EPA recommended that total PFAS concentrations should be less than 70 parts per trillion (ppt) if the groundwater has the potential to be used as drinking water, with several jurisdictions implementing standards that are lower still.

PFAS compounds are synthetic compounds that are comprised of a carbon chain which is saturated with fluorine atoms in lieu of hydrogen atoms. Figure 1 shows PFOS as an example PFAS molecule.

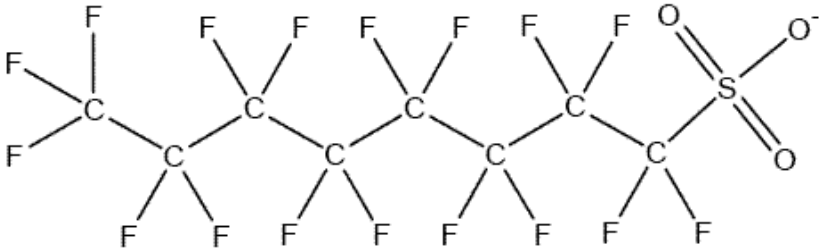


Figure 1. Perfluorooctanesulfonate (PFOS) Chemical Structure

The carbon-fluorine (C-F) bond is one of the strongest single covalent bonds known, and the presence of numerous C-F bonds in any given PFAS molecule makes these compounds very difficult to degrade. As a result of their resilience, PFAS compounds have commonly become known as "forever chemicals" due to their persistence in the environment.

Therefore, there are few remedial methods that are both cost-effective and remove PFAS entirely from the environment. Many remedial methods rely on immobilizing PFAS in situ via adsorption of activated charcoal or co-precipitation with metal ions. While these solutions limit the mobility of PFAS in the environment, the PFAS is not removed or destroyed meaning that sites will require on going risk management and eventual remediation once suitable technologies are developed. Other water treatment technologies are being developed which utilize exotic materials and high energies, limiting their practicality in field deployment.

PFAS compounds are generally considered to be resistant to biodegradation due to their chemical stability, although some limited biotransformations of PFAS are known in wastewater systems that typically lead to the formation of PFOS from other fluorinated compounds.

In 2019 the Fixed Earth team began the study of methods which could be used to identify PFAS degrading microorganisms. The goal of this work is to demonstrate that bioremediation of PFAS is possible and can result in a more complete removal of PFAS from the environment at a lower cost than other emerging methods. Our research in this area is ongoing and we are always striving to further our understanding of this challenging pollutant. The end of this document provides an overview of our ongoing research in this area with the remainder of the document describing our work-to-date.

Initial Desktop Study

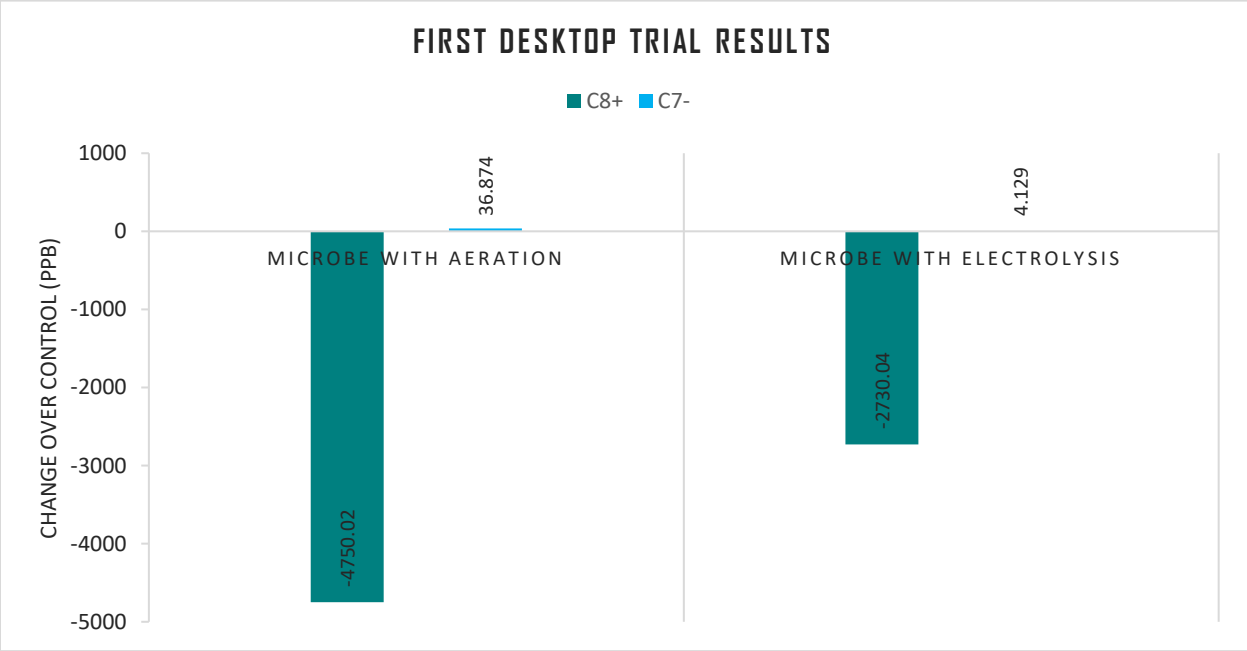
In early 2019 our team developed a method to obtain microbes that we believed were capable of degrading PFAS substances. Our first PFAS degrading microbe was collected from an industrial site in southern Alberta, Canada.

To confirm or refute the ability of the microbe to degrade PFAS, we initiated a low-complexity desktop experiment in which tap water was sterilized and spiked with approximately 8 parts per million (ppm) for PFOS which also contained trace PFOA. Two one-liter subsamples of the spiked water were taken, and the microbe was added to each sample with low concentrations of molasses to act as a supplementary carbon source.

One sample was aerated by gently bubbling the sample continuously while the second sample was aerated through electrolysis of water. Electrolysis of water was chosen for this experiment as this technology is routinely deployed by our partner companies to generate aerobic environments in the subsurface, allowing for more effective biodegradation of organic pollutants. Electrolysis was carried out by placing two titanium electrodes into the sample and applying a six-volt direct current continuously.

Following two weeks of treatment, samples were collected and analysed for PFAS via the Total Oxidizable Precursor Assay (TOPA). This method was chosen as it was believed it would account for partially degraded PFAS molecules and liberate any PFAS that had simply become bound to biological material in solution.

The results of this sampling are shown in the graph below, which shows the change in PFAS compounds when compared to the control sample. For simplicity in interpretation, PFAS compounds have been shown as two fractions: Compounds eight or more carbons in length and compounds with seven or fewer carbons.



In both treatment groups, a significant decrease in the concentration of PFAS compounds containing eight or more carbons was observed. A slight increase in PFAS compounds containing less than seven carbons was also observed. It is believed that these short-chain PFAS compounds are either formed as a by-product of the reaction or are intermediates in more complete PFAS degradation. As the increase

in short-chain PFAS compounds was not comparable to the decrease in longer chain compounds, we believe that these are most likely intermediates in the reaction and are further degraded by the microbes in time.

