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Fluorotelomer Alcohol Biodegradation Yields Poly- and Perfluorinated Acids

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The widespread detection of environmentally persistent perfluorinated acids (PFCAs) such as perfluorooctanoic acid (PFOA) and its longer chained homologues (C9>C15) in biota has instigated a need to identify potential sources. It has recently been suggested that fluorinated telomer alcohols (FTOHs) are probable precursor compounds that may undergo transformation reactions in the environment leading to the formation of these potentially toxic and bioaccumulative PFCAs. This study examined the aerobic biodegradation of the 8:2 telomer alcohol (8:2 FTOH, $\text{CF}_3(\text{CF}_2)_7\text{CH}_2\text{CH}_2\text{OH}$) using a mixed microbial system. The initial measured half-life of the 8:2 FTOH was ~ 0.2 days mg^{-1} of initial biomass protein. The degradation of the telomer alcohol was monitored using a gas chromatograph equipped with an electron capture detector (GC/ECD). Volatile metabolites were identified using gas chromatography/mass spectrometry (GC/MS), and nonvolatile metabolites were identified and quantified using liquid chromatography/tandem mass spectrometry (LC/MS/MS). Telomer acids ($\text{CF}_3(\text{CF}_2)_7\text{CH}_2\text{COOH}$; $\text{CF}_3(\text{CF}_2)_6\text{CFCHCOOH}$) and PFOA were identified as metabolites during the degradation, the unsaturated telomer acid being the predominant metabolite measured. The overall mechanism involves the oxidation of the 8:2 FTOH to the telomer acid via the transient telomer aldehyde. The telomer acid via a β -oxidation mechanism was further transformed, leading to the unsaturated acid and ultimately producing the highly stable PFOA. Telomer alcohols were demonstrated to be potential sources of PFCAs as a consequence of biotic degradation. Biological transformation may be a major degradation pathway for fluorinated telomer alcohols in aquatic systems.

Introduction

The extensive use of perfluorinated organic compounds, in both commercial and industrial applications, has recently prompted research into the disposition, fate, persistence, and overall environmental impact of this class of compounds. Their widespread application is attributed to the unique properties that the perfluoroalkyl chain imparts upon the compound. Many of these compounds have been found to

be highly stable in the environment due to the strength of the carbon–fluorine bond (1).

Extensive biological monitoring studies in recent years have revealed widespread global distribution of perfluorinated acids such as perfluoroalkane sulfonate, perfluorooctane sulfonate (PFOS), and perfluorinated carboxylic acids (PFCAs) of which perfluorooctanoic acid (PFOA) and perfluorodecanoic acid (PFDA) are examples (2–5). Some long chained homologues of PFCAs were first reported in fish samples collected from a creek after a large spill of aqueous film forming foam (AFFF) (6); subsequent monitoring of fish from this and nearby creeks suggests that the PFCAs, other than PFOA, did not arise from the spill (7). More recently, biota samples collected from the Canadian arctic (8) were shown to contain the full suite of PFCAs (C9–C15). PFOA has also been detected in trace concentrations from human serum samples worldwide (9). Long chain perfluorinated acids have been found to be environmentally persistent, bioaccumulative (10, 11), and potentially toxic (12, 13).

Perfluorinated acids are stronger acids as compared to their hydrocarbon counterparts and the correspondingly lower pK_a (i.e., PFOA is 2.80) (14) results in the dominance of the anionic form with little propensity to escape via volatilization. To explain the occurrence of PFCAs in remote regions, we have postulated that another class of more volatile neutral compounds might serve as atmospheric precursors. These would undergo environmental decomposition either biotically, or abiotically, to the more persistent acids (15).

Fluorotelomer alcohols (FTOHs) are polyfluorinated compounds typically characterized by even numbered perfluorinated carbons and two nonfluorinated carbons adjacent to a hydroxyl group. FTOHs are typically used as precursor compounds in the production of fluorinated polymers used in paper and carpet treatments and have similar applications as those of PFOS-based products (14). They are also used in the manufacture of a wide range of products such as paints, adhesives, waxes, polishes, metals, electronics, and caulks (14). During the years 2000–2002, an estimated 5×10^6 kg year^{-1} of these compounds was produced worldwide, 40% of which was in North America (15). Their name is derived from the telomerization process from which they are produced. FTOHs are given nomenclature based upon the number of perfluorinated carbons in relation to the number of hydrogenated carbons they possess (i.e., 8:2 FTOH; Table 1). Measured vapor pressures of FTOHs range from 140 to 990 Pa (16). The calculated dimensionless Henry's law constants for this class of compounds (i.e., 270 for 8:2 FTOH) using the limited data available for water solubility and vapor pressure reveals the propensity of these compounds to partition into air. This is supported by a recent air sampling campaign in which FTOHs were detected at tropospheric concentrations typically ranging from 17 to 135 pg m^{-3} (17, 18) with urban locations apparently having higher concentrations than rural areas. A study by Ellis et al. shows that the atmospheric lifetime of short chain FTOHs as determined by its reaction with OH radicals is approximately 20 days (15). These results demonstrate that fluorotelomer alcohols are widely disseminated in the troposphere and are capable of long-range atmospheric transport. Sources of these compounds are currently unknown, although it is likely that they may be released from the decomposition of polymeric or nonpolymeric materials that incorporate FTOHs or from the release of residual amounts of the fluorotelomer alcohols themselves that failed to be covalently linked to polymers during production (15). If polyfluorinated polymers are indeed a source for these compounds, then a potential fate

