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6:2 Fluorotelomer alcohol biotransformation in an aerobic river sediment system

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HIGHLIGHTS

- A novel aerobic sediment system was developed to study FTOH biodegradation in river sediment.
- 5:3 Acid is a predominant product during 6:2 FTOH aerobic biotransformation in river sediment.
- PFPeA, PFHxA, and PFBA also are formed during 6:2 FTOH biotransformation in river sediment.
- 5:3 Acid was sediment-bound and can only be recovered by post-treatment with NaOH and ENVI-Carb™.
- The 5:3 acid can be further degraded to 4:3 acid in river sediment via one-carbon removal pathways.

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ABSTRACT

The 6:2 FTOH [$F(CF_2)_6CH_2CH_2OH$] is a major raw material being used to replace 8:2 FTOH [$F(CF_2)_8CH_2CH_2OH$] to make FTOH-based products for industrial and consumer applications. A novel aerobic sediment experimental system containing 20 g wet sediment and 30 mL aqueous solution was developed to study 6:2 FTOH biotransformation in river sediment. 6:2 FTOH was dosed into the sediment to follow its biotransformation and to analyze transformation products over 100 d. The primary 6:2 FTOH biotransformation in the aerobic sediment system was rapid ($T_{1/2}$ < 2 d). 5:3 acid [$F(CF_2)_5CH_2CH_2COOH$] was observed as the predominant polyfluorinated acid on day 100 (22.4 mol%), higher than the sum of perfluoropentanoic acid (10.4 mol%), perfluorohexanoic acid (8.4 mol%), and perfluorobutanoic acid (1.5 mol%). Perfluoroheptanoic acid was not observed during 6:2 FTOH biotransformation. The 5:3 acid can be further degraded to 4:3 acid [$F(CF_2)_4CH_2CH_2COOH$, 2.7 mol%]. This suggests that microbes in the river sediment selectively degraded 6:2 FTOH more toward 5:3 and 4:3 acids compared with soil. Most of the observed 5:3 acid formed bound residues with sediment organic components and can only be quantitatively recovered by post-treatment with NaOH and ENVI-Carb™ carbon. The 6:2 FTCA [$F(CF_2)_6CH_2COOH$], 6:2 FTUCA [$F(CF_2)_5CF=CHCOOH$], 5:2 ketone [$F(CF_2)_5C(O)CH_3$], and 5:2 sFTOH [$F(CF_2)_5CH(OH)CH_3$] were major transient intermediates during 6:2 FTOH biotransformation in the sediment system. These results suggest that if 6:2 FTOH or 6:2 FTOH-based materials were released to the river or marine sediment, poly- and per-fluorinated carboxylates could be produced.

1. Introduction

Perfluoroalkyl sulfonates (PFSAs) and perfluoroalkyl carboxylates (PFCAs) are persistent substances detected in the environment, biota, and humans (Harada et al., 2007; Shoeib et al., 2010; Ahrens, 2011; Goosey and Harrad, 2011; Zhang et al., 2011). In particular, these perfluorinated substances are detected in surface waters (Takagi et al., 2008; Quinete et al., 2009; Li et al., 2011) and sediment (Pan and You, 2010; Li et al., 2011). The sources of PFSAs and PFCAs detected in the environment including aquatic sediment may come from direct emission or indirect precursor abiotic and biodegradation (Prevedouros et al., 2006; Cousins et al., 2011). Fluorotelomer alcohols [FTOHs, $F(CF_2)_nCH_2CH_2OH$, n = 6, 8, 10] are raw materials used to manufacture FTOH-based polymeric and surfactant products with broad commercial and industrial applications (Kissa, 2001; Prevedouros et al., 2006). For example, FTOH-based products are used in textiles, papers, carpet, paint, and lubricating oil due to their water- and oil-repellent properties (Kissa, 2001). FTOHs and perfluoroalkane sulfonamido alcohols are some of the potential precursors and can lead to PFSAs and PFCAs from their abiotic (Ellis et al., 2004; Martin et al., 2006) and aerobic

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biodegradation in soil and activated sludge (Wang et al., 2005a,b, 2009; Liu et al., 2007b, 2010a,b; Rhoads et al., 2008).

Historically, 8:2 FTOH [$F(CF_2)_8CH_2CH_2OH$] was a major FTOH raw material which has been used since the 1970s to manufacture FTOH-based products (Prevedouros et al., 2006). Previous reports show that 8:2 FTOH can be biotransformed in various environmental matrices (e.g., activated sludge and soil) to form perfluorooctanoic acid (Wang et al., 2005a,b, 2009; Liu et al., 2007b), a PFCA with a long elimination half-life in biota. Recently, 6:2 FTOH [$F(CF_2)_6CH_2CH_2OH$] is being used to replace 8:2 FTOH as an industrial raw material (Ritter, 2010) to eliminate the potential formation of long-chain ($\geqslant$8 carbons) PFCAs in the environment (OECD, 2012). If 6:2 FTOH-based products were released to the environment, these products could biodegrade to 6:2 FTOH. The 6:2 FTOH then could be further degraded in various environmental matrices or to be partitioned to the surface soil, water, sediment, or to be transported to the atmosphere. The 6:2 FTOH residual raw material content in 6:2 FTOH-based polymeric products is generally less than 0.1%, which is much less than that of the historic 8:2 FTOH-based products (Russell et al., 2008).

