Title
Feasibility of Biodegradation of Polyfluoroalkyl and Perfluoroalkyl Substances

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Feasibility of Biodegradation of Polyfluoroalkyl
and Perfluoroalkyl Substances

A thesis submitted in partial satisfaction of the
requirements for the degree Master of Science in
Civil Engineering

by

Nancy Shiao-lynn Tseng

2012
ABSTRACT OF THE THESIS

Feasibility of Biodegradation of Polyfluoroalkyl and Perfluoroalkyl Substances

by

Nancy Shiao-lynn Tseng

Master of Science in Civil Engineering

University of California, Los Angeles, 2012

Professor Shaily Mahendra, Chair

Polyfluoroalkyl and perfluoroalkyl substances (PFASs) are highly stable organic compounds, which contain multiple carbon-fluorine bonds. They are used in various commercial products, including aqueous fire-fighting foams (AFFF) and products with non-stick coatings. However, these compounds are reproductive and developmental toxicants, endocrine disrupters, and potential human carcinogens. They are found globally as emerging contaminants in groundwater and surface water resources. The two most persistent and widely detected PFASs are perfluorooctanoate (PFOA) and perfluorooctane sulfonic acid (PFOS). Other fluorinated compounds, such as fluorotelomer alcohols (FTOHs), can transform to PFOA and PFOS in the environment via biological and physico-chemical processes. Current methods to remove PFASs from waste streams and contaminated environments (e.g. activated carbon adsorption, sonolysis, photodegradation, and reverse osmosis) are expensive, impractical for in situ removal, use high
pressures and temperatures, or result in toxic waste. In contrast, biodegradation may lead to a cost-effective, *in-situ* remediation strategy for PFASs. Bioremediation has been successfully used for other recalcitrant contaminants, including chlorinated volatile organic compounds.

This thesis investigated the biodegradation potential of PFASs. The two groups of laboratory strains tested in this study were ligninolytic fungi (*Phanerochaete chrysosporium* and *Aspergillus niger*) and oxygenase-expressing bacteria (*Pseudonocardia dioxanivorans* CB1190, *Methylosinus trichosporium* OB3b, *Burkholderia cepacia* G4, and *Pseudomonas putida* F1). All strains are known to degrade xenobiotic compounds. In addition, 5 fungal strains and 2 strains of aerobic bacteria isolated from an aqueous fire-fighting foam (AFFF)-contaminated site were also evaluated for their PFAS biodegradation potential. Results indicate that *P. chrysosporium* was able to transform about 50% 6:2 FTOH and 70% 8:2 FTOH in 28 days. Major metabolites of 6:2 FTOH included 5:3 polyfluorinated acid (40%), 5:2 sFTOH (10%), PFHxA (4%), and others (about 1% each). Fewer metabolites were produced after 8:2 FTOH degradation, such as 7:2 sFTOH (6%), PFOA (5%), 7:2 Ft ketone (3%), and others (< 1% each). In contrast to *P. chrysosporium*, *A. niger* did not transform 6:2 FTOH during 35 days. Among the environmental fungal isolates, Envi 5 and Envi 7 transformed about 20% PFOS within 28 or 14 days, respectively, and only Envi 7 could partially transform about 20% PFOA within 14 days. There was a small increase in fluoride ions, but no metabolites were measured. None of the tested bacteria were able to transform PFOA within 7 days. This study demonstrated that fungi would be likely candidates for bioremediation of PFASs. Consequently, ongoing research is investigating the metabolites, enzymatic reaction kinetics, and conditions favorable for in-situ bioremediation.
The thesis of Nancy Shiao-lynn Tseng is approved.

Michael K. Stenstrom

Jenny A. Jay

Shaily Mahendra, Committee Chair

University of California, Los Angeles

2012
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<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Per- and polyfluoroalkyl substances</td>
<td>PFASs</td>
</tr>
<tr>
<td>Aqueous Fire-Fighting Foams</td>
<td>AFFFS</td>
</tr>
<tr>
<td>Perfluoroalkyl sulfonates</td>
<td>PFSAs</td>
</tr>
<tr>
<td>Perfluoroalkyl carboxylates</td>
<td>PFCAs</td>
</tr>
<tr>
<td>Electrochemical fluorination</td>
<td>ECF</td>
</tr>
<tr>
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<td>TFE</td>
</tr>
<tr>
<td>Fluorotelomer alcohol</td>
<td>FTOH</td>
</tr>
<tr>
<td>Saturated fluorotelomer carboxylic acids</td>
<td>FTCAs</td>
</tr>
<tr>
<td>Unsaturated fluorotelomer carboxylic acids</td>
<td>FTUCAs</td>
</tr>
<tr>
<td>Lignin peroxidase</td>
<td>LiP</td>
</tr>
<tr>
<td>Manganese peroxidase</td>
<td>MnP</td>
</tr>
<tr>
<td>1H,1H,2H,2H,8H,8H-perfluorododecanol</td>
<td>DTFA</td>
</tr>
<tr>
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<td>TCE</td>
</tr>
<tr>
<td>Dichloroethylene</td>
<td>DCE</td>
</tr>
<tr>
<td>Perfluorooctanesulfonic acid potassium salt</td>
<td>PFOS·K⁺</td>
</tr>
<tr>
<td>Perfluoro-n-[1,2,3,4-¹³C]octanoic acid</td>
<td>MPFOA</td>
</tr>
<tr>
<td>Sodium perfluoro-1-[1,2,3,4-¹³C]octane sulfonate</td>
<td>MPFOS</td>
</tr>
<tr>
<td>Perfluoroalkyl acids</td>
<td>PFAAs</td>
</tr>
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<td>PCR</td>
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<tr>
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</tr>
<tr>
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<td>YPD</td>
</tr>
<tr>
<td>Potato Dextrose</td>
<td>PD</td>
</tr>
<tr>
<td>Difco-Czapek Dox</td>
<td>DCD</td>
</tr>
<tr>
<td>Luria Broth</td>
<td>LB</td>
</tr>
<tr>
<td>Ammonium Mineral Salts</td>
<td>AMS</td>
</tr>
</tbody>
</table>
Gas Chromatography-Flame Ionization Detector  
Total Ionic Strength Adjustment Buffer  
Liquid Chromatography-Tandem Mass Spectrometer

Perfluorobutane sulfonic acid  
Perfluorobutyric acid  
Perfluoropentanoic acid  
Perfluorohexanoic acid  
Perfluoroheptanoic acid  
Perfluorooctanoic acid  
Perfluoronoanoic acid  
Perfluoroctane sulfonic acid  
7:2 Polyfluorinated ketone  
5:2 Polyfluorinated ketone  
8:2 Fluorotelomer unsaturated carboxylic acid  
6:2 Fluorotelomer unsaturated carboxylic acid  
8:2 Fluorotelomer saturated carboxylic acid  
6:2 Fluorotelomer saturated carboxylic acid  
7:3 Polyfluorinated acid  
5:3 Polyfluorinated acid  
4:3 Polyfluorinated acid  
3:3 Polyfluorinated acid  
6:2 Fluorotelomer alcohol  
8:2 Fluorotelomer alcohol  
7:3 Polyfluorinated unsaturated acid  
5:3 Polyfluorinated unsaturated acid  
7:2 Secondary polyfluorinated alcohol  
5:2 Secondary polyfluorinated alcohol

Perfluorobutane sulfonic acid  
Perfluorobutyric acid  
Perfluoropentanoic acid  
Perfluorohexanoic acid  
Perfluoroheptanoic acid  
Perfluoroctanoic acid  
Perfluoronoanoic acid  
Perfluoroctane sulfonic acid  
7:2 Polyfluorinated ketone  
5:2 Polyfluorinated ketone  
8:2 Fluorotelomer unsaturated carboxylic acid  
6:2 Fluorotelomer unsaturated carboxylic acid  
8:2 Fluorotelomer saturated carboxylic acid  
6:2 Fluorotelomer saturated carboxylic acid  
7:3 Polyfluorinated acid  
5:3 Polyfluorinated acid  
4:3 Polyfluorinated acid  
3:3 Polyfluorinated acid  
6:2 Fluorotelomer alcohol  
8:2 Fluorotelomer alcohol  
7:3 Polyfluorinated unsaturated acid  
5:3 Polyfluorinated unsaturated acid  
7:2 Secondary polyfluorinated alcohol  
5:2 Secondary polyfluorinated alcohol

F(CF\(_2\))\(_4\)SO\(_3\)H  
F(CF\(_2\))\(_3\)COOH  
F(CF\(_2\))\(_4\)COOH  
F(CF\(_2\))\(_5\)COOH  
F(CF\(_2\))\(_6\)COOH  
F(CF\(_2\))\(_7\)COOH  
F(CF\(_2\))\(_8\)COOH  
F(CF\(_2\))\(_4\)SO\(_3\)H  
F(CF\(_2\))\(_7\)C(O)CH\(_3\)  
F(CF\(_2\))\(_5\)C(O)CH\(_3\)  
F(CF\(_2\))\(_7\)CF=CHCOOH  
F(CF\(_2\))\(_5\)CF=CHCOO  
F(CF\(_2\))\(_8\)CH\(_2\)COOH  
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F(CF\(_2\))\(_3\)CH\(_2\)CH\(_2\)COOH  
F(CF\(_2\))\(_6\)CH\(_2\)CH\(_2\)OH  
F(CF\(_2\))\(_8\)CH\(_2\)CH\(_2\)OH  
F(CF\(_2\))\(_7\)CH=CHCOOH  
F(CF\(_2\))\(_5\)CH=CHCOOH  
F(CF\(_2\))\(_7\)CH(OH)CH\(_3\)  
F(CF\(_2\))\(_5\)CH(OH)CH\(_3\)

PFBS  
PFBA  
PFPeA  
PFHxA  
PFHpA  
PFOA  
PFNA  
PFOS  
7:2 Ketone  
5:2 Ketone  
8:2 FTUA  
6:2 FTUA  
8:2 FTA  
6:2 FTA  
7:3 acid  
5:3 Acid  
4:3 Acid  
3:3 Acid  
6:2 FTOH  
8:2 FTOH  
7:3 Uacid  
5:3 Uacid  
7:2 sFTOH  
5:2 sFTOH

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First and foremost, I would like to thank my advisor, Professor Shaily Mahendra, and my committee members, Professor Michael Stenstrom and Professor Jennifer Jay. Professor Mahendra introduced me to environmental microbiology and biodegradation. It is incredibly exciting to explore how bacteria and fungi can help clean up hazardous pollutants from the environment. Professors at UCLA in Civil and Environmental Engineering, Chemical and Biomolecular Engineering, and the School of Public Health helped me build a strong foundation for my Masters thesis research by offering advice, knowledge, and laboratory resources. Professor Yi Tang’s laboratory (UCLA) generously donated Aspergillus niger.

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Introduction

Toxic compounds, including natural and man-made contaminants, have polluted the world for decades. However, society was not aware of the global extent of pollution until after World War II when a series of pollution-related disasters and scientific studies occurred. Since then, a plethora of regulation protecting the environment and human health have passed along with the formation of the United States Environmental Protection Agency (USEPA). Although there have been significant improvements, pollutants continue to be produced and released into the environment, including 1,4-dioxane, polychlorinated biphenyls (PCBs), volatile organic compounds (VOCs), munitions waste, and synthetic dyes. One way to remove these compounds from the environment is through bioremediation, which utilizes microorganisms naturally found in the environment to degrade contaminants to less toxic or non-toxic substances. The objective of this research is to determine whether biodegradation of perfluoroalkyl and polyfluoroalkyl substances (PFASs) can occur.

Introduction to Perfluoroalkyl and Polyfluoroalkyl (PFASs) Substances

PFASs are highly stable compounds that have been mass-produced since the late 1940s due to their chemically and thermally inert structure. These compounds consist of a hydrophilic head group and a hydrophobic, aliphatic chain with varying number of carbon-fluorine (C-F) bonds.
bonds (Figure 1). Buck et al. [1] published a comprehensive classification of PFASs. Fluorine has unique properties that lend stability to the structure, such as high oxidation potential, high ionization energy, high electron affinity, and high electronegativity [2]. The C-F bond also has the strongest dissociation energy at 450 kJ mol\(^{-1}\). In comparison, carbon-chlorine and carbon-bromine bonds have a dissociation energy of 330 kJ mol\(^{-1}\) and 194 kJ mol\(^{-1}\), respectively [3, 4]. The number of C-F bonds is usually between 4-16 carbons where longer chain PFASs are more persistent and toxic in the environment. Perfluoroalkyl compounds (only C-F bonds) also tend to be more persistent and toxic in the environment when compared to polyfluoroalkyl compounds (some C-H bonds). Polyfluoroalkyl compounds tend to be precursors of perfluoroalkyl compounds and can be abiotically or biotically transformed in the environment to perfluoroalkyl compounds.

*Production and Transport of PFASs*

PFASs are used in a variety of products and industrial applications for its ability to resist soil, stain, grease, and water. These include non-stick coatings, aqueous fire-fighting foams (AFFFs), insulation, electronics, and textiles [5]. A recent estimate of the total production of perfluoroalkyl sulfonates (PFSAs) from 1970 to 2002 amounts to about 4500 tonnes [6]. This estimate has decreased to about 1000 tonnes due to the termination of perfluorooctane sulfonic acid (PFOS) production by the major U.S. producer, 3M. However, PFOS is still manufactured in other countries, such as China. An estimate for the total production of perfluoroalkyl carboxylates (PFCAs) from 1951 to 2004 amounts to about 4400 to 8000 tonnes [7]. In the past
decade, shorter-chain PFASs and polyfluoroalkyl compounds have begun to replace longer-chain PFASs. As a result, the global production of fluorotelomers was 9000 tonnes in 2006 with the US accounting for more than 50% of the production [8]. Fluorotelomers are mainly used for textiles, such as carpet products, apparel, paints and coatings on consumer products [8].

PFASs are produced via 2 main manufacturing processes: electrochemical fluorination (ECF) and telomerization. ECF uses the Simon method [9, 10] to perfluorinate organic compounds in one step by exposing the organic compound to electrolysis with anhydrous hydrogen fluoride in solution at cell voltages of 5-6 V [11]. This method results in a mixture of linear and branched perfluorinated isomers and homologues [12]. For PFOA and PFOS production, about 70-80% of isomers produced are linear while 20-30% are branched [13-16]. The second manufacturing process is telomerization, where a telogen, such as perfluoroalkyl iodide ($C_{m}F_{2m+1}I$, PFAI), is reacted with a taxogen, such as tetrafluoroethylene ($CF_2=CF_2$, TFE). This produces longer perfluorinated PFAI chains (Telomer A) that is then reacted in a second step to insert ethylene in the compound (Telomer B). Telomer A and B are then used to create fluorotelomer-based surfactant and polymer products, such as fluorotelomer alcohol (FTOH) [1]. Linear products are produced when a linear telogen and taxogen are used while branched and/or odd C number products are produced when branched and/or odd C number telogens are used.