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TABLE 1. Acronym, Structure, and Molecular Weight of Perfluorinated Compounds of Interest

compound	acronym	structure	molecular wt. (amu)
8:2 fluorotelomer alcohol	8:2 FTOH	$\text{CF}_3(\text{CF}_2)_7\text{CH}_2\text{CH}_2\text{OH}$	464
8:2 fluorotelomer aldehyde	8:2 FTAL	$\text{CF}_3(\text{CF}_2)_7\text{CH}_2\text{CHO}$	462
8:2 fluorotelomer acid	8:2 FTCA	$\text{CF}_3(\text{CF}_2)_7\text{CH}_2\text{C}(\text{O})\text{O}^-$	477
8:2 fluorotelomer unsaturated acid	8:2 FTUCA	$\text{CF}_3(\text{CF}_2)_6\text{CF}=\text{CHC}(\text{O})\text{O}^-$	457
allylic 8:2 fluorotelomer alcohol	allylic 8:2 FTOH	$\text{CF}_3(\text{CF}_2)_6\text{CF}=\text{CHCH}_2\text{OH}$	444
perfluorooctanoic acid	PFOA	$\text{CF}_3(\text{CF}_2)_6\text{C}(\text{O})\text{O}^-$	413

of these materials is to end up in an aqueous environment such as sewage treatment plants as a result of routine activities such as carpet or upholstery cleaning. This type of an environment would subject these polymers to potential microbial degradation, possibly releasing FTOHs to the aqueous systems where they too are subjected to biodegradation.

An earlier study by Hagen et al. (19) showed compelling evidence for FTOH biotransformation. They identified 2H-, 2H-perfluorodecanoic acid (8:2 FTCA, Table 1) and PFOA as metabolites in rats given a single dose of 8:2 FTOH using ^{19}F NMR and a gas chromatograph equipped with a microwave plasma detector (GC/MPD) (19). They have also suggested the unsaturated form of the acid (8:2 FTUCA, Table 1) as another metabolite using retention time matching of a synthesized standard by gas chromatography using an electron capture detector (GC/ECD). A β -oxidation mechanism was proposed to be involved in this biotransformation. This rat metabolism study suggests that FTOHs subjected to biotic reactions are sources of the more stable perfluorinated acids. It has also been reported that a mixture of fluorotelomer alcohols were biodegraded when exposed to municipal wastewater treatment sludge (20). This screening study has shown the production of both even and odd chained perfluorinated acids after a 16 day incubation period and proposes that β - and α -oxidation mechanisms may be involved in the degradation pathway. Transient species detected by this study using LC/MS/MS included the unsaturated telomer acids of the corresponding alcohols (C8, C9, C10, and C12).

In our investigation, we demonstrated that fluorotelomer alcohols could undergo biodegradation under aerobic conditions using 8:2 FTOH (Table 1) as a model telomer in a microbial enrichment culture known to degrade ethanol. We hypothesized that fluorotelomer alcohols can be oxidized to the corresponding aldehyde (Table 1), subsequently to the related acids, and ultimately leading to the production of persistent PFCAs similar to what was initially shown by Hagen et al. in rats (19) and that of the screening study by Lange (20). To test this hypothesis, we developed a method to measure 8:2 FTOH via headspace using solid-phase microextraction (SPME) coupled with GC/ECD. Methods were developed to identify volatile metabolites using gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) for the nonvolatile metabolites. Unlike the use of gas chromatography for the analysis of PFCAs and telomer acids, applying the technique of LC/MS/MS involves little sampling preparation and eliminates the need for derivatization. It also provides enhanced confidence in chemical identification from MS/MS spectra.

Experimental Procedures

Media and Chemicals. The enrichment culture used in the experiments was routinely grown in a defined mineral medium which contained the following constituents added to distilled and deionized water to make one liter: 65 mL of phosphate buffer (27.2 g of KH_2PO_4 and 38.4 g of K_2HPO_4 L^{-1}), 10 mL of salt solution (53.5 g of NH_4Cl , 7.0 g of $\text{CaCl}_2 \cdot 6$

H_2O , 2.0 g of $\text{FeCl}_2 \cdot 4 \text{H}_2\text{O}$ L^{-1}), 2 mL of trace mineral solution (0.3 g of H_3BO_3 , 0.1 g of ZnCl_2 , 0.1 g of $\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$, 0.75 g of $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$, 1.0 g of $\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$, 0.1 g of $\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$, 1.5 g of $\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$, 0.02 g of Na_2SeO_3 , 0.1 g of $\text{Al}_2(\text{SO}_4)_3 \cdot 18 \text{H}_2\text{O}$, and 1 mL of concentrated H_2SO_4 L^{-1}), 2 mL of $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$ solution (48.8 g L^{-1}), and 10 mg of yeast extract. The mixture was autoclaved for 20 min at a temperature of 120 °C and pressure of 18 psi. The pH was subsequently adjusted to approximately 7 by the addition of 1N HCl.

The 8:2 FTOH (97%) was purchased from Oakwood Research Chemicals (West Columbia, SC). The 8:2 FTOH telomer aldehyde (8:2 FTAL) was synthesized as described by Napoli et al. (21), and the 8:2 FTOH telomer acid (8:2 FTCA) and the 8:2 FTOH unsaturated acid (8:2 FTUCA) were synthesized as described by Achilefu et al. (22). Characterization of these synthesized standards was done using ^{13}C , ^{19}F , and ^1H NMR along with high-resolution electron impact mass spectrometry, negative chemical ionization, and positive chemical ionization mass spectrometry. Purity of the 8:2 FTAL, 8:2 FTCA, and 8:2 FTUCA was >95%. PFOA (96%) and mercuric chloride were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Protein Analysis. Protein measurements were carried out using the Bradford method, using a microassay kit (Bio-Rad Laboratories, Hercules, CA) and bovine serum albumin (BSA) as a standard.