Water Chemistry Changes

In addition to the measurement of PFAS, samples were also analyzed for basic salinity parameters, including pH, carbonate, basic anions, and basic cations. In the samples treated with microbes, it was found that pH, carbonate, and bicarbonate increased while fluoride, magnesium, and calcium decreased. This data is shown in Table 1.

Table 1. Basic Salinity Results

Parameter	Control	Microbes with Aeration	Microbes with Electrolysis
pH	7.54	8.67	8.39
Fluoride (mg/L)	1.58	1.01	1.01
Bicarbonate (mg/L)	201	345	568
Carbonate (mg/L)	<5	49.8	16.2
Magnesium (mg/L)	12	11.4	10.5
Calcium (mg/L)	52.6	39.6	41.5

In general, it was expected to see an increase of fluoride as a function of PFAS degradation. However, in the presence of divalent ions, such as calcium and magnesium, fluoride minerals such as fluorite will form and are of very low solubility in water. While no precipitates were visible in the samples, the concentrations of fluoride which would be generated during PFAS degradation are low and no significant accumulation of fluoride minerals is anticipated.

A stoichiometric mass balance was attempted which assumed eight carbon PFAS molecules were degraded in entirety. From this, we find that a total of 1.9×10^{-4} moles of fluoride is unaccounted for in solution following PFAS degradation, including fluoride which was lost when compared to the control. Should the formation of CaF_2 (fluorite) be occurring, this would account for the loss of 7.7 mg of calcium ions. As 13 mg of calcium was lost, other minerals, such as carbonates, may be forming and resulting in the observed loss of divalent ions.

A notable increase in pH was also observed in both the solution which was aerated and the solution in which electrolysis was applied. With this observation and the unaccounted-for fluoride, a second possible reaction series was proposed:

$$PFOS \rightarrow CO_2 + F^-$$
$$CO_2 + H_2O \rightarrow H_2CO_3$$
$$H_2CO_3 + F^- \rightarrow HF\ (g\uparrow) + HCO_3^-(aq)$$

This reaction series would account for the loss of fluoride, increase in pH, increase in carbonates, and loss of divalent ions (as carbonate minerals) observed in the experiment. As the solution at hand is a complex mixture of ions, it was not possible to calculate if the change in pH matches the proposed mechanism.

While this preliminary study confirmed the ability of the microbial strains to degrade PFAS, the mechanism of degradation, by-products formed, and PFAS degradation at environmentally relevant concentrations have not been determined. Further studies will be required to make these determinations.

Second Desktop Study

In late 2019 our team wanted to further validate the method utilized to obtain putative PFAS degrading microorganisms. In this study, we obtained three additional microbes from a variety of environments. Gram staining of the microbes was performed and are shown in Figure 2.

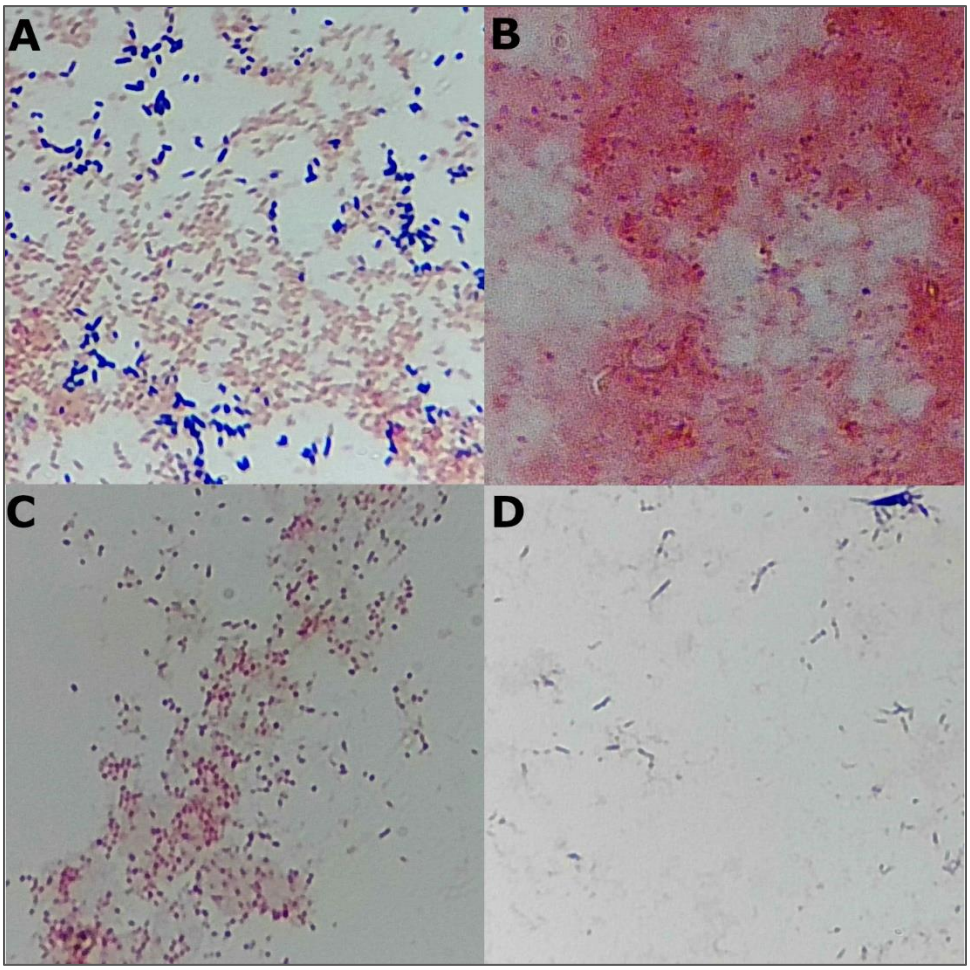


Figure 2. Putative PFAS degrading microorganisms.

Microbe 1 (A) – This microbe was collected from an industrial site in southern Alberta, Canada and was identified as being two organisms. One which is gram positive (purple) and the second as being gram negative (pink). Both are believed to be motile. This microbe was previously utilized in the first desktop study.

Microbe 2 (B) – This microbe was collected from an industrial site in central Alberta, Canada. The isolate consists of a gram-positive bacteria which produces significant extracellular polymeric substances (EPS) which are shown as pink in the image.

Microbe 3 (C) – This microbe was collected from a tropical site in Trinidad to confirm the method worked on soils from warm climates. The obtain was identified as being gram positive and produces trace EPS.

Microbe 4 (D) – This isolate was collected from a site in far northern British Columbia, Canada which possesses complex geochemical conditions. The microbe was identified as being gram positive and motile.