Limited information is available on 6:2 FTOH biodegradation potential and biotransformation pathways in the environment. So far, only two reports are available on 6:2 FTOH biotransformation in soil and bacterial enrichment culture (Liu et al., 2010a,b). These studies show that perfluoropentanoic acid (PFPeA) is the predominant product (~30 mol%) in aerobic soil, followed by 5:3 acid [$F(CF_2)_5CH_2CH_2COOH$, 15 mol%], perfluorohexanoic acid (PFHxA, 8 mol%), and perfluorobutanoic acid (PFBA, 2 mol%) from 6:2 FTOH biotransformation in soil. Additionally, it was found that ~25% of 6:2 FTOH starting material was conjugated to soil organic components to form soil-bound residues, which may not be available for further biotransformation by soil microbes (Liu et al., 2010b).

Sediment is an important environmental sink for most organic pollutants. During the life cycle, 6:2 FTOH-based products may be emitted into surface water and sediment via direct discharge or effluent release from wastewater treatment plants and potentially impact water quality and aquatic environment. For example, unreacted 6:2 FTOH and transformation products could be discharged into a river, where partitioning into the sediment and further transformation of these compounds may occur. Another route of entry is the potential runoff of 6:2 FTOH and transformation products from biosolids applied to surface soil. No information is available on the biodegradability of FTOHs such as 6:2 FTOH in a sediment environment. Sediment microbial populations may be different from those in soils and other environmental matrices (Nealson, 1997; Tate, 2000). The 6:2 FTOH biotransformation in sediment may yield different transformation products that are relevant to potential environmental exposure. Furthermore, the unique combination of aqueous and sediment phases in a river sediment system may affect 6:2 FTOH air-aqueous-sediment partitioning behavior with potential to form sediment-bound residues. Therefore, it is essential to understand 6:2 FTOH biotransformation in sediment systems to understand the potential 6:2 FTOH contribution to environmental loadings of PFCAs and to identify novel transformation products that may impact aquatic life and water quality. Recently, the aquatic toxicity of a number of FTOH biotransformation products has been presented (Hoke et al., 2012).

The traditional flow-through method (OECD, 2002) designed to study biodegradation of other water soluble and non-volatile industrial chemicals in river sediment is not suitable to study 6:2 FTOH, which is volatile (Krusic et al., 2005), has low water solubility, and is highly absorptive to organic matter in environmental matrices (Liu and Lee, 2007a). The experimental system with multiple tubing and trapping devices described in the OECD guideline is very cumbersome and hinders recovery of fluorinated chemicals with a propensity to be absorbed to the tube surfaces. To alleviate these potential problems that would affect mass balance and study integrity, a modified novel method (Fig. 1) was developed to achieve efficient aeration and simultaneously capture of volatile transformation products during 6:2 FTOH biotransformation in a sediment system as required in the OECD study guideline.

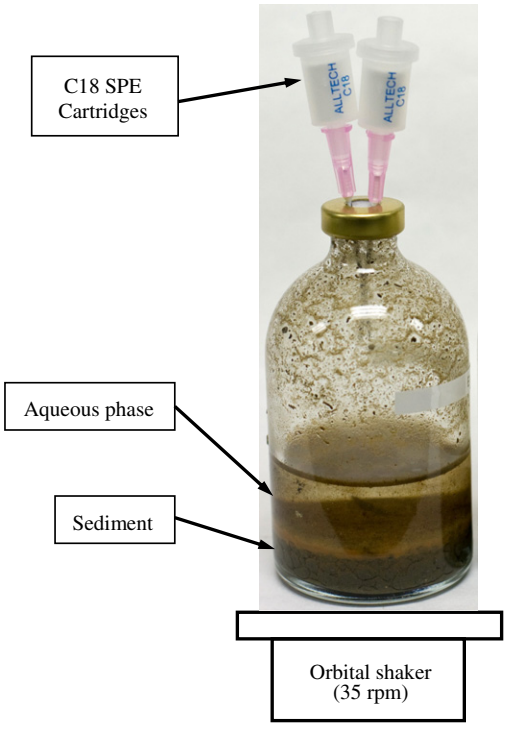

Fig. 1. Illustration of experimental devices of an aerobic sediment system. The main feature of the system is the use of two C18 SPE cartridges to capture volatile parent and transformation products from the headspace of a 119-mL glass serum bottle and also to ensure aeration of the aqueous phase. An orbital shaker (not drawn to scale) with slow motion (~35 rpm) further kept the aqueous phase being constantly aerated but not disturbed the sediment.

The objective of this study was to investigate 6:2 FTOH aerobic biotransformation potential in a sediment system, to identify transformation products, and to determine whether or not 6:2 FTOH biotransformation in sediment environment is different from that in soil. This is the first report of 6:2 FTOH aerobic biotransformation in a river sediment system.