Growth Conditions and Culture Preparation for Degradation Experiments. The enrichment culture was obtained from sediment and groundwater taken from a contaminated site and had been enriched on 1,2-dichloroethane and subsequently maintained using ethanol as the sole carbon source (23). This mixed culture was chosen because it was acclimated to degradation of chlorinated alkanes and alcohols and therefore may also be active on fluorinated alcohols. Cells were harvested by centrifugation, washed with defined mineral medium, and resuspended in 2% of the total liquid volume used in the experiments. Degradation experiments were performed in triplicate using 1 L glass vessels (Pyrex) filled with 950 mL of defined mineral medium and sealed with mininert caps. The 8:2 FTOH was added to the culture vessels by adding 14 μL of a concentrated stock solution (50 μg μL^{-1}) made up in ethanol to attain a target aqueous concentration of 50 μg L^{-1} . The vessels were allowed to equilibrate for 24 h and sampled for time zero concentrations prior to adding the inoculum. Sterile controls were prepared similarly except that 500 mg of mercuric chloride was added to inhibit microbial activity. All cultures were stored in the dark at room temperature on a shaker at 95 rpm to allow for continued mixing and to enhance mass transfer of oxygen from the headspace to the liquid phase. For degradation experiments using sewage treatment plant samples, activated sludge obtained from Ashbridges Bay Treatment Plant (Toronto, ON) was used as the inoculum and prepared as described previously without acclimation to ethanol.

GC/ECD and GC/MS Analysis of 8:2 FTOH and Volatile Transformation Products. The degradation of the 8:2 FTOH was monitored using solid-phase microextraction (SPME). A 30 μm fiber with poly(dimethylsiloxane) (PDMS) coating (Supelco, Bellefonte, PA) was exposed to the headspace of

TABLE 2. Optimized MS/MS Conditions for Metabolite Confirmation

compound	parent ion (<i>m/z</i>)	cone vol. (V)	daughter ion (<i>m/z</i>)	collision energy (eV)
8:2 FTCA	477	15	393	15
			63	8
			39	10
8:2 FTUCA	457	15	393	15
PFOA	413	15	369	10
			219	15
			169	18

the sealed culture vessels and was allowed to equilibrate for 5 min. Headspace analysis was used to address the volatility of these fluoroalcohols. Stock et al. have measured the vapor pressure of the 8:2 FTOH at 212 Pa (16), and the water solubility was measured to be $148 \mu\text{g L}^{-1}$ (24) from which we calculated a Henry's law constant value of 270. Aqueous concentrations of the 8:2 FTOH were then determined, to ensure that the system was below the water solubility, from the following relationship:

$$M_{\text{tot}} = C_L(V_L + H \times V_g) \quad (1)$$

where M_{tot} is the total mass of the compound, C_L is the aqueous concentration, H is the Henry's law constant, and V_g is the headspace volume; typical aqueous concentrations were $\sim 50 \mu\text{g L}^{-1}$.

Analysis was done using a Hewlett-Packard 5890 Series II gas chromatograph equipped with an electron capture detector (Agilent Technologies, Wilmington, DE) and a $30 \text{ m} \times 0.5 \text{ mm} \times 250 \mu\text{m}$ DB-35 column (Phenomenex, Mississauga, ON). The injector temperature was 250°C , and the detector temperature was set at 320°C . The GC oven program was initially held at 45°C for 2 min followed by a $10^\circ\text{C min}^{-1}$ ramp to 95°C and held for 5 min and a final ramp of $30^\circ\text{C min}^{-1}$ to 250°C . The carrier gas was hydrogen at a pressure of 5 psi, and the make-up gas was nitrogen. External standards were used for calibration. Standards used had aqueous concentrations ranging from 2 to $55 \mu\text{g L}^{-1}$, and response was linear with r^2 typically >0.99 .

A Hewlett-Packard 6890 gas chromatograph coupled to a mass selective detector (Agilent Technologies, Wilmington, DE) was used under full scan positive chemical ionization mode to identify the volatile metabolites observed in the degradation. The carrier gas was helium, and methane was used as the ionizing gas at a flow rate of 1 mL min^{-1} . The source temperature was 250°C , and the electron energy was at 100 eV. Gas chromatographic separation was performed using a DB-Wax column ($30 \text{ m} \times 0.25 \text{ mm} \times 250 \mu\text{m}$) (J&W Scientific, Folsom, CA). The initial oven temperature was 45°C for 5 min and ramped at $15^\circ\text{C min}^{-1}$ to 210°C . Pulsed $1 \mu\text{L}$ splitless injections were performed at an initial pressure of 25 psi and 220°C , returning to 10 psi at 1.2 min, and followed by an injector purge.

LC/MS/MS Analysis of Nonvolatile Metabolites. Prior to analysis, 3 mL samples were obtained from each experimental vessel and centrifuged to remove biomass. One mL of methanol and 1 mL of supernatant were then transferred to polypropylene autosampler vials for analysis. Measurement and identification of target nonvolatile metabolites were performed using a Waters 717 autosampler along with an Alltech 426 isocratic pump equipped with an Alltech Econosil C18 column ($5 \mu\text{m}$, $4.6 \times 250 \text{ mm}$) at a flow rate of $400 \mu\text{L min}^{-1}$. Gradient elution was not applied because of contamination problems for PFOA with the available gradient pump. Isocratic elution proved to be an adequate and faster alternative for the analysis using a mobile phase comprised of 70% Optima grade methanol and 30% 18M Ω deionized water. Samples were injected at a volume of $20 \mu\text{L}$, and the

HPLC column eluate entered the mass spectrometer ion source without splitting. All analytes of interest eluted in less than 10 min.

Acquisition of the mass spectra was carried out using a Micromass Quattro micro Triple Quadrupole Mass Spectrometer (Micromass; Manchester, UK) operated under negative electrospray ionization mode. A standard with a concentration of $500 \mu\text{g L}^{-1}$ in methanol was infused through the equipped syringe pump at a flow rate of $30 \mu\text{L min}^{-1}$ for positioning of the ion sprayer and tuning of the mass spectrometer. The capillary voltage was 2.9 kV, while the cone voltage was set at 15 V. Specific operating parameters are listed in Table 2. The source block and desolvation temperatures were 110 and 300°C , respectively, and the dwell time was 0.5 s. The nebulizer and desolvation gas flow rates were 20 and 260 L h^{-1} , respectively. For tandem mass spectrometric analysis, argon was used as the collision gas ($2.86 \times 10^{-3} \text{ mbar}$), and collision energies (Table 2) were varied to optimize for the sensitivity of each compound.