Since PFASs are used often, it has been found globally, even in locations that do not manufacture or use PFASs. PFASs have been found in China [17:20], the United States [21], Europe [22-28], Japan [29-32], Australia [33], Singapore [34], Canada [35-37], the deep sea [38], and the Arctic [39]. Several studies have even confirmed PFASs in Arctic polar bears (>4000 ug kg$^{-1}$), indicating bioaccumulation through the food chain [40, 41]. In addition, Canada has no
known sources of PFASs production [42]. The major sources of PFASs are from food, drinking water, house dust, and indoor air [43, 44]. Studies have found PFASs in wastewater treatment plant effluent [45-49] and food items like apples [50], milk [51], butter [51], olive oil [51], microwave popcorn [51], beef [52], and fish [52]. PFASs are also found in indoor air (~450 ng m\(^{-3}\)), house dust (~10 - 40 µg g\(^{-1}\)), ambient air (~800 pg m\(^{-3}\)) [53-60], lakes and rivers (0.3 - 2600 ng L\(^{-1}\)) [61, 62], and drinking water (0.1 - 70 ng L\(^{-1}\)) [63-67].

PFASs can be found globally due to abiotic and biotic processes. Abiotic processes that affect the concentration of PFASs include (1) atmospheric transport and oxidation of volatile precursors, such as fluorotelomer alcohols [68, 69], (2) marine aerosols [40], and (3) direct transport through atmospheric transport [69] or ocean currents [31]. Volatile precursors can be oxidated via hydroxyl radicals to form perfluoroalkyl substances [68] and can be biotically degraded via metabolic pathways in animals and microbes [70]. These precursors include fluorotelomer alcohols [68, 70-73], fluorotelomer acrylates [74], fluorotelomer iodides [75], fluorotelomer olefins [76], N-alkyl perfluoroalkane sulfonamidoethanols [77, 78], N-ethyl perfluoroctane sulfonamidoethanol [79], and perfluoroalkyl phosphates [77, 80].

Health Effects of PFASs

Since PFASs are persistent in the environment and found globally, several studies have found that PFASs are reproductive and developmental toxins, endocrine disrupters, likely carcinogens, and bioaccumulative. The half life of PFOA is about 3 years and for PFOS, about 5.4 years in humans [81]. PFASs have been found to be carcinogenic only in animal studies [82],
but there may still be significant health implications due to bioaccumulation [83-87]. Bioaccumulation of PFASs is positively correlated with the carbon chain length where longer chain ($C \geq 8$) PFASs are especially bioaccumulative [86, 87]. PFASs tend to bind to proteins and can accumulate in blood serum, the kidney, and the liver [88, 89]. When PFASs bind to proteins, it can interfere with organ functions and prevent natural compounds that are important for signaling duties from binding.

Currently, the two most persistent and toxic PFASs are perfluorooctane sulfonic acid (PFOS, $C_{8}F_{17}SO_{3}H$) and perfluorooctanoic acid (PFOA, $C_{7}F_{15}COOH$). PFOS and PFOA are highly persistent in the environment and are terminal products of the abiotic and biotic degradation of polyfluoroalkyl compounds. PFOS may affect cardiac development [90, 91], thyroid development [91], and cause developmental malformations and reduced body length [83, 91, 93]. Perfluorooctanoic acid (PFOA) exhibits similar affects as PFOS in terms of placental development and fetal growth [83, 93, 94]. However, several studies have found that PFOA does not bioaccumulate in biota [95-100].

The toxicity of PFASs may differ between species and gender. There have been more toxicity studies on animals than humans. One study found that rats and mice more effectively eliminated PFOS than monkeys [99]. Several studies found that adult men have a higher concentration of PFOS than adult women [100-102] since women may excrete PFOS during menstrual bleeding, pregnancy, and lactation [102-105]. However, this indicates that there is fetal uptake of PFASs [98, 105]. PFOS, PFOA, and other perfluoroalkyl substances have been found in cord serum [103, 104, 106-109], but there is no indication of negative correlation towards gestation age [106], newborn length [106], or birthweight [109]. There may be a positive
correlation between the concentration of PFOS in the fetus and increasing age of the mother, and male fetuses may have higher PFOS concentrations than female fetuses [105]. Once the fetus is born, breast milk is the likely contributing source of most PFASs to breast-fed infants where two times more PFOA was transferred to the infant compared to PFOS [98].

Regulation of PFASs

The toxicity, persistence, and widespread occurrence of PFASs has caused several regulatory bodies to publish health-based guidelines. The US EPA provisional health-based guideline for drinking water is 0.4 µg L⁻¹ for PFOA, 0.3 - 7 µg L⁻¹ for C4-C7 PFASs, and 0.2 µg L⁻¹ for PFOS [8]. Other places have also developed provisional tolerable daily intakes (pTDI) and drinking water guidelines, including Germany [110], the European Food Safety Authority [111], Minnesota [112], New Jersey [113], and the United Kingdom [114]. Eight major global companies have also committed to eliminate PFOA production by 2015 under the 2010/15 PFOA Stewardship Program [8], and PFOS production has already been eliminated in the United States since 2003. PFOS was added to Annex B of the Stockholm Convention list of persistent organic pollutants in 2009 [115] and has been classified as persistent, bioaccumulative, and toxic [116]. Placement on Annex B permits the production and use of PFOS only for specific purposes. Several reports indicate PFOS concentrations have decreased globally in the environment and humans [117, 118], but there is an increase in the shorter chain PFASs, such as perfluorobutanoic acid (PFBA) and perfluorobutane sulfonic acid (PFBS) [40, 117-119]. Industries are producing
shorter chain PFASs to replace longer chain ones since these are more degradable and less bioaccumulative and persistent.

*Removal of PFASs*

Removal of PFASs from the environment is either difficult or expensive using current physico-chemical treatment methods. Most treatment methods use extreme conditions (e.g. high temperatures and pressure) that increase the cost of treatment, including photolytic degradation, sonolysis, microwave thermal treatment, and electrochemical oxidation [120-127]. Other treatment methods utilize activated carbon, ion exchange, or other adsorption materials to adsorb PFASs, but this does not transform PFASs to less toxic compounds and leaves waste contaminated with PFASs [128-132]. In addition, these physico-chemical treatment methods cannot remove PFASs *in situ* in a cost-efficient manner.

Compared to current physico-chemical treatment methods, bioremediation may be a more cost-effective approach to remove PFASs from the environment. Bioremediation uses naturally-occurring microorganisms to degrade contaminants to less toxic or nontoxic forms. For example, *Pseudomonas putida* F1 can degrade toluene – a compound found in gasoline and other petroleum fuels – and use it as a sole source of carbon and energy for growth [133]. *P. putida* F1 produces a dioxygenase enzyme that can degrade toluene and other similar structures. Bioremediation has been used for a wide variety of pollutants since many microorganisms have metabolic functions and produce enzymes that degrade naturally-occurring compounds. In addition, these microbes may acclimate to pollutants and begin using them as a carbon and
energy source. Bioremediation is a cost-effective clean-up method that is already used at industrial sites, wastewater treatment plants, and other contaminated sites to clean up hazardous waste and prevent the release of toxic compounds.

Microorganisms have been found to degrade some PFASs, especially the polyfluorinated compounds. Aerobic bacteria can transform 6:2 fluorotelomer sulfonate [134], 6:2 fluorotelomer alcohol [135], N-ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE) [136], and 8:2 FTOH [137]. In another study, *Pseudomonas* strain D2 was found to partially degrade sulfonates with hydrogen, such as H-PFOS and 2,2,2-trifluoroethene, by defluorinating them in anaerobic, sulfur-limiting conditions [138]. Myers and Mabury [139] tested soil-water microcosms for the degradation of saturated (FTCA) and unsaturated (FTUCAs) fluorotelomer carboxylic acids. After 50 days, 8:2 FTCA was transformed to about 30% PFOA. However, previous studies have shown that PFOS and PFOA cannot be degraded under the conditions tested. 3M, the major PFASs producer, conducted studies using activated sludge and 4 pure cultures but did not observe PFOS and PFOA degradation in short-term experiments [140]. There have been a limited number of studies tested to determine whether PFOS and PFOA can be degraded via microorganisms. More research needs to be done to identify optimum conditions and capable microorganisms.

<table>
<thead>
<tr>
<th>The objective of this research was to determine whether biodegradation of PFASs can occur by:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Microorganisms known to degrade halogenated compounds.</td>
</tr>
<tr>
<td>2. Microorganisms isolated from a site contaminated with PFASs.</td>
</tr>
</tbody>
</table>

8
This thesis will discuss four experiments: (1) fungal degradation of 6:2 FTOH, (2) degradation of 6:2 FTOH, 8:2 FTOH, and PFOA by \textit{Phanerochaete chrysosporium}, (3) degradation of PFOA and PFOS by environmental fungal isolates, and (4) bacterial degradation of PFOA.

\textbf{Introduction to Fungal Degradation}

Fungi have not been tested for their ability to degrade PFASs, but they are known to degrade a wide variety of toxic compounds, especially those belonging to the wood-rotting fungal groups. These compounds include organochlorines such as DDT and DDE \cite{141, 142}, organophosphate pesticides such as chlorpyrifos, fonofos, and terbufos \cite{142, 143}, polychlorinated biphenyls \cite{144-148} polycyclic aromatic hydrocarbons \cite{149-150}, munition wastes \cite{151, 153-156}, nitroglycerin \cite{157}, RDX \cite{158}, endocrine disrupters \cite{152, 159} \cite{5,35}, and pharmaceuticals \cite{160-170}. However, fungi are still an unexplored area for PFASs biodegradation and all except one study \cite{171} have focused on bacterial biodegradation.

Fungal degradation differs from bacterial degradation. Remediation has mainly focused on bacterial degradation due to the low cost, resilience, and fast growth of bacteria. In addition, bacteria may use the organic pollutant as an energy and carbon source while fungi usually need an energy and carbon source for degradation to occur (a.k.a. co-metabolism). The source of energy and carbon may already be found in the environment or can be provided with inexpensive lignocellulosic sources, such as rice straw, wheat straw, or wood chips. Fungi degrade toxic compounds by producing extracellular enzymes, allowing them to degrade a wide diversity of toxic compounds over a large concentration range. In addition, filamentous fungi can gain access
to contaminants over a large area due to hyphal extension.

Wood-rotting fungi can be further divided into white-, soft-, and brown-rot fungi. White- and soft-rot fungi can degrade lignin, cellulose, and hemicellulose while brown-rot fungi can only degrade cellulose and hemicellulose. Lignin, cellulose, and hemicelluloses are all plant polymers that provide higher plants stiffness and protection [172]. Cellulose is the principal chemical constituent in plants and forms highly ordered crystalline structures made up of glucose polymers. Hemicellulose are a group of carbohydrates that contribute to the bonding between cellulose and lignin. Out of all three plant polymers, lignin is the most resistant to microbial degradation and consists of highly branched, phenylpropanoid polymers with many C-O-C and C-C bonds (Figure 2).

Wood-rotting fungi are likely candidates for PFASs degradation. All identified brown-rot and most white-rot fungi belong to the phylum Basidiomycota while most soft-rot fungi belong to the phylum Ascomycota. White-rot fungi are capable of mineralizing lignin to CO$_2$ and H$_2$O [173]. Soft-rot fungi can only oxidize and mineralize syringyl lignin [174]. Brown-rot fungi can only degrade hemicellulose and cellulose. These fungi use oxidative mechanisms to degrade

---

**Figure 2. Structure of Lignin** [203]. Lignin is a complex structure made up of highly branched, phenylpropanoid polymers with many C-O-C and C-C bonds.
these polymers via extracellular and metal-containing oxidoreductases, such as lignin peroxidase (LiP), manganese peroxidase (MnP), laccase, cellulases, and hemicellulases.

LiP and MnP play an important role in lignin degradation. These are heme-containing proteins that are activated by $\text{H}_2\text{O}_2$ and can catalyze the oxidation of lignin through an unstable intermediate (LiP) or $\text{Mn}^{3+}$ (MnP) [175]. LiP and MnP have redox potentials of about 1.2 V (pH 3) [176] and 0.8 V (pH 4.5) [176], respectively. The native or resting states of MnP and LiP
have a Fe$^{3+}$ atom. Under catalysis, Fe$^{3+}$ is oxidized by H$_2$O$_2$ to form Fe$^{4+}$ (Compound I) (Figure 3). This is followed by two 1-electron transfers to form Compound II and subsequent conversion back to the native compound. For MnP, the preferred electron donor is Mn$^{2+}$, and MnP catalysis forms Mn$^{3+}$, which can then diffuse through the medium and penetrate small pores. For LiP, the preferred electron donor varies. Both LiP and MnP are more electron deficient compared to other fungal peroxidases due to their heme environment [177] and are highly likely to degrade a wide range of organic compounds.

Laccase are a family of copper-containing proteins that catalyze demethylation of lignin components and oxidation of substrate molecules. The preferred co-substrate is O$_2$, but laccase usually needs to interact with a mediator compound to react with a wider range of substrates [175]. Laccase contains 4 copper atoms that are all involved in laccase catalysis. Catalysis begins with the reduction of 1 copper atom by a reducing substrate, followed by an electron transfer to the other copper atoms and reduction of O$_2$ to H$_2$O (Figure 4). The redox potential is about 0.8 V when undergoing one electron oxidation of phenolic compounds [178].