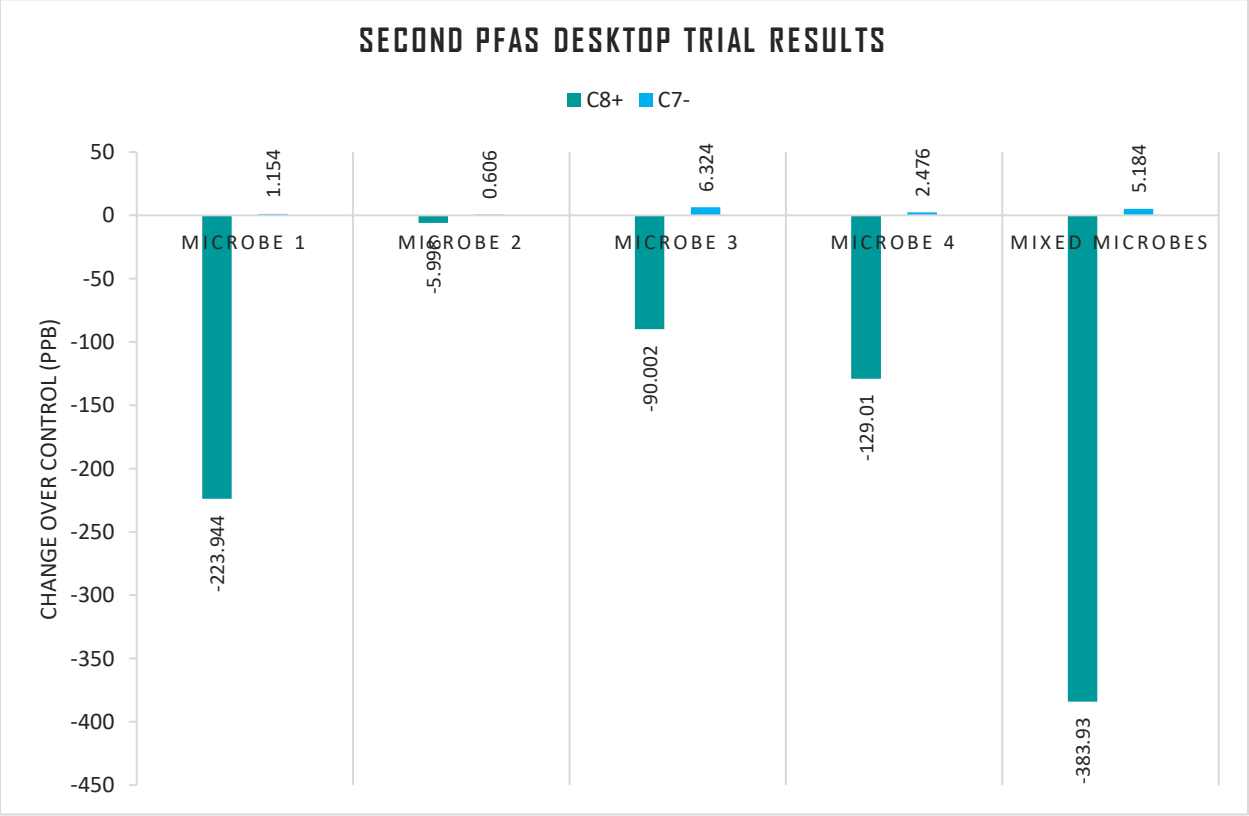
In addition to obtaining additional microbes, this desktop study utilized PFAS concentrations which were an order of magnitude lower. As Microbe 1 was utilized in the previous study, it would offer a general comparison in performance between concentrations. This study would also utilize a combination of PFNA and PFOS as opposed to PFOS alone.

In this experiment, a stock growth media was prepared by spiking tap water with approximately 500 ug/L (ppb) of both PFNA and PFOS, for approximately 1000 ppb of total PFAS. Additional nutrients such as dilute sucrose and mineral nutrients (NPK) were added to enhance microbial growth. Florence flasks were each filled with 500 mL of the stock solution, plugged with cotton, and autoclaved for 35 minutes.

Each flask was inoculated with its respective microbe, with the control remaining uninoculated and a sixth flask was inoculated with all four microbes. The flasks were placed onto a rotary shaker and agitated at 25 RPM for a period of two weeks to maintain aerobic conditions. Sterile distilled water was periodically added to maintain the solution volume.

After the incubation period, samples were collected and submitted to ALS for analysis of PFAS parameters. Although the samples were also analyzed by TOPA, a 20% loss of PFOS was observed in the control sample when it was subjected to TOPA. As a result, the TOPA data was not considered to be reliable enough to measure microbe performance and was disregarded.

The results of this sampling are shown in the graph below, which shows the change in PFAS compounds when compared to the control sample. For simplicity in interpretation, PFAS compounds have been shown as two fractions: Compounds eight or more carbons in length and compounds with seven or fewer carbons.



Of the four microbes tested, it was found that three were capable of degrading PFAS compounds, with the fourth microbe showing negligible change in PFAS concentrations (<5 ppb). The best performance was observed when all four microbes were utilized together.

As was observed in the previous study, a slight increase in short-chain (seven carbons and less) PFAS compounds was observed. The increase in short-chain PFAS was not proportionate to the decrease in longer chain compounds. Insufficient data is available to determine if these compounds are formed as a by-product of the degradation or are intermediates in the biodegradation of longer-chain compounds.

General conclusions are that the microbial acquisition method is applicable to a variety of environmental and geochemical conditions and that PFAS biodegradation can occur at concentrations in the range of parts per billion. Further studies are warranted to determine if PFAS degradation can occur in the low concentration of parts per trillion (ng/L), as is commonly observed in environmental media (groundwater).

Third Desktop Study

In early 2020 we initiated our third desktop study of PFAS degrading organisms. In this study we worked with a client in the United States with access to a site which was impacted by AFFFs during an industrial fire. The scope of this study included the acquisition of site-specific microbes, testing of microbes in tap water spiked with PFOA and PFOS, testing of the microbes in PFAS impacted groundwater samples from the site, and testing of the microbes in PFAS impacted soil samples from the site.

A large portion of the samples from this trial are still undergoing analysis as of the time of this publication. However, preliminary results from the early stages of the experiment are available for discussion. This publication will be updated as additional results become available.

A total of six microbes were obtained from the site and are all believed to be unique based on their morphology. Further identification and characterization of the microbes is planned for summer 2020.