2. Materials and methods

2.1. 2.1 Chemicals

The purity of 6:2 FTOH and other fluorinated chemicals and analytical standards were all above 97%. The details of these chemical standards including acronyms, structures, CAS numbers and sources were listed in an early study (Liu et al., 2010a). The 4:3 acid [$F(CF_2)_4CH_2CH_2COOH$] used in this study was newly synthesized and has a purity of 96%. Stable isotope quantification internal standards used in LC/MS/MS (liquid chromatography/tandem mass spectrometry) analysis were [1,1,2,2-D;3-^{13}C] 6:2 FTOH [$F(CF_2)_5{}^{13}CF_2CD_2CD_2OH$] (DuPont, Wilmington, DE) and [1,2-^{13}C] PFHxA [$F(CF_2)_4{}^{13}CF_2{}^{13}COOH$] (Wellington Laboratories, Ontario, Canada). All solvents were HPLC grade or higher and all other chemicals referenced were at least reagent grade. De-ionized water ($\geqq$18 MΩ cm) was from a Barnstead E-Pure system used throughout all the experiment. Omnisov® water (EMD Chemicals, Gibbstown, NJ) was used For LC/MS/MS analysis.

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2.2. Sediment and river water collection

The river sediment was collected from Brandywine Creek, PA at the position of Latitude 39° 51 min 34 s, longitude 75° 35 min 55 s, and 78 m above sea level. River water was also collected from the same location. The cobblestones and decayed leaves were discarded from the sediment after bringing the sediment to the laboratory. The sediment physicochemical characteristics were determined by Agvise Laboratories (Northwood, ND). The collected sediment was sandy loam (67% sand, 24% silt, and 9% clay) with 5.3% organic matter or 3.1% organic carbon content. The pH of the sediment was 6.9 and the cation exchange capacity (CEC) was 86 mmol kg^{-1} sediment. The detailed characteristics of the sediment are listed in the Supplementary material (Table S1). Some sediment and river water were autoclaved as sterile control to assess the integrity of the experiment.

2.3. 6:2 FTOH biotransformation in the sediment system

The biotransformation was conducted in 119-mL glass serum bottles. Twenty grams wet sediment containing 9.3 g dry weight and 10.7 mL river water, 25 mL pure river water, and 5 mL mineral media were added sequentially into each test vessel. The mineral medium solution contained 85 mg L^{-1} of KH_2PO_4, 218 mg L^{-1} of K_2HPO_4, 334 mg L^{-1} of $Na_2HPO_4{\cdot}2H_2O$, 5 mg L^{-1} of NH_4Cl, 36.4 mg L^{-1} of $CaCl_2{\cdot}2H_2O$, 22.5 mg L^{-1} of $MgSO_4{\cdot}7H_2O$, and 0.25 mg L^{-1} of $FeCl_3{\cdot}6H_2O$ with a pH of 7.0. For the sterile control, autoclaved sediment and river water were used instead and triple antibiotics (kanamycin, chloramphenicol, and cycloheximide) were added to the sterile sediment to a final concentration of 200 mg kg^{-1} sediment. The sample bottles containing live and sterile sediment were crimp-sealed with butyl rubber stoppers and aluminum caps and incubated at room temperature for 5 d before dosing 6:2 FTOH to initiate the experiment.

After the pre-incubation, each bottle was inverted and 10 μL of 6:2 FTOH stock solution (5000 mg L^{-1}) made in 50% ethanol (v/v, ethanol:water = 1:1) was injected into the sediment with a 10-μL glass microsyringe. The initial 6:2 FTOH dosing concentration was determined based on the LC/MS/MS detection limit that would allow low levels (1–2% of initially applied 6:2 FTOH) of transformation products to be quantified and environmentally relevant. Some of the live sediment sample bottles after pre-incubation were only dosed with 10 μL of 50% ethanol into each bottle as live matrix control for monitoring headspace O_2 content during the biotransformation and also serving as background blank for LC/MS/MS analysis. After dosing with 6:2 FTOH or 50% ethanol, the bottle was gently shaken to disperse the dosed solution throughout the sediment system and then the bottle was inverted back to upright position (Fig. 1). Two C18 SPE cartridges (0.6 g sorbent each, Alltech, Deerfield, IL) pre-activated with acetonitrile (CH_3CN), were coupled with two 18-gauge needles, which were pushed into the headspace of each sample bottle. These two cartridges enabled air exchange between headspace and ambient air and also captured 6:2 FTOH and other volatile intermediates during biotransformation. After dosing with 6:2 FTOH or 50% ethanol and connecting with C18 cartridges, the sample bottles were kept static for 10–15 min to settle the sediment before the bottles were shaken continuously at about 35 rpm on an orbital shaker at room temperature. The 35 rpm low-velocity motion ensured aqueous phase aeration but did not disturb the settled sediment phase. At each sampling time (days 0, 2, 7, 14, 28, 56, and 100), three live, three sterile control, and two live matrix control bottles were sacrificed for sampling and processing to analyze 6:2 FTOH and transformation products. The day 0 sample bottles were processed within 15 min after dosing with 6:2 FTOH stock solution or 50% ethanol.

2.4. Sampling and sample processing

At each sampling time except day 0, the O_2 content in the headspace of the live matrix control bottles (dosed with only 50% Ethanol) was measured using a headspace Oxygen Analyzer Model 905 (Quantek Instruments, Grafton, MA) to estimate the aerobic condition of the live sediment system. The C18 cartridges were disconnected from all the three live, three sterile control, and two live matrix control bottles and each was eluted with 5 mL CH_3CN for LC/MS/MS analysis. Each of the butyl rubber stoppers from the bottles was removed and transferred to a glass vial containing 5 mL CH_3CN to extract 6:2 FTOH and other volatile products. The aqueous phase from each bottle was decanted to a glass bottle containing 60 mL CH_3CN for extraction of 6:2 FTOH and transformation products. Forty milliliters of CH_3CN was added to the remaining sediment of each bottle, which was immediately crimp-sealed with a fresh butyl rubber stop and aluminum cap for the extraction. All extractions were carried out at 50 °C for 2–5 d on an orbital shaker kept at 200 rpm. The extract solution (first extract) from each of the sediment sample bottles was decanted to a glass container after centrifugation at ~1000 rpm (162*g*) with a Sorval GSA rotor for 15–20 min. The remaining sediment was extracted again with 40 mL CH_3CN plus 40 μL of 5 M NaOH at 50 °C overnight and the extract solution (2nd extract) was decanted to a glass container after the centrifugation. All the processed sample solutions described above were filtrated through nylon filters (0.45 μm pore) and stored at −10 °C before subject to LC/MS/MS analysis.