Two well known fungi that can degrade toxic contaminants are the white-rot fungi *Phanerochaete chrysosporium* and the brown-rot fungi *Aspergillus niger* (Table 1). P.
**chrysosporium** is the most studied white-rot fungi [178], known to degrade a wide variety of contaminants, such as butyl benzyl phthalate [179], HMX [180], Congo Red [181], azo and heterocyclic dyes [182], polymeric dyes [183], olive mill wastes [184], chlorinated compounds [185-188], phenanthrene [189], TNT [190], polycyclic hydrocarbons [191, 192], and more. It can also mineralize lignin to CO$_2$ and H$_2$O via production of LiP, MnP, and other enzymes. *A. niger* is known to degrade triphenylmethane dye [193], chlorimuron-ethyl [194], tannins [195], crude oil [196], chlorinated compounds [197], hexadecane [198], Congo Red [199], olive mill wastes [200], indole [201], and more.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Classification</th>
<th>Enzyme</th>
<th>*Contaminants Known to Degrade</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phanerochaete</em></td>
<td>White-rot</td>
<td>LiP and MnP</td>
<td>Butyl benzyl phthalate, HMX, dyes, olive mill wastes, chlorinated compounds, phenanthrene, TNT, polycyclic hydrocarbons</td>
</tr>
<tr>
<td><em>chrysosporium</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>Brown-rot</td>
<td>Dioxygenase, xylanases, cellulases, hemicellulases</td>
<td>Dyes, chlorimuron-ethyl, tannins, crude oil, chlorinated compounds, hexadecane, olive mill wastes, indole</td>
</tr>
</tbody>
</table>

* = not a complete list

**Introduction to Bacterial Degradation**

Many aerobic bacteria that degrade other recalcitrant and halogenated pollutants are likely candidates to transform PFASs. There are many studies that report aerobic degradation of a variety of fluorinated organic compounds, including PFOA and PFOS precursors (e.g. 6:2 FTOH and 8:2 FTOH) [70, 72, 73, 134, 135]. Possible mechanisms of aerobic biodegradation of fluorinated organic compounds include catalysis by cytochrome P450 monooxygenase,
transaminase metabolism, and beta-oxidation [70, 72, 73, 134, 135]. A bacterial inoculum from activated sludge was able to degrade 1H,1H,2H,2H,8H,8H-perfluorododecanol (DTFA) without PFOA or PFOS as end products [204]. In addition, a bacterium isolated from a fluoroacetate-producing plant could completely defluorinate fluoroacetate and convert it to CO₂ at a rate of 25.53 mg/10⁹ cells per hour [205].

Aerobic bacteria that produce oxygenase enzymes may degrade PFASs. Oxygenase enzymes have broad substrate specificity and have been shown to degrade methane [206], toluene [207, 208], and chlorinated compounds [209]. Oxygenase enzymes were discovered in 1955 [210, 211] and are split into mono- and dioxygenase groups. Both enzymes use oxygen as an oxidant, but the monooxygenase enzyme will incorporate one oxygen atom into the product while the dioxygenase enzyme will incorporate both oxygen atoms into the product [212]. Examples of possible aerobic bacteria known to degrade toxic contaminants are discussed below and summarized in Table 2.

*Pseudonocardia dioxanivorans* strain CB1190 (NCBI taxonomy 675635) is a Gram-positive actinomycete isolated from sludge contaminated with 1,4-dioxane. This bacteria can use 1,4-dioxane as its sole carbon and energy source and can degrade 1,4-dioxane to CO₂ [233-235]. It can also grow on other ethers, alcohols, and benzene [236]. The genome of strain CB1190 was recently sequenced [237], and it was determined that it contains eight putative gene clusters encoding bacterial multicomponent monooxygenases. This indicates that strain CB1190 may degrade a wide variety of organic compounds, including PFASs, by using them as carbon and energy sources.
Methylosinus trichosporium strain OB3b is an obligate methanotroph capable of using C\textsubscript{1} compounds as growth substrates, such as methane, methanol, methylated amines, formate, formaldehyde, and carbon monoxide. Strain OB3b was isolated on Whittenbury media with 100 other methanotrophs [222] and can also transform chlorinated compounds [208, 223-227], naphthalene [228], phenanthrene [228], vanillyl alcohols [229], veratryl alcohols [229], and cyclopropane [226] via sMMO, pMMO, and methanol dehydrogenase [229]. However, this bacteria cannot grow on carbon-carbon bonds and may only be capable of co-metabolizing PFASs.

Methane monooxygenase enzyme has been shown to transform many other pollutants. It is present in two forms: soluble (sMMO) and particulate methane monooxygenase (pMMO). sMMO can degrade a wider variety of compounds compared to pMMO [231] and can oxidize alkanes, alkenes, ethers, halogenated methanes, and aromatic compounds [226, 232]. sMMO is also a NADH-dependent monooxygenase and incorporates oxygen into methane, producing methanol and eventually, CO\textsubscript{2}.

Burkholderia cepacia strain G4 and Pseudomonas putida strain F1 are Gram-negative nonmotile, chemoorganotrophic, rod-shaped bacteria. P. putida F1 was isolated from soil using ethylbenzene as the carbon source [213] while B. cepacia G4 was isolated from water samples contaminated with organochlorine compounds in Pensacola, Florida [214]. These bacteria have been shown to co-metabolize trichloroethylene (TCE) and three isomers of dichloroethylene [208, 215] with toluene, benzene, o-cresol, or other aromatic compounds as carbon and energy sources [133, 208, 215-218]. B. cepacia G4 utilizes toluene monooxygenase to degrade TCE [219, 220] while P. putida F1 utilizes toluene dioxygenase to degrade TCE [208, 215, 221].
### Table 2. Selected Aerobic Bacteria Known to Degrade Toxic Contaminants.

<table>
<thead>
<tr>
<th>Aerobic Bacteria</th>
<th>Enzyme</th>
<th>*Contaminants Known to Degrade</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudonocardia dioxanivorans</em> CB1190</td>
<td>Monooxygenase</td>
<td>1,4-dioxane, tetrohydrofuran, benzene, toluene</td>
</tr>
<tr>
<td><em>Methylosinus trichosporium</em> OB3b</td>
<td>Methane monooxygenase</td>
<td>Chlorinated compounds, naphthalene, phenanthrene, vanillyl alcohols, veratryl alcohols, cyclopropane</td>
</tr>
<tr>
<td><em>Burkholderia cepacia</em> G4</td>
<td>Toluene monooxygenase</td>
<td>Trichloroethylene (TCE), dichloroethylene (DCE), toluene, benzene, o-cresol, other aromatic compounds</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em> F1</td>
<td>Toluene dioxygenase</td>
<td>Trichloroethylene (TCE), dichloroethylene (DCE), toluene, benzene, o-cresol, other aromatic compounds</td>
</tr>
</tbody>
</table>

* = not a complete list
Materials and Methods

Chemicals

All media components had a purity ≥ 98.0% and were bought from Sigma-Aldrich or Fisher Scientific. Perfluorooctanesulfonic acid potassium salt (PFOS·K+) was ≥ 98.0% purity (Sigma-Aldrich, Missouri, USA). Perfluorooctanoic acid (PFOA) was ≥ 97.0% analytical grade (Oakwood Products, Inc., South Carolina, USA). All solvents, including acetonitrile, water, and methanol, were HPLC grade or higher. Internal standards for LC/MS/MS analysis were perfluoro-n-[1,2,3,4-13C]octanoic acid (MPFOA) and sodium perfluoro-1-[1,2,3,4-13C]octanesulfonate (MPFOS) (Wellington Laboratories, Ontario, Canada).

Groundwater Samples

Groundwater samples were obtained anaerobically in amber glass 1 L bottles from a fire-training site where aqueous film forming foam (AFFF) was used extensively. Samples were transferred to polypropylene serum bottles, aluminum crimped with butyl rubber stopper, and kept under anaerobic condition at 4°C. Samples contained high concentrations of a wide variety of perfluoroalkyl acids (PFAAs), up to 90 mg L⁻¹. In addition, samples had high concentrations of iron (up to 223 mg L⁻¹), manganese (up to 25 mg L⁻¹), ammonia (up to 58 mg L⁻¹), and methane (unknown concentration).
Isolation of PFASs-tolerant Microbes

Aerobic bacteria and fungi were isolated from the PFAAs-contaminated groundwater samples on agar plates. The entire microbial community of each sample was obtained by streaking 100 µL groundwater sample onto nutrient-rich agar and incubating at 30°C for 7:30 days. Several different media were used to isolate a wide variety of microbes (Table 3). Afterwards, morphologically different colonies were isolated on nutrient-rich agar to obtain a large sample of each colony and incubated at 30°C for 7:30 days. Each colony was then transferred to selective media with 10 mg L^{-1} of PFOA or PFOS and incubated at 30°C for 7:30 days. Those colonies that could tolerate and grow on 10 mg L^{-1} PFOA or PFOS were then transferred to the appropriate selective, liquid media containing 10 mg L^{-1} PFOA or PFOS.

Table 3. Media Conditions for Isolating Microorganisms.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Nutrient-Rich Media</th>
<th>Selective Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi</td>
<td>Malt Extract (ME)</td>
<td>Kirk/YMPG [238]</td>
</tr>
<tr>
<td></td>
<td>Yeast Peptone Dextrose (YPD)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Potato Dextrose (PD)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Difco-Czapek Dox (DCD)</td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>Luria Broth (LB)</td>
<td>Whittenbury with addition of carbon source</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ammonium Mineral Salts (AMS) with addition of carbon source</td>
</tr>
</tbody>
</table>
The DNA of those bacteria and fungi that could grow and tolerate PFOA or PFOS in liquid media were then sequenced. DNA was extracted using ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research). Polymerase chain reaction (PCR) was performed on the 16S rRNA for bacteria and the internal transcribed spacer (ITS) region encompassing the 5.8S rRNA and 28S rRNA for fungi using a Promega GoTaq Kit (Promega). The primers used for bacteria were 16s rRNA for and rev (ReadyMade Primers, Integrated DNA Technologies) while the primers used for fungi were ITS1-F and ITS4-R (Prewit2008, Embong2008 in DNASequencing protocol, Integrated DNA Technologies). The PCR conditions for bacteria contained 30 cycles: initial denaturation temperature 95°C for 2 min, melt temperature 95°C for 1 min, annealing temperature 50°C for 1 min, extending temperature 72°C for 2 min, final extension temperature 72°C for 5 min, hold temperature 4°C. The PCR conditions for fungi followed touchdown PCR conditions and contained 40 cycles: initial denaturation temperature 95°C for 35 sec, melt temperature 95°C for 35 sec, annealing temperature of (1) 64°C to 55°C (-1°C per cycle) for 55 sec (10 cycles) and (2) 54°C for 55 sec (30 cycles), extending temperature of 72°C for 1 min, final extension temperature 72°C for 10 min, hold temperature 4°C. The PCR product was verified using DNA gel electrophoresis and cleaned using the UltraClean PCR Clean-Up Kit (MoBio). The cleaned up PCR product was sequenced at the UCLA Genotyping and Sequencing Core.

**Media Components**
All media were either autoclaved at 121°C for 30 min or filter-sterilized through a 0.22 µm filter. For fungal media, 0.1% chloramphenicol was added after autoclaving to prevent growth of bacteria. For agar preparation, 15 g L⁻¹ agar was added to the medium.

Malt Extract (ME) contained (per liter) glucose 10 g, malt extract 10 g, Bacto peptone 2 g, yeast extract 2 g, asparagine 1 g, KH₂PO₄ 2 g, MgSO₄·7H₂O 1 mL of 1 g L⁻¹, and thiamine 1 mL of 1 g L⁻¹. Yeast Peptone Dextrose (YPD) (per liter) contained Bacto peptone 20 g, yeast extract 10 g, and dextrose 20 g.

Potato Dextrose (PD) (per liter) contained potato starch 4 g and dextrose 20 g.

Difco-Czapek Dox (DCD) (per liter) contained sucrose 30 g, NaNO₃ 3 g, MgSO₄·7H₂O 0.5 g, KCl 0.5 g, and FeSO₄·7H₂O 0.01 g. After autoclaving those components, 10 mL of 100 g L⁻¹ filter-sterilized K₂HPO₄ was added to the media. The pH was adjusted to 6.8 ± 0.1 before autoclaving.

Kirk/YMPG was obtained from Ramirez et al. [238]. Stock reagents were prepared and stored at 4°C. Glucose 2 g L⁻¹, diammonium tartrate 8.78 g L⁻¹, acetate buffer (0.2 mM), and Tween 80 (1.0% v/v) were autoclaved. Trace elements, thiamin (1.00 g L⁻¹), veratryl alcohol (67.28 g L⁻¹), Basal III media, and MnSO₄ (1200 mg L⁻¹) were filter-sterilized through a 0.22 µm filter. Trace elements (per liter) contained MgSO₄·7H₂O 3 g, NaCl 1.0 g, FeSO₄·7H₂O 0.1 g, CoCl₂·6H₂O 0.19 g, ZnSO₄·7H₂O 1 g, CuSO₄·7H₂O 0.156 g, AlK(SO₄)₂·12H₂O 0.01 g, H₃BO₃ 0.01 g, Na₂MoO₄·2H₂O, 0.01 g, and EDTA disodium salt 1.5 g. The pH was adjusted to 6.5 with 1 M KOH. Basal III media (per liter) contained KH₂PO₄ 20 g, MgSO₄·7H₂O 14.5 g, CaCl₂·2H₂O 1.32 g. For stationary cultures, stock reagents were combined (per liter): 100 mL glucose, 25 mL diammonium tartrate, 70 mL trace elements, 1 mL thiamine, 100 mL acetate buffer, 1 mL
veratryl alcohol, and 100 mL basal medium. For shaking cultures, stock reagents were combined (per liter): 100 mL glucose, 25 mL diammonium tartrate, 70 mL trace elements, 1 mL thiamine, 100 mL acetate buffer, 1 mL veratryl alcohol, 100 mL basal medium, 50 mL Tween 80, and 10 mL MnSO$_4$.

Luria Broth (LB) (per liter) contained tryptone 10 g, yeast extract 5 g, and NaCl 10 g.

Whittenbury (per liter) contained MgSO$_4$·7H$_2$O 1 g, KNO$_3$ 1 g, CaCl$_2$·2H$_2$O 0.2 g, EDTA-Ferric sodium salt 0.95 mL of 1 g L$^{-1}$, Na$_2$MoO$_4$·2H$_2$O 0.145 mL of 1 g L$^{-1}$, and trace metals 1 mL. After autoclaving, 10 mL of filter sterilized phosphate buffer was added. The pH was adjusted to 6.8 - 7 before autoclaving. Trace metals (per liter) contained FeSO$_4$·7H$_2$O 0.0005 g, ZnSO$_4$·7H$_2$O 0.0004 g, MnCl$_2$·4H$_2$O 0.00002 g, CoCl$_2$·6H$_2$O 0.00005, NiCl$_2$·6H$_2$O 0.00001 g, H$_3$BO$_3$ 0.000015 g, and EDTA-Ferric sodium salt 0.00025 g. Phosphate buffer (per liter) contained K$_2$HPO$_4$ 33 g and KH$_2$PO$_4$ 26 g.