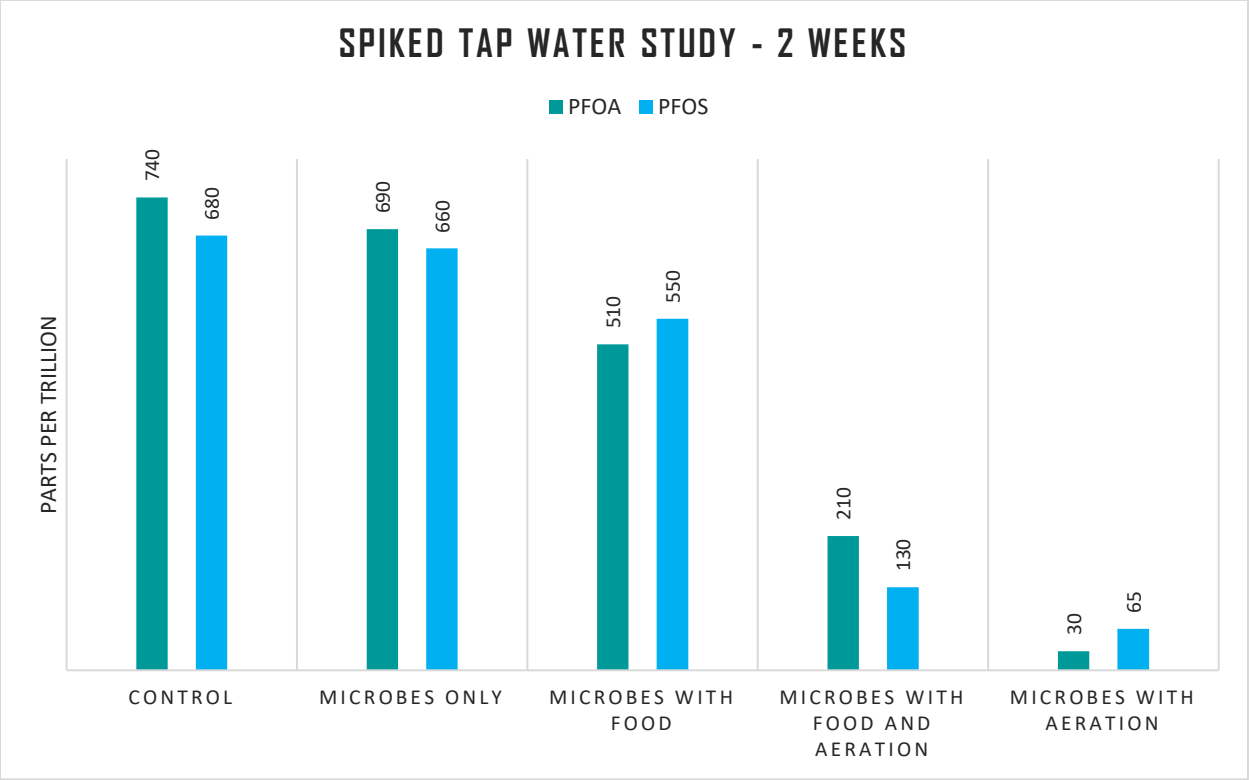
In this desktop experiment, sterilized tap water was spiked with 1000 parts per trillion (ppt) of both PFOA and PFOS. Analytical standards were purchased as the source of both compounds to ensure accuracy in the spiked concentration. Six treatment groups were utilized to give different combinations of microbes, supplemental carbon, and aeration as well as an untreated control. Additionally, groundwater samples were obtained from the site and had three treatments applied: Untreated control, microbes alone, and microbes with supplemental carbon and aeration.

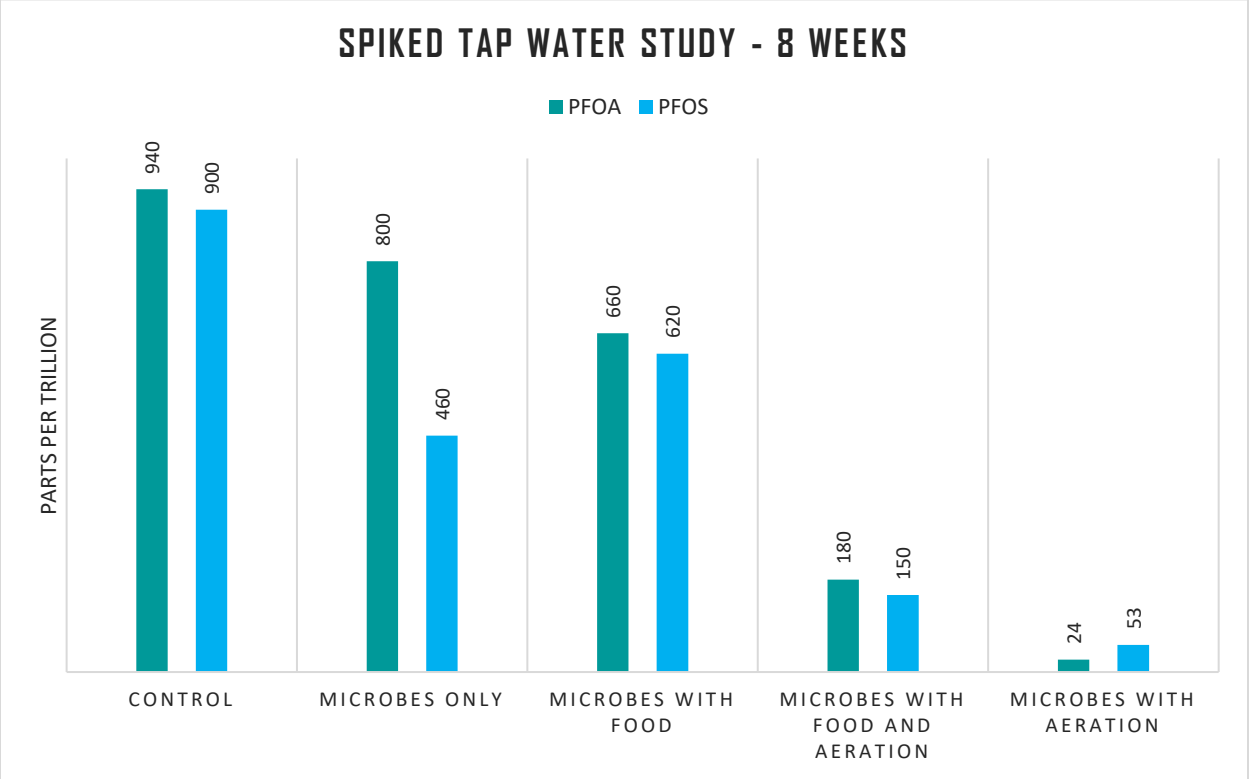
Each water sample was treated for two weeks prior to the first sampling event. Water volume was maintained by mass with sterilized distilled water being periodically added to maintain the appropriate volume. Water samples were analyzed via EPA Method 537 and were preserved by the analytical laboratory with formic acid upon receipt. The goal of preservation was to prevent further microbial activity while the samples were being stored prior to analysis.

Soil samples from the site were homogenized and treated in three groups: Untreated control, microbes alone, and microbes with supplemental carbon. Soil samples were not submitted for analysis at the two-week sampling event and will be analyzed at a later date. Analysis of the water samples collected during this study are currently undergoing analysis by advanced methods to further validate the observations.