Quantification was achieved under multiple reaction monitoring (MRM) mode and by calibrating the primary daughter ion peak area versus the concentration. Four-point matrix-matched calibration curves were generated daily by using freshly prepared standards. Standard concentrations ranged from 100 to $1000 \mu\text{g L}^{-1}$, and r^2 was typically >0.99 . Cone voltages and collision energies were optimized by standards made up in methanol for each individual compound to ensure the sensitivity of MS/MS analysis. Late in the study, when 8:2 FTUCA concentrations were declining, and correspondingly, PFOA concentrations were rising, some suppression of the PFOA signal, was revealed. Standard additions were performed for PFOA at the final time point of the experiment (day 81) so that an accurate mass balance was determined; no suppression was observed for the other analytes of interest during the investigation. Along with the hypothesized metabolites, other prospective products such as perfluorononanoic acid (PFNA) and perfluoroheptanoic acid (PFHpA) were also monitored although neither were detected in any samples.

QA/QC of LC/MS/MS Analysis. To guarantee data quality, a reagent blank (methanol) was injected after each time-point sample group (four samples/group) to reveal any problems of carryover. A typical chromatogram is shown in Figure 1. Three parent-daughter transitions were monitored for 8:2 FTCA and PFOA to confirm their identity and only one for 8:2 FTUCA since no further fragmentation was observed for this compound. Each ion was monitored under its own optimal condition listed in Table 2. The ratio of the peak areas of the two less intense product ions to the strongest ion was calculated and compared between samples and standards. As outlined by the European Analytical Guidelines, the repeatability of the product-ion ratios obtained in the confirmation procedure is the average ratio of three replicate injections of both the standards and the samples (Figure 2); the difference between samples and standards in this study was within the recommended tolerance set by the guidelines (25).

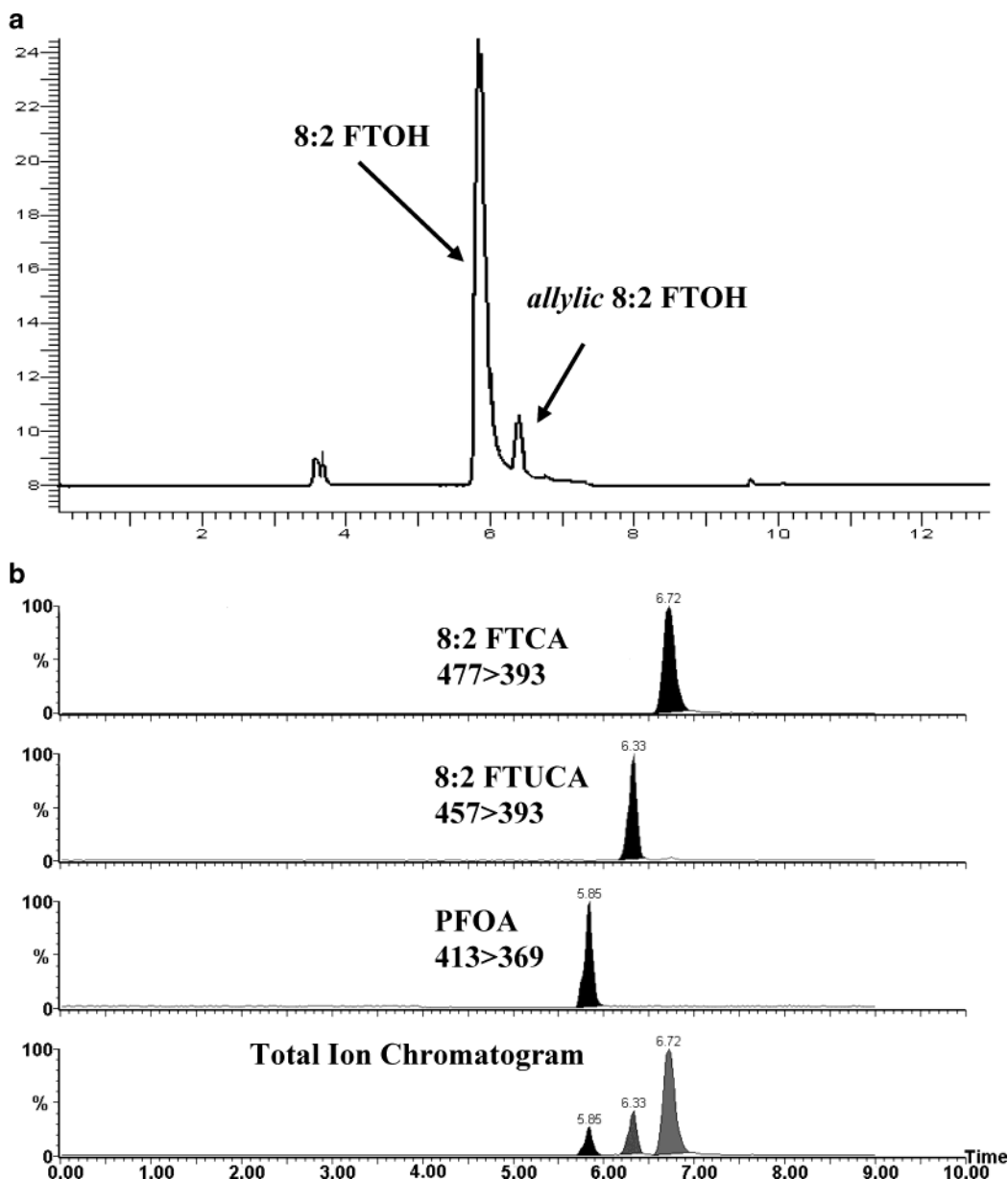


FIGURE 1. (a) Typical GC/ECD chromatogram showing the 8:2 FTOH and the impurity allylic 8:2 FTOH. (b) Typical LC/MS/MS chromatogram of the acid metabolites.