Ammonium Mineral Salts (AMS) (per liter) contained (NH$_4$)$_2$SO$_4$ 0.66 g, MgSO$_4$·7H$_2$O 1 g, CaCl$_2$·2H$_2$O 0.015 g, trace elements 1 mL, and stock A 1 mL. After autoclaving, 20 mL of 1.0 M filter sterilized phosphate buffer was added. The pH was adjusted to 6.8 - 7.0 ± 0.1 before autoclaving. Trace elements (per liter) contained FeSO$_4$·7H$_2$O 0.5 g, ZnSO$_4$·7H$_2$O 0.4 g, MnSO$_4$·H$_2$O 0.02 g, H$_3$BO$_3$ 0.015 g, NiCl$_2$·6H$_2$O 0.01 g, EDTA-Ferric sodium salt 0.25 g, CoCl$_2$·6H$_2$O 0.05 g, and CuCl$_2$·2H$_2$O 0.005 g. Phosphate buffer (per liter) contained K$_2$HPO$_4$ 113 g and KH$_2$PO$_4$ 47 g. Stock A (per liter) contained EDTA-Ferric sodium salt 5 g and Na$_2$MoO$_4$·2H$_2$O 2 g.
Fungal 6:2 FTOH Degradation Experiments

Two fungi - *Phanerochaete chrysosporium* (ATCC: 24725) and *Aspergillus niger* (ATCC: 1015) - were tested for their ability to degrade 6:2 FTOH over a 35-day period. For more information on these fungi, refer back to Table 1. *Aspergillus niger* was generously donated from Professor Yi Tang’s laboratory at the University of California, Los Angeles (UCLA). These experiments were maintained at 30°C, 150 rpm.

Fungi were grown on PD agar plates for 1 week at 22°C to obtain enough spores for degradation experiments. Spores were collected by re-suspending in 8 mL sterile DI water and filtering through sterile glass wool. The density of filtered spores was then counted on a hemacytometer and about $10^4$ spores were used to inoculate degradation experiments. Degradation experiments were conducted in 120 mL serum bottles crimped with rubber butyl stoppers. The fungi were grown in 10 mL of their respective medium (Table 4) and exposed to 3 mg L$^{-1}$ 6:2 FTOH. To all bottles, 50 mg C18 powder, collected from C18 cartridges (Grace Davison Discovery Sciences, Maxi-Clean SPE 600 mg), was added to prevent vaporization of 6:2 FTOH. Controls included an exposed sterile control and biotic (6:2 FTOH Free). The exposed sterile control was autoclaved at the start of the experiment. The experiment was done in triplicate. Cultures were aerated every 2-3 days for 60 min. Two C18 cartridges were inserted into the bottle via a sterile 18G needle (BD PrecisionGlide Needle) to collect any possible volatile intermediates. Bottles were sacrificed on day 0, 14, and 35 to measure fluoride ions, 6:2 FTOH and metabolites, and biomass. See measurements section for more details.
Table 4. Media Conditions for Fungi Exposed to 6:2 FTOH.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Media</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. chrysosporium</em></td>
<td>Kirk/YMPG (2 g glucose L(^{-1}), 200 mg cellulose L(^{-1}), and 50 mg yeast L(^{-1}))</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>DCD</td>
</tr>
<tr>
<td></td>
<td>(2 g sucrose L(^{-1}))</td>
</tr>
</tbody>
</table>

*Phanerochaete chrysosporium* 6:2 FTOH, 8:2 FTOH, and PFOA Degradation Experiments

In independent experiments at DuPont (Newark, DE), *P. chrysosporium* was tested for its ability to degrade 6:2 FTOH, 8:2 FTOH, and PFOA. *P. chrysosporium* was grown at UCLA on PD agar plates for 1 week at 22°C to obtain enough spores. Spores were harvested as described in the section titled Fungal 6:2 FTOH Degradation Experiments and grown in Kirk/YMPG medium with 2 g glucose L\(^{-1}\) at 30°C, 150 rpm. After two weeks, *P. chrysosporium* was then transferred to 1 L bottles and sent to DuPont (Newark, DE) with new Kirk/YMPG medium (without glucose) for further degradation experiments.

Briefly, once received, the culture was grown in new medium for about 6 days at 30°C. Afterwards, the culture was aseptically blended, centrifuged, and resuspended in 350 mL of new Kirk/YMPG medium (from UCLA) containing either (1) 10 g L\(^{-1}\) processed Timothy hay and 50 mg C18 powder or (2) 10 g L\(^{-1}\) processed Timothy hay, 20% yeast extract, 1 g L\(^{-1}\) glucose, 0.2 g L\(^{-1}\) cellulose, and 50 mg C18 powder. 10 mL of culture was then aliquoted into serum bottles, crimped with rubber butyl stopper. Similar to experiments done at UCLA, two C18 cartridges were inserted into the stopper to adsorb any volatile compounds. Controls included an exposed
sterile control and a matrix control (one replicate for each time point). For all exposed conditions, either 3.17 mg L\(^{-1}\) 6:2 FTOH, 3 mg L\(^{-1}\) 8:2 FTOH, or 8 mg L\(^{-1}\) PFOA was added to each bottle. Bottles were aerated at 30 mLs min\(^{-1}\) for 15 min when oxygen fell below 18%. Bottles were sacrificed on Day 0, 7, 14, and 28 and measured for the parent compound and possible metabolites. See Table 5 for a list of experiments tested.

**Table 5.** Conditions for Three Independent Experiments Conducted at DuPont (Newark, DE): *Phanerochaete chrysosporium* Exposed to 6:2 FTOH, 8:2 FTOH, and PFOA.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Exposed*</th>
<th>Exposed (G+C)**</th>
<th>Exposed Sterile Control</th>
<th>Matrix Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>6:2 FTOH</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>8:2 FTOH</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>PFOA</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

*Exposed contain processed Timothy hay and C18 powder  
**Exposed (G+C) contain processed Timothy hay, yeast extract, glucose, cellulose, and C18 powder

**Environmental Fungal Isolates - PFOA and PFOS Degradation Experiments**

Environmental isolate 5 (Envi 5), environmental isolate 6 (Envi 6), environmental isolate 7 (Envi 7), and environmental isolate 8 (Envi 8) were tested for their ability to degrade PFOA and PFOS over a 14 or 28 day period. These experiments were maintained at 30°C, 150 rpm.

Fungi were grown on PD agar plates for 2 weeks at 30°C to obtain enough spores for degradation experiments. Spores were collected by re-suspending in 8 mL sterile DI water and filtering through sterile glass wool. The concentration of filtered spores were then counted on a hemacytometer and about 10^4 spores were used to inoculate degradation experiments.
Degradation experiments were conducted in 60 mL serum bottles, crimped with rubber butyl stoppers. The fungi were grown in 15 mL of their respective medium (Table 6) and exposed to 100 mg L$^{-1}$ PFOA or PFOS. Controls included an abiotic (no fungi), biotic (PFOA or PFOS Free), and matrix (no fungi or PFOA or PFOS). Envi 6, 7, and 8 were aerated every 2-3 days for 15 min while Envi 5 was oxygenated every 2-3 days for 15 min. A C18 cartridge (Grace Davison Discovery Sciences, Maxi-Clean SPE 600 mg) was inserted into the bottle via a sterile 18G needle (BD PrecisionGlide Needle) to collect any possible volatile intermediates. Bottles were sacrificed on day 0, 7, 14, and/or 28 to measure for fluoride ions, PFOA or PFOS concentration, and biomass. See measurements section for more details.

**Table 6.** Media Conditions for Isolated Fungi Exposed to PFOA or PFOS.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Media</th>
<th>Headspace</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental isolate 5</td>
<td>Kirk/YMPG (2 g L$^{-1}$ glucose)</td>
<td>Oxygen</td>
</tr>
<tr>
<td>Environmental isolate 6</td>
<td>DCD (2 g L$^{-1}$ glucose)</td>
<td>Air</td>
</tr>
<tr>
<td>Environmental isolate 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Environmental isolate 8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Short-term Bacteria PFOA Degradation Experiments**

Short-term PFOA degradation experiments were conducted to determine whether aerobic bacteria (1) can tolerate high concentrations of PFOA or (2) use PFOA as a carbon source and transform it to shorter carbon-chain products. These were conducted over 7 days and maintained at 30°C, 150 rpm.
Growing aerobic bacteria

*Pseudonocardia dioxanivorans* CB1190, *Methylosinus trichosporium* OB3b, *Burkholderia cepacia* G4, *Pseudomonas putida* F1, Envi 1, and Envi 2 were exposed to PFOA. For more information on the pure cultures, refer back to Table 2. These bacteria were grown in 30 mL of their respective media and given the appropriate carbon source to obtain enough biomass for PFOA degradation experiments (Table 7). All bacteria except for *P. dioxanivorans* CB1190 were grown in 120 mL serum bottles crimped with rubber butyl stoppers to avoid loss of toluene or methane. *P. dioxanivorans* CB1190 was grown in 100 mL Pyrex glass bottles since 1,4-dioxane is not volatile under experimental conditions. For toluene or 1,4-dioxane, 3 µL was added to the bottle. For methane, 50% (v/v head space) of air was removed via sterilized syringe before adding 50% (v/v head space) filtered methane to the bottle. Protein was measured as an indicator for biomass using Coomassie Plus Protein Assay Kit (Thermo Scientific). See measurements section for more details on protein measurements.

The concentration of toluene, 1,4-dioxane, and methane in the bottles were measured with a gas chromatograph equipped with flame ionization detector (GC-FID). See measurements section for more details on GC-FID method. When the concentration of carbon source was low, more carbon source was added. Roughly, toluene and 1,4-dioxane were added every 2-3 days while methane was added once a week. When adding more methane, the bottle was aerated for 15 min to remove buildup of carbon dioxide before removing 50% (v/v head space) air and adding 50% (v/v head space) methane.
Degradation Experiments

Once protein concentrations were greater than 100 µg mL\(^{-1}\), the bacteria were re-suspended in the appropriate media without any carbon source and exposed to 10 mg L\(^{-1}\) PFOA for 7 days. Controls included an abiotic (no fungi), biotic (PFOA Free), and matrix (no fungi or PFOA). Bottles were sacrificed on day 0 and day 7 to measure for protein concentration, fluoride ion concentration, and PFOA concentration. See measurements section for more details.

Table 7. Media Conditions for Bacteria Exposed to PFOA.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Media</th>
<th>Carbon Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. cepacia G4</td>
<td>Whittenbury</td>
<td>Toluene</td>
</tr>
<tr>
<td>P. putida F1</td>
<td>Toluene</td>
<td></td>
</tr>
<tr>
<td>M. trichosporium OB3b</td>
<td>Methane</td>
<td></td>
</tr>
<tr>
<td>Environmental isolate 1</td>
<td>AMS</td>
<td>Toluene</td>
</tr>
<tr>
<td>Environmental isolate 2</td>
<td>Toluene</td>
<td></td>
</tr>
<tr>
<td>P. dioxanivorans CB1190</td>
<td>1,4-dioxane</td>
<td></td>
</tr>
</tbody>
</table>

Measurements

Gas Chromatography-Flame Ionization Detector (GC-FID) Measurements

1,4-dioxane, methane, and toluene were measured by a Hewlett-Packard 6890 Chromatograph (GC) analyzer equipped with a Flame Ionization Detector (FID) and a Restek
Stabilwax-DB capillary column (30 m x 0.53 mm id x 1 \(\mu\)m). Injector and detector were maintained at 220˚C and 250˚C, respectively. Refer to Table 8 for more details on the method.

For 1,4-dioxane, samples were analyzed by injecting 2 \(\mu\)L filtered liquid (0.22 \(\mu\)m syringe filter). The oven was programmed at initial temperature of 80˚C (3 min) and ramp at 20˚C min\(^{-1}\) to 140˚C (1 min). The peak was obtained at 3.7 min. (Table 8).

For methane and toluene, samples were analyzed by injecting 100 \(\mu\)L gaseous head space. (Table 8). The oven program for toluene began at an initial temperature of 45˚C (1 min) and ramped to 200˚C at a rate of 100˚C min\(^{-1}\) (3 min). The oven program for methane ran for 2 min at 40˚C. The peak was obtained at 2.55 min for toluene and 1.42 min for methane. The concentration in the gas and liquid phase were calculated by the equation:

\[
M = C_w V_w + C_g V_g = C_w (V_w + H_c V_g)
\]

where \(M\) is the total compound mass (mol), \(C_w\) is the concentration of compound in the aqueous phase (\(\mu\)M), \(C_g\) is the concentration of compound in the gaseous phase (\(\mu\)M), \(V_w\) is the volume of liquid phase in the bottle (L), \(V_g\) is the volume of gaseous head space in the bottle (L), and \(H_c\) is dimensionless Henry’s Law constant [239, 240]
**Table 8.** GC-FID Conditions for 1,4-Dioxane, Toluene, and Methane analysis.