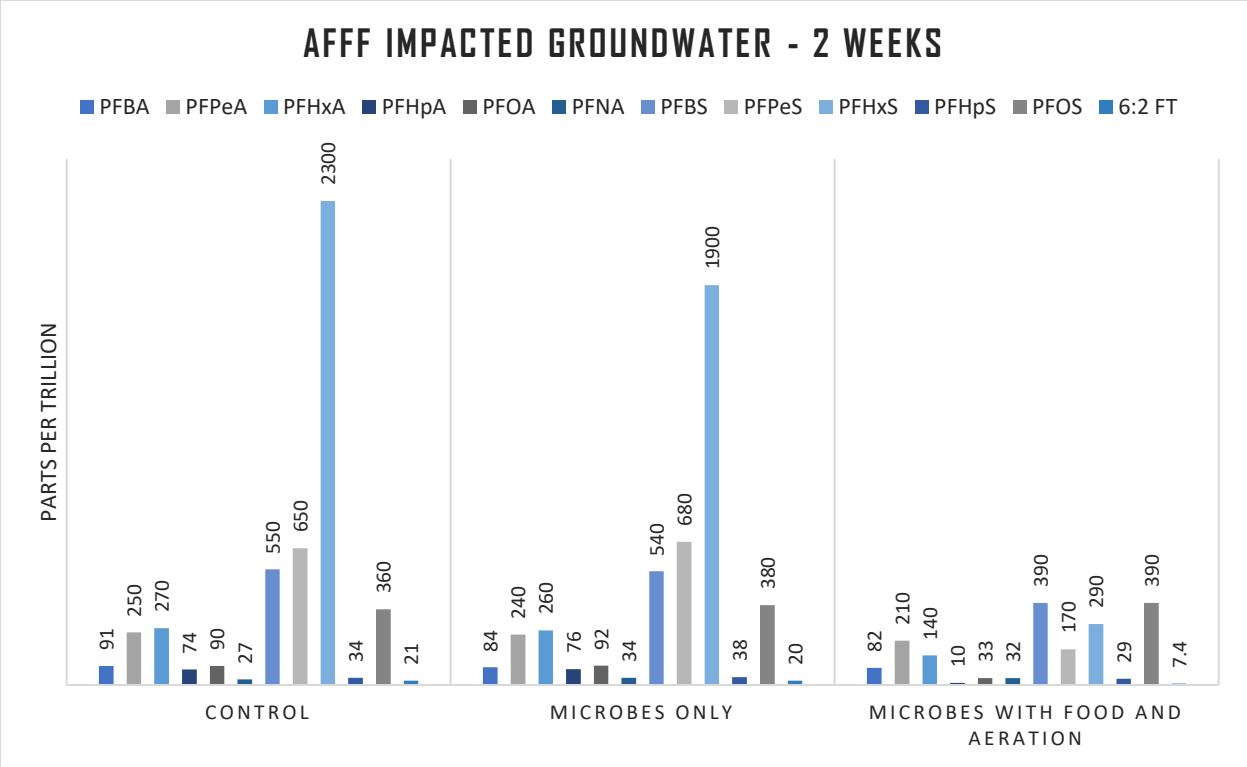
The following pages shows four figures: Two for the PFAS spiked tap water samples and two for the groundwater samples from site showing observations made at each sampling event. Each PFAS compound is shown as an individual bar on the graphs to show the response of each compound during the treatment.