2.5. Sediment extract clean-up to recover sediment-bound 5:3 acid

Previous work (Liu et al., 2010b) demonstrated that 5:3 acid formed during 6:2 FTOH biotransformation in soil was conjugated to soil organic components as bound residues and could only be partially recovered for quantification by aggressively digesting the sample extract with base plus ENVI-Carb™ graphitized carbon (Supelco, Bellefonte, PA) to break up the bound residues. Similar post-treatment procedures were used to quantitatively recover 5:3 acid that may have been bound to sediment organic components. Typically, 0.4 mL sediment extract (1st or 2nd extract) was mixed with 1.2 mL CH_3CN plus 45 μL of 1 M NaOH and 25–50 mg ENVI-Carb™ graphitized carbon. The mixture was incubated for 3–6 h at 50 °C in an orbital shaker at ~200 rpm. After centrifugation at ~2000 rpm, the supernatant was decanted and filtered through nylon filters (0.45 μm pore) for LC/MS/MS analysis. Previous work (Liu et al., 2010b and unpublished work) demonstrated that 6:2 FTOH, 5:2 sFTOH, 6:2 FTUCA, and 5:3 acid are stable in 50 mM NaOH solution at 50 °C for at least 7 d. However, under such conditions, 5:2 ketone was converted to 5:2 sFTOH and 6:2 FTCA to 6:2 FTUCA. Therefore, the base treatment plus ENVI-Carb™ clean-up was only used to recover and account for the portion of 5:3 acid that was not quantifiable in acetonitrile extracts without such treatment.

2.6. LC/MS/MS quantitative analysis

All the processed sample solutions from C18 cartridge eluent, stoppers, aqueous phase, and sediment extractions were analyzed individually. Before analysis, each processed sample solution from sediment and aqueous extracts and C18 cartridge and septa eluent was spiked with internal standards at 50 μL mL^{-1} containing 200 μg L^{-1} of [1, 2-^{13}C] PFHxA and 5000 μg L^{-1} of [1,1,2,2-D; 3-^{13}C] 6:2 FTOH for quantifying 6:2 FTOH precursor and transformation products. The analysis was done with a 2795 HPLC/Micromass Quattro Micro-system (Waters, (Milford, MA) performed in negative electro-spray ionization mode with multiple reaction monitoring. Detailed information on the instrumental parameters

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is provided in previous study (Liu et al., 2010a) and Table S2. The mobile phases consisted of two parts: A, 0.15% acetic acid in nano-pure water; B, 0.15% acetic acid in CH_3CN. Twenty microliter of a calibration standards or sample were injected to the system and the separation was accomplished by an Agilent Zorbax RX-C8 column (150 mm × 2.1 mm, 5 μm particle size, pore size 80 Å, not end-capped, carbon loading 5.5%) at a flow rate of 0.4 mL min^{-1} of gradient mobile phases (Table S2).

3. Results and discussion

3.1. Experimental system and mass balance

Fig. 1 shows a novel aerobic sediment experimental system used in this study. The O_2 content in the headspace of the sterile controls was about 21% (v/v) and of live matrix controls averaged about 19% during the 100-d period. Because the aqueous phase was under constant shaking and aeration, the aqueous phase and surface sediment were aerobic. However, the sediment near the bottom might be anoxic. Such setting is as close in a river sediment environment as a laboratory-simulation system can achieve given the unique property of the 6:2 FTOH used. The 6:2 FTOH transformation rate and observed transformation products (see Section 3.2) confirmed the system overall was aerobic similar to that of soil (Liu et al., 2010a). This suggests that our modified experimental system can achieve aeration as efficiently as that of a flow-through system as described in the OECD test guideline 308 (OECD, 2002). Because multiple tubing and trapping devices were replaced by two C18 cartridges, sample processing was simplified and potential loss of 6:2 FTOH and other transformation products was minimized.

Quantitative recovery was achieved for 6:2 FTOH from sterile controls and for 6:2 FTOH and quantifiable transformation products from live sediment system. In sterile sediment control samples, only 6:2 FTOH was detected and ranged between 86 and 98 mol% of day 0 concentration (Fig. 2) over 100 d. The recovery of 6:2 FTOH and quantifiable transformation products in live samples ranged 71–88 mol% of initially applied 6:2 FTOH (Fig. 2) over 100 d. The bound residues formed between live sediment and 6:2 FTOH or 5:3 acid catalyzed by microbial enzymes may explain the slightly lower recovery in live sediment system versus sterile controls, as also occurred in soil (Liu et al., 2010b). For example, bound residues of ^{14}C-labeled 6:2 FTOH or 5:3 acid formed in live soil accounted for about 25 mol% of initially applied ^{14}C and cannot be quantified by LC/MS/MS analysis (Liu et al., 2010b).