<table>
<thead>
<tr>
<th>GC-FID Conditions</th>
<th>1,4-dioxane</th>
<th>Toluene</th>
<th>Methane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet (Splitless injection)</td>
<td>2 µL liquid</td>
<td>100 µL head space</td>
<td>100 µL head space</td>
</tr>
<tr>
<td>Heater</td>
<td>220°C</td>
<td>220°C</td>
<td>220°C</td>
</tr>
<tr>
<td>Pressure</td>
<td>5.1 psi</td>
<td>5.1 psi</td>
<td>4.3 psi</td>
</tr>
<tr>
<td>Total Flow</td>
<td>12.3 mL min⁻¹</td>
<td>12.3 mL min⁻¹</td>
<td>23.2 mL min⁻¹</td>
</tr>
<tr>
<td>Carrier Gas</td>
<td>Helium</td>
<td>Helium</td>
<td>Helium</td>
</tr>
</tbody>
</table>

Oven Program

<table>
<thead>
<tr>
<th></th>
<th>1,4-dioxane</th>
<th>Toluene</th>
<th>Methane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Temp</td>
<td>80°C (3 min)</td>
<td>45°C (1 min)</td>
<td>40°C (2 min)</td>
</tr>
<tr>
<td>Ramp</td>
<td>20°C min⁻¹ to 140°C (1 min)</td>
<td>100°C min⁻¹ to 200°C (3 min)</td>
<td>--</td>
</tr>
</tbody>
</table>

Detector

<table>
<thead>
<tr>
<th></th>
<th>1,4-dioxane</th>
<th>Toluene</th>
<th>Methane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heater</td>
<td>250°C</td>
<td>250°C</td>
<td>250°C</td>
</tr>
<tr>
<td>H₂ flow</td>
<td>50 mL min⁻¹</td>
<td>50 mL min⁻¹</td>
<td>50 mL min⁻¹</td>
</tr>
<tr>
<td>Air flow</td>
<td>300 mL min⁻¹</td>
<td>300 mL min⁻¹</td>
<td>300 mL min⁻¹</td>
</tr>
<tr>
<td>N₂ flow</td>
<td>30 mL min⁻¹</td>
<td>30 mL min⁻¹</td>
<td>30 mL min⁻¹</td>
</tr>
<tr>
<td>Retention time</td>
<td>3.7 min</td>
<td>2.55 min</td>
<td>1.42 min</td>
</tr>
</tbody>
</table>

**Biomass**

Bacteria - Protein: Protein was measured by following the Coomassie Plus Protein Assay Kit (Thermo Scientific). Briefly, 0.5 mL sample was collected and added to 0.1 mL of 5 M NaOH. After vortexing, the sample was boiled at 98°C for 10 min. The digested cells were then centrifuged for 15 min at 13,200 rpm and 50 µL sample was reacted with 1.5 mL Coomassie Plus Reagent. A calibration curve ranging from 1 to 250 µg mL⁻¹ was made by using bovine serum albumin as the standard in the appropriate medium. Absorbance was read at 595 nm on the NanoDrop 2000c (Thermo Scientific) spectrophotometer.
Fungi - Dry Weight: Dry weight was collected after collecting 10 mL of sample for LC/MS/MS quantification (see LC/MS/MS section for more details). The whole sample was filtered through a pre-weighed 0.47 μm glass fiber (Whatman GF/C) filter. The filter was then dried in an oven at 80°C for 2 days and weighed.

Fluoride ions

Fluoride ion concentrations were measured by using a fluoride ion-selective electrode (Thermo Scientific, 9609BNWP) connected to an Orion 5-Star meter (Thermo Scientific). Briefly, 1 mL sample was taken before LC/MS/MS preparation and mixed with 1 mL low-level total ionic strength adjustment buffer (TISAB). Samples were shaken for 15 min before analyzing. A calibration curve ranging from 10 to 410 μg L⁻¹ was made by using sodium fluoride as the standard. Each sample was measured for 7 min. In between samples, the electrode was soaked in a solution of 50% TISAB and 50% DI water for 1 min.

Liquid Chromatography-Tandem Mass Spectrometer (LC/MS/MS)

Concentrations of PFOA, 6:2 FTOH, and respective metabolites in each sample were measured by LC/MS/MS. To ensure full recovery of all PFASs in each bottle, two extractions were conducted and any volatile intermediates were extracted from the C18 cartridge using 5 mL HPLC-grade acetonitrile. The cartridge elution was stored in serum bottles crimped with butyl rubber stoppers plus aluminum caps. Each butyl rubber stopper was also extracted
with 10 mL HPLC-grade acetonitrile to recover any PFASs sorbed to the septum surface. The first extract was prepared by adding HPLC-grade acetonitrile to bottles in a 1:1 liquid volume ratio. Afterwards, the bottles were shaken overnight at 50°C, 150 rpm. 10 mL was collected into scintillation vials. The second extract was prepared by adding 5 mL HPLC-grade acetonitrile and 200 µL of 1 M NaOH to the cell pellets after centrifugation and decanting the first extract, followed by overnight shaking at 50°C, 150 rpm. This solution was stored in scintillation vials.

For testing bacterial degradation of PFASs, the first extract was analyzed at UCLA using LC/MS/MS (Applied Biosystems-MDS Sciex Q Trap coupled with hybrid triple-quad linear ion trap analyzer). From the 10 mL sample collected into scintillation vials, 1 mL was filtered through Whatman GD/X polypropylene filters, diluted with HPLC-grade acetonitrile, and used for analysis. A C18 column (Atlantis® T3 5 µm 2.1x150mm, Waters) equipped with a C18 guard column (Waters) was used to elute PFASs and their respective internal standards. The mass spectrometer was operated in negative electrospray ionization mode using scheduled multiple reaction monitoring (MRM). Ion transitions were identified as 413 > 369 (PFOA) and 417 > 372 (MPFOA). Samples were injected via an autosampler and eluted with 10 mM ammonium acetate in HPLC grade water (solvent A) and 80:20 methanol:acetonitrile solution with 10 mM ammonium acetate (solvent B) at a flow rate of 300 µL min⁻¹. The gradient started with 40% A and 60% B (0.50 min), followed by 0% A and 100% B (2.00 to 5.00 min), and ended with 40% A and 60% B (5.50 min to 7.00 min). The injection volume was 20 µL. A six-point calibration curve ranging 0.5 - 100 µg L⁻¹ was analyzed and performed at the beginning, middle, and end of every sample sequence. Solvent blanks and a quality control sample were analyzed every 4
samples. PTFE and other fluoro-polymer materials were avoided as much as possible to prevent background contamination. See Table 9 for more information on LC/MS/MS parameters.

For assessing PFASs degradation by fungi, 1.5 mL sample of the first extract, second extract, cap extract, and C18 cartridge extract were sent to DuPont for further analysis using LC/MS/MS. Before analyzing the samples via LC/MS/MS, 1 mL sample was filtered and spiked with internal standards at 50 µL mL⁻¹ containing 0.200 mg L⁻¹ of [1, 2-¹³C] PFHxA and 5 mg L⁻¹ of [1, 1, 2, 2-D; 3-¹³C] 6:2 FTOH. The analysis of 6:2 FTOH and its possible transformation products were done with a Model 2795 HPLC/Micromass Quattro Micro tandem mass spectrometry system (Waters, Milford, MA). A C8 column (Agilent Zorbax RX-C8 5 µm 2.1x150mm, Agilent) was used to elute 6:2 FTOH, its possible metabolites, and its respective internal standards. The mass spectrometer was operated in negative electrospray ionization mode using scheduled multiple reaction monitoring (MRM). Ion transitions are listed in Table 6. Samples were injected via an autosampler and eluted with 0.15% acetic acid in HPLC grade water (solvent A) and 0.15% acetic acid in HPLC grade acetonitrile (solvent B) at a flow rate of 400 µL min⁻¹. The gradient started with 90% A and 10% B (1.00 min), followed by 45% A and 0% B (1.10 to 2.00 min), 20% A and 80% B (2.00 to 7.50 min), 90% A and 10% B (7.50 to 7.60 min), and ending with 90% A and 10% B (7.60 to 8.00 min). The injection volume was 20 µL. A seven-point calibration curve ranging 0.33 LOQ to 75 LOQ was analyzed. PTFE and other fluoro-polymer materials were avoided as much as possible to prevent background contamination. See Table 10 for more information on LC/MS/MS parameters. A similar method was used for analysis of 8:2 FTOH and PFOA (Table 11).
All extractions were also directly infused into the mass spectrometer to determine whether any metabolites formed.
**Table 9.** LC/MS/MS Conditions for PFOA Analysis, used for Short-Term Bacterial Degradation Experiment.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Applied Biosystems-MDS Sciex Q Trap coupled with hybrid triple-quad linear ion trap analyzer. The mass spectrometer was operated in negative electrospray ionization mode using scheduled multiple reaction monitoring (MRM).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Column</td>
<td>C18 column (Atlantis® T3 5 µm 2.1x150mm, Waters) equipped with a C18 guard column (Waters)</td>
</tr>
<tr>
<td>Mobile Phases</td>
<td>A: 10 mM ammonium acetate in water</td>
</tr>
<tr>
<td></td>
<td>B: 80:20 methanol:acetonitrile with 10 mM ammonium acetate</td>
</tr>
<tr>
<td>Gradient Profile</td>
<td><strong>Time (min)</strong></td>
</tr>
<tr>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td>5.50</td>
</tr>
<tr>
<td></td>
<td>7.00</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>300 μL min⁻¹</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>20 μL</td>
</tr>
<tr>
<td>Monitored Ion Transitions</td>
<td><strong>Transitions</strong></td>
</tr>
<tr>
<td></td>
<td>413 &gt; 369</td>
</tr>
<tr>
<td></td>
<td>417 &gt; 372</td>
</tr>
<tr>
<td>LC/MS/MS Analog Parameters</td>
<td><strong>Curtain gas</strong></td>
</tr>
<tr>
<td></td>
<td><strong>CAD gas</strong></td>
</tr>
<tr>
<td></td>
<td><strong>IonSpray Voltage</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Temp</strong></td>
</tr>
<tr>
<td>Instrument</td>
<td>Waters Model 2795 High Performance Liquid Chromatograph with a Waters Quattro Micro Mass Spectrometer equipped with an electro-spray source. The mass spectrometer was operated in the negative ion multiple reaction-monitoring mode.</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Analytical Column</td>
<td>Agilent Zorbax RX-C8 (150 mm x 2.1 mm, 5 µm particle size, pore size 80 Å, not end-capped, carbon loading 5.5%)</td>
</tr>
</tbody>
</table>
| Mobile Phases | A: 0.15% acetic acid in nanopure water  
B: 0.15% acetic acid in acetonitrile |
<p>| Gradient Profile | Time (min) | A(%) |
| | 0.00 | 90 |
| | 1.00 | 90 |
| | 1.10 | 45 |
| | 2.00 | 45 |
| | 7.50 | 20 |
| | 7.60 | 90 |
| | 8.00 | 90 |
| Flow Rate | 400 µL min$^{-1}$ |
| Injection Volume | 20 µL |
| Monitored Ion Transitions | Transitions | Compound (LOQ*) | Cone Voltage, V | Collision Energy |
| | 213 &gt; 169 | PFBA (0.50) | 14 | 8 |
| | 263 &gt; 219 | PFPeA (0.50) | 14 | 8 |
| | 313 &gt; 269 | PFHxA (0.50) | 14 | 8 |
| | 363 &gt; 319 | PFHpA (0.20) | 16 | 10 |
| | 315 &gt; 270 | [M+2] PFHxA | 14 | 8 |
| | 389 &gt; 311 | 5:2 Ketone (10) | 8 | 10 |
| | 377 &gt; 293 | 6:2 FTCA (2.1) | 16 | 16 |
| | 357 &gt; 293 | 6:2 FTUCA (1.0) | 16 | 14 |
| | 291 &gt; 187 | 4:3 acid (3.0) | 18 | 13 |
| | 339 &gt; 295 | 5:3U acid (3.0) | 16 | 14 |
| | 341 &gt; 237 | 5:3 acid (1.9) | 18 | 13 |</p>
<table>
<thead>
<tr>
<th>M/z</th>
<th>Compound Description</th>
<th>Q1:</th>
<th>Q2:</th>
</tr>
</thead>
<tbody>
<tr>
<td>357 &gt; 205</td>
<td>α-OH 5:3 acid (1.0)</td>
<td>18</td>
<td>unit resolution</td>
</tr>
<tr>
<td>373 &gt; 59</td>
<td>5:2 sFTOH (11)</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>423 &gt; 59</td>
<td>6:2 FTOH (11)</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>428 &gt; 59</td>
<td>[M+5] 6:2 FTOH</td>
<td>12</td>
<td>8</td>
</tr>
</tbody>
</table>

*LOQ: Limit of Quantitation defined as the lowest calibration standard in µg L⁻¹ multiplied by a dilution factor to the sample solution being analyzed

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary</td>
<td>3.50 kV</td>
</tr>
<tr>
<td>Extractor</td>
<td>0 V</td>
</tr>
<tr>
<td>RF Lens</td>
<td>0 V</td>
</tr>
<tr>
<td>Source Temp</td>
<td>120°C</td>
</tr>
<tr>
<td>Desolvation Temp</td>
<td>250°C</td>
</tr>
<tr>
<td>Cone Gas Flow</td>
<td>50 L Hr⁻¹</td>
</tr>
<tr>
<td>Desolvation Gas Flow</td>
<td>500 L Hr⁻¹</td>
</tr>
</tbody>
</table>

**LC/MS/MS Analog Parameters**

- Capillary: 3.50 kV
- Q1: Unit resolution
- Extractor: 0 V
- Ion Energy 1: 0.6
- RF Lens: 0 V
- Entrance: -1
- Source Temp: 120°C
- Exit: 0
- Desolvation Temp: 250°C
- Q2: Unit resolution
- Cone Gas Flow: 50 L Hr⁻¹
- Ion Energy 2: 0.6
- Desolvation Gas Flow: 500 L Hr⁻¹
- Multiplier: 700 V
**Table 11. LC/MS/MS Conditions for 8:2 FTOH and PFOA Analysis, used for Fungal Degradation Experiments.**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Waters Model 2795 High Performance Liquid Chromatograph with a Waters Quattro Micro Mass Spectrometer equipped with an electro-spray source. The mass spectrometer was operated in the negative ion multiple reaction-monitoring mode.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Column</td>
<td>Agilent Zorbax RX-C8 (150 mm x 2.1 mm, 5 µm particle size, pore size 80 Å, not end-capped, carbon loading 5.5%)</td>
</tr>
</tbody>
</table>
| Mobile Phases | A: 0.15% acetic acid in nanopure water  
B: 0.15% acetic acid in acetonitrile |
| Gradient Profile |  
| Time (min) | A (%)  
| 0.00 | 90  
| 1.00 | 90  
| 1.10 | 45  
| 2.00 | 45  
| 7.50 | 20  
| 8.00 | 10  
| 8.50 | 10  
| 9.00 | 90  
| 10.00 | 90  
| Flow Rate | 400 µL min⁻¹ |
| Injection Volume | 20 µL |
| Monitored Ion Transitions |  
| Transitions | Compound (LOQ*) | Cone Voltage, V | Collision Energy  
| 313 > 269 | PFHxA (0.50) | 14 | 8  
| 363 > 319 | PFHpA (0.20) | 16 | 10  
| 413 > 369 | PFOA (0.5) | 16 | 10  
| 415 > 370 | [M+2] PFOA | 20 | 10  
| 463 > 419 | PFNA (0.5) | 15 | 10  
| 489 > 411 | 7:2 Ketone (10) | 8 | 10  
| 477 > 393 | 8:2 FTA (2.1) | 16 | 16  
| 457 > 393 | 8:2 FTUA (1.0) | 16 | 14  
| 439 > 369 | 7:3 u acid (3.0) | 16 | 14  

37
<table>
<thead>
<tr>
<th>Mass Transition</th>
<th>Molecule</th>
<th>Base Peak (m/z)</th>
<th>Q1</th>
<th>Q2</th>
</tr>
</thead>
<tbody>
<tr>
<td>441 &gt; 337</td>
<td>7:3 acid (3.0)</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>473 &gt; 59</td>
<td>7:2 sFTOH (10)</td>
<td>12</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>523 &gt; 59</td>
<td>8:2 FTOH (10)</td>
<td>12</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>528 &gt; 59</td>
<td>[M+5] 8:2 FTOH</td>
<td>12</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

*LOQ: Limit of Quantitation defined as the lowest calibration standard in µg L\(^{-1}\) multiplied by a dilution factor to the sample solution being analyzed

**LC/MS/MS Analog Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary</td>
<td>3.50 kV</td>
</tr>
<tr>
<td>Extractor</td>
<td>0 V</td>
</tr>
<tr>
<td>RF Lens</td>
<td>0 V</td>
</tr>
<tr>
<td>Source Temp</td>
<td>120°C</td>
</tr>
<tr>
<td>Desolavation Temp</td>
<td>250°C</td>
</tr>
<tr>
<td>Cone Gas Flow</td>
<td>50 L Hr(^{-1})</td>
</tr>
<tr>
<td>Desolavation Gas Flow</td>
<td>500 L Hr(^{-1})</td>
</tr>
</tbody>
</table>

**Ion Energy**

- Ion Energy 1: 0.6
- Ion Energy 2: 0.6
- Multiplier: 700 V
Results and Discussion

Isolates

A total of seven isolates (two bacteria and five fungi) were purified from the PFASs-contaminated groundwater by first streaking the groundwater on nutrient-rich media, followed by streaking each morphologically different colony on defined media with 10 mg L\(^{-1}\) PFOA or PFOS. All isolates could tolerate at least 10 mg L\(^{-1}\) of PFOA or PFOS.