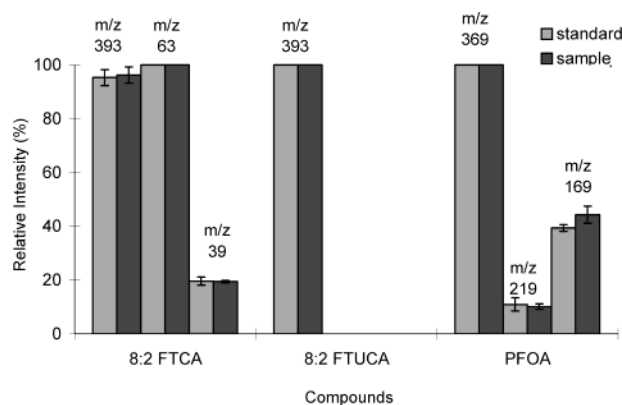


FIGURE 2. Confirmation of metabolites observed from the degradation of 8:2 FTOH. Comparison of transition parent–daughter m/z ratios in samples and standards. Base peak set at 100%.

Results and Discussion

Despite the rising number of published studies looking at the detection of perfluorinated acids in the environment,

only a small number have examined their potential sources. Ellis et al. have identified thermolysis of fluoropolymers as an abiotic mechanism that can potentially lead to the production of these persistent compounds (26), while Hagen et al. have been the first to observe the production of PFOA from the 8:2 telomer alcohol in a biotic system (19), and more recently, the production of perfluorocarboxylic acids were observed from a telomer alcohol biodegradation screening study by Lange (20). The study presented here not only provides further evidence that telomer alcohols are a potential source of PFCAs through biotransformation reactions, but it also presents a plausible biotic mechanism in a microbial system.

In this laboratory study, an initial mass of 750 μg (1.5 μmol) of 8:2 FTOH was added to vessels (1 L) inoculated with microorganisms. As seen in Figure 3, the 8:2 FTOH spiked was 85% degraded as of day 7 and was below detection limit levels (2 $\mu\text{g/L}$) by day 16. Triplicate vessels showed similar trends, although rates differed by as much as 35%. The initial half-life of the 8:2 FTOH was calculated to be ~ 0.2 days mg^{-1} of initial biomass protein followed by a second half-life of

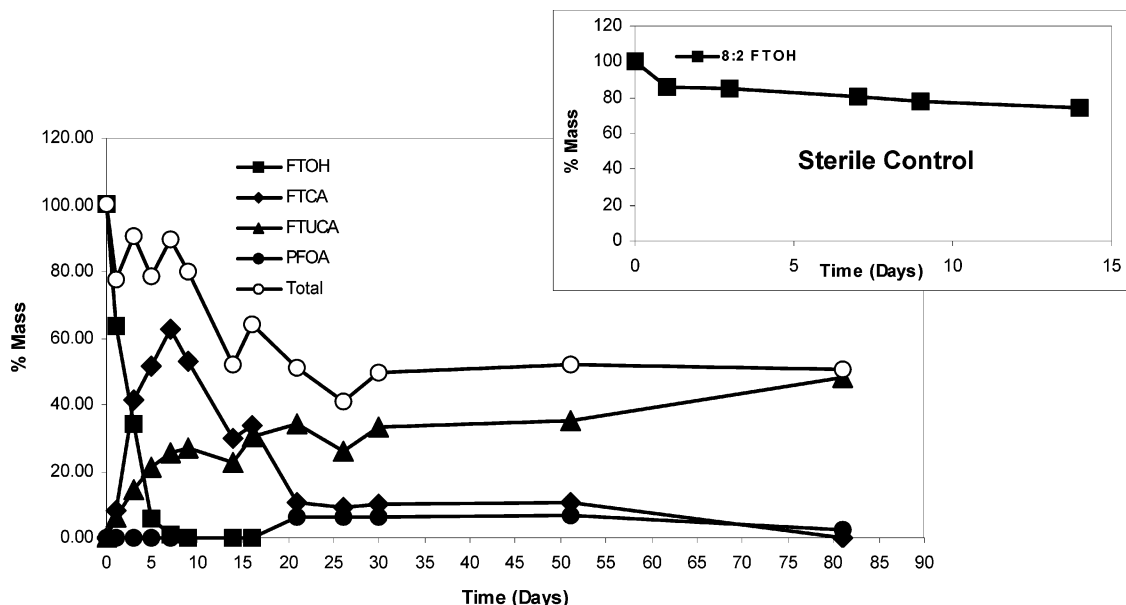


FIGURE 3. Typical transformation kinetics and mass balance of metabolites observed in degradation experiments; degradation of 8:2 FTOH in active microcosm (vessel B). Loss of 8:2 FTOH, production of 8:2 FTCA, 8:2 FTUCA, PFOA, and overall mass balance. No observable loss of 8:2 FTOH in sterile control ($n = 1$) (inset). PFOA values were obtained using standard additions at day 81.

~ 0.8 days mg^{-1} . The complex kinetics observed in the degradation of 8:2 FTOH maybe attributed to a couple of probable explanations. First, there may have been a change in the activity of specific microbial populations comprising the mixed inoculum during the experiment that could explain the observed change in the rate of the degradation after day 3. Second, more than one mechanism of degradation may be operative, resulting in varying rates of reaction involved; this study, however, could only provide evidence for one dominant mechanism.