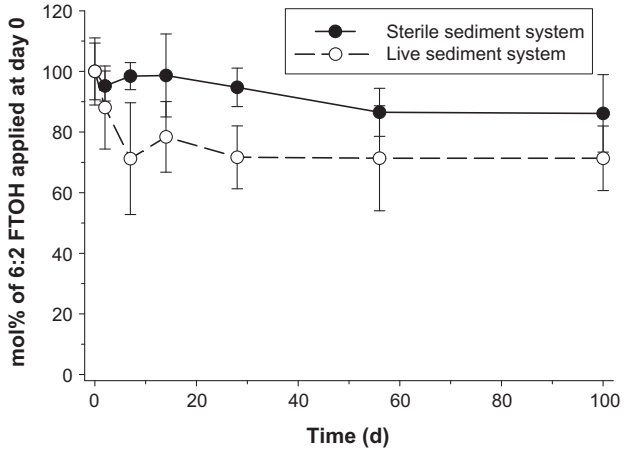

Fig. 2. Total mol percentage recovery (mass balance) of 6:2 FTOH and quantified transformation products in live aerobic sediment system ($n = 3$) and sterile sediment system ($n = 3$) over 100 d. Fifty microgram of 6:2 FTOH was applied to each 119-mL glass serum bottle containing 20 g wet sediment, 25 mL river water, and 5 mL mineral medium. The mass balance was calculated based on the total moles of 6:2 FTOH and transformation products recovered from the sediment, aqueous phase, and headspace during 6:2 FTOH biotransformation in comparison with that at day 0.

The partition of sediment-dosed 6:2 FTOH and subsequently formed transformation products are shown in Fig. S1 of Supplementary material. In sterile controls, 51 mol% of the sterile sediment-dosed 6:2 FTOH still remained in the sediment phase, 3.4 mol% was partitioned to the aqueous phase, and 32 mol% was volatized to the headspace on day 100. In live samples, 39 mol% of the sediment-dosed 6:2 FTOH and formed transformation products still remained in the sediment phase, 16 mol% was partitioned to the aqueous phase, and 15 mol% was volatized to the headspace on day 100. The biotransformation of 6:2 FTOH in live sediment system to non-volatile poly- and per-fluorinated carboxylates under neutral pH explains the lower levels of volatiles captured in the headspace of live samples. The non-recoverable portion of the 5:3 acid or 6:2 FTOH bound residues was not accounted for in this study and will be investigated in the future using a similar sediment system dosed with ^{14}C-labeled 6:2 FTOH [$F(CF_2)_6{}^{14}CH_2{}^{14}CH_2OH$], which allows the detection of such bound residues by either thermal combustion or harsh concentrated hydrochloric acid extraction.

3.2. 6:2 FTOH biotransformation products and molar yields

For sediment not dosed with 6:2 FTOH (live matrix control), PFCAs were detected: PFBA was at ~8 ng g^{-1} dry sediment, PFPeA and PFHxA both at ~3 ng g^{-1}, and PFHpA at ~2 ng g^{-1}. No other potential transformation products were detected above the limit of detection which is described in Table S2.

At least 9 transformation products were observed and quantified during 6:2 FTOH biotransformation in the sediment system (Fig. 3). The 6:2 FTOH half-life in live sediment system was estimated to be 1.8 d, similar to that observed in aerobic soil (1.3 d) and mixed bacterial culture (1.6 d) (Liu et al., 2010a). This suggests that microbes in the sediment system rapidly transformed 6:2 FTOH to form various transient intermediates and poly- and perfluorinated carboxylic acids. After the initially rapid decrease, the 6:2 FTOH level was relatively constant after day 28, accounting for 3.9–6.1 mol% of initially applied concentration at day 0.

The 6:2 FTCA [$F(CF_2)_6CH_2COOH$], 6:2 FTUCA [$F(CF_2)_5CF = CHCOOH$], 5:2 ketone [$F(CF_2)_5C(O)CH_3$], and 5:2 sFTOH [$F(CF_2)_5CH(OH)CH_3$] are major transient intermediates observed during 6:2 FTOH biotransformation in the sediment system. The 5:3 Uacid [$F(CF_2)_5CH = CHCOOH$] was not quantified due to lack of authentic analytical standard but is expected to be a 5:3 acid precursor (Liu et al., 2010a). The levels of 6:2 FTCA and 6:2 FTUCA peaked on day 2 with 23.5 mol% and 7 mol%, respectively, and decreased markedly afterward due to further transformation to other downstream products in the 6:2 FTOH biotransformation pathways (Fig. 5). This is in sharp contrast to soil, where no 6:2 FTCA and 6:2 FTUCA were observed due to their rapid transformation to down steam products (Liu et al., 2010a). The volatile 5:2 ketone level peaked on day 7 at 7.1 mol% and stayed relative constant until day 56 and then decreased to 1.5 mol% on day 100 due to its near complete transformation to 5:2 sFTOH and PFCAs (Figs. 3 and 5). The 5:2 sFTOH is the dominant transient intermediate in the sediment, accounting for 34.6 mol% at day 28 and then decreased steadily to 20.2 mol% at day 100 (Fig. 3). Most of the 5:2 sFTOH observed at day 100 was captured by C18 cartridges from the headspace and thus was not likely to be available for further biodegradation in the test system to form PFCAs. Similarly, 15 mol% of 5:2 sFTOH was observed in the headspace at day 90 in soil dosed with 6:2 FTOH at day 90 (Liu et al., 2010a).