The two bacteria isolates were grown on the nutrient defined medium, AMS, with toluene as the carbon source to induce expression of the oxygenase enzyme (Figure 5). Envi 1 (Figure 5a) was 99% (1371 bp) related to *Brevibacterium* sp. (Genbank Accession numbers: HQ188605.1, JF905608.1, HQ188606.1, JF905607.1, DQ784785.1, DQ2177787.1), *Brevibacterium sanguinis* (Genbank Accession numbers: HM584237.1, NR_028016.1, AJ564859.1, AJ628351.1), and *Brevibacterium celere* (Genbank Accession number: NR_025727.1). *Brevibacterium* sp. are Actinobacteria (Gram positive) that have been found in a wide variety of locations, including the East Sea (Korea) [241](Cho2010), humans [242], soil (root-colonizing bacteria) [243], deep-sea sediments (unpublished), and Andean lakes [245]. It has been found to degrade thallus [246] and BTEX (Benzene, Toluene, Ethyl Benzene, and Xylene) [247] and is a heavy metal resistant bacterium [244].

Envi 2 (Figure 5b) was 99% (1403 bp) related to *Pseudoxanthomonas* sp. (Genbank Accession numbers: HQ588834.1, JQ396581.1, JQ396572.1, JQ396597.1), *Pseudoxanthomonas yeongjuensis* (Genbank Accession numbers: AB682413.1, NR_043812.1), *Pseudoxanthomonas ginsengisoli* (Genbank Accession numbers: AB245360.1, AB245361.1, JN637330.1), and
Xanthomonas sp. (Genbank Accession numbers: JF778676.1, DQ177466.2). These are classified as Gammaproteobacteria (Gram negative). *Pseudoxanthomonas* sp. has been found in soil contaminated with hydrocarbons [247], Arctic soil (root-colonizing bacteria) (unpublished data), ginseng field (unpublished data), and permafrost (unpublished data). *Xanthomonas* sp. has been found in similar conditions as *Pseudoxanthomonas* sp., including permafrost and soil contaminated with hydrocarbons (Antarctica) [248].

![Figure 5a. Envi 1](image1)
![Figure 5b. Envi 2](image2)

**Figure 5. Photographs of Plates Containing Envi 1 and Envi 2.** Photographs of plates containing Envi 1 and 2, streaked onto the nutrient-rich media, Luria Broth, to check for uniform colony morphology. These bacteria were originally isolated on the defined nutrient medium, AMS, with toluene as the carbon source.

DNA sequencing results determined that the five isolated fungi belong to the fungal phylum Ascomycota (Figure 6). However, there are currently no known reports on whether these fungi are capable of degrading toxic compounds. This study hypothesized that fungi producing laccase and lignin and manganese peroxidase will be able to degrade PFASs. Unfortunately, there are fewer fungi categorized under Ascomycota that are known for producing lignin and
manganese peroxidase. Most white-rot fungi are categorized as Basidiomycota, with only a few categorized as Ascomycota. Brown- and soft-rot fungi are mostly Ascomycota, but these can only degrade hemicellulose and cellulose or only degrade lignin slowly and hemicellulose and cellulose quickly, respectively. In comparison, white-rot fungi are known to mineralize lignin to CO$_2$ and H$_2$O and may produce a combination of ligninolytic enzymes.

Envi 4 and 5 were 99% (568/564 bp) related to *Pseudeurotium* isolates, which are known ectomycorrhizal fungi (Genbank Accession numbers FJ378726.1 and GU934582.1). This indicates that these fungi were important in nutrient-limited environments and probably grow on the roots of trees. It is likely that Envi 4 and 5 were closely related isolates under *Pseudeurotium*, but there are some morphological differences (Figure 6a and 6b).

Envi 6 (Figure 6c) was 100% (550 bp) related to *Cadophora malorum* (Genbank Accession numbers JQ796752.1, GU212431, GU212430.1, GU212386.1, GU212378.1, GU212375.1, and DQ317328.1), a known soft-rot fungi that causes wood decay and discoloration [249-252].

Envi 7 (Figure 2d) was 99% (936 bp) related to *Geomyces* isolates (Genbank Accession numbers JF439475.1, JF439475, FJ362278.1), which have been shown to exhibit hemicellulase activity [253] and may have algicidal ability [254]. In addition, this fungus has been shown to be an endophyte, growing on the roots of grasses [255].

Envi 8 (Figure 6e) was 99% (561 bp) related to uncultured soil fungus from forest soil in a northern temperate forest (Genbank Accession number JQ666401) and from a fungus known to deteriorate stone (Accession number JQ666330.1).
Figure 6e. Envi 8

Figure 6. Photographs of Plates Containing Envi 4, 5, 6, 7, and 8.
All fungi were plated with a 5 mm plug taken from the outer diameter of a previous plate and grown for two weeks on Kirk/YMPG Medium (Envi 4 and 5) or DCD Medium (Envi 6, 7, and 8).

Fungal 6:2 FTOH Degradation Experiments

Two pure fungal cultures, *Phanerochaete chrysosporium* and *Aspergillus niger*, were exposed to 3 mg L⁻¹ of 6:2 FTOH over 35 days. *P. chrysosporium* is the most studied white-rot fungus while *A. niger* is a soft-rot fungus that are both known to degrade contaminants (See Introduction section). Both of these cultures have been exposed to 100 mg L⁻¹ PFOA over 28 days in Kirk/YMPG medium (*P. chrysosporium*) and DCD medium (*A. niger*) containing 2 g L⁻¹ glucose or sucrose, respectively. However, there was no indication of PFOA transformation for *P. chrysosporium* (Figure 7) and *A. niger* (data not shown). Although this data suggests PFOA cannot be degrade by wood-rotting fungi, an independent study was done using only lignin and
manganese peroxidase. Both enzymes were exposed to 500 mg L⁻¹ PFOA, and after 10 days, there was about a 54 % increase in fluoride ions (Figure 8). PFOA concentrations could not be tested for this study, but the huge increase in fluoride ions suggests there was some transformation occurring. Since *P. chrysosporium* is known to produce these two enzymes, conditions may need to be optimized so that it produces a high concentration of ligninolytic enzymes.

To positively determine whether the fungal culture can degrade fluorinated compounds, *P. chrysosporium* and *A. niger* were exposed to 6:2 FTOH, a polyfluorinated compound. This compound is likely to be degraded easily in comparison. After 35 days, *P. chrysosporium* was able to transform 6:2 FTOH (Figure 9). The concentration of 6:2 FTOH decreased from 105.36 ± 0.00 % nmol on Day 0 to 74.32 ± 14.66 % nmol on Day 35. However, 6:2 FTOH concentration for Day 14 (75.88 ± 4.71 % nmol) was about the same as Day 35 concentration. This indicates that *P. chrysosporium* may need more nutrients to continue degrading 6:2 FTOH. The exposed sterile control and the biotic control (6:2 FTOH Free) had about the same concentration throughout the experiment. Fluoride ion concentrations also increased from 0.13 ± 0.01 % nmol on Day 0 to 0.21 ± 0.04 % nmol on Day 35, suggesting possible transformation of metabolites produced. The fluoride ion concentrations remained about the same for the exposed sterile controls and the biotic control (6:2 FTOH Free). In addition, there was one known metabolite and unknown metabolite(s) formed. 5:3 polyfluorinated acid (CF₃(CF₂)₄CH₂CH₂COOH, CAS: 914637-49-3, DuPont, Newark, DE) was found in only the exposed bottles at 1.45 ± 0.35 % nmol on Day 14 and at 1.15 ± 0.49 % nmol on Day 35. For Day 14, there were still 21.63 ± 4.54 % nmol unknown metabolite(s) unaccounted for, and on Day 35, there were still 23.00 ± 14.63 % nmol.
nmol unknown metabolite(s) unaccounted for. These unknown metabolite(s) could actually be conjugated metabolites, such as glucoronide and fluorinated amines, and could not be analyzed by LC/MS/MS. More detailed analysis will be done to identify the unknown metabolite(s).

Unlike *P. chrysosporium*, *A. niger* was not able to transform 6:2 FTOH. There was no change in 6:2 FTOH concentration over 35 days (Figure 10) and the fluoride ion concentrations remained below detection levels (data not shown).

These results indicate that white-rot fungi can transform polyfluorinated compounds, such as 6:2 FTOH and may be able to transform perfluoroalkyl substances with changes in medium composition and long-term acclimatization to perfluoroalkyl substances.
Figure 7. Lack of Degradation of PFOA by *Phanerochaete chrysosporium*. 
*P. chrysosporium* was unable to transform 100 mg L⁻¹ PFOA over 28 days in Kirk/YMPG medium. Error bars represent standard deviation of triplicate samples.
Figure 8. Increase in Fluoride ions after PFOA was Incubated with Lignin (LiP) and Manganese (MnP) Peroxidase.
When the purified enzymes, LiP and MnP, were exposed to 500 mg L$^{-1}$ PFOA, a 50% increase in fluoride ions was observed after 10 days. This suggests that fungi producing ligninolytic enzymes can degrade PFOA under lignolysis-inducing conditions. Error bars represent standard deviation of triplicate samples.
Figure 9. Degradation of 6:2 FTOH by *Phanerochaete chrysosporium*. *P. chrysosporium* was able to transform 3 mg L\(^{-1}\) 6:2 FTOH within 14 days exposure (Figure 9a). By Day 35, there was minimal further degradation of 6:2 FTOH, indicating *P. chrysosporium* needed additional nutrients to continue transforming 6:2 FTOH. Fluoride ions increased by Day 35 (% nmol) (Figure 9b), suggesting possible transformation of unknown metabolites. Approximately 1.5% of 6:2 FTOH was converted to 5:3 acid and less than 1% to other measured metabolites. Error bars represent standard deviation of triplicate samples.
Figure 10. Lack of Degradation of 6:2 FTOH by *Aspergillus niger*.
*A. niger* was not able to transform 3 mg L\(^{-1}\) 6:2 FTOH within 35 days exposure. There was no change in 6:2 FTOH or fluoride ion concentrations. Error bars represent standard deviation of triplicate samples.
Phanerochaete chrysosporium 6:2 FTOH, 8:2 FTOH, and PFOA Degradation Experiments

*P. chrysosporium* was also independently tested at DuPont (Newark, DE) for its ability to degrade 6:2 FTOH, 8:2 FTOH, and PFOA. *P. chrysosporium* was grown at UCLA and shipped to DuPont along with glucose-free Kirk/YMPG medium for degradation experiments. Two conditions were tested for 6:2 FTOH and PFOA: (1) addition of 10 g L\(^{-1}\) processed Timothy hay and 50 mg C18 powder (Exposed) and (2) addition of 10 g L\(^{-1}\) processed Timothy hay, 20% yeast extract, 1 g L\(^{-1}\) glucose, 0.2 g L\(^{-1}\) cellulose, and 50 mg C18 powder (Exposed (G+C)). Compared to the experiment done at UCLA, these conditions were more conducive to degradation of 6:2 FTOH and production of measured metabolites.

*P. chrysosporium - 6:2 FTOH Degradation Experiments*

*P. chrysosporium* was able to transform 3.17 mg L\(^{-1}\) 6:2 FTOH under both conditions within 28 days. However, the Exposed (G+C) condition (47.81 ± 3.31 % nmol 6:2 FTOH) resulted in about 5% more transformation of 6:2 FTOH compared to the Exposed condition (51.73 ± 18.70 % nmol) by Day 28 (Figure 11a). In addition, 6:2 FTOH was transformed faster under the Exposed (G+C) condition (about 40% decrease by Day 7) versus the Exposed condition (about 15% decrease by Day 7).

Several metabolites were produced (Figure 11b). The metabolites that were measured include perfluorobutyric acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), 5:2 polyfluorinated ketone (5:2 Ketone), 6:2 fluorotelomer unsaturated carboxylic acid
(6:2 FTUA), 5:3 polyfluorinated acid (5:3 Acid), 6:2 fluorotelomer saturated carboxylic acid (6:2 FTA), 5:2 secondary polyfluorinated alcohol (5:2 sFTOH), 5:3 polyfluorinated unsaturated acid (5:3 u Acid), perfluoroheptanoic acid (PFHpA), 4:3 polyfluorinated acid (4:3 Acid), 3-3 polyfluorinated acid (3-3 Acid), and 5:3 Acid-OH (See abbreviations for structure). For the Exposed (G+C) condition, all metabolites could be accounted for while the Exposed condition led to about 16% unknown metabolites (data not shown for Exposed condition). Similar to the experiment done at UCLA, this indicates that these metabolites are either (1) unknown or (2) are conjugated and could not be measured by LC/MS/MS. The Exposed (G+C) condition also led to more production of 5:3 Acid by Day 14 (43.09 ± 3.86 % nmol) than the Exposed condition (16.82 ± 3.06 % nmol). After Day 14, there was a decrease in 5:3 Acid in the Exposed (G+C) condition (32.20 ± 4.99 % nmol) and an increase in other metabolites (Table 12).