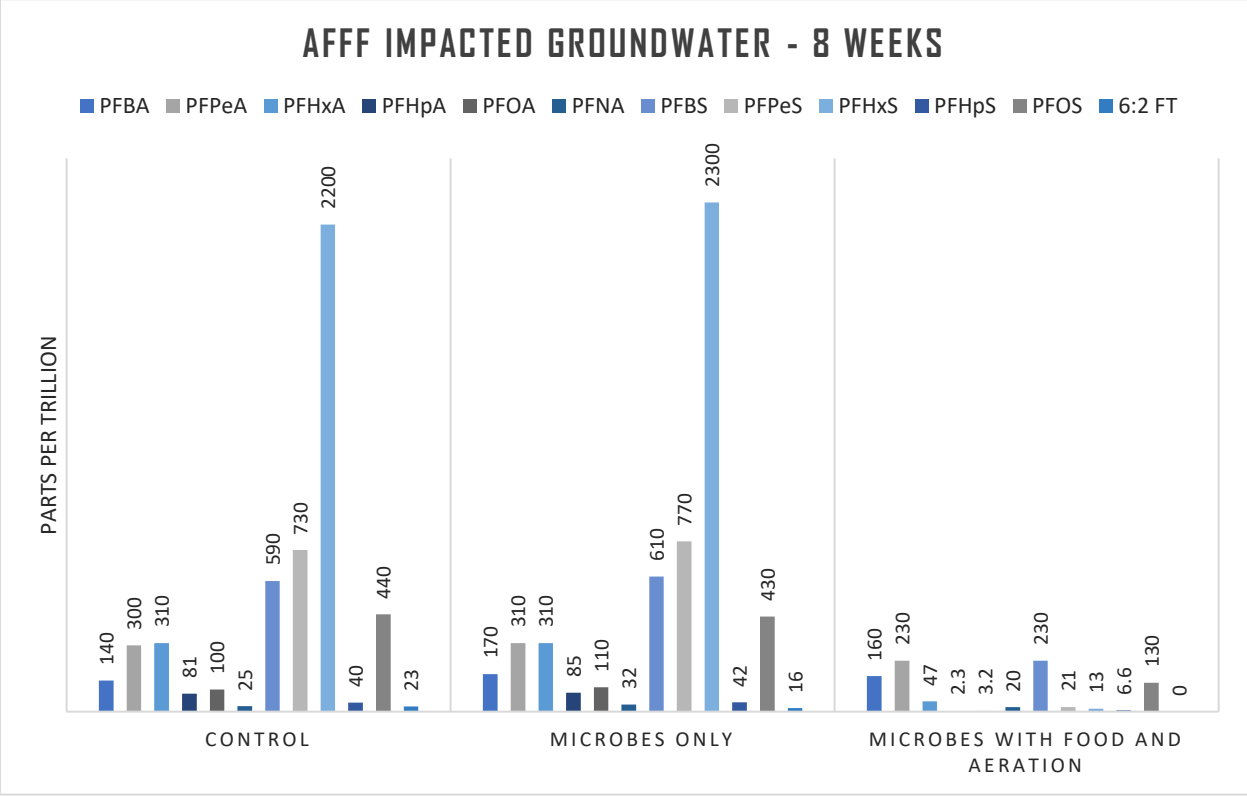
Spiked Tap Water Results





Aqueous Film Forming Foams Impacted Groundwater Results



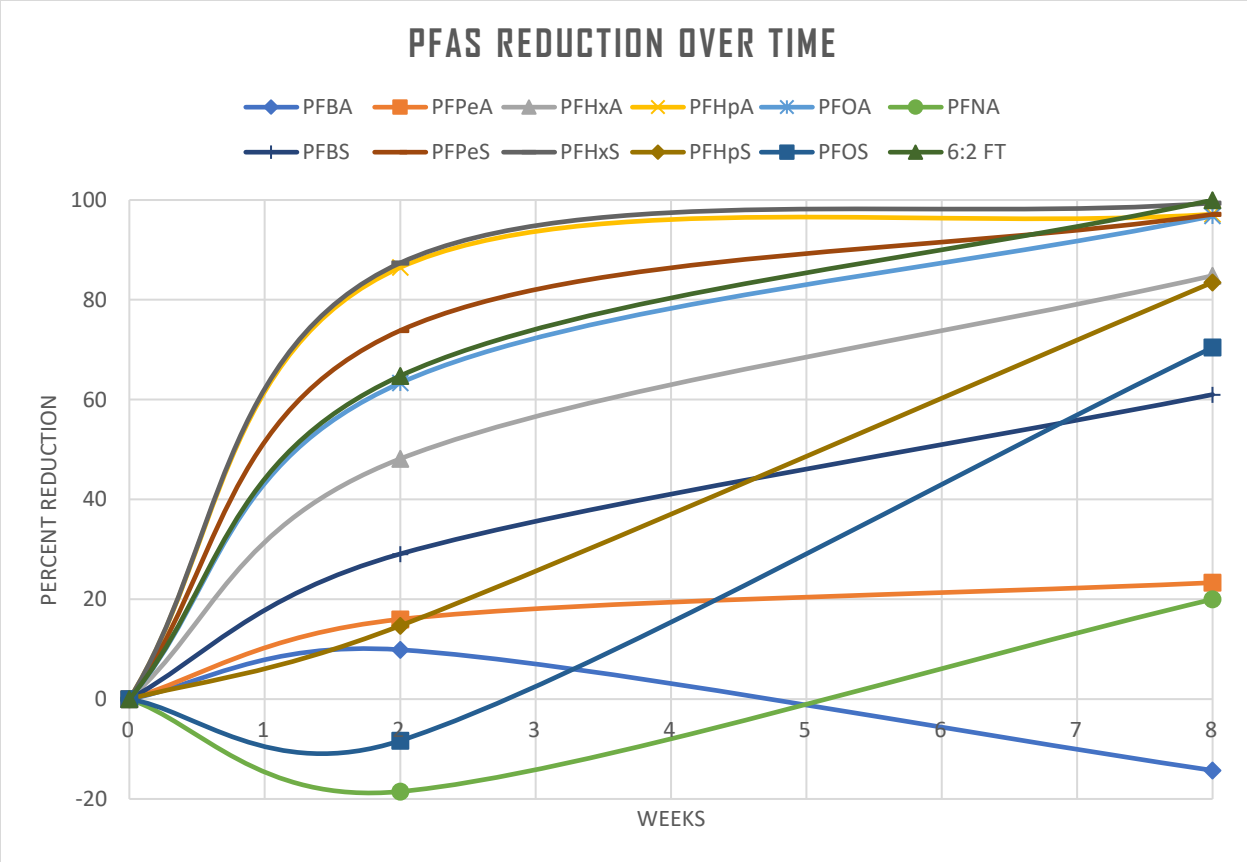


Discussion of EPA 537 Results

In the portion of the study with spiked tap water, PFOS and PFOA removals exceeding 90% occurred in the two-week treatment period when treated with microbes and aeration alone. Good performance in PFAS removal was also observed when supplemental carbon and aeration were provided. However, as PFAS removal was less complete, this may be a possible indication that the microbes preferentially consume non-fluorinated compounds. This will require further investigation to further refine future field deployment methods regarding the incorporation of supplemental carbon sources. Similar trends were observed at the eight-week sampling event. It is noted that PFAS removal rates declined, as is expected in microbial degradation of pollutants.

Significantly less PFAS removal occurred without aeration regardless of time, indicating that oxygen is required for microbial growth. It is unknown at this time if oxygen is required for PFAS catabolism directly or is simply required for other cellular processes.

In the treatment of impacted groundwater samples, significant removal of PFAS was again observed only when aeration was also supplied. The most significant change in concentration observed was with PFHxS, which decreased from 2300 ppt to 290 ppt during the two-week treatment and had further decreased to 13 ppt after eight weeks of treatment. Following eight weeks of treatment the majority of PFAS compounds had significantly decreased in the treated groundwater. It is noted that PFBA increased slightly following eight weeks of treatment. The graph below shows PFAS removal rates in treated AFFF impacted groundwater.



During the initial sampling event, a slight increase in PFOS and PFNA was observed, followed by a reduction after additional treatment. Other compounds ranging from five to eight carbons in size were found to rapidly decrease, with the majority of the degradation occurring within the first two weeks. It was noted that shorter chain compounds, specifically PFBA, PFPeA, and PFBS, were slower to degrade. At this time, it is unknown if these are formed during the breakdown of larger PFAS compounds or are more resistant to microbial action than longer-chain molecules. Additional research is warranted to clarify this observation.

Further studies of the samples shown above are ongoing so that we can better determine the mechanism or microbial action and the by-products formed during PFAS biodegradation.

Degradation of Branched and Linear PFOS

A slight increase in PFOS was observed, increasing from 360 ppt to 390 ppt. The current hypothesis is that branched longer chain PFAS compounds may form PFOS as they are degraded (i.e. fluorinated methane is removed from branched PFNS, resulting in PFOS).

This hypothesis has been supported through a re-evaluation of the laboratory data to determine the branched verses linear proportions of PFOS. It was noted that the ratio of branched to linear PFOS increased significantly over the course of the study, as shown in Table 2 below.

Table 2. Branched vs Linear PFOS

Parameter	Control	Microbes Alone	Microbes with Carbon and Aeration
PFOS (ppt)	360	380	390
Branched to Linear Ratio	4.01	3.52	7.03

The only plausible hypothesis which has been developed to date is that complex (branched) long-chain PFAS compounds are being degraded to form branched PFOS as they are degraded. This is supported both by the overall increase in PFOS concentrations as well as the shift in the branched to linear ratio. Following eight weeks of sample treatment, PFOS concentrations were found to decline, suggesting that branched PFOS is eventually degraded. Branched vs linear data for this time point is pending.

VOC Scan and Total Oxidizable Precursor Results

The mechanisms and by-products of microbial PFAS degradation are unknown. As part of the third desktop study, additional analysis was conducted to determine if volatile fluorinated VOCs or partially defluorinated compounds are formed.

Volatile Organic Compound Scan

To better understand the by-products formed by microbes during PFAS degradation, a scan for volatile organic compounds (VOCs) was conducted. It was previously hypothesized that microbes may chemically alter PFAS compounds by cleaving the carbon-carbon bonds, forming one and two carbon fluorinated compounds. As the toxicity and environmental fate of these compounds is still being researched, formation of these compounds would be considered undesirable. Figure 3 shows examples of hypothesized fluorinated VOCs

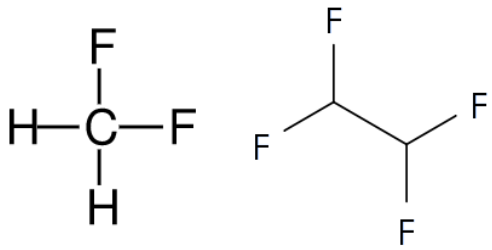


Figure 3. Theorized fluorinated VOC by-products.