The degradation of the 8:2 FTOH was presumably due to microbial activity since the sterile control showed little to no transformation during the experimental period as shown in Figure 3 (inset). The small decrease in the concentration observed in the sterile control is attributed to losses of volatiles from the vessel during sampling. The sterile control was not routinely sampled for 8:2 FTOH past day 16 of the study. Further evidence that the degradation of the telomer alcohol was driven by microbial activity in active bottles was the observed and quantified acids in the active bottles and their absence in the sterile control (Figure 3). The degradation of the 8:2 FTOH occurred concurrently with the production of the telomer acid, 8:2 FTCA and the telomer unsaturated acid, 8:2 FTUCA. This transformation step occurred via the formation of the telomer aldehyde, 8:2 FTAL. This volatile metabolite was detected and confirmed in sewage treatment plant samples by matching gas chromatograph peak retention times with synthesized standards as well as with mass spectral data (Figure 4a). The synthesized standard of the 8:2 FTAL showed a distinctive double peak for its molecular ion m/z 463 ($M + 1$), the presence of which was also confirmed in the samples. This observation may be due to the existence of two different conformations of the compound in the gas phase. Studies are ongoing to further investigate this observation. The 8:2 FTOH aldehyde appeared to be a transient intermediate, and confident quantification was not possible with our current method. The 8:2 FTCA and 8:2 FTUCA were more stable in the system.

Upon the depletion of the 8:2 FTOH in the system, the loss of the 8:2 FTCA was observed coincident with an increase in the production of 8:2 FTUCA. There are potentially two mechanisms by which the observed 8:2 FTUCA was produced in the system, via abiotic hydrolysis where the 8:2 FTCA loses

an -HF , or biotically, where perhaps an acyl-coA dehydrogenase type of enzyme oxidizes the $\text{C}\alpha\text{-C}\beta$ bond. This reaction proceeds via the removal of the α -proton, followed by hydride transfer of the β -proton presumably to a cofactor such as flavin adenine dinucleotide (FAD). Other experiments in our laboratory indicate that the abiotic elimination of HF from FTCAs are slower (half-lives > 1 week) than observed in these biological systems, although it is likely that both pathways were involved as the experiment progressed.

The production of the unsaturated acid can also be attributed to the degradation of the allylic 8:2 FTOH present as an impurity in the 8:2 FTOH alcohol (Figure 1a). Mass spectral data from previous studies within the group have identified the allylic form of the 8:2 FTOH as the only impurity present since the purity of the 8:2 FTOH used in this study was of 97% (27), and we can assume that the allylic 8:2 FTOH comprised at most 3% of the total mass of FTOH initially. This impurity would likely be metabolized in analogous fashion as the saturated alcohol, presumably forming the unsaturated acid via the unsaturated aldehyde. The detection of the 8:2 FTUCA early in the experiment (day 2–5) at approximately the same time as the detection of 8:2 FTCA may be a consequence of the oxidation of the allylic form of the 8:2 FTOH.

PFOA was detected in the system at very low concentrations beginning at day 16. A sample chromatogram is presented in Figure 4 of all nonvolatile metabolites detected in samples and their absence in the blank and in the sterile control. It appears that the highest concentrations of PFOA detected in the system within the duration of the experiment occurred at the peak concentration of the 8:2 FTUCA. By day 81, PFOA was detected at approximately 3% of the total mass. This production of PFOA may be attributed to the degradation of the earlier produced 8:2 FTUCA, and we suggest that further degradation of the 8:2 FTUCA in the system may lead to an increase in the production of this perfluorinated acid. Current studies are looking at degradation products when only the 8:2 FTUCA is spiked in a microbial system. Hagen et al. (19) in their earlier study identified 8:2 FTUCA in their system but were unable to definitively show that this was the initial step in the biotransformation process prior to forming PFOA. Lange et al. (18) reported the detection of 6–7% of PFOA at the conclusion of their biodegradation study of telomer