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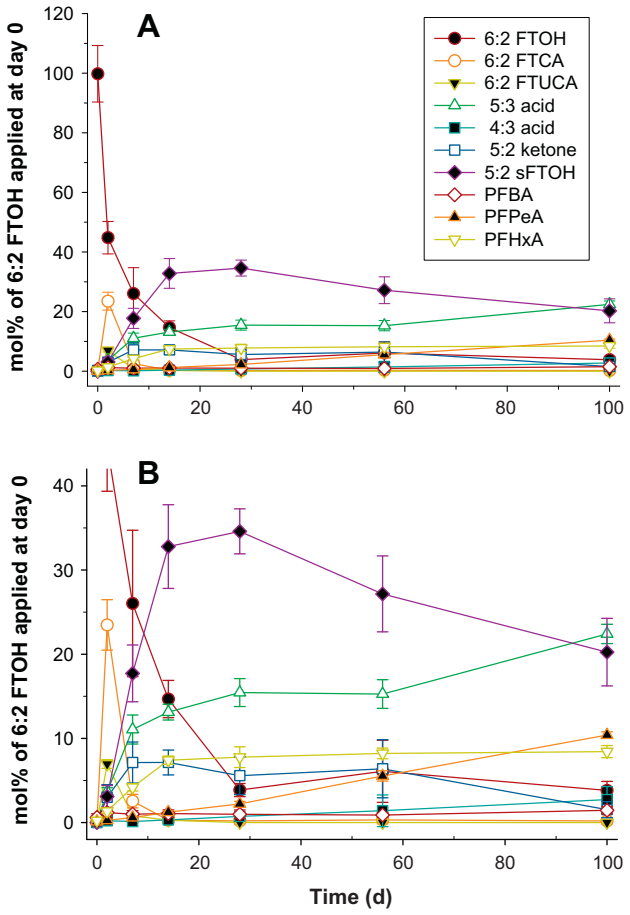

Fig. 3. The molar yield of individual transformation products during 6:2 FTOH biodegradation in aerobic sediment system ($n = 3$) over 100 d. Graph B is a zoom view of (A) to show the trend of individual transformation products over time. The molar yield was calculated based on the total moles of 6:2 FTOH or individual transformation products recovered from the sediment, aqueous phase, and headspace during 6:2 FTOH biotransformation in comparison with that at day 0.

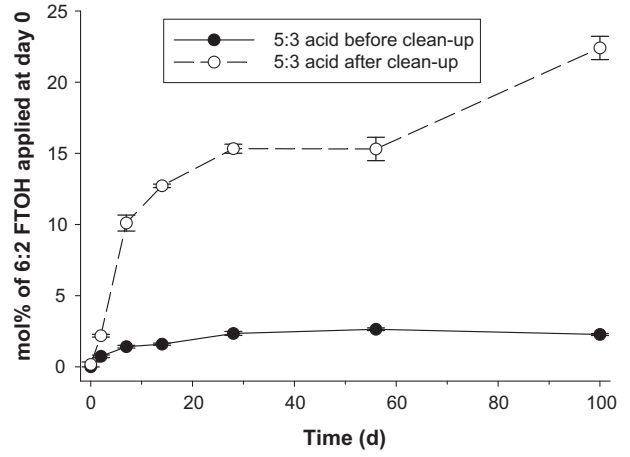

Fig. 4. Post-treatment of live sediment extraction solution with NaOH (~37 mM final concentration) plus ENVI-Carb™ graphitized carbon (25–50 mg) to enhance recovery of sediment-bound 5:3 acid. Some of the error bars are not visible as they are smaller than the symbol height.

The 5:3 acid [$F(CF_2)_5CH_2CH_2COOH$] is the most abundant polyfluorinated x:3 acid formed during 6:2 FTOH biotransformation in the sediment. Its level steadily increased over time and on day 100 reached 22.4 mol% of initially applied 6:2 FTOH, higher than the sum (20.3 mol%) of PFBA, PFPeA, and PFHxA. Although we cannot rule out the possible variation of 5:3 acid molar yield in other sediment systems, this is a first study showing highest 5:3 acid yield in environmental matrices studied so far. This suggests that the river sediment system may have different microbial populations to selectively degrade 6:2 FTOH more toward the *x*:3 acids (e.g., 5:3 acid and 4:3 acid) than in soil, where 6:2 FTOH was biodegraded more toward PFCAs with molar yields about 2.5 times higher than that of the *x*:3 acids (Liu et al., 2010a). Prokaryotes such as bacteria are more dominant in aquatic or marine sediment environment (Nealson, 1997) whereas bacterial and fungus populations co-exist in soils (Tate, 2000).