These results indicate that *P. chrysosporium* can degrade polyfluorinated compounds, especially with the addition of a lignocellulosic substrate and glucose. Further studies are being conducted to determine the optimum conditions, the degradation pathway, and enzymatic kinetics.
Table 12. Metabolites Produced (% nmol) when *Phanerochaete chrysosporium* was Exposed to 6:2 FTOH under G+C Conditions (Significant Metabolites are Plotted in Figure 11b)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFBA</td>
<td>1.32 ± 0.13</td>
<td>1.27 ± 0.16</td>
<td>0.98 ± 0.30</td>
</tr>
<tr>
<td>PFPeA</td>
<td>0.49 ± 0.34</td>
<td>0.33 ± 0.17</td>
<td>1.48 ± 0.95</td>
</tr>
<tr>
<td>PFHxA</td>
<td>2.38 ± 0.83</td>
<td>2.24 ± 0.30</td>
<td>4.19 ± 1.74</td>
</tr>
<tr>
<td>5:2 Ketone</td>
<td>0.64 ± 0.18</td>
<td>0.64 ± 0.25</td>
<td>1.21 ± 1.13</td>
</tr>
<tr>
<td>6:2 FTUA</td>
<td>0.89 ± 0.43</td>
<td>1.31 ± 0.21</td>
<td>0.83 ± 0.19</td>
</tr>
<tr>
<td>5:3 Acid</td>
<td>38.16 ± 8.80</td>
<td>43.09 ± 3.86</td>
<td>32.20 ± 4.99</td>
</tr>
<tr>
<td>6:2 FTA</td>
<td>0.32 ± 0.03</td>
<td>0.29 ± 0.22</td>
<td>0.04 ± 0.05</td>
</tr>
<tr>
<td>5:2 sFTOH</td>
<td>4.06 ± 1.80</td>
<td>6.81 ± 1.98</td>
<td>9.44 ± 1.23</td>
</tr>
<tr>
<td>5:3 u Acid</td>
<td>1.42 ± 0.43</td>
<td>1.27 ± 0.54</td>
<td>0.88 ± 0.23</td>
</tr>
<tr>
<td>PFHpA</td>
<td>0.03 ± 0.04</td>
<td>0.01 ± 0.01</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>4:3 Acid</td>
<td>0.53 ± 0.49</td>
<td>0.77 ± 0.72</td>
<td>0.90 ± 0.53</td>
</tr>
<tr>
<td>3-3 Acid</td>
<td>0.08 ± 0.15</td>
<td>0.16 ± 0.28</td>
<td>0.36 ± 0.25</td>
</tr>
<tr>
<td>5:3 Acid-OH</td>
<td>0.18 ± 0.07</td>
<td>0.13 ± 0.09</td>
<td>0.15 ± 0.09</td>
</tr>
</tbody>
</table>
Figure 11. Degradation of 6:2 FTOH by *Phanerochaete chrysosporium* and Identification of Metabolites.

6:2 FTOH concentrations (% nmol) (Figure 11a) and metabolites formed under Exposed (G+C) condition (Figure 11b) for *P. chrysosporium* grown in Kirk/YMPG medium with 3.17 mg L⁻¹ 6:2 FTOH for 28 days. Error bars represent standard deviation of triplicate samples.
Figure 11a: When *P. chrysosporium* was grown with Timothy hay, yeast extract, cellulose, and glucose (Exposed (G+C)), there was more transformation of 6:2 FTOH, especially at Day 7, when compared to *P. chrysosporium* grown only on Timothy hay (Exposed). In addition, all metabolites for Exposed (G+C) could be accounted for and about 47% of the initial 6:2 FTOH concentration was left. In contrast, about 16% of metabolites produced were unaccounted for under the Exposed condition and about 51% of the initial 6:2 FTOH concentration was left (data not shown). Symbols: Exposed (diamond), Exposed (G+C) (square), Exposed Sterile Control (triangle), and Matrix (asterisk).

Figure 11b: After 28 days exposure to 6:2 FTOH, *P. chrysosporium* was able to transform 6:2 FTOH to 9 different compounds. For Exposed (G+C), about 40% of metabolites produced was 5:3 acid, 10% was 5:2s FTOH, 4% was PFHxA, and 6% all other metabolites (1% contribution each). Under Exposed conditions (data not shown), fewer metabolites could be accounted for with about 16% of metabolites produced as 5:3 acid. 6:2 FTOH concentration (diamond) corresponds with Figure 11a Exposed (diamond). Metabolites produced are in various colors: PFBA (red), PFPeA (dark green), PFHxA (dark purple), 5:2 Ketone (Turquoise), 6:2 FTUA (orange), 5:3 acid (light blue), 5:2 sFTOH (pink), 5:3u Acid (light green), and 4:3 acid (light purple).
**P. chrysosporium - 8:2 FTOH & PFOA Degradation Experiments**

*P. chrysosporium* was also able to transform 3 mg L\(^{-1}\) 8:2 FTOH within 28 days. By Day 7, the concentration of 8:2 FTOH decreased to 44.22 ± 2.65 % nmol and continued to decrease by Day 14 (29.87 ± 17.18 % nmol). However, by Day 28, the rate of transformation slowed and there was still 27.93 ± 2.37 % nmol 6:2 FTOH.

Several metabolites were identified (Figure 12b and Table 13). The metabolites measured include PFHxA, 6:3 FTA, perfluoroheptanoic acid (PFHpA), PFOA, PFNA, 7:2 sFTOH, 7:2 Ft Ketone, 8:2 FTA, 7:3 FTA, 8:2 FTUA, and 7:3 FTUA (See abbreviations for structure). There were about 56.13 % nmol unknown metabolite(s) produced by Day 28. Similar to the experiment done at UCLA, these metabolites are either (1) unknown or (2) are conjugated and could not be measured by LC/MS/MS. Based on the measured metabolites, *P. chrysosporium* could transform 8:2 FTOH to PFOA, and by Day 28, there was already 4.51 ± 0.45 % nmol PFOA. PFOA was a likely terminal product based on previous studies and experiments. *P. chrysosporium* was unable to transform PFOA after 28 days exposure (Figure 7) under conditions tested by UCLA and DuPont (data not shown). In previous studies, aerobic bacteria could degrade precursors, such as 8:2 FTCA [139], 8:2 FTOH [72, 137], to PFOA, but further degradation was not observed. This suggests that there has yet to be a microbe identified that can degrade PFOA.

These results indicate that *P. chrysosporium* can degrade polyfluorinated compounds, such as 6:2 FTOH and 8:2 FTOH. However, *P. chrysosporium* was unable to degrade perfluoroalkyl substances under the present conditions. Further studies are being conducted to
determine the optimum conditions, the degradation pathway, and enzymatic kinetics for degradation of 6:2 and 8:2 FTOH.
Table 13. Metabolites Produced (% nmol) when *Phanerochaete chrysosporium* was Exposed to 8:2 FTOH under G+C Conditions (Significant Metabolites are Plotted in Figure 12b)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOA</td>
<td>1.61 ± 0.16</td>
<td>2.97 ± 1.99</td>
<td>4.51 ± 0.45</td>
</tr>
<tr>
<td>PFNA</td>
<td>0.03 ± 0.02</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>PFHxA</td>
<td>0.26 ± 0.06</td>
<td>0.37 ± 0.20</td>
<td>0.65 ± 0.09</td>
</tr>
<tr>
<td>7:2 sFTOH</td>
<td>3.88 ± 0.50</td>
<td>7.66 ± 1.07</td>
<td>5.81 ± 1.91</td>
</tr>
<tr>
<td>7:2 Ft Ketone</td>
<td>3.01 ± 1.15</td>
<td>3.88 ± 0.54</td>
<td>3.08 ± 3.16</td>
</tr>
<tr>
<td>8:2 FTA</td>
<td>0.15 ± 0.02</td>
<td>0.10 ± 0.15</td>
<td>0.08 ± 0.11</td>
</tr>
<tr>
<td>6:3 FTA</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.01</td>
<td>0.03 ± 0.04</td>
</tr>
<tr>
<td>7:3 FTA</td>
<td>2.51 ± 0.16</td>
<td>0.61 ± 0.05</td>
<td>0.97 ± 0.22</td>
</tr>
<tr>
<td>8:2 FTUA</td>
<td>1.26 ± 0.06</td>
<td>0.54 ± 0.35</td>
<td>0.11 ± 0.05</td>
</tr>
<tr>
<td>7:3 FTUA</td>
<td>1.60 ± 0.18</td>
<td>0.13 ± 0.19</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>PFHpA</td>
<td>0.17 ± 0.00</td>
<td>0.71 ± 0.62</td>
<td>1.25 ± 0.18</td>
</tr>
</tbody>
</table>
Figure 12. Degradation of 8:2 FTOH by *Phanerochaete chrysosporium* Exposed and Identification of Metabolites.

8:2 FTOH concentrations (% nmol) (Figure 12a) and metabolites formed under Exposed (G+C) condition (Figure 12b) for *P. chrysosporium* grown in Kirk/YMPG medium with 3 mg L\(^{-1}\) 8:2 FTOH for 28 days. Error bars represent standard deviation of triplicate samples.
Figure 12a: After 28 days exposure, *P. chrysosporium* was able to transform 8:2 FTOH. By day 28, only about 27% of the initial 8:2 FTOH concentration was left. However, not all metabolites could be accounted for and about 56% of metabolites produced were unknown. Symbols: Exposed (square) and Matrix (triangle).

Figure 12b: *P. chrysosporium* was able to transform 8:2 FTOH to 8 known metabolites. By Day 28, roughly 6% of metabolites produced was 7:2s FTOH, 5% was PFOA, 3% 7:2 Ft Ketone, and < 5% other metabolites (≤ 1% contribution each). Symbols: 8:2 FTOH concentration (square) corresponds with Figure 12a Exposed (square). Metabolites produced are in various colors: 7:3 FTUA (pink), PFHxA (dark blue), PFHpA (red), PFOA (green), 7:2 sFTOH (purple), 7:2 Ft Ketone (turquoise), 7:3 FTA (orange), and 8:2 FTUA (light blue).
Environmental Fungal Isolates - PFOA and PFOS Degradation Experiments

Four environmental fungi were tested for their ability to transform 10 mg L$^{-1}$ PFOA and PFOS over 14 or 28 days. These isolates include Envi 5, Envi 6, Envi 7, and Envi 8. Envi 5 was grown in Kirk/YMPG medium for 28 days while Envi 6, 7, and 8 were grown in DCD medium for 14 days. Both medium contained only 2 g L$^{-1}$ glucose to limit the carbon source. For all samples, the PFOA and PFOS concentration (% nmol) for the abiotic control (PFOA or PFOS Free) and the matrix control remained at about 0 - 8 % nmol for the whole experiment.

PFOA Degradation Experiments

When the four environmental fungi isolates were exposed to 100 mg L$^{-1}$ PFOA, there was some transformation for Envi 7 (Figure 15). However, there was no indication of PFOA transformation for Envi 5 (Figure 13), Envi 6 (Figure 14), and Envi 8 (Figure 16). For Envi 7, PFOA concentration decreased from $128.26 \pm 15.04$ % nmol on Day 0 to $78.15 \pm 12.79$ % nmol on Day 14 (Figure 15a). Comparatively, the abiotic control (No Fungi) stayed roughly the same at $98.96 \pm 8.61$ % nmol on Day 0 and $114.29 \pm 15.85$ % nmol on Day 14. However, fluoride ion concentrations only increased slightly from $0.004 \pm 0.000$ % pmol on Day 0 to $0.005 \pm 0.000$ % nmol on Day 14. In addition, these concentrations were about the same concentration as the biotic control (PFOA Free) for both days (Figure 15b). This indicates that any possible transformation of PFOA did not occur via breaking the C-F bond and occurred by breaking C-C bonds.
For Envi 6 (Figure 14a) and Envi 8 (Figure 16a), PFOA concentrations increased slightly from Day 0 to Day 14. The slight increase in PFOA concentration is the result of inaccurate addition of PFOA at the start of the experiment since all bottles were sacrificed at each time point. For Envi 6, fluoride ion concentrations remained below detection limit (data not shown). For Envi 8 (Figure 16b), fluoride ion concentrations increased slightly from 0.004 ± 0.000 % nmol on Day 0 to 0.005 ± 0.000 % nmol. However, the biotic control (PFOA Free) had about the same concentration of fluoride ions, suggesting that the slight increase in fluoride ion concentration was not the result of PFOA transformation.

For Envi 5 (Figure 13a), there was no change in PFOA concentration over 28 days. In addition, the fluoride ion concentration change from Day 0 to Day 28 was similar to Envi 7 and Envi 8.

These results indicate that there may be possible transformation of PFOA when Envi 7 is exposed to 100 mg L⁻¹ PFOA over 14 days. A longer study needs to be conducted to give more conclusive evidence of PFOA transformation. Information is also needed on the metabolites produced and the enzymes produced. One possible enzyme that could be involved in PFOA transformation for Envi 7 is hemicellulase [253].
Figure 13a.

Figure 13b.