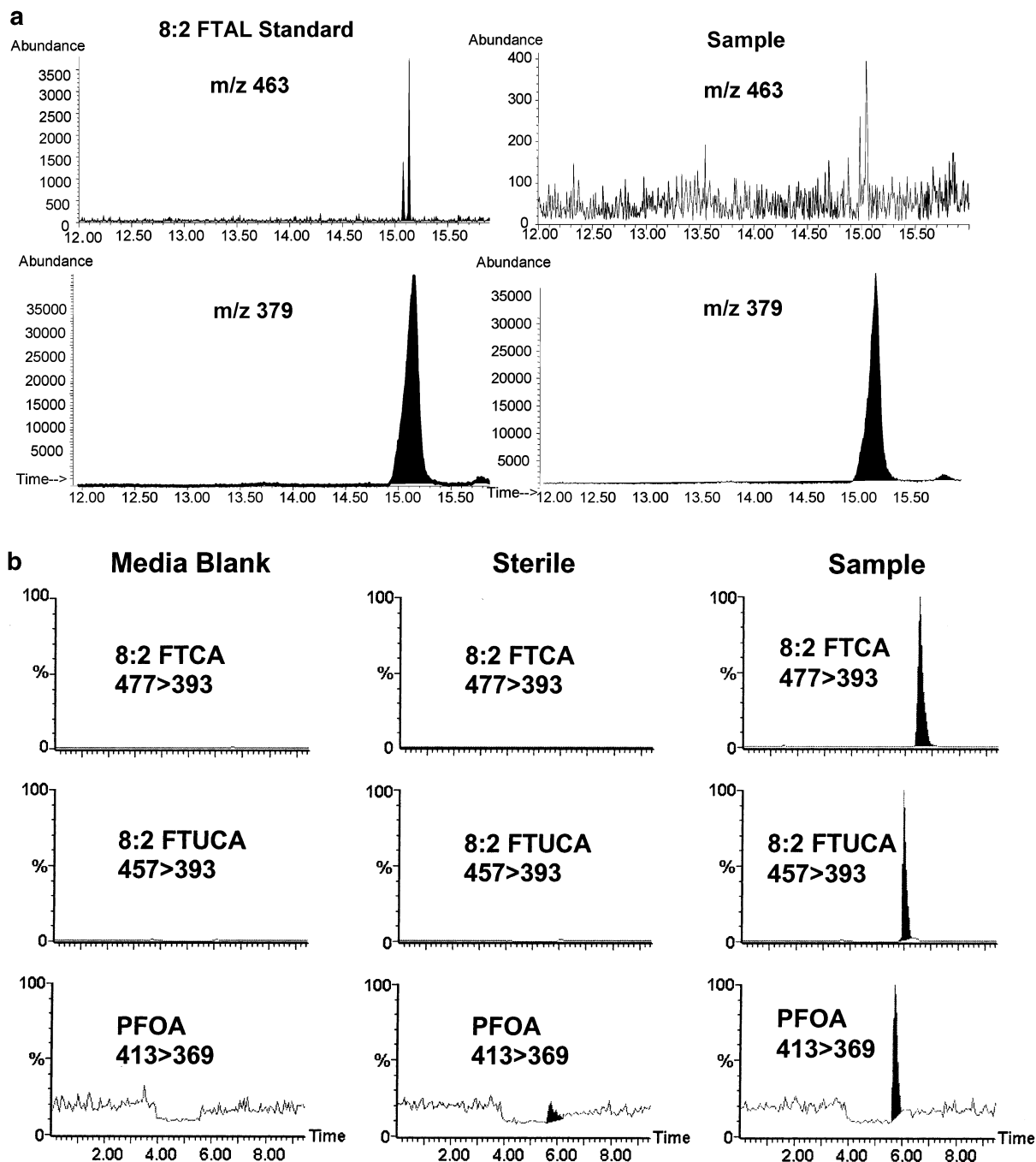


FIGURE 4. (a) GC/MS (PCI) extracted chromatograms of synthesized 8:2 FTAL standard and 8:2 FTAL detected in samples inoculated with sludge obtained from a sewage treatment plant. Distinctive double peak for m/z 463 ($M + 1$) in standard was also detected in samples. (b) LC/MS/MS chromatogram of sample taken from a blank, sterile control, and an active bottle. 8:2 FTCA, 8:2 FTUCA, and PFOA were detected in the active sample and were absent in the blank and sterile control.

alcohol mixtures and have also identified the unsaturated acid as an intermediate in the degradation process.

An assessment of the mass balance between the parent compound and the nonvolatile metabolites resulted in approximately 55% of products accounted for at the conclusion of the study (Figure 3), with 8:2 FTUCA being the most abundant metabolite along with PFOA. During the time interval, day 1–5, there was also a noticeable loss in the total mass measured (Figure 3), which suggests that metabolite(s) produced early on in the pathway was unaccounted. This apparent loss in mass may be due to our inability to quantify the 8:2 FTAL, along with other observed but unidentified volatile metabolites. Analyses via GC/ECD have shown other unknown chromatographic peaks produced in the early stage

of the degradation in experimental bottles and their absence in the sterile control. By day 81, the observed 45% loss of the products may be due to a number of reasons. As previously alluded to, other volatile metabolites observed in the degradation that were left unidentified may account for partial loss in measured products, as well as that volatile metabolites may have been lost during routine sampling. It is also possible the unaccounted mass could arise from the unsaturated metabolites (i.e., 8:2 FTUCA and 8:2 unsaturated aldehyde) being covalently bound by biological macromolecules such as extrapolymeric substances (EPS) produced extensively by most bacteria leading to its perceived loss. The unsaturated metabolites are presumably quite electrophilic and hence susceptible to attack by endogenous

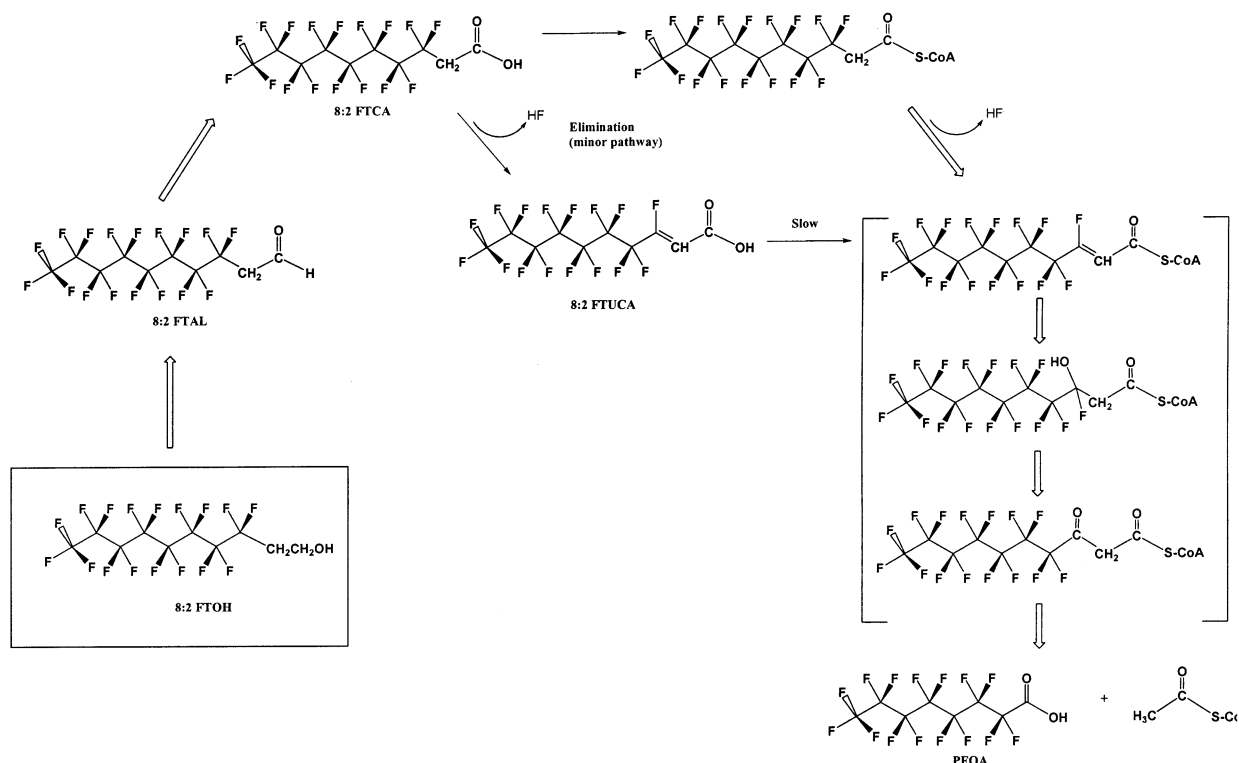


FIGURE 5. Proposed biodegradation pathway and products of 8:2 FTOH based upon laboratory experiments. Structures in brackets are proposed transitional intermediates and were not determined in this study.

nucleophiles present in biological systems. The observation that the unsaturated acid was the dominant metabolite produced from telomer alcohol biodegradation may be of significance since it may very well be a toxic metabolite for organisms.