Most of the 5:3 acid in the sediment formed bound residues with sediment organic components catalyzed by microbial enzymes, as also occurred in soil (Liu et al., 2010b). The 5:3 acid in the sediment-5:3 acid bound residues can only be recovered by post-treatment with NaOH and ENVI-Carb™ graphitized carbon to break up the bound residues. As a result, the recovery of 5:3 acid was enhanced up to 10 times for day 100 live sediment samples (Fig. 4). Such enhancement in 5:3 acid recovery is much less pronounced than that in soil with up to 38-fold (Liu et al., 2010b), suggesting that the formation of sediment-5:3 acid bound residues is less extensive and 5:3 acid is more available for further biodegradation compared with soil. This is because there is larger amount of polar groups in soil organic matter with plant origin as compared to sediment organic matter derived largely from animal residues (Schwarzenbach et al., 2003). The polar groups are responsible for the forming of bound residues with polar organic chemicals. Indeed, the 5:3 acid can be further degraded to 4:3 acid (2.7 mol%) via novel "one-carbon removal pathways" to remove one $-CF_2-$ group as occurred in activated sludge (Wang et al., 2012). The 4:3 acid conversion ratio from 5:3 acid is 12.1 mol% (2.7 mol%/22.4 mol%), which is close to that in activated sludge at 14.2 mol% (Wang et al., 2012) and is much faster than that in soil at 2.3 mol% (Liu et al., 2010a). The α-OH 5:3 acid [$F(CF_2)_5CH_2CH(OH)COOH$], a newly identified major transient intermediate of 5:3 acid biotransformation (Wang et al., 2012), was not observed in this study due to its rapid conversion to 4:3 acid via the one-carbon removal pathways.

The PFCA profile from 6:2 FTOH biodegradation in sediment is similar to that in soil (Table 1), with PFPeA accounting for 10.4 mol%, PFHxA for 8.4 mol%, and PFBA for 1.5 mol%. However, PFPeA molar yield in the sediment system dosed with 6:2 FTOH is about three times lower than that in soil at 30 mol% (Liu et al., 2010a), indicating that microbes in the sediment may prefer to metabolizing 6:2 FTUCA to 5:3 acid (see Section 3.4 for detailed discussion). This means that *x*:3 acids (e.g., 5:3 and 4:3 acids) may be present in sediment formed from biotransformation of 6:2 FTOH that has entered the aquatic environment. Perfluoroheptanoic acid (PFHpA) was not detected in the sediment system with the limit of detection at 0.2 μg L^{-1} during 6:2 FTOH biotransformation as also not occurred in soil (Liu et al., 2010a), suggesting that 6:2 FTOH biotransformation may not contribute to PFHpA detected in the environment.

3.3. The x:3 acids are unique transformation products from FTOH biodegradation

8:2 FTOH and 6:2 FTOH biodegradation in soil, activated sludge, and *Pseudomonas olevoran* pure bacterial culture leads to two major classes of transformation products, PFCAs and *x*:3 acids [$F(CF_2)_xCH_2CH_2COOH$, $x = 3, 4, 5, 7$] (Wang et al., 2009; Liu et al., 2110a; Kim et al., 2012). Although PFCAs detected in the environment can come from both direct emission or indirect precursor degradation (Buck et al., 2011), these *x*:3 acids can only come from

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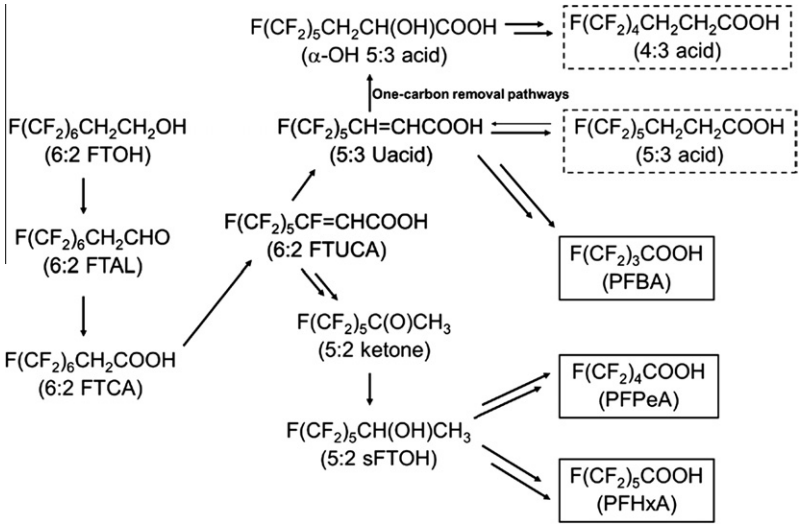

Fig. 5. 6:2 FTOH biotransformation pathways in aerobic sediment system. The solid arrows indicate transformation steps based on observed transformation products in this study. The double arrows indicate multiple transformation steps. The transformation products inside the solid rectangular boxes are perfluorinated carboxylic acids and inside the dashed rectangular boxes are *x*:3 acids.

Table 1
Comparison of molar yields of *x*:3 acids and PFCAs from 6:2 FTOH biodegradation in aerobic soil (Liu et al., 2010a) and aerobic sediment system (this study).

Transformation Product	Soil (mol%)	Sediment system (mol%)
X:3 acids		
5:3 acid	15	22.4
4:3 acid	1.0	2.7
Sum	16	25
PFCAs		
PFHxA	8	8.4
PFPeA	30	10.4
PFBA	2	1.5
Sum	40	20

6:2 FTOH and 8:2 FTOH biotransformation, not from abiotic or industrial sources. For example, 8:2 FTOH biotransformation leads to 7:3 acid but not 6:3 acid (Wang et al., 2009) and 6:2 FTOH leads to 3:3, 4:3, and 5:3 acids (Liu et al., 2010a; Wang et al., 2012).