VOCs were analyzed by both traditional means as well as an open scan for peaks not identified by the normal analysis package. All identified peaks were fingerprinted and compared to applicable libraries of volatile compounds. Samples analyzed included control (untreated) samples as well as samples where significant PFAS degradation was observed after two weeks of treatment. This included a sample containing very high (60 mg/L) concentrations of PFAS, as it was anticipated that degradation by-products would be easiest to detect in this sample. No fluorinated VOCs were detected in any of the analyzed samples. This suggests that fluorinated VOCs are not formed as a PFAS degradation product by the microbes or are formed in non-detectable quantities.

Total Oxidizable Precursor Assay

It was also hypothesized that partial defluorination of PFAS compounds may occur during microbial degradation. While this would mask the PFAS from the EPA 537 method, it represents a pool of PFAS precursor compounds that may form short-chain PFAS compounds under oxidizing conditions. For example, microbes may partially defluorinate PFOS to form the 6:2 Fluorotelomer Sulfonate (6:2 FtS). The structure of 6:2 FtS, as an example PFAS precursor, is shown in Figure 4.

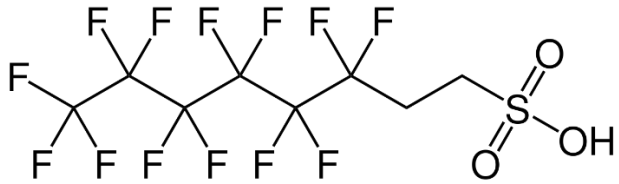


Figure 4. 6:2 Fluorotelomer Sulfonate (6:2 FtS) Chemical Structure.

The Total Oxidizable Precursor Assay (TOPA) attempts to account for PFAS precursor compounds by oxidizing them to perfluoroalkyl carboxylates, which would be detectable by the EPA 537 method. For example, 6:2 FtS would be oxidized to perfluoroheptanoic acid (PFHpA). As such, if the microbes were partially defluorinating long-chain PFAS, an increase in short-chain PFAS would be observed when analyzed via TOPA. However, TOPA is not capable of accounting for all fluorinated compounds in a sample and simply provides one possible way to observe potential by-products of microbial action.

Four samples were analyzed via TOPA and included two control samples (AFFF impacted groundwater and spiked tap water) and two samples treated via microbes where appreciable degradation of PFAS was observed in groundwater and spiked tap water. Table 3 shows the difference in PFAS concentrations before and after the oxidation step. Positive changes in concentration are indicative of precursor presence. Positive values have been highlighted for ease of interpretation.

Table 3. TOPA Difference Between Oxidized and Oxidized Samples

Parameter	Groundwater Control	Treated Groundwater	Tap Water Control	Treated Tap Water
PFBA (ug/L)	-0.0083	-0.05	0	0
PFPeA(ug/L)	0.0095	-0.014	0	0
PFHxA (ug/L)	-0.008	-0.0032	0	0
PFHpA (ug/L)	-0.00071	0	0	0
PFOA (ug/L)	0.0033	0	-0.072	-0.023
PFNA (ug/L)	-0.021	0	0	0
PFBS (ug/L)	0.02	-0.024	0	0
PFHxS (ug/L)	-0.047	0	0	0
PFHpS (ug/L)	-0.034	0	0	0
PFOS (ug/L)	-0.02	-0.046	-0.024	0.0012

In the control (untreated) AFFF impacted groundwater sample, an increase in PFPeA, PFOA, and PFBS was observed when compared to the unoxidized sample. This indicates that the groundwater likely contains precursor compounds. However, in the microbe-treated AFFF impacted groundwater sample, no increase in any PFAS compound was observed. This indicates that precursor compounds are not present in detectable quantities and suggests that the microbes are also capable of degrading some precursor compounds.

In the spiked tap water control sample, no increase in PFAS was observed, indicating that no detectable precursor compounds are present in the sample. In the microbe-treated tap water sample, a slight increase in PFOS was detected. This indicates that PFOS precursors may be formed during microbial degradation of longer-chain compounds. However, it is established that the microbes are capable of degrading of PFOS and it is expected that any PFOS formed from precursors will be further degraded over time.

Cleavage of the Carbon-Fluorine Bond

In spring 2020 Fixed Earth commenced a qualitative study using a unique colorimetric assay to determine if cleavage of the carbon-fluorine bond was occurring in any of the six microbes obtained during the third desktop study. This assay is unique in that it was originally developed to test for heavy metals in aqueous samples. However, the fluoride ion was found to strongly interfere with the assay and is one of few interferences known to the assay. As a result, the assay, when using stable concentrations of a heavy metal, can be used to assess the presence of fluoride ions.

In this assay, media is prepared as a solution of sugars, mineral nutrients (NPK), and PFAS (in this case, a mix of PFBS and PFNA was utilized). The media was solidified by adding 1.5% (w/w) molecular grade agarose as typical microbiology-grade agar was found to interfere with the assay. Microbes are inoculated onto the media and allowed a minimum of seven days to grow. The assay is completed when low melting point agar is poured over the petri plate containing a known concentration of heavy metals and an indicator dye under acidic conditions. Following the pour-over, the agar is allowed 10 minutes to solidify and develop colour.

The metal-dye complex in the absence of fluoride is pink to purple in colour, but changes to yellow in the presence of low concentrations of fluoride. Two microliters of sodium fluoride solution are placed onto the petri plate as a positive control for the assay.

This assay was performed with each of the six microbes obtained during the third desktop trial and the assay was performed on duplicate petri plates with differing positioning of microbes relative to one another. The results of this preliminary qualitative study indicate that the microbe "2RA" forms fluoride ions when grown in the presence of PFNA and PFBS as the sole source of fluorine atoms.

Figure 2 shows the positive assay test. The yellow halo surrounding the colonies of "2RA" (dark spheres) can be seen, as can the positive controls (yellow areas in the top/bottom center of the image), and a negative test (no yellow halo) can be seen at microbe "2RB" for comparison. An identical result was observed on the duplicate petri plate.