Ion chromatography was used in an attempt to quantify inorganic fluorine (F^-) in the system that may account for a small part of the observed loss in mass. If the telomer acids and PFOA were the only primary metabolites of telomer alcohol degradation as observed in this study, then it is suggested that these compounds fail to undergo extensive defluorination; hence, expected fluoride concentrations would be low. The high amounts of chloride present in the matrix (mineral medium) prevented the detection limits for fluoride to be any lower than 1 mg L^{-1} . The use of a fluoride specific electrode was also considered, although a similar type of interference was expected from the matrix. This limit of detection for fluoride was too high for the telomer alcohol concentration used in this study; the initial spike of $750 \mu\text{g}$ of 8:2 FTOH would have produced approximately $60 \mu\text{g/L}$ of fluoride (1:2 molar ratio) from the hypothesized pathway where 1 equiv is produced upon generation of the unsaturated acid. The concentration used in this study for dosing the FTOH was chosen to be well below its saturated water solubility, which made it impossible to determine the evolved fluoride. It should be noted that if the FTOH underwent complete defluorination, the resulting fluoride concentration ($>1 \text{ mg/L}$) would have been observable.

A proposed biodegradation scheme (Figure 5) is based upon results of this laboratory study and built on earlier results presented by Hagen et al. (19). Under this proposed pathway, 8:2 FTOH can be oxidized by an alcohol dehydrogenase enzyme, fairly common in bacteria (28–30), to form the 8:2 FTAL. Subsequent oxidation of the terminal carbon leads to the formation of the 8:2 FTCA perhaps via an aldehyde dehydrogenase type of enzyme. Murphy et al. reported the isolation of an aldehyde dehydrogenase enzyme capable of converting fluoroacetaldehyde to fluoroacetate in *Strepto-*

myces cattleya (31). Although this study was looking at the probable enzymes involved in the biosynthesis of fluorinated compounds, it confirms that bacteria indeed possess enzymes capable of mediating such metabolic transformations. This proposed degradation pathway for the fluorotelomer alcohols involves reactions similar to dehydrogenation reactions seen in the conversion of ethanol to acetic acid in the absence of molecular oxygen (32). Dehydrogenation reactions also require the coenzyme nicotinamide adenine dinucleotide (NAD^+), which serves as hydrogen carriers. However, in the presence of molecular oxygen, typical oxidation reactions producing similar products as stated previously may be aided by mixed function oxidases (MFO) or monooxygenase type of enzymes such as cytochrome P450, also widespread in microorganisms, animals, and humans (32). Despite performing the biodegradation experiments under aerobic conditions, the oxygen concentration was not measured; hence, we are unable to definitively determine which of these two types of reactions is involved. The transformation of the 8:2 FTCA leading to its unsaturated form and ultimately to PFOA is an example of a β -oxidation mechanism as previously proposed by Hagen et al. (19). Several critical enzymes are possibly involved in such a mechanism. We suggest that enzymes such as acyl-CoA synthases and crotonases may be required. The oxidation step from the unsaturated acid to PFOA is thermodynamically costly and hence is expected to be slow. This hypothesis was consistent with our observation that PFOA was first detected in the system in the latter phase of the experiment (day 16). This suggests that oxidation of the FTUCA β -carbon is difficult given its high electrophilicity, although it appears to be transformed to the highly persistent perfluorocarboxylic acid (e.g., PFOA). Corollary experiments using the activated sludge from a sewage treatment plant showed that a similar pathway was operable with all the identified degradation products indicated in Figure 5 observed (data not shown). The previous observations suggest that other telomer alcohols may degrade analogously producing their corresponding even perfluorocarboxylic acid

under the proposed mechanism (i.e., 10:2 FTOH may biodegrade producing the perfluorodecanoic acid, and the 6:2 FTOH would form the perfluorohexanoic acid).

The current study showed that telomer alcohols readily biodegrade, producing telomer acids and perfluorinated acids, with the unsaturated telomer acid being the predominant metabolite; we are currently investigating whether the FTUCAs are commonly observed in environmental samples. Microbial transformation reactions such as demonstrated by these experiments have strong implications for other biological transformations since microorganisms can be seen as surrogates for metabolic reactions of higher organisms. Thus, these reactions may serve as probable sources of PFOA and other carboxylic fluorinated acids detected widely in biota and as previously demonstrated by Hagen et al. (19). There are likely several pathways under which telomer alcohols can biodegrade when released into the environment, although it appears that the β -oxidation pathway described previously is a principal fate for these compounds. There exists the potential for α -oxidation of the FTOHs to yield the odd numbered FTCAs recently detected in biota (8); our investigation, however, indicated no evidence for this pathway being operable under these microbial conditions. Further studies are underway to determine the identity of other volatile metabolites observed in these experiments. Hagen et al. (19) were also unable to identify a major metabolite in their experiments. The identity of these unknown volatile metabolites may provide further clues to the existence of alternative degradation pathways. Microbial degradation of telomer alcohols may very well be a primary fate for these compounds since the potential sources for these compounds (i.e., polymers) often end up in sewage treatment plants.

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