The *x*:3 acids would likely to be detected if FTOHs were released to the environment, because the molar yields of these *x*:3 acids are in the same order of magnitude as that of PFCAs during FTOH biotransformation as described before. Also, *x*:3 acids can be immobilized to the sediment and soil via sediment- or soil-bound residues as described previously whereas PFCAs, as the ionized form under normal environmental conditions (Webster and Ellis, 2011), have much higher mobility. Thus *x*:3 acids may be selectively enriched in and bound to sediment or soils compared with PFCAs if FTOHs were released there. Consequentially, simultaneous detection of both PFCAs and *x*:3 acids in different environmental matrices would indicate at least partial contribution of FTOHs to the PFCAs observed. Conversely, if detected PFCA levels were much higher than that of *x*:3 acids, most of the PFCAs found in the environment would likely come from PFCA direct emission or other sources.

3.4. 6:2 FTOH biotransformation pathways in aerobic sediment

The 6:2 FTOH biotransformation pathways in the river sediment are similar to that in soil although the molar yields of the PFCAs and *x*:3 acids are different as described earlier. Fig. 5 is based on elucidated biotransformation pathways in soil (Liu et al., 2010a) and most recent work on 5:3 acid biotransformation pathways (Wang et al., 2012). The 6:2 FTOH was initially transformed to 6:2 FTUCA via three enzymatic steps as described (Liu et al., 2010a). The 6:2 FTUCA is an important transient intermediate which can be converted eventually either to *x*:3 acids or to PFCAs.

For *x*:3 acids to be formed, 6:2 FTUCA was first defluorinated using the reducing energy [NAD(P)H] in microbial cells to form 5:3 Uacid, which was then converted to 5:3 acid (Liu et al., 2010a,b). The 4:3 acid was formed via "one-carbon removal pathways" (Wang et al., 2012), in which 5:3 Uacid was hydroxylated on α-carbon to form α-OH 5:3 acid. The α-OH 5:3 acid can be rapidly converted to 4:3 acid via 5 additional enzymatic steps along the "one-carbon removal pathways" (Wang et al., 2012). It was hypothesized (Liu et al., 2010a) that 5:3 Uacid in soil can be converted to ß-OH 5:3 acid (or 3-OH 5:3 acid), whose further degradation pathways were not understood. It is found recently that 5:3 Uacid can only be hydroxylated on α-carbon to form α-OH 5:3 acid (Wang et al., 2012) and therefore ß-OH 5:3 acid was unlikely a transient intermediate formed during 6:2 FTOH biotransformation in the river sediment.

For PFCAs to be formed, 6:2 FTUCA was transformed to 5:2 ketone via multiple enzymatic steps yet to be elucidated and 5:2 ketone can be further reduced to 5:2 sFTOH in one enzymatic step (Liu et al., 2010a). The 5:2 sFTOH is a direct precursor to PFPeA and PFHxA (Liu et al., 2010a), although the multiple enzymatic reactions converting 5:2 sFTOH to these PFCAs are still unknown. The 5:2 sFTOH is very volatile and most of it was detected in the headspace on day 100 in the river sediment system and was not likely available for further biodegradation to PFPeA and PFHxA. Therefore, future studies to investigate 5:2 sFTOH atmospheric degradation are warranted.

The more selective degradation of 6:2 FTOH in the river sediment toward 5:3 and 4:3 acids compared with soil is rather desirable since both *x*:3 acids can be further degraded whereas PFCAs formed are likely persistent in the environment. Microbial communities in the sediment system dominated with potential *x*:3 acid degraders could be able to draw 6:2 FTUCA metabolism toward *x*:3 acids and thereby reduce PFCA production if 6:2 FTOH were released to the environment.

4. Conclusions

6:2 FTOH biotransformation in an aerobic sediment system led to 5:3 acid as the major transformation product on day 100 with

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molar yields higher than the sum of PFCAs, suggesting that microbes in the sediment system selectively degraded 6:2 FTOH more toward the *x*:3 acids (e.g., 5:3 acid and 4:3 acid) compared with soil. Most of the 5:3 acid in the sediment formed bound residues with sediment organic components and can only be quantitatively recovered by post-treatment with NaOH and ENVI-Carb™ graphitized carbon to break up the bound residues. The 5:3 acid can be further degraded to 4:3 acid via novel "one-carbon removal pathways" to remove one $-CF_2-$ group. The PFPeA, PFHxA, and PFBA were steadily formed during 6:2 FTOH biotransformation along with the major transient intermediate 5:2 sFTOH. PFHpA was neither observed during 6:2 FTOH biotransformation in the sediment, nor in soil previously, indicating that 6:2 FTOH is not a source of PFHpA in the environment via biotransformation. The *x*:3 acids (e.g., 7:3, 5:3, and 4:3 acids) are unique transformation products from FTOH biodegradations and may be used as indicators of emission sources from FTOH-based chemistry in the environment. Our work provides new insights and practical knowledge to conduct environmental monitoring and to understand the sources and fate of poly- and per-fluorinated chemicals in the environment. Future work needs to investigate 6:2 FTOH biodegradation potential in other aerobic environmental matrices such as activated sludge from WWTPs and under anaerobic conditions in marine sediment and anaerobic digester under sulfate-reducing and methanogenic conditions, respectively. It is also desirable to isolate and identify *x*:3 acid degrading microbes to reduce PFCA loadings to the environment from 6:2 FTOH biodegradation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.06.035.

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