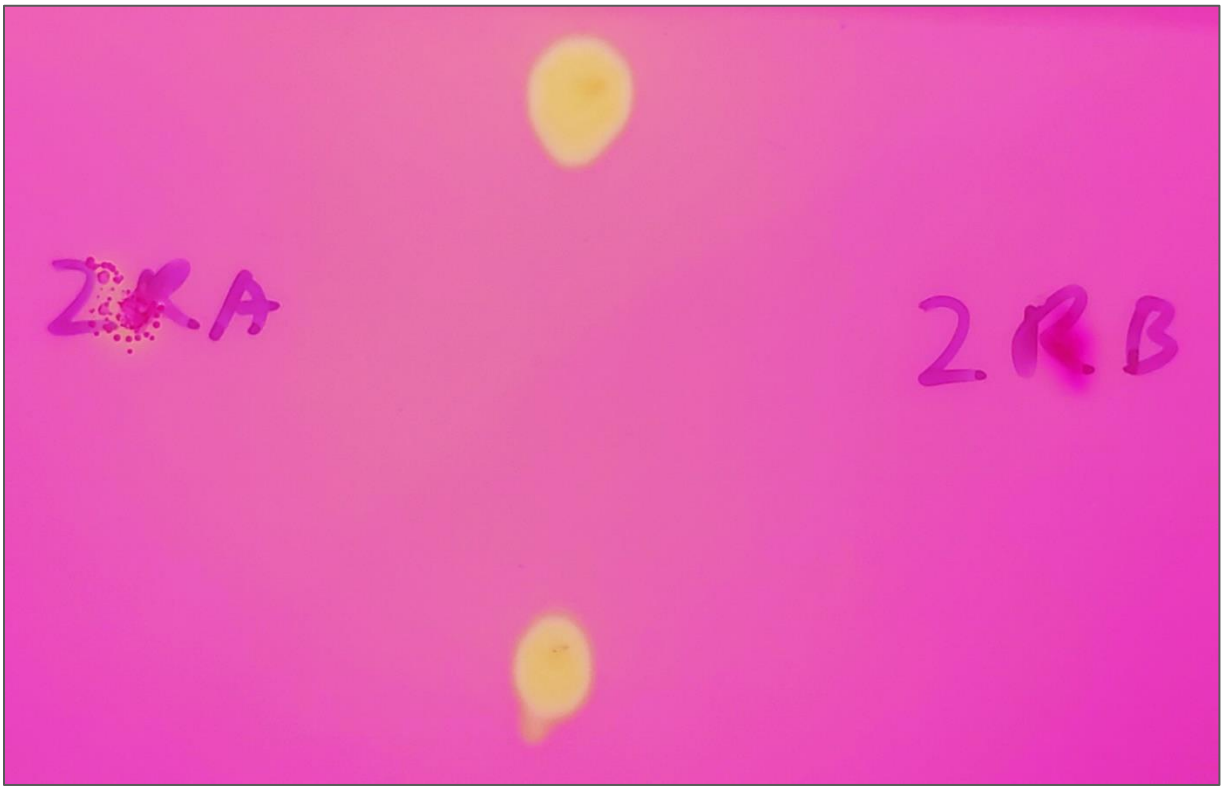


Figure 2. Fluoride Detection Assay Results

This experiment was replicated using a higher density of microbes, but otherwise utilizing similar assay conditions. Microbe 2RA was further confirmed to generate fluoride and microbe 2DB was also found to generate fluoride when grown at higher densities. Inconclusive results were observed for microbe 10A.

Water Chemistry Changes

During the third desktop trial, AFFF impacted groundwater was analyzed for dissolved metals and basic salinity parameters under each treatment group to determine how microbial degradation of PFAS may alter water chemistry at future sites. Additionally, groundwater was analyzed from the site serving as the source of impacted groundwater to determine what chemical changes, if any, occurred during shipment of the samples. Table 4 summarizes parameters where a significant change in parameter concentration was observed. Parameters in which no significant change occurred are not shown. However, this data is available upon request.

Table 4. Water Chemistry Changes

Decreasing Parameters					
Parameter	**Unit**	**GW Average**	**Control**	**Microbes Only**	**Microbes, Food, Aeration**
Aluminum	ug/L	51	8	5	<2
Barium	ug/L	75	55	37	20
Bicarbonate	mg/L	470	710	444	210
Calcium	mg/L	170	184	121	58.6
Iron	ug/L	7563	540	310	40
Manganese	ug/L	1143	1280	390	<5
Strontium	ug/L	486	552	447	371
Increasing Parameters					
Parameter	**Unit**	**GW Average**	**Control**	**Microbes Only**	**Microbes, Food, Aeration**
Nitrate	mg/L	<0.01	<0.01	<0.01	9.78
Sulfur	mg/L	-	16	35	36
Sulfate	mg/L	100	47.9	105	108
Tin	ug/L	-	<1	<1	388

Overall, no unexpected changes to water chemistry were observed. The primary chemistry changes observed between the control and treated samples appear to be primarily affiliated with aeration of the solution and not known to be linked to PFAS degradation. For example, it is expected that iron and manganese will precipitate under aerobic conditions, binding other metals such as strontium and barium into the precipitates.

An increase in sulfate was observed in samples containing microbes and is believed to be affiliated with the microbial growth media that was used to inoculate the samples. The increase in nitrate observed in the "Microbes, Food, and Aeration" treatment group is believed to be affiliated with the supplemental nutrients that were provided to the microbes as part of this treatment group.

Tin was observed to increase in the aerated sample. The source of the tin is unknown but is believed to be affiliated with the air stone used for aeration of the sample.

Current Research and Future Directions

Our work on PFAS biodegradation is ongoing and we seek to better understand the mechanism(s) employed by the microbes as well as assessing methods that can be used to enhance microbe performance. Below is a brief overview of PFAS related topics we are researching.

The Advantages and Disadvantages of Supplemental Carbon

Preliminary data from our desktop studies indicates that the addition of supplemental carbon sources during PFAS biodegradation may reduce the rate of PFAS consumption. Further studies are required to determine how to best deploy these microbes in the field to provide the maximum rate of PFAS consumption. Results from this study are expected in fall 2020.

Additives for the Enhancement of Microbial Performance

Although the precise mechanism of PFAS degradation remains unknown at this time, we have begun to research additives which may assist the microbes in defluorination. This work is based on known enzymatic pathways and the metabolism of other challenging pollutants. Further literature review is

required to determine the potential list of additives and a desktop study will be required to evaluate their efficacy. Results from the first desktop study are expected in late summer 2020.

PFAS Degradation in Soil

Our research to-date has focused on biodegradation of PFAS in water samples. However, PFAS contamination of soil can remain a significant concern at many sites. Our third desktop trial included a subset of data in which impacted soil was treated with our microbial technology to confirm or refute PFAS degradation in this environmental media. Results from this study are expected in summer 2020.

Enzyme Location

At this time, it is unknown if the enzyme(s) required for PFAS degradation occurs within the cell or if they are exuded into environmental media. In order to better understand the performance and field application of these microbes, additional studies will be required to determine the location of these proteins. However, we must first develop a suitable enzyme assay to study PFAS degradation. This study is not expected to commence until late 2020.

In addition to the items listed above, we are always working to streamline our microbial acquisition process and further validate microbial performance at varying concentrations of PFAS. This document will be updated periodically as additional studies in soil and water are completed.

Further Information

If you have any questions or inquiries regarding the technologies or data discussed in this paper, please feel free to contact the author directly via email: tim@fixed.earth

For further information about our company and our other ongoing research and development efforts, visit our website: www.fixed.earth

The processes and technologies outlined in this paper are not yet widely commercially available. However, we are happy to discuss potential applications on field sites, pilot scale projects, or the possibility of undertaking additional client-driven desktop studies. For more information please contact us.