**Figure 13. Lack of Degradation of PFOA by Envi 5.**
Envi 5 was exposed to 100 mg L\(^{-1}\) PFOA in Kirk/YMPG medium with 2 g L\(^{-1}\) glucose. After 28 days exposure, there was no transformation of PFOA (Figure 13a) even though fluoride ions increased (Figure 13b). Error bars represent standard deviation of triplicate samples.
Figure 14. Lack of Degradation of PFOA by Envi 6.
Envi 6 was exposed to 100 mg L\(^{-1}\) PFOA in DCD medium with 2 g L\(^{-1}\) sucrose. After 14 days exposure, there was no transformation of PFOA or production of fluoride ions (data not shown), indicating no transformation of PFOS. Error bars represent standard deviation of triplicate samples.
Envi 7 was exposed to 100 mg L⁻¹ PFOA in DCD medium with 2 g L⁻¹ sucrose. After 14 days exposure, PFOA concentrations decreased (Figure 15a). However, fluoride ions increased slightly and was similar to the concentration found in the PFOA Free control (Figure 15b). This indicates that possible transformation of PFOA occurred without breaking any C-F bonds. Error bars represent standard deviation of triplicate samples.
**Figure 16a.**

**Figure 16b.**

**Figure 16. Lack of Degradation of PFOA by Envi 8.**

Envi 8 was exposed to 100 mg L\(^{-1}\) PFOA in DCD medium with 2 g L\(^{-1}\) sucrose. After 14 days exposure, there was no transformation of PFOA (Figure 16a). Fluoride ions increased for both Envi 8 Exposed and PFOA Free control (Figure 16b), indicating no transformation of PFOS. Error bars represent standard deviation of triplicate samples.
PFOS Degradation Experiments

When the four environmental fungal isolates were exposed to 100 mg L$^{-1}$ PFOS, there was some transformation for Envi 5 (Figure 17) and Envi 7 (Figure 19). However, there was no indication of PFOS transformation for Envi 6 (Figure 18) and Envi 8 (Figure 20). For Envi 5, PFOS concentration decreased from 95.53 ± 11.70 % nmol on Day 0 to 67.65 ± 2.20 % nmol for Day 28 (Figure 17a). In addition, the fluoride ion concentration increased from 0.008 ± 0.000 % nmol on Day 0 to 0.015 ± 0.001 % nmol on Day 28 (Figure 17b). The concentration for all other controls remained about the same throughout the experiment. However, the error bars for Day 28 No Fungi overlaps with the decrease in PFOS concentration, so it is unclear whether the decrease in PFOS concentration was correlated with PFOS transformation. Ongoing experiments are being conducted to determine whether transformation occurred.

For Envi 7, PFOS concentration decreased from 95.00 ± 10.63 % nmol on Day 0 to 78.20 ± 12.13 % nmol on Day 14 (Figure 19a). Comparatively, the abiotic control (No Fungi) stayed roughly the same at 120.41 ± 4.51 % nmol on Day 0 and 114.39 ± 0.83 % nmol on Day 14. In addition, the fluoride ion concentration increased from 0.006 ± 0.001 % nmol on Day 0 to 0.011 ± 0.000 % nmol on Day 14 (Figure 19b). However, the concentration of fluoride ions in the abiotic control (No Fungi) increased from below detection levels (0.002 % nmol) to 0.006 ± 0.000 % nmol on Day 14. This suggests that there may not be any C-F bonds breaking.

For Envi 6 (Figure 18a) and Envi 8 (Figure 20a), PFOS concentrations remained the same throughout the experiment. For both Envi 6 and 8, fluoride ion concentrations increased for both the abiotic control (No Fungi) and the exposed.
These results indicate that there may be possible transformation of PFOS when Envi 5 and Envi 7 were exposed to 100 mg L\(^{-1}\) PFOS over 14 or 28 days, respectively. A longer study needs to be conducted to give more conclusive evidence of PFOS transformation. Information is also needed on the metabolites and enzymes produced. Envi 7 was 99% similar to *Geomyces* isolates that produce hemicellulase [253] (Genbank Accession numbers JF439475.1, JF439475, FJ362278.1) and both fungi are endophytes [255] (Genbank Accession numbers FJ378726.1 and GU934582.1). This indicates that these fungi are likely candidates to degrade PFOS compared to the other three fungal isolates found.
Figure 17. Possible Transformation of PFOS by Envi 5.
Envi 5 was exposed to 100 mg L\(^{-1}\) PFOS in Kirk/YMPG medium with 2 g L\(^{-1}\) glucose. After 28 days exposure, decrease of PFOS (Figure 17a) combined with an increase in fluoride ions was observed (Figure 17b). However, the data were not statistically significant. Error bars represent standard deviation of triplicate samples.
Figure 18. Lack of Degradation of PFOS by Envi 6.
Envi 6 was exposed to 100 mg L\(^{-1}\) PFOS in DCD medium with 2 g L\(^{-1}\) sucrose. After 14 days exposure, there was no transformation of PFOS (Figure 18a). Fluoride ions increased for both Envi 6 Exposed and No Fungi (Figure 18b), indicating no transformation of PFOS. Error bars represent standard deviation of triplicate samples.
Figure 19. Possible Transformation of PFOS by Envi 7.
Envi 7 was exposed to 100 mg L\(^{-1}\) PFOS in DCD medium with 2 g L\(^{-1}\) sucrose. After 14 days exposure, decrease in PFOS (Figure 19a) combined with an increase in fluoride ions was observed (Figure 19b). This could represent possible transformation of PFOS. Error bars represent standard deviation of triplicate samples.
Figure 20a.

Figure 20b.

**Figure 20. Lack of Degradation of PFOS by Envi 8.**
Envi 8 was exposed to 100 mg L⁻¹ PFOS in DCD medium with 2 g L⁻¹ sucrose. After 14 days exposure, there was no transformation of PFOS (Figure 20a) even though there was a slight increase in fluoride ions (Figure 20b). Error bars represent standard deviation of triplicate samples.
Short-term Experiments Assessing PFOA Degradation by Aerobic Bacteria

Six aerobic cultures were tested over 7 days to determine whether aerobic bacteria with oxygenase enzymes can degrade PFOA within a short timeframe. These cultures include *P. dioxanivorans* CB1190, *M. trichosporium* OB3b, *B. cepacia* G4, *P. putida* F1, and two environmental isolates (Envi 1 and 2). These bacteria were first grown in Whittenbury or AMS media with carbon and energy source to increase biomass. After the biomass concentrations were greater than 100 µg mL\(^{-1}\) protein, the cultures were washed in media free of carbon and energy source and grown in media with 10 mg L\(^{-1}\) PFOA without any carbon and energy source. Fluoride ion concentration, PFOA concentration, and biomass were measured.

Results indicate that these bacteria cannot transform PFOA and that the oxygenase enzymes are unlikely candidates to degrade perfluoroalkyl substances over 7 days. PFOA concentrations stayed the same over 7 days for all bacteria (Figure 21, 22a, 23a, 24a, 25a, 26a). Protein concentrations (Figure 22c, 23c, 24c, 25c, 26c) were stagnant or decreased below 100 µg mL\(^{-1}\) for all bacteria by day 7 except for *M. trichosporium* OB3b, which increased from 96.99 ± 30.29 to 191.80 ± 37.57 µg mL\(^{-1}\) (Figure 22b). The increase in protein concentration for strain OB3b was likely the result of nutrients carried over inside its cells. *P. dioxanivorans* CB1190 protein concentrations were low (38.38 ± 20.88 µg mL\(^{-1}\) for day 0 and 24.68 ± 3.69 µg mL\(^{-1}\) for day 7; data not shown) due to the heterogeneous nature of *P. dioxanivorans* CB1190, resulting in inaccurate and imprecise protein measurements.

Fluoride ion concentrations (Figure 23b, 24b, 25b, 26b) either stayed unchanged, remained below detection limit, or decreased. For *P. dioxanivorans* CB1190 and *M.*
trichosporium OB3b, fluoride ion concentrations remained below detection limit (data not shown). For *B. cepacia* G4 (Figure 23b), *P. putida* F1 (Figure 24b), and Envi 1 (Figure 25b), there was a decrease in fluoride ions. This decrease may be attributed to an error in day 0 fluoride ion measurements. For Envi 2 (Figure 6b), fluoride ion concentrations remained the same.

These results indicate that the aerobic bacteria tested did not transform 10 mg L\(^{-1}\) PFOA over 7 days.
Figure 21. Lack of PFOA Degradation by *Pseudomonas dioxanivorans* CB1190.

*P. dioxanivorans* CB1190 was first grown to 100 µg mL\(^{-1}\) protein in PFOA-free AMS medium with 1,4-dioxane as the carbon source. Afterwards, strain CB1190 was exposed to 10 mg L\(^{-1}\) PFOA in 30 mL AMS medium without 1,4-dioxane. After 7 days exposure, there was no transformation of PFOA (Figure 1a), fluoride ion concentrations remained below detection limit (data not shown) and protein concentrations were unreliable (data not shown). Unreliable protein concentrations were a result of the heterogeneous morphology of strain CB1190. Error bars represent standard deviation of triplicate samples.
Figure 22. Lack of PFOA Degradation by *Methylosinus trichosporium* OB3b.

*M. trichosporium* OB3b was first grown to 100 µg mL\(^{-1}\) protein in PFOA-free Whittenbury medium with methane as the carbon source. Afterwards, strain OB3b was exposed to 10 mg L\(^{-1}\) PFOA in 30 mL Whittenbury medium without methane. After 7 days exposure, PFOA was not transformed (Figure 22a), fluoride ion concentrations remained unchanged (data not shown), and protein concentrations increased (Figure 22b). Error bars represent standard deviation of triplicate samples.
**Figure 23. Lack of PFOA Degradation by *Burkholderia cepacia* G4.**

*B. cepacia* G4 was first grown to 100 µg mL\(^{-1}\) protein in PFOA-free Whittenbury medium with toluene as the carbon source. Afterwards, strain G4 was exposed to 10 mg L\(^{-1}\) PFOA in 30 mL Whittenbury medium without toluene. After 7 days exposure, concentration of PFOA remained unchanged (Figure 23a), fluoride ion concentrations decreased slightly (Figure 23b) and protein concentrations decreased (Figure 23c). Error bars represent standard deviation of triplicate samples.
Figure 24. Lack of PFOA Degradation by *Pseudomonas putida* F1.

*P. putida* F1 was first grown to 100 µg mL⁻¹ protein in PFOA-free Whittenbury medium with toluene as the carbon source. Afterwards, strain F1 was exposed to 10 mg L⁻¹ PFOA in 30 mL Whittenbury medium without toluene. After 7 days exposure, concentration of PFOA remained unchanged (Figure 24a), fluoride ion concentrations decreased slightly (Figure 24b) and protein concentrations decreased (Figure 24c). Error bars represent standard deviation of triplicate samples.
Figure 25. Lack of PFOA Degradation by Envi 1.
Envi 1 was first grown to 100 µg mL⁻¹ protein in PFOA-free AMS medium with toluene as the carbon source. Afterwards, Envi 1 was exposed to 10 mg L⁻¹ PFOA in 30 mL AMS medium without toluene. After 7 days exposure, there was no transformation of PFOA (Figure 25a), fluoride ion concentrations decreased (Figure 25b) and protein concentrations decreased (Figure 25c). There was a decrease in PFOA concentrations for Envi 1 exposed to PFOA, but since bottles were sacrificed, Day 0 bottles for Envi 1 could be due to inconsistent preparation. Error bars represent standard deviation of triplicate samples.
Figure 26. Lack of PFOA Degradation by Envi 2.
Envi 2 was first grown to 100 µg mL⁻¹ protein in PFOA-free AMS medium with toluene as the carbon source. Afterwards, Envi 2 was exposed to 10 mg L⁻¹ PFOA in 30 mL AMS medium without toluene. After 7 days exposure, there was no transformation of PFOA (Figure 26a), fluoride ion concentrations remained the same (Figure 26b) and protein concentrations decreased (Figure 26c). Error bars represent standard deviation of triplicate samples.
Summary and Ongoing Work

PFASs are highly stable compounds that are difficult to remove from the environment using common treatment technologies. Current PFASs removal methods may be expensive, use high pressures and temperatures, produce PFASs-contaminated waste, and are impractical for in situ remediation. In contrast, biodegradation may be a feasible removal approach, especially for in situ treatment. Although successful biodegradation of perfluoroalkyl substances has not been previously reported, those studies did not explore a wide range of microbial activities and mostly focused on bacterial degradation. The objective of this research was to determine whether microorganisms could degrade PFASs, especially, FTOH, PFOA, and PFOS.

This research examined the feasibility of aerobic bacteria and fungi to degrade per- and polyfluoroalkyl substances. The bacteria tested included *P. dioxanivorans* CB1190, *M. trichosporium* OB3b, *B. cepacia* G4, and *P. putida* F1. Pure cultures of fungi tested were *P. chrysosporium* and *A. niger*. Bacteria and fungi were also isolated from PFASs-contaminated groundwater to determine whether any naturally occurring microbes historically exposed to PFASs could degrade PFASs. Five fungi and two aerobic bacteria could tolerate high concentrations of PFOA and PFOS and were used to test degradation in laboratory experiments. All bacteria produced oxygenase enzymes and used toluene, 1,4-dioxane, or methane as a carbon and energy source in this study. *P. chrysosporium* and *A. niger* are known to produce ligninolytic enzymes (e.g. lignin peroxidase, manganese peroxidase, cellulase, hemicellulase, etc.), which can degrade lignin and other recalcitrant compounds. Bacteria were exposed to 10 mg L\(^{-1}\) PFOA for 7 days without any addition of carbon or energy source while fungi were exposed to 100 mg L\(^{-1}\) PFOA, 3 or 3.17 mg L\(^{-1}\) 6:2 FTOH, or 3 mg L\(^{-1}\) 8:2 FTOH.
Fungal degradation of per- and polyfluoroalkyl substances was promising. Results indicated possible transformation of both PFOA and PFOS for Envi 7 and PFOS for Envi 5. Ongoing studies are being conducted to determine transformation rates, metabolites, and enzymes catalyzing the reaction. In addition, *P. chrysosporium* was able to degrade 6:2 and 8:2 FTOH, producing several metabolites, including 5:3 acid, PFBA, PFPeA, PFHxA, 5:2 Ketone, 6:2 FTUA, 5:2 sFTOH, 5:3 uAcid, and 4:3 acid. However, when exposed to 8:2 FTOH, production of PFOA occurred, which is a likely terminal metabolite. Further analysis will determine other possible metabolite(s) and enzymes involved. Although *P. chrysosporium* was unable to transform PFOA under these conditions, it is possible that with medium modifications, degradation can occur. When studies were conducted with purified ligninolytic enzymes, 54% increase in fluoride ions was measured within 10 days. Future experiments will modify the medium to promote production of ligninolytic enzymes for assessing degradation of PFOA and PFOS.

Neither PFOA nor PFOS was transformed by bacteria in 7 days. Future experiments will determine whether the aerobic bacteria tested in this study can acclimatize to PFOA or PFOS over 1 year and begin to transform these compounds.

The results of this research encourage further exploration of fungi as likely candidates to biodegrade PFASs. Studies are underway to determine conditions favorable for PFASs biodegradation, the degradation pathway of polyfluorinated compounds, and the enzymes involved in catalyzing critical steps in the biodegradation reaction